Toxicological profile for

Sugars (fructose)

This ingredient has been assessed to determine potential human health effects for the consumer. It was considered not to increase the inherent toxicity of the product and thus is acceptable under conditions of intended use.

1. Name of substance and physico-chemical properties

1.1. IUPAC systematic name

(3S,4R,5R)-1,3,4,5,6-Pentahydroxyhexan-2-one (PubChem)

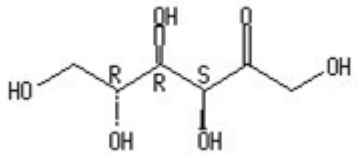
1.2. Synonyms

AI3-23514; Advantose FS 95; CCRIS 3335; D-(-)-Fructose; D-(-)-Levulose; EINECS 200-333-3; Fructose; Fructose, D-; Fruit sugar; Furucton; Hi-Fructo 970; Krystar 300; Levulose; Nevulose; Sugar, fruit; UNII-6YSS42VSEV; arabino-Hexulose; D-Fructose; Fructose, pure; Fructose solution (ChemIDplus)

1.3. Molecular formula

$C_6H_{12}O_6$

1.4. Structural Formula



1.5. Molecular weight (g/mol)

180.16

1.6. CAS registration number

57-48-7

1.7. Properties

1.7.1. Melting point

(°C):100-104 (decomposition) (IUCLID, 2000); 134.5 (EPISuite, 2017); decomposes at 103–105 (D-fructose) (CIR, 2014); 129–130 (DL-fructose) (CIR, 2014); 105–110 or 119 (ChemSpider a)

1.7.2. Boiling point

(°C):372.5 (estimated) (EPISuite, 2017); 440.1 (ChemSpider a)

1.7.3. Solubility

790 g/L at 20°C (IUCLID, 2000); 778 g/L at 20°C (ChemIDplus; EPISuite, 2017); 399 g/L (PubChem)

1.7.4. pKa

12.06 (ChemIDplus)

1.7.5. Flashpoint

(°C): 220±28.7 (estimated) (ChemSpider a); 196.4±28.7 (estimated) (ChemSpider b)

1.7.6. Flammability limits (vol/vol%)

No data available to us at this time

1.7.7. (Auto)ignition temperature

(°C): 360 (PubChem)

1.7.8. Decomposition temperature

(°C):100-105 (IUCLID, 2000; PubChem)

1.7.9. Stability

Stable under ordinary conditions of use and storage.

1.7.10. Vapor pressure

1.91E-008 mm Hg at 25°C (estimated) (EPISuite, 2017); 0±2.4 mmHg at 25°C (estimated) (ChemSpider a); 0.0±2.1 mmHg at 25°C (estimated) (ChemSpider b)

1.7.11. log Kow

-1.55 (estimated) (ChemIDplus; EPISuite, 2017); -1.47 (estimated) (ChemSpider a); -0.33 (estimated) (ChemSpider b)

2. General information

2.1. Exposure

Cosmetics	Yes (Cosmetics Bench Ref.1996)
Food	Yes (Ash, 1995)
Environment	Yes (Merck, 2014)
Pharmaceuticals	Yes (Martindale, 1999)
Tobacco products	In tobaco naturally (Stedman, 1968) and in burnt part

FDA Maximum (Recommended) Daily Dose: ActivityCategory_MCASE_mg	Low
ActivityCategory_MRDD_mmol	Low
ChemClass_MRDD_grouping	Fructose
ChemicalReplicateCount	1 of 2
Dose_MRDD_mg	417.0 mg/kg-bw/day
Dose_MRDD_mmol	2.32 mmol/kg-bw/day

Endpoint	Maximum Recommended Daily Dose
LOGINV_MRDD_mmol	-0.36 mmol
N100_MRDD_mmol	7.6 mmol
Note_FDAMDD	form of drug administered not known
Species	human
StudyType	Clinical Reports
TherapeuticCategory	fluid and nutrient replenisher; ferric form as hematinic

As taken from the US EPA ACToR database, 2015.

An individual's estimated mean intake of fructose is about 13-19 g/day.

Average daily intake of fructose by age and sex	
Aged 1.5 years and over	*National Diet and Nutrition Survey years 1-3 combined (2008/09 - 2010/11)*
Fructose	

	Sex and age group (years)												
	Boys		**Men**		**Girls**		**Women**		**Total**				
	4-10	11-18	19-64	65+	4-10	11-18	19-64	65+	1.5-3	4-10	11-18	19-64	65+
g/day													
Mean	17.2	19.0	17.5	16.8	15.7	15.9	15.5	15.0	12.8	16.5	17.5	16.5	15.8
Median	15.9	16.8	15.6	14.7	14.6	14.2	14.0	12.9	11.3	15.1	15.9	14.6	13.5
sd	9.0	10.4	11.5	10.5	7.8	9.6	9.7	9.5	7.9	8.5	10.1	10.7	9.9
Upper 2.5 percentile	41.1	46.1	47.9	50.8	35.8	39.1	39.9	44.3	32.9	36.8	42.3	43.0	44.3
Lower 2.5 percentile	4.9	3.7	3.2	2.4	3.1	2.9	1.9	2.4	2.4	4.0	2.9	2.7	2.4
% food and drink energy (excluding energy from alcohol)													
Mean	4.1	3.6	3.3	3.4	3.9	3.8	3.8	3.8	4.2	4.0	3.7	3.5	3.6
Median	3.7	3.4	2.8	3.2	3.5	3.4	3.4	3.3	3.8	3.6	3.4	3.1	3.2
sd	2.1	1.8	2.1	1.9	1.9	2.1	2.1	2.2	2.3	2.0	1.9	2.1	2.1
Upper 2.5 percentile	10.1	7.8	8.2	8.2	9.0	8.8	9.0	10.2	10.5	9.2	8.7	8.6	9.3
Lower 2.5 percentile	1.2	0.9	0.8	0.6	0.9	0.8	0.6	0.8	1.1	1.1	0.9	0.7	0.7
% total energy (including energy from alcohol)													
Mean	4.1	3.6	3.1	3.3	3.9	3.7	3.6	3.7	4.2	4.0	3.7	3.4	3.5
Median	3.7	3.4	2.6	3.0	3.5	3.4	3.2	3.1	3.8	3.6	3.4	2.9	3.1
sd	2.1	1.8	2.1	1.8	1.9	2.0	2.0	2.1	2.3	2.0	1.9	2.0	2.0

Upper 2.5 percentile	10.1	7.2	7.8	7.7	9.0	8.8	8.1	9.8	10.5	9.2	8.1	8.0	8.9
Lower 2.5 percentile	1.2	0.9	0.7	0.5	0.9	0.8	0.6	0.8	1.1	1.1	0.9	0.7	0.7
Number of participants (unweighted)	*311*	*342*	*519*	*131*	*302*	*324*	*667*	*174*	*303*	*613*	*666*	*1186*	*305*

As taken from SACN, 2015.

In the EU, fructose is used as a humectant in cosmetics (CosIng. Cosmetic Substances and Ingredients Database). Available at http://ec.europa.eu/growth/tools-databases/cosing/

Fructose (CAS RN 57-48-7) is listed as an ingredient in personal care and pesticide products by the US Department of Health and Human Services (2019).

Added to ice cream to prevent sandiness. Used as a preservative and as an intravenous infusion in parenteral feeding. Used as an additive to food/foodstuffs and in pharmaceuticals. Used as flavoring agent or adjuvant, formulation aid, nutritive sweetener, processing aid, solvent, stabilizer or thickener and texturizer for foods (Haz-Map, 2018).

Frequency and concentration of use (in cosmetics) according to duration and type of exposure:

	# of Uses	**Max. Conc. Of Use (%)**
Totals*	172	1.0001-20
Duration of use		
Leave on	144	0.0002-2
Rinse off	28	0.0001-20
Diluted for (bath) use	NR	NR
Exposure type		
Eye area	10	0.002-0.075
Incidental ingestion	1	NR

Incidental inhalation-spray	1; 62[a]; 50[b]	0.23; aerosol: 0.0002 0.08-2[a]
Incidental inhalation-powder	50[b]	0.002
Dermal contact	153	0.0003-20
Deodorant (underarm)	NR	NR
Hair - Non-coloring	18	0.0001-0.1
Hair - Coloring	NR	NR
Nail	NR	NR
Mucous membrane	4	0.0015-0.002
Baby products	NR	NR

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses
[a] Includes products that can be sprays, but it is not known whether the reported uses are sprays
[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
[c] Includes products that can be powders, but it is not known whether the reported uses are powders NR – Not reported

As taken from CIR, 2014.

“BACKGROUND: Measurement error in self-reported sugars intake may be obscuring the association between sugars and cancer risk in nutritional epidemiologic studies. METHODS: We used 24-hour urinary sucrose and fructose as a predictive biomarker for total sugars, to assess measurement error in self-reported sugars intake. The Nutrition and Physical Activity Assessment Study (NPAAS) is a biomarker study within the Women's Health Initiative (WHI) Observational Study that includes 450 postmenopausal women ages 60 to 91 years. Food Frequency Questionnaires (FFQ), four-day food records (4DFR), and three 24-hour dietary recalls (24HRs) were collected along with sugars and energy dietary biomarkers. RESULTS: Using the biomarker, we found self-reported sugars to be substantially and roughly equally misreported across the FFQ, 4DFR, and 24HR. All instruments were associated with considerable intake- and person-specific bias. Three 24HRs would provide the least attenuated risk estimate for sugars (attenuation factor, AF = 0.57), followed by FFQ (AF = 0.48) and 4DFR (AF = 0.32), in studies of energy-adjusted sugars and disease risk. In calibration models, self-reports explained little variation in true intake (5%-6% for absolute sugars and 7%-18% for sugars density). Adding participants' characteristics somewhat improved the percentage variation explained (16%-18% for

absolute sugars and 29%-40% for sugars density). CONCLUSIONS: None of the self-report instruments provided a good estimate of sugars intake, although overall 24HRs seemed to perform the best. IMPACT: Assuming the calibrated sugars biomarker is unbiased, this analysis suggests that measuring the biomarker in a subsample of the study population for calibration purposes may be necessary for obtaining unbiased risk estimates in cancer association studies." As taken from Tasevska N et al. 2014a. Cancer Epidemiol. Biomarkers Prev. 23(12), 2874-83. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25234237

It has been recommended that the dietary intake of "free sugars" (all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and unsweetened fruit juices) does not exceed 5% of total dietary energy (SACN, 2015; WHO, 2015).

"Fructose and sorbitol are used as excipients in their capacity as sweeteners, flavour constituents, modifier of viscosity, humectant, emollient/plasticisers and protein stabiliser in some medicinal products for parenteral use. Both sugars are used in high concentrations as active pharmaceutical ingredients in parenteral nutrition formulations (energy source), as laxatives and osmotic diuretics."

As taken from EMA, 2017.

National Occupational Exposure Survey (1981 - 1983)

Estimated Numbers of Employees Potentially Exposed to Fructose (CAS RN 57-48-7) by Occupation*

Code	Occupation Description (1980)	Total # Employees (Male & Female)	Total # Female Employees
073	CHEMISTS, EXCEPT BIOCHEMISTS	1,555	812
078	BIOLOGICAL AND LIFE SCIENTISTS	229	188
095	REGISTERED NURSES	7,291	6,990
096	PHARMACISTS	331	186
203	CLINICAL LABORATORY TECHNOLOGISTS AND TECHNICIANS	1,466	1,127
216	ENGINEERING TECHNICIANS, N.E.C.	2,401	1,624
223	BIOLOGICAL TECHNICIANS	850	402

225	SCIENCE TECHNICIANS, N.E.C.	775	55
335	FILE CLERKS	227	
368	WEIGHERS, MEASURERS, AND CHECKERS	19	
436	COOKS, EXCEPT SHORT ORDER	86	
446	HEALTH AIDES, EXCEPT NURSING	462	190
453	JANITORS AND CLEANERS	2,241	624
547	SPECIFIED MECHANICS AND REPAIRERS, N.E.C.	831	
633	SUPERVISORS, PRODUCTION OCCUPATIONS	112	46
754	PACKAGING AND FILLING MACHINE OPERATORS	7,354	663
756	MIXING AND BLENDING MACHINE OPERATORS	3,358	693
759	PAINTING AND PAINT SPRAYING MACHINE OPERATORS	78	
764	WASHING, CLEANING, AND PICKLING MACHINE OPERATORS	2	
777	MISCELLANEOUS MACHINE OPERATORS, N.E.C.	615	145
779	MACHINE OPERATORS, NOT SPECIFIED	486	
797	PRODUCTION TESTERS	19	
856	INDUSTRIAL TRUCK AND TRACTOR EQUIPMENT OPERATORS	292	

859	MISCELLANEOUS MATERIAL MOVING EQUIPMENT OPERATORS	156	
888	HAND PACKERS AND PACKAGERS	847	706
889	LABORERS, EXCEPT CONSTRUCTION	6,590	
TOTAL		38,673	14,453

*(1) The estimates for each occupation apply across the surveyed industries in which the agent was observed. Not all industries were surveyed, and not all agents were observed in all surveyed industries. (2) When using the estimates, standard errors associated with estimates should be considered. (3) Potential exposures to a chemical agent are categorized as actual (i.e., the surveyor observed the use of the specific agent) or tradename (i.e., the surveyor observed the use of a tradename product known to contain the specific agent). The estimates presented in the table combine both categories.

As taken from NIOSH, available at https://web.archive.org/web/20111028132002/http://www.cdc.gov/noes/noes2/a1082occ.html

“Introduction: Sugars are major constituents and additives in traditional tobacco products, but little is known about their content or related toxins (formaldehyde, acetaldehyde, and acrolein) in electronic cigarette (e-cigarette) liquids. This study quantified levels of sugars and aldehydes in e-cigarette liquids across brands, flavors, and nicotine concentrations (n = 66). Methods: Unheated e-cigarette liquids were analyzed using liquid chromatography mass spectrometry and enzymatic test kits. Generalized linear models, Fisher's exact test, and Pearson's correlation coefficient assessed sugar, aldehyde, and nicotine concentration associations. Results: Glucose, fructose and sucrose levels exceeded the limits of quantification in 22%, 53% and 53% of the samples. Sucrose levels were significantly higher than glucose [$\chi2(1) = 85.9$, $p < .0001$] and fructose [$\chi2(1) = 10.6$, $p = .001$] levels. Formaldehyde, acetaldehyde, and acrolein levels exceeded the limits of quantification in 72%, 84%, and 75% of the samples. Acetaldehyde levels were significantly higher than formaldehyde [$\chi2(1) = 11.7$, $p = .0006$] and acrolein [$\chi2(1) = 119.5$, $p < .0001$] levels. Differences between nicotine-based and zero-nicotine labeled e-cigarette liquids were not statistically significant for sugars or aldehydes. We found significant correlations between formaldehyde and fructose (-0.22, $p = .004$) and sucrose (-0.25, $p = .002$) and acrolein and fructose (-0.26, $p = .0006$) and sucrose (-0.21, $p = .0006$). There were no significant correlations between acetaldehyde and any of the sugars or any of the aldehydes and glucose. Conclusions: Sugars and related aldehydes were identified in unheated e-cigarette liquids and their composition may influence experimentation in naïve users and their potential toxicity. Implications: The data can inform the regulation of specific flavor constituents in tobacco products as a strategy to protect young people from using e-cigarettes, while balancing FDA's interest in how these emerging products could potentially benefit adult smokers who are seeking to safely quit cigarette smoking. The data can also

be used to educate consumers about ingredients in products that may contain nicotine and inform future FDA regulatory policies related to product standards and accurate and comprehensible labeling of e-cigarette liquids." As taken from Fagan P et al. 2018. Nicotine Tob. Res. 20(8), 985-992. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29182761

2.2. Combustion products

This ingredient was investigated in a pyrolysis study. Results are given in JTI Study Report (s).

Compound	Two stage heating		One stage heating	
	Abundance	Area%	Abundance	Area%
furfural	351929489	38.96	290153592	41.05
5-methylfurfural	10937298	1.21	13920833	1.97
4,5-dimethylfurfural	24719116	2.74	17184828	2.43
5-hydroxymethylfurfural	426916324	47.27	277783215	39.30
5,5'-oxy-dimethylene-bis(2-furaldehyde)	13223804	1.46	1492170	0.19
Total ion chromatogram	905740000	100	785352631.6	100

This ingredient was investigated in a pyrolysis study. Results are given in Baker R and Bishop L. (2005). J. Anal. Appl. Pyrolysis 74, 145–170.

IngredientName &CAS Number	Max. cig. appln. level (ppm)	Purity of Sample (%)	Composition of pyrolysate Compound, %)	Max. level in smoke (µg)
d-Fructose 57-48-7	2100	na	Hydroxymethylfurfural (71.1) Furfural (7.9) Dihydrodihydroxymethyl pyranone (2.9)	750 80 30

			Acetic acid (1.3) Methylfurfural (1.2)	14 13

The amount of carbonyl compounds formed from added fructose was analysed in smoke by Shelar et al. (1992):

	µg carbonyl in smoke/cigarette				
Sample	**Formaldehyde**	**Acetaldehyde**	**Acetone**	**Acrolein**	**Total carbonyls**
K1 control	16.0	506.7	368.9	117.5	909.1
K1 4% fructose	18.2	590.7	333.0	135.5	1076.9
K1 8% fructose	19.1	593.8	351.6	144.0	1108.5
K1 12% fructose	24.5	572.4	354.2	161.0	1112.1
K1 16% fructose	27.4	667.6	432.6	189.7	1137.3
K2 control	11.2	577.6	306.3	117.8	1148.9
K2 4% fructose	12.0	652.0	330.4	130.3	1124.7
K2 8% fructose	21.7	626.4	339.8	138.4	1190.3
K2 12% fructose	20.3	637.8	344.3	141.8	1144.2
K2 16% fructose	27.4	799.4	415.5	177.6	1419.9

K1: low-mid stalk Burley tobacco, 2.4% nicotine

K2: mid-upper stalk Burley tobacco, 3.6% nicotine

However, conflicting results have been reported by other investigators about whether the concentration of reducing sugars in tobacco is associated with the levels of carbonyls in mainstream smoke (reviewed by Talhout et al., 2006).

2.3. Ingredient(s) from which it originates

Natural sources of fructose include fruits, vegetables (including sugar cane), and honey. Fructose is often further concentrated from these sources. The highest dietary sources of fructose, besides pure crystalline fructose, are foods containing table sugar (sucrose), high-fructose corn syrup, agave nectar, honey, molasses, maple syrup, and fruit juices, as these have the highest percentages of fructose (including fructose in sucrose) per serving compared to other common foods and ingredients. Fructose exists in foods either as a free monosaccharide, or bound to glucose as sucrose, a disaccharide. As taken from Park, KY; Yetley AE (1993). Intakes and food sources of fructose in the United States. American Journal of Clinical Nutrition 58 (5 Suppl). PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/8213605

Prepared from sucrose by enzymic conversion (Merck, 2014).

Prepared by adding absolute alcohol to the syrup obtained from the acid hydrolysis of inulin; prepared from dextrose; obtained from glucose in corn syrup by the use of glucose isomerase (CIR, 2014).

Occurs naturally in acerola, aconite, alfalfa, angelica, avocado, carob (13%), chicory root, codonopsis, comfrey, cranberry, dandelion root, Echinacea purpurea roots, euphorbia, gentian, ginkgo nuts, Asian ginseng, Gotu kola, Crataegus laevigata and C. monogyna, juniper berries, Kava rootstock, poria, psyllium, Chinese rhubarbs, rose hips, senna leaves and turmeric.

As taken from Khan IA and Abourashed EA, 2010.

3. Status in legislation and other official guidance

States approving use in tobacco	UK, EU and USA
Food	UK, EU and USA
ADI	No formal ADI identified.
Codex Alim.	Not listed
C of E no.	Not listed
FEMA no.	Not identified
TLV (ACGIH)	Not listed

Cosmetics (UK)	Not listed in Schedule 1

This material appears on the list of “Permitted Additives to Tobacco Products in the United Kingdom" at a maximum level permitted for inclusion in cigarettes/RYO and cigars of 10.0% w/w and pipe tobacco of 15.0% w/w (Department of Health, 2003).

Fructose (CAS RN 57-48-7; levulose) is included on the FDA’s inventory of “Substances Added to Food (formerly EAFUS)” as a flavor enhancer, flavoring agent or adjuvant, formulation aid, nutritive sweetener, processing aid, solvent or vehicle, stabilizer or thickener and texturizer; it is included under 21 CFR sections 131.111 (acidified milk); 131.112 (cultured milk); 131.170 (eggnog); 131.200 (yogurt); 131.203 (lowfat yogurt); 131.206 (nonfat yogurt); 133.179 (Pasteurized process cheese spread); 146.132 (grapefruit juice); 155.170 (canned peas), and also under 101.9 (nutrition labeling of food) (FDA, 2019a,b).

Pre-registered under REACH (“envisaged registration deadline 30 November 2010”) (ECHA, 2018).

Glucose is listed in the European Union inventory of cosmetic ingredients and REACH Annex IV. Annex IV “sets out substances that are exempted from the registration, evaluation and downstream user provisions of REACH as sufficient information is known about these substances that they are considered to cause minimum risk because of their intrinsic properties.”The CIR Expert Panel concluded that fructose is safe in the present practices of use and concentration in cosmetics.

Purity specifications:

Food use: not more than (NMT) 0.018% chloride; NMT 0.1 mg/kg lead; NMT 0.5% glucose; NMT 0.1% hydroxy-methylfurfural, calculated on the dried ash and free-ash basis; NMT 0.5% loss on drying; NMT 0.5% residue on ignition (sulfated ash).

USP: NMT 1 ppm arsenic;; NMT 5 ppm heavy metals: NMT 0.5% loss on drying; NMT 0.5% residue on ignition.

As taken from CIR, 2014.

D-Fructose (CAS RN 57-48-7) is listed in the US EPA InertFinder Database (2019) as approved for food and non-food use pesticide products. For food use, it is regulated under 40 CFR Part 180.950a (Tolerances and Exemptions for Pesticide Chemical Residues in Food. Tolerance exemptions for minimal risk active and inert ingredients) (EPA, 2019).

D-Fructose (CAS RN 57-48-7) is listed in the US EPA Toxic Substances Control Act (TSCA) inventory, and also in the US EPA 2012 CDR and 2016 CDR Partial Exempt lists (Chemical Data Reporting Rule). The CDR regulation requires companies that manufacture (including import) certain chemicals at certain volumes in the U.S. to report to EPA every four years through its CDR.

The TSCA inventory, and 2012 CDR and 2016 CDR Partial Exempt lists are available at https://iaspub.epa.gov/sor_internet/registry/substreg/searchandretrieve/searchbylist/search.do

Fructose (CAS RN 57-48-7) is not classified for packaging and labelling under Regulation (EC) No. 1272/2008 (ECHA, 2019).

“EFSA will provide scientific advice on the daily intake of added sugar in food by early 2020. The Authority aims to establish a science-based cut-off value for daily exposure to added sugars from all sources which is not associated with adverse health effects. The work will be carried out following a request from Denmark, Finland, Iceland, Norway and Sweden.

Added sugars from all sources comprise sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup, and other sugar preparations consumed as such or added during food preparation and manufacturing.

The adverse health effects under consideration will include body weight, glucose intolerance and insulin sensitivity, type-2-diabetes, cardiovascular risk factors, as well as dental caries. In its assessment, EFSA will look at the general healthy population, including children, adolescents, adults and the elderly.

The advice will guide Member States when establishing recommendations for the consumption of added sugars and in planning food-based dietary guidelines.”

As taken from EFSA, 2017.

“The Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA published in 2005 an opinion paper on a request from the Commission related to the evaluation of fructose for labelling purposes. They stated that the spectrum of individual fructose tolerance in HFI appears to depend on age (infants being more sensitive than adults) and on the respective gene mutation and that there are no scientifically established and generally accepted triggering doses for patients with HFI.

There is no scientifically established and generally accepted safe dose for patients with HFI (hereditary fructose intolerance). Nevertheless, a threshold dose of 5 mg/kg/day was derived for oral and parenteral (other than IV) products as a limit above which a warning in the PIL is deemed necessary and useful. An oral, subcutaneous or intramuscular dose of 5 mg/kg/day is expected to result in a systemic exposure level (Cmax and AUC) not higher than that reached after an oral dietary intake of 10 mg/kg/day, which is the lowest level of restriction for the oral fructose intake in patients with HFI. This dose level corresponds to 1/8 of the internationally recommended upper level of oral fructose intake in HFI of 40 mg/kg/day and corresponds to 1/50 of the challenge dose of intravenous fructose (0.25 g/kg) applied in the fructose tolerance test which has been used in the past for diagnosis of HFI. This conservative approach was chosen in view of the potentially fatal condition and the fact that the intake from a medicinal product will always be additive to the dietary intake.” As taken from EMA, 2017.

Excipients and information for the package leaflet:

Name	Updated on	Route of administration	Threshold	Information for the Package Leaflet	Comments
Fructose	09/10/2017	Oral, parenteral	Zero	This medicine contains x mg fructose in each >.	The additive effect of concomitantly administered products containing fructose (or sorbitol) and dietary intake of fructose (or

					sorbitol) should be taken into account.
Fructose	09/10/2017	Oral	Zero	[If the medicine is in contact with teeth (e.g. oral liquids, lozenges or chewable tablets) and is intended for long term use:] Fructose may damage teeth.	Oral products used frequently or over a long period of time, e.g. for two weeks or longer
Fructose	09/10/2017	Intravenous (IV)	Zero	If you (or your child) have hereditary fructose intolerance (HFI), a rare genetic disorder, you (or your child) must not receive this medicine. Patients with HFI cannot break down fructose in this medicine, which may cause serious side effects. You must tell your doctor before receiving this medicine if you (or your child) have HFI or if your child can no longer take sweet foods or drinks because they feel sick, vomit or get unpleasant effects such as bloating, stomach cramps or diarrhoea.	Patients with hereditary fructose intolerance (HFI) must not be given this medicine unless strictly necessary. Babies and young children (below 2 years of age) may not yet be diagnosed with hereditary fructose intolerance (HFI). Medicines (containing fructose) given intravenously may be lifethreatening and must be contraindicated in this population unless there is an overwhelming clinical need and no alternatives are available. A detailed history with regard to HFI symptoms has to be taken of each patient prior to being given this medicinal product.
Fructose	09/10/2017	Oral, parenteral (other than IV)	5 mg/kg/day	If your doctor has told you that you (or your child) have an intolerance to some sugars or if you have been diagnosed with hereditary fructose intolerance (HFI), a rare genetic disorder in which a person cannot break down fructose, talk to your doctor before you (or your child) take or receive this medicine.	Patients with hereditary fructose intolerance (HFI) should not take/be given this medicinal product.

As taken from EMA, 2018.

D-Fructose (CAS RN 57-48-7) is included on the New Zealand Inventory of Chemicals and may be used as a single component chemical under an appropriate group standard (NZ EPA, 2006).

Fructose (CAS RN 57-48-7) is “not considered to pose an unreasonable risk to the health of workers and public health on the basis of the Tier I IMAP assessment” and has been “identified as low concern to human health by application of expert validated rules” (NICNAS, 2018).

Fructose is included on the US FDA’s list of inactive ingredients for approved drug products. It is permitted for use as ingredients in various products, at the following maximum potency per unit doses:

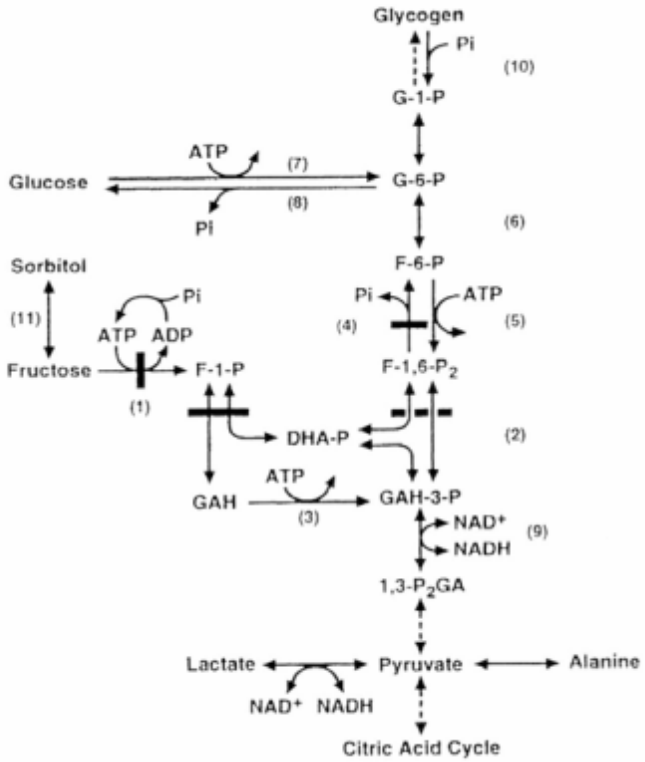
Inactive ingredient	**Route**	**Dosage Form**	**CAS Number**	**UNII**	**Maximum Potency oer unit dose**
Fructose	Iv(infusion)	Powder, for injection solution, lyophilized	57487	6YSS42VSEV	5%
Fructose	Oral	Bar, chewable	57487	6YSS42VSEV	na
Fructose	Oral	Powder, for suspension	57487	6YSS42VSEV	1376mg/5ml
Fructose	Oral	Solution	57487	6YSS42VSEV	2481mg/5ml
Fructose	Oral	Suspension	57487	6YSS42VSEV	20mg/1ml
Fructose	Oral	Suspension, sustained action	57487	6YSS42VSEV	na
Fructose	Oral	Syrup	57487	6YSS42VSEV	1732.5mg/5ml
Fructose	Oral	Tablet	57487	6YSS42VSEV	64.92mg
Fructose	Rectal	Solution	57487	6YSS42VSEV	1%

As taken from FDA, 2019c.

4. Metabolism/Pharmacokinetics

4.1. Metabolism/metabolites

EFSA (2005) provides the following diagram of the metabolic pathways of fructose:



“Fructose is mainly metabolized by a specialized pathway found in the liver, kidney cortex and small intestinal mucosa and composed of fructokinase (1), aldolase B (2) and triokinase (3). Aldolase B also intervenes in theglycolytic-gluconeogenic pathway, which also includes the following enzymes: fructose-1,6-bisphosphatase (4),phosphofructokinase (5), glucose-6-phosphate isomerase (6), glucokinase and hexokinase (7), glucose-6-phosphatase (8) and glyceraldehyde-3-phosphate dehydrogenase (9). Also shown are glycogen phophorylase (10) and sorbitol dehydrogenase (11). 1,3-P2GA, 1,3-bisphosphoglycerate; DHA-P, dihydroxyacetone phosphate;F, fructose; G, glucose; GAH, glyceraldehyde; P, phosphate; Pi, inorganic phosphate. The three enzyme defects in fructose metabolism are depicted by solid bars across the arrows; the diminished activity of aldolase B toward fructose-1,6-bisphosphate in hereditary fructose intolerance is depicted by a broken bar” (EFSA, 2005).

Metabolism of 13C-enriched D-fructose in hepatocytes from Goto-Kakizaki rats (Abstract). This study aims at assessing the conversion of exogenous D-[1-13C]fructose, D-[2-13C]fructose or D-[6-13C]-fructose (10 mM) to 13C-enriched and either hydrogenated or deuterated D-glucose, L-lactate and L-alanine released by rat liver cells prepared from Goto-Kakizaki rats and incubated for 120 min in the presence of unlabelled D-glucose (also 10 mM) and D2O. The results of this study are relevant to the relative contribution of fructokinase and hexokinase isoenzyme to the phosphorylation of D-fructose, the capacity of D-glucose to confer to glucokinase positive cooperativity towards D-fructose, the circulation of D-fructose 6-phosphate in the pentose phosphate pathway, the regulation of the cytosolic NADD/NADH ratio, the respective fate of D-fructose-derived D-glyceraldehyde

and dihydroxyacetone phosphate, the deuteration of fructose-derived glycolytic intermediates at the phosphoglucoisomerase, phosphomannoisomerase, enolase, pyruvate kinase and glutamate-alanine transaminase levels, and the unequal generation of L-[1-13C] lactate by cells exposed to D-[1-13C]fructose or D-[6-13C]fructose versus D-[2-13C]-fructose. As taken from Malaisse WJ et al. Int J Mol Med. 2004 May;13(5):697-703. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15067373&query_hl=39&itool=pubmed_docsum

Metabolism of D-[1-(13)C]fructose, D-[2-(13)C]fructose, and D-[6-(13)C]fructose in rat hepatocytes incubated in the presence of H(2)O or D(2)O (Abstract). Isolated hepatocytes from fed rats were exposed for 120 min to D-[1-(13)C]fructose, D-[2-(13)C]fructose, or D-[6-(13)C]fructose in the presence of H(2)O or D(2)O. The identification and quantification of (13)C-enriched metabolites (D-glucose, L-lactate) in the incubation medium and the measurement of their deuterated isotopomers indicated that the ketohexose was phosphorylated predominantly at the intervention of fructokinase and that the majority of the D-glyceraldehyde molecules generated from d-fructose 1-phosphate were further metabolized, e.g., after phosphorylation to D-glyceraldehyde 3-phosphate. It is proposed that the present procedure may help to further characterize the regulation of D-fructose metabolism in both hepatocytes and other cell types. As taken from Malaisse WJ et al. Mol Genet Metab. 2002 Feb;75(2):162-7. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=11855935&query_hl=39&itool=pubmed_docsum

Absorption and metabolism of fructose in the small intestine of conventional and germ-free piglets in vitro (Abstract). The aerobic uptake and metabolism of D-fructose in the rings of the small intestine of conventional and germ-free piglets during development was studied. No accumulation of fructose in the intracellular water against its concentration gradient was found. In both groups of animals the intracellular concentration of fructose decreases with increasing age, being highest in the newborn intestine and the sharpest decrease being observed on the first two days of post-natal life. Lactic acid was found to be the main metabolic product of fructose; a considerable amount of it was formed also from endogenous sources of the small intestine. In the newborn piglet fructose does not affect the formation of lactic acid, on the first two days after birth this effect of fructose is already apparent and more pronounced in germ-free piglets than in conventional ones. Conventional and germ-free animals differ markedly in endogenous lactic acid production. While in the former with increasing age endogenous lactate formation decreases, in the latter it rises after a sharp drop on the first post-natal day. As taken from Talafantová M and Kolínská J. Folia Biol (Praha). 1977 Jan;23(1):76-80. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=844630&query_hl=42&itool=pubmed_DocSum

Dangers of intravenous fructose (Abstract). Fructose has been recommended as an intravenous energy source during parenteral nutrition for patients with hepatic disease, uncontrolled diabetes mellitus, and in the postoperative state. Fructose is metabolised in the liver where it causes increased lactate formation, high-energy-phosphate depletion, increased uric-acid formation, and inhibition of protein synthesis. This results in increased concentrations of blood-lactate and serum-uric-acid. In hepatic disease and anoxic states fructose infusion can lead to lactic acidosis. In diabetic ketoacidosis the rises in blood lactate and uric acid which normally occur will be exacerbated. Great caution should be

exercised before using intravenous fructose. As taken from Woods H.F. and Alberti K.G.M.M. Lancet 1972 Dec 23, vol. 300, 1354 - 57 The Lancet, 2011 available at http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(72)92791-2/abstract

"…because fructose is not metabolized in the central nervous system, it does not exert the acute neuronal depression experienced by those imbibing ethanol…." As taken from Lustig RH. 2013. Adv. Nutr. 4(2), 226-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493539?dopt=AbstractPlus

"Both controversy and confusion exist concerning fructose, sucrose, and high-fructose corn syrup (HFCS) with respect to their metabolism and health effects. These concerns have often been fueled by speculation based on limited data or animal studies. In retrospect, recent controversies arose when a scientific commentary was published suggesting a possible unique link between HFCS consumption and obesity. Since then, a broad scientific consensus has emerged that there are no metabolic or endocrine response differences between HFCS and sucrose related to obesity or any other adverse health outcome. This equivalence is not surprising given that both of these sugars contain approximately equal amounts of fructose and glucose, contain the same number of calories, possess the same level of sweetness, and are absorbed identically through the gastrointestinal tract. Research comparing pure fructose with pure glucose, although interesting from a scientific point of view, has limited application to human nutrition given that neither is consumed to an appreciable degree in isolation in the human diet. Whether there is a link between fructose, HFCS, or sucrose and increased risk of heart disease, metabolic syndrome, or fatty infiltration of the liver or muscle remains in dispute with different studies using different methodologies arriving at different conclusions. Further randomized clinical trials are needed to resolve many of these issues. The purpose of this review is to summarize current knowledge about the metabolism, endocrine responses, and potential health effects of sucrose, HFCS, and fructose." As taken from Rippe JM & Angelopoulos TJ. 2013. Adv. Nutr. 4(2), 236-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493540?dopt=AbstractPlus

"….Fructose is a major component of added sugars and is distinct from other sugars in its ability to cause intracellular ATP depletion, nucleotide turnover, and the generation of uric acid. In this article, we revisit the hypothesis that it is this unique aspect of fructose metabolism that accounts for why fructose intake increases the risk for metabolic syndrome…." As taken from Johnson RJ et al. 2013. Diabetes 62(10), 3307-15. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24065788

"….Outcomes attributed to fructose - obesity, high triglycerides and other features of metabolic syndrome - are not found in every experimental test and may be more reliably caused by increased total carbohydrate. In this review, we try to put fructose in perspective by looking at the basic metabolic reactions. We conclude that fructose is best understood as part of carbohydrate metabolism. The pathways of fructose and glucose metabolism converge at the level of the triose-phosphates and, therefore, any downstream effects also occur with glucose. In addition, a substantial part of ingested fructose is turned to glucose. Regulation of fructose metabolism per se, is at the level of substrate control - the lower Km of fructokinase compared to glucokinase will affect the population of triose-phosphates. Generally deleterious effects of administering fructose alone suggest that fructose metabolism is normally controlled in part by glucose…." As taken from Feinman RD & Fine

EJ. 2013. Nutr. Metab. (Lond.) 10(1), 45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23815799

"Fructose consumption has dramatically increased in the last 30 years. The principal form has been in the form of high-fructose corn syrup found in soft drinks and processed food. The effect of excessive fructose consumption on human health is only beginning to be understood. Fructose has been confirmed to induce several obesity-related complications associated with the metabolic syndrome. Here we present an overview of fructose metabolism and how it contrasts with that of glucose. In addition, we examine how excessive fructose consumption can affect de novo lipogenesis, insulin resistance, inflammation, and reactive oxygen species production. Fructose can also induce a change in the gut permeability and promote the release of inflammatory factors to the liver, which has potential implications in increasing hepatic inflammation. Moreover, fructose has been associated with colon, pancreas, and liver cancers, and we shall discuss the evidence for these observations. Taken together, data suggest that sustained fructose consumption should be curtailed as it is detrimental to long-term human health." As taken from Charrez B et al. 2015. Horm. Mol. Biol. Clin. Investig. 22, 79-89. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25965509

"Fructose is one of the main sweetening agents in the human diet and its ingestion is increasing globally. Dietary sugar has particular effects on those whose capacity to metabolize fructose is limited. If intolerance to carbohydrates is a frequent finding in children, inborn errors of carbohydrate metabolism are rare conditions. Three inborn errors are known in the pathway of fructose metabolism; (1) essential or benign fructosuria due to fructokinase deficiency; (2) hereditary fructose intolerance; and (3) fructose-1,6-bisphosphatase deficiency. In this review the focus is set on the description of the clinical symptoms and biochemical anomalies in the three inborn errors of metabolism. The potential toxic effects of fructose in healthy humans also are discussed. Studies conducted in patients with inborn errors of fructose metabolism helped to understand fructose metabolism and its potential toxicity in healthy human. Influence of fructose on the glycolytic pathway and on purine catabolism is the cause of hypoglycemia, lactic acidosis and hyperuricemia. The discovery that fructose-mediated generation of uric acid may have a causal role in diabetes and obesity provided new understandings into pathogenesis for these frequent diseases." As taken from Tran C. 2017. Nutrients 9(4), E356. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28368361

"High fructose consumption in the Western diet correlates with disease states such as obesity and metabolic syndrome complications, including type II diabetes, chronic kidney disease, and non-alcoholic fatty acid liver disease. Liver and kidneys are responsible for metabolism of 40-60% of ingested fructose, while the physiological fate of the remaining fructose remains poorly understood. The primary metabolic pathway for fructose includes the fructose-transporting solute-like carrier transport proteins 2a (SLC2a or GLUT), including GLUT5 and GLUT9, ketohexokinase (KHK), and aldolase. Bioinformatic analysis of gene expression encoding these proteins (glut5, glut9, khk, and aldoC, respectively) identifies other organs capable of this fructose metabolism. This analysis predicts brain, lymphoreticular tissue, placenta, and reproductive tissues as possible additional organs for fructose metabolism. While expression of these genes is highest in liver, the brain is predicted to have expression levels of these genes similar to kidney. RNA in situ hybridization of coronal slices of adult mouse brains validate the in silico expression of glut5, glut9, khk, and aldoC, and show expression across many regions of the brain, with

the most notable expression in the cerebellum, hippocampus, cortex, and olfactory bulb. Dissected samples of these brain regions show KHK and aldolase enzyme activity 5-10 times the concentration of that in liver. Furthermore, rates of fructose oxidation in these brain regions are 15-150 times that of liver slices, confirming the bioinformatics prediction and in situ hybridization data. This suggests that previously unappreciated regions across the brain can use fructose, in addition to glucose, for energy production." As taken from Oppelt SA et al. 2017. Brain Res. 1657, 312-322. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28034722

"Although altered glucose metabolism is a well-studied feature of malignant cells, little is known about the direct metabolism of fructose. In this issue of Cancer Cell, Chen et al. report that AML cells consume fructose and use it to maintain viability, especially when glucose is scarce." As taken from Cairns RA and Mak TW. 2016. Cancer Cell 30(5), 660-662. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27846386

"Whether fructose (FRU), as the sole energy source, confers a metabolic advantage on cancer cells against noxious stimuli is unknown. The aim of this study was to evaluate the effects of low (11 mM), moderate (25 mM), and high (55 mM) FRU concentrations alone or in combination with rotenone (ROT) or doxorubicin (DOX) in Jurkat cells, an acute lymphoblastic leukemia cell model. Glucose (GLU) was used as a control. Using different cell analysis techniques, we demonstrated that FRU was predominantly metabolized via oxidative phosphorylation (~95%) (i.e., lactate production was reduced >120-fold), resulting in endogenous oxidative stress-induced conditions. The cells were characterized by generation of O2·- (43%)/ H2 O2 (40%) and activation of NF-κB (~95-fold increase, fi), c-Jun-N terminal kinase (JNK), p53 (40-fi), and c-Jun (9-fi). In addition, we observed a loss of ΔΨm (10%), activation of caspase-3 (50-fi) and apoptosis-inducing factor (AIF, 2-fi), and condensation and fragmentation of the nuclei [20% by acridine orange/ethidium bromide/Hoechst (AO/EB/H) staining, 15% by flow cytometry] compared to those of GLU 11 at 24 h. Although DOX killed Jurkat cells independent of sugar content in the culture medium, leukemic cells in low, but not high, FRU were extremely sensitive to ROT. Taken together, our findings suggest that Jurkat cells are more susceptible to cell death if forced to shift from GLU metabolism (i.e., aerobic glycolysis) to FRU metabolism (i.e., oxidative phosphorylation) after treatment with mitochondria-targeting molecules. These observations may help elucidate the cell death mechanism of leukemic cells cultured in FRU." As taken from Diaz-Aguirre V et al. 2016. Cell Biol. Int. 40(11), 1162-1173. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27486090

"Fructose in the form of sucrose and high fructose corn syrup is absorbed by the intestinal transporter and mainly metabolized in the small intestine. However, excess intake of fructose overwhelms the absorptive capacity of the small intestine, leading to fructose malabsorption. Carbohydrate response element-binding protein (ChREBP) is a basic helix-loop-helix leucine zipper transcription factor that plays a key role in glycolytic and lipogenic gene expression in response to carbohydrate consumption. While ChREBP was initially identified as a glucose-responsive factor in the liver, recent evidence suggests that ChREBP is essential for fructoseinduced lipogenesis and gluconeogenesis in the small intestine as well as in the liver. We recently identified that the loss of ChREBP leads to fructose intolerance via insufficient induction of genes involved in fructose transport and metabolism in the intestine. As fructose consumption is increasing and closely associated with metabolic and gastrointestinal diseases, a comprehensive understanding of cellular fructose sensing and metabolism via ChREBP may uncover new therapeutic opportunities.

In this mini review, we briefly summarize recent progress in intestinal fructose metabolism, regulation and function of ChREBP by fructose, and delineate the potential mechanisms by which excessive fructose consumption may lead to irritable bowel syndrome." As taken from Lee HJ and Cha JY. 2018. BMB Rep. 51(9), 429-436. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30158026

"The liver is one of the most essential organs in metabolism and is responsible for metabolizing a wide variety of molecules from amino acids to sugars. Although it is responsible for many essential metabolic processes, it is one of the most severely affected by metabolic disease because, in many cases, it is the first to be exposed to the toxic intermediates. The metabolism of galactose, fructose, and tyrosine involve the liver and although there are systemic findings in metabolic disease involved with these substrates, severe hepatopathy is a common presenting aspect of galactosemia, hereditary fructose intolerance, and tyrosinemia type I." As taken from Demirbas D et al. 2018. Pediatr. Clin. North Am. 65(2), 337-352. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29502917

4.2. Absorption, distribution and excretion

Incomplete intestinal absorption of fructose (Abstract). Intestinal D-fructose absorption in 31 children was investigated using measurements of breath hydrogen. Twenty five children had no abdominal symptoms and six had functional bowel disorders. After ingestion of fructose (2 g/kg bodyweight), 22 children (71%) showed a breath hydrogen increase of more than 10 ppm over basal values, indicating incomplete absorption: the increase averaged 53 ppm, range 12 to 250 ppm. Four of these children experienced abdominal symptoms. Three of the six children with bowel disorders showed incomplete absorption. Seven children were tested again with an equal amount of glucose, and in three of them also of galactose, added to the fructose. The mean maximum breath hydrogen increases were 5 and 10 ppm, respectively, compared with 103 ppm after fructose alone. In one boy several tests were performed with various sugars; fructose was the only sugar incompletely absorbed, and the effect of glucose on fructose absorption was shown to be dependent on the amount added. It is concluded that children have a limited absorptive capacity for fructose. We speculate that the enhancing effect of glucose and galactose on fructose absorption may be due to activation of the fructose carrier. Apple juice in particular contains fructose in excess of glucose and could lead to abdominal symptoms in susceptible children. As taken from Kneepkens CM et al. Arch Dis Child. 1984 Aug;59(8):735-8. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=6476870&query_hl=44&itool=pubmed_docsum

Evaluation of 13CO2 breath tests for the detection of fructose malabsorption (Abstract).

Breath hydrogen (H2) studies have made clear that small intestinal absorption of fructose is limited, especially in toddlers. Malabsorption of fructose may be a cause of recurrent abdominal pain and chronic nonspecific diarrhea (toddler's diarrhea). Fructose absorption is facilitated by equimolar doses of glucose and, as we have found, amino acids (especially L-

alanine); the mechanism underlying this effect remains unclear. To study fructose absorption in a more direct way, we combined breath H2 studies with breath 13CO2 studies. Gastric emptying was studied by using L-glycine-1-13C in 4 children from 12.1 to 16.0 years of age. After 25 gm of fructose and 27.5 gm of glucose, when given together, gastric emptying was significantly (p<0.05) slower than with either sugar alone. In a second series of experiments, 5 children from 12.0 to 15.9 years of age were tested with 25 gm of fructose, alone and with equimolar doses of glucose and L-alanine, and 4 younger children from 3.1 to 6.1 years of age were tested with 2 gm/kg (max 37.5 gm) fructose, alone or with an equimolar dose of L-alanine. All fructose solutions were enriched with 15 mg of D-fructose-13C-6. In all 9 children, fructose was malabsorbed as judged by breath H2 increases > or = 20 ppm, and the addition of glucose or L-alanine resulted in significantly lower breath H2 increases (p < or = 0.005 for glucose, p < or = 0.001 for alanine). In contrast, the addition of alanine or glucose did not change the pattern of breath 13CO2 excretion in the 5 older children, whereas in the 4 younger children (with relatively higher doses), L-alanine addition resulted in significantly lower increases in breath 13CO2. In the latter group, for each time point, breath H2 and 13CO2 concentrations after fructose were compared with those after fructose plus L-alanine; in 20 out of 24 points, both H2 and 13CO2 were higher after fructose. These results suggest that 13CO2 not only originated from the oxidation of absorbed substrate but also, at least in part, from colonic bacterial metabolism. For the detection of [correction of or] fructose malabsorption--as opposed to, for instance, lactose--the 13CO2 breath test seems to be of limited value. As taken from Hoekstra Jhet al. J Lab Clin Med. 1996 Mar;127(3):303-9. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=9273364&query_hl=37&itool=pubmed_docsum

Kinetic characterization of apical D-fructose transport in chicken jejunum (Abstrac). In mammals, D-fructose transport takes place across the brush-border membrane of the small intestine through GLUT5, a member of the facilitative glucose transporter family. In the present paper, we describe and characterize for the first time the apical transport of D-fructose in chicken intestine. Brush-border membrane vesicles (BBMV) were obtained from jejunum of 5- to 6-wk-old chickens. D-Fructose uptake by BBMV from chicken jejunum comprises a saturable component and a simple diffusion process. The maximal rate of transport (V(max)) for D-fructose was 2.49 nmol.(mg prot)(-1).s(-1), the Michaelis constant (K(m)) was 29 mM, and the diffusion constant (K(d)) was 25 nl.(mg prot)(-1).s(-1). The apical transport of D-fructose was Na(+)-independent, phlorizin-, phloretin-, and cytochalasin B-insensitive, and did not show cis-inhibition by D-glucose or D-galactose. These properties, together with the detection of specific GLUT5 mRNA, indicate the presence of a low-affinity high-capacity GLUT5-type carrier in the chicken jejunum, responsible for the entry of D-fructose across the brush-border membrane of enterocytes. As taken from Garriga C et al. J Membr Biol. 2004 Jan 1;197(1):71-6. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15014919&query_hl=45&itool=pubmed_docsum

Fructose absorption (Abstract). Fructose found in modern diets as a constituent of the disaccharide sucrose is absorbed by a well-characterized absorptive system integrating enzymatic hydrolysis of the disaccharide and transfer of the resulting two monosaccharides through the apical membrane of the epithelial cell. The increasing use of high-fructose syrups and crystalline fructose prompted new studies aimed at the determination of the absorptive capacity for free fructose in the human gut. Results indicate that the capacity for

fructose absorption is small compared with that for sucrose and glucose and is much less than previously estimated. The unexpected finding that the simultaneous ingestion of glucose can prevent fructose malabsorption suggests that the pair of monosaccharides might be absorbed by the disaccharidase-related transport system as if they were the product of the enzymatic hydrolysis of sucrose. This absorptive mechanism might not be able to transport fructose when ingested without glucose. As taken from Riby JE et al. Am J Clin Nutr. 1993 Nov;58(5 Suppl):748S-753S. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/8213606

Urinary sucrose and fructose as biomarkers for sugar consumption (Abstract). The use of 24-hour urinary sucrose and fructose as potential biomarkers for sugars consumption was investigated in two studies of 21 healthy participants living in a volunteer suite where dietary intake was known and all specimens collected. The dose-response was assessed in 12 males using a randomized crossover design of three diets containing constant levels of 63, 143, and 264 g of sugars for 10 days each. Both sugars and sucrose intake were significantly correlated with the sum of sucrose and fructose concentration in urine (0.888; P < 0.001). To assess effects with volunteers consuming their habitual varying diets, seven males and six females were fed their usual diet (assessed beforehand from four consecutive self-completed 7-day food diaries) for 30 days under controlled conditions in the volunteer suite. The mean (+/-SD) calculated total sugars intake was 202 +/- 69 g/d, 41% from sucrose. Mean (+/-SD) urinary sucrose and fructose were 36.6 +/- 16.6 and 61.8 +/- 61.3 mg/d, respectively. The sum of sucrose and fructose in urine was significantly correlated with sugars (0.841; P < 0.001) and sucrose intake (0.773; P = 0.002). In the regression, 200 g of sugars intake predicted approximately 100 mg of sucrose and fructose in urine. The correlation between individual means of randomized 16 days of sugars intake and 8 days of sugars excretion data (as used in validation studies) remained as high as that obtained with the means of 30-day measurements and the regression estimates were very similar. Twenty-four-hour urinary sucrose and fructose could be grouped into a new category of biomarkers, predictive biomarkers, that can be used in studies determining the structure of dietary measurement error in free living individuals and to relate sugars intake to disease risk. As taken from Tasevska N et al. Cancer Epidemiol Biomarkers Prev. 2005 May; 14(5):1287-94. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=pubmed&cmd=Retrieve&dopt=Abstract&list_uids=15894688&query_hl=4&itool=pubmed_docsum

Intestinal fructose transport and malabsorption in humans (Abstract). Fructose is a hexose sugar that is being increasingly consumed in its monosaccharide form. Patients who exhibit fructose malabsorption can present with gastrointestinal symptoms that include chronic diarrhoea and abdominal pain. However, with no clearly established gastrointestinal mechanism for fructose malabsorption, patient analysis by the proxy of a breath hydrogen test (BHT) is controversial. The major transporter for fructose in intestinal epithelial cells is thought to be the facilitative transporter GLUT5. Consistent with a facilitative transport system, we show here by analysis of past studies on healthy adults that there is a significant relationship between fructose malabsorption and fructose dose (r=0.86, p<0.001). Thus, there is a dose-dependent and limited absorption capacity even in healthy individuals. Changes in fructose malabsorption with age have been observed in human infants and this may parallel the developmental regulation of GLUT5 expression. Moreover, a GLUT5 knockout mouse has displayed the hallmarks associated with profound fructose malabsorption. Fructose malabsorption appears to be partially modulated by the amount of glucose ingested. While solvent drag and passive diffusion have been proposed to explain

the effect of glucose on fructose malabsorption, this could possibly be a result of the facilitative transporter GLUT2. GLUT5 and GLUT2 mRNA have been shown to be rapidly upregulated by the presence of fructose and GLUT2 mRNA is also upregulated by glucose, but in humans the distribution and role of GLUT2 in the brush border membrane is yet to be definitively decided. Understanding the relative roles of these transporters in humans will be crucial for establishing a mechanistic basis for fructose malabsorption in gastrointestinal patients. As taken from Jones HF et al. American Journal of Physiology: Gastrointestinal and Liver Physiology. APS, 2011 available at http://ajpgi.physiology.org/content/early/2010/12/03/ajpgi.00457.2010.reprint

"Fructose at 70 g or less can most likely be fully absorbed" (Wang et al., 1981).

"Urinary sugars excretion has been proposed as a potential biomarker for intake of sugars. In this study, we compared 2 analytical methods (gas chromatography [GC] and enzymatic reactions-UV absorption) for quantifying urinary fructose and sucrose using 24-hour urine samples from a randomized crossover controlled feeding study. All samples were successfully quantified by the GC method; however, 21% and 1.9% of samples were below the detection limit of the enzymatic method for sucrose and fructose, respectively. Although the correlation between the 2 methods was good for fructose (Pearson correlation, 0.71), the correlation was weak for sucrose (Pearson correlation, 0.27). We favor the GC method because of its better sensitivity, simplicity, and the ability to quantify fructose and sucrose directly in the same run. Of the 106 samples from 53 participants with complete urine collection after 2 study diets, 24-hour urinary fructose excretion was significantly associated with fructose intake. The sum of 24-hour urinary fructose and sucrose was significantly associated with total sugars consumption. However, variation in intakes of sugars explained only a modest amount of variation in urinary sugars excretion. In the unadjusted models, fructose intake explained 24.3% of urinary fructose excretion, and intake of total sugars explained 16.3% of the sum of urinary fructose and sucrose. The adjusted models explained 44.3% of urinary fructose excretion and 41.7% of the sum of urinary fructose and sucrose. Therefore, we caution using these biomarkers to predict sugars consumption before other factors that determine urinary sugars excretion are understood." As taken from Song X et al. 2013. Nutr. Res. 33(9), 696-703. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24034568

"The SLC2 family of facilitative Glucose transporters (Gluts) contains 14 isoforms divided into three classes based on amino acid sequence. While the majority of these proteins transport glucose, a subset can transport fructose. Recently, fructose and the Gluts responsible for fructose uptake have received increased interest due to the correlation between high fructose consumption and early onset of metabolic syndrome. In addition, the up-regulation of Gluts in certain cancers has made possible the development of a number of fructose probes for imaging cancer. Although structure activity data has defined some aspects of fructose-specific uptake, a far more detailed clarification of the variables governing the onset and progression of fructose-correlated diseases is still needed. Here, we summarize what is known about molecular structure and fructose uptake as it relates to the correlation of fructose and disease." As taken from McQuade DT et al. 2013. Org. Biomol. Chem. 11(30), 4909-20. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23784005

"Although excessive fructose intake is epidemiologically linked with dyslipidemia, obesity, and diabetes, the mechanisms regulating plasma fructose are not well known. Cells

transfected with sodium/glucose cotransporter 5 (SGLT5), which is expressed exclusively in the kidney, transport fructose in vitro; however, the physiological role of this transporter in fructose metabolism remains unclear. To determine whether SGLT5 functions as afructose transporter in vivo, we established a line of mice lacking the gene encoding SGLT5. Sodium-dependent fructose uptake disappeared in renal brush border membrane vesicles from SGLT5-deficient mice, and the increased urinary fructose in SGLT5-deficient mice indicated that SGLT5 was the major fructose reabsorption transporter in the kidney. From this, we hypothesized that urinary fructose excretion induced by SGLT5 deficiency would ameliorate fructose-induced hepatic steatosis. To test this hypothesis we compared SGLT5-deficient mice with wild-type mice under conditions of long-term fructose consumption. Paradoxically, however, fructose-induced hepatic steatosis was exacerbated in the SGLT5-deficient mice, and the massive urinary fructose excretion was accompanied by reduced levels of plasma triglycerides and epididymal fat but fasting hyperinsulinemia compared with fructose-fed wild-type mice. There was no difference in food consumption, water intake, or plasma fructose between the two types of mice. No compensatory effect by other transporters reportedly involved in fructose uptake in the liver and kidney were indicated at the mRNA level. These surprising findings indicated a previously unrecognized link through SGLT5 between renal fructose reabsorption and hepatic lipid metabolism." As taken from Fukuzawa T et al. 2013. PLoS One 8(2), e56681. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23451068

"Fructose intake has increased dramatically since humans were hunter-gatherers, probably outpacing the capacity of human evolution to make physiologically healthy adaptations. Epidemiological data indicate that this increasing trend continued until recently. Excessive intakes that chronically increase portal and peripheral blood fructose concentrations to >1 and 0.1 mm, respectively, are now associated with numerous diseases and syndromes. The role of the fructose transporters GLUT5 and GLUT2 in causing, contributing to or exacerbating these diseases is not well known. GLUT5 expression seems extremely low in neonatal intestines, and limited absorptive capacities for fructose may explain the high incidence of malabsorption in infants and cause problems in adults unable to upregulate GLUT5 levels to match fructose concentrations in the diet. GLUT5- and GLUT2-mediated fructose effects on intestinal electrolyte transporters, hepatic uric acid metabolism, as well as renal and cardiomyocyte function, may play a role in fructose-induced hypertension. Likewise, GLUT2 may contribute to the development of non-alcoholic fatty liver disease by facilitating the uptake of fructose. Finally, GLUT5 may play a role in the atypical growth of certain cancers and fat tissues. We also highlight research areas that should yield information needed to better understand the role of these GLUTs in fructose-induced diseases" As taken from Douard V & Ferraris RP. 2013. J. Physiol. 591(Pt 2), 401-14. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23129794?dopt=AbstractPlus

"Free fructose, ingested orally either as monosaccharide or enzymatically cleaved from sucrose, is absorbed directly by the intestine. It is transported through the intestinal epithelium by GLUT-5 (passive) and GLUT-2 (active) transporters. Studies indicate that fructose absorption is dose-dependent and facilitated by the simultaneous ingestion of glucose. Saturation of absorptive capacity is reached with doses of about 0.5 g/kg body weight, above which a significant proportion of subjects show signs of fructose malabsorption. Plasma fructose concentration time courses are available from 19 healthy subjects after peroral intake of 50 g fructose, and from 8 healthy adults after a test meal of 0.5 g/kg fructose. Both showed a peak in plasma fructose after 30 min (tmax).

...since there are no PK data on subcutaneous or intramuscular absorption of fructose/sorbitol available, it cannot be excluded that absorption from these routes might be even faster than the oral absorption and Cmax reached in plasma after subcutaneous administration might be higher than after an oral application of the same dose/kg."

As taken from EMA, 2017.

"Excessive consumption of sweets is a risk factor for metabolic syndrome. A major chemical feature of sweets is fructose. Despite strong ties between fructose and disease, the metabolic fate of fructose in mammals remains incompletely understood. Here we use isotope tracing and mass spectrometry to track the fate of glucose and fructose carbons in vivo, finding that dietary fructose is cleared by the small intestine. Clearance requires the fructose-phosphorylating enzyme ketohexokinase. Low doses of fructose are ~90% cleared by the intestine, with only trace fructose but extensive fructose-derived glucose, lactate, and glycerate found in the portal blood. High doses of fructose (≥1 g/kg) overwhelm intestinal fructose absorption and clearance, resulting in fructose reaching both the liver and colonic microbiota. Intestinal fructose clearance is augmented both by prior exposure to fructose and by feeding. We propose that the small intestine shields the liver from otherwise toxic fructose exposure." As taken from Jang C et al. 2018. Cell Metab. 27(2), 351-361. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29414685

4.3. Interactions

"BACKGROUND: Intrauterine growth restriction (IUGR) is known to affect the risk of adult diseases. Consumption of lipogenic fructose is increasing, and it is used as an enhancer of metabolic syndrome in rat experiments. The effects of IUGR, postnatal fructose diet, and their interaction on the lipid profile and adiposity were studied in adult rats. METHODS: IUGR was induced by providing pregnant rats with 50% of daily food intake. From 1 mo onward, half of the offspring received a fructose-rich diet and were then followed to the age of 1 and 6 mo, when plasma lipid, glucose, and insulin levels were measured. The adipose tissue was visualized by magnetic resonance imaging at the age of 6 mo. RESULTS: IUGR and fructose diet decreased body weight in adult rats. IUGR increased low-density lipoprotein cholesterol in 6-mo-old rats. The fructose diet evoked hypertriglyceridemia and hyperinsulinemia in both the sexes and decreased fasting glucose levels in female rats. Postnatal fructose diet increased lipid content percentage in the retroperitoneal and intra-abdominal adipose tissues in male rats. Interactions between IUGR and postnatal fructose diet were observed in adult weight in males. CONCLUSION: These results demonstrate the importance of IUGR and fructose diet in adverse changes in lipid and glucose metabolism." As taken from Malo E et al. 2013. Pediatr. Res. 73(2), 155-62. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23174704?dopt=AbstractPlus

"INTRODUCTION: People with diet-induced metabolic diseases, such as obesity and diabetes, may be susceptible to the health effects of air pollutants. The present study was designed to compare the nasal toxicity caused by ambient air pollutant exposures in rats fed a normal or high-fructose diet (ND and HFD, respectively) for 10 weeks. METHODS: Repeated inhalation exposures of male Sprague Dawley rats to ozone (O3; 0.5 ppm) and/or concentrated ambient fine particles (CAPs, 400 µg/m3) were conducted in a mobile air

research laboratory located in an industrial area of Dearborn, MI during the summer 2011. Rats were exposed for 9 days, 8 h/day (4-5 days/week). All rats were sacrificed 24 h after the last exposure. Nasal tissues were processed for light microscopic examination and morphometric analysis. RESULTS: ND and HFD rats exposed to O3 or O3/CAPs, but not control ND rats exposed to filtered air alone, had an acute rhinitis with marked hyperplasia of nasal epithelium (NE). HFD rats, but not ND rats, exposed to CAPs also had an acute rhinitis but no epithelial hyperplasia. The severity of the inflammatory cell response (numeric density of neutrophils) was not different among the rats with rhinitis. Of all the air pollutant-exposed groups, the HFD rats coexposed to O3/CAPs had the greatest numeric cell density of NE (54, 45, 12 and 12% greater than CAPs-exposed HFD, air-exposed HFD, O3/CAPs-exposed ND, and O3-exposed HFD rats, respectively). Though we have previously reported that repeated O3 exposure will cause rhinitis with nasal epithelial hyperplasia, this is the first report of a diet-related enhancement of air pollutant-induced nasal toxicity. Underlying mechanisms responsible for HFD enhancement of O3- and/or CAPsinduced nasal epithelial or inflammatory lesions are yet to be determined." As taken from Hotchkiss IP et al. 2012. The Toxicologist. SOT 2012 Annual Meeting. Available at http://www.toxicology.org/AI/PUB/Tox/2012Tox.pdf

"People with diet-induced metabolic maladies may be more susceptible to the adverse effects of air pollution (AP). The present study evaluated the effect of AP on facets of diet-induced metabolic dysfunction. Mature male Sprague Dawley rats were fed normal chow (NC) or a high fructose diet (HF) for 8 weeks prior to and during AP exposure. Inhalation exposures to ozone (O3; 0.5 ppm) and/or concentrated ambient fine particles (CAPs, 400 µg/m3) were conducted in a mobile air research laboratory located in an industrial area of Dearborn, MI during summer 2011. Rats were exposed for 9 days, 8 h/day (4-5 days/week). Rats were sacrificed 24h after the end of exposure. Prior to sacrifice, blood was collected for homeostatic model assessment–insulin resistance (HOMA-IR) analysis. At necropsy, hepatic tissues were collected for light microscopy and gene expression analyses using qRTPCR and whole-genome arrays. Though HF feeding did not induce obesity, HOMA-IR was increased concurrent with periportal steatosis. HF-induced gene expression of stearoyl-Coenzyme A desaturase (Scd1) was potentiated after exposure to O3, CAPs and O3/CAPs (~21-fold) relative to filtered air/HF group (~14-fold). Scd1 expression was repressed by O3 (~3.4-fold) and O3/CAPs (~2.5-fold) in rats fed NC. Low density lipoprotein receptor (Ldlr) was also induced in HF-fed rats that were exposed to O3 and O3/CAPs (~1.6-fold) compared to filtered air-exposed NC and HF rats. In contrast, HF feeding caused a significant decrease in phosphoenolpyruvate carboxykinase 1 (Pck1), suggesting inhibition of gluconeogenesis. Furthermore, CAPs exposure down-regulated Pck1 in NC-fed rats (~1.7- fold) and further suppressed Pck1 on a HF diet (~6-fold). Collectively, these data suggest that acute AP exposure may exacerbate facets of the metabolic syndrome." As taken from Allen K et al. 2012. The Toxicologist. SOT 2012 Annual Meeting. Available at http://www.toxicology.org/AI/PUB/Tox/2012Tox.pdf

5. Toxicity

5.1. Single dose toxicity

Effects of fructose infusion on lactate and uric acid metabolim (Abstract). Ten healthy volunteers received 10% IV fructose (0.5 g/kg/bw/hr) for 2 hours following a one hour control period in which physiological saline solution was infused IV. Blood lactate rose significantly during fructose administration; and this change was accompanied by a fall in serum bicarbonate and a rise in serum glucose, which fell to hypoglycemic concentrations an hour after infusion with no alteration of urinary pH. The lack of hyperuricemia suggests that the hyperuricemic effect of fructose previously reported is dose related and associated only with larger than physiological concentrations of fructose infused under experimental conditions. While fructose seems to remain contraindicated in clinical settings where lactate accumulation must be avoided (diabetic ketoacidosis, hypoxia, postoperative perio, and uremia), it can be safely administered in physiological concentrations without fear of hyperuricemia. As taken from Hamid Sahebjami and Raymond Scalettar Lancet. 1971 Feb 20;1(7695):366-9. The Lancet, 2011 available at http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(71)92208-2/abstract

Plasma D-glucose, D-fructose and insulin responses after oral administration of D-glucose, D-fructose and sucrose to normal rats (Abstract). Oral D-glucose (1.7, 3.5, 6.9 or 13.9 micromol/g body weight), D-fructose (6.9 micromol/g), both D-glucose and D-fructose (1.7 or 3.5 micromol/g of each hexose) or sucrose (3.7 micromol/g) were administered intragastrically to overnight fasted rats and the plasma concentration of D-glucose, D-fructose and insulin measured over the ensuing 120 minutes. Control experiments were conducted after oral administration of H(2)O or saline. The administration of D-fructose, given as the free hexose or as sucrose, instead of augmenting the plasma D-glucose concentration evoked by the concomitant administration of D-glucose, tended both to improve the insulin response of the pancreatic B-cell and to minimize hyperglycemia, when compared to the results of experiments including the administration of equimolar amounts of D-glucose alone. For instance, the area under the plasma D-glucose curve was comparable in the rats receiving both D-glucose and D-fructose (3.5 micromol/g of each hexose) and the rats receiving only D-glucose (3.5 micromol/g), averaging respectively 836 +/- 32 and 850 +/- 34 mM . min each. Likewise, the paired ratio between the areas under the plasma insulin and D-glucose curves, when corrected for the threshold concentration for the insulinotropic action of the hexose (2.05 +/- 0.10 mM), averaged 44.3 +/- 3.0 nmol/mol in the 16 rats receiving D-fructose alone, sucrose alone or both D-glucose and D-fructose, as compared to 37.7 +/- 2.9 nmol/mol in the 22 rats receiving increasing amounts of D-glucose alone. The intake of D-fructose, as the free hexose or as sucrose, favours D-glucose homeostasis. This is likely to be attributable to the reciprocal effects of the aldose and ketose upon their respective phosphorylation by glucokinase in both hepatocytes and insulin-producing pancreatic islet cells. As taken from Prieto PG et al. J Am Coll Nutr. 2004 Oct;23(5):414-9. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/15466948?dopt=Abstract

“Described are 2 cases in which severe metabolic acidosis developed following administration of large amounts of Aminosol (preotein hydrolysate)/fructose/ethanol post-surgically. Both patients died” (Rickett et al., 1993).

“Twelve men with normal glucose tolerance received 50 mL of 62.5% fructose or an equivalent amount of 50% glucose over 5 minutes. They served as control group. Ninety minutes later, the other sugar was given to half the controls in crossover fashion. Seven diabetic patients received only the fructose. The 12 controls receiving fructose with normal

glucose tolerance demonstrated a rise in plasma insulin and corticoid levels. Similar but lesser changes were observed in the diabetics, who also demonstrated a significant increase in blood glucose. A catabolite of fructose may have been reponsible for these changes." (Aitken et al., 1969).

"In general, there is no adverse effect other than osmotic diarrhea after oral administration of these sugars. Transient hyperuricemia was seen in some humans" (Wang et al., 1981)

Non-human

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
LD50 - Lethal dose, 50 percent kill	Intravenous	Rodent - rabbit	15 gm/kg	Details of toxic effects not reported other than lethal dose value	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: AD-A132-656
TDLo - Lowest published toxic dose	Oral	Rodent - rat	4 gm/kg	Details of toxic effects not reported other than lethal dose value	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 79,17,2002
TDLo - Lowest published toxic dose	Oral	Rodent - rat	4 gm/kg	Kidney/Ureter/Bladder - other changes in urine composition	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 79,17,2002

As taken from RTECS, 2018.

"The safety of rare sugar syrup obtained from high-fructose corn syrup under slightly alkaline conditions was studied. …... Oral administration of single dose (15,000 mg/kg) of rare sugar syrup to rats caused no abnormalities, suggesting no adverse effect of rare sugar syrup. In humans, the acute non-effect level of rare sugar syrup for causing diarrhea was estimated as 0.9 g/kg body weight as dry solid base in both males and females." As taken from Yamada T et al. 2015. Shokuhin Eiseigaku Zasshi 56, 211-6. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26537651

5.2. Repeated dose toxicity

Human

Lactic Acidosis Complicating Liver Failure after Intravenous Fructose (Summary). The occurence of lactic acidosis in 69-year-old woman undergoing therapy with fructose is presented. The patient's carbohydrate intake was 400g. Daily, given as IV fructose 40%. Therapy was continued for 4 days. In this patient, it was believed that lactate formation resulting from fructose breakdown initiated the lactic acidosis, since this coincided with fructose therapy and improved slightly when glucose was substituted and the serum magnesium was corrected. As taken from Craig GM and Crane CW. Br Med J. 1971 Oct 23;4(781):211-2. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1799234/pdf/brmedj02672-0047.pdf

"The metabolic effects of fructose (I) 15%, administered IV at a rate of 1 g/kg/hr for 2.5 hr to 8 infants, aged 3-8 months, hospitalized for acute gastroenteritis, were monitored. Significant elevation of total reducing substances, lactic acid, and uric acid blood levels was found. These results confirm similar observations in other age groups, and suggest that close biochemical monitoring is essential when I is used as an energy source during IV alimentation" (Zahavi et al.,1981).

Non-human

Type of Test	Route of Exposure	Species Observed	Dose Data	Toxic Effects	Reference
TDLo - Lowest published toxic dose	Oral	Rodent - rat	119 mg/kg/14D (intermittent)	Blood - other changes Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - true cholinesterase Related to Chronic Data - death	NTIS** National Technical Information Service. (Springfield, VA 22161) Formerly U.S. Clearinghouse for Scientific & Technical Information. Volume(issue)/page/year: AD-A132-656
TDLo - Lowest published toxic dose	Oral	Rodent - rat	140 gm/kg/2W (intermittent)	Liver - other changes Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Metabolism (Intermediary) - lipids including transport	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1-1909/10- Volume(issue)/page/year: 302,232,2002
TDLo - Lowest	Oral	Rodent - rat	70 gm/kg/1W	Behavioral - fluid intake	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub.

published toxic dose			(continuous)		Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 83,183,2002
TDLo - Lowest published toxic dose	Oral	Rodent - rat	70 gm/kg/3W (continuous)	Behavioral - food intake (animal) Cardiac - pulse rate increase, without fall in BP Vascular - BP elevation not characterized in autonomic section	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979- Volume(issue)/page/year: 83,183,2002
TDLo - Lowest published toxic dose	Oral	Rodent - rat	42 gm/kg/1W (continuous)	Vascular - BP elevation not characterized in autonomic section	BPBLEO Biological and Pharmaceutical Bulletin. (Pharmaceutical Society of Japan, 2-12-15-201 Shibuya Shibuya-ku, Tokyo 150, Japan) V.16- 1993- Volume(issue)/page/year: 27,1779,2004
TDLo - Lowest published toxic dose	Oral	Rodent - rat	168 gm/kg/4W (continuous)	Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - transaminases Endocrine - other changes	BPBLEO Biological and Pharmaceutical Bulletin. (Pharmaceutical Society of Japan, 2-12-15-201 Shibuya Shibuya-ku, Tokyo 150, Japan) V.16- 1993- Volume(issue)/page/year: 27,1779,2004
TDLo - Lowest published toxic dose	Oral	Rodent - rat	700000 mg/kg/35D (continuous)	Gastrointestinal - other changes Liver - changes in liver weight	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 46,752,2008
TDLo - Lowest published toxic dose	Oral	Rodent - rat	2160 gm/kg/60D (intermittent)	Endocrine - hyperglycemia Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol) Biochemical - Metabolism (Intermediary) - lipids including transport	SIMEJ* Singapore medical journal(Singapore, Singapore Medical Assn.) V.1- 1960- Volume(issue)/page/year: 47,858,2006

TDLo - Lowest published toxic dose	Oral	Rodent - rat	605.25 gm/kg/8W (continuous)	Vascular - BP elevation not characterized in autonomic section Endocrine - other changes Biochemical - Metabolism (Intermediary) - lipids including transport	BPBLEO Biological and Pharmaceutical Bulletin. (Pharmaceutical Society of Japan, 2-12-15-201 Shibuya Shibuya-ku, Tokyo 150, Japan) V.16- 1993- Volume(issue)/page/year: 31,2103,2008
TDLo - Lowest published toxic dose	Oral	Rodent - rat	140 gm/kg/2W (continuous)	Cardiac - cardiac output Cardiac - other changes	BJPCBM British Journal of Pharmacology. (Macmillan Press Ltd., Houndmills, Basingstoke, Hants. RG21 2XS, UK) V.34- 1968- Volume(issue)/page/year: 151,341,2007
TDLo - Lowest published toxic dose	Oral	Rodent - rat	700 gm/kg/8W (continuous)	Vascular - BP elevation not characterized in autonomic section Vascular - contraction (isolated tissues) Vascular - relaxation (isolated tissues)	BCLPT* Basic & clinical pharmacology & toxicology (Copenhagen, Denmark : Nordic Pharmacological Society Oxford, UK : Distributed by Blackwell Munksgaard) V.94- 2004- Volume(issue)/page/year: 102(Suppl 1),54,2008
TDLo - Lowest published toxic dose	Oral	Rodent - rat	140 gm/kg/2W (continuous)	Vascular - BP elevation not characterized in autonomic section Endocrine - other changes Biochemical - Metabolism (Intermediary) - lipids including transport	JPETAB Journal of Pharmacology and Experimental Therapeutics. (Williams & Wilkins Co., 428 E. Preston St., Baltimore, MD 21202) V.1- 1909/10- Volume(issue)/page/year: 328,610,2009
TDLo - Lowest published toxic dose	Oral	Rodent - rat	2376 gm/kg/60D (intermittent)	Endocrine - hyperglycemia Endocrine - other changes Biochemical - Metabolism (Intermediary) -	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982-

				lipids including transport	Volume(issue)/page/year: 47,2224,2009
TDLo - Lowest published toxic dose	Oral	Rodent - rat	560 gm/kg/8W (continuous)	Kidney/Ureter/Bladder - renal function tests depressed Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - xanthine oxidases Biochemical - Metabolism (Intermediary) - xanthine, purine or nucleotides including urate	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 135,399,2011
TDLo - Lowest published toxic dose	Oral	Rodent - rat	840 gm/kg/12W (continuous)	Vascular - BP elevation not characterized in autonomic section Endocrine - hyperglycemia Endocrine - other changes	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 49,3007,2011
TDLo - Lowest published toxic dose	Oral	Rodent - rat	257.6 gm/kg/14D (continuous)	Liver - hepatitis (hepatocellular necrosis), diffuse Liver - changes in liver weight Biochemical - Metabolism (Intermediary) - lipids including transport	TXAPA9 Toxicology and Applied Pharmacology. (Academic Press, Inc., 1 E. First St., Duluth, MN 55802) V.1- 1959- Volume(issue)/page/year: 251,32,2011
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	4373.6 gm/kg/8W (continuous)	Liver - fatty liver degeneration Biochemical - Metabolism (Intermediary) - lipids including transport	TOLED5 Toxicology Letters. (Elsevier Science Pub. B.V., POB 211, 1000 AE Amsterdam, Netherlands) V.1- 1977- Volume(issue)/page/year: 212,229,2012
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	2520 gm/kg/6W (continuous)	Kidney/Ureter/Bladder - renal function tests depressed Kidney/Ureter/Bladder - other changes Biochemical - Metabolism	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1- 1979- Volume(issue)/page/year: 169,49,2015

				(Intermediary) - other proteins	
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	882 mg/kg/21D (continuous)	Liver - fatty liver degeneration Liver - other changes Biochemical - Enzyme inhibition, induction, or change in blood or tissue levels - cytochrome oxidases (including oxidative phosphorylation)	FCTOD7 Food and Chemical Toxicology. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.20- 1982- Volume(issue)/page/year: 103,111,2017
TDLo - Lowest published toxic dose	Oral	Rodent - rat	840 gm/kg/12W (continuous)	Blood - other changes Biochemical - Metabolism (Intermediary) - effect on inflammation or mediation of inflammation	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,1977,2015
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	5040 gm/kg/12W (continuous)	Kidney/Ureter/Bladder - renal function tests depressed Endocrine - other changes Blood - changes in serum composition (e.g. TP, bilirubin, cholesterol)	PYTOEY Phytomedicine. (Gustav Fischer Verlag, Postfach 720143, D-70577 Stuttgart, Germany) V.1- 1994- Volume(issue)/page/year: 23,1504,2016

As taken from RTECS, 2018.

Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols (Abstract). In search of an adequate model for the human metabolic syndrome, the metabolic characteristics of Wistar rats were analysed after being submitted to different protocols of high fructose ingestion. First, two adult rat groups (aged 90 d) were studied: a control group (C1; n 6) received regular rodent chow (Labina, Purina) and a fructose group (F1; n 6) was fed on regular rodent chow. Fructose was administered as a 10 % solution in drinking water. Second, two adult rat groups (aged 90 d) were evaluated: a control group (C2; n 6) was fed on a balanced diet (AIN-93G) and a fructose group (F2; n 6) was fed on a purified 60 % fructose diet. Finally, two young rat groups (aged 28 d) were analysed: a control group (C3; n 6) was fed on the AIN-93G diet and a fructose group (F3; n 6) was fed on a 60 % fructose diet. After 4-8 weeks, the animals were evaluated. Glucose tolerance, peripheral insulin sensitivity, blood lipid profile and body fat were analysed. In the fructose groups F2 and F3 glucose tolerance and insulin sensitivity were lower, while triacylglycerolaemia was higher than the respective controls C2 and C3 ($P < 0.05$). Blood total cholesterol, HDL and LDL as well as body fat showed change only in the second protocol. In conclusion, high fructose intake is more effective at producing the signs of the metabolic syndrome in adult than in young Wistar rats. Additionally, diet seems to be a

more effective way of fructose administration than drinking water. As taken from de Moura RF et al. Br J Nutr. 2009 Apr; 101(8):1178-84. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/19007450

"BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015." As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24552754

"Clinical studies have linked the increased consumption of fructose to the development of obesity, dyslipidemia, and impaired glucose tolerance, and a role in hepatosteatosis development is presumed. Fructose can undergo a nonenzymatic reaction from which advanced glycation end products (AGEs) are derived, leading to the formation of dysfunctional, fructosylated proteins; however, the in vivo formation of AGEs from fructose is still less known than that from glucose. In the present study C57Bl/6J mice received 15% (wt/vol) fructose (FRT) or 15% (wt/vol) glucose (GLC) in water to drink for 30 wk, resembling human habit to consume sugary drinks. At the end of the protocol both FRT- and GLC-drinking mice had increased fasting glycemia, glucose intolerance, altered plasma lipid profile, and marked hepatosteatosis. FRT mice had higher hepatic triglycerides deposition than GLC, paralleled by a greater increased expression and activity of the sterol regulatory element-binding protein 1 (SREBP1), the transcription factor responsible for the de novo lipogenesis, and of its activating protein SCAP. LC-MS analysis showed a different pattern of AGE production in liver tissue between FRT and GLC mice, with larger amount of carboxymethyl lysine (CML) generated by fructose. Double immunofluorescence and coimmunoprecipitation analysis revealed an interaction between CML and SCAP that could lead to prolonged activation of SREBP1. Overall, the high levels of CML and activation of SCAP/SREBP pathway associated to high fructose exposure here reported may suggest a key role of this signaling pathway in mediating fructose-induced lipogenesis." As taken from

Mastrocola R et al. 2013. Am. J. Physiol. Gastrointest. Liver Physiol. 305(6), G398-407. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23868406

"In the present study, we tested the effect of OS (oxidative stress) inhibition in rats fed on an FRD [fructose-rich diet; 10% (w/v) in drinking water] for 3 weeks. Normal adult male rats received a standard CD (commercial diet) or an FRD without or with an inhibitor of NADPH oxidase, APO (apocynin; 5 mM in drinking water; CD-APO and FRD-APO). We thereafter measured plasma OS and metabolic-endocrine markers, AAT (abdominal adipose tissue) mass and cell size, FA (fatty acid) composition (content and release), OS status, LEP (leptin) and IRS (insulin receptor substrate)-1/IRS-2 mRNAs, ROS (reactive oxygen species) production, NADPH oxidase activity and LEP release by isolated AAT adipocytes. FRD-fed rats had larger AAT mass without changes in body weight, and higher plasma levels of TAG (triacylglycerol), FAs, TBARS (thiobarbituric acid-reactive substance) and LEP. Although no significant changes in glucose and insulin plasma levels were observed in these animals, their HOMA-IR (homoeostasis model assessment of insulin resistance) values were significantly higher than those of CD. The AAT from FRD-fed rats had larger adipocytes, higher saturated FA content, higher NADPH oxidase activity, greater ROS production, a distorted FA content/release pattern, lower insulin sensitivity together with higher and lower mRNA content of LEP and IRS-1-/2 respectively, and released a larger amount of LEP. The development of all the clinical, OS, metabolic, endocrine and molecular changes induced by the FRD were significantly prevented by APO co-administration. The fact that APO treatment prevented both changes in NADPH oxidase activity and the development of all the FRD-induced AAT dysfunctions in normal rats strongly suggests that OS plays an important role in the FRD-induced MS (metabolic syndrome) phenotype." As taken from Fariña JP et al. 2013. Clin. Sci. (Lond.) 125(2), 87-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23384123?dopt=AbstractPlus

"The use of high D(+)-fructose corn syrup has increased over the past several decades in the developed countries, while overweight and obesity rates and the related diseases have risen dramatically. However, we found that feeding a high D(+)-fructose diet (80% D(+)-fructose as part of the diet) to weaning rats for 21 days led to reduced food intake (50% less, $P < 0.0001$) and thus delayed the weight gains in the body (40% less, $P < 0.0001$) and testes (40% less, $P < 0.0001$) compared to the no D(+)-fructose diet…." As taken from Shibata K & Fukuwatari T. 2013. Nutr. Metab. Insights 6, 29-34. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23935370

"Consumption of added sugar has increased over recent decades and is correlated with numerous diseases. Rodent models have elucidated mechanisms of toxicity, but only at concentrations beyond typical human exposure. Here we show that comparatively low levels of added sugar consumption have substantial negative effects on mouse survival, competitive ability, and reproduction. Using Organismal Performance Assays--in which mice fed human-relevant concentrations of added sugar (25% kcal from a mixture of fructose and glucose, modeling high fructose corn syrup) and control mice compete in seminatural enclosures for territories, resources and mates--we demonstrate that fructose/glucose-fed females experience a twofold increase in mortality while fructose/glucose-fed males control 26% fewer territories and produce 25% less offspring. These findings represent the lowest level of sugar consumption shown to adversely affect mammalian health. Clinical defects of fructose/glucose-fed mice were decreased glucose clearance and increased fasting cholesterol. Our data highlight that physiological adversity can exist when clinical disruptions are minor, and suggest that Organismal Performance Assays represent a

promising technique for unmasking negative effects of toxicants." Ruff JS et al. 2013. Nat. Commun. 4, 2245. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23941916?dopt=AbstractPlus

"Sugar intake in the United States has increased by >40 fold since the American Revolution. The health concerns that have been raised about the amounts of sugar that are in the current diet, primarily as beverages, are the subject of this review. Just less than 50% of the added sugars (sugar and high-fructose corn syrup) are found in soft drinks and fruit drinks. The intake of soft drinks has increased 5-fold between 1950 and 2000. Most meta-analyses have shown that the risk of obesity, diabetes, cardiovascular disease, and metabolic syndrome are related to consumption of beverages sweetened with sugar or high-fructose corn syrup. Calorically sweetened beverage intake has also been related to the risk of nonalcoholic fatty liver disease, and, in men, gout. Calorically sweetened beverages contribute to obesity through their caloric load, and the intake of beverages does not produce a corresponding reduction in the intake of other food, suggesting that beverage calories are "add-on" calories. The increase in plasma triglyceride concentrations by sugar-sweetened beverages can be attributed to fructose rather than glucose in sugar. Several randomized trials of sugar-containing soft drinks versus low-calorie or calorie-free beverages show that either sugar, 50% of which is fructose, or fructose alone increases triglycerides, body weight, visceral adipose tissue, muscle fat, and liver fat. Fructose is metabolized primarily in the liver. When it is taken up by the liver, ATP decreases rapidly as the phosphate is transferred to fructose in a form that makes it easy to convert to lipid precursors. Fructose intake enhances lipogenesis and the production of uric acid. By worsening blood lipids, contributing to obesity, diabetes, fatty liver, and gout, fructose in the amounts currently consumed is hazardous to the health of some people." As taken from Bray GA. 2013. Adv. Nutr. 4(2), 220-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493538?dopt=AbstractPlus

Glucose, fructose or lactose (g/day) and type 2 diabetes mellitus
né+ No association né+ Limited evidence

As taken from SACN, 2015.

"This study investigates the possible protective effects of levocetirizine against fructose-induced insulin resistance, hepatic steatosis and vascular dysfunction, in comparison to pioglitazone, a standard insulin sensitizer. Male Sprague Dawley rats (150-200 g) were divided into 4 groups. Three groups were fed on high fructose diets (HFD) containing 60% w/w fructose, while the fourth control group was fed on standard laboratory food for 8 weeks. AUCOGTT, AUCITT, fasting glucose, HOMA-IR, hepatic glutathione (GSH) and malondialdehyde (MDA) levels, serum total cholesterol, LDL-C, C-reactive protein (CRP) level and lactate dehydrogenase (LDH) activity and liver steatosis scores were significantly higher in HFD group compared to control group. Moreover, body weight gain, food intake, feeding efficiency, HOMA-β, Emax and pEC50 of acetylcholine-induced relaxations of aortic rings and hepatic superoxide dismutase (SOD) activity were significantly lower in HFD group than in control group. Treatment with levocetirizine caused significant decreases in AUCOGTT, AUCITT, HOMA-IR, hepatic GSH and MDA levels and serum CRP level and

LDH activity and significant increases in hepatic SOD activity and HOMA-β when compared with the HFD group. Although levocetirizine failed to alter TC and LDL-C levels, it produced a significant increase in HDL-C level relative to control group. Levocetirizine was also able to improve acetylcholine-induced relaxations of aortic rings, indicating a protective effect against insulin resistance-induced endothelial damage comparable to that offered by pioglitazone. Moreover, levocetirizine substantially attenuated insulin resistance-associated liver macrovesicular steatosis. These findings demonstrate that levocetirizine ameliorates insulin resistance, improves glucose tolerance and attenuates insulin resistance-linked hepatic steatosis and vascular damage." As taken from Shawkey NM et al. 2014. Eur. J. Pharmacol. 740, 353-63. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25064340

"Currently, the metabolic syndrome (MS) is occurring at growing rates worldwide, raising extensive concerns on the mechanisms and therapeutic interventions for this disorder. Herein, we described a novel method of establishing MS model in rodents. Male Institute of Cancer Research (ICR) mice were fed with high-fat-high-fructose (HFHF) diet or normal chow (NC) respectively for 12 weeks. Metabolic phenotypes were assessed by glucose tolerance test, insulin tolerance test and hyperinsulinemic-euglycemic clamp. Blood pressure was measured by a tail-cuff system. At the end of the experiment, mice were sacrificed, and blood and tissues were harvested for subsequent analysis. Serum insulin levels were measured by ELISA, and lipid profiles were determined biochemically. The HFHF diet-fed ICR mice exhibited obvious characteristics of the components of MS, including obvious obesity, severe insulin resistance, hyperinsulinemia, dislipidemia, significant hypertension and hyperuricemia. Our data suggest that HFHF diet-fed ICR mice may be a robust and efficient animal model that could well mimic the basic pathogenesis of human MS." As taken from Zhuhua Z et al. 2015. Exp. Anim. 64, 435-42. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26134356

"BACKGROUND: Intake of added sugar has been shown to correlate with many human metabolic diseases, and rodent models have characterized numerous aspects of the resulting disease phenotypes. However, there is a controversy about whether differential health effects occur because of the consumption of either of the two common types of added sugar-high-fructose corn syrup (fructose and glucose monosaccharides; F/G) or table sugar (sucrose, a fructose and glucose disaccharide). OBJECTIVES: We tested the equivalence of sucrose- vs. F/G-containing diets on mouse (Mus musculus) longevity, reproductive success, and social dominance. METHODS: We fed wild-derived mice, outbred mice descended from wild-caught ancestors, a diet in which 25% of the calories came from either an equal ratio of F/G or an isocaloric amount of sucrose (both diets had 63% of total calories as carbohydrates). Exposure lasted 40 wk, starting at weaning (21 d of age), and then mice (104 females and 56 males) were released into organismal performances assays-seminatural enclosures where mice competed for territories, resources, and mates for 32 wk. Within enclosures all mice consumed the F/G diet. RESULTS: Females initially fed the F/G diet experienced a mortality rate 1.9 times the rate (P = 0.012) and produced 26.4% fewer offspring than females initially fed sucrose (P = 0.001). This reproductive deficiency was present before mortality differences, suggesting the F/G diet was causing physiologic performance deficits prior to mortality. No differential patterns in survival, reproduction, or social dominance were observed in males, indicating a sex-specific outcome of exposure. CONCLUSION: This study provides experimental evidence that the consumption of human-relevant levels of F/G is more deleterious than an isocaloric amount of sucrose for key organism-level health measures in female mice." As

taken from Ruff JS et al. 2015. J. Nutr. 145, 434-41. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733457

“BACKGROUND: The effect of Hibiscus sabdariffa calyx extract was evaluated in high-fructose-induced metabolic syndrome rats. Insulin resistance, hyperglycemia, dyslipidemia and oxidative rout were induced in rats using high-fructose diet. High-fructose diet-fed rats were administered 100 and 200 mg kg(-1) body weight of H. sabdariffa extract for 3 weeks, starting from week 7 of high-fructose diet treatment. RESULTS: High-fructose diet significantly (P < 0.05) increased the serum levels of blood glucose, insulin, total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDLc) and very-low-density lipoprotein cholesterol (VLDLc), with a concomitant reduction in high-density lipoprotein cholesterol (HDLc). These alterations were significantly ameliorated by the extract. High-fructose diet-mediated decreases in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-red) and glucose 6-phosphate dehydrogenase (Glc 6-PD) were significantly (P < 0.05) attenuated. Altered levels of reduced glutathione (GSH) and glutathione disulfide (GSSG) were significantly (P < 0.05) restored to normal. High-fructose diet-mediated increases in the concentrations of malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl and percentage fragmented DNA were significantly (P < 0.05) lowered by the Hibiscus extract. CONCLUSION: Overall, aqueous extract of H. sabdariffa palliates insulin resistance, hyperglycemia, dyslipidemia and oxidative rout in high-fructose-induced metabolic syndrome rats.” As taken from Ajiboye TO et al. 2016. J. Sci. Food Agric. 96(5), 1522-31. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/25965053

“AIM: To investigate the effects of fructose consumption on the antioxidant capacity in heart and kidney, cardiovascular disease risk factors, and evaluation of these variables after its removal. METHODS: Male Wistar rats (n = 36) were divided into control group (n = 12): standard chow + water or F group: standard chow + fructose in drinking water (30%) for 15 weeks. After, F group was divided to continue receiving standard chow + fructose in drinking water (30%) (n = 12) or standard chow + water (Ex group, n = 12) for 9 weeks. Water, chow and caloric diaries intake, final body weight, adiposity index, plasma glucose and triacylglycerol, systolic blood pressure, and cardiac and renal hydrophilic antioxidant capacity were analyzed. RESULTS: Control and Ex groups consumed less chow and water compared to F group. Caloric intake was higher in control group. There was no difference in final body weight and adiposity index. Systolic blood pressure and cardiac and renal hydrophilic antioxidant capacity were worst in F group. CONCLUSION: Prolonged exposure to fructose induces oxidative stress, systolic blood pressure, and increase in triacylglycerol. When stopped fructose consumption, Ex group presented improvement in these variables, suggesting the toxicity effect of fructose when consumed in high amounts and prolonged exposure.” As taken from Francisqueti FV et al. 2016. SAGE Open Med. 4, 2050312116684294. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28348741

“BACKGROUND: Sugar-sweetened beverage (SSB) consumption and low-grade chronic inflammation are both independently associated with type 2 diabetes and cardiovascular disease. Fructose, a major component of SSBs, may acutely trigger inflammation, which may be one link between SSB consumption and cardiometabolic disease. OBJECTIVE: We sought to determine whether beverages sweetened with fructose, high-fructose corn syrup (HFCS), and glucose differentially influence systemic inflammation [fasting plasma C-reactive protein and interleukin-6 (IL-6) as primary endpoints] acutely and before major

changes in body weight. Secondary endpoints included adipose tissue inflammation, intestinal permeability, and plasma fetuin-A as potential mechanistic links between fructose intake and low-grade inflammation. DESIGN: We conducted a randomized, controlled, double-blind, crossover design dietary intervention (the Diet and Systemic Inflammation Study) in 24 normal-weight to obese adults without fructose malabsorption. Participants drank 4 servings/d of fructose-, glucose-, or HFCS-sweetened beverages accounting for 25% of estimated calorie requirements while consuming a standardized diet ad libitum for three 8-d periods. RESULTS: Subjects consumed 116% of their estimated calorie requirement while drinking the beverages with no difference in total energy intake or body weight between groups as reported previously. Fasting plasma concentrations of C-reactive protein and IL-6 did not differ significantly at the end of the 3 diet periods. We did not detect a consistent differential effect of the diets on measures of adipose tissue inflammation except for adiponectin gene expression in adipose tissue (P = 0.005), which was lowest after the glucose phase. We also did not detect consistent evidence of a differential impact of these sugars on measures of intestinal permeability (lactulose:mannitol test, plasma zonulin, and plasma lipopolysaccharide-binding protein). CONCLUSION: Excessive amounts of fructose, HFCS, and glucose from SSBs consumed over 8 d did not differentially affect low-grade chronic systemic inflammation in normal-weight to obese adults." As taken from Kuzma JN et al. 2016. Am. J. Clin. Nutr. 104(2), 306-14. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27357093

Quantitative Risk Type - Not calculated
Quantitative Risk Value - Not calculated
Product Use – Not specified
Safety Evaluation Owner - COSMOS TTC (NON-CANCER)
POD Method - LOAEL
POD Value – 888.9 mg/kg bw/day
POD Owner - COSMOS TTC (NON-CANCER)
Adjustment factors used in calculations: Adjustment factor: Study: Dose Duration: 6.0 (6)
Adjustment factor: Study: LOEL-NOEL Extrapolation: 3.0 (3)
Critical study: RAT (Short Term Toxicity) Oral exposure for 50 day
NOAEL/LOAEL Owner - PAFA
Original NOAEL - Not established
Original LOAEL - 16000.0 mg/kg bw/day
Critical Effects - BODY WEIGHT CHANGES

As taken from the COSMOS database available at http://www.cosmostox.eu/what/COSMOSdb/

5.3. Reproduction toxicity

Influence of sorbitol accumulation on growth and development of embryos cultured in elevated levels of glucose and fructose (Abstract). Day-9 rat embryos, cultured for 48 hours in 67 mmol/l D-glucose, showed impaired growth, increased malformation rate and elevated concentration of sorbitol compared to embryos cultured in medium without additional glucose supplement. Supplementing the high-glucose cultures with an aldose reductase inhibitor markedly decreased the sorbitol levels without affecting the malformations or the retarded growth of the embryos. Since embryos cultured in 6.6 mmol/l D-fructose showed normal growth and morphology despite increased accumulation of sorbitol, this study suggests a dissociation between raised sorbitol levels and glucose-induced maldevelopment in rat embryos. As taken from Eriksson UJ et al. Diabetes Res. 1989 May;11(1):27-32. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/2515932?dopt=Abstract

Effects of D-glucose concentration, D-fructose, and inhibitors of enzymes of the pentose phosphate pathway on the development and sex ratio of bovine blastocysts (Abstract). Here, we have determined the threshold of embryo sensitivity to glucose, whether substitution of D-fructose for glucose reduces the selective loss of female embryos, and whether inhibition of an X-linked gene product, glucose 6-phosphate dehydrogenase (G6PD), normalizes sex ratio among bovine blastocysts. IVP zygotes were cultured in glucose-free medium to 72 hr post-insemination, at which time 8-cell embryos were selected for treatment and cultured until 144 hr post-insemination. Addition of 4 mM glucose at the 8-cell stage reduced ($P < 0.05$) the number of blastocyst that formed, whereas 2.5 and 1 mM glucose had no effect. Sex ratio in the presence of 4 and 2.5 mM glucose differed significantly from 0.5, while 1 mM glucose had no effect. D-fructose (5.6 mM) did not compromise development to blastocyst and did not alter the sex ratio of blastocysts that formed. When G6PD inhibitors (dehydroepiandrosterone: DHEA and 6-aminonicotinamide: 6-AN), which effectively inhibit the reduction of the NADPH-sensitive dye, brilliant cresyl blue (BCB) in bovine morulae, were added to the culture medium containing 4 mM glucose, embryo development was moderately reduced, but sex ratio skewing was corrected (with 6-AN) or lowered (with DHEA). In conclusion, glucose above 2.5 mM impairs bovine embryo development and increases sex ratio, most likely as a result of increased pentose-phosphate (PP) pathway activity in female embryos. As taken from Kimura K et al. Mol Reprod Dev. 2005 Oct;72(2):201-7. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/15968626?dopt=Abstract

Fructose in fetal cord blood and its relationship with maternal and 48-hour-newborn blood concentrations (Abstract).

BACKGROUND: Studies have suggested that different non-glucose sugars and sugar alcohols play a role in placental and fetal metabolism. However, the role of fructose in the fetal and newborn metabolism is unclear and studies are scarce.

AIM: Our objective was to investigate the presence of fructose in umbilical cord blood in full-term gestation and its relationship with maternal and 48-hour-old- newborn blood concentrations, to evaluate fructose production by the fetus and newborn infant.

METHODS: Blood fructose and glucose concentrations were determined by HPLC in 26 paired samples of maternal blood, umbilical cord vein, and peripheral newborn blood at 48h after birth. ANOVA, the Friedman Analysis of Variance on Ranks and the Pearson correlation with $p<0.05$ were used.

RESULTS: Fructose concentration in umbilical cord blood was higher than maternal blood (p=0.024), suggesting endogenous fructose production by the fetal-placental unit via the sorbitol pathway. Fructose concentrations were higher in newborns at 48h after birth than in the fetal umbilical cord blood (p=0.004), suggesting that fructose production is a continuous process from fetus to newborn.

CONCLUSIONS: Fructose production by the sorbitol pathway, present in the fetus and newborn, is an alternative pathway in glucose metabolism probably used to maintain redox balance in the fetus. We suggest that endogenous fructose, similar to dietary ingested fructose, under physiological conditions produces the backbone for triacylglycerol and lipid synthesis in the fetus and newborn. Therefore the route for metabolizing fructose is already present in the early steps of human development. As taken from Trindade CE, Barreiros RC et al. Early Hum Dev. 2011 Jan 26. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/21276669

Effects of feeding sows fat or fructose during late gestation and lactation (Abstract). The effects of dietary fat or fructose supplementation during late gestation and lactation on sow milk production and composition and on progeny were examined. On d 88 of gestation, 24 sows were allotted by parity to three dietary treatments (eight sows/treatment). Treatments were 1) a 12.5% crude protein, corn-soybean meal control, 2) the control + 10% added fat or 3) the control + 23% high fructose corn syrup. All treatments were fed to supply 1.82 kg/d of the control diet from d 89 of gestation to parturition with sows in treatments 2 or 3 receiving .18 kg of additional fat or .53 kg of additional high fructose corn syrup, respectively. Feed was gradually increased from d 1 to 7 of lactation to 4.54 kg/d of the control diet (plus .45 kg of added fat and 1.33 kg of added fructose for treatments 2 and 3) and remained at these levels for the remainder of the 21 d lactation period. All treatments were iso-nitrogenous; treatments 2 and 3 were iso-caloric. Litter birth weights, number of pigs born alive, weaning weights and piglet survival rate were not affected by sow treatment. Stillbirths were less (P less than .05) for sows fed fat. Lipid content of milk 24 h post-farrowing was greater (P less than .05) from sows fed fat compared with sows fed fructose. Milk production estimates indicated that multiparous sows fed fat produced more (P less .05) milk than sows fed the control diet. On d 112 of gestation and d 15 of lactation, serial blood samples were drawn to monitor sow response to a glucose challenge (1 g/kg body weight). As taken from Coffey MT et al. J Anim Sci. 1987, Nov; 65(5):1249-56. PubMed, 2010 avaiable at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=3320005&dopt=AbstractPlus

Yield and composition of milk and weight gain of nursing pigs from sows fed diets containing fructose or dextrose (Abstract). Yield and composition of milk and growth of nursing pigs in response to dietary treatment were estimated from 25 lactating sows during a 22-d period. Eight sows were fed 6 kg/d of a corn-soybean control diet (C sows). Nine were fed the control diet in which approximately 6.5 g X kg body weight (BW-1) X d-1 of carbohydrate was supplied by fructose corn syrup (F sows) and eight were fed the control diet containing equivalent carbohydrate supplied by powdered dextrose (D sows). Blood samples collected via jugular cannulae were analyzed for plasma concentrations of fructose, glucose and insulin. Concentrations of fructose and glucose from F sows were significantly higher throughout the study than that from D and C sows, while insulin concentration was approximately 2.5-fold lower. Milk yield from F sows on d 14 and 21 was significantly higher and pigs weaned on d 21 were heavier than those from D and C sows.

Sows fed the diet containing fructose experienced significant BW loss during lactation. Coefficients of gross correlation across treatments showed milk yield and litter weight gain to be negatively associated with percentages of protein, lipids and total solids in milk, but positively associated with concentrations of lactose and gross energy. Nursing pig weight gain at weaning was more responsive to total yields of milk and milk nutrients than to composition. These data support the hypothesis that source of metabolizable energy (ME) affects milk yield, composition and efficiency at which the sow converts dietary nutrients into milk. As taken from White CE et al. J Anim Sci. 1984, Jul; 59(1):141-50. PubMed, 2010 available at

http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=6378870&dopt=AbstractPlus

Glucose and fructose as functional modulators of overall dog, but not boar sperm function (Abstract). The main aim of the present work was to test the effects of glucose and fructose on the phosphorylation levels of proteins linked to the control of overall sperm function in two species with very different metabolic characteristics, dog and boar. Incubation of dog spermatozoa with 10mM glucose increased serine phosphorylation of proteins related to cell cycle and signal transduction including cyclins B and E, Cdk2, Cdk6, Cdc6, PYK2, c-kit, Raf-1, TRK and several protein phosphatases. Incubation of dog spermatozoa with 10mM fructose decreased serine phosphorylation levels of cyclins B and D3, Cdk1/Cdc2, Cdk2, Cdk6, Akt, PI3 kinase, ERK-1 and protein kinase C. Incubation of boar spermatozoa with glucose or fructose did not modify any of the phosphorylation patterns studied. Given that one important difference between dog and boar spermatozoa is the presence of glucokinase (GK) in dog but not in boar, GK-transfected COS7 cells were incubated with either 10mM glucose or 10mM fructose. Incubation of GK-transfected cells with fructose decreased serine phosphorylation of cyclin A, ERK-2 and Hsp-70. In contrast, incubation of control COS7 cells with fructose increased serine phosphorylation of Cdk6, Cdk1/Cdc2, protein kinase C and Hsp-70. Incubation with glucose did not induce any significant effect. Our results indicate that monosaccharides act as signalling compounds in dog spermatozoa after ejaculation through changes in the phosphorylation levels of specific proteins. One of the factors that may be related to the action of sugars is the equilibrium of the total sperm hexokinase activity, in which the presence or absence of GK appears to be relevant. Taken from Fernández-Novell JM et al. (2011). Reprod. Fertil. Dev. 23(3):468-80, available at http://www.ncbi.nlm.nih.gov/pubmed/21426864?dopt=AbstractPlus

“Fructose is an increasingly common constituent of the Westernized diet due to cost and production efficiencies. Although an integral component of our pre-industrial revolution diet, over the past two decades human and animal studies have highlighted that excessive fructose intake appears to be associated with adverse metabolic effects. Excessive intake of fructose is the combined result of increased total energy consumption and increased portion sizes of foods, which often incorporate the fructose-containing sugars sucrose and high-fructose corn-syrup (HFCS). The adverse metabolic effects following excessive fructose consumption have become a hot topic in mainstream media and there is now rigorous scientific debate regarding periods of exposure, dosage levels, interactive effects with other sugars and fats and mechanisms underlying the actions of fructose. There is still a degree of controversy regarding the extent to which sugars such as sucrose and HFCS have contributed to the current epidemic of obesity and diabetes. Furthermore, an increasing number of infants are being exposed to sugar-sweetened food and beverages before birth and during early postnatal life, highlighting the importance of determining the

long-term effects of this perinatal exposure on the developing offspring. There are limited human observational and controlled studies identifying associations of excessive sweetened food and beverage consumption with poor pregnancy outcomes. Animal research has demonstrated an increased incidence of gestational diabetes as well as altered maternal, fetal and offspring metabolic function, although the long-term effects and the mechanism underlying these perturbations are ill defined. This review aims to understand the role of early life fructose exposure in modifying postnatal risk of disease in the offspring, focusing on fructose intake during pregnancy and in early postnatal life." As taken from Regnault TR et al. 2013. Clin. Exp. Pharmacol. Physiol. 40(11), 824-37. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24033459?dopt=AbstractPlus

"Accumulating evidence suggests that both an adverse prenatal and early postnatal environment increase susceptibility to renal and metabolic dysfunction later in life; however, whether exposure to adverse conditions during both prenatal and postnatal development act synergistically to potentiate the severity of renal and metabolic injury remains unknown. Sprague-Dawley rats were fed either a standard diet or a diet high in fat/fructose throughout pregnancy and lactation. After being weaned, female offspring were randomized to either standard diet or the high-fat/high-fructose diet, resulting in the following treatment groups: NF-NF, offspring of mothers fed a standard diet and fed a standard diet postnatally; NF-HF, offspring of mothers fed a standard diet and fed a high-fat/fructose diet postnatally; HF-NF, offspring of mothers fed a high-fat/fructose diet and fed a standard diet postnatally; HF-HF, offspring of mothers fed a high-fat/fructose diet and fed a high-fat/fructose diet postnatally. At the time of euthanasia (17 wk of age), HF-HF offspring weighed 30% more and had 110% more visceral fat than NF-NF offspring. The HF-HF offspring also had elevated blood glucose levels, glucose intolerance, 286% increase in urine albumin excretion, and 60% increase in glomerulosclerosis compared with NF-NF. In addition, HF-HF offspring exhibited a 100% increase in transforming growth factor-β protein expression and 116% increase in the abundance of infiltrated macrophages compared with the NF-NF offspring. These observations suggest that high-fat/fructose feeding during prenatal and throughout postnatal life increases the susceptibility to renal and metabolic injury later in life." As taken from Flynn ER et al. 2013. Am. J. Physiol. Regul. Integr. Comp. Physiol. 304(4), R278-85. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23255587?dopt=AbstractPlus

"This review article endeavoured to ascertain the facts about fructose, a mono-saccharide sugar being increasingly used in daily diet. Human and animal studies suggest that fructose ingestion produces deleterious effects on the cardiovascular system, such as increase in blood pressure and also has adverse metabolic effects, for example insulin resistance and hypertriglyceridaemia. On the other hand it has also been determined that fructose, especially that is contained in natural sources as apples, dates and honey may produce beneficial effects on human health; smaller amounts of fructose may even help improve diabetic control and that adequate seminal fructose concentrations help promote male fertility. In conclusion, fructose appears to be not all that bad; is essential for male fertility, small amounts of fructose, specifically which comes from dietary sources appears to be beneficial for health." As taken from Memon MQ & Kumar A. 2013. Pak. J. Pharm. Sci. 26(6), 1241-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24191333?dopt=AbstractPlus

“Obesogens are compounds that disrupt the function and development of adipose tissue or the normal metabolism of lipids, leading to an increased risk of obesity and associated diseases. Evidence for the adverse effects of industrial and agricultural obesogens, such as tributyltin, bisphenol A and other organic pollutants is well-established. Current evidence suggests that high maternal consumption of fat promotes obesity and increased metabolic risk in offspring, but less is known about the effects of other potential nutrient obesogens. Widespread increase in dietary fructose consumption over the past 30 years is associated with chronic metabolic and endocrine disorders and alterations in feeding behaviour that promote obesity. In this Perspectives, we examine the evidence linking high intakes of fructose with altered metabolism and early obesity. We review the evidence suggesting that high fructose exposure during critical periods of development of the fetus, neonate and infant can act as an obesogen by affecting lifelong neuroendocrine function, appetite control, feeding behaviour, adipogenesis, fat distribution and metabolic systems. These changes ultimately favour the long-term development of obesity and associated metabolic risk.” As taken from Goran MI et al. 2013. Nat. Rev. Endocrinol. 9(8), 494-500. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23732284?dopt=AbstractPlus

“BACKGROUND: Energy-containing beverages are widely consumed among premenopausal women, but their association with reproductive hormones is not well understood. OBJECTIVE: The objective was to assess the association of energy-containing beverages, added sugars, and total fructose intake with reproductive hormones among ovulatory cycles and sporadic anovulation in healthy premenopausal women. DESIGN: Women (n = 259) in the BioCycle Study were followed for up to 2 menstrual cycles; they provided fasting blood specimens during up to 8 visits/cycle and four 24-h dietary recalls/cycle. RESULTS: Women who consumed ≥1 cup (1 cup = 237 mL) sweetened soda/d had 16.3% higher estradiol concentrations compared with women who consumed less sweetened soda (86.5 pg/mL compared with 74.4 pg/mL, P = 0.01) after adjustment for age, BMI, race, dietary factors, and physical activity. Similarly elevated estradiol concentrations were found for ≥1 cup cola/d and noncola soda intake. Neither artificially sweetened soda nor fruit juice intake ≥1 cup/d was significantly associated with reproductive hormones. Added sugar above the average US woman's intake (≥73.2 g/d) or above the 66th percentile in total fructose intake (≥41.5 g/d) was associated with significantly elevated estradiol but not consistently across all models. No associations were found between beverages, added sugars, or total fructose intake and anovulation after multivariate adjustment. CONCLUSIONS: Even at moderate consumption amounts, sweetened soda is associated with elevated follicular estradiol concentrations among premenopausal women but does not appear to affect ovulatory function. Further research into the mechanism driving the association between energy-containing beverages and reproductive hormones, and its potential implications for women's health, is warranted.” As taken from Schliep KC et al. 2013. Am. J. Clin. Nutr. 97(3), 621-30. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23364018?dopt=AbstractPlus

“Consumption of added sugar has increased over recent decades and is correlated with numerous diseases. Rodent models have elucidated mechanisms of toxicity, but only at concentrations beyond typical human exposure. Here we show that comparatively low levels of added sugar consumption have substantial negative effects on mouse ... reproduction. Using Organismal Performance Assays--in which mice fed human-relevant concentrations of added sugar (25% kcal from a mixture of fructose and glucose, modeling high fructose corn syrup) and control mice compete in seminatural enclosures for ... mates--we demonstrate that .... fructose/glucose-fed males .... produce 25% less offspring. These

findings represent the lowest level of sugar consumption shown to adversely affect mammalian health. Clinical defects of fructose/glucose-fed mice were decreased glucose clearance and increased fasting cholesterol. Our data highlight that physiological adversity can exist when clinical disruptions are minor, and suggest that Organismal Performance Assays represent a promising technique for unmasking negative effects of toxicants." Ruff JS et al. 2013. Nat. Commun. 4, 2245. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23941916?dopt=AbstractPlus

"Fructose has recently been observed to affect brain metabolism and cognitive function in adults. Yet, possible late-onset effects by gestational fructose exposure have not been examined. We evaluated mitochondrial function in the brain of aging (15 months) male offspring of Fischer F344 rat dams fed a high-fructose diet (50% energy from fructose) during gestation and lactation. Maternal fructose exposure caused a significantly lower body weight of the offspring throughout life after weaning, while birth weight, litter size, and body fat percentage were unaffected. Isolated brain mitochondria displayed a significantly increased state 3 respiration of 8%, with the substrate combinations malate/pyruvate, malate/pyruvate/succinate, and malate/pyruvate/succinate/rotenone, as well as a significant decrease in the P/O2 ratio, compared with the control. Uncoupling protein 5 (UCP5) protein levels increased in the fructose group compared with the control (P=0.03) and both UCP5 mRNA and protein levels were inversely correlated with the P/O2 ratio (P=0.008 and 0.03, respectively), suggesting that UCP5 may have a role in the observed decreased phosphorylation efficiency. In conclusion, maternal high-fructose diet during gestation and lactation has long-term effects (fetal programming) on brain mitochondrial function in aging rats, which appears to be linked to an increase in UCP5 protein levels." As taken from Mortensen OH et al. 2014. J. Cereb. Blood Flow Metab. 34(7), 1205-11. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24756078

"The consumption of artificially sweetened processed foods, particularly high in fructose or high fructose corn syrup, has increased significantly in the past few decades. As such, interest into the long term outcomes of consuming high levels of fructose has increased significantly, particularly when the exposure is early in life. Epidemiological and experimental evidence has linked fructose consumption to the metabolic syndrome and associated comorbidities-implicating fructose as a potential factor in the obesity epidemic. Yet, despite the widespread consumption of fructose-containing foods and beverages and the rising incidence of maternal obesity, little attention has been paid to the possible adverse effects of maternal fructose consumption on the developing fetus and long term effects on offspring. In this paper we review studies investigating the effects of fructose intake on metabolic outcomes in both mother and offspring using human and experimental studies." As taken from Sloboda DM et al. 2014. J. Obes. 2014, 203474. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24864200?dopt=AbstractPlus

"Excess consumption of added sugars, including sucrose and high fructose corn syrup (HFCS-55), have been implicated in the global epidemics of obesity and type 2 diabetes. This study aimed to investigate and compare the impact of maternal consumption of sucrose or HFCS-55 during pregnancy and lactation on the metabolic health of the dam and her offspring at birth. Female Albino Wistar rats were given access to chow and water, in addition to a sucrose or HFCS-55 beverage (10% w/v) before, and during pregnancy and lactation. Maternal glucose tolerance was determined throughout the study, and a postmortem was conducted on dams following lactation, and on offspring within 24 h of birth. Sucrose and HFCS-55 consumption resulted in increased total energy intake

compared with controls, however the increase from sucrose consumption was accompanied by a compensatory decrease in chow consumption. There was no effect of sucrose or HFCS-55 consumption on body weight, however sucrose consumption resulted in increased adiposity and elevated total plasma cholesterol in the dam, while HFCS-55 consumption resulted in increased plasma insulin and decreased plasma non-esterified fatty acids (NEFA). Maternal HFCS-55 consumption was associated with decreased relative liver weight and plasma NEFA in the offspring at birth. There was no effect of either treatment on pup weight at birth. These findings suggest that both sucrose and HFCS-55 consumption during pregnancy and lactation have the potential to impact negatively on maternal metabolic health, which may have adverse consequences for the long-term health of the offspring." As taken from Toop CR et al. 2015. J. Dev. Orig. Health Dis. 6, 38-46. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25523154

"BACKGROUND: Intake of added sugar has been shown to correlate with many human metabolic diseases, and rodent models have characterized numerous aspects of the resulting disease phenotypes. However, there is a controversy about whether differential health effects occur because of the consumption of either of the two common types of added sugar-high-fructose corn syrup (fructose and glucose monosaccharides; F/G) or table sugar (sucrose, a fructose and glucose disaccharide). OBJECTIVES: We tested the equivalence of sucrose- vs. F/G-containing diets on mouse (Mus musculus) longevity, reproductive success, and social dominance. METHODS: We fed wild-derived mice, outbred mice descended from wild-caught ancestors, a diet in which 25% of the calories came from either an equal ratio of F/G or an isocaloric amount of sucrose (both diets had 63% of total calories as carbohydrates). Exposure lasted 40 wk, starting at weaning (21 d of age), and then mice (104 females and 56 males) were released into organismal performances assays-seminatural enclosures where mice competed for territories, resources, and mates for 32 wk. Within enclosures all mice consumed the F/G diet. RESULTS: Females initially fed the F/G diet experienced a mortality rate 1.9 times the rate (P = 0.012) and produced 26.4% fewer offspring than females initially fed sucrose (P = 0.001). This reproductive deficiency was present before mortality differences, suggesting the F/G diet was causing physiologic performance deficits prior to mortality. No differential patterns in survival, reproduction, or social dominance were observed in males, indicating a sex-specific outcome of exposure. CONCLUSION: This study provides experimental evidence that the consumption of human-relevant levels of F/G is more deleterious than an isocaloric amount of sucrose for key organism-level health measures in female mice." As taken from Ruff JS et al. 2015. J. Nutr. 145, 434-41. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25733457

"The aim of this work was to determine the effect of a fructose rich diet (FRD) consumed by the pregnant mother on the endocrine-metabolic and in vivo and in vitro adipose tissue (AT) functions of the male offspring in adulthood. At 60 days of age, rats born to FRD-fed mothers (F) showed impaired glucose tolerance after glucose overload and high circulating levels of leptin (LEP). Despite the diminished mass of retroperitoneal AT, this tissue was characterized by enhanced LEP gene expression, and hypertrophic adipocytes secreting in vitro larger amounts of LEP. Analyses of stromal vascular fraction composition by flow cytometry revealed a reduced number of adipocyte precursor cells. Additionally, 60 day-old control (C) and F male rats were subjected to control diet (CC and FC animals) or FRD (CF and FF rats) for three weeks. FF animals were heavier and consumed more calories. Their metabolic-endocrine parameters were aggravated; they developed severe hyperglycemia, hypertriglyceridemia, hyperleptinemia and augmented AT mass with hypertrophic

adipocytes. Our study highlights that manipulation of maternal diet induced an offspring phenotype mainly imprinted with a severely unhealthy adipogenic process with undesirable endocrine-metabolic consequences, putting them at high risk for developing a diabetic state." As taken from Alzamendi A et al. 2016. Nutrients 8(3), 178. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27011203

"The most significant increase in metabolic syndrome over the previous decade occurred in women of reproductive age, which is alarming given that metabolic syndrome is associated with reproductive problems including subfertility and early pregnancy loss. Individuals with metabolic syndrome often consume excess fructose, and several studies have concluded that excess fructose intake contributes to metabolic syndrome development. Here, we examined the effects of increased fructose consumption on pregnancy outcomes in mice. Female mice fed a high-fructose diet (HFrD) for 6 weeks developed glucose intolerance and mild fatty liver but did not develop other prominent features of metabolic syndrome such as weight gain, hyperglycemia, and hyperinsulinemia. Upon mating, HFrD-exposed mice had lower pregnancy rates and smaller litters at midgestation than chow-fed controls. To explain this phenomenon, we performed artificial decidualization experiments and found that HFrD consumption impaired decidualization. This appeared to be due to decreased circulating progesterone as exogenous progesterone administration rescued decidualization. Furthermore, HFrD intake was associated with decreased bone morphogenetic protein 2 expression and signaling, both of which were restored by exogenous progesterone. Finally, expression of forkhead box O1 and superoxide dismutase 2 [Mn] proteins were decreased in the uteri of HFrD-fed mice, suggesting that HFrD consumption promotes a prooxidative environment in the endometrium. In summary, these data suggest that excess fructose consumption impairs murine fertility by decreasing steroid hormone synthesis and promoting an adverse uterine environment." As taken from Saben JL et al. 2016. Endocrinology 157(2), 956-68. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26677880

"PURPOSE: Fructose intake from added sugars correlates with the epidemic rise in metabolic syndrome and cardiovascular diseases. However, consumption of beverages containing fructose is allowed during gestation. Recently, we found that an intake of fructose (10 % wt/vol) throughout gestation produces impaired fetal leptin signaling and hepatic steatosis. Therefore, we have investigated whether fructose intake during pregnancy produces subsequent changes in the progeny, when adult. METHODS: Fed 261-day-old male and female descendants from fructose-fed, control or glucose-fed mothers were used. Plasma was used to analyze glucose, insulin, leptin, and adiponectin. Hepatic expression of proteins related to insulin signaling was determined. RESULTS: Fructose intake throughout pregnancy did not produce alterations in the body weight of the progeny. Adult male progeny of fructose-fed mothers had elevated levels of insulin without a parallel increase in phosphorylation of protein kinase B. However, they displayed an augmented serine phosphorylation of insulin receptor substrate-2, indicating reduced insulin signal transduction. In agreement, adiponectin levels, which have been positively related to insulin sensitivity, were lower in male descendants from fructose-fed mothers than in the other two groups. Furthermore, mRNA levels for insulin-responsive genes were not affected (phosphoenolpyruvate carboxykinase, glucose-6-phosphatase) or they were decreased (sterol response element-binding protein-1c) in the livers of male progeny from fructose-supplemented rats. On the contrary, adult female rats from fructose-fed mothers did not exhibit any of these disturbances. CONCLUSION: Maternal fructose, but not glucose, intake confined to the prenatal stage provokes impaired insulin signal transduction,

hyperinsulinemia, and hypoadiponectinemia in adult male, but not female, progeny." As taken from Rodriguez L et al. 2016. Eur. J. Nutr. 55(2), 665-674. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/25808117

"BACKGROUND: Consumption of fructose-rich diets in the United States is on the rise and thought to be associated with obesity and cardiometabolic diseases. OBJECTIVE: We sought to determine the effects of antenatal exposure to high-fructose diet on offspring's development of metabolic syndrome-like phenotype and other cardiovascular disease risk factors later in life. STUDY DESIGN: Pregnant C57BL/6J dams were randomly allocated to fructose solution (10% wt/vol, n = 10) or water (n = 10) as the only drinking fluid from day 1 of pregnancy until delivery. After weaning, pups were started on regular chow, and evaluated at 1 year of life. We measured percent visceral adipose tissue and liver fat infiltrates using computed tomography, and blood pressure using CODA nonivasive monitor. Intraperitoneal glucose tolerance testing with corresponding insulin concentrations were obtained. Serum concentrations of glucose, insulin, triglycerides, total cholesterol, leptin, and adiponectin were measured in duplicate using standardized assays. Fasting homeostatic model assessment was also calculated to assess insulin resistance. P values <.05 were considered statistically significant. RESULTS: Maternal weight, pup number, and average weight at birth were similar between the 2 groups. Male and female fructose group offspring had higher peak glucose and area under the intraperitoneal glucose tolerance testing curve compared with control, and higher mean arterial pressure compared to control. Female fructose group offspring were heavier and had higher percent visceral adipose tissue, liver fat infiltrates, homeostatic model assessment of insulin resistance scores, insulin area under the intraperitoneal glucose tolerance testing curve, and serum concentrations of leptin, and lower concentrations of adiponectin compared to female control offspring. No significant differences in these parameters were noted in male offspring. Serum concentrations of triglycerides or total cholesterol were not different between the 2 groups for either gender. CONCLUSION: Maternal intake of high fructose leads to fetal programming of adult obesity, hypertension, and metabolic dysfunction, all risk factors for cardiovascular disease. This fetal programming is more pronounced in female offspring. Limiting intake of high fructose-enriched diets in pregnancy may have significant impact on long-term health." As taken from Saad AF et al. 2016. Am. J. Obstet. Gynecol. 215(3), 378. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27060421

"The Western diet is typically high in salt and fructose, which have pressor activity. Maternal diet can affect offspring blood pressure, but the extent to which maternal intake of excess salt and fructose may influence cardiovascular function of the offspring is unknown. We sought to determine the effect of moderate maternal dietary intake of salt and/or fructose on resting and stimulated cardiovascular function of the adult male and female offspring. Pregnant rats were fed purified diets (± 4% salt) and water (± 10% fructose) before and during gestation and through lactation. Male and female offspring were weaned onto standard laboratory chow. From 9 to 14 weeks of age, cardiovascular parameters (basal, circadian and stimulated) were assessed continuously by radiotelemetry. Maternal salt intake rendered opposite-sex siblings with a 25-mmHg difference in blood pressure as adults; male offspring were hypertensive (15 mmHg mean arterial pressure (MAP)) and female offspring were hypotensive (10 mmHg MAP) above and below controls, respectively. Sex differences were unrelated to endothelial nitric oxide activity in vivo, but isolation-induced anxiety revealed a significantly steeper coupling between blood pressure and heart rate in salt-exposed male offspring but not in female offspring. MAP of all offspring was refractory to salt loading but sensitive to subsequent dietary fructose, an effect exacerbated

in female offspring from fructose-fed dams. Circadian analyses of pressure in all offspring revealed higher mean set-point for heart rate and relative non-dipping of nocturnal pressure. In conclusion, increased salt and fructose in the maternal diet has lasting effects on offspring cardiovascular function that is sex-dependent and related to the offspring's stress-response axis." As taken from Gray C et al. 2016. Br. J. Nutr. 115(4), 594-604. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26653028

"It has become increasingly clear that maternal nutrition can strongly influence the susceptibility of adult offspring to cardiometabolic disease. For decades, it has been thought that excessive intake of fructose, such as sugar-sweetened beverages and foods, has been linked to increased risk of obesity, type 2 diabetes, and cardiovascular disease in various populations. These deleterious effects of excess fructose consumption in adults are well researched, but limited data are available on the long-term effects of high fructose exposure during gestation, lactation, and infancy. This review aims to examine the evidence linking early life fructose exposure during critical periods of development and its implications for long-term cardiometabolic health in offspring." As taken from Zheng J et al. 2016. Nutrients 8(11), E685. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27809266

"Hypertension originates from early-life insults by so-called "developmental origins of health and disease" (DOHaD). Studies performed in the previous few decades indicate that fructose consumption is associated with an increase in hypertension rate. It is emerging field that tends to unfold the nutrient-gene interactions of maternal high-fructose (HF) intake on the offspring which links renal programming to programmed hypertension. Reprogramming interventions counteract disturbed nutrient-gene interactions induced by maternal HF intake and exert protective effects against developmentally programmed hypertension. Here, we review the key themes on the effect of maternal HF consumption on renal transcriptome changes and programmed hypertension. We have particularly focused on the following areas: metabolic effects of fructose on hypertension and kidney disease; effects of maternal HF consumption on hypertension development in adult offspring; effects of maternal HF consumption on renal transcriptome changes; and application of reprogramming interventions to prevent maternal HF consumption-induced programmed hypertension in animal models. Provision of personalized nutrition is still a faraway goal. Therefore, there is an urgent need to understand early-life nutrient-gene interactions and to develop effective reprogramming strategies for treating hypertension and other HF consumption-related diseases." As taken from Tain YL et al. 2016. Nutrients 8(12), E757. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27897982

"BACKGROUND: Maternal exposure to overnutrition during fetal development contributes to metabolic and renal damage in offspring. Adiponectin plays a protective role against obesity-related renal injury. However, role of adiponectin in renal injury of offspring exposed to maternal overnutrition remains unknown. We addressed the issue. METHODS: Female Sprague-Dawley rats were fed either a standard (N) or a high-fat and high-fructose (HFF)-diet for 6 weeks before mating, and kept each diet during the gestation and lactation period. After 4 weeks postpartum, all the offspring were fed N diet, and followed by 12 weeks. Kidney weight, urinary albumin excretion, blood pressure, and blood chemistry, including adiponectin and malondialdehyde, a marker of oxidative stress, were evaluated in the offspring. RESULTS: Compared with N-offspring, serum adiponectin levels of 1-day- and 4-week-old HFF-offspring were significantly lower, the latter of which was inversely associated with malondialdehyde. Kidney weight was significantly decreased in 1-day-old HFF-offspring, whereas increased in 4-week-old HFF-offspring. Urinary albumin excretion levels

of HFF-offspring at 8, 12, and 16-week old were significantly higher than those of N-offspring at the same age, whose levels at 16-week old were inversely correlated with plasma adiponectin. Compared with N-offspring, HFF-offspring at 16-week old exhibited glomerulosclerosis, hyperglycemia, and high mean blood pressure associated with reduced podocin and increased transforming growth factor-β1 expression in the kidneys. CONCLUSIONS: Our present study suggests that exposure to maternal HFF-diet during fetal and early postnatal development induces hypoadiponectinemia in offspring, which might cause renal injury and metabolic derangements later in life." As taken from Yamada-Obara N et al. 2016. Clin. Exp. Nephrol. 20(6), 853-861. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27179663

"Impairment of learning and memory has been documented in the later life of offspring to maternal consumption with high energy diet. Environmental stimulation enhances the ability of learning and memory. However, potential effects of environmental stimulation on the programming-associated deficit of learning and memory have not been addressed. Here, we examined the effects of enriched-housing on hippocampal learning and memory in adult female offspring rats from mother fed with 60% high fructose diet (HFD) during pregnancy and lactation. Impairment of spatial learning and memory performance in HFD group was observed in offspring at 3-month-old. Hippocampal brain-derived neurotrophic factor (BDNF) was decreased in the offspring. Moreover, the HFD group showed an up-regulation of histone deacetylase 4 (HDAC4) in the nuclear fractions of hippocampal neurons. Stimulation to the offspring for 4weeks after winning with an enriched-housing environment effectively rescued the decrease in cognitive function and hippocampal BDNF level; alongside a reversal of the increased distribution of nuclear HDAC4. Together these results suggest that later life environmental stimulation effectively rescues the impairment of hippocampal learning and memory in female offspring to maternal HFD intake through redistributing nuclear HDAC4 to increase BDNF expression." As taken from Wu KL et al. 2016. Neurobiol. Learn. Mem. 130, 105-17. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26872592

"AIMS: This study examined the interaction between maternal high fructose diet and neonatal inflammation in neonates (P7), juveniles (P26-34) and adults on measures of anxiety-like behavior and cognition. The study aimed to assess the potential synergistic effects of these two forms of early-life inflammation. MAIN METHODS: We fed Sprague-Dawley dams with high fructose (60%) diet or normal chow. Each litter was treated with either saline or lipopolysaccharide (LPS) on postnatal day (P)3 and P5 and two pups were tested for USVs after maternal separation on P7. Post-weaning, juveniles were tested on the elevated zero maze (EZM) and in a context-object discrimination (COD) task prior to tissue harvest. Adults were tested on the EZM and the COD task as well. Immunohistochemistry and ELISA were used to assess molecular and cellular changes in the offspring. KEY FINDINGS: This study demonstrates that maternal diet and neonatal inflammation altered peripheral inflammation in neonates, altered anxiety-like behavior in juveniles, and altered anxiety-like behavior in adulthood. Maternal diet and sex increased juvenile peripheral inflammation and altered memory on the context-discrimination task. SIGNIFICANCE: Maternal diet has a profound impact on fetal and neonatal development, especially as obesity rates are on the rise worldwide. Together, these findings reveal enduring effects of maternal diet on offspring, support the findings on the effects of neonatal inflammation on anxiety-like behaviors in later-life periods, and add to the complex relationship between gestational and neonatal inflammation and anxiety." As taken from

Bukhari SHF et al. 2018. Life Sci. 197, 114-121. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29428599

“RATIONALE: Cross-sectional studies have linked intake of high-fructose corn syrup-sweetened beverages with asthma in schoolchildren. OBJECTIVES: To examine associations of maternal prenatal and early childhood intake of sugar-sweetened beverages and fructose with current asthma in midchildhood (median age, 7.7 yr). METHODS: We assessed maternal pregnancy (first- and second-trimester average) and child (median age, 3.3 yr) intake of sugar-sweetened beverages and total fructose using food frequency questionnaires in 1,068 mother-child pairs from Project Viva, a prospective prebirth cohort. In a multivariable analysis, we examined associations of quartiles of maternal and child sugar-sweetened beverage, juice, and total fructose intake with child current asthma in midchildhood, assessed by questionnaire as ever having doctor-diagnosed asthma plus taking asthma medications or reporting wheezing in the past 12 months. RESULTS: Higher maternal pregnancy sugar-sweetened beverage consumption (mean, 0.6 servings/d; range, 0-5) was associated with younger maternal age, nonwhite race/ethnicity, lower education and income, and higher prepregnancy body mass index. Adjusting for prepregnancy body mass index and other covariates, comparing quartile 4 with quartile 1, higher maternal pregnancy intake of sugar-sweetened beverages (odds ratio, 1.70; 95% confidence interval, 1.08-2.67) and total fructose (odds ratio, 1.58; 95% confidence interval, 0.98-2.53) were associated with greater odds of midchildhood current asthma (prevalence, 19%). Higher early childhood fructose intake (quartile 4 vs. quartile 1) was also associated with midchildhood current asthma in models adjusted for maternal sugar-sweetened beverages (odds ratio, 1.79; 95% confidence interval, 1.07-2.97) and after additional adjustment for midchildhood body mass index z-score (odds ratio, 1.77; 95% confidence interval, 1.06-2.95). CONCLUSIONS: Higher sugar-sweetened beverage and fructose intake during pregnancy and in early childhood was associated with childhood asthma development independent of adiposity.” As taken from Wright LS et al. 2018. Ann. Am. Thorac. Soc. 15(2), 217-224. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29219619

“BACKGROUND: Maternal dietary choices throughout preconception, pregnancy, and lactation irreversibly affect the development of fetal tissues and organs, known as fetal programming. Recommendations tend to emphasize reducing added sugars. However, the impact of maternal dietary free or bound fructose in added sugars on developmental programming of lipogenesis is unknown. METHODS: Virgin Sprague-Dawley rats were randomly divided into five groups. Rats were given feed and plain water (control) or water containing maltodextrin (vehicle), fructose, high-fructose corn syrup (HFCS) containing 55% fructose, sucrose (20% w/v) for 12 weeks before mating and throughout the pregnancy and lactation periods. Body weight, water, and feed intake were measured throughout the study. At the end of the lactation period, blood was drawn to determine the fasting levels of glucose, insulin, triglycerides, and non-esterified fatty acids (NEFA) in blood. Triglycerides and acetyl Co-A Carboxylase-1 (ACC1) levels in livers were analyzed, and insulin resistance was calculated. RESULTS: The energy intake of dams in the HFCS group was higher than in the fructose group, while weight gain was less in the HFCS group than in the fructose group. HFCS resulted in greater insulin resistance in dams, whereas free fructose had a robust effect on the fetal programming of insulin resistance. Free fructose and HFCS in the maternal diet increased blood and liver triglycerides and NEFA content in pups. Furthermore, fructose and HFCS exposure increased phosphorylated ACC1 as compared to maltodextrin and control, indicating greater fatty acid synthesis in pups and dams.

CONCLUSION: Different types of added sugar in the maternal diet have different metabolic effects on the developmental programming of lipogenesis. Consequently, high fructose intake via processed foods may increase the risk for chronic diseases, and free fructose might contribute to developmental programming of chronic diseases more than bound fructose." As taken from Yuruk AA & Nergiz-Unal R. 2017. Lipids Health Dis. 16(1), 226. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29191195

5.4. Mutagenicity

Test system	**Test conditions**	**Endpoint**	**Activation**	**Result**	**References**
Chinese hamster ovary cells	Cells incubated with fructose at 15 mg/ml for 3 hr, and examined for chromosome breaks and exchanges A caramelized solution (a 38% fructose solution heated at 180ºC for 1 hr) was similarly tested	Chromosome damage Chromosome damage	Without Without	-ve +ve	Stich *et al.* 1981
Salmonella typhimurium strains TA97, TA98 and TA100	Test on pyrolyzed fructose (heated in a sealed test tube under vacuum at 700°C for 1 minute)	Mutation	With and without S9	+ve	Kuroda *et al.* 1985
Salmonella typhimurium strains TA98 and TA100	Test on pyrolyzed fructose (heated in a sealed test tube under vacuum at 700°C for 1 minute)	Mutation	With S9	-ve	Okamoto *et al.* 1979
Salmonella typhimurium strains TA98 and TA100	Test on condensate from cigarettes with and without added fructose. 1 g fructose was added per cigarette, by soaking a cellophane-wrapped cigarette in fructose solution for 10 minutes and then drying it completely at 80°C for 18 hr	Anti-mutagenicity	With and without S9	Inhibitory effect in the presence of S9 only. Other sugars gave similar	Sato *et al.* 1979
Salmonella typhimurium strains TA98 and TA100	Testing effect of fructose and pyrolyzed fructose on the mutagenic activity of a known mutagen (2-AAF). Pyrolyzed	Co-mutagenicity	With S9	Fructose did not affect the mutagenic	Okamoto *et al.* 1979

	fructose was prepared by heating in a sealed test tube under vacuum at 700°C for 1 minute			effect of 2-AAF pyrolyzed fructose increased 5-fold the number of revertants induced by AAF	
Salmonella typhimurium strain DG2670	Testing effect of a number of carbon sources, including fructose (1% w/v), on the mutagenic activity of 9-aminoacridine (a known mutagen)	Anti-mutagenicity	without	Fructose reduced the effect of the known mutagen (protective effect)	Kopsidas & MacPhee, 1994
Salmonella typhimurium strain TA98	Testing effect of various sugars, including fructose (up to 20 mg/ml), on the mutagenic activity of Trp-p-1 (a known mutagen)	Anti-mutagenicity	With S9	The effect of Trp-p-1 was reduced by fructose (protective effect)	Wang *et al.* 2002
Salmonella typhimurium strain hisG46	Testing effect of monosaccharides, including fructose (2-hr exposure at 15 mM), on the mutagenic activity of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)	Co-mutagenicity	without	Fructose increased 3-fold the number of revertants induced by MNNG	Shirai *et al.* 1980
Salmonella typhimurium strain TA100	Testing effect of monosaccharides, including fructose (4-hr exposure at 150 mM), on the mutagenic activity of 2-chloro-4-methylthiobutanoic acid	Co-mutagenicity	without	Fructose increased the mutagenic potency of the mutagen	Kimura *et al.* 2000

"Pyridoxal 5'-phosphate (PLP), the active form of vitamin B6, has been implicated in preventing human pathologies, such as diabetes and cancer. However, the mechanisms underlying the beneficial effects of PLP are still unclear. Using Drosophila as a model system, we show that PLP deficiency, caused either by mutations in the pyridoxal kinase-coding gene (dPdxk) or by vitamin B6 antagonists, results in chromosome aberrations (CABs). The CAB frequency in PLP-depleted cells was strongly enhanced by sucrose, glucose or fructose treatments, and dPdxk mutant cells consistently displayed higher glucose contents than their wild type counterparts, an effect that is at least in part a consequence of an acquired insulin resistance. Together, our results indicate that a high intracellular level of glucose has a dramatic clastogenic effect if combined with PLP deficiency. This is likely due to an elevated level of Advanced Glycation End-products (AGE) formation. Treatment of dPdxk mutant cells with α-lipoic acid (ALA) lowered both

AGE formation and CAB frequency, suggesting a possible AGE-CAB cause-effect relationship. The clastogenic effect of glucose in PLP-depleted cells is evolutionarily conserved. RNAi-mediated silencing of PDXK in human cells or treatments with PLP inhibitors resulted in chromosome breakage, which was potentiated by glucose and reduced by ALA. These results suggest that patients with concomitant hyperglycemia and vitamin B6 deficiency may suffer chromosome damage. This might impact cancer risk, as CABs are a well-known tumorigenic factor." As taken from Marzio A et al. 2014. PLoS Genet. 10(3), e1004199. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24651653

"The safety of rare sugar syrup obtained from high-fructose corn syrup under slightly alkaline conditions was studied. Mutagenicity of rare sugar syrup was assessed by a reverse mutation assay using Salmonella typhimurium and Escherichia coli, and an in vitro chromosomal aberration assay using Chinese hamster lung cell line (CHL/IU). No mutagenicity of rare sugar syrup was detected under these experimental conditions. ……" As taken from Yamada T et al. 2015. Shokuhin Eiseigaku Zasshi 56, 211-6. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26537651

"Patients with type 2 diabetes mellitus (T2DM) are associated with an elevated, but poorly understood baseline of genomic instability (GIN). Expert panels are still debating on whether hyperglycemia is the key element in conferring this high GIN. Since high blood glucose and low blood folate are prevalent in T2DM, we hypothesized that high glucose may work with low folate to induce GIN. Using NCM460, CCD841 and L02 cell lines as in vitro cell models, we investigated the genotoxic effects of high sugars (HS; 1-2% glucose, fructose, galactose or sucrose) alone or in combination with folate deficiency (23 nM, FD) over a course of 7 days by the cytokinesis block micronucleus assay. We found that HS is nongenotoxic to NCM460, CCD841 and L02 cells. However, the combination of HS and FD induced significantly higher levels of micronuclei, nucleoplasmic bridges and nuclear buds. Our in vitro work demonstrates that HS is non-genotoxic under folate repletive condition, but is genotoxic under FD condition. These results provide preclinical proof of concept that concomitant hyperglycemia and low folate may explain, at least in part, the high baseline of GIN in T2DM patients, suggesting that folate levels should be kept under control in order to limit the risk of GIN and carcinogenesis in T2DM." As taken from Guo X et al. 2019. Mutat. Res. 814, 15-22. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30682723

5.5. Cytotoxicity

Hepatocyte inflammation model for cytotoxicity research: fructose or glycolaldehyde as a source of endogenous toxins (Abstract). Insulin resistance and hepatotoxicity induced in high fructose fed rats may involve fructose derived endogenous toxins formed by inflammation. Thus fructose was seventy-fold more toxic if hepatocytes were exposed to non-toxic levels of hydrogen peroxide (H(2)O(2)) released by inflammatory cells. This was prevented by iron (Fe) chelators, hydroxyl radical scavengers, and increased by Fe, copper (Cu) or catalase inhibition. Fructose or glyceraldehyde/dihydroxyacetone metabolites were oxidized by Fenton radicals to glyoxal. Glyoxal (15 microM) cytotoxicity was increased about 200-fold by H(2)O(2). Glycolaldehyde was enzymically formed from glyceraldehyde, the fructokinase/aldolase B product of fructose. Glycolaldehyde cytotoxicity was increased 20-fold by H(2)O(2). The oxidative stress cytotoxicity induced was attributed to the Fenton

oxidation of glycolaldehyde forming glycolaldehyde radicals and glyoxal, since cytotoxicity was prevented by aminoguanidine (glyoxal trap) or Fenton inhibitors. Glyoxal was also the Fenton product responsible for glycolaldehyde protein carbonylation as carbonylation was prevented by aminoguanidine or Fenton inhibitors. As taken from Feng CYet al. Arch Physiol Biochem. 2009 May; 115(2):105-11. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/19485706

Fructose and carbonyl metabolites as endogenous toxins (Abstract). Dietary fructose consumption is one of the environmental factors contributing to the development of obesity and a fatty liver (hepatic steatosis). A two-hit hypothesis has been proposed for progression of hepatic steatosis to the more serious non-alcoholic steatosis (NASH), with the first hit being hepatic steatosis, and the second hit being inflammation and associated oxidative stress caused by reactive oxygen species (ROS) formation. As well, fructose-fed rats develop insulin resistance and serum levels of methylglyoxal, a glycolytic metabolite, are increased. Previously we reported that glyoxal-induced hepatocyte cytotoxicity could be attributed to mitochondrial toxicity as mitochondrial membrane potential was decreased and cytotoxicity was increased several orders of magnitude by low non-cytotoxic doses of H2O2 (hepatocyte inflammation model). In this study, we have assessed the toxicity of fructose towards hepatocytes and investigated the molecular cytotoxic mechanisms involved. Fructose itself was only toxic at 1.5 M, whereas 12 mM caused 50% cell death in 2 h if the hepatocytes were exposed to a non-cytotoxic dose of H2O2 continuously generated by glucose and glucose oxidase. The cytotoxic mechanism involved oxidative stress as ROS and H2O2 formation preceded cytotoxicity, and cytotoxicity was prevented by radical scavengers, lipid antioxidants and ROS scavengers. It is proposed that the highly potent Fenton derived ROS catalyse the oxidation of fructose and particularly its carbonyl metabolites glycolaldehyde, dihydroxyacetone, glyceraldehyde. The carbon radicals and glyoxal formed compromise the cell's resistance to H2O2. As taken from Lee O et al., Chem Biol Interact 2009, vol. 178, no1-3, pp. 332-339. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/19000661

“The effects of high (H)-fructose (FR) diet (D) (HFRD) on hepatic lipid homeostasis, oxidative stress, inflammation and hepatocyte apoptosis were investigated in 6-week old female C57BL/6J mice fed a regular chow (ContD) or HFRD (35% fructose-derived calories) for 3 weeks. HFRD-fed mice exhibited increased levels of hepatic steatosis with a significant elevation of serum levels of triglyceride, cholesterol and TNFα compared to ContD-fed mice (P<0.05). HFRD-fed mice exhibited ~2.7- fold higher levels FAS along with significantly decreased protein levels of adiponection-R2 (~30%), P-AMPK (~60%), P-ACC (~70%) and RXR-α (~55%), suggesting decreased hepatic fat oxidation compared to controls. Interestingly, hepatic fatty acid uptake into hepatocytes and lipolysis were significantly increased in HFRD-fed mice, as shown by decreased CD36 and fatty acid transporter protein-2, and increased adipose triglyceride lipase, respectively (P<0.05). Increased hepatic levels of iNOS and GSSG/GSH suggest elevated oxidative stress with a higher number of macrophages in the adipose tissue in HFRD-fed mice (P<0.05). Significantly elevated rates of hepatocyte apoptosis (~2.4-fold), as determined by TUNEL analysis with increased Bax/Bcl2 ratio and PARP-1 levels (~2- and 1.5-fold, respectively), were observed in HFRD-fed mice. Thus, HFRD exposure increased hepatic steatosis accompanied by oxidative stress and inflammation, leading to hepatocyte apoptosis.” As taken from Choi Y et al. 2017. Food Chem. Toxicol. 103, 111-121. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28257781

“Whether fructose (FRU), as the sole energy source, confers a metabolic advantage on cancer cells against noxious stimuli is unknown. The aim of this study was to evaluate the effects of low (11 mM), moderate (25 mM), and high (55 mM) FRU concentrations alone or in combination with rotenone (ROT) or doxorubicin (DOX) in Jurkat cells, an acute lymphoblastic leukemia cell model. Glucose (GLU) was used as a control. Using different cell analysis techniques, we demonstrated that FRU was predominantly metabolized via oxidative phosphorylation (~95%) (i.e., lactate production was reduced >120-fold), resulting in endogenous oxidative stress-induced conditions. The cells were characterized by generation of O2·- (43%)/ H2 O2 (40%) and activation of NF-κB (~95-fold increase, fi), c-Jun-N terminal kinase (JNK), p53 (40-fi), and c-Jun (9-fi). In addition, we observed a loss of ΔΨm (10%), activation of caspase-3 (50-fi) and apoptosis-inducing factor (AIF, 2-fi), and condensation and fragmentation of the nuclei [20% by acridine orange/ethidium bromide/Hoechst (AO/EB/H) staining, 15% by flow cytometry] compared to those of GLU 11 at 24 h. Although DOX killed Jurkat cells independent of sugar content in the culture medium, leukemic cells in low, but not high, FRU were extremely sensitive to ROT. Taken together, our findings suggest that Jurkat cells are more susceptible to cell death if forced to shift from GLU metabolism (i.e., aerobic glycolysis) to FRU metabolism (i.e., oxidative phosphorylation) after treatment with mitochondria-targeting molecules. These observations may help elucidate the cell death mechanism of leukemic cells cultured in FRU.” As taken from Diaz-Aguirre V et al. 2016. Cell Biol. Int. 40(11), 1162-1173. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27486090

5.6. Carcinogenicity

Species	**Test conditions**	**Evidence of carcinogenicity**	**Reference**
Human, 88,000 women in US Nurses’ Health Study	180 were diagnosed with cancer of the pancreas during 18 years of follow-up. Using food frequency questionnaires submitted in 1980, sucrose, fructose and carbohydrate intakes were estimated.	No convincing evidence. For women in the fifth quintile of fructose intake (>35 g/day, median 45 g/day), a slight (statistically non-significant) increase in pancreatic cancer risk was observed (relative risk (RR) 1.57; 95% CI 0.95-2.57). Body mass, exercise and glycemic load are potential confounding factors	Michaud *et al.* 2002
Human, 35,000 American women, aged 55-69 years	A prospective study of diet and colon cancer risk. The women completed dietary	None[high sucrose intake was associated with an	Bostick *et al.* 1994

	questionnaires in 1986 212 had developed colon cancer by 1990	increased risk of colon cancer]	
Human, 1993 Americans with colon cancer and 2410 healthy controls	A case-control study. Subjects answered questions about their diet in the calendar year 2 yrs prior to diagnosis or selection as a control	None [high sucrose intake was associated with an increased risk of colon cancer]	Slattery *et al.* 1997
Human	Effect of high fructose consumption on the risk of stomach cancer	High fructose consumption was associated with a reduced risk of stomach cancer [t his study is not further described as it is not an adequate investigation of fructose's potential to induce cancer]	De Stefani *et al.* 1999
Human	Effect of high fructose consumption on the risk of prostate cancer	High fructose consumption was associated with a reduced risk of prostate cancer [this study is not further described as it is not an adequate investigation of fructose's potential to induce cancer]	Giovannucci *et al.* 1998
Rat (Sprague-Dawley, at least 30 males/group)	Fructose at 0 or 120 g/L in drinking water for 30 wks, liver examined microscopically	None Inadequate study – modern protocols involve microscopic examination of a comprehensive range of tissues and organs from about 50 animals per sex, exposed daily for 2 years	Enzmann *et al.* 1989
Human	Effect of high fructose consumption on the risk of stomach cancer	High fructose consumption was associated with a reduced risk of stomach cancer [this study is not further described as it is not an adequate investigation of fructose's potential to induce cancer]	De Stefani *et al.* 1999

Human	Effect of high fructose consumption on the risk of prostate cancer	High fructose consumption was associated with a reduced risk of prostate cancer [this study is not further described as it is not an adequate investigation of fructose's potential to induce cancer]	Giovannucci *et al.* 1998
Rat (Sprague-Dawley, at least 30 males/group)	Fructose at 0 or 120 g/L in drinking water for 30 wks, liver examined microscopically	None Inadequate study – modern protocols involve microscopic examination of a comprehensive range of tissues and organs from about 50 animals per sex, exposed daily for 2 years	Enzmann *et al.* 1989
Mouse (CF1, Charles River, groups of 20 females) [This outbred strain is particularly sensitive to colon carcinogenesis]	Administration of 0 or 10 g/kg bw by stomach tube, examination of the colonic epithelium 16 hr later Administration of 0 or 10 g/kg bw by stomach tube followed, 14 hr later, by a dose of a known carcinogen (azoxymethane)	Increased mitotic cells per crypt (0.5 in controls, around 4.5 in fructose-treated mice, $p<0.001$), biological significance unclear Increased number of aberrant crypt foci per colon (7.5 in controls, 12.5 in fructose-treated mice, $p<0.05$), possibly indicative of tumour initiating activity?	Stamp *et al.* 1993

A tumor promotion study was conducted in Sprague-Dawley male rats in which 100 mg/kg/wk doses of N-nitrosomorpholine were given orally for 7 weeks. Two days after the last carcinogen dose, 0 or 120 g fructose/L in drinking water (*ad libitum*) were administered. Hepatocellular carcinomas were observed.

As taken from CCRIS, 1992.

Sucrose, glucose and fructose have similar genotoxicity in the rat colon and affect the metabolome (Abstract). We have shown previously that a high sucrose intake increases the background level of somatic mutations and the level of bulky DNA adducts in the colon epithelium of rats. The mechanism may involve either glucose or fructose formed by hydrolysis of sucrose. Male Big Blue rats were fed 30% sucrose, glucose, fructose or potato starch as part of the diet. Mutation rates and bulky DNA adduct levels were determined in colon and liver. The concentration of short-chain fatty acids and pH were determined in caecum, C-peptide was determined in plasma, biomarkers for oxidative damage and proliferation were determined in colon, and a metabonomic analysis was performed in plasma and urine. The sugars increased the mutation rates in colon and the bulky adduct levels in colon and liver to a similar extent. All sugars decrease the caecal concentration of acetic acid and propionic acid. The metabonomic studies indicated

disturbed amino acid metabolism and decrease in plasma and urinary acetate as a common feature for all sugars and confirmed triglyceridemic effects of fructose. In conclusion, the genotoxicity may be related to the altered chemical environment in the caecum and thereby also in the colon but we found no related changes in insulin resistance or oxidative stress. As taken from Hansen M et al. Food Chem Toxicol. 2008 Feb;46(2):752-60. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/17988776

Dietary glycemic load, added sugars, and carbohydrates as risk factors for pancreatic cancer: the Multiethnic Cohort Study (Abstract).

BACKGROUND: Because elevated blood glucose concentrations have been shown to be associated with greater risk of pancreatic cancer, a high dietary glycemic load, which is based on an empirical measure of blood glucose response after food consumption, has been hypothesized as a pancreatic cancer risk factor. However, results so far are scarce and inconsistent.

OBJECTIVE: We analyzed data for 162 150 participants in the Hawaii-Los Angeles Multiethnic Cohort Study to investigate associations between glycemic load, dietary carbohydrates, sucrose, fructose, total sugars, and added sugars and the risk of pancreatic cancer.

DESIGN: Dietary intake was assessed at baseline by using a quantitative food-frequency questionnaire. During 8 y of follow-up, 434 incident pancreatic cancer cases occurred.

RESULTS: Glycemic load and added sugars were not significantly associated with pancreatic cancer risk. The risk increased with higher intakes of total sugars, fructose, and sucrose, and the association with fructose was significant when the highest and lowest quartiles were compared (relative risk: 1.35; 95% CI: 1.02, 1.80; P for trend = 0.046). A significant association was found with fruit and juices intake (1.37; 1.02, 1.84; P for trend = 0.04) but not with soda intake. Statistical evidence of a significant interaction with body mass index was present only for sucrose intake (P = 0.04). A comparison of the highest and lowest quartiles of sucrose intake in overweight or obese participants gave a relative risk of 1.46 (0.95-2.25; P for trend = 0.04), but the comparison was not significant in normal-weight participants.

CONCLUSIONS: High fructose and sucrose intakes may play a role in pancreatic cancer etiology. Conditions such as overweight or obesity in which a degree of insulin resistance may be present may also be important. As taken from Nöthlings et al. American Journal of Clinical Nutrition 86, 1495–501. Available at http://www.ajcn.org/content/86/5/1495.full.pdf+html

Dietary glycemic load, carbohydrate, sugar, and colorectal cancer risk in men and women (Abstract). Hyperinsulinemia may explain excess colorectal cancer among individuals who are overweight or inactive. Recent studies have observed elevated colorectal cancer risk among individuals with elevated insulin levels 2 hours after oral glucose challenge or with elevated plasma C-peptide levels. The effect of consuming a high glycemic diet on colorectal risk, however, remains uncertain. Two prospective cohort studies, the Nurses' Health Study and the Health Professionals Follow-up Study, contributed up to 20 years of follow-up. After exclusions, 1,809 incident colorectal cancers were available for analyses. Dietary glycemic load (GL) was calculated as a function of glycemic index (postprandial blood glucose response as compared with a reference food), carbohydrate content, and frequency of intake of individual foods reported on food frequency questionnaires. Multivariable Cox proportional hazards models were used to

adjust for potential confounders. Intakes of dietary carbohydrate, GL, overall glycemic index, sucrose, and fructose were not associated with colorectal cancer risk in women. A small increase in risk was observed in men with high dietary GL (multivariate relative risk, 1.32; 95% confidence interval, 0.98-1.79; highest versus lowest quintile), sucrose or fructose (multivariate relative risk, 1.37; 95% confidence interval, 1.05-1.78; highest versus lowest quintile of fructose, P = 0.008). Associations were slightly stronger among men with elevated body mass index (z25 kg/m2). Results among women were similar after stratifying by body mass index or physical activity. High intakes of GL, fructose, and sucrose were related to an elevated colorectal cancer risk among men. For women, however, these factors did not seem to increase the risk of colorectal cancer. As taken from Michaud et al. Cancer Epidemiology, Biomarkers and Prevention 14, 138–43 available at http://cebp.aacrjournals.org/content/14/1/138.full.pdf

Fructose transporter Glut5 expression in clear renal cell carcinoma (Abstract). Renal cell carcinomas (RCC) can be subclassified for general purposes into clear cell, papillary cell, chromophobe cell carcinomas and oncocytomas. Other tumours such as collecting duct, medullary, mucinous tubular and spindle cell and associated with Xp 11.2 translocations/TFE 3 gene fusion, are much less common. There is also a residual group of unclassified cases. Previous studies have shown that RCC has high glycolytic rates, and expresses GLUT transporters, but no distinction has been made among the different subtypes of renal cell tumours and their grades of malignancy. In clear renal cell carcinoma (cRCC) glycogen levels increase, glycolysis is activated and gluconeogenesis is reduced. The clear cell subtype of RCC is characterized histologically by a distinctive pale, glassy cytoplasm and this appearance of cRCC is due to abnormalities in carbohydrate and lipid metabolism, and this abnormality results in glycogen and sterol storage. Several isoforms of glucose carriers (GLUTs) have been identified. We show here in a panel of 80 cRCC samples a significant correlation between isoform 5 (GLUT5) and many pathological parameters such as grade of differentiation, pelvis invasion and breaking capsule. GLUT5 expression also appears to associate more strongly with the clear cell RCC subtype. These data suggest a role for the GLUT5 isoform in fructose uptake that takes place in cRCC cells and which subsequently leads to the malignant RCC progression. As taken from Villaamil et al. Oncology Reports 25, 315-323 available at http://www.spandidos-publications.com/or/25/2/315

Fructose induces transketolase flux to promote pancreatic cancer growth Abstract). Carbohydrate metabolism via glycolysis and the tricarboxylic acid cycle is pivotal for cancer growth, and increased refined carbohydrate consumption adversely affects cancer survival. Traditionally, glucose and fructose have been considered as interchangeable monosaccharide substrates that are similarly metabolized, and little attention has been given to sugars other than glucose. However, fructose intake has increased dramatically in recent decades and cellular uptake of glucose and fructose uses distinct transporters. Here, we report that fructose provides an alternative substrate to induce pancreatic cancer cell proliferation. Importantly, fructose and glucose metabolism are quite different; in comparison with glucose, fructose induces thiamine-dependent transketolase flux and is preferentially metabolized via the nonoxidative pentose phosphate pathway to synthesize nucleic acids and increase uric acid production. These findings show that cancer cells can readily metabolize fructose to increase proliferation. They have major significance for cancer patients given dietary refined fructose consumption, and indicate that efforts to reduce refined fructose intake or inhibit fructose-mediated actions may disrupt cancer growth. As taken from Liu et al. Cancer Research 70, 6368–76.

Available carbohydrates, glycemic load, and pancreatic cancer: is there a link? (Abstract). High-carbohydrate diets have been linked to pancreatic cancer risk in case-control studies, but prospective studies have shown mostly null results. The authors investigated the associations of glycemic load, glycemic index, and carbohydrate intake with pancreatic cancer risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Dietary intake was assessed by using a self-administered questionnaire. Between 1998 and 2006 (median follow-up = 6.5 years), 266 incident, confirmed pancreatic cancers were identified among 109,175 participants. Hazards ratios and 95% confidence intervals were adjusted for sex, smoking, body mass index, and total energy. Overall, elevated risks for pancreatic cancer were observed in the 90th versus 10th percentile of glycemic load (hazards ratio (HR) = 1.45, 95% confidence interval (CI): 1.05, 2.00), available carbohydrate (HR = 1.47, 95% CI: 1.05, 2.06), and sucrose (HR = 1.37, 95% CI: 0.99, 1.89) intake. The positive association for available carbohydrate intake was observed during the first 4 years of follow-up (HR(<2 years) = 2.60, 95% CI: 1.34, 5.06; HR(2-<4 years) = 1.94, 95% CI: 1.06, 3.55) but not subsequently (HR = 0.86, 95% CI: 0.52, 1.44); the opposite pattern was observed for total fat and saturated fat intake. Rather than being causal, the short-term increase in pancreatic cancer risk associated with high available carbohydrate and low fat intake may be capturing dietary changes associated with subclinical disease. As taken from Meinhold et al. (2010). Am. J. Epidemiol. 171, 1174-1182. Abstract available via PubMed at http://www.ncbi.nlm.nih.gov/pubmed/20452999

Refined fructose and cancer (Abstract).

Introduction: Caloric excess, including increased refined carbohydrate intake, is associated with higher cancer risk emphasizing the importance of improved understanding of cancer cell metabolism in tumor survival and metastasis.
Areas covered: This article reviews the relationship between increased dietary refined sugar and cancer risk, with specific emphasis on the monosaccharide fructose. Cancer cell metabolism is reviewed, and the potential mechanisms by which dietary sugars contribute to the tumor microenvironment are discussed. Recent observations indicate that cancer cells readily utilize fructose to support proliferation and preferentially use fructose for nucleic acid synthesis. This review discusses the potential role of how dietary fructose can promote cancer growth by a variety of mechanisms, including altered cellular metabolism, increased reactive oxygen species, DNA damage and inflammation. Preliminary insights into potential therapeutic strategies by which fructose-mediated cancer effects may be abrogated are presented.
Expert opinion: Other sugars (particularly fructose, given its abundance in the modern diet) must be considered with reference to cancer cell metabolism. Cancer cells utilize similar sugars in distinct ways, which may present important new therapeutic avenues of targeting cancer. As taken from Liu and Heaney (2011). Expert Opin. Ther. Targets 15(9): 1049-59. Epub 2011 May 31, available at http://www.ncbi.nlm.nih.gov/pubmed/21623683?dopt=AbstractPlus

“BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item

NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015." As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24552754

"OBJECTIVE: A diet high in sugars may promote colorectal carcinogenesis, but it remains uncertain whether high intake of sugars or sucrose confers increased risk of colorectal cancer. The authors investigated the associations of sugars and sucrose intake with colorectal cancer risk in a community-based case-control study in Japan. METHODS: The study subjects comprised 816 incident cases of colorectal cancer and 815 community controls. Consumption frequencies and portion sizes of 148 food and beverage items were ascertained by a computer-assisted interview. The authors used the consumption of 29 food items to estimate sugars and sucrose intake. The odds ratios of colorectal cancer risk according to intake categories were obtained using a logistic regression model with adjustment for potential confounding variables. RESULTS: Overall, intakes of sugars and sucrose were not related to colorectal cancer risk either in men or women. The association between sugars intake and colorectal cancer risk differed by smoking status and alcohol use in men, but not in women. In men, sugars intake tended to be associated with colorectal cancer risk inversely among never-smokers and positively among male ever-smokers (interaction p=0.01). Sugars intake was associated with an increased risk among men with no alcohol consumption, but was unrelated to the risk among male alcohol drinkers (interaction p=0.02). Body mass index did not modify the association with sugars intake in either men or women. CONCLUSION: Sugars intake was associated with increased risk of colorectal cancer among smokers and non-alcohol drinkers in men selectively." As taken from Wang Z et al. 2014. Scand. J. Gastroenterol. 49(5), 581-8. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24716480

"Sugar intake has dramatically increased during the last few decades. Specifically, there has been a clear trend towards higher consumption of fructose and high fructose corn syrup, which are the most common added sugars in processed food, soft drinks and other sweetened beverages. Although still controversial, this rising trend in simple sugar consumption has been positively associated with weight gain and obesity, insulin resistance and type 2 diabetes mellitus and non-alcoholic fatty liver disease. Interestingly, all of these metabolic alterations have also been related to the development of hepatocellular carcinoma. The purpose of this review is to discuss the evidence coming from epidemiological studies and data from animal models relating the consumption of simple sugars, and specifically fructose, with an increased risk of hepatocellular carcinoma and to

gain insight into the putative molecular mechanisms involved." As taken from Laguna et al. 2014. Nutrients 6(12), 5933-54. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25533006

"Epidemiologic studies have shown that dietary sugar intake has a significant impact on the development of breast cancer. One proposed mechanism for how sugar impacts cancer development involves inflammation. In the current study, we investigated the impact of dietary sugar on mammary gland tumor development in multiple mouse models, along with mechanisms that may be involved. We found that sucrose intake in mice comparable with levels of Western diets led to increased tumor growth and metastasis, when compared with a nonsugar starch diet. This effect was ascribed in part to increased expression of 12-lipoxygenase (12-LOX) and its arachidonate metabolite 12-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12-HETE). We determined that fructose derived from the sucrose was responsible for facilitating lung metastasis and 12-HETE production in breast tumors. Overall, our data suggested that dietary sugar induces 12-LOX signaling to increase risks of breast cancer development and metastasis" As taken from Jiang Y et al. 2016. Cancer Res. 76, 24-9. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26729790

"Epidemiological studies have demonstrated clear associations between specific dietary and environmental risk factors and incidence of colorectal cancer, but the mechanisms responsible for these associations are not known. An animal model could facilitate such an understanding. Both genotoxic and nongenotoxic carcinogens induce aberrant crypt foci (ACF) in the colons of F344 rats. F344 rats were provided with diets that contained putative risk factors for CRC: low calcium and low vitamin D, high iron, high fructose, and decreased light (UV) exposure or a control diet for 14 wk. The rats were then assessed with biochemical measures and by topological examination for evidence of colon abnormalities. Circulating ionized calcium was decreased from 2.85 to 1.69 mmol/L, and ACF were increased from 0.7 to 13.6 lesions/colon (both $P < 0.001$). Rats exposed to the multiple environmental conditions associated with colon cancer, developed ACF similar to the heterogeneous or ill-defined ACF in the human colon. Heterogeneous ACF are the most frequently seen in humans and are also seen in rats shortly after exposure to the non-genotoxic colon carcinogen, dextransulfate sodium. The rodent model could be used to assess the pathways from diet and environment to colon cancer and to provide guidance for clinical studies." As taken from Yang K et al. 2016. Nutr. Cancer 68, 94-104. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26709971

"Fructose consumption has dramatically increased in the last 30 years. The principal form has been in the form of high-fructose corn syrup found in soft drinks and processed food. The effect of excessive fructose consumption on human health is only beginning to be understood. Fructose has been confirmed to induce several obesity-related complications associated with the metabolic syndrome. Here we present an overview of fructose metabolism and how it contrasts with that of glucose. In addition, we examine how excessive fructose consumption can affect de novo lipogenesis, insulin resistance, inflammation, and reactive oxygen species production. Fructose can also induce a change in the gut permeability and promote the release of inflammatory factors to the liver, which has potential implications in increasing hepatic inflammation. Moreover, fructose has been associated with colon, pancreas, and liver cancers, and we shall discuss the evidence for these observations. Taken together, data suggest that sustained fructose consumption should be curtailed as it is detrimental to long-term human health." As taken from Charrez B

et al. 2015. Horm. Mol. Biol. Clin. Investig. 22, 79-89. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25965509

“Although altered glucose metabolism is a well-studied feature of malignant cells, little is known about the direct metabolism of fructose. In this issue of Cancer Cell, Chen et al. report that AML cells consume fructose and use it to maintain viability, especially when glucose is scarce.” As taken from Cairns RA and Mak TW. 2016. Cancer Cell 30(5), 660-662. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27846386

“Whether fructose (FRU), as the sole energy source, confers a metabolic advantage on cancer cells against noxious stimuli is unknown. The aim of this study was to evaluate the effects of low (11 mM), moderate (25 mM), and high (55 mM) FRU concentrations alone or in combination with rotenone (ROT) or doxorubicin (DOX) in Jurkat cells, an acute lymphoblastic leukemia cell model. Glucose (GLU) was used as a control. Using different cell analysis techniques, we demonstrated that FRU was predominantly metabolized via oxidative phosphorylation (~95%) (i.e., lactate production was reduced >120-fold), resulting in endogenous oxidative stress-induced conditions. The cells were characterized by generation of O2·- (43%)/ H2 O2 (40%) and activation of NF-κB (~95-fold increase, fi), c-Jun-N terminal kinase (JNK), p53 (40-fi), and c-Jun (9-fi). In addition, we observed a loss of ΔΨm (10%), activation of caspase-3 (50-fi) and apoptosis-inducing factor (AIF, 2-fi), and condensation and fragmentation of the nuclei [20% by acridine orange/ethidium bromide/Hoechst (AO/EB/H) staining, 15% by flow cytometry] compared to those of GLU 11 at 24 h. Although DOX killed Jurkat cells independent of sugar content in the culture medium, leukemic cells in low, but not high, FRU were extremely sensitive to ROT. Taken together, our findings suggest that Jurkat cells are more susceptible to cell death if forced to shift from GLU metabolism (i.e., aerobic glycolysis) to FRU metabolism (i.e., oxidative phosphorylation) after treatment with mitochondria-targeting molecules. These observations may help elucidate the cell death mechanism of leukemic cells cultured in FRU.” As taken from Diaz-Aguirre V et al. 2016. Cell Biol. Int. 40(11), 1162-1173. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27486090

“The consumption of fructose, including the use of high-fructose corn syrup as a sweetener, has increased continuously in recent decades. Although the involvement of fructose in the development of metabolic diseases has been emphasized recently, whether fructose intake increases susceptibility to steatosis-associated hepatocellular carcinoma (HCC) is unclear. Here, we investigated this issue using mice lacking a circulating protein, apoptosis inhibitor of macrophage (AIM, encoded by cd5l). AIM does not induce carcinogenesis of hepatocytes, but provokes necrotic death specifically in AIM-bound cancer cells through complement cascade activation, thereby preventing HCC tumor development in wild-type mice. When subjected to a high-fructose diet (HFrD), AIM-deficient (AIM-/-) mice showed liver steatosis and subsequent liver inflammation as well as fibrosis, but at much milder levels compared with mice fed a high-fat diet. However, AIM-/- mice were markedly susceptible to HCC tumor development, whereas no wild-type mice developed the disease. Systemic metabolic states, including obesity and insulin resistance, were similar in both types of mice after HFrD challenge, indicating no influence of AIM on HFrD-induced metabolic changes. Our results suggest that dietary fructose increases the risk for liver carcinogenesis and that individuals with low blood AIM levels may be susceptible to HCC under chronic fructose intake.” As taken from Ozawa T et al. 2016. Genes Cells 21(12), 1320-1332. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27813205

“Rapid proliferation and Warburg effect make cancer cells consume plenty of glucose, which induces a low glucose micro-environment within the tumor. Up to date, how cancer cells keep proliferating in the condition of glucose insufficiency still remains to be explored. Recent studies have revealed a close correlation between excessive fructose consumption and breast cancer genesis and progression, but there is no convincing evidence showing that fructose could directly promote breast cancer development. Herein, we found that fructose, not amino acids, could functionally replace glucose to support proliferation of breast cancer cells. Fructose endowed breast cancer cells with the colony formation ability and migratory capacity as effective as glucose. Interestingly, although fructose was readily used by breast cancer cells, it failed to restore proliferation of non-tumor cells in the absence of glucose. These results suggest that fructose could be relatively selectively employed by breast cancer cells. Indeed, we observed that a main transporter of fructose, GLUT5, was highly expressed in breast cancer cells and tumor tissues but not in their normal counterparts. Furthermore, we demonstrated that the fructose diet promoted metastasis of 4T1 cells in the mouse models. Taken together, our data show that fructose can be used by breast cancer cells specifically in glucose-deficiency, and suggest that the high-fructose diet could accelerate the progress of breast cancer in vivo.” As taken from Fan X et al. 2017. PeerJ 5, e3804. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/28970966

“The metabolism of cancer cells is highly plastic. Cancer cells can change their preference for nutrient uptake under nutrient stress. Fructose is one of the most common carbohydrates in diet and its metabolism is also involved in the development and progression of tumors. GLUT5, encoded by SLC2A5, is the specific fructose transporter in mammalian cells. In this study, we found that SLC2A5 is significantly upregulated in lung adenocarcinoma (LUAD) patients and overexpression of SLC2A5 is highly correlated with poor prognosis of LUAD patients. The expression of SLC2A5 determined fructose uptake and utilization efficacy in LUAD cells. GLUT5 is critical for the survival of LUAD cells in fructose-containing culture medium. Depletion of SLC2A5 undermined cell proliferation and invasion meanwhile increased cell apoptosis. Overexpression of SLC2A5 enhances cell proliferation, migration, invasion, and tumorigenic. Compared to glucose, fructose is prone to strengthen intracellular-free fatty acid accumulation and ATP production. Moreover, inhibition of GLUT5 by specific small chemical inhibitor sensitizes LUAD cells to paclitaxel treatment. Taken together, our results suggest that GLUT5 could be a potential target alone or combination with other treatment for lung cancer therapy.” As taken from Weng Y et al. 2018. Cell Death Discov. 4, 38. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29531835

“Many studies have pinpointed that fructose could be utilized as a carbon source in some cancers, and we also defined that glioma cells could utilize fructose to maintain themselves survival and proliferation depending on GLUT5 expression recently. However, ketohexokinase (KHK), as a key enzyme involved in fructose catabolism, drew less attention in cancers, especially in glioma. In the present study, we first analyzed the expression levels of KHK in glioma tissues and the correlations of KHK expression with clinicopathological variables of patients with glioma. Meanwhile, we detected the effect of silencing KHK on the biological functions of glioma cells in fructose medium. From the results, we found that KHK was expressed at significantly higher level in glioma tissues than in non-tumor brain, and KHK expression was significantly correlated with tumor malignancy and poor survival of glioma patients ($p < 0.01$). Functionally, knockdown of KHK could significantly inhibit cell proliferation and migration of glioma cells in fructose medium.

Furthermore, we investigated the KHK expression level after long-time treatment with fructose, and detected the change of cell biological behaviour, then we found that the expression level of KHK was significantly increased and these cells showed more malignant properties. Taken together, our results suggest that high fructose diet and KHK overexpression are correlated with glioma malignant progression and patients' poor survival, and we believe this hypothesis would open the door for novel therapeutic agents and mentalities for glioma." As taken from Gao W et al. 2018. Biochem. Biophys. Res. Commun. 503(3), 1298-1306. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30031605

"High sugar intake may increase cancer risk by promoting insulin-glucose dysregulation, oxidative stress, inflammation, and body adiposity, but epidemiologic evidence is unclear. Associations between dietary sugars and lifestyle-related cancer risk from longitudinal studies were evaluated. We systematically searched PubMed, Embase, and CINAHL and identified 37 prospective cohort studies (1990-2017) reporting multivariable adjusted risk estimates for dietary sugars in relation to cancer. Of 15 and 14 studies on total sugar and sucrose respectively, 11 reported a null association in relation to cancer. Of 14 studies on fructose, 8 reported null associations, and 2 reported protective and 4 reported detrimental associations. In two of five studies on added sugars, a 60-95% increased cancer risk was observed with higher intakes. In 8 of 15 studies on sugary foods and beverages, a 23-200% higher cancer risk was observed with higher sugary beverage consumption. In conclusion, most studies were indicative of a null association, but suggestive detrimental associations were reported for added sugars and sugary beverages." As taken from Makarem N et al. 2018a. Annu. Rev. Nutr. 38, 17-39. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29801420

"Cancer metastasis accounts for the majority of cancer-related deaths and remains a clinical challenge. Metastatic cancer cells generally resemble cells of the primary cancer, but they may be influenced by the milieu of the organs they colonize. Here, we show that colorectal cancer cells undergo metabolic reprogramming after they metastasize and colonize the liver, a key metabolic organ. In particular, via GATA6, metastatic cells in the liver upregulate the enzyme aldolase B (ALDOB), which enhances fructose metabolism and provides fuel for major pathways of central carbon metabolism during tumor cell proliferation. Targeting ALDOB or reducing dietary fructose significantly reduces liver metastatic growth but has little effect on the primary tumor. Our findings suggest that metastatic cells can take advantage of reprogrammed metabolism in their new microenvironment, especially in a metabolically active organ such as the liver. Manipulation of involved pathways may affect the course of metastatic growth." As taken from Bu P et al. 2018. Cell Metab. 27(6), 1249-1262.e4. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29706565

"Background: Higher sugar consumption may increase cancer risk by promoting insulin-glucose dysregulation, oxidative stress, hormonal imbalances, and excess adiposity. This prospective study investigates the association between dietary sugars (fructose and sucrose) and sugary foods and beverages in relation to combined and site-specific (breast, prostate, colorectal) adiposity-associated cancers. Methods: The analytic sample consisted of 3,184 adults, aged 26-84 years, from the Framingham Offspring cohort. Diet data were first collected between 1991 and 1995 using a food frequency questionnaire. Intakes of fructose, sucrose, sugary foods, and sugary beverages (fruit juice and sugar-sweetened beverages) were derived. Participants were followed up until 2013 to ascertain cancer

incidence; 565 doctor-diagnosed adiposity-related cancers, including 124 breast, 157 prostate, and 68 colorectal cancers occurred. Multivariable-adjusted Cox proportional hazards models were used to evaluate associations. Tests for interaction with BMI and waist circumference were conducted. Results: No associations were observed between fructose, sucrose, sugary food consumption, and combined incidence of adiposity-related cancers or the examined site-specific cancers. While total consumption of sugary beverages was not associated with site-specific cancer risk, higher intakes of fruit juice were associated with 58% increased prostate cancer risk (HR: 1.58; 95% CI, 1.04-2.41) in multivariable-adjusted models. In exploratory stratified analyses, higher sugary beverage intakes increased overall adiposity-related cancer risk by 59% in participants with excessive central adiposity (HR: 1.59; 95% CI, 1.01-2.50; Ptrend = 0.057).Conclusions: In this cohort of American adults, higher sugary beverage consumption was associated with increased cancer risk among participants with central adiposity. Impact: These analyses suggest that avoiding sugary beverages represents a simple dietary modification that may be used as an effective cancer control strategy." As taken from Makarem N et al. 2018b. Cancer Prev. Res. (Phila). 11(6), 347-358. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29674390

"Fructose is now such an important component of human diets, and several studies have found that some cancer cells could utilize fructose to overcome low glucose micro-environment, but the study on the role of fructose in glioma is rare. To explore the role of fructose in glioma, we detected the proliferation and colony formation ability of glioma cells in fructose medium, and found that the abilities of proliferation and colony formation of glioma cells in fructose medium were similar with abilities in glucose medium, however, fructose just partly restored proliferation ability of normal glial cells. To explore the mechanism, we compared the expression level of GLUT5 (Glucose transporter type 5) in these cell lines, and the results showed that glioma cell lines had higher GLUT5 expression than normal glial cell lines. And knockdown of GLUT5 could significantly inhabit cell proliferation of glioma cells in fructose medium. Furthermore, we found that GLUT5 was also higher expressed in glioma tissues, and GLUT5 expression correlated significantly with glioma malignancy and poor survival of glioma patients ($p < 0.01$). In addition, we also demonstrated that knockdown of GLUT5 could significantly inhabit tumor proliferation in vivo, and intake fructose could increase tumor volume prominently. Taken together, our data show that fructose can be used by glioma cells, and restrict the fructose intake or targeting GLUT5 could be efficacious strategies in glioma." As taken from Su C et al. 2018. Biochem. Biophys. Res. Commun. 500(2), 462-469. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29660339

5.7. Irritation/immunotoxicity

Fructose is not an allergen (EFSA, 2005).

May cause irritation or mechanical irritation (Haz-Map, 2018).

"INTRODUCTION: People with diet-induced metabolic diseases, such as obesity and diabetes, may be susceptible to the health effects of air pollutants. The present study was

designed to compare the nasal toxicity caused by ambient air pollutant exposures in rats fed a normal or high-fructose diet (ND and HFD, respectively) for 10 weeks. METHODS: Repeated inhalation exposures of male Sprague Dawley rats to ozone (O3; 0.5 ppm) and/or concentrated ambient fine particles (CAPs, 400 µg/m3) were conducted in a mobile air research laboratory located in an industrial area of Dearborn, MI during the summer 2011. Rats were exposed for 9 days, 8 h/day (4-5 days/week). All rats were sacrificed 24 h after the last exposure. Nasal tissues tissues were processed for light microscopic examination and morphometric analysis. RESULTS: ND and HFD rats exposed to O3 or O3/CAPs, but not control ND rats exposed to filtered air alone, had an acute rhinitis with marked hyperplasia of nasal epithelium (NE). HFD rats, but not ND rats, exposed to CAPs also had an acute rhinitis but no epithelial hyperplasia. The severity of the inflammatory cell response (numeric density of neutrophils) was not different among the rats with rhinitis. Of all the air pollutant-exposed groups, the HFD rats coexposed to O3/CAPs had the greatest numeric cell density of NE (54, 45, 12 and 12% greater than CAPs-exposed HFD, air-exposed HFD, O3/CAPs-exposed ND, and O3-exposed HFD rats, respectively). Though we have previously reported that repeated O3 exposure will cause rhinitis with nasal epithelial hyperplasia, this is the first report of a diet-related enhancement of air pollutant-induced nasal toxicity. Underlying mechanisms responsible for HFD enhancement of O3- and/or CAPsinduced nasal epithelial or inflammatory lesions are yet to be determined." As taken from Hotchkiss IP et al. 2012. The Toxicologist. SOT 2012 Annual Meeting. Available at http://www.toxicology.org/AI/PUB/Tox/2012Tox.pdf

"We recently showed that T regulatory lymphocytes (Treg), which are immune suppressors of inflammatory responses, play a role blunting the development of hypertension-induced injury. Treg are unchanged or decreased in children with metabolic syndrome, and therefore, their role in metabolic syndrome remains unclear. We hypothesized that Treg number or function would be depressed in a high-fructose diet-induced metabolic syndrome-like model in rats. Sprague-Dawley rats were fed normal chow or a high-fructose diet for 5 weeks. The high-fructose diet-induced a 3.8-fold increase in plasma triglycerides and a 14% reduction in high-density lipoprotein cholesterol (P<0.001). The high-fructose diet increased reactive oxygen species in aorta and periaortic adipose tissue 2.8-fold (P<0.05), and reduced nicotinamide adenine dinucleotide phosphate oxidase activity 1.9-fold in aorta, and 2.5-fold in the heart (P<0.05). It also increased plasma nitric oxide metabolite levels 6.4-fold (P<0.001). Western blots showed that the high-fructose diet increased ≥2.3-fold vascular and in platelet endothelial cell adhesion molecule 1 in aorta (P<0.01). It did not affect monocyte/macrophage aortic infiltration but caused a 2.4-fold increase in collagen deposition in the aortic media (P<0.01). No change in plasma interleukin-10 was detected. The percentage of spleen CD4+ CD25- and Treg (CD4+ CD25(high)) cells was unaltered by the high-fructose diet. However, cultured Treg from high-fructose diet-fed rats secreted 62% less interleukin-10 than control cells (P<0.05), suggesting a decreased Treg function, which could play a role in the development of cardiovascular complications of the metabolic syndrome." As taken from Leibowitz A et al. 2013. Hypertension 61(6), 1316-21. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23529169

"CONTEXT: Metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) are the emerging co-morbidities of skin inflammation. Occurrence of skin inflammation such as psoriasis is substantially higher in NAFLD patients than normal. Currently, there are no animal models to study the interaction between these co-morbidities. OBJECTIVE: The present study seeks to develop a simple mouse model of NAFLD-enhanced skin

inflammation and to study the effect of NAFLD on different parameters of skin inflammation. MATERIALS AND METHOD: Metabolic syndrome and NAFLD were induced in C57BL/6 mice by feeding high-fat diet (HFD, 60% kcal) and high fructose liquid (HFL, 40% kcal) in drinking water. Skin inflammation was induced by repeated application of oxazolone (1% sensitization and repeated 0.5% challenge) in both normal and NAFLD mice and various parameters of skin inflammation and NAFLD were measured. RESULTS: HFD and HFL diet induced obesity, hyperglycemia, hyperinsulinemia, and histological features of NAFLD in mice. Oxazolone challenge significantly increased ear thickness, ear weight, MPO activity, NF-κB activity, and histological features of skin inflammation in NAFLD mice as compared with normal mice. Overall, induction of oxazolone-induced skin inflammation was more prominent in NAFLD mice than normal mice. Hence, HFD and HFL diet followed by topical oxazolone application develops metabolic syndrome, NAFLD, and enhanced skin inflammation in mice. DISCUSSION AND CONCLUSION: This simple model can be utilized to evaluate a therapeutic strategy for the treatment of metabolic syndrome and NAFLD with skin inflammation and also to understand the nexus between these co-morbidities." As taken from Kulkami NM et al. 2015. Pharm. Biol. 53(8), 1110-7. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25430922

5.8. All other relevant types of toxicity

"The field of sugar metabolism, and fructose metabolism in particular, has experienced a resurgence of interest in the past decade. The "fructose hypothesis" alleges that the fructose component common to all major caloric sweeteners (sucrose, high-fructose corn syrup, honey, and fruit juice concentrates) plays a unique and causative role in the increasing rates of cardiovascular disease, hypertension, diabetes, cancer, and nonalcoholic fatty liver disease. This review challenges the fructose hypothesis by comparing normal U.S. levels and patterns of fructose intake with contemporary experimental models and looking for substantive cause-and-effect evidence from real-world diets. It is concluded that 1) fructose intake at normal population levels and patterns does not cause biochemical outcomes substantially different from other dietary sugars and 2) extreme experimental models that feature hyperdosing or significantly alter the usual dietary glucose-to-fructose ratio are not predictive of typical human outcomes or useful to public health policymakers. It is recommended that granting agencies and journal editors require more physiologically relevant experimental designs and clinically important outcomes for fructose research." As taken from White JS. 2013. Adv. Nutr. 4(2), 246-56. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493541

"Excessive fructose consumption and elevated glucocorticoids contribute to metabolic syndrome. We show that fructose as the only carbohydrate source is sufficient for the differentiation of 3T3-L1 fibroblasts into adipocytes. Differentiation of cells in fructose containing medium resulted in increased 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) expression and activity. Experiments with transfected HEK-293 cells suggested more efficient NADPH generation by fructose compared with glucose in the endoplasmic reticulum (ER). Adipocytes differentiated in the presence offructose showed increased FABP4 expression, C/EBPα to C/EBPβ ratio and lipolysis. Thus, excessive fructose may cause adverse metabolic effects by enhancing 11β-HSD1 activity and increasing lipolysis in

adipocytes." As taken from Legeza B et al. 2014. FEBS Lett. 588(3), 490-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24374344

"….Recent studies show that fructose-induced uric acid generation causes mitochondrial oxidative stress that stimulates fat accumulation independent of excessive caloric intake. These studies challenge the long-standing dogma that "a calorie is just a calorie" and suggest that the metabolic effects of food may matter as much as its energy content. The discovery that fructose-mediated generation of uric acid may have a causal role in diabetes and obesity provides new insights into pathogenesis and therapies for this important disease." As taken from Johnson RJ et al. 2013. Diabetes 62(10), 3307-15. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24065788

"Classically, leptin resistance has been associated with increased body fat and circulating leptin levels, and the condition is believed to contribute to the onset and/or maintenance of obesity. Although a great deal is known about the central nervous system mechanisms mediating leptin resistance, considerably less is known about the role of diet in establishing and maintaining this altered hormonal state. An exciting new finding has recently been published demonstrating the existence of leptin resistance in normal-weight rats with lean leptin levels by feeding them a high-concentration-fructose diet. This finding has opened the possibility that specific macronutrients may be capable of inducing leptin resistance, independently of the amount of body fat or circulating leptin present in the treated animals. This review describes several lines of research that have recently emerged indicating that specific types of dietary sugars and fats are capable of inducing leptin resistance in experimental rodent models…." As taken from Vasselli JR et al. 2013. Adv. Nutr. 4(2), 164-75. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493533?dopt=AbstractPlus

"BACKGROUND AND AIMS: Normoglucosetolerants (NGT) are considered at low risk, even if a 1-h post-load glucose (PLG) value ≥ 155 mg dl(-1) identifies NGTs at high risk of type-2 diabetes (T2D) and sub-clinical organ damage. Specific dietary factors may affect insulin sensitivity and the risk of T2D. However, it is unknown whether dietary components affect 1-h PLG in hypertensive NGT. Therefore, we investigate the effect of dietary patterns on 1-h PLG. METHODS AND RESULTS: We selected 188 subjects (94 NGTs < 155 mg dl(-1) and 94 NGTs ≥ 155 mg dl(-1) PLG), well matched for age, gender and body mass index (BMI). Insulin sensitivity was evaluated using the Matsuda index. Dietary intake was quantified by a semiquantitative food frequency questionnaire (FEQ) validated in the European Investigation into Cancer and Nutrition (EPIC) study. The NGT ≥ 155 group had significantly reduced insulin sensitivity (40.3 ± 19.8 vs. 73.3 ± 28.8; P < 0.0001). With the exclusion of total calories, lipids, alcohol and fiber consumption we observed a significant difference, between groups, in starch (214.1 ± 52.4 vs. 268.8 ± 71.8 g; P < 0.0001), saturated (27.4 ± 8.7 vs. 24.1 ± 8.5 g; P = 0.009), monounsaturated (45.5 ± 8.9 vs. 48.8 ± 10.7 g; P = 0.023) and polyunsaturated fatty acids (FAs) (14.5 ± 4.0 vs. 16.8 ± 4.7 g; P < 0.0001), fructose (14.5 ± 5.3 vs. 11.2 ± 4.8 g; P < 0.0001), and oligosaccharides (103.2 ± 26.6 vs. 89.9 ± 29.2 g; P = 0.001) consumption. In the whole population, starch was the major predictor of 1-h PLG, explaining 23.2% of variation (P < 0.0001). In the NGT < 155 group, fructose was the strongest predictor, accounting for 15.4% of the variation; BMI, gender and polyunsaturated FAs added another 6.6%, 3.6% and 3.2%, respectively. In the NGT ≥ 155 group, saturated and polyunsaturated FAs were retained as the major predictors of 1-h PLG, explaining 18.2% and 11.4% of the variation. CONCLUSIONS: The present data demonstrate that dietary patterns affect 1-h PLG, remarking the importance of both

quantitative and qualitative composition of a diet." As taken from Sciacqua A et al. 2014. Nutr. Metab. Cardiovasc. Dis. 24(5), 547-53. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24462366

"OBJECTIVE: The aim of this study was to review the current corpus of human studies to determine the association of various doses and durations of fructose consumption on metabolic syndrome. METHODS: We searched human studies in PubMed, Scopus, Ovid, ISI Web of Science, Cochrane library, and Google Scholar databases. We searched for the following keywords in each paper: metabolic syndrome x, insulin resistance, blood glucose, blood sugar, fasting blood sugar, triglycerides, lipoproteins, HDL, cholesterol, LDL, blood pressure, mean arterial pressure, systolic blood pressure, diastolic blood pressure, hypertens*, waist circumference, and fructose, sucrose, high-fructose corn syrup, or sugar. RESULTS: Overall, 3102 articles were gathered. We excluded studies on natural fructose content of foods, non-clinical trials, and trials in which fructose was recommended exclusively as sucrose or high-fructose corn syrup. Overall, 3069 articles were excluded. After review by independent reviewers, 15 studies were included in the meta-analysis. Fructose consumption was positively associated with increased fasting blood sugar (FBS; summary mean difference, 0.307; 95% confidence interval [CI], 0.149-0.465; P = 0.002), elevated triglycerides (TG; 0.275; 95% CI, 0.014-0.408; P = 0.002); and elevated systolic blood pressure (SBP; 0.297; 95% CI, 0.144-0.451; P = 0.002). The corresponding figure was inverse for high-density lipoprotein (HDL) cholesterol (-0.267; 95% CI, -0.406 to -0.128; P = 0.001). Significant heterogeneity existed between studies, except for FBS. After excluding studies that led to the highest effect on the heterogeneity test, the association between fructose consumption and TG, SBP, and HDL became non-significant. The results did not show any evidence of publication bias. No missing studies were identified with the trim-and-n¼üll method. CONCLUSION: Fructose consumption from industrialized foods has significant effects on most components of metabolic syndrome." As taken from Kelishadi R et al. 2014. Nutrition 30(5), 503-10. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24698343?dopt=AbstractPlus

"The impact of fructose, commonly consumed with sugars by humans, on blood pressure and uric acid has yet to be defined. A total of 267 weight-stable participants drank sugar-sweetened milk every day for 10 weeks as part of their usual, mixed-nutrient diet. Groups 1 and 2 had 9% estimated caloric intake from fructose or glucose, respectively, added to milk. Groups 3 and 4 had 18% of estimated caloric intake from high fructose corn syrup or sucrose, respectively, added to the milk. Blood pressure and uric acid were determined prior to and after the 10-week intervention. There was no effect of sugar type on either blood pressure or uric acid (interaction P>.05), and a significant time effect for blood pressure was noted (P<.05). The authors conclude that 10 weeks of consumption of fructose at the 50th percentile level, whether consumed as pure fructose or with fructose-glucose-containing sugars, does not promote hyperuricemia or increase blood pressure." As taken from Angelopoulos TJ et al. 2015. J. Clin. Hypertens. (Greenwich) 17(2), 87-94. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25496265

"The modern Western society lifestyle is characterized by a hyperenergetic, high sugar containing food intake. Sugar intake increased dramatically during the last few decades, due to the excessive consumption of high-sugar drinks and high-fructose corn syrup. Current evidence suggests that high fructose intake when combined with overeating and adiposity promotes adverse metabolic health effects including dyslipidemia, insulin resistance, type II diabetes, and inflammation. Similarly, elevated glucocorticoid levels,

especially the enhanced generation of active glucocorticoids in the adipose tissue due to increased 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) activity, have been associated with metabolic diseases. Moreover, recent evidence suggests that fructose stimulates the 11β-HSD1-mediated glucocorticoid activation by enhancing the availability of its cofactor NADPH. In adipocytes, fructose was found to stimulate 11β-HSD1 expression and activity, thereby promoting the adipogenic effects of glucocorticoids. This article aims to highlight the interconnections between overwhelmed fructose metabolism, intracellular glucocorticoid activation in adipose tissue, and their metabolic effects on the progression of the metabolic syndrome." As taken from Legeza B et al. 2017. Nutrients 9(5), E426. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28445389

"The potential impact on health of diets rich in free sugars, and particularly fructose, is of major concern. The focus of this review is the impact of these sugars on insulin resistance and obesity, and the associated risk of developing type 2 diabetes. Much of the concern is focussed on specific metabolic effects of fructose, which are argued to lead to increased fat deposition in the liver and skeletal muscle with subsequent insulin resistance and increased risk of diabetes. However, much of the evidence underpinning these arguments is based on animal studies involving very large intakes of the free sugars. Recent human studies, in the past 5 years, provide a rather different picture, with a clear dose response link between fructose intake and metabolic changes. In particular, the most marked effects are observed when a high sugars intake is accompanied by an excess energy intake. This does not mean that a high intake of free sugars does not have any detrimental impact on health, but rather that such an effect seems more likely to be a result of the high sugars intake increasing the chances of an excessive energy intake rather than it leading to a direct detrimental effect on metabolism." As taken from Macdonald IA. 2016. Eur. J. Nutr. 55(Suppl 2), 17-23. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27882410

"The objective of the current study was to explore our hypothesis that average consumption of fructose and fructose containing sugars would not increase risk factors for cardiovascular disease (CVD) and the metabolic syndrome (MetS). A randomized, double blind, parallel group study was conducted where 267 individuals with BMI between 23 and 35 kg/m² consumed low fat sugar sweetened milk, daily for ten weeks as part of usual weight-maintenance diet. One group consumed 18% of calories from high fructose corn syrup (HFCS), another group consumed 18% of calories from sucrose, a third group consumed 9% of calories from fructose, and the fourth group consumed 9% of calories from glucose. There was a small change in waist circumference (80.9 ± 9.5 vs. 81.5 ± 9.5 cm) in the entire cohort, as well as in total cholesterol (4.6 ± 1.0 vs. 4.7 ± 1.0 mmol/L, $p < 0.01$), triglycerides (TGs) (11.5 ± 6.4 vs. 12.6 ± 8.9 mmol/L, $p < 0.01$), and systolic (109.2 ± 10.2 vs. 106.1 ± 10.4 mmHg, $p < 0.01$) and diastolic blood pressure (69.8 ± 8.7 vs. 68.1 ± 9.7 mmHg, $p < 0.01$). The effects of commonly consumed sugars on components of the MetS and CVD risk factors are minimal, mixed and not clinically significant." As taken from Angelopoulos TJ et al. 2016. Nutrients 8(4), 179. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27023594

"The impact of sugar consumption on health continues to be a controversial topic. The objective of this review is to discuss the evidence and lack of evidence that allows the controversy to continue, and why resolution of the controversy is important. There are plausible mechanisms and research evidence that supports the suggestion that consumption of excess sugar promotes the development of cardiovascular disease (CVD) and type 2 diabetes (T2DM) both directly and indirectly. The direct pathway involves the

unregulated hepatic uptake and metabolism of fructose, leading to liver lipid accumulation, dyslipidemia, decreased insulin sensitivity and increased uric acid levels. The epidemiological data suggest that these direct effects of fructose are pertinent to the consumption of the fructose-containing sugars, sucrose and high fructose corn syrup (HFCS), which are the predominant added sugars. Consumption of added sugar is associated with development and/or prevalence of fatty liver, dyslipidemia, insulin resistance, hyperuricemia, CVD and T2DM, often independent of body weight gain or total energy intake. There are diet intervention studies in which human subjects exhibited increased circulating lipids and decreased insulin sensitivity when consuming high sugar compared with control diets. Most recently, our group has reported that supplementing the ad libitum diets of young adults with beverages containing 0%, 10%, 17.5% or 25% of daily energy requirement (Ereq) as HFCS increased lipid/lipoprotein risk factors for CVD and uric acid in a dose-response manner. However, un-confounded studies conducted in healthy humans under a controlled, energy-balanced diet protocol that enables determination of the effects of sugar with diets that do not allow for body weight gain are lacking. Furthermore, recent reports conclude that there are no adverse effects of consuming beverages containing up to 30% Ereq sucrose or HFCS, and the conclusions from several meta-analyses suggest that fructose has no specific adverse effects relative to any other carbohydrate. Consumption of excess sugar may also promote the development of CVD and T2DM indirectly by causing increased body weight and fat gain, but this is also a topic of controversy. Mechanistically, it is plausible that fructose consumption causes increased energy intake and reduced energy expenditure due to its failure to stimulate leptin production. Functional magnetic resonance imaging (fMRI) of the brain demonstrates that the brain responds differently to fructose or fructose-containing sugars compared with glucose or aspartame. Some epidemiological studies show that sugar consumption is associated with body weight gain, and there are intervention studies in which consumption of ad libitum high-sugar diets promoted increased body weight gain compared with consumption of ad libitum low- sugar diets. However, there are no studies in which energy intake and weight gain were compared in subjects consuming high or low sugar, blinded, ad libitum diets formulated to ensure both groups consumed a comparable macronutrient distribution and the same amounts of fiber. There is also little data to determine whether the form in which added sugar is consumed, as beverage or as solid food, affects its potential to promote weight gain. It will be very challenging to obtain the funding to conduct the clinical diet studies needed to address these evidence gaps, especially at the levels of added sugar that are commonly consumed. Yet, filling these evidence gaps may be necessary for supporting the policy changes that will help to turn the food environment into one that does not promote the development of obesity and metabolic disease." As taken from Stanhope KL. 2016. Crit. Rev. Clin. Lab. Sci. 53(1), 52-67. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26376619

"OBJECTIVE: The calorie distribution of macronutrients affects individuals' health. Quantity and source of macronutrients may play major roles in waist circumference (WC) and hip circumference (HC). This study's purpose is to investigate the association between the quantity and source of macronutrients and the change in WC and HC over 9 years. METHODS: Participants (N = 11,343) were from the Atherosclerosis Risk in Community Study. Those diagnosed with cancer or a decrease in WC or HC of 15 cm or more over 9 years were excluded. Change scores were created for anthropometrics between clinic visits over 9 years. Macronutrient intakes were assessed by a food frequency questionnaire at visit 1 and presented as a percentage of energy intake. Linear regressions were performed

with quartiles of dietary components on change scores for WC and HC with controlling cofactors. Gender subgroup analysis was performed. RESULTS: A larger increase in WC was associated with higher intakes of total carbohydrates, dietary fiber, and fructose (p for trend < 0.005). A smaller increase in WC was associated with higher intakes of sucrose, total protein, animal protein, and alcohol (p for trend < 0.02). A larger increase in HC was associated with higher intakes of total carbohydrate, sucrose, fructose, animal protein, and vegetable fat (p for trend < 0.05). A smaller increase in HC was associated with higher intakes of animal fat, total fat, and total protein (p for trend < 0.05). In males, changes in WC and HC were associated with fructose, sucrose, total fat, and total protein. In females, changes in WC and HC were associated with dietary fiber, fructose, alcohol, animal protein, total protein, animal fat, and vegetable fat. CONCLUSION: Macronutrient source and quantity play a significant role in individuals' adiposity and effects on WC and HC. Overall, an increase in WC and HC was seen over the 11 years. The source and quantity of the macronutrients play a significant role in WC and HC. Further research needs to be conducted to see the exact effect that macronutrients play on WC and HC." As taken from Lofley AC and Root MM. 2017. J. Am. Coll. Nutr. 36(1), 57-63. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27797648

"Although calorically equivalent to glucose, fructose appears to be more lipogenic, promoting dyslipidemia, fatty liver disease, cardiovascular disease, and diabetes. To better understand how fructose induces lipogenesis, we compared the effects of fructose and glucose on mammalian target of rapamycin complex 1 (mTORC1), which appeared to have both positive and negative effects on lipogenic gene expression. We found that fructose acutely and transiently suppressed mTORC1 signaling in vitro and in vivo The constitutive activation of mTORC1 reduced hepatic lipogenic gene expression and produced hypotriglyceridemia after 1 week of fructose feeding. In contrast, glucose did not suppress mTORC1, and the constitutive activation of mTORC1 failed to suppress plasma triglycerides after 1 week of glucose feeding. Thus, these data reveal fundamental differences in the signaling pathways used by fructose and glucose to regulate lipid metabolism." As taken from Hu Y et al. 2018. J. Biol. Chem. 293(6), 2006-2014. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29222328

"INTRODUTION AND OBJECTIVE: Increased fructose consumption is associated with various metabolic changes that favor the onset of obesity and related comorbidities. The objective of this study was to assess the effects of chronic fructose consumption on body weight and adipose tissue, as well as on serum glucose and triglyceride levels. METHODS: Thirty-day-old Wistar rats were divided into two groups: fructose (F) and control (C), which had free access to commercial chow and either water or a 20% fructose solution. Body mass was measured weekly and food consumption at 30, 60 and 90 days. At 90 days, the animals were killed by decapitation and fat deposits (mesenteric, epididymal and retroperitoneal) were removed and blood collected for measurement of glucose and triglyceride levels. RESULTS: There was no significant difference in body weight gain, but the percentage of body fat was higher in group F. This group also consumed less feed at 60 and 90 days and had higher consumption of fructose solution than water in group C at 30 and 60 days. This meant higher calorie intake in group F and lower feed efficiency. Retroperitoneal and epididymal fat deposits and triglycerides were higher in group F than in group C. CONCLUSION: Consumption of fructose solution for eight weeks, while not directly reflected in body weight gain, did increase abdominal fat in group F compared to group C, as well as changing triglyceride levels. These two factors increase risk of

cardiovascular disease." As taken from Ramos VW et al. 2017. Rev. Port. Cardiol. 36(12), 937-941. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29221682

Type of Test	Route of Exposure or Administration	Species/Test System	Dose Data	Toxic Effects	Reference
ICLo - Inhibitor Concentration Low	In vitro	Rat - muscular	40 mmol/L/30M	Vascular - other changes	TIVIEQ Toxicology In Vitro. (Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, NY 10523) V.1- 1987- Volume(issue)/page/year: 29,2030,2015

As taken from RTECS, 2018.

6. Functional effects on

6.1. Broncho/pulmonary system

"There is growing evidence that intakes of high-fructose corn syrup (HFCS), HFCS-sweetened soda, fruit drinks and apple juice - a high-fructose 100 % juice - are associated with asthma, possibly because of the high fructose:glucose ratios and underlying fructose malabsorption, which may contribute to enteral formation of pro-inflammatory advanced glycation end products, which bind receptors that are mediators of asthma. Cox proportional hazards models were used to assess associations between intakes of these beverages and asthma risk, with data from the Framingham Offspring Cohort. Diet soda and orange juice - a 100 % juice with a 1:1 fructose:glucose ratio - were included for comparison. Increasing intake of any combination of HFCS-sweetened soda, fruit drinks and apple juice was significantly associated with progressively higher asthma risk, plateauing at 5-7 times/week v. never/seldom, independent of potential confounders (hazard ratio 1·91, P<0·001). About once a day consumers of HFCS-sweetened soda had a 49 % higher risk (P<0·011), moderate apple juice consumers (2-4 times/week) had a 61 % higher risk (P<0·007) and moderate fruit drink consumers had a 58 % higher risk (P<0·009), as compared with never/seldom consumers. There were no associations with diet soda/orange juice. These associations are possibly because of the high fructose:glucose ratios, and fructose malabsorption. Recommendations to reduce consumption may be inadequate to address asthma risk, as associations are evident even with moderate intake of these beverages, including apple juice - a 100 % juice. The juice reductions in the US Special Supplemental Nutrition Program for Women, Infants, and Children in 2009, and the plateauing/decreasing asthma prevalence (2010-2013), particularly among non-Hispanic black children, may be related. Further research regarding the consequences of fructose malabsorption is needed."

As taken from DeChristopher LR & Tucker KL, 2018. Br. J. Nutr. 119(10), 1157-1167. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29587887

"RATIONALE: Cross-sectional studies have linked intake of high-fructose corn syrup-sweetened beverages with asthma in schoolchildren. OBJECTIVES: To examine associations of maternal prenatal and early childhood intake of sugar-sweetened beverages and fructose with current asthma in midchildhood (median age, 7.7 yr). METHODS: We assessed maternal pregnancy (first- and second-trimester average) and child (median age, 3.3 yr) intake of sugar-sweetened beverages and total fructose using food frequency questionnaires in 1,068 mother-child pairs from Project Viva, a prospective prebirth cohort. In a multivariable analysis, we examined associations of quartiles of maternal and child sugar-sweetened beverage, juice, and total fructose intake with child current asthma in midchildhood, assessed by questionnaire as ever having doctor-diagnosed asthma plus taking asthma medications or reporting wheezing in the past 12 months. RESULTS: Higher maternal pregnancy sugar-sweetened beverage consumption (mean, 0.6 servings/d; range, 0-5) was associated with younger maternal age, nonwhite race/ethnicity, lower education and income, and higher prepregnancy body mass index. Adjusting for prepregnancy body mass index and other covariates, comparing quartile 4 with quartile 1, higher maternal pregnancy intake of sugar-sweetened beverages (odds ratio, 1.70; 95% confidence interval, 1.08-2.67) and total fructose (odds ratio, 1.58; 95% confidence interval, 0.98-2.53) were associated with greater odds of midchildhood current asthma (prevalence, 19%). Higher early childhood fructose intake (quartile 4 vs. quartile 1) was also associated with midchildhood current asthma in models adjusted for maternal sugar-sweetened beverages (odds ratio, 1.79; 95% confidence interval, 1.07-2.97) and after additional adjustment for midchildhood body mass index z-score (odds ratio, 1.77; 95% confidence interval, 1.06-2.95). CONCLUSIONS: Higher sugar-sweetened beverage and fructose intake during pregnancy and in early childhood was associated with childhood asthma development independent of adiposity." As taken from Wright LS et al. 2018. Ann. Am. Thorac. Soc. 15(2), 217-224. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29219619

6.2. Cardiovascular system

High-fructose diet elevates myocardial superoxide generation in mice in the absence of cardiac hypertrophy (Abstract). OBJECTIVE: Dietary fructose intake has increased considerably in recent decades and this has been paralleled by an increase in the incidence of insulin resistance, especially in children and adolescents. The impact of a high-fructose diet on the myocardium is not fully understood. The aims of this study were to characterize the murine metabolic and cardiac phenotypes associated with a high-fructose diet and to determine whether this diet imparts differential effects with age.

METHODS: Juvenile (4 wk) and adult (14 wk) C57Bl/6 mice were fed a 60% fructose diet or isoenergetic control (starch) diet for 6 wk.

RESULTS: At completion of the dietary intervention (at ages 10 and 20 wk), fructose-fed

mice were normotensive; hyperinsulinemia and cardiac hypertrophy were not evident. Interestingly, fructose-fed mice exhibited lower blood glucose levels (10 wk: 4.81+/-0.28 versus 5.42+/-0.31 mmol/L; 20 wk: 4.88+/-0.30 versus 5.96+/-0.42 mmol/L, P<0.05) compared with controls. Nicotinamide adenosine dinucleotide phosphate-driven myocardial superoxide production was significantly increased in fructose-fed mice at both ages (by approximately 29% of control at 10 wk of age and 16% at 20 wk, P<0.01). No increase in aortic superoxide production was observed. Fructose feeding did not alter gene expression of the antioxidant thioredoxin-2, suggesting an imbalance between myocardial reactive oxygen species generation and antioxidant induction.

CONCLUSION: These findings indicate that increased myocardial superoxide production may represent an early and primary cardiac pathologic response to the metabolic challenge of excess dietary fructose in juveniles and adults that can be detected in the absence of cardiac hypertrophy and hypertension. As taken from Mellor et al., 2010b.Nutrition 26, 842-8.

Elevated dietary sugar and the heart: experimental models and myocardial remodeling (Abstract). A dramatic rise in the prevalence of insulin resistance has been paralleled by increasing dietary consumption of sugar. The use of added sweeteners containing fructose (sucrose and high-fructose corn syrup) has increased by 25% over the past 3 decades. High fructose intake has the potential to adversely influence systemic and cellular metabolism via insulin resistance and glycolytic dysregulation. As a tissue that is both insulin sensitive and glycolysis dependent, the heart may be especially vulnerable to fructose over-consumption. In this review, experimental studies of elevated dietary sugar intake are evaluated, including sucrose and fructose dietary manipulation models. The possible role of the GLUT5 transporter as a mediator of cardiomyocyte fructose uptake is considered. The impact of dietary sucrose and fructose on cardiac insulin-dependent signaling in the context of perturbed systemic metabolic response is detailed. Myocardial dysfunction, modified growth, and oxidative stress responses associated with high dietary sugar intake are discussed. Finally, the involvement of the renin-angiotensin system in mediating fructose cardiopathology is considered. This review highlights the importance of obtaining new mechanistic data that can contribute to a more developed understanding of how high sugar intake directly contributes to structural and functional cardiomyopathy. As taken from Mellor et al. 2010a. Canadian Journal of Physiology and Pharmacology 88, 525-40 available at http://article.pubs.nrc-cnrc.gc.ca/ppv/RPViewDoc?issn=0008-4212&volume=88&issue=5&startPage=525

Effects of intragastric fructose and dextrose on mesenteric microvascular inflammation and postprandial hyperemia in the rat (Abstract). OBJECTIVES: Fructose superfused on the mesenteric venules of rats induces microvascular inflammation via oxidative stress. It is unknown whether intragastric fructose exerts a similar effect and whether fructose impairs postprandial hyperemia (PPH). The goals were to determine whether intragastric fructose administration promotes leukocyte adherence and whether fructose, owing to its oxidative properties, may also impair nitric oxide-dependent PPH in the mesenteric microcirculation of rats. METHODS: Leukocyte adherence to mesenteric venules, arteriolar velocity, and diameter were measured in Sprague-Dawley rats before and 30 minutes after intragastric (1 mL 0.5 M, ~0.3 g/kg) dextrose (n = 5), fructose (n = 6), and fructose after intravenous injection of the antioxidant α-lipoic acid (ALA, n = 6). RESULTS: Only fructose increased leukocyte adherence: control 2.3 ± 0.3 per 100 µm;

fructose 9.7 ± 1.4 per 100 µm (P < .001). This effect was independent of changes in venular shear rate: control 269 ± 48 s(-1); fructose 181 ± 27 s(-1) (P > .05, r(2) = 0.083 for shear rate vs leukocyte adherence). Dextrose had no effect on leukocyte adherence: control 1.52 ± 0.13 per 100 µm; dextrose 2.0 ± 0.7 per 100 µm (P > .05). ALA prevented fructose-induced leukocyte adherence: control 1.9 ± 0.2 per 100 µm; fructose + ALA 1.8 ± 0.3 per 100 µm (P > .05). Neither fructose nor dextrose induced PPH: arteriolar velocity: control 3.3 ± 0.49 cm/s, fructose 3.06 ± 0.34 cm/s (P > .05); control 3.3 ± 1.0 cm/s, dextrose 3.15 ± 1.1 cm/s (P > .05); arteriolar diameter: control 19.9 ± 1.10 µm, fructose 19.7 ± 1.0 µm (P > .05); control 21.5 ± 2.6, dextrose 20.0 ± 2.7 µm (P > .05). CONCLUSIONS: Intragastric fructose induced leukocyte adherence via oxidative stress. Neither dextrose nor fructose induced PPH, likely because of the inhibitory effect of anesthesia on splanchnic vasomotor tone. As taken from Mattioli et al. (2011). J. Parenter. Enteral Nutr. 35(2): 223-8, available at http://www.ncbi.nlm.nih.gov/pubmed/21378252?dopt=AbstractPlus

Dietary fructose and hypertension (Abstract). The association between fructose and increased blood pressure is still incompletely defined, because experimental studies have produced dissimilar conclusions. Amplified vasopressor responses to minimal stimuli and differing responses to fructose in peripheral versus central sites may explain the controversy. Fructose induces systemic hypertension through several mechanisms mainly associated with deleterious effects on target organs (kidney, endothelium, heart) exerted by the byproducts of its metabolism, such as uric acid. The kidney is particularly sensitive to the effects of fructose because high loads of this sugar reach renal tissue. In addition, fructose increases reabsorption of salt and water in the small intestine and kidney; thus the combination of salt and fructose has a synergistic effect in the development of hypertension. Clinical and epidemiologic studies have also linked fructose consumption with hypertension. Further studies are warranted in order to understand the role of fructose in the development of hypertension. As taken from Madero et al. (2011). Curr. Hypertens. Rep. 13(1):29-35, available at http://www.ncbi.nlm.nih.gov/pubmed/20957458?dopt=AbstractPlus

Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial (Abstract). Background: Sugar-sweetened beverages (SSBs) have unfavorable effects on glucose and lipid metabolism if consumed in high quantities by obese subjects, but the effect of lower doses in normal-weight subjects is less clear. OBJECTIVE: The aim was to investigate the effects of SSBs consumed in small to moderate quantities for 3 wk on LDL particle distribution and on other parameters of glucose and lipid metabolism as well as on inflammatory markers in healthy young men. Design: Twenty-nine subjects were studied in a prospective, randomized, controlled crossover trial. Six 3-wk interventions were assigned in random order as follows: 600 mL SSBs containing 1)40 g fructose/d [medium fructose (MF)], 2) 80 g fructose/d [high fructose (HF)], 3) 40 g glucose/d [medium glucose (MG)], 4) 80 g glucose/d [high glucose (HG)], 5) 80 g sucrose/d [high sucrose (HS)], or 6) dietary advice to consume low amounts of fructose. Outcome parameters were measured at baseline and after each intervention. Results: LDL particle size was reduced after HF by -0.51 nm (95% CI: -0.19, -0.82 nm) and after HS by -0.43 nm (95% CI: -0.12, -0.74; P < 0.05 for both). Similarly, a more atherogenic LDL subclass distribution was seen when fructose-containing SSBs were consumed (MF, HF, and HS: P < 0.05). Fasting glucose and high-sensitivity C-reactive protein (hs-CRP) increased significantly after all interventions (by 4-9% and 60-109%, respectively; P < 0.05); leptin increased during interventions with SSBs

containing glucose only (MG and HG: P < 0.05). CONCLUSION: The present data show potentially harmful effects of low to moderate consumption of SSBs on markers of cardiovascular risk such as LDL particles, fasting glucose, and hs-CRP within just 3 wk in healthy young men, which is of particular significance for young consumers. This trial was registered at clinicaltrials.gov as NCT01021969. As taken from Aeberli I et al. Am J Clin Nutr. 2011 Aug;94(2):479-85. Epub 2011 Jun 15. Available via PubMed at http://www.ncbi.nlm.nih.gov/pubmed/21677052

Sugar-sweetened beverage link to cardiovascular risk factors is unsupported (no abstract). White JS. Am J Clin Nutr. 2012 Mar;95(3):773; author reply 773-4. http://www.ncbi.nlm.nih.gov/pubmed/22350364?dopt=AbstractPlus

Sugar-sweetened beverage, sugar intake of individuals, and their blood pressure: international study of macro/micronutrients and blood pressure (Abstract). The obesity epidemic has focused attention on relationships of sugars and sugar-sweetened beverages (SSBs) to cardiovascular risk factors. Here we report cross-sectional associations of SSBs, diet beverages, and sugars with blood pressure (BP) for United Kingdom and US participants of the International Study of Macro/Micronutrients and Blood Pressure. Data collected include four 24-hour dietary recalls, two 24-hour urine collections, 8 BP readings, and questionnaire data for 2696 people ages 40 to 59 years of age from 10 US/United Kingdom population samples. Associations of SSBs, diet beverages, and sugars (fructose, glucose, and sucrose) with BP were assessed by multiple linear regression. SSB intake related directly to BP, with P values of 0.005 to <0.001 (systolic BP) and 0.14 to <0.001 (diastolic BP). SSB intake higher by 1 serving per day (355 mL/24 hours) was associated with systolic/diastolic BP differences of +1.6/+0.8 mm Hg (both P<0.001) and +1.1/+0.4 mm Hg (P<0.001/<0.05) with adjustment for weight and height. Diet beverage intake was inversely associated with BP (P 0.41 to 0.003). Fructose- and glucose-BP associations were direct, with significant sugar-sodium interactions: for individuals with above-median 24-hour urinary sodium excretion, fructose intake higher by 2 SD (5.6% kcal) was associated with systolic/diastolic BP differences of +3.4/+2.2 mm Hg (both P<0.001) and +2.5/+1.7 mm Hg (both P=0.002) with adjustment for weight and height. Observed independent, direct associations of SSB intake and BP are consistent with recent trial data. These findings, plus adverse nutrient intakes among SSB consumers, and greater sugar-BP differences for persons with higher sodium excretion lend support to recommendations that intake of SSBs, sugars, and salt be substantially reduced. As taken from Brown IJ et al. Hypertension. 2011 Apr;57(4):695-701. Epub 2011 Feb 28. Available via PubMed at http://www.ncbi.nlm.nih.gov/pubmed/21357284?dopt=AbstractPlus

“BACKGROUND: Although previous studies have linked intake of sugars with incidence of cancer and other chronic diseases, its association with mortality remains unknown. OBJECTIVE: We investigated the association of total sugars, added sugars, total fructose, added fructose, sucrose, and added sucrose with the risk of all-cause, cardiovascular disease, cancer, and other-cause mortality in the NIH-AARP Diet and Health Study. DESIGN: The participants (n = 353,751), aged 50-71 y, were followed for up to 13 y. Intake of individual sugars over the previous 12 mo was assessed at baseline by using a 124-item NIH Diet History Questionnaire. RESULTS: In fully adjusted models (fifth quartile compared with first quartile), all-cause mortality was positively associated with the intake of total sugars [HR (95% CI): 1.13 (1.06, 1.20); P-trend < 0.0001], total fructose [1.10 (1.04, 1.17); P-trend < 0.0001], and added fructose [1.07 (1.01, 1.13); P-trend = 0.005) in women and total fructose [1.06 (1.01, 1.10); P-trend = 0.002] in men. In men, a weak inverse

association was found between other-cause mortality and dietary added sugars (P-trend = 0.04), sucrose (P-trend = 0.03), and added sucrose (P-trend = 0.006). Investigation of consumption of sugars by source showed that the positive association with mortality risk was confined only to sugars from beverages, whereas the inverse association was confined to sugars from solid foods. CONCLUSIONS: In this large prospective study, total fructose intake was weakly positively associated with all-cause mortality in both women and men, whereas added sugars, sucrose, and added sucrose intakes were inversely associated with other-cause mortality in men. In our analyses, intake of added sugars was not associated with an increased risk of mortality. The NIH-AARP Diet and Health Study was registered at clinicaltrials.gov as NCT00340015." As taken from Tasevska N et al. 2014b. Am. J. Clin. Nutr. 99(5), 1077-88. PubMed, 2015 available at http://www.ncbi.nlm.nih.gov/pubmed/24552754

"We recently showed that T regulatory lymphocytes (Treg), which are immune suppressors of inflammatory responses, play a role blunting the development of hypertension-induced injury. Treg are unchanged or decreased in children with metabolic syndrome, and therefore, their role in metabolic syndrome remains unclear. We hypothesized that Treg number or function would be depressed in a high-fructose diet-induced metabolic syndrome-like model in rats. Sprague-Dawley rats were fed normal chow or a high-fructose diet for 5 weeks. The high-fructose diet-induced a 3.8-fold increase in plasma triglycerides and a 14% reduction in high-density lipoprotein cholesterol (P<0.001). The high-fructose diet increased reactive oxygen species in aorta and periaortic adipose tissue 2.8-fold (P<0.05), and reduced nicotinamide adenine dinucleotide phosphate oxidase activity 1.9-fold in aorta, and 2.5-fold in the heart (P<0.05). It also increased plasma nitric oxide metabolite levels 6.4-fold (P<0.001). Western blots showed that the high-fructose diet increased ≥2.3-fold vascular and in platelet endothelial cell adhesion molecule 1 in aorta (P<0.01). It did not affect monocyte/macrophage aortic infiltration but caused a 2.4-fold increase in collagen deposition in the aortic media (P<0.01). No change in plasma interleukin-10 was detected. The percentage of spleen CD4+ CD25- and Treg (CD4+ CD25(high)) cells was unaltered by the high-fructose diet. However, cultured Treg from high-fructose diet-fed rats secreted 62% less interleukin-10 than control cells (P<0.05), suggesting a decreased Treg function, which could play a role in the development of cardiovascular complications of the metabolic syndrome." As taken from Leibowitz A et al. 2013. Hypertension 61(6), 1316-21. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23529169

"This review article endeavoured to ascertain the facts about fructose, a mono-saccharide sugar being increasingly used in daily diet. Human and animal studies suggest that fructose ingestion produces deleterious effects on the cardiovascular system, such as increase in blood pressure and also has adverse metabolic effects, for example insulin resistance and hypertriglyceridaemia. On the other hand it has also been determined thatfructose, especially that is contained in natural sources as apples, dates and honey may produce beneficial effects on human health; smaller amounts of fructose may even help improve diabetic control and that adequate seminal fructose concentrations help promote male fertility. In conclusion, fructose appears to be not all that bad; is essential for male fertility, small amounts of fructose, specifically which comes from dietary sources appears to be beneficial for health." As taken from Memon MQ & Kumar A. 2013. Pak. J. Pharm. Sci. 26(6), 1241-5. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24191333?dopt=AbstractPlus

“BACKGROUND: High ambient levels of ozone (O3) and fine particulate matter (PM2.5) are associated with cardiovascular morbidity and mortality, especially in people with preexisting cardiopulmonary diseases. Enhanced susceptibility to the toxicity of air pollutants may include individuals with metabolic syndrome (MetS). OBJECTIVE: We tested the hypothesis that cardiovascular responses to O3 and PM2.5 will be enhanced in rats with diet-induced MetS. METHODS: Male Sprague-Dawley rats were fed a high-fructose diet (HFrD) to induce MetS and then exposed to O3, concentrated ambient PM2.5, or the combination of O3 plus PM2.5 for 9 days. Data related to heart rate (HR), HR variability (HRV), and blood pressure (BP) were collected. RESULTS: Consistent with MetS, HFrD rats were hypertensive and insulin resistant, and had elevated fasting levels of blood glucose and triglycerides. Decreases in HR and BP, which were found in all exposure groups, were greater and more persistent in HFrD rats compared with those fed a normal diet (ND). Coexposure to O3 plus PM2.5 induced acute drops in HR and BP in all rats, but only ND rats adapted after 2 days. HFrD rats had little exposure-related changes in HRV, whereas ND rats had increased HRV during O3 exposure, modest decreases with PM2.5, and dramatic decreases during O3 plus PM2.5 coexposures. CONCLUSIONS: Cardiovascular depression in O3- and PM2.5-exposed rats was enhanced and prolonged in rats with HFrD-induced MetS. These results in rodents suggest that people with MetS may be prone to similar exaggerated BP and HR responses to inhaled air pollutants.” As taken from Wagner JG et al. 2014. Environ. Health Perspect. 122(1), 27-33. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24169565

“The study evaluates the effects of genistein on blood pressure (BP) and ultrastructural changes in kidney of fructose-fed hypertensive rats. Male Wistar rats were fed a diet containing 60 % starch or 60 % fructose as the source of carbohydrate. After 15 d, rats in each dietary group were divided into two groups and were treated with either genistein (1 mg/kg per d) in dimethylsulfoxide (DMSO) or 30 % DMSO alone. BP, pressor mechanisms, protein kinase C-βII (PKC-βII) expression, endothelial NO synthase (eNOS) expression and renal ultrastructural changes were evaluated after 60 d.Fructose-fed rats displayed significant elevation in BP and heart rate. Significant increase in plasma angiotensin-converting enzyme (ACE) activity, alterations in renal lipid profile, nitrite and kallikrein activity, enhanced expression of membrane-associated PKC-βII and decreased expression of eNOS were observed in them. Histology and electron microscopic studies showed structural changes in the kidney….” As taken from Palanisamy N & Venkataraman AC. 2013. Br. J. Nutr. 109(10), 1806-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23116847

“Evidence that fructose intake may modify blood pressure is generally limited to adult populations. This study examined cross-sectional associations between dietary intake of fructose, serum uric acid and blood pressure in 814 adolescents aged 13-15 years participating in the Western Australian Pregnancy Cohort (Raine) Study. Energy-adjusted fructose intake was derived from 3-day food records, serum uric acid concentration was assessed using fasting blood and resting blood pressure was determined using repeated oscillometric readings. In multivariate linear regression models, we did not see a significant association between fructose and blood pressure in boys or girls. In boys, fructose intake was independently associated with serum uric acid ($P<0.01$), and serum uric acid was independently associated with systolic blood pressure ($P<0.01$) and mean arterial pressure ($P<0.001$). Although there are independent associations, there is no direct relationship between fructose intake and blood pressure. Our data suggest that gender may influence these relationships in adolescence, with significant associations observed more frequently

in boys than girls." As taken from Bobridge KS et al. 2013. J. Hum. Hypertens. 27(4), 217-24. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22971754?dopt=AbstractPlus

"AIM: To determine the therapeutic effects of adrenomedullin (ADM) on vascular calcification and related molecular mechanism in fructose-induced insulin resistance rats. METHODS: Rats received ordinary drinking water or 10% fructose in drinking water for 12 weeks and subcutaneous injection of normal saline or ADM (3.6 μg kg(-1)) twice a day for the last 4 weeks. Levels of ADM, calcitonin receptor-like receptors (CRLR), receptor activity-modifying proteins (RAMP) as well as calcium content, alkaline phosphatase (ALP) activity, osteoblastic and contractile smooth muscle markers in aortic media were measured. RESULTS: The levels of ADM, CRLR, RAMP2 and RAMP3 in aortic media were increased in fructose-fed rats. ADM treatment attenuated thefructose-induced insulin resistance, increased blood pressure, fasting glucose, insulin, triglycerides and cholesterol levels. It improved VSMCs proliferation and disordered arrangement and hyperplasia of elastic fibres in fructose-fed rats. Calcium deposits, calcium content and ALP activity in the aortic media were increased in fructose-fed rats, which were attenuated by ADM treatment. The osteoblastic markers such as osteopontin (OPN), bone morphogenetic protein 2 (BMP2) proteins and core binding factor alpha-1 (Cbfα-1) protein and mRNA expressions were increased in fructose-fed rats. ADM treatment increased the OPN protein expression, but reduced the BMP2 protein, Cbfα-1 protein and mRNA expression. Contractile smooth muscle markers such as α-actin and smooth muscle 22α (SM-22α) were downregulated in fructose-fed rats, which were recovered by ADM treatment. CONCLUSION: Administration of ADM attenuates insulin resistance, calcium deposition and osteogenic transdifferentiation in aortic media infructose-fed rats." As taken from Zhou YB et al. 2013. Acta Physiol. (Oxf.) 207(3), 437-46. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23121999?dopt=AbstractPlus

"Fructose-rich diets (FRD) cause cardiac insulin resistance manifested by impairment of Akt/endothelial NO synthase (eNOS) signalling. In contrast, oestradiol (E2) activates this signalling pathway in the heart. To study the ability of E2 to revert the detrimental effect of fructose on cardiac Akt/eNOS, female rats were subjected to a FRD and ovariectomy followed with or without E2 replacement. We also analysed the effects of the FRD and E2 on cardiac extracellular signal-regulated kinase (Erk 1/2) signalling related to their role in cardiac hypertrophy development. Expression of Akt, eNOS and Erk 1/2, as well as regulatory phosphorylations of these molecules were determined. The protein expression of cardiac Akt and eNOS was not affected by the diet or E2 treatment. However, the FRD was accompanied by a decrease in Akt phosphorylation at Ser(473) and Thr(308), and eNOS at Ser(1177), while the phosphorylation of eNOS at Thr(495) was increased. E2 replacement in ovariectomised fructose-fed rats caused a reversion of the diet effect on Akt and eNOS serine phosphorylation, but mostly had no effect on threonine phosphorylation of the molecules. The FRD and E2 treatment did not influence Erk 1/2 expression and phosphorylation and heart mass as well. The data show that E2 selectively suppress the negative effects of a FRD on Akt/eNOS signalling and probably point to the different effects of E2 on kinase/phosphatase pathways responsible for phosphorylation/dephosphorylation of Akt and eNOS. Furthermore, the results suggest that the heart of females in the reproductive period is partially protected against the damaging effects of increased fructose intake." As taken from Romic S et al. 2013. Br. J. Nutr. 109(11), 1940-8. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23069112?dopt=AbstractPlus

“Excessive fructose intake from high-fructose corn syrup (HFCS) and sucrose has been implicated as a driving force behind the increasing prevalence of obesity and its downstream cardiometabolic complications including hypertension, gout, dyslidpidemia, metabolic syndrome, diabetes, and non-alcoholic fatty liver disease (NAFLD). Most of the evidence to support these relationships draws heavily on ecological studies, animal models, and select human trials of fructose overfeeding. There are a number of biological mechanisms derived from animal models to explain these relationships, including increases in de novo lipogenesis and uric acid-mediated hypertension. Differences between animal and human physiology, along with the supraphysiologic level at which fructose is fed in these models, limit their translation to humans. Although higher level evidence from large prospective cohorts studies has shown significant positive associations comparing the highest with the lowest levels of intake of sugar-sweetened beverages (SSBs), these associations do not hold true at moderate levels of intake or when modeling total sugars and are subject to collinearity effects from related dietary and lifestyle factors. The highest level of evidence from controlled feeding trials has shown a lack of cardiometabolic harm of fructose and SSBs under energy-matched conditions at moderate levels of intake. It is only when fructose-containing sugars or SSBs are consumed at high doses or supplement diets with excess energy that a consistent signal for harm is seen. The available evidence suggests that confounding by excess energy is an important consideration in assessing the role of fructose-containing sugars and SSBs in the epidemics of hypertension and other cardiometabolic diseases.” As taken from Ha V et al. 2013. Curr. Hypertens. Rep. 15(4), 281-97. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23793849

“AIMS: High fructose levels are found in diabetes mellitus, associated with high corn syrup diets, and have been claimed to cause hypertension. As the direct effects on conduit and resistance arteries have not been previously reported, we measured these in vivo in the anaesthetized pig with instrumented iliac arteries. METHODS: Experiments were performed on the iliac artery preparation in the anaesthetized pig: blood flow, diameter and pressure were measured in the iliac. RESULTS: The change in diameter of an occluded iliac artery segment filled with hyperfructosemic (15 μm) blood was 89.5 ± 22.1 μm (mean ± SE), contrasted with 7.7 ± 13.06 μm control (P = 0.005, paired t-test, n = 6). There was no significant difference when compared with blood containing both hyperfructosemic blood and the nitric oxide synthesis inhibitor, N(G)-nitro-l-arginine methyl ester (250 μg mL(-1)). Step changes in pressure and flow were achieved by progressive arterial stenosis during control saline and 15 μm min(-1) fructose downstream intra-arterial infusions. Linear regression of the step changes in blood pressure versus the instantaneous step changes in blood flow showed a statistically significant decrease in slope of the conductance (P < 0.001, analysis of covariance), indicating an increase in instantaneous peripheral vascular resistance. Peripheral autoregulation and conduit artery shear-stress-mediated dilatation were not significantly altered. CONCLUSION: An elevated level of fructose caused dilatation of a conduit artery but constriction of resistance vessels. The latter effect could account, if maintained long-term, for the hypertension claimed to be due to hyperfuctosemia.” As taken from Ruane-O’Hara T et al. 2013. Acta Physiol. (Oxf.) 209(4), 254-61. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24102866

“The current epidemic of obesity and type 2 diabetes is attributed to a high carbohydrate diet, containing mainly high fructose corn syrup and sucrose. More than two thirds of diabetic patients have hypertension. Methylglyoxal is a highly reactive dicarbonyl generated during glucose and fructose metabolism, and a major precursor of advanced glycation end products (AGEs). Plasma methylglyoxal levels are increased in hypertensive rats and

diabetic patients. Our aim was to examine the levels of methylglyoxal, mediators of the renin angiotensin system and blood pressure in male Sprague-Dawley rats treated with a high fructose diet (60% of total calories) for 4 months. The thoracic aorta and kidney were used for molecular studies, along with cultured vascular smooth muscle cells (VSMCs). HPLC, Western blotting and Q-PCR were used to measure methylglyoxal and reduced glutathione (GSH), proteins and mRNA, respectively. Fructose treated rats developed a significant increase in blood pressure. Methylglyoxal level and protein and mRNA for angiotensin II, AT1 receptor, adrenergic α1D receptor and renin were significantly increased, whereas GSH levels were decreased, in the aorta and/or kidney of fructose fed rats. The protein expression of the receptor for AGEs (RAGE) and NF-κB were also significantly increased in the aorta of fructose fed rats. MG treated VSMCs showed increased protein for angiotensin II, AT1 receptor, and α1D receptor. The effects of methylglyoxal were attenuated by metformin, a methylglyoxal scavenger and AGEs inhibitor. In conclusion, we report a strong association between elevated levels of methylglyoxal, RAGE, NF-κB, mediators of the renin angiotensin system and blood pressure in high fructose diet fed rats." As taken from Dhar I et al. 2013. PLoS One. 8(9), e74212. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24040205

"In the current study, effects of acute short term administration of fructose on cardiac arrhythmias and myocardial infarction size following ischemia/reperfusion were investigated in isolated rat heart. The hearts were subjected to 30 min zero flow global ischemia followed by 120 min reperfusion. In the control group, the hearts were perfused by normal drug free Krebs-Henseleit (K/H) solution throughout the experiments, while in the treated groups (2-4), they were perfused with fructose containing K/H solution at 12, 24 and 48 mM concentrations during stabilization and reperfusion time, respectively. Cardiac arrhythmias were determined based on the Lambeth conventions and the infarct size was measured by computerized planimetry. Myocardial infarction size was 22 ± 3% in the control group, however administration of fructose (12, 24 and 48 mM) reduced it to 15 ± 3 ($P<0.05$), 7±2 ($P<0.001$) and 4 ± 2% ($P<0.001$), respectively. A direct linear correlation between fructose concentrations and infarction size reduction was observed ($R(2)=0.970$). In addition, total number of ventricular ectopic beats were significantly decreased by all used concentrations of fructose ($P<0.01$ for group 2, $P<0.001$ for groups 3 and 4, respectively). Fructose also produced significant decrease in the number, incidence and duration of ventricular tachycardia compared to the control ($P<0.05$). The data showed that acute short term administration of fructose can protect isolated rat heart against ischemia/reperfusion injuries as reduction of infarct size and cardiac arrhythmias. Alterations in glycogen storage and/or glycolytic efficiency may probably involve in these cardioprotective effects. Also it is possible that fructose can act as a pharmacological preconditioning agent." As taken from Haghighat Azari M & Najafi M 2014. Iran. J. Pharm. Res. 13(4), 1303-11. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25587319

"The impact of fructose, commonly consumed with sugars by humans, on blood pressure and uric acid has yet to be defined. A total of 267 weight-stable participants drank sugar-sweetened milk every day for 10 weeks as part of their usual, mixed-nutrient diet. Groups 1 and 2 had 9% estimated caloric intake from fructose or glucose, respectively, added to milk. Groups 3 and 4 had 18% of estimated caloric intake from high fructose corn syrup or sucrose, respectively, added to the milk. Blood pressure and uric acid were determined prior to and after the 10-week intervention. There was no effect of sugar type on either blood pressure or uric acid (interaction $P>.05$), and a significant time effect for blood pressure was noted ($P<.05$). The authors conclude that 10 weeks of consumption of

fructose at the 50th percentile level, whether consumed as pure fructose or with fructose-glucose-containing sugars, does not promote hyperuricemia or increase blood pressure." As taken from Angelopoulos TJ et al. 2015. J. Clin. Hypertens. (Greenwich) 17(2), 87-94. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25496265

"BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is a common liver disease in obese children. Diets high in added fructose (high fructose corn syrup; HFCS) and glycemic index (GI)/glycemic load (GL) are associated with increased risk of NAFLD. Lifestyle modification is the main treatment, but no guidelines regarding specific dietary interventions for childhood NAFLD exist. We hypothesized that reductions in dietary fructose (total, free, and HFCS)/GI/GL over 6 months would result in improvements in body composition and markers of liver dysfunction and cardiometabolic risk in childhood NAFLD. METHODS: Children and adolescents with NAFLD (n = 12) and healthy controls (n = 14) 7-18 years were studied at baseline and 3 and 6 months post-dietary intervention. Plasma markers of liver dysfunction (ALT, AST, γGT), cardiometabolic risk (TG, total cholesterol, LDL-HDL cholesterol, Apo-B100, Apo-B48, Apo-CIII, insulin, homeostasis model of assessment of insulin resistance [HOMA-IR]), inflammation (TNF-α, IL-6, IL-10), anthropometric, and blood pressure (BP) were studied using validated methodologies. RESULTS: Significant reductions in systolic BP (SBP), percentage body fat (BF), and plasma concentrations of ALT (P = .04), Apo-B100 (P < .001), and HOMA-IR were observed in children with NAFLD at 3 and 6 months (P < .05). Dietary reductions in total/free fructose/HFCS and GL were related to reductions in SBP (P = .01), ALT (P = .004), HOMA-IR (P = .03), and percentage BF in children with NAFLD. Reductions in dietary GI were associated with reduced plasma Apo-B100 (P = .02) in both groups. With the exception of Apo-B100, no changes in laboratory variables were observed in the control group. CONCLUSION: Modest reductions in fructose (total/free, HFCS) and GI/GL intake result in improvements of plasma markers of liver dysfunction and cardiometabolic risk in childhood NAFLD." As taken from Mager DR et al. 2015. JPEN J. Parenter. Enteral. Nutr. 39(1), 73-84. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/23976771

"OBJECTIVE: The aim of this study was to review the current corpus of human studies to determine the association of various doses and durations of fructose consumption on metabolic syndrome. METHODS: We searched human studies in PubMed, Scopus, Ovid, ISI Web of Science, Cochrane library, and Google Scholar databases. We searched for the following keywords in each paper: metabolic syndrome x, insulin resistance, blood glucose, blood sugar, fasting blood sugar, triglycerides, lipoproteins, HDL, cholesterol, LDL, blood pressure, mean arterial pressure, systolic blood pressure, diastolic blood pressure, hypertens*, waist circumference, and fructose, sucrose, high-fructose corn syrup, or sugar. RESULTS: Overall, 3102 articles were gathered. We excluded studies on natural fructose content of foods, non-clinical trials, and trials in which fructose was recommended exclusively as sucrose or high-fructose corn syrup. Overall, 3069 articles were excluded. After review by independent reviewers, 15 studies were included in the meta-analysis. Fructose consumption was positively associated with increased fasting blood sugar (FBS; summary mean difference, 0.307; 95% confidence interval [CI], 0.149-0.465; P = 0.002), elevated triglycerides (TG; 0.275; 95% CI, 0.014-0.408; P = 0.002); and elevated systolic blood pressure (SBP; 0.297; 95% CI, 0.144-0.451; P = 0.002). The corresponding figure was inverse for high-density lipoprotein (HDL) cholesterol (-0.267; 95% CI, -0.406 to -0.128; P = 0.001). Significant heterogeneity existed between studies, except for FBS. After excluding studies that led to the highest effect on the heterogeneity test, the association between fructose consumption and TG, SBP, and HDL became non-significant. The results

did not show any evidence of publication bias. No missing studies were identified with the trim-and-n¼üll method. CONCLUSION: Fructose consumption from industrialized foods has significant effects on most components of metabolic syndrome." As taken from Kelishadi R et al. 2014. Nutrition 30(5), 503-10. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24698343?dopt=AbstractPlus

"OBJECTIVES: Although most controlled feeding trials have failed to show an adverse effect of fructose on blood pressure, concerns continue to be raised regarding the role of fructose in hypertension. To quantify the association between fructose-containing sugar (high-fructose corn syrup, sucrose, and fructose) intake and incident hypertension, a systematic review and meta-analysis of prospective cohort studies was undertaken. METHODS: MEDLINE, EMBASE, CINAHL and the Cochrane Library (through February 5, 2014) were searched for relevant studies. Two independent reviewers reviewed and extracted relevant data. Risk estimates were aggregated comparing the lowest (reference) quintile with highest quintile of intake using inverse variance random effect models and expressed as risk ratios (RR) with 95% confidence intervals (CIs). Interstudy heterogeneity was assessed (Cochran Q statistic) and quantified (I(2) statistic). The Newcastle-Ottawa Scale assessed study quality. Clinicaltrials.gov NCT01608620. RESULTS: Eligibility criteria were met by 3 prospective cohorts (n = 37,375 men and 185,855 women) with 58,162 cases of hypertension observed over 2,502,357 person-years of follow-up. Median fructose intake was 5.7-6.0% total energy in the lowest quintile and 13.9-14.3% total energy in the highest quintile. Fructose intake was not associated with incident hypertension (RR = 1.02, 95% CI, 0.99-1.04), with no evidence of heterogeneity (I(2) = 0%, p = 0.59). Spline curve modeling showed a U-shaped relationship with a negative association at intakes ≤50th percentile (~10% total energy) and a positive association at higher intakes. CONCLUSIONS: Total fructose intake was not associated with an increased risk of hypertension in 3 large prospective cohorts of U.S. men and women." As taken from Jayalath VH et al. 2014. J. Am. Coll. Nutr. 33(4), 328-39. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25144126

"This study investigates the possible protective effects of levocetirizine against fructose-induced insulin resistance, hepatic steatosis and vascular dysfunction, in comparison to pioglitazone, a standard insulin sensitizer. Male Sprague Dawley rats (150-200 g) were divided into 4 groups. Three groups were fed on high fructose diets (HFD) containing 60% w/w fructose, while the fourth control group was fed on standard laboratory food for 8 weeks. AUCOGTT, AUCITT, fasting glucose, HOMA-IR, hepatic glutathione (GSH) and malondialdehyde (MDA) levels, serum total cholesterol, LDL-C, C-reactive protein (CRP) level and lactate dehydrogenase (LDH) activity and liver steatosis scores were significantly higher in HFD group compared to control group. Moreover, body weight gain, food intake, feeding efficiency, HOMA-β, Emax and pEC50 of acetylcholine-induced relaxations of aortic rings and hepatic superoxide dismutase (SOD) activity were significantly lower in HFD group than in control group. Treatment with levocetirizine caused significant decreases in AUCOGTT, AUCITT, HOMA-IR, hepatic GSH and MDA levels and serum CRP level and LDH activity and significant increases in hepatic SOD activity and HOMA-β when compared with the HFD group. Although levocetirizine failed to alter TC and LDL-C levels, it produced a significant increase in HDL-C level relative to control group. Levocetirizine was also able to improve acetylcholine-induced relaxations of aortic rings, indicating a protective effect against insulin resistance-induced endothelial damage comparable to that offered by pioglitazone. Moreover, levocetirizine substantially attenuated insulin resistance-associated liver macrovesicular steatosis. These findings demonstrate that levocetirizine ameliorates

insulin resistance, improves glucose tolerance and attenuates insulin resistance-linked hepatic steatosis and vascular damage." As taken from Shawkey NM et al. 2014. Eur. J. Pharmacol. 740, 353-63. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25064340

"OBJECTIVES: The objective of this study is to develop an experimental model of hyperlipidemia and insulin resistance (IR), markers of coronary heart disease (CHD) using high fat and high sugar (HFHS) diet and to evaluate the efficacy of the model using atorvastatin, a known antihyperlipidemic drug, pioglitazone, a known insulin sensitizer, and Tinospora cordifolia (Tc), an antidiabetic plant. MATERIALS AND METHODS: Following Institutional Animal Ethics Committee permission, the study was conducted in male Wistar rats (200-270 g). The model was developed using a high fat (vanaspati ghee: coconut oil, 3:1) oral diet along with 25% fructose (high sugar) added in drinking water over a period of 6 weeks. Atorvastatin (2.1 mg/kg/day), pioglitazone (2.7 mg/kg/day) and Tc (200 mg/kg/day) were administered 3 weeks after initiation of HFHS diet and continued for another 3 weeks. Parameters assessed were weight, lipid profile, fasting blood glucose, insulin, and gastric emptying. Serum malondialdehyde (MDA) and catalase were assessed as markers of oxidative stress. RESULTS: Administration of HFHS diet demonstrated a significant increase in blood glucose, insulin, total and low density lipoprotein cholesterol and triglycerides with a decrease in high density lipoprotein cholesterol. Treatment with test drugs decreased blood sugar, insulin, lipid parameters, increased gastric emptying rate, decreased MDA levels, and catalase activity when compared to HFHS diet group, confirming the efficacy of the model. Atherogenic index of all the test drugs (0.48, 0.57, and 0.53) was significantly lower as compared to HFHS diet group (1.107). CONCLUSION: This study confirms the development of a diet based cost-effective and time efficient experimental model, which can be used to study two important markers of cardiovascular disease that is, hyperlipidemia and IR and to explore the efficacy of new molecules in CHD." As taken from Munshi RP et al. 2014. Indian J. Pharmacol. 46(3), 270-6. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24987172

"The aim of this study was to evaluate the role of α-lipoic acid (α-LA) on oxidative damage and inflammation that occur in endothelium of aorta and heart while constant consumption of high-fructose corn syrup (HFCS). The rats were randomly divided into three groups with each group containing eight rats. The groups include HFCS, HFCS + α-LA treatment, and control. HFCS was given to the rats at a ratio of 30% of F30 corn syrup in drinking water for 10 weeks. α-LA treatment was given to the rats at a dose of 100 mg/kg/day orally for the last 6 weeks. At the end of the experiment, the rats were killed by cervical dislocation. The blood samples were collected for biochemical studies, and the aortic and cardiac tissues were collected for evaluation of oxidant-antioxidant system, tissue bath, and pathological examination. HFCS had increased the levels of malondialdehyde, creatine kinase MB, lactate dehydrogenase, and uric acid and showed significant structural changes in the heart of the rats by histopathology. Those changes were improved by α-LA treatment as it was found in this treatment group. Immunohistochemical expressions of tumor necrosis factor α and inducible nitric oxide synthase were increased in HFCS group, and these receptor levels were decreased by α-LA treatment. All the tissue bath studies supported these findings. Chronic consumption of HFCS caused several problems like cardiac and endothelial injury of aorta by hyperuricemia and induced oxidative stress and inflammation. α-LA treatment reduced uric acid levels, oxidative stress, and corrected vascular responses. α-LA can be added to cardiac drugs due to its cardiovascular protective effects against the

cardiovascular diseases." As taken from Saygin M et al. 2016. Hum. Exp. Toxicol. 35, 194-204. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25825413

"BACKGROUND: Debate over the role of fructose in mediating cardiovascular risk remains active. To update the evidence on the effect of fructose on established therapeutic lipid targets for cardiovascular disease (low-density lipoprotein cholesterol [LDL]-C, apolipoprotein B, non-high-density lipoprotein cholesterol [HDL-C]), and metabolic syndrome (triglycerides and HDL-C), we conducted a systematic review and meta-analysis of controlled feeding trials. METHODS AND RESULTS: MEDLINE, EMBASE, CINHAL, and the Cochrane Library were searched through July 7, 2015 for controlled feeding trials with follow-up ≥7 days, which investigated the effect of oral fructose compared to a control carbohydrate on lipids (LDL-C, apolipoprotein B, non-HDL-C, triglycerides, and HDL-C) in participants of all health backgrounds. Two independent reviewers extracted relevant data. Data were pooled using random effects models and expressed as mean difference with 95% CI. Interstudy heterogeneity was assessed (Cochran Q statistic) and quantified (I(2) statistic). Eligibility criteria were met by 51 isocaloric trials (n=943), in which fructose was provided in isocaloric exchange for other carbohydrates, and 8 hypercaloric trials (n=125), in which fructose supplemented control diets with excess calories compared to the control diets alone without the excess calories. Fructose had no effect on LDL-C, non-HDL-C, apolipoprotein B, triglycerides, or HDL-C in isocaloric trials. However, in hypercaloric trials, fructose increased apolipoprotein B (n=2 trials; mean difference = 0.18 mmol/L; 95% CI: 0.05, 0.30; P=0.005) and triglycerides (n=8 trials; mean difference = 0.26 mmol/L; 95% CI: 0.11, 0.41; P<0.001). The study is limited by small sample sizes, limited follow-up, and low quality scores of the included trials. CONCLUSIONS: Pooled analyses showed that fructose only had an adverse effect on established lipid targets when added to existing diets so as to provide excess calories (+21% to 35% energy). When isocalorically exchanged for other carbohydrates, fructose had no adverse effects on blood lipids. More trials that are larger, longer, and higher quality are required." As taken from Chiavaroli L et al. 2015. J. Am. Heart Assoc. 4, e001700. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26358358

"Currently, the metabolic syndrome (MS) is occurring at growing rates worldwide, raising extensive concerns on the mechanisms and therapeutic interventions for this disorder. Herein, we described a novel method of establishing MS model in rodents. Male Institute of Cancer Research (ICR) mice were fed with high-fat-high-fructose (HFHF) diet or normal chow (NC) respectively for 12 weeks. Metabolic phenotypes were assessed by glucose tolerance test, insulin tolerance test and hyperinsulinemic-euglycemic clamp. Blood pressure was measured by a tail-cuff system. At the end of the experiment, mice were sacrificed, and blood and tissues were harvested for subsequent analysis. Serum insulin levels were measured by ELISA, and lipid profiles were determined biochemically. The HFHF diet-fed ICR mice exhibited obvious characteristics of the components of MS, including obvious obesity, severe insulin resistance, hyperinsulinemia, dislipidemia, significant hypertension and hyperuricemia. Our data suggest that HFHF diet-fed ICR mice may be a robust and efficient animal model that could well mimic the basic pathogenesis of human MS." As taken from Zhuhua Z et al. 2015. Exp. Anim. 64, 435-42. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26134356

"AIM: To investigate the effects of fructose consumption on the antioxidant capacity in heart and kidney, cardiovascular disease risk factors, and evaluation of these variables after its

removal. METHODS: Male Wistar rats (n = 36) were divided into control group (n = 12): standard chow + water or F group: standard chow + fructose in drinking water (30%) for 15 weeks. After, F group was divided to continue receiving standard chow + fructose in drinking water (30%) (n = 12) or standard chow + water (Ex group, n = 12) for 9 weeks. Water, chow and caloric diaries intake, final body weight, adiposity index, plasma glucose and triacylglycerol, systolic blood pressure, and cardiac and renal hydrophilic antioxidant capacity were analyzed. RESULTS: Control and Ex groups consumed less chow and water compared to F group. Caloric intake was higher in control group. There was no difference in final body weight and adiposity index. Systolic blood pressure and cardiac and renal hydrophilic antioxidant capacity were worst in F group. CONCLUSION: Prolonged exposure to fructose induces oxidative stress, systolic blood pressure, and increase in triacylglycerol. When stopped fructose consumption, Ex group presented improvement in these variables, suggesting the toxicity effect of fructose when consumed in high amounts and prolonged exposure." As taken from Francisqueti FV et al. 2016. SAGE Open Med. 4, 2050312116684294. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28348741

"Insulin resistance is associated with accelerated atherosclerosis. Although high fructose is known to induce insulin resistance, it remains unclear as to how fructose regulates insulin receptor signaling and proliferative phenotype in vascular smooth muscle cells (VSMCs), which play a major role in atherosclerosis. Using human aortic VSMCs, we investigated the effects of high fructose treatment on insulin receptor substrate-1 (IRS-1) serine phosphorylation, insulin versus platelet-derived growth factor (PDGF)-induced phosphorylation of Akt, S6 ribosomal protein, and extracellular signal-regulated kinase (ERK), and cell cycle proteins. In comparison with PDGF (a potent mitogen), neither fructose nor insulin enhanced VSMC proliferation and cyclin D1 expression. d-[14C(U)]fructose uptake studies revealed a progressive increase in fructose uptake in a time-dependent manner. Concentration-dependent studies with high fructose (5-25mM) showed marked increases in IRS-1 serine phosphorylation, a key adapter protein in insulin receptor signaling. Accordingly, high fructose treatment led to significant diminutions in insulin-induced phosphorylation of downstream signaling components including Akt and S6. In addition, high fructose significantly diminished insulin-induced ERK phosphorylation. Nevertheless, high fructose did not affect PDGF-induced key proliferative signaling events including phosphorylation of Akt, S6, and ERK and expression of cyclin D1 protein. Together, high fructose dysregulates IRS-1 phosphorylation state and proximal insulin receptor signaling in VSMCs, but does not affect PDGF-induced proliferative signaling. These findings suggest that systemic insulin resistance rather than VSMC-specific dysregulation of insulin receptor signaling by high fructose may play a major role in enhancing atherosclerosis and neointimal hyperplasia." As taken from Osman I et al. 2016. Eur. J. Pharmacol. 791, 703-710. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27729247

"It has become increasingly clear that maternal nutrition can strongly influence the susceptibility of adult offspring to cardiometabolic disease. For decades, it has been thought that excessive intake of fructose, such as sugar-sweetened beverages and foods, has been linked to increased risk of obesity, type 2 diabetes, and cardiovascular disease in various populations. These deleterious effects of excess fructose consumption in adults are well researched, but limited data are available on the long-term effects of high fructose exposure during gestation, lactation, and infancy. This review aims to examine the evidence linking early life fructose exposure during critical periods of development and its implications for

long-term cardiometabolic health in offspring." As taken from Zheng J et al. 2016. Nutrients 8(11), E685. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27809266

"The objective of the current study was to explore our hypothesis that average consumption of fructose and fructose containing sugars would not increase risk factors for cardiovascular disease (CVD) and the metabolic syndrome (MetS). A randomized, double blind, parallel group study was conducted where 267 individuals with BMI between 23 and 35 kg/m² consumed low fat sugar sweetened milk, daily for ten weeks as part of usual weight-maintenance diet. One group consumed 18% of calories from high fructose corn syrup (HFCS), another group consumed 18% of calories from sucrose, a third group consumed 9% of calories from fructose, and the fourth group consumed 9% of calories from glucose. There was a small change in waist circumference (80.9 ± 9.5 vs. 81.5 ± 9.5 cm) in the entire cohort, as well as in total cholesterol (4.6 ± 1.0 vs. 4.7 ± 1.0 mmol/L, $p < 0.01$), triglycerides (TGs) (11.5 ± 6.4 vs. 12.6 ± 8.9 mmol/L, $p < 0.01$), and systolic (109.2 ± 10.2 vs. 106.1 ± 10.4 mmHg, $p < 0.01$) and diastolic blood pressure (69.8 ± 8.7 vs. 68.1 ± 9.7 mmHg, $p < 0.01$). The effects of commonly consumed sugars on components of the MetS and CVD risk factors are minimal, mixed and not clinically significant." As taken from Angelopoulos TJ et al. 2016. Nutrients 8(4), 179. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27023594

"Dietary guidelines continue to recommend restricting intake of saturated fats. This recommendation follows largely from the observation that saturated fats can raise levels of total serum cholesterol (TC), thereby putatively increasing the risk of atherosclerotic coronary heart disease (CHD). However, TC is only modestly associated with CHD, and more important than the total level of cholesterol in the blood may be the number and size of low-density lipoprotein (LDL) particles that contain it. As for saturated fats, these fats are a diverse class of compounds; different fats may have different effects on LDL and on broader CHD risk based on the specific saturated fatty acids (SFAs) they contain. Importantly, though, people eat foods, not isolated fatty acids. Some food sources of SFAs may pose no risk for CHD or possibly even be protective. Advice to reduce saturated fat in the diet without regard to nuances about LDL, SFAs, or dietary sources could actually increase people's risk of CHD. When saturated fats are replaced with refined carbohydrates, and specifically with added sugars (like sucrose or high fructose corn syrup), the end result is not favorable for heart health. Such replacement leads to changes in LDL, high-density lipoprotein (HDL), and triglycerides that may increase the risk of CHD. Additionally, diets high in sugar may induce many other abnormalities associated with elevated CHD risk, including elevated levels of glucose, insulin, and uric acid, impaired glucose tolerance, insulin and leptin resistance, non-alcoholic fatty liver disease, and altered platelet function. A diet high in added sugars has been found to cause a 3-fold increased risk of death due to cardiovascular disease, but sugars, like saturated fats, are a diverse class of compounds. The monosaccharide, fructose, and fructose-containing sweeteners (e.g., sucrose) produce greater degrees of metabolic abnormalities than does glucose (either isolated as a monomer, or in chains as starch) and may present greater risk of CHD. This paper reviews the evidence linking saturated fats and sugars to CHD, and concludes that the latter is more of a problem than the former. Dietary guidelines should shift focus away from reducing saturated fat, and from replacing saturated fat with carbohydrates, specifically when these carbohydrates are refined. To reduce the burden of CHD, guidelines should focus particularly on reducing intake of concentrated sugars, specifically the fructose-containing sugars like sucrose and high-fructose corn syrup in the form of ultra-processed foods and beverages." As taken from DiNicolantonio JJ et al. 2016. Prog.

Cardiovasc. Dis. 58(5), 464-72. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26586275

“The impact of sugar consumption on health continues to be a controversial topic. The objective of this review is to discuss the evidence and lack of evidence that allows the controversy to continue, and why resolution of the controversy is important. There are plausible mechanisms and research evidence that supports the suggestion that consumption of excess sugar promotes the development of cardiovascular disease (CVD) and type 2 diabetes (T2DM) both directly and indirectly. The direct pathway involves the unregulated hepatic uptake and metabolism of fructose, leading to liver lipid accumulation, dyslipidemia, decreased insulin sensitivity and increased uric acid levels. The epidemiological data suggest that these direct effects of fructose are pertinent to the consumption of the fructose-containing sugars, sucrose and high fructose corn syrup (HFCS), which are the predominant added sugars. Consumption of added sugar is associated with development and/or prevalence of fatty liver, dyslipidemia, insulin resistance, hyperuricemia, CVD and T2DM, often independent of body weight gain or total energy intake. There are diet intervention studies in which human subjects exhibited increased circulating lipids and decreased insulin sensitivity when consuming high sugar compared with control diets. Most recently, our group has reported that supplementing the ad libitum diets of young adults with beverages containing 0%, 10%, 17.5% or 25% of daily energy requirement (Ereq) as HFCS increased lipid/lipoprotein risk factors for CVD and uric acid in a dose-response manner. However, un-confounded studies conducted in healthy humans under a controlled, energy-balanced diet protocol that enables determination of the effects of sugar with diets that do not allow for body weight gain are lacking. Furthermore, recent reports conclude that there are no adverse effects of consuming beverages containing up to 30% Ereq sucrose or HFCS, and the conclusions from several meta-analyses suggest that fructose has no specific adverse effects relative to any other carbohydrate. Consumption of excess sugar may also promote the development of CVD and T2DM indirectly by causing increased body weight and fat gain, but this is also a topic of controversy. Mechanistically, it is plausible that fructose consumption causes increased energy intake and reduced energy expenditure due to its failure to stimulate leptin production. Functional magnetic resonance imaging (fMRI) of the brain demonstrates that the brain responds differently to fructose or fructose-containing sugars compared with glucose or aspartame. Some epidemiological studies show that sugar consumption is associated with body weight gain, and there are intervention studies in which consumption of ad libitum high-sugar diets promoted increased body weight gain compared with consumption of ad libitum low- sugar diets. However, there are no studies in which energy intake and weight gain were compared in subjects consuming high or low sugar, blinded, ad libitum diets formulated to ensure both groups consumed a comparable macronutrient distribution and the same amounts of fiber. There is also little data to determine whether the form in which added sugar is consumed, as beverage or as solid food, affects its potential to promote weight gain. It will be very challenging to obtain the funding to conduct the clinical diet studies needed to address these evidence gaps, especially at the levels of added sugar that are commonly consumed. Yet, filling these evidence gaps may be necessary for supporting the policy changes that will help to turn the food environment into one that does not promote the development of obesity and metabolic disease.” As taken from Stanhope KL. 2016. Crit. Rev. Clin. Lab. Sci. 53(1), 52-67. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26376619

“Insulin resistance (IR) and impaired glucose tolerance (IGT) are the first manifestations of diet-induced metabolic alterations leading to Type 2 diabetes, while hypertension is the deadliest risk factor of cardiovascular disease. The roles of dietary fat and fructose in the development of IR, IGT, and hypertension are controversial. We tested the long-term effects of an excess of fat or sucrose (fructose/glucose) on healthy male Wistar-Kyoto (WKY) rats. Fat affects IR and IGT earlier than fructose through low-grade systemic inflammation evidenced by liver inflammatory infiltration, increased levels of plasma IL-6, PGE2, and reduced levels of protective short-chain fatty acids without triggering hypertension. Increased populations of gut Enterobacteriales and Escherichia coli may contribute to systemic inflammation through the generation of lipopolysaccharides. Unlike fat, fructose induces increased levels of diacylglycerols (lipid mediators of IR) in the liver, urine F2-isoprostanes (markers of systemic oxidative stress), and uric acid, and triggers hypertension. Elevated populations of Enterobacteriales and E. coli were only detected in rats given an excess of fructose at the end of the study. Dietary fat and fructose trigger IR and IGT in clearly differentiated ways in WKY rats: early low-grade inflammation and late direct lipid toxicity, respectively; gut microbiota plays a role mainly in fat-induced IR, and hypertension is independent of inflammation-mediated IR. The results provide evidence that suggests that the combination of fat and sugar is potentially more harmful than fat or sugar alone when taken in excess.” As taken from Ramos-Romero S et al. 2018. Am. J. Physiol. Endocrinol. Metab. 314(6), E552-E563. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29351480

“The consumption of high amounts of fructose can have adverse effects on the metabolism. In concrete terms, it can contribute to metabolic syndrome as well as lipometabolic disorders, fatty liver, obesity and diabetes mellitus type 2. In addition, there are known intolerances to fructose.”

As taken from BfR, 2018.

“We have recently shown that type of supplemented simple sugar, not merely calorie intake, determines adverse effects on metabolism and aortic endothelial function in female rats. The aim of the current study was to investigate and compare the effects of high consumption of glucose or fructose on mesenteric arterial reactivity and systolic blood pressure (SBP). Sprague-Dawley female rats were supplemented with 20% w/v glucose or fructose in drinking water for 8 weeks. Here, we show that both sugars alter insulin signaling in mesenteric arteries (MA), assessed by a reduction in phosphorylated Akt, and increase in SBP. Furthermore, ingestion of glucose or fructose enhances inducible nitric oxide synthase (iNOS) expression and contractile responses to endothelin and phenylephrine in MA of rats. The endothelium-dependent vasodilation to acetylcholine and bradykinin as well as the relaxation responses to the nitric oxide donor sodium nitroprusside are impaired in MA of fructose- but not glucose-supplemented rats. In contrast, only glucose supplementation increases the expression of phosphorylated endothelial NOS (eNOS) in MA of rats. In conclusion, this study reveals that supplementation with fructose or glucose in liquid form enhances vasocontractile responses and increases iNOS expression in MA, effects which are accompanied by increased SBP in those groups. On the other hand, the preserved vasodilatory responses in MA from glucose-supplemented rats could be attributed to the enhanced level of phosphorylated eNOS expression in this group.” As taken from Shaligram S et al. 2018. J. Nutr. Biochem. 57, 136-144. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29727795

6.3. Nervous system

Brain functional magnetic resonance imaging response to glucose and fructose infusions in humans (Abstract). Aims: In animals, intracerebroventricular glucose and fructose have opposing effects on appetite and weight regulation. In humans, functional brain magnetic resonance imaging (fMRI) studies during glucose ingestion or infusion have demonstrated suppression of hypothalamic signalling, but no studies have compared the effects of glucose and fructose. We therefore sought to determine if the brain response differed to glucose vs. fructose in humans independently of the ingestive process. Methods: Nine healthy, normal weight subjects underwent blood oxygenation level dependent (BOLD) fMRI measurements during either intravenous (IV) glucose (0.3 mg/kg), fructose (0.3 mg/kg) or saline, administered over 2 min in a randomized, double-blind, crossover study. Blood was sampled every 5 min during a baseline period and following infusion for 60 min in total for glucose, fructose, lactate and insulin levels. Results: No significant brain BOLD signal changes were detected in response to IV saline. BOLD signal in the cortical control areas increased during glucose infusion ($p = 0.002$), corresponding with increased plasma glucose and insulin levels. In contrast, BOLD signal decreased in the cortical control areas during fructose infusion ($p = 0.006$), corresponding with increases of plasma fructose and lactate. Neither glucose nor fructose infusions significantly altered BOLD signal in the hypothalamus. Conclusion: In normal weight humans, cortical responses as assessed by BOLD fMRI to infused glucose are opposite to those of fructose. Differential brain responses to these sugars and their metabolites may provide insight into the neurologic basis for dysregulation of food intake during high dietary fructose intake. As taken from Purnell JQ et al. Diabetes Obes Metab. 2011 Mar; 13(3):229-234. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/21205113

"….Fructose also promotes reactive oxygen species formation, which leads to cellular dysfunction and aging, and promotes changes in the brain's reward system, which drives excessive consumption. Thus, fructose can exert detrimental health effects beyond its calories and in ways that mimic those of ethanol, its metabolic cousin. Indeed, the only distinction is that because fructose is not metabolized in the central nervous system, it does not exert the acute neuronal depression experienced by those imbibing ethanol…." As taken from Lustig RH. 2013. Adv. Nutr. 4(2), 226-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493539?dopt=AbstractPlus

"Glucagon-like peptide-1 (GLP-1) suppresses food intake via activation of a central (i.e., brain) GLP-1 receptor (GLP-1R). Central AMP-activated protein kinase (AMPK) is a nutrient-sensitive regulator of food intake that is inhibited by anorectic signals. The anorectic effect elicited by hindbrain GLP-1R activation is attenuated by the AMPK stimulator AICAR. This suggests that central GLP-1R activation suppresses food intake via inhibition of central AMPK. The present studies examined the mechanism(s) by which central GLP-1R activation inhibits AMPK. Supporting previous findings, AICAR attenuated the anorectic effect elicited by intracerebroventricular (icv) administration of the GLP-1R agonist exendin-4 (Ex-4)….We also tested whether other carbohydrates affect the anorectic response to Ex-4. Intracerebroventricular pretreatment with the sucrose metabolite fructose, an AMPK activator, attenuated the anorectic effect of Ex-4. This potentially explains the increased food intake observed in sucrose-fed mice. In summary, we propose a model whereby

activation of the central GLP-1R reduces food intake via glucose metabolism-dependent inhibition of central AMPK. We also suggest that fructose stimulates food intake by impairing central GLP-1R action. This has significant implications given the correlation between sugar consumption and obesity." As taken from Burmeister MA et al. 2013. Am. J. Physiol. Endocrinol. Metab. 304(7), E677-85. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23341495?dopt=AbstractPlus

"Western diets are characterized by both dietary omega-3 fatty acid deficiency and increased fructose intake. The latter found in high amounts in added sugars such as sucrose and high fructose corn syrup (HFCS). Both a low intake of omega-3 fatty acids or a high fructose intake contribute to metabolic syndrome, liver steatosis or non-alcoholic fatty liver disease (NAFLD), promote brain insulin resistance, and increase the vulnerability to cognitive dysfunction. Insulin resistance is the core perturbation of metabolic syndrome. Multiple cognitive domains are affected by metabolic syndrome in adults and in obese adolescents, with volume losses in the hippocampus and frontal lobe, affecting executive function….This review presents the current status of the mechanisms involved in the development of the metabolic syndrome, brain insulin resistance, and NAFLD a most promising area of research in Nutrition for the prevention of these conditions, chronic diseases, and improvement of Public Health." As taken from Simopoulos AP. 2013. Nutrients 5(8), 2901-23. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23896654

"….Given the increase in the incidence of Alzheimer's disease (AD), characterized by an age-related decline in memory and cognitive functioning, in this report we review the effects of obesity on cognitive performance and the impact of high fructose intake in promoting cognitive decline. The paper then considers the effects of omega-3 fatty acids (FAs), which have been linked to promising results in cognitive function including ameliorating the impact of a high-fructose diet." As taken from Lakhan SE & Kirchgessner A. 2013. Nutr. J. 12, 114. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23924506

"Several hypotheses for the causes of the obesity epidemic in the US have been proposed. One such hypothesis is that dietary intake patterns have significantly shifted to include unprecedented amounts of refined sugar. We set out to determine if different sugars might promote changes in the hypothalamic mechanisms controlling food intake by measuring several hypothalamic peptides subsequent to overnight access to dilute glucose, sucrose, high fructose corn syrup, or fructose solutions. Rats were given access to food, water and a sugar solution for 24h, after which blood and tissues were collected. Fructose access (as opposed to other sugars that were tested) resulted in a doubling of circulating triglycerides. Glucose consumption resulted in upregulation of 7 satiety-related hypothalamic peptides whereas changes in gene expression were mixed for remaining sugars. Also, following multiple verification assays, 6 satiety related peptides were verified as being affected by sugar intake. These data provide evidence that not all sugars are equally effective in affecting the control of intake." As taken from Colley DL & Castonguay TW. 2015. Physiol. Behav. 139, 202-9. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25449399

"The intake of free fructose has increased substantially since the development of high-fructose corn syrup. This has not only been associated with metabolic disorders but recent evidence also indicates that chronic fructose consumption can affect neuronal and cognitive function. In this study we investigated the effects of fructose consumption on serotonergic signaling and neuronal activity in the mouse submucous plexus. Male mice were put on a

control or fructose (23% solution) diet for 6 weeks or were assigned to a recovery group that received normal water (2 weeks) after 4 weeks of fructose. At the end of the diet, gene expressions and enteric neuronal activity, after depolarization with high K(+) and 5-HT, were measured using Ca(2+) imaging and RT-qPCR, respectively. Even in the lack of gain weight and the absence of changes in duodenal permeability, the total number of 5-HT-responding neurons and the depolarization and 5-HT-evoked Ca(2+) amplitudes were significantly lower after fructose consumption. Expression of synaptobrevin CaV 2.1 and CaV 2.2 mRNA did not differ after fructose intake; however, CaV 2.1 mRNA levels were significantly higher in the recovery animals. SERT mRNA concentration, isolated from submucosal plexus containing mucosal epithelium, was significantly decreased after fructose consumption. Chronic fructose consumption impairs serotonergic signaling in the mouse submucous plexus, prior to weight gain and detectable intestinal permeability problems." As taken from Lowette K et al. 2016. Neurogastroenterol. Motil. 28(9), 1438-42. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27098706

"AIMS: This study examined the interaction between maternal high fructose diet and neonatal inflammation in neonates (P7), juveniles (P26-34) and adults on measures of anxiety-like behavior and cognition. The study aimed to assess the potential synergistic effects of these two forms of early-life inflammation. MAIN METHODS: We fed Sprague-Dawley dams with high fructose (60%) diet or normal chow. Each litter was treated with either saline or lipopolysaccharide (LPS) on postnatal day (P)3 and P5 and two pups were tested for USVs after maternal separation on P7. Post-weaning, juveniles were tested on the elevated zero maze (EZM) and in a context-object discrimination (COD) task prior to tissue harvest. Adults were tested on the EZM and the COD task as well. Immunohistochemistry and ELISA were used to assess molecular and cellular changes in the offspring. KEY FINDINGS: This study demonstrates that maternal diet and neonatal inflammation altered peripheral inflammation in neonates, altered anxiety-like behavior in juveniles, and altered anxiety-like behavior in adulthood. Maternal diet and sex increased juvenile peripheral inflammation and altered memory on the context-discrimination task. SIGNIFICANCE: Maternal diet has a profound impact on fetal and neonatal development, especially as obesity rates are on the rise worldwide. Together, these findings reveal enduring effects of maternal diet on offspring, support the findings on the effects of neonatal inflammation on anxiety-like behaviors in later-life periods, and add to the complex relationship between gestational and neonatal inflammation and anxiety." As taken from Bukhari SHF et al. 2018. Life Sci. 197, 114-121. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29428599

"Excessive sugar intake has been related to cognitive alterations, but it remains unclear whether these effects are related exclusively to increased energy intake, and the molecular mechanisms involved are not fully understood. We supplemented Sprague-Dawley female rats with 10% w/v fructose in drinking water or with isocaloric glucose solution for 7 months. Cognitive function was assessed through the Morris water maze (MWM) and the novel object recognition (NOR) tests. Plasma parameters and protein/mRNA expression in the frontal cortex and hippocampus were determined. Results showed that only fructose-supplemented rats displayed postprandial and fasting hypertriglyceridemia (1.4 and 1.9-fold, $p < 0.05$) and a significant reduction in the discrimination index in the NOR test, whereas the results of the MWM test showed no differences between groups. Fructose-drinking rats displayed an abnormal glucose tolerance test and impaired insulin signaling in the frontal cortex, as revealed by significant reductions in insulin receptor substrate-2 protein levels (0.77-fold, $p < 0.05$) and Akt phosphorylation (0.72-fold, $p < 0.05$), and increased insulin-

degrading enzyme levels (1.86-fold, $p < 0.001$). Fructose supplementation reduced the expression of antioxidant enzymes and altered the amount of proteins involved in mitochondrial fusion/fission in the frontal cortex. In conclusion, cognitive deficits induced by chronic liquid fructose consumption are not exclusively related to increased caloric intake and are correlated with hypertriglyceridemia, impaired insulin signaling, increased oxidative stress and altered mitochondrial dynamics, especially in the frontal cortex." As taken from Sangüesa G et al. 2018a. Mol. Neurobiol. 55(8), 6984-6999. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29372547

"Oxidative stress (OS) contributes to the neurological and cardio/pulmonary effects caused by adverse metabolic states and air pollutants such as ozone (O_3). This study explores the interactive effects of O_3 and diet (high-fructose (FRUC) or high⁻fat (FAT)) on OS in different rat brain regions. In acute exposure, there was a decrease in markers of reactive oxygen species (ROS) production in some brain regions by diet and not by O_3. Total antioxidant substances (TAS) were increased in the cerebellum (CER) and frontal cortex (FC) and decreased in the striatum (STR) by both diets irrespective of O_3 exposure. Protein carbonyls (PC) and total aconitase decreased in some brain regions irrespective of exposure. Following subacute exposure, an increase in markers of ROS was observed in both diet groups. TAS was increased in the FC (FAT only) and there was a clear O_3 effect where TAS was increased in the FC and STR. Diet increased PC formation within the CER in the FAT group, while the hippocampus showed a decrease in PC after O_3 exposure in controls. In general, these results indicate that diet/O_3 did not have a global effect on brain OS parameters, but showed some brain region- and OS parameter-specific effects by diets." As taken from Valdez JM et al. 2018. Int. J. Mol. Sci. 20(1), pii: E11. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30577502

"Fructose-, compared to glucose-, sweetened beverages increase liver triglyceride content in the short-term, prior to weight gain. In secondary analyses of a randomized cross-over design study during which 24 healthy adults consumed 25% of their estimated energy requirement in the form of glucose-, fructose-, and high-fructose corn syrup-sweetened beverages in addition to an identical ad libitum diet for three periods of 8 days each, we investigated the hypothesis that fructose in sweetened beverages also triggers insulin resistance in the short term. Total energy intake, body weight, and fasting glucose did not differ among diet phases. However, there was a significant trend for higher fasting insulin ($p = 0.042$ for trend) and, among normal-weight participants, homeostasis model assessment index of insulin resistance ($p = 0.034$ for diet × adiposity interaction) according to the glucose content of the beverages. In conclusion, in contrast to our hypothesis, insulin resistance was increased with higher glucose vs. fructose content of the beverages in this short-term trial." As taken from Kuzma JN et al. 2019. Eur. J. Clin. Nutr. 73(3), 487-490. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30166639

6.4. Other organ systems, dependent on the properties of the substance

Fructose consumption: recent results and their potential implications (Abstract). In addition to acquiring a better understanding of foods that may have intrinsic health benefits,

increasing our knowledge of dietary components that may adversely impact health and wellness, and the levels of consumption at which these adverse effects may occur, should also be an important priority for the Foods for Health initiative. This review discusses the evidence that additional research is needed to determine the adverse effects of consuming added sugars containing fructose. Current guidelines recommend limiting sugar consumption in order to prevent weight gain and promote nutritional adequacy. However, recent data suggest that fructose consumption in human results in increased visceral adiposity, lipid dysregulation, and decreased insulin sensitivity, all of which have been associated with increased risk for cardiovascular disease and type 2 diabetes. A proposed model for the differential effects of fructose and glucose is presented. The only published study to directly compare the effects of fructose with those of commonly consumed dietary sweeteners, high fructose corn syrup and sucrose, indicates that high fructose corn syrup and sucrose increase postprandial triglycerides comparably to pure fructose. Dose-response studies investigating the metabolic effects of prolonged consumption of fructose by itself, and in combination with glucose, on lipid metabolism and insulin sensitivity in both normal weight and overweight/obese subjects are needed. As taken from Stanhope KL; Havel PJ. Ann N Y Acad Sci. 2010, Mar; 1190(1):15-24. PubMed, 2010 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=retrieve&db=pubmed&list_uids=20388133&dopt=AbstractPlus

Immediate and delayed effects of D-fructose upon insulin, somatostatin, and glucagon release by the perfused rat pancreas (Abstract). Novel information was recently provided concerning the reciprocal effects of D-glucose and D-fructose upon their respective metabolism in rat pancreatic islets. In the light of such findings, this study aims at comparing the effects of D-glucose and D-fructose on insulin, somatostatin, and glucagon release from the isolated perfused rat pancreas. A rise in D-glucose concentration from 3.3 to 5.0 or 7.3 mM or the administration of D-fructose (17 and 40 mM) in the presence of 3.3 mM D-glucose stimulated insulin release in a concentration-related manner, but failed to affect somatostatin output. The secretion of glucagon was decreased in all cases. The secretory response to L-arginine (5 mM), 25 min after restoring the basal concentration of D-glucose, was more markedly affected, in terms of potentiation of insulin and somatostatin release and reduction of glucagon output, after prior administration of D-fructose than after a prior increase in D-glucose concentration. These findings argue against any major role for a paracrine regulation of hormonal release and, instead, are consistent with a causal link between metabolic and secretory events in the islet cells. Nevertheless, the present results emphasize differences in the response of distinct pancreatic endocrine cell types to the same or distinct hexoses. As taken from Sener A at al. Endocrine. 2004 Jun;24(1):73-81. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=pubmed&dopt=Abstract&list_uids=15249706&query_hl=40&itool=pubmed_docsum

Dietary fructose causes tubulointerstitial injury in the normal rat kidney (Abstract). Recent studies suggest that the metabolic syndrome is associated with renal disease. We previously reported that a high-fructose diet, but not a high-glucose diet, can induce metabolic syndrome and accelerate chronic renal disease in rats. We now examined the effects of a high-fructose diet on normal rat kidneys. Three groups of Sprague-Dawley rats were pair fed a special diet containing 60% fructose, 60% glucose, or control standard rat chow for 6 wk, and then histological studies were performed. The effect of fructose to induce cell proliferation in cultured proximal tubular cells was also performed. Fructose diet, but not glucose diet, significantly increased kidney weight by 6 wk. The primary finding was

tubular hyperplasia and proliferation involving all segments of the proximal tubules while glomerular changes were not observed. This is the same site where the fructose transporters (GLUT2 and -5) as well as the key enzyme in fructose metabolism (ketohexokinase) were expressed. Consistently, fructose also induced proliferation of rat proximal tubular cells in culture. In vivo, tubular proliferation was also associated with focal tubular injury, with type III collagen deposition in the interstitium, an increase in alpha-smooth muscle actin positive myofibroblasts, and an increase in macrophage infiltration. In conclusion, a high-fructose diet induces cell proliferation and hyperplasia in proximal tubules, perhaps via a direct metabolic effect. The effect is independent of total energy intake and is associated with focal tubulointerstitial injury. These studies may provide a mechanism by which metabolic syndrome causes renal disease. As taken from Nakayama T et al . Am J Physiol Renal Physiol. 2010 Mar;298(3):F712-20. PubMed, 2011 available at http://www.ncbi.nlm.nih.gov/pubmed/20071464

Fructose impairs glucose-induced hepatic triglyceride synthesis (Abstract). Obesity, type 2 diabetes and hyperlipidemia frequently coexist and are associated with significantly increased morbidity and mortality. Consumption of refined carbohydrate and particularly fructose has increased significantly in recent years and has paralleled the increased incidence of obesity and diabetes. Human and animal studies have demonstrated that high dietary fructose intake positively correlates with increased dyslipidemia, insulin resistance, and hypertension. Metabolism of fructose occurs primarily in the liver and high fructose flux leads to enhanced hepatic triglyceride accumulation (hepatic steatosis). This results in impaired glucose and lipid metabolism and increased proinflammatory cytokine expression. Here we demonstrate that fructose alters glucose-stimulated expression of activated acetyl CoA carboxylase (ACC), pSer hormone sensitive lipase (pSerHSL) and adipose triglyceride lipase (ATGL) in hepatic HepG2 or primary hepatic cell cultures in vitro. This was associated with increased de novo triglyceride synthesis in vitro and hepatic steatosis in vivo in fructose- versus glucose-fed and standard-diet fed mice. These studies provide novel insight into the mechanisms involved in fructose-mediated hepatic hypertriglyceridemia and identify fructose-uptake as a new potential therapeutic target for lipid-associated diseases. As taken from Huang et al. 2011 Lipids in Health and Disease 10:20.

The effect of fructose on renal biology and disease (Abstract). Dietary fructose intake is increasing. It is increasing primarily from added sugars, including sucrose and high fructose corn syrup, and correlates epidemiologically with the rising prevalence of metabolic syndrome and hypertension worldwide. The administration of fructose to animals and humans increases BP and the development of metabolic syndrome. These changes occur independently of caloric intake because of the effect of fructose on ATP depletion and uric acid generation. Fructose ingestion may also be a risk factor for kidney disease that includes glomerular hypertension, renal inflammation, and tubulointerstitial injury in animals. We suggest excessive fructose intake should be considered an environmental toxin with major health implications. As taken from Johnson et al., 2010. Journal of the American Society of Nephrology 21, 2036-9.

Evidence-based review on the effect of normal dietary consumption of fructose on blood lipids and body weight of overweight and obese individuals (Abstract). Although some investigators have hypothesized that ingestion of fructose from foods and beverages is responsible for the development of hyperlipidemia or obesity, a recent evidence-based review demonstrated that there was no relationship between the

consumption of fructose in a normal dietary manner and the development of hyperlipidemia or increased weight in normal weight individuals. Because overweight and obese individuals may exhibit metabolic abnormalities such as insulin resistance, impaired glucose tolerance, hyperlipedemia, and/or alterations in gut hormones involved in appetite regulation, the findings of fructose studies performed in normal weight subjects may not be particularly relevant for overweight or obese subjects. A systematic assessment of the strength and quality of the studies and their relevance for overweight or obese humans ingesting fructose in a normal dietary manner has not been performed. The purpose of this review was to critically evaluate the existing database for a causal relationship between the ingestion of fructose in a normal, dietary manner and the development of hyperlipidemia or increased body weight in overweight or obese humans, using an evidence-based approach. The results of the analysis indicate that there is no evidence which shows that the consumption of fructose at normal levels of intake causes biologically relevant changes in triglycerides (TG) or body weight in overweight or obese individuals. As taken from Dolan et al., 2010. Critical Reviews in Food Science and Nutrition 50, 889-918.

Fructose induced lipogenesis: from sugar to fat to insulin resistance (Abstract). Increasing consumption of sugars is one of the contributing factors to the obesity epidemic. Both cane sugar and high-fructose corn syrup contain glucose and fructose. Fructose, in contrast to glucose, is known to potently stimulate lipogenesis, but the mechanisms responsible are not yet fully known. This paper reviews several possible pathways that might be involved, such as activation of pyruvate dehydrogenase, and transcriptional activation of sterol regulatory element binding protein 1c by key regulators such as peroxisome proliferator activated receptor-γ co-activator 1β and the splice variant of X-box binding protein 1. Together, these pathways might establish a feed forward cycle that can rapidly increase hepatic lipogenesis. As a result, dietary fructose might promote the development of nonalcoholic fatty liver disease, which in and of itself, can result in hepatic insulin resistance, a key feature of type 2 diabetes mellitus. As taken from Samuel (2011). Trends Endocrinol. Metab. 22(2): 60-5. Epub 2010 Nov 9, available at http://www.ncbi.nlm.nih.gov/pubmed/21067942?dopt=AbstractPlus

“BACKGROUND: Controversy exists regarding the causative role of dietary fructose in obesity and fatty liver diseases. Clinical trials have indicated that negative health consequences may occur only when fructose is consumed within excess calories. Animal studies have suggested that fructose impairs intestinal integrity and leads to hepatic steatosis (HS). OBJECTIVES:We assessed nonhuman primates after chronic ad libitum and short-term calorically controlled consumption of a high-fructose (HFr), low-fat diet (24% of calories). Microbial translocation (MT), microbiome, and metabolic health indexes were evaluated. DESIGN: Seventeen monkeys fed 0.3–7 y of an HFr ad libitum diet were compared with 10 monkeys fed a low-fructose, low-fat diet (control). Ten middle-aged, weight-stable, fructose-naive monkeys were stratified into HFr and control groups fed for 6 wk at caloric amounts required to maintain weight stability. Metabolic endpoints, feces, liver, small and large intestinal biopsies, and portal blood samples were collected. RESULTS: Monkeys allowed ad libitum HFr developed HS in contrast to the control diet, and the extent of ectopic fat was related to the duration of feeding. Diabetes incidence also increased. Monkeys that consumed calorically controlled HFr showed significant increases in biomarkers of liver damage, endotoxemia, and MT indexes and a trend for greater hepatitis that was related to MT; however, HS did not develop. CONCLUSIONS: Even in the absence of weight gain, fructose rapidly causes liver damage that we suggest is secondary

to endotoxemia and MT. HS relates to the duration of fructose consumption and total calories consumed. These data support fructose inducing both MT and ectopic fat deposition in primates." As taken from Kavanagh K et al. 2013. Am. J. Clin. Nutr. 98(2), 349-57. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23783298?dopt=AbstractPlus

"The current epidemic of obesity and type 2 diabetes is attributed to a high carbohydrate diet, containing mainly high fructose corn syrup and sucrose. More than two thirds of diabetic patients have hypertension. Methylglyoxal is a highly reactive dicarbonyl generated during glucose and fructose metabolism, and a major precursor of advanced glycation end products (AGEs). Plasma methylglyoxal levels are increased in hypertensive rats and diabetic patients. Our aim was to examine the levels of methylglyoxal, mediators of the renin angiotensin system and blood pressure in male Sprague-Dawley rats treated with a high fructose diet (60% of total calories) for 4 months. The thoracic aorta and kidney were used for molecular studies, along with cultured vascular smooth muscle cells (VSMCs). HPLC, Western blotting and Q-PCR were used to measure methylglyoxal and reduced glutathione (GSH), proteins and mRNA, respectively. Fructose treated rats developed a significant increase in blood pressure. Methylglyoxal level and protein and mRNA for angiotensin II, AT1 receptor, adrenergic α1D receptor and renin were significantly increased, whereas GSH levels were decreased, in the aorta and/or kidney of fructose fed rats. The protein expression of the receptor for AGEs (RAGE) and NF-κB were also significantly increased in the aorta of fructose fed rats. MG treated VSMCs showed increased protein for angiotensin II, AT1 receptor, and α1D receptor. The effects of methylglyoxal were attenuated by metformin, a methylglyoxal scavenger and AGEs inhibitor. In conclusion, we report a strong association between elevated levels of methylglyoxal, RAGE, NF-κB, mediators of the renin angiotensin system and blood pressure in high fructose diet fed rats." As taken from Dhar I et al. 2013. PLoS One. 8(9), e74212. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24040205

"Although excessive fructose intake is epidemiologically linked with dyslipidemia, obesity, and diabetes, the mechanisms regulating plasma fructose are not well known. Cells transfected with sodium/glucose cotransporter 5 (SGLT5), which is expressed exclusively in the kidney, transport fructosein vitro; however, the physiological role of this transporter in fructose metabolism remains unclear. To determine whether SGLT5 functions as afructose transporter in vivo, we established a line of mice lacking the gene encoding SGLT5. Sodium-dependent fructose uptake disappeared in renal brush border membrane vesicles from SGLT5-deficient mice, and the increased urinary fructose in SGLT5-deficient mice indicated that SGLT5 was the major fructose reabsorption transporter in the kidney. From this, we hypothesized that urinary fructose excretion induced by SGLT5 deficiency would ameliorate fructose-induced hepatic steatosis. To test this hypothesis we compared SGLT5-deficient mice with wild-type mice under conditions of long-term fructose consumption. Paradoxically, however, fructose-induced hepatic steatosis was exacerbated in the SGLT5-deficient mice, and the massive urinary fructose excretion was accompanied by reduced levels of plasma triglycerides and epididymal fat but fasting hyperinsulinemia compared with fructose-fed wild-type mice. There was no difference in food consumption, water intake, or plasma fructose between the two types of mice. No compensatory effect by other transporters reportedly involved in fructose uptake in the liver and kidney were indicated at the mRNA level. These surprising findings indicated a previously unrecognized link through SGLT5 between renal fructose reabsorption and hepatic lipid metabolism." As taken from

Fukuzawa T et al. 2013. PLoS One 8(2), e56681. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23451068

“….Fructose consumption has increased worldwide, paralleling the obesity and chronic metabolic disease pandemic. Sugar (i.e., fructose-containing mixtures) has been vilified by nutritionists for ages as a source of "empty calories," no different from any other empty calorie. However, fructose is unlike glucose. In the hypercaloric glycogen-replete state, intermediary metabolites from fructose metabolism overwhelm hepatic mitochondrial capacity, which promotes de novo lipogenesis and leads to hepatic insulin resistance, which drives chronic metabolic disease. Fructose also promotes reactive oxygen species formation, which leads to cellular dysfunction and aging, and promotes changes in the brain's reward system, which drives excessive consumption. Thus, fructose can exert detrimental health effects beyond its calories and in ways that mimic those of ethanol, its metabolic cousin. Indeed, the only distinction is that because fructose is not metabolized in the central nervous system, it does not exert the acute neuronal depression experienced by those imbibing ethanol. These metabolic and hedonic analogies argue that fructose should be thought of as "alcohol without the buzz." As taken from Lustig RH. 2013. Adv. Nutr. 4(2), 226-35. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493539?dopt=AbstractPlus

“BACKGROUND AND GOALS: Dietary fructose intake in the United States has been increasing, and fructose intake has been associated with the metabolic syndrome and hepatic steatosis. This study aimed to determine whether dietary fructose intake is associated with advanced hepatic fibrosis and inflammation in an hepatitis C virus (HCV)-infected male population. STUDY: We conducted a cross-sectional study of HCV-infected male veterans. The main exposure variable was daily dietary fructose calculated from the National Cancer Institute Diet History Questionnaire and the main outcome variables were FibroSURE-ActiTest determined hepatic fibrosis (F0-F3=mild vs. F3/F4-F4=advanced) and inflammation (A0-A2=mild vs. A2/A3-A3=advanced). We examined this association in logistic regression adjusting for demographic, clinical, and other dietary variables. RESULTS: Among 313 HCV* males, 103 (33%) had advanced fibrosis and 89 (28%) had advanced inflammation. Median daily fructose intake was 46.8 g (interquartile range, 30.4 to 81.0). Dietary fructose intake across quartiles among males with advanced versus mild fibrosis was 21.4% versus 25.2%, 32.0% versus 24.8%, 24.3% versus 25.2%, and 22.3% versus 24.8%, respectively, and among males with advanced versus mild inflammation was 20.2% versus 25.5%, 41.6% versus 21.4%, 22.5% versus 25.9%, and 15.7% versus 27.2%, respectively. In multivariate analysis, there were no significant associations between daily fructose intake and advanced fibrosis. There was a significant association only between the second quartile of daily fructose intake (30 to 48 g) and advanced inflammation. CONCLUSIONS: There were no significant associations between dietary fructose intake and hepatic fibrosis risk, as assessed by FibroSURE, in HCV-infected males. Additional research is needed to clarify the potential role of fructose intake and HCV-related hepatic inflammation.” As taken from Tyson GL et al. 2013. J. Clin. Gastroenterol. 47(6), 545-52. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23426443

“We studied the effect of feeding normal adult male rats with a commercial diet supplemented with fructose added to the drinking water (10% w/v;fructose-rich diet, FRD) on the adipogenic capacity of stromal-vascular fraction (SVF) cells isolated from visceral adipose tissue (VAT) pads. Animals received either the commercial diet or FRD ad libitum for 3 weeks; thereafter, we evaluated the in vitro proliferative and adipogenic capacities of

their VAT SVF cells. FRD significantly increased plasma insulin, triglyceride and leptin levels, VAT mass/cell size, and the in vitro adipogenic capacity of SVF cells. Flow cytometry studies indicated that the VAT precursor cell population number did not differ between groups; however, the accelerated adipogenic process could result from an imbalance between endogenous pro- and anti-adipogenic SVF cell signals, which are clearly shifted towards the former. The increased insulin milieu and its intracellular mediator (insulin receptor substrate-1) in VAT pads, as well as the enhanced SVF cell expression of Zpf423 and peroxisome proliferator receptor-γ2 (all pro-adipogenic modulators), together with a decreased SVF cell concentration of anti-adipogenic factors (pre-adipocyte factor-1 and wingless-type MMTV-10b), strongly supports this assumption. We hypothesize that the VAT mass expansion recorded in FRD rats results from the combination of initial accelerated adipogenesis and final cell hypertrophy. It remains to be determined whether FRD administration over longer periods could perpetuate both processes, or whether cell hypertrophy itself remains responsible for a further VAT mass expansion, as observed in advanced/morbid obesity." As taken from Zubiría MG et al. 2013. FEBS J. 280(22), 5864-74. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23992485

"High fructose consumption is commonly associated with insulin resistance, disturbed glucose homeostasis and low-grade inflammation. Increased glucocorticoid production within adipose tissue has been implicated in the pathogenesis of fructose-induced metabolic syndrome. Immunosuppressive actions of glucocorticoids can be counter-regulated by macrophage migration inhibitory factor (MIF), which is recognised as a key molecule in metabolic inflammation. In the present study, we hypothesised that coordinated action of glucocorticoids and MIF can mediate the effects of a high-fructose diet on adipose tissue and liver inflammation. We examined the effects of long-term consumption of a 10% fructose solution on corticosterone (CORT) and MIF levels in rat blood plasma, liver and adipose tissue, as well as MIF and TNF-a mRNA expression and NF-kB activation in the same tissues. The high-fructose diet led to an increase in both CORT and MIF in the adipose tissue, and a highly significant positive correlation between their levels was observed. The attenuated NF-kB activation and unaltered TNF-a mRNA expression noticed in the adipose tissue could be interpreted as an outcome of the opposing actions of CORT and MIF. In contrast to adipose tissue, inflammation in the liver was characterised by NF-kB activation, an increased TNF-a mRNA level and unchanged levels of MIF protein, MIF mRNA and CORT. Overall, these findings suggest that a high-fructose diet differently affects the levels of glucocorticoids and MIF in the adipose tissue and liver, implicating that fructose over-consumption has tissue-specific effects on regulation of metabolic inflammation." As taken from Veli-ìkovi-ç N et al. 2013. Br. J. Nutr. 110(3), 456-65. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23286672?dopt=AbstractPlus

"People with diet-induced metabolic maladies may be more susceptible to the adverse effects of air pollution (AP). The present study evaluated the effect of AP on facets of diet-induced metabolic dysfunction. Mature male Sprague Dawley rats were fed normal chow (NC) or a high fructose diet (HF) for 8 weeks prior to and during AP exposure. Inhalation exposures to ozone (O3; 0.5 ppm) and/or concentrated ambient fine particles (CAPs, 400 µg/m3) were conducted in a mobile air research laboratory located in an industrial area of Dearborn, MI during summer 2011. Rats were exposed for 9 days, 8 h/day (4-5 days/week). Rats were sacrificed 24h after the end of exposure. Prior to sacrifice, blood was collected for homeostatic model assessment–insulin resistance (HOMA-IR) analysis. At necropsy, hepatic tissues were collected for light microscopy and gene expression analyses using qRTPCR and whole-genome arrays. Though HF feeding did not induce obesity, HOMA-IR

was increased concurrent with periportal steatosis. HF-induced gene expression of stearoyl-Coenzyme A desaturase (Scd1) was potentiated after exposure to O3, CAPs and O3/CAPs (~21-fold) relative to filtered air/HF group (~14-fold). Scd1 expression was repressed by O3 (~3.4-fold) and O3/CAPs (~2.5-fold) in rats fed NC. Low density lipoprotein receptor (Ldlr) was also induced in HF-fed rats that were exposed to O3 and O3/CAPs (~1.6-fold) compared to filtered air-exposed NC and HF rats. In contrast, HF feeding caused a significant decrease in phosphoenolpyruvate carboxykinase 1 (Pck1), suggesting inhibition of gluconeogenesis. Furthermore, CAPs exposure down-regulated Pck1 in NC-fed rats (~1.7- fold) and further suppressed Pck1 on a HF diet (~6-fold). Collectively, these data suggest that acute AP exposure may exacerbate facets of the metabolic syndrome." As taken from Allen K et al. 2012. The Toxicologist. SOT 2012 Annual Meeting. Available at http://www.toxicology.org/AI/PUB/Tox/2012Tox.pdf

"Clinical studies have linked the increased consumption of fructose to the development of obesity, dyslipidemia, and impaired glucose tolerance, and a role in hepatosteatosis development is presumed. Fructose can undergo a nonenzymatic reaction from which advanced glycation end products (AGEs) are derived, leading to the formation of dysfunctional, fructosylated proteins; however, the in vivo formation of AGEs from fructose is still less known than that from glucose. In the present study C57Bl/6J mice received 15% (wt/vol) fructose (FRT) or 15% (wt/vol) glucose (GLC) in water to drink for 30 wk, resembling human habit to consume sugary drinks. At the end of the protocol both FRT- and GLC-drinking mice had increased fasting glycemia, glucose intolerance, altered plasma lipid profile, and marked hepatosteatosis. FRT mice had higher hepatic triglycerides deposition than GLC, paralleled by a greater increased expression and activity of the sterol regulatory element-binding protein 1 (SREBP1), the transcription factor responsible for the de novo lipogenesis, and of its activating protein SCAP. LC-MS analysis showed a different pattern of AGE production in liver tissue between FRT and GLC mice, with larger amount of carboxymethyl lysine (CML) generated by fructose. Double immunofluorescence and coimmunoprecipitation analysis revealed an interaction between CML and SCAP that could lead to prolonged activation of SREBP1. Overall, the high levels of CML and activation of SCAP/SREBP pathway associated to high fructose exposure here reported may suggest a key role of this signaling pathway in mediating fructose-induced lipogenesis." As taken from Mastrocola R et al. 2013. Am. J. Physiol. Gastrointest. Liver Physiol. 305(6), G398-407. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23868406

"The study evaluates the effects of genistein on blood pressure (BP) and ultrastructural changes in kidney of fructose-fed hypertensive rats. Male Wistar rats were fed a diet containing 60 % starch or 60 % fructose as the source of carbohydrate. After 15 d, rats in each dietary group were divided into two groups and were treated with either genistein (1 mg/kg per d) in dimethylsulfoxide (DMSO) or 30 % DMSO alone. BP, pressor mechanisms, protein kinase C-βII (PKC-βII) expression, endothelial NO synthase (eNOS) expression and renal ultrastructural changes were evaluated after 60 d.Fructose-fed rats displayed significant elevation in BP and heart rate. Significant increase in plasma angiotensin-converting enzyme (ACE) activity, alterations in renal lipid profile, nitrite and kallikrein activity, enhanced expression of membrane-associated PKC-βII and decreased expression of eNOS were observed in them. Histology and electron microscopic studies showed structural changes in the kidney…." As taken from Palanisamy N & Venkataraman AC. 2013. Br. J. Nutr. 109(10), 1806-12. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23116847

“We recently discovered that chronic high fructose intake by lactating rats prevented adaptive increases in rates of active intestinal Ca(2+) transport and in levels of 1,25-(OH)2D3, the active form of vitamin D. Since sufficient Ca(2+) absorption is essential for skeletal growth, our discovery may explain findings that excessive consumption of sweeteners compromises bone integrity in children. We tested the hypothesis that 1,25-(OH)2D3 mediates the inhibitory effect of excessive fructose intake on active Ca(2+) transport. First, compared with those fed glucose or starch, growing rats fed fructose for 4 wk had a marked reduction in intestinal Ca(2+) transport rate as well as in expression of intestinal and renal Ca(2+) transporters that was tightly associated with decreases in circulating levels of 1,25-(OH)2D3, bone length, and total bone ash weight but not with serum parathyroid hormone (PTH). Dietary fructose increased the expression of 24-hydroxylase (CYP24A1) and decreased that of 1α-hydroxylase (CYP27B1), suggesting that fructose might enhance the renal catabolism and impair the synthesis, respectively, of 1,25-(OH)2D3. Serum FGF23, which is secreted by osteocytes and inhibits CYP27B1 expression, was upregulated, suggesting a potential role of bone in mediating the fructose effects on 1,25-(OH)2D3 synthesis. Second, 1,25-(OH)2D3 treatment rescued the fructose effect and normalized intestinal and renal Ca(2+) transporter expression. The mechanism underlying the deleterious effect of excessive fructose intake on intestinal and renal Ca(2+) transporters is a reduction in serum levels of 1,25-(OH)2D3. This finding is significant because of the large amounts of fructose now consumed by Americans increasingly vulnerable to Ca(2+) and vitamin D deficiency.” As taken from Douard V et al. 2013. Am. J. Physiol. Endocrinol. Metab. 304(12), E1303-13. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23571713?dopt=AbstractPlus

“PURPOSE: We explored the pathophysiologic mechanisms of long-term fructose-induced lower urinary tract symptoms (LUTS) in rats. METHODS: Male Wistar rats were fed with fructose for 3 or 6 months. Biochemical and transcystometric parameters were compared between fructose-fed and age-matched normal-diet rats. Pelvic nerve and external urethral sphincter-electromyogram activity recordings were performed to investigate fructose effects on neural control of bladders. Mitochondrial structure, ATP and acetylcholine content and purinergic and muscarinic cholinergic receptors were examined. Cytosolic cytochrome C staining by Western blot and immunocytochemistry for mitochondrial injury and PGP 9.5 stain for nerve density were also determined. RESULTS: The fructose-fed rats with higher plasma triglyceride, LDL and fasting glucose levels displayed LUTS with increased frequency and suppressed voiding contractile amplitude in phase 1 and phase 2 duration versus normal-diet control. Fructose feeding altered the firing types in pelvic afferent and efferent nerves and external urethral sphincter-electromyogram activity. Increased mast cell number, disrupted and swollen mitochondria, increased cytosolic cytochrome C stain and expression and decreased nerve density in bladder smooth muscle layers appeared in the fructose-fed rats. Fructose feeding also significantly reduced ATP and acetylcholine content and enhanced protein expression of postsynaptic P(2)X(1), P(2)X(2) and P(2)X(3) purinergic receptors and M(2) and M(3) muscarinic cholinergic receptors expression in the smooth muscles of urinary bladder. CONCLUSION: Long-term fructose feeding induced neuropathy and myopathy in the urinary bladders. Impaired mitochondrial integrity, reduced nerve density, ATP and acetylcholine content and upregulation of purinergic and muscarinic cholinergic receptors expression may contribute to the bladder dysfunction of fructose-fed animals.” As taken from Chung SD et al. 2013. Eur. J. Nutr. 52(1), 347-59. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22426756?dopt=AbstractPlus

“Both controversy and confusion exist concerning fructose, sucrose, and high-fructose corn syrup (HFCS) with respect to their metabolism and health effects. These concerns have often been fueled by speculation based on limited data or animal studies. In retrospect, recent controversies arose when a scientific commentary was published suggesting a possible unique link between HFCS consumption and obesity. Since then, a broad scientific consensus has emerged that there are no metabolic or endocrine response differences between HFCS and sucrose related to obesity or any other adverse health outcome. This equivalence is not surprising given that both of these sugars contain approximately equal amounts of fructose and glucose, contain the same number of calories, possess the same level of sweetness, and are absorbed identically through the gastrointestinal tract. Research comparing pure fructose with pure glucose, although interesting from a scientific point of view, has limited application to human nutrition given that neither is consumed to an appreciable degree in isolation in the human diet. Whether there is a link between fructose, HFCS, or sucrose and increased risk of heart disease, metabolic syndrome, or fatty infiltration of the liver or muscle remains in dispute with different studies using different methodologies arriving at different conclusions. Further randomized clinical trials are needed to resolve many of these issues. The purpose of this review is to summarize current knowledge about the metabolism, endocrine responses, and potential health effects of sucrose, HFCS, and fructose.” As taken from Rippe JM & Angelopoulos TJ. 2013. Adv. Nutr. 4(2), 236-45. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23493540?dopt=AbstractPlus

“Sugar consumption, especially in the form of fructose, has been hypothesized to cause kidney disease. This review provides an overview of the epidemiologic evidence that sugar consumption increases CKD risk. Research supports a causal role of sugar in several kidney disease risk factors, including increasing serum uric acid levels, diabetes, and obesity. Sugar may also harm the kidney via other mechanisms. There is no evidence thatsucrose is any safer for the kidney than high fructose corn syrup (HFCS) because both are similar in composition. To date, 5 epidemiologic studies have directly evaluated the relationship between sugar consumption (in the form of sugar-sweetened beverages) and CKD. Although most studies suggest that the risk of CKD is elevated among consumers of sugar-sweetened beverages, only 2 studies report statistically significant associations. Three studies have also examined diet soda consumption, with two reporting positive and significant associations. Confounding by unmeasured lifestyle factors may play a role in the positive results whereas poor measurement of sugar and artificial sweetener intake could explain null results. Nevertheless, the hypothesis that sugar causes kidney disease remains plausible, and alternative research designs may be needed.” As taken from Karalius VP & Shoham DA. 2013. Adv. Chronic Kidney Dis. 20(2), 157-64. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23439375?dopt=AbstractPlus

“OBJECTIVE: Adverse effects of hypercaloric, high-fructose diets on insulin sensitivity and lipids in human subjects have been shown repeatedly. The implications of fructose in amounts close to usual daily consumption, however, have not been well studied. This study assessed the effect of moderate amounts of fructose and sucrose compared with glucose on glucose and lipid metabolism. RESEARCH DESIGN AND METHODS: Nine healthy, normal-weight male volunteers (aged 21-25 years) were studied in this double-blind, randomized, cross-over trial. All subjects consumed four different sweetened beverages (600 mL/day) for 3 weeks each: medium fructose (MF) at 40 g/day, and high fructose (HF), high glucose (HG), and high sucrose (HS) each at 80 g/day. Euglycemic-hyperinsulinemic clamps with [6,6]-(2)H(2) glucose labeling were used to measure endogenous glucose

production. Lipid profile, glucose, and insulin were measured in fasting samples. RESULTS: Hepatic suppression of glucose production during the clamp was significantly lower after HF (59.4 ± 11.0%) than HG (70.3 ± 10.5%, P < 0.05), whereas fasting glucose, insulin, and C-peptide did not differ between the interventions. Compared with HG, LDL cholesterol and total cholesterol were significantly higher after MF, HF, and HS, and free fatty acids were significantly increased after MF, but not after the two other interventions (P < 0.05). Subjects' energy intake during the interventions did not differ significantly from baseline intake. CONCLUSIONS: This study clearly shows that moderate amounts of fructose and sucrose significantly alter hepatic insulin sensitivity and lipid metabolism compared with similar amounts of glucose." As taken from Aeberli I et al. 2013. Diabetes Care 36(1), 150-6. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/22933433

"We aimed to investigate whether increased consumption of fructose is linked to the increased prevalence of fatty liver. The prevalence of nonalcoholic steatohepatitis (NASH) is 3% and 20% in nonobese and obese subjects, respectively. Obesity is a low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin-6, adiponectin, leptin, and tumor necrosis factor-α may play important roles in the development of nonalcoholic fatty liver disease (NAFLD). Additionally, the prevalence of NASH associated with both cirrhosis and hepatocellular carcinoma was reported to be high among patients with type 2 diabetes with or without obesity. Our research group previously showed that consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes in mice, the American Lifestyle-Induced Obesity Syndrome model, which included consumption of a high-fructose corn syrup in amounts relevant to that consumed by some Americans. The observation reinforces the concerns about the role of fructose in the obesity epidemic. Increased availability of fructose (e.g., high-fructose corn syrup) increases not only abnormal glucose flux but also fructose metabolism in the hepatocyte. Thus, the anatomic position of the liver places it in a strategic buffering position for absorbed carbohydrates and amino acids. Fructose was previously accepted as a beneficial dietary component because it does not stimulate insulin secretion. However, since insulin signaling plays an important role in central mechanisms of NAFLD, this property of fructose may be undesirable. Fructose has a selective hepatic metabolism, and provokes a hepatic stress response involving activation of c-Jun N-terminal kinases and subsequent reduced hepatic insulin signaling. As high fat diet alone produces obesity, insulin resistance, and some degree of fatty liver with minimal inflammation and no fibrosis, the fast food diet which includes fructose and fats produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress and lipoapoptosis. Hepatic de novo lipogenesis (fatty acid and triglyceride synthesis) is increased in patients with NAFLD. Stable-isotope studies showed that increased de novo lipogenesis (DNL) in patients with NAFLD contributed to fat accumulation in the liver and the development of NAFLD. Specifically, DNL was responsible for 26% of accumulated hepatic triglycerides and 15%-23% of secreted very low-density lipoprotein triglycerides in patients with NAFLD compared to an estimated less than 5% DNL in healthy subjects and 10% DNL in obese people with hyperinsulinemia. In conclusion, understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease." As taken from Basaranoglu M et al. 2013. World J. Gastroenterol. 19(8), 1166-72. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23482247

"Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in adults and children. A number of genetic and environmental factors are known to predispose individuals to NAFLD. Certain dietary sugars, particularly fructose, are

suspected to contribute to the development of NAFLD and its progression. The increasing quantity of fructose in the diet comes from sugar additives (most commonly sucrose and high fructosecorn syrup) in beverages and processed foods. Substantial links have been demonstrated between increased fructose consumption and obesity, dyslipidemia, and insulin resistance. Growing evidence suggests that fructose contributes to the development and severity of NAFLD. In human studies, fructose is associated with increasing hepatic fat, inflammation, and possibly fibrosis. Whether fructose alone can cause NAFLD or if it serves only as a contributor when consumed excessively in the setting of insulin resistance, positive energy balance, and sedentary lifestyle is unknown. Sufficient evidence exists to support clinical recommendations that fructose intake be limited through decreasing foods and drinks high in added (fructose-containing) sugars." As taken from Vos MB & Lavine JE. 2013. Hepatology 57(6), 2525-31. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23390127

"….Researchers around the world have come together in a just-published study that offers new ideas about how fructose consumption results in obesity and metabolic syndrome, which can lead to diabetes. In this review, we discuss that how fructose causes fatty liver, obesity and insulin resistance. We also discuss the effects of consumption of high fructose corn syrup, dietary fructose, fructose-induced changes in metabolism." As taken from Akram M & Hamid A. 2013. Obes. Res. Clin. Pract. 7(2), e89-94. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/24331770

"OBJECTIVE: Metabolic disturbances are common in patients with renal function impairment and are related to high rates of cardiovascular incidents and mortality. Kidney transplantation leads to improved survival but may lead to additional metabolic alterations caused by immunosuppressive drugs and improved nutrition. MATERIALS AND METHODS: The short-term effect of oral fructose load on serum uric acid (UA), plasma lipids, and blood pressure (BP) was studied in 85 patients with chronic kidney disease (CKD) and impairment of renal function (glomerular filtration rate 50-65 ml/min per 1.73 m(2)), comprising 55 renal transplant recipients (RTR) treated with standard triple immunosuppressive therapy including a calcineurin inhibitor (CNI) cyclosporine A (CsA) or tacrolimus (Tac) and 30 non-transplanted patients with CKD. Both non-transplanted CKD patients and RTR had stable renal function and a comparable degree of kidney dysfunction. All subjects received orally 70 g of fructose dissolved in 200 ml of water. Serum UA, lipids, and blood pressure were measured at baseline and 60, 120, 180, and 240 minutes after fructose administration. RESULTS: There was a significant increase of serum UA concentration ($p<0.001$) in both CKD patients and RTR--CsA- or Tac-treated patients comparable in the latter. Total cholesterol (TC), LDL, and HDL cholesterol significantly decreased and serum triglycerides (TG) markedly increased in RTR, whereas in CKD patients all serum lipid fractions increased. Blood pressure was unaffected by fructose intake. CONCLUSION: Both non-transplanted and transplanted patients with mild renal function impairment show similar acute purine metabolic disturbances following oral administration of fructose but in the latter dietary fructose may induce a smaller hyperlipidemic response." As taken from Zawiasa A & Nowicki M. 2013. Metabolism 62(10), 1462-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23866980

"PURPOSE: Dietary factors have a role in stone disease in adults but little is known about the relationship in children. We tested whether diet could alter urine and serum parameters in a pediatric mouse model. MATERIALS AND METHODS: We randomized 30 female BALB/c mice at age 3 weeks to mouse chow, a complex carbohydrate Western diet or a

high fructose, high sodium Western diet. Body weight was measured twice weekly. Urine was periodically collected and mice were sacrificed on day 30. Samples were pooled to analyze serum and urine electrolyte factors. RESULTS: There was no significant difference in body weights among the groups at any time or in kidney weight at sacrifice (each p >0.15). There was no observable increase in urine stone forming analytes across the arms except uric acid, which tended to be higher in the Western diet and high fructose, high sodium Western diet groups. There was a substantial decrease in urinary stone inhibitors (magnesium and citrate), a decrease in urinary potassium and an increase in serum calcium in the 2 Western diet groups. CONCLUSIONS: This pilot study suggests that consuming a Western diet, especially one composed of high fructose and sodium, may alter parameters that predispose to stone formation in children by decreasing stone inhibitors. Results require validation in larger studies and ultimately in humans." As taken from Ross SS et al. 2013. J. Urol. 190(4 Suppl.), 1484-9. PubMed, 2014 available at http://www.ncbi.nlm.nih.gov/pubmed/23473903

"CONTEXT: Metabolic syndrome and non-alcoholic fatty liver disease (NAFLD) are the emerging co-morbidities of skin inflammation. Occurrence of skin inflammation such as psoriasis is substantially higher in NAFLD patients than normal. Currently, there are no animal models to study the interaction between these co-morbidities. OBJECTIVE: The present study seeks to develop a simple mouse model of NAFLD-enhanced skin inflammation and to study the effect of NAFLD on different parameters of skin inflammation. MATERIALS AND METHOD: Metabolic syndrome and NAFLD were induced in C57BL/6 mice by feeding high-fat diet (HFD, 60% kcal) and high fructose liquid (HFL, 40% kcal) in drinking water. Skin inflammation was induced by repeated application of oxazolone (1% sensitization and repeated 0.5% challenge) in both normal and NAFLD mice and various parameters of skin inflammation and NAFLD were measured. RESULTS: HFD and HFL diet induced obesity, hyperglycemia, hyperinsulinemia, and histological features of NAFLD in mice. Oxazolone challenge significantly increased ear thickness, ear weight, MPO activity, NF-κB activity, and histological features of skin inflammation in NAFLD mice as compared with normal mice. Overall, induction of oxazolone-induced skin inflammation was more prominent in NAFLD mice than normal mice. Hence, HFD and HFL diet followed by topical oxazolone application develops metabolic syndrome, NAFLD, and enhanced skin inflammation in mice. DISCUSSION AND CONCLUSION: This simple model can be utilized to evaluate a therapeutic strategy for the treatment of metabolic syndrome and NAFLD with skin inflammation and also to understand the nexus between these co-morbidities." As taken from Kulkami NM et al. 2015. Pharm. Biol. 53(8), 1110-7. PubMed, 2016 available at: http://www.ncbi.nlm.nih.gov/pubmed/25430922

"The current study evaluates the impact of high fructose feeding in rat model of gentamicin induced nephrotoxicity. Sprague-Dawley rats weighing 180-200 g were randomized into four groups; (C) received standard rodents chow with free access to ad libitum drinking water for 8 weeks and was considered as control, (F) received standard rodents chow with free access to drinking water supplemented with 20% (W/V) fructose for the same abovementioned period, (FG) was fed as group F and was given 80 mg/kg (body weight)/day gentamicin sulphate intraperitoneally during the last 20 days of the feeding period, and (G) was given gentamicin as above and fed as group C. Renal function was assessed at the end of the treatment period through measuring serum creatinine, uric acid and albumin, creatinine clearance, absolute and fractional excretion of both sodium and potassium, twenty-four-hour urinary excretion of albumin, and renal histology. For metabolic syndrome assessment, fasting plasma glucose and insulin were measured and oral glucose

tolerance test was performed throughout the treatment period. Results showed that gentamicin enhances progression of fructose induced metabolic syndrome. On the other hand, fructose pretreatment before gentamicin injection produced a comparable degree of renal dysfunction to those which were given fructose-free water but the picture of nephrotoxicity was somewhat altered as it was characterized by higher extent of glomerular congestion and protein urea. Overall, more vigilance is required when nephrotoxic drugs are prescribed for patients with fructose induced metabolic syndrome." As taken from Ibraheem ZO et al. 2014. Biomed. Res. Int. 2014, 823879. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25045706

"We investigated the capacity of dietary (-)-epicatechin (EC) to mitigate insulin resistance through the modulation of redox-regulated mechanisms in a rat model of metabolic syndrome. Adolescent rats were fed a regular chow diet without or with high fructose (HFr; 10% w/v) in drinking water for 8 weeks, and a group of HFr-fed rats was supplemented with EC in the diet. HFr-fed rats developed insulin resistance, which was mitigated by EC supplementation. Accordingly, the activation of components of the insulin signaling cascade (insulin receptor, IRS1, Akt, and ERK1/2) was impaired, whereas negative regulators (PKC, IKK, JNK, and PTP1B) were upregulated in the liver and adipose tissue of HFr rats. These alterations were partially or totally prevented by EC supplementation. In addition, EC inhibited events that contribute to insulin resistance: HFr-associated increased expression and activity of NADPH oxidase, activation of redox-sensitive signals, expression of NF-κB-regulated proinflammatory cytokines and chemokines, and some sub-arms of endoplasmic reticulum stress signaling. Collectively, these findings indicate that EC supplementation can mitigate HFr-induced insulin resistance and are relevant for defining interventions that can prevent/mitigate MetS-associated insulin resistance." As taken from Bettajeb A et al. 2014. Free Radic. Biol. Med. 72, 247-56. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24746618

"BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) is a common liver disease in obese children. Diets high in added fructose (high fructose corn syrup; HFCS) and glycemic index (GI)/glycemic load (GL) are associated with increased risk of NAFLD. Lifestyle modification is the main treatment, but no guidelines regarding specific dietary interventions for childhood NAFLD exist. We hypothesized that reductions in dietary fructose (total, free, and HFCS)/GI/GL over 6 months would result in improvements in body composition and markers of liver dysfunction and cardiometabolic risk in childhood NAFLD. METHODS: Children and adolescents with NAFLD (n = 12) and healthy controls (n = 14) 7-18 years were studied at baseline and 3 and 6 months post-dietary intervention. Plasma markers of liver dysfunction (ALT, AST, γGT), cardiometabolic risk (TG, total cholesterol, LDL-HDL cholesterol, Apo-B100, Apo-B48, Apo-CIII, insulin, homeostasis model of assessment of insulin resistance [HOMA-IR]), inflammation (TNF-α, IL-6, IL-10), anthropometric, and blood pressure (BP) were studied using validated methodologies. RESULTS: Significant reductions in systolic BP (SBP), percentage body fat (BF), and plasma concentrations of ALT (P = .04), Apo-B100 (P < .001), and HOMA-IR were observed in children with NAFLD at 3 and 6 months (P < .05). Dietary reductions in total/free fructose/HFCS and GL were related to reductions in SBP (P = .01), ALT (P = .004), HOMA-IR (P = .03), and percentage BF in children with NAFLD. Reductions in dietary GI were associated with reduced plasma Apo-B100 (P = .02) in both groups. With the exception of Apo-B100, no changes in laboratory variables were observed in the control group. CONCLUSION: Modest reductions in fructose (total/free, HFCS) and GI/GL intake result in improvements of plasma markers of liver dysfunction and cardiometabolic risk in childhood NAFLD." As taken from Mager DR et

al. 2015. JPEN J. Parenter. Enteral. Nutr. 39(1), 73-84. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/23976771

“BACKGROUND: Concerns have been raised about the concurrent temporal trend between simple sugar intakes, especially of fructose or high-fructose corn syrup (HFCS), and rates of nonalcoholic fatty liver disease (NAFLD) in the United States. OBJECTIVE: We examined the effect of different amounts and forms of dietary fructose on the incidence or prevalence of NAFLD and indexes of liver health in humans. DESIGN: We conducted a systematic review of English-language, human studies of any design in children and adults with low to no alcohol intake and that reported at least one predetermined measure of liver health. The strength of the evidence was evaluated by considering risk of bias, consistency, directness, and precision. RESULTS: Six observational studies and 21 intervention studies met the inclusion criteria. The overall strength of evidence for observational studies was rated insufficient because of high risk of biases and inconsistent study findings. Of 21 intervention studies, 19 studies were in adults without NAFLD (predominantly healthy, young men) and 1 study each in adults or children with NAFLD. We found a low level of evidence that a hypercaloric fructose diet (supplemented by pure fructose) increases liver fat and aspartate aminotransferase (AST) concentrations in healthy men compared with the consumption of a weight-maintenance diet. In addition, there was a low level of evidence that hypercaloric fructose and glucose diets have similar effects on liver fat and liver enzymes in healthy adults. There was insufficient evidence to draw a conclusion for effects of HFCS or sucrose on NAFLD. CONCLUSIONS: On the basis of indirect comparisons across study findings, the apparent association between indexes of liver health (ie, liver fat, hepatic de novo lipogenesis, alanine aminotransferase, AST, and γ-glutamyl transpeptase) and fructose or sucrose intake appear to be confounded by excessive energy intake. Overall, the available evidence is not sufficiently robust to draw conclusions regarding effects of fructose, HFCS, or sucrose consumption on NAFLD.” As taken from Chung M et al. 2014. Am. J. Clin. Nutr. 100(3), 833-49. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25099546

“AIM: To develop an animal model that encompasses the different facets of non-alcoholic steatohepatitis (NASH), which has been a challenge. METHODS: In this study, we used a high fat diet (HFD) feeding supplemented with fructose and sucrose in the water mimicking the high-fructose corn syrup that is abundant in the diet in the United States. We used C57Bl/6 wild-type mice for short and long-term feedings of 6 and 16 wk respectively, and evaluated the extent of liver damage, steatosis, and inflammasome activation. Our methods included histopathological analysis to assess liver damage and steatosis, which involved H and E and oil-red-o staining; biochemical studies to look at ALT and triglyceride levels; RNA analysis using quantitative polymerase chain reaction; and cytokine analysis, which included the enzyme-linked immunosorbent assay method to look at interleukin (IL)-1β and tumor necrosis factor-α (TNFα) levels. Furthermore, at each length of feeding we also looked at insulin resistance and glucose tolerance using insulin tolerance tests (ITT) and glucose tolerance tests. RESULTS: There was no insulin resistance, steatosis, or inflammasome activation at 6 wk. In contrast, at 16 wk we found significant insulin resistance demonstrated by impaired glucose and ITT in male, but not female mice. In males, elevated alanine aminotransferase and triglyceride levels, indicated liver damage and steatosis, respectively. Increased liver TNFα and monocyte chemoattractant protein-1 mRNA and protein, correlated with steatohepatitis. The inflammasome components, adaptor molecule, Aim2, and NOD-like receptor 4, increased at the mRNA level, and functional inflammasome activation was indicated by increased caspase-1 activity and IL-1β

protein levels in male mice fed a long-term HFD. Male mice on HFD had increased α-smooth muscle actin and pro-collagen-1 mRNA indicating evolving fibrosis. In contrast, female mice displayed only elevated triglyceride levels, steatosis, and no fibrosis. CONCLUSION: Our data indicate gender differences in NASH. Male mice fed a long-term HFD display steatohepatitis and inflammasome activation, whereas female mice have steatosis without inflammation." As taken from Ganz M et al. 2014. World J. Gastroenterol. 20(26), 8525-34. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25024607

"This review examines the current evidence of the relationship between sugar consumption and the development of retinal and other eye diseases including diabetic retinopathy, hypertensive retinopathy, age-related macular degeneration, non-arteritic anterior ischaemic optic neuropathy and cataract. Sucrose is comprised of fructose and glucose. Sugar consumption has increased five-fold over the last century, with high quantities of sucrose and high-fructose corn syrup found in processed food and soft drinks. This increased consumption is increasingly recognized as a central factor in the rapidly rising rates of obesity and type 2 diabetes. The body metabolizes fructose and glucose differently, with fructose appearing to have the greater propensity to contribute to the metabolic syndrome. This review examines the effect of high rates of dietary consumption of refined carbohydrates on the eye, including the effect of chronic hyperglycaemia on microvascular disease in diabetic retinopathy, and the pathophysiological changes in the retinal circulation in hypertensive retinopathy." As taken from Kearney FM et al. 2014. Clin. Experiment. Ophthalmol. 42(6), 564-73. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24373051

"This study investigates the possible protective effects of levocetirizine against fructose-induced insulin resistance, hepatic steatosis and vascular dysfunction, in comparison to pioglitazone, a standard insulin sensitizer. Male Sprague Dawley rats (150-200 g) were divided into 4 groups. Three groups were fed on high fructose diets (HFD) containing 60% w/w fructose, while the fourth control group was fed on standard laboratory food for 8 weeks. AUCOGTT, AUCITT, fasting glucose, HOMA-IR, hepatic glutathione (GSH) and malondialdehyde (MDA) levels, serum total cholesterol, LDL-C, C-reactive protein (CRP) level and lactate dehydrogenase (LDH) activity and liver steatosis scores were significantly higher in HFD group compared to control group. Moreover, body weight gain, food intake, feeding efficiency, HOMA-β, Emax and pEC50 of acetylcholine-induced relaxations of aortic rings and hepatic superoxide dismutase (SOD) activity were significantly lower in HFD group than in control group. Treatment with levocetirizine caused significant decreases in AUCOGTT, AUCITT, HOMA-IR, hepatic GSH and MDA levels and serum CRP level and LDH activity and significant increases in hepatic SOD activity and HOMA-β when compared with the HFD group. Although levocetirizine failed to alter TC and LDL-C levels, it produced a significant increase in HDL-C level relative to control group. Levocetirizine was also able to improve acetylcholine-induced relaxations of aortic rings, indicating a protective effect against insulin resistance-induced endothelial damage comparable to that offered by pioglitazone. Moreover, levocetirizine substantially attenuated insulin resistance-associated liver macrovesicular steatosis. These findings demonstrate that levocetirizine ameliorates insulin resistance, improves glucose tolerance and attenuates insulin resistance-linked hepatic steatosis and vascular damage." As taken from Shawkey NM et al. 2014. Eur. J. Pharmacol. 740, 353-63. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/25064340

“High-fructose feeding impairs copper status and leads to low copper availability, which is a novel mechanism in obesity-related fatty liver. Copper deficiency-associated hepatic iron overload likely plays an important role in fructose-induced liver injury. Excess iron in the liver is distributed throughout hepatocytes and Kupffer cells (KCs). The aim of this study was to examine the role of KCs in the pathogenesis of nonalcoholic fatty liver disease induced by a marginal-copper high-fructose diet (CuMF). Male weanling Sprague-Dawley rats were fed either a copper-adequate or a marginally copper-deficient diet for 4 wk. Deionized water or deionized water containing 30% fructose (wt/vol) was also given ad libitum. KCs were depleted by intravenous administration of gadolinium chloride (GdCl3) before and/or in the middle of the experimental period. Hepatic triglyceride accumulation was completely eliminated with KC depletion in CuMF consumption rats, which was associated with the normalization of elevated plasma monocyte chemoattractant protein-1 (MCP-1) and increased hepatic sterol regulatory element binding protein-1 expression. However, hepatic copper and iron content were not significantly affected by KC depletion. In addition, KC depletion reduced body weight and epididymal fat weight as well as adipocyte size. Plasma endotoxin and gut permeability were markedly increased in CuMF rats. Moreover, MCP-1 was robustly increased in the culture medium when isolated KCs from CuMF rats were treated with LPS. Our data suggest that KCs play a critical role in the development of hepatic steatosis induced by marginal-copper high-fructose diet.” As taken from Song M et al. 2015. Am. J. Physiol. Gastrointest. Liver Physiol. 308, G934-45. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25813056

“Fructose consumption has dramatically increased in the last 30 years. The principal form has been in the form of high-fructose corn syrup found in soft drinks and processed food. The effect of excessive fructose consumption on human health is only beginning to be understood. Fructose has been confirmed to induce several obesity-related complications associated with the metabolic syndrome. Here we present an overview of fructose metabolism and how it contrasts with that of glucose. In addition, we examine how excessive fructose consumption can affect de novo lipogenesis, insulin resistance, inflammation, and reactive oxygen species production. Fructose can also induce a change in the gut permeability and promote the release of inflammatory factors to the liver, which has potential implications in increasing hepatic inflammation. Moreover, fructose has been associated with colon, pancreas, and liver cancers, and we shall discuss the evidence for these observations. Taken together, data suggest that sustained fructose consumption should be curtailed as it is detrimental to long-term human health.” As taken from Charrez B et al. 2015. Horm. Mol. Biol. Clin. Investig. 22, 79-89. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/25965509

“The obesity epidemic in the U.S. has led to extensive research into potential contributing dietary factors, especially fat and fructose. Recently, increased consumption of soybean oil, which is rich in polyunsaturated fatty acids (PUFAs), has been proposed to play a causal role in the epidemic. Here, we designed a series of four isocaloric diets (HFD, SO-HFD, F-HFD, F-SO-HFD) to investigate the effects of saturated versus unsaturated fat, as well as fructose, on obesity and diabetes. C57/BL6 male mice fed a diet moderately high in fat from coconut oil and soybean oil (SO-HFD, 40% kcal total fat) showed statistically significant increases in weight gain, adiposity, diabetes, glucose intolerance and insulin resistance compared to mice on a diet consisting primarily of coconut oil (HFD). They also had fatty livers with hepatocyte ballooning and very large lipid droplets as well as shorter colonic crypt length. While the high fructose diet (F-HFD) did not cause as much obesity or diabetes as SO-HFD, it did cause rectal prolapse and a very fatty liver, but no balloon injury. The

coconut oil diet (with or without fructose) increased spleen weight while fructose in the presence of soybean oil increased kidney weight. Metabolomics analysis of the liver showed an increased accumulation of PUFAs and their metabolites as well as γ-tocopherol, but a decrease in cholesterol in SO-HFD. Liver transcriptomics analysis revealed a global dysregulation of cytochrome P450 (Cyp) genes in SO-HFD versus HFD livers, most notably in the Cyp3a and Cyp2c families. Other genes involved in obesity (e.g., Cidec, Cd36), diabetes (Igfbp1), inflammation (Cd63), mitochondrial function (Pdk4) and cancer (H19) were also upregulated by the soybean oil diet. Taken together, our results indicate that in mice a diet high in soybean oil is more detrimental to metabolic health than a diet high in fructose or coconut oil." As taken from Deol P et al. 2015. PLoS One 10, e0132672. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26200659

"Currently, the metabolic syndrome (MS) is occurring at growing rates worldwide, raising extensive concerns on the mechanisms and therapeutic interventions for this disorder. Herein, we described a novel method of establishing MS model in rodents. Male Institute of Cancer Research (ICR) mice were fed with high-fat-high-fructose (HFHF) diet or normal chow (NC) respectively for 12 weeks. Metabolic phenotypes were assessed by glucose tolerance test, insulin tolerance test and hyperinsulinemic-euglycemic clamp. Blood pressure was measured by a tail-cuff system. At the end of the experiment, mice were sacrificed, and blood and tissues were harvested for subsequent analysis. Serum insulin levels were measured by ELISA, and lipid profiles were determined biochemically. The HFHF diet-fed ICR mice exhibited obvious characteristics of the components of MS, including obvious obesity, severe insulin resistance, hyperinsulinemia, dislipidemia, significant hypertension and hyperuricemia. Our data suggest that HFHF diet-fed ICR mice may be a robust and efficient animal model that could well mimic the basic pathogenesis of human MS." As taken from Zhuhua Z et al. 2015. Exp. Anim. 64, 435-42. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26134356

"BACKGROUND: Debate over the role of fructose in mediating cardiovascular risk remains active. To update the evidence on the effect of fructose on established therapeutic lipid targets for cardiovascular disease (low-density lipoprotein cholesterol [LDL]-C, apolipoprotein B, non-high-density lipoprotein cholesterol [HDL-C]), and metabolic syndrome (triglycerides and HDL-C), we conducted a systematic review and meta-analysis of controlled feeding trials. METHODS AND RESULTS: MEDLINE, EMBASE, CINHAL, and the Cochrane Library were searched through July 7, 2015 for controlled feeding trials with follow-up ≥7 days, which investigated the effect of oral fructose compared to a control carbohydrate on lipids (LDL-C, apolipoprotein B, non-HDL-C, triglycerides, and HDL-C) in participants of all health backgrounds. Two independent reviewers extracted relevant data. Data were pooled using random effects models and expressed as mean difference with 95% CI. Interstudy heterogeneity was assessed (Cochran Q statistic) and quantified (I(2) statistic). Eligibility criteria were met by 51 isocaloric trials (n=943), in which fructose was provided in isocaloric exchange for other carbohydrates, and 8 hypercaloric trials (n=125), in which fructose supplemented control diets with excess calories compared to the control diets alone without the excess calories. Fructose had no effect on LDL-C, non-HDL-C, apolipoprotein B, triglycerides, or HDL-C in isocaloric trials. However, in hypercaloric trials, fructose increased apolipoprotein B (n=2 trials; mean difference = 0.18 mmol/L; 95% CI: 0.05, 0.30; P=0.005) and triglycerides (n=8 trials; mean difference = 0.26 mmol/L; 95% CI: 0.11, 0.41; P<0.001). The study is limited by small sample sizes, limited follow-up, and low quality scores of the included trials. CONCLUSIONS: Pooled analyses showed that fructose only had an adverse effect on established lipid targets when added to existing diets so as to

provide excess calories (+21% to 35% energy). When isocalorically exchanged for other carbohydrates, fructose had no adverse effects on blood lipids. More trials that are larger, longer, and higher quality are required." As taken from Chiavaroli L et al. 2015. J. Am. Heart Assoc. 4, e001700. PubMed, 2016 available at http://www.ncbi.nlm.nih.gov/pubmed/26358358

"BACKGROUND: The effect of Hibiscus sabdariffa calyx extract was evaluated in high-fructose-induced metabolic syndrome rats. Insulin resistance, hyperglycemia, dyslipidemia and oxidative rout were induced in rats using high-fructose diet. High-fructose diet-fed rats were administered 100 and 200 mg kg(-1) body weight of H. sabdariffa extract for 3 weeks, starting from week 7 of high-fructose diet treatment. RESULTS: High-fructose diet significantly (P < 0.05) increased the serum levels of blood glucose, insulin, total cholesterol (TC), triacylglycerol (TAG), low-density lipoprotein cholesterol (LDLc) and very-low-density lipoprotein cholesterol (VLDLc), with a concomitant reduction in high-density lipoprotein cholesterol (HDLc). These alterations were significantly ameliorated by the extract. High-fructose diet-mediated decreases in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione reductase (GSH-red) and glucose 6-phosphate dehydrogenase (Glc 6-PD) were significantly (P < 0.05) attenuated. Altered levels of reduced glutathione (GSH) and glutathione disulfide (GSSG) were significantly (P < 0.05) restored to normal. High-fructose diet-mediated increases in the concentrations of malondialdehyde, conjugated dienes, lipid hydroperoxides, protein carbonyl and percentage fragmented DNA were significantly (P < 0.05) lowered by the Hibiscus extract. CONCLUSION: Overall, aqueous extract of H. sabdariffa palliates insulin resistance, hyperglycemia, dyslipidemia and oxidative rout in high-fructose-induced metabolic syndrome rats." As taken from Ajiboye TO et al. 2016. J. Sci. Food Agric. 96(5), 1522-31. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/25965053

"With people aging, osteoporosis is expected to increase notably. Nutritional status is a relatively easily-modified risk factor, associated with many chronic diseases, and is involved in obesity, diabetes, and coronary heart disease (CHD), along with osteoporosis. Nutrients, such as fats, sugars, and proteins, play a primary function in bone metabolism and maintaining bone health. In Western nations, diets are generally high in saturated fats, however, currently, the nutritional patterns dominating in China continue to be high in carbohydrates from starch, cereals, and sugars. Moreover, high fat or high sugar (fructose, glucose, or sucrose) impart a significant impact on bone structural integrity. Due to diet being modifiable, demonstrating the effects of nutrition on bone health can provide an approach for osteoporosis prevention. Most researchers have reported that a high-fat diet consumption is associated with bone mineral density (BMD) and, as bone strength diminishes, adverse microstructure changes occur in the cancellous bone compartment, which is involved with lipid metabolism modulation disorder and the alteration of the bone marrow environment, along with an increased inflammatory environment. Some studies, however, demonstrated that a high-fat diet contributes to achieving peak bone mass, along with microstructure, at a younger age. Contrary to these results, others have shown that a high-fructose diet consumption leads to stronger bones with a superior microarchitecture than those with the intake of a high-glucose diet and, at the same time, research indicated that a high-fat diet usually deteriorates cancellous bone parameters, and that the incorporation of fructose into a high-fat diet did not aggravate bone mass loss. High-fat/high-sucrose diets have shown both beneficial and detrimental influences on bone metabolism. Combined, these studies showed that nutrition exerts different effects on bone

health. Thus, a better understanding of the regulation between dietary nutrition and bone health might provide a basis for the development of strategies to improve bone health by modifying nutritional components." As taken from Tian L and Yu X. 2017. Nutrients 9(5), E506. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28513571

"The modern Western society lifestyle is characterized by a hyperenergetic, high sugar containing food intake. Sugar intake increased dramatically during the last few decades, due to the excessive consumption of high-sugar drinks and high-fructose corn syrup. Current evidence suggests that high fructose intake when combined with overeating and adiposity promotes adverse metabolic health effects including dyslipidemia, insulin resistance, type II diabetes, and inflammation. Similarly, elevated glucocorticoid levels, especially the enhanced generation of active glucocorticoids in the adipose tissue due to increased 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) activity, have been associated with metabolic diseases. Moreover, recent evidence suggests that fructose stimulates the 11β-HSD1-mediated glucocorticoid activation by enhancing the availability of its cofactor NADPH. In adipocytes, fructose was found to stimulate 11β-HSD1 expression and activity, thereby promoting the adipogenic effects of glucocorticoids. This article aims to highlight the interconnections between overwhelmed fructose metabolism, intracellular glucocorticoid activation in adipose tissue, and their metabolic effects on the progression of the metabolic syndrome." As taken from Legeza B et al. 2017. Nutrients 9(5), E426. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28445389

"AIM: To investigate the effects of fructose consumption on the antioxidant capacity in heart and kidney, cardiovascular disease risk factors, and evaluation of these variables after its removal. METHODS: Male Wistar rats (n = 36) were divided into control group (n = 12): standard chow + water or F group: standard chow + fructose in drinking water (30%) for 15 weeks. After, F group was divided to continue receiving standard chow + fructose in drinking water (30%) (n = 12) or standard chow + water (Ex group, n = 12) for 9 weeks. Water, chow and caloric diaries intake, final body weight, adiposity index, plasma glucose and triacylglycerol, systolic blood pressure, and cardiac and renal hydrophilic antioxidant capacity were analyzed. RESULTS: Control and Ex groups consumed less chow and water compared to F group. Caloric intake was higher in control group. There was no difference in final body weight and adiposity index. Systolic blood pressure and cardiac and renal hydrophilic antioxidant capacity were worst in F group. CONCLUSION: Prolonged exposure to fructose induces oxidative stress, systolic blood pressure, and increase in triacylglycerol. When stopped fructose consumption, Ex group presented improvement in these variables, suggesting the toxicity effect of fructose when consumed in high amounts and prolonged exposure." As taken from Francisqueti FV et al. 2016. SAGE Open Med. 4, 2050312116684294. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28348741

"A high consumption of fat and simple sugars, especially fructose, has been related to the development of insulin resistance, but the mechanisms involved in the effects of these nutrients are not fully understood. This study investigates the effects of a Western-type diet and liquid fructose supplementation, alone and combined, on insulin signalling and inflammation in low-density lipoprotein (LDL) receptor-deficient mice (LDL-R-/-). LDL-R-/- mice were fed chow or Western diet ±15% fructose solution for 12 weeks. Plasma glucose and insulin, and the expression of genes related to inflammation in the liver and visceral white adipose tissue (vWAT), were analysed. V-akt murine thymoma viral oncogene homolog-2 (Akt) activation was measured in the liver of the mice after a single injection of

saline or insulin. None of the dietary interventions caused inflammation in vWAT, whereas the Western diet induced hepatic inflammation, which was further enhanced by liquid fructose, leading also to a significant increase in fibrogenesis markers. However, there was no change in plasma glucose or insulin, or insulin-induced Akt phosphorylation. In conclusion, hepatic inflammation and fibrogenesis markers induced by a Western diet supplemented with liquid fructose in LDL-R-/- mice are not associated with a significant impairment of hepatic insulin signalling." As taken from Sangüesa G et al. 2017. Nutrients 9(3), E278. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28294959

"The effects of high (H)-fructose (FR) diet (D) (HFRD) on hepatic lipid homeostasis, oxidative stress, inflammation and hepatocyte apoptosis were investigated in 6-week old female C57BL/6J mice fed a regular chow (ContD) or HFRD (35% fructose-derived calories) for 3 weeks. HFRD-fed mice exhibited increased levels of hepatic steatosis with a significant elevation of serum levels of triglyceride, cholesterol and TNFα compared to ContD-fed mice (P<0.05). HFRD-fed mice exhibited ~2.7- fold higher levels FAS along with significantly decreased protein levels of adiponection-R2 (~30%), P-AMPK (~60%), P-ACC (~70%) and RXR-α (~55%), suggesting decreased hepatic fat oxidation compared to controls. Interestingly, hepatic fatty acid uptake into hepatocytes and lipolysis were significantly increased in HFRD-fed mice, as shown by decreased CD36 and fatty acid transporter protein-2, and increased adipose triglyceride lipase, respectively (P<0.05). Increased hepatic levels of iNOS and GSSG/GSH suggest elevated oxidative stress with a higher number of macrophages in the adipose tissue in HFRD-fed mice (P<0.05). Significantly elevated rates of hepatocyte apoptosis (~2.4-fold), as determined by TUNEL analysis with increased Bax/Bcl2 ratio and PARP-1 levels (~2- and 1.5-fold, respectively), were observed in HFRD-fed mice. Thus, HFRD exposure increased hepatic steatosis accompanied by oxidative stress and inflammation, leading to hepatocyte apoptosis." As taken from Choi Y et al. 2017. Food Chem. Toxicol. 103, 111-121. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28257781

"Glucose is a major energy source for the entire body, while fructose metabolism occurs mainly in the liver. Fructose consumption has increased over the last decade globally and is suspected to contribute to the increased incidence of non-alcoholic fatty liver disease (NAFLD). NAFLD is a manifestation of metabolic syndrome affecting about one-third of the population worldwide and has progressive pathological potential for liver cirrhosis and cancer through non-alcoholic steatohepatitis (NASH). Here we have reviewed the possible contribution of fructose to the pathophysiology of NAFLD. We critically summarize the current findings about several regulators, and their potential mechanisms, that have been studied in humans and animal models in response to fructose exposure. A novel hypothesis on fructose-dependent perturbation of liver regeneration and metabolism is advanced. Fructose intake could affect inflammatory and metabolic processes, liver function, gut microbiota, and portal endotoxin influx. The role of the brain in controlling fructose ingestion and the subsequent development of NAFLD is highlighted. Although the importance for fructose (over)consumption for NAFLD in humans is still debated and comprehensive intervention studies are invited, understanding of how fructose intake can favor these pathological processes is crucial for the development of appropriate noninvasive diagnostic and therapeutic approaches to detect and treat these metabolic effects. Still, lifestyle modification, to lessen the consumption of fructose-containing products, and physical exercise are major measures against NAFLD. Finally, promising drugs against fructose-induced insulin resistance and hepatic dysfunction that are emerging from studies in rodents are reviewed, but need further validation in human patients." As taken from

Alwahsh SM and Gebhardt R. 2017. Arch. Toxicol. 91(4), 1545-1563. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27995280

"BACKGROUND: A high intake of fructose increases the risk for hyperuricemia. It has been reported that long-term fructose consumption suppressed renal uric acid excretion and increased serum uric acid level. However, the effect of single administration of fructose on excretion of uric acid has not been clarified. METHODS: We used male Wistar rats, which were orally administered fructose (5g/kg). Those rats were used in each experiment at 12h after administration. RESULTS: Single administration of fructose suppressed the function of ileal uric acid excretion and had no effect on the function of renal uric acid excretion. Breast cancer resistance protein (BCRP) predominantly contributes to intestinal excretion of uric acid as an active homodimer. Single administration of fructose decreased BCRP homodimer level in the ileum. Moreover, diphenyleneiodonium (DPI), an inhibitor of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Nox), recovered the suppression of the function of ileal uric acid excretion and the Bcrp homodimer level in the ileum of rats that received single administration of fructose. CONCLUSIONS: Single administration of fructose decreases in BCRP homodimer level, resulting in the suppression the function of ileal uric acid excretion. The suppression of the function of ileal uric acid excretion by single administration of fructose is caused by the activation of Nox. The results of our study provide a new insight into the mechanism of fructose-induced hyperuricemia." As taken from Kaneko C et al. 2017. Biochim. Biophys. Acta 1861(3), 559-566. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27913188

"BACKGROUND: Sugar-sweetened beverage (SSB) consumption and low-grade chronic inflammation are both independently associated with type 2 diabetes and cardiovascular disease. Fructose, a major component of SSBs, may acutely trigger inflammation, which may be one link between SSB consumption and cardiometabolic disease. OBJECTIVE: We sought to determine whether beverages sweetened with fructose, high-fructose corn syrup (HFCS), and glucose differentially influence systemic inflammation [fasting plasma C-reactive protein and interleukin-6 (IL-6) as primary endpoints] acutely and before major changes in body weight. Secondary endpoints included adipose tissue inflammation, intestinal permeability, and plasma fetuin-A as potential mechanistic links between fructose intake and low-grade inflammation. DESIGN: We conducted a randomized, controlled, double-blind, crossover design dietary intervention (the Diet and Systemic Inflammation Study) in 24 normal-weight to obese adults without fructose malabsorption. Participants drank 4 servings/d of fructose-, glucose-, or HFCS-sweetened beverages accounting for 25% of estimated calorie requirements while consuming a standardized diet ad libitum for three 8-d periods. RESULTS: Subjects consumed 116% of their estimated calorie requirement while drinking the beverages with no difference in total energy intake or body weight between groups as reported previously. Fasting plasma concentrations of C-reactive protein and IL-6 did not differ significantly at the end of the 3 diet periods. We did not detect a consistent differential effect of the diets on measures of adipose tissue inflammation except for adiponectin gene expression in adipose tissue (P = 0.005), which was lowest after the glucose phase. We also did not detect consistent evidence of a differential impact of these sugars on measures of intestinal permeability (lactulose:mannitol test, plasma zonulin, and plasma lipopolysaccharide-binding protein). CONCLUSION: Excessive amounts of fructose, HFCS, and glucose from SSBs consumed over 8 d did not differentially affect low-grade chronic systemic inflammation in normal-weight to obese adults." As taken from Kuzma JN et al. 2016. Am. J. Clin. Nutr. 104(2), 306-14. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27357093

“BACKGROUND: Obesity and the metabolic syndrome occur in approximately one-third of patients with alcoholic liver disease (ALD). The increased consumption of fructose parallels the increased prevalence of obesity and the metabolic syndrome in the United States and worldwide. In this study, we investigated whether dietary high fructose potentiates chronic alcohol-induced liver injury, and explored potential mechanism(s). METHODS: Six-week-old male C57BL/6J mice were assigned to 4 groups: control, high fructose, chronic ethanol (EtOH), and high fructose plus chronic alcohol. The mice were fed either control diet or high-fructose diet (60%, w/w) for 18 weeks. Chronic alcohol-fed mice were given 20% (v/v) ethanol (Meadows-Cook model) ad libitum as the only available liquid from the 9th week through the 18th week. Liver injury, steatosis, hepatic inflammatory gene expression, and copper status were assessed. RESULTS: High-fructose diet and chronic alcohol consumption alone each induce hepatic fat accumulation and impair copper status. However, the combination of dietary high fructose plus chronic alcohol synergistically induced liver injury as evidenced by robustly increased plasma alanine aminotransferase and aspartate aminotransferase, but the combination did not exacerbate hepatic fat accumulation nor worsen copper status. Moreover, FE-fed mice were characterized by prominent microvesicular steatosis. High-fructose diet and chronic alcohol ingestion together led to a significant up-regulation of Kupffer cell (KC) M1 phenotype gene expression (e.g., tumor necrosis factor-α and monocyte chemoattractant protein-1), as well as Toll-like receptor 4 (TLR4) signaling gene expression, which is also associated with the up-regulation of KCs and activation marker gene expression, including Emr1, CD68, and CD163. CONCLUSIONS: Our data suggest that dietary high fructose may potentiate chronic alcohol consumption-induced liver injury. The underlying mechanism might be due to the synergistic effect of dietary high fructose and alcohol on the activation of the TLR4 signaling pathway, which in turn leads to KC activation and phenotype switch toward M1 polarization. This study suggests that alcohol-fructose combination contributes to ALD progression.” As taken from Song M et al. 2016. Alcohol Clin. Exp. Res. 40(3), 518-28. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26858005

“OBJECTIVE: Increased fructose consumption is a contributor to the burgeoning epidemic of non-alcoholic fatty liver disease (NAFLD). Recent evidence indicates that the metabolic hormone FGF21 is regulated by fructose consumption in humans and rodents and may play a functional role in this nutritional context. Here, we sought to define the mechanism by which fructose ingestion regulates FGF21 and determine whether FGF21 contributes to an adaptive metabolic response to fructose consumption. METHODS: We tested the role of the transcription factor carbohydrate responsive-element binding protein (ChREBP) in fructose-mediated regulation of FGF21 using ChREBP knockout mice. Using FGF21 knockout mice, we investigated whether FGF21 has a metabolic function in the context of fructose consumption. Additionally, we tested whether a ChREBP-FGF21 interaction is likely conserved in human subjects. RESULTS: Hepatic expression of ChREBP-β and Fgf21 acutely increased 2-fold and 3-fold, respectively, following fructose gavage, and this was accompanied by increased circulating FGF21. The acute increase in circulating FGF21 following fructose gavage was absent in ChREBP knockout mice. Induction of ChREBP-β and its glycolytic, fructolytic, and lipogenic gene targets were attenuated in FGF21 knockout mice fed high-fructose diets, and this was accompanied by a 50% reduction in de novo lipogenesis a, 30% reduction VLDL secretion, and a 25% reduction in liver fat compared to fructose-fed controls. In human subjects, serum FGF21 correlates with de novo lipogenic rates measured by stable isotopic tracers (R = 0.55, P = 0.04) consistent with conservation of a ChREBP-FGF21 interaction. After 8 weeks of high-fructose diet, livers from FGF21

knockout mice demonstrate atrophy and fibrosis accompanied by molecular markers of inflammation and stellate cell activation; whereas, this did not occur in controls. CONCLUSIONS: In summary, ChREBP and FGF21 constitute a signaling axis likely conserved in humans that mediates an essential adaptive response to fructose ingestion that may participate in the pathogenesis of NAFLD and liver fibrosis." As taken from Fisher FM et al. 2016. Mol. Metab. 6(1), 14-21. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/28123933

"The potential impact on health of diets rich in free sugars, and particularly fructose, is of major concern. The focus of this review is the impact of these sugars on insulin resistance and obesity, and the associated risk of developing type 2 diabetes. Much of the concern is focussed on specific metabolic effects of fructose, which are argued to lead to increased fat deposition in the liver and skeletal muscle with subsequent insulin resistance and increased risk of diabetes. However, much of the evidence underpinning these arguments is based on animal studies involving very large intakes of the free sugars. Recent human studies, in the past 5 years, provide a rather different picture, with a clear dose response link between fructose intake and metabolic changes. In particular, the most marked effects are observed when a high sugars intake is accompanied by an excess energy intake. This does not mean that a high intake of free sugars does not have any detrimental impact on health, but rather that such an effect seems more likely to be a result of the high sugars intake increasing the chances of an excessive energy intake rather than it leading to a direct detrimental effect on metabolism." As taken from Macdonald IA. 2016. Eur. J. Nutr. 55(Suppl 2), 17-23. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27882410

"Purpose: Relatively little is known about gender-dependent susceptibility to hepatic injury induced by nutritional factors. In the current study, we investigated dietary fructose-induced hepatic degeneration and roles of endothelial nitric oxide synthase (eNOS), insulin receptor (IRβ) and substrate-1 (IRS-1) expressions in association with inflammatory markers in male and female rats. Moreover, we examined potential effect of resveratrol on fructose-induced changes. Methods: Male and female rats were divided into 4 groups as control, resveratrol, fructose and resveratrol plus fructose. All rats were fed with a standard diet with or without resveratrol (500 mg/kg). Fructose was given as 10% in drinking waterfor 24 weeks. Results: Long-term dietary fructose caused parenchymal degeneration and hyperemia in association with impaired eNOS mRNA/protein expressions in liver of male and female rats. This dietary intervention also led to increases in hepatic triglyceride content, TNFα and IL-1β levels in both genders. Gender-related differences to consequence of fructose consumption were not obvious. Resveratrol supplementation markedly attenuated hepatic degeneration, hyperemia and triglyceride content in association with reduced TNFα and IL-1β levels, but enhanced IRβ mRNA and IRS-1 protein, in male and female rats upon fructose feeding. Conclusion: Long-term dietary fructose causes hepatic degeneration possibly via a decrease in eNOS, but increase in TNFα and IL-1β, in both genders. Resveratrol supplementation improved fructose-induced hepatic injury." As taken from Pektas MB et al. 2017. Drug Res. (Stuttg.) 67(2), 103-110. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/27824430

"Nonalcoholic fatty liver disease (NAFLD) is a liver manifestation of metabolic syndrome. Overconsumption of high-fat diet (HFD) and increased intake of sugar-sweetened beverages are major risk factors for development of NAFLD. Today the most commonly consumed sugar is high fructose corn syrup. Hepatic lipids may be derived from dietary intake, esterification of plasma free fatty acids (FFA) or hepatic de novo lipogenesis (DNL).

A central abnormality in NAFLD is enhanced DNL. Hepatic DNL is increased in individuals with NAFLD, while the contribution of dietary fat and plasma FFA to hepatic lipids is not significantly altered. The importance of DNL in NAFLD is further established in mouse studies with knockout of genes involved in this process. Dietary fructose increases levels of enzymes involved in DNL even more strongly than HFD. Several properties of fructose metabolism make it particularly lipogenic. Fructose is absorbed via portal vein and delivered to the liver in much higher concentrations as compared to other tissues. Fructose increases protein levels of all DNL enzymes during its conversion into triglycerides. Additionally, fructose supports lipogenesis in the setting of insulin resistance as fructose does not require insulin for its metabolism, and it directly stimulates SREBP1c, a major transcriptional regulator of DNL. Fructose also leads to ATP depletion and suppression of mitochondrial fatty acid oxidation, resulting in increased production of reactive oxygen species. Furthermore, fructose promotes ER stress and uric acid formation, additional insulin independent pathways leading to DNL. In summary, fructose metabolism supports DNL more strongly than HFD and hepatic DNL is a central abnormality in NAFLD. Disrupting fructose metabolism in the liver may provide a new therapeutic option for the treatment of NAFLD." As taken from Softic S et al. 2016. Dig. Dis. Sci. 61(5), 1282-93. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26856717

"PURPOSE: In recent years, the increasing consumption of soft drinks containing high-fructose corn syrup or sucrose has caused a rise in fructose intake, which has been related to the epidemic of metabolic diseases. As fructose and glucose intake varies in parallel, it is still unclear what the effects of the increased consumption of the two single sugars are. In the present study, the impact of chronic consumption of glucose or fructose on skeletal muscle of healthy mice was investigated. METHODS: C57BL/6J male mice received water (C), 15 % fructose (ChF) or 15 % glucose (ChG) to drink for up to 7 months. Lipid metabolism and markers of inflammation and autophagy were assessed in gastrocnemius muscle. RESULTS: Increased body weight and gastrocnemius muscle mass, as well as circulating glucose, insulin, and lipid plasma levels were observed in sugar-drinking mice. Although triglycerides increased in the gastrocnemius muscle of both ChF and ChG mice (+32 and +26 %, vs C, respectively), intramyocellular lipids accumulated to a significantly greater extent in ChF than in ChG animals (ChF +10 % vs ChG). Such perturbations were associated with increased muscle interleukin-6 levels (threefold of C) and with the activation of autophagy, as demonstrated by the overexpression of LC3B-II (ChF, threefold and ChG, twofold of C) and beclin-1 (ChF, sevenfold and ChG, tenfold of C). CONCLUSIONS: The present results suggest that intramyocellular lipids and the pro-inflammatory signaling could contribute to the onset of insulin resistance and lead to the induction of autophagy, which could be an adaptive response to lipotoxicity." As taken from De Stefanis D et al. 2017. Eur. J. Nutr. 56(1), 363-373. PubMed, 2017 available at https://www.ncbi.nlm.nih.gov/pubmed/26487451

Type of Test	**Route of Exposure**	**Species Observed**	**Dose Data**	**Toxic Effects**	**Reference**
TDLo - Lowest published toxic dose	Oral	Rodent - mouse	2520 gm/kg/6W (continuous)	Kidney/Ureter/Bladder - renal function tests depressed Kidney/Ureter/Bladder - other changes Biochemical - Metabolism	JOETD7 Journal of Ethnopharmacology. (Elsevier Scientific Pub. Ireland Ltd., POB 85, Limerick, Ireland) V.1-1979-

				(Intermediary) - other proteins	Volume(issue)/page/year: 169,49,2015

As taken from RTECS, 2018.

“Fatal parenteral administrations of fructose and sorbitol cause acute hepatorenal failure associated with bleeding.

When taken orally in larger amounts sorbitol (and to a less extent fructose) can also cause laxative effects and gastrointestinal discomfort.”

“HFI is a hereditary autosomal recessive deficit in fructose 1,6-bisphosphate aldolase (aldolase B), the main enzyme responsible for hepatic metabolism of fructose. It is expressed in the liver, small intestine, and proximal renal tubule where it facilitates assimilation of dietary fructose by catalysing the cleavage of fructose-1-phosphate. A lack of functional aldolase B leads to an accumulation of toxic fructose 1-phosphate in liver cells, ultimately resulting in hepatic decompensation.

Clinical symptoms usually include vomiting, anorexia, gastro-intestinal disorders, apathy, height and weight retardation and hepatomegaly. Forced ingestion of a large amount of fructose may lead to acute hepatic decompensation, with major hepatocellular insufficiency, haemorrhagic syndrome and jaundice. The reason for the severe adverse events with infusion of fructose solution, including death, is not hypoglycaemia but the intracellular accumulation of fructose 1-phosphate which is highly toxic. Biochemical signs are postprandial hypoglycaemia, hypophosphatemia, hyperlactacidemia and hyperuricaemia. Prolongation of coagulation time, increased hepatic enzymes and increased bilirubin are also often present. Methionine and plasma tyrosin are often elevated. A proximal and distal renal tubulopathy is frequent.

Patients with HFI [hereditary fructose intolerance] develop a natural defence mechanism against fructose and sorbitol by vomiting any food containing either of the two substances. Since this defence mechanism is bypassed as soon as fructose or sorbitol is delivered intravenously, a much higher risk is associated with solutions for parenteral (especially intravenous) use.

In the fructose tolerance test which has been used in the past for diagnosis of HFI [hereditary fructose intolerance], patients are challenged IV with fructose 0.25 g/kg body weight. As a result glycogenolysis is inhibited and gluconeogenesis is impaired. Diagnosis by a fructose tolerance test leads to the same acute symptoms as HFI and can be life threatening. It can be deduced that this dose can definitely be regarded as toxic and should therefore be above a potential threshold.

A publication by Barshop et al. 2003 indicates that short-term (2 days) dietary intake of 4.7 mg of fructose/kg/day was safe and well tolerated among individuals with diagnosed HFI. Five subjects with HFI (ages 14–52 years; 4 males, 1 female) participated in a prospective, nonrandomised open challenge with FOS at 6 g/m2 /d for 2 days. Diet records were maintained for the 48-hr period and analysed for dietary fructose. Tolerance was assessed through evaluation of serum AST, ALT, GGT, glucose, bilirubin, uric acid, phosphorus, and electrolytes, upon initiation and at 12-hr intervals during the challenge. Blood chemistry values were within normal ranges and did not change appreciably during the study period, except for two patients with slight elevations of uric acid. One subject reported gastric discomfort on day 2 of the FOS challenge. These data suggest that approximately 4.7 mg

fructose/kg bw/day for 2 days is safe and well tolerated among individuals with diagnosed HFI."

As taken from EMA, 2017.

"Despite clinical findings suggesting that the form (liquid versus solid) of the sugars may significantly affect the development of metabolic diseases, no experimental data are available on the impact of their formulations on gut microbiota, integrity and hepatic outcomes. In the present sudy, C57Bl/6j mice were fed a standard diet plus water (SD), a standard diet plus 60% fructose syrup (L-Fr) or a 60% fructose solid diet plus water (S-Fr) for 12 weeks. Gut microbiota was characterized through 16S rRNA phylogenetic profiling and shotgun sequencing of microbial genes in ileum content and related volatilome profiling. Fructose feeding led to alterations of the gut microbiota depending on the fructose formulation, with increased colonization by Clostridium, Oscillospira and Clostridiales phyla in the S-Fr group and Bacteroides, Lactobacillus, Lachnospiraceae and Dorea in the L-Fr. S-Fr evoked the highest accumulation of advanced glycation end products and barrier injury in the ileum intestinal mucosa. These effects were associated to a stronger activation of the lipopolysaccharide-dependent proinflammatory TLR4/NLRP3 inflammasome pathway in the liver of S-Fr mice than of L-Fr mice. In contrast, L-Fr intake induced higher levels of hepatosteatosis and markers of fibrosis than S-Fr. Fructose-induced ex novo lipogenesis with production of SCFA and MCFA was confirmed by metagenomic analysis. These results suggest that consumption of fructose under different forms, liquid or solid, may differently affect gut microbiota, thus leading to impairment in intestinal mucosa integrity and liver homeostasis." As taken from Mastrocola R et al. 2018. J. Nutr. Biochem. 55, 185-199. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29539590

"PURPOSE: Sugar-sweetened beverage intake is a risk factor for insulin resistance, dyslipidemia, fatty liver, and steatohepatitis (NASH). Sub-chronic supplementation of liquid fructose, but not glucose, in female rats increases liver and plasma triglycerides without inflammation. We hypothesized that chronic supplementation of fructose would cause NASH and liver insulin resistance. METHODS: We supplemented female Sprague-Dawley rats with water or either fructose or glucose 10% w/v solutions under isocaloric conditions for 7 months. At the end, plasma analytes, insulin, and adiponectin were determined, as well as liver triglyceride content and the expression of key genes controlling inflammation, fatty acid synthesis and oxidation, endoplasmic reticulum stress, and plasma VLDL clearance, by biochemical and histological methods. RESULTS: Although sugar-supplemented rats increased their energy intake by 50-60%, we found no manifestation of liver steatosis, fibrosis or necrosis, unchanged plasma or tissue markers of inflammation or fibrosis, and reduced liver expression of gluconeogenic enzymes, despite both sugars increased fatty acid synthesis, mTORC1, and IRE1 activity, while decreasing fatty acid oxidation and PPARα activity. Only fructose-supplemented rats were hypertriglyceridemic, showing a reduced expression of VLDL receptor and lipoprotein lipase in skeletal muscle and vWAT. Glucose-supplemented rats showed increased adiponectinemia, which would explain the different metabolic outcomes of the two sugars. CONCLUSIONS: Chronic liquid simple sugar supplementation, as the sole risk factor, is not enough for female rats to develop NASH and increased liver gluconeogenesis. Nevertheless, under isocaloric conditions, only fructose induced hypertriglyceridemia, thus confirming that also the type of nutrient matters in the development of metabolic diseases." As taken from Sangüesa G et al. 2019a. Eur. J. Nutr 58(3), 1283-1297. PubMed, 2019 available at https://www.ncbi.nlm.nih.gov/pubmed/29516226

“Diabetic nephropathy (DN) is one of the complications of diabetes and is now the most common cause of end-stage renal disease. Fructose is a simple carbohydrate that is present in fruits and honey and is used as a sweetener because of its sweet taste. Fructose has been reported to have the potential to progress diabetes and DN in humans even though fructose itself does not increase postprandial plasma glucose levels. In this study, we investigated the effects of high fructose intake on the kidney of the Spontaneously Diabetic Torii (SDT) rats which have renal lesions similar to those in DN patients and compared these with the effects in normal SD rats. This study revealed that a 4-week feeding of the high fructose diet increased urinary excretion of kidney injury makers for tubular injury and accelerated mainly renal tubular and interstitial lesions in the SDT rats but not in normal rats. The progression of the nephropathy in the SDT rats was considered to be related to increased internal uric acid and blood glucose levels due to the high fructose intake. In conclusion, high fructose intake exaggerated the renal lesions in the SDT rats probably due to effects on the tubules and interstitium through metabolic implications for uric acid and glucose.” As taken from Toyoda K et al. 2018. J. Toxicol. Sci. 43(1), 45-58. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29415951

“The intake of excess dietary fructose most often leads to non-alcoholic fatty liver disease (NAFLD). Fructose is metabolized mainly in the liver and its chronic consumption results in lipogenic gene expression in this organ. However, precisely how fructose is involved in NAFLD progression is still not fully understood, limiting therapy. Lipocalin-2 (LCN2) is a small secreted transport protein that binds to fatty acids, phospholipids, steroids, retinol, and pheromones. LCN2 regulates lipid and energy metabolism in obesity and is upregulated in response to insulin. We previously discovered that LCN2 has a hepatoprotective effect during hepatic insult, and that its upregulation is a marker of liver damage and inflammation. To investigate if LCN2 has impact on the metabolism of fructose and thereby arising liver damage, we fed wild type and Lcn2-/- mice for 4 or 8 weeks on diets that were enriched in fructose either by adding this sugar to the drinking water (30% w/v), or by feeding a chow containing 60% (w/w) fructose. Body weight and daily intake of food and water of these mice was then measured. Fat content in liver sections was visualized using Oil Red O stain, and expression levels of genes involved in fat and sugar metabolism were measured by qRT-PCR and Western blot analysis. We found that fructose-induced steatosis and liver damage was more prominent in female than in male mice, but that the most severe hepatic damage occurred in female mice lacking LCN2. Unexpectedly, consumption of elevated fructose did not induce de novo lipogenesis or fat accumulation. We conclude that LCN2 acts in a lipid-independent manner to protect the liver against fructose-induced damage.” As taken from Lambertz J et al. 2017. Front. Physiol. 8, 964. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29234288

“Phase-II enzymes are important in metabolizing many xenobiotics including prescription drugs and chemical carcinogens. Whereas it is known that diet can alter the expression of phase-II conjugation enzymes, the previous studies are limited in using only two or three diets and examining only a few enzymes. 2. Adult male C57BL6 mice were fed one of nine diets for 3 weeks. Of the 87 genes encoding major hepatic phase-II enzymes, approximately one-half (43) were altered by at least one diet. Diet restriction altered the hepatic expression of the most genes encoding phase-II enzymes (27), followed by lab chow (15), atherogenic diet (13), high-fat diet (10), western diet (7), high-fructose diet (5), and essential fatty acid-deficient diet (3), whereas the low n-3 fatty acid diet had no effect on the hepatic expression of these phase-II enzymes. 3. This comprehensive study provides detailed information on which conjugation enzymes are changed by these diets,

and these data can be used to further investigate the mechanism for these changes in messenger RNAs, and whether these changes result in alterations in enzyme activity and drug action." As taken from Guo Y et al. 2017. Xenobiotica 47(8), 645-654. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27686132

"Dramatic increases in obesity and diabetes have occurred worldwide over the past 30 years. Some investigators have suggested that these increases may be due, in part, to increased added sugars consumption. Several scientific organizations, including the World Health Organization, the Scientific Advisory Council on Nutrition, the Dietary Guidelines Advisory Committee 2015, and the American Heart Association, have recommended significant restrictions on upper limits of sugars consumption. In this review, the scientific evidence related to sugars consumption and its putative link to various chronic conditions such as obesity, diabetes, heart disease, nonalcoholic fatty liver disease, and the metabolic syndrome is examined. While it appears prudent to avoid excessive calories from sugars, the scientific basis for restrictive guidelines is far from settled." As taken from Rippe JM et al. 2017. Nutr. Rev. 75(1), 18-36. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/27974597

"Non-alcoholic fatty liver disease (NAFLD) is the hepatic manifestation of metabolic syndrome; its rising prevalence parallels the rise in obesity and diabetes. Historically thought to result from overnutrition and a sedentary lifestyle, recent evidence suggests that diets high in sugar (from sucrose and/or high-fructose corn syrup [HFCS]) not only increase the risk of NAFLD, but also non-alcoholic steatohepatitis (NASH). Herein, we review the experimental and clinical evidence that fructose precipitates fat accumulation in the liver, due to both increased lipogenesis and impaired fat oxidation. Recent evidence suggests that the predisposition to fatty liver is linked to the metabolism of fructose by fructokinase C, which results in ATP consumption, nucleotide turnover and uric acid generation that mediate fat accumulation. Alterations to gut permeability, the microbiome, and associated endotoxemia contribute to the risk of NAFLD and NASH. Early clinical studies suggest that reducing sugary beverages and total fructose intake, especially from added sugars, may have a significant benefit on reducing hepatic fat accumulation. We suggest larger, more definitive trials to determine if lowering sugar/HFCS intake, and/or blocking uric acid generation, may help reduce NAFLD and its downstream complications of cirrhosis and chronic liver disease." As taken from Jensen T et al. 2018. J. Hepatol. 68(5), 1063-1075. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29408694

"Introduction International studies show an increased incidence of chronic kidney disease (CKD) in patients with metabolic syndrome (MS). It is assumed that the major components of MS - obesity, insulin resistance, dyslipidemia, and hypertension - are linked to renal damage through the systemic release of several pro-inflammatory mediators, such as uric acid (UA), C-reactive protein (CRP), and generalized oxidative stress. The aim of the present study was to investigate the extent of kidney impairment and manifestations of dysfunction in rats with fructose-induced MS. Methods We used a model of high-fructose diet in male Wistar rats with 35% glucose-fructose corn syrup in drinking water over a duration of 16 weeks. The experimental animals were divided into two groups: control and high-fructose drinking (HFD). Serum samples were obtained from both groups for laboratory study, and the kidneys were extracted for observation via light microscopy examination. Results All HFD rats developed obesity, hyperglycemia, hypertriglyceridemia, increased levels of CRP and UA (when compared to the control group), and oxidative stress with high levels of malondialdehyde and low levels of reduced glutathione. The kidneys of the HFD

group revealed a significant increase in kidney weight in the absence of evidence of renal dysfunction and electrolyte disturbances. Under light microscopy, the kidneys of the HFD group revealed amyloid deposits in Kimmelstiel-Wilson-like nodules and the walls of the large caliber blood vessels, early-stage atherosclerosis with visible ruptures and scarring, hydropic change (vacuolar degeneration) in the epithelial cells covering the proximal tubules, and increased eosinophilia in the distant tubules when compared to the control group. Conclusion Under the conditions of a fructose-induced metabolic syndrome, high serum UA and CRP correlate to the development of early renal disorders without a clinical manifestation of renal dysfunction. These phenomena are of particular importance for assessing the risk of developing future CKD." As taken from Bratoevka K et al. 2017. Cureus 9(11), e1826. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29321950

"Fatty liver disease affects up to one out of every two adults in the western world. Data from animal and human studies implicate added sugars (eg, sucrose and high-fructose corn syrup) in the development of fatty liver disease and its consequences. Added fructose in particular, as a component of added sugars, may pose the greatest risk for fatty liver disease. Considering that there is no requirement for added sugars in the diet, dietary guidelines should recommend reducing the intake of added sugars to just 5% of total calories in order to decrease the prevalence of fatty liver disease and its related consequences." As taken from DiNicolantonio JJ et al. 2017. Open Heart 4(2), e000631. PubMed, 2018 available at https://www.ncbi.nlm.nih.gov/pubmed/29118995

"Fructose intake is known to induce obesity, insulin resistance, metabolic syndrome and non-alcoholic fatty liver disease (NAFLD). We aimed to evaluate the effects of fructose drinking on gut leakiness, endotoxemia, and NAFLD and study the underlying mechanisms in rats, mice, and T84 colon cells. The levels of ileum junctional proteins, oxidative stress markers and apoptosis-related proteins in rodents, T84 colonic cells and human ileums were determined by immunoblot, immunoprecipitation, and immunofluorescence analyses. Fructose drinking caused microbiome change, leaky gut, and hepatic inflammation/fibrosis with increased levels of nitroxidative stress marker proteins cytochrome P450-2E1 (CYP2E1), inducible nitric oxide synthase, and nitrated proteins in small intestine and liver of rodents. Fructose drinking significantly elevated plasma bacterial endotoxin levels likely resulting from decreased levels of intestinal tight junction (TJ) proteins (ZO-1, occludin, claudin-1, and claudin-4), adherent junction (AJ) proteins (β-catenin and E-cadherin), and desmosome plakoglobin along with α-tubulin in wild-type rodents but not in the fructose-exposed Cyp2e1-null mice. Consistently, decreased intestinal TJ/AJ proteins and increased hepatic inflammation with fibrosis were observed in autopsied obese people compared to lean individuals. Furthermore, histological and biochemical analyses showed markedly elevated hepatic fibrosis markers proteins in fructose-exposed rats compared to controls. Immunoprecipitation followed by immunoblot analyses revealed that intestinal TJ proteins were nitrated and ubiquitinated, leading to their decreased levels in the fructose-exposed rats. CONCLUSION: These results showed for the first time that fructose intake causes protein nitration of intestinal TJ and AJ proteins, resulting in increased gut leakiness, endotoxemia and steatohepatitis with liver fibrosis in a CYP2E1-dependent manner." As taken from Cho YE et al. 2019. Hepatology. Epub ahead of print. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30959577

“One of the most important threats to global human health is the increasing incidences of metabolic pathologies (including obesity, type 2 diabetes and non-alcoholic fatty liver disease), which is paralleled by increasing consumptions of hypercaloric diets enriched in simple sugars. The challenge is to identify the metabolic pathways affected by the excessive consumption of these dietary components when they are consumed in excess, to unravel the molecular mechanisms leading to metabolic pathologies and identify novel therapeutic targets to manage them. Mechanistic (mammalian) target of rapamycin (mTOR) has emerged as one of the key molecular nodes that integrate extracellular signals, such as energy status and nutrient availability, to trigger cell responses that could lead to the above-mentioned diseases through the regulation of lipid and glucose metabolism. By activating mTOR signalling, excessive consumption of simple sugars (such as fructose and glucose), could modulate hepatic gluconeogenesis, lipogenesis and fatty acid uptake and catabolism and thus lipid deposition in the liver. In the present review we will discuss some of the most recent studies showing the central role of mTOR in the metabolic effects of excessive simple sugar consumption.” As taken Sangüesa G et al. 2019b. Int. J. Mol. Sci. 20(5), pii: E1117. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30841536

“Scientific evidence has identified that the excessive consumption of products made from high-fructose corn syrup is a trigger for obesity, whose prevalence increased in recent years. Due to the metabolic characteristics of fructose, a rapid gastric emptying is produced, altering signals of hunger-satiety and decreasing the appetite. In addition to the hepatic level during catabolism, triose phosphate is generated and adenosine triphosphate (ATP) is reduced, producing uric acid. Triose phosphate triggers the synthesis of fatty acids that increase the production and accumulation of triglycerides, diacylglycerols and ceramides that induce insulin resistance. Hyperlipidemia, insulin resistance and hyperuricemia contribute to the development of hypertension, cardiovascular disease, kidney failure, non-alcoholic fatty liver disease and some kinds of cancer. Understanding the molecular mechanisms and signaling pathways altered by the consumption of fructose is relevant to understand the development of metabolic diseases, as well as to seek therapeutic strategies to improve quality of life.” As taken from Loza-Medrano SS et al. 2019. Rev. Med. Inst. Mex. Seguro Soc. 56(5), 491-504. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30777418

“Non-alcoholic fatty liver disease (NAFLD) is an increasingly common and potentially serious condition, which has emerged with the obesity epidemic. This disease can progress to cirrhosis and hepatocellular cancer. Associated comorbidities, such as cardiovascular disease and type 2 diabetes, are common. Obesity is the key risk factor and diet appears to be a critical factor in the pathogenesis of NAFLD. We reviewed studies undertaken on human subjects investigating which dietary components initiate excess hepatic triglyceride deposition. Most experimental diets used high-calorie excesses, or extreme proportions of fat or carbohydrate, not typical of current dietary patterns. Hypercaloric diets, where the additional calories were predominantly either fat or carbohydrates, increased intrahepatocellular lipids. The type of fat appeared important, with diets high in saturated fatty acids favoring hepatic fat accumulation which was substantially lower with polyunsaturated fatty acids. The effect of dietary fructose on markers of NAFLD did not appear to be worse than that of glucose. The initiation of excess hepatic triglycerides is likely to be a complex interaction of energy and nutrients with more than one dietary factor involved. It was not possible to disentangle the hepatic effects of excess energy from that of different macronutrient distributions in current literature. Further investigation is needed to determine the type of diet that is likely to lead to the development of NAFLD. A better

understanding of the contribution of diet to pathogenesis of NAFLD would better inform prevention strategies." As taken from Sharp KPH et al. 2018 JGH Open 2(2), 59-74. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30483565

"This review will focus on the question of whether dietary sugars are a relevant determinant in the global rise of overweight and obesity in adults, adolescents, and children. Initially, the review describes the current definitions for sugars in the diet and makes reference to them while analyzing their role in overweight and obesity as well as diet-related diseases, including type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease and cancer. Second, it will focus particularly on sucrose and the question of whether it is the molecular composition of sucrose (glucose and fructose) or its energy content that promotes body weight gain and diet-related diseases. Finally, the review will clarify the molecular characteristics of sucrose regarding the release of the gastrointestinal glucose-dependent insulinotropic peptide (GIP) compared to other energy-providing nutrients and its relevance in metabolic diseases. Current data indicates that dietary sugars are only associated with an increase in obesity when consumed as an excess source of calories and with that an increase in the risk of diet-related diseases. Furthermore, it was shown that a diet rich in fat will stimulate GIP secretion more than a diet rich in sucrose. Taken together, current scientific evidence does not support the conclusion that dietary sugars per se are detrimental to human health." As taken from Prinz P. 2019. Eur. J. Clin. Nutr. Epub ahead of print. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30787473/

"SCOPE: The effect of chronic supplementation with simple-sugar solutions on leptin signaling in liver, hypothalamus, and visceral white adipose tissue (vWAT) is studied, which is designed to mimic the temporal pattern of consumption by humans. METHODS AND RESULTS: Solutions of fructose or glucose are isocalorically supplemented (7 months) in female Sprague-Dawley rats consuming ad libitum rodent chow. After sacrifice, plasma and tissue samples (liver, hypothalamus, and vWAT) are collected. Zoometric parameters, plasma analytes, and the tissue expression and activity of markers of leptin signaling are determined by biochemical and molecular biological methods. The two sugars cause different types of adiposopathy. Both sugars induce increases in plasma nonesterified fatty acids, and leptin resistance in the liver and the hypothalamus. Only fructose-supplemented rats show hyperleptinemia, and increased body weight due to a hypertrophy of vWAT, with no signs of leptin-mediated lipolysis. Glucose-supplemented rats show no significant changes in these parameters but present elevated plasma adiponectin concentrations, lipolysis, and inflammatory markers in vWAT, indicating a shift to a nonexpandable adipose tissue phenotype. CONCLUSION: Chronic consumption of fructose places a greater burden on metabolic homeostasis than equivalent consumption of glucose, inducing hyperleptinemia, generalized leptin resistance, and increased body weight due to expanded, hypertrophic vWAT." As taken from Sangüesa G et al. 2018b. Mol. Nutr. Food Res. 62(22), e1800777. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/30260587

"Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by aldolase B (ALDOB) deficiency resulting in an inability to metabolize fructose. The toxic accumulation of intermediate fructose-1-phosphate causes multiple metabolic disturbances, including postprandial hypoglycemia, lactic acidosis, electrolyte disturbance, and liver/kidney dysfunction. The clinical presentation varies depending on the age of exposure and the load of fructose. Some common infant formulas contain fructose in various forms, such as sucrose, a disaccharide of fructose and glucose. Exposure to formula containing

fructogenic compounds is an important, but often overlooked trigger for severe metabolic disturbances in HFI. Here we report four neonates with undiagnosed HFI, all caused by the common, homozygous mutation c.448G>C (p.A150P) in ALDOB, who developed life-threatening acute liver failure due to fructose-containing formulas. These cases underscore the importance of dietary history and consideration of HFI in cases of neonatal or infantile acute liver failure for prompt diagnosis and treatment of HFI." As taken from Li H et al. 2018. Mol. Genet. Metab. 123(4), 428-432. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29510902

"PURPOSE: Carbohydrate intake increases postprandial insulin secretion and may affect breast density, a strong risk factor for breast cancer, early in life. We examined associations of adolescent and early adulthood intakes of total carbohydrates, glycemic index/load, fiber, and simple sugars with breast density among 182 young women. METHODS: Diet was assessed using three 24-h recalls at each of five Dietary Intervention Study in Children (DISC) clinic visits when participants were age 10-19 years and at the DISC06 Follow-Up Study clinic visit when participants were age 25-29 years. Associations between energy-adjusted carbohydrates and MRI-measured percent dense breast volume (%DBV) and absolute dense breast volume (ADBV) at 25-29 years were quantified using multivariable-adjusted mixed-effects linear models. RESULTS: Adolescent sucrose intakes and premenarcheal total carbohydrates intakes were modestly associated with higher %DBV (mean %DBVQ1 vs Q4, 16.6 vs 23.5% for sucrose; and 17.2 vs 22.3% for premenarcheal total carbohydrates, all Ptrend ≤ 0.02), but not with ADBV. However, adolescent intakes of fiber and fructose were not associated with %DBV and ADBV. Early adulthood intakes of total carbohydrates, glycemic index/load, fiber, and simple sugars were not associated with %DBV and ADBV. CONCLUSIONS: Insulinemic carbohydrate diet during puberty may be associated with adulthood breast density, but our findings need replication in larger studies." As taken from Jung S et al. 2018. Cancer Causes Control 29(7), 631-642. PubMed, 2019 available at: https://www.ncbi.nlm.nih.gov/pubmed/29802491

7. Addiction

JTI is not aware of any information that demonstrates that this ingredient has any addictive effect.

8. Burnt ingredient toxicity

This ingredient was considered as part of an overall safety assessment of ingredients added to tobacco in the manufacture of cigarettes. An expert panel of toxicologists reviewed the open literature and internal toxicology data of 5 tobacco companies to evaluate a

composite list of ingredients used in the manufacture of cigarettes. The conclusion of this report was that these ingredients did not increase the inherent biological activity of tobacco cigarettes, and are considered to be acceptable under conditions of intended use (Doull et al., 1994 & 1998).

Tobacco smoke condensates from cigarettes containing sugars (Fructose) and an additive free, reference cigarettes were tested in a battery of in vitro and/or in vivo test(s). Within the sensitivity and specificity of the bioassay(s) the activity of the condensate was not changed by the addition of sugars (Fructose). Table below provides tested level(s) and specific endpoint(s).

Endpoint	**Tested level (ppm)**	**Reference**
Smoke chemistry	15,000	JTI KB Study Report(s)
In vitro genotoxicity	48,000	fGLH Study Report (2010)
In vitro cytotoxicity	48,000	fGLH Study Report (2010)

A water pipe transfer study showed that 0.010% of fructose (CAS RN: 57-48-7) transfers intact.

When an aqueous fructose solution (5%) was sprayed onto tobacco the influence on mainstream smoke components was as follows (Burton & Benner 1972):

TPM: increase 4%

Tar: increase 4%

Nicotine: decrease 5%

Phenol: increase 14%

o-Cresol: increase 39%

m-, p-Cresol: increase 19%

B[a]P: decrease 27%

When a 12.8% aqueous solution of fructose was sprayed onto Burley tobacco during the manufacture of cigarettes, a decrease in TPM, alkaloids and volatile aldehydes was observed in comparison with the control (no fructose). Levels of carbonyls, volatile carbonyls, 2-furfural, total acids and volatile acids increased in comparison with the control (Thornton & Massey 1975).

Fructose contributions to mainstream smoke catechol: 1.4% (Carmella *et al* 1984).

Sugars, such as sucrose or invert sugar, have been used as tobacco ingredients in American-blend cigarettes to replenish the sugars lost during curing of the Burley component of the blended tobacco in order to maintain a balanced flavor. Chemical-analytical studies of the mainstream smoke of research cigarettes with various sugar application levels revealed that most of the smoke constituents determined did not show any sugar-related changes in yields (per mg nicotine), while ten constituents were found to either increase (formaldehyde, acrolein, 2-butanone, isoprene, benzene, toluene, benzo[*k*]fluoranthene) or decrease (4-aminobiphenyl, N-nitrosodimethylamine, N-nitrosonornicotine) in a statistically significant manner with increasing sugar application levels. Such constituent yields were modelled into constituent uptake distributions using simulations of nicotine uptake distributions generated on the basis of published nicotine biomonitoring data, which were multiplied by the constituent/nicotine ratios determined in the current analysis. These simulations revealed extensive overlaps for the constituent uptake distributions with and without sugar application. Moreover, the differences in smoke

composition did not lead to relevant changes in the activity in *in vitro* and *in vivo* assays. The potential impact of using sugars as tobacco ingredients was further assessed in an indirect manner by comparing published data from markets with predominantly American-blend or Virginiatype (no added sugars) cigarettes. No relevant difference was found between these markets for smoking prevalence, intensity, some markers of dependence, nicotine uptake, or mortality from smoking-related lung cancer and chronic obstructive pulmonary disease. In conclusion, thorough examination of the data available suggests that the use of sugars as ingredients in cigarette tobacco does not increase the inherent risk and harm of cigarette smoking (Roemer et al., 2012).

"We set out to determine whether the addition of an aryl hydrocarbon receptor (AHR) antagonist has an effect on glucose/fructose utilization in the spermatocyte when exposed to cigarette smoke condensate (CSC). We exposed male germ cells to 5 and 40 µg/mL of CSC ± 10 µmol/L of AHR antagonist at various time points. Immunoblot expression of specific glucose/fructose transporters was compared to control. Radiolabeled uptake of 2-deoxyglucose (2-DG) and fructose was also performed. Spermatocytes utilized fructose nearly 50-fold more than 2-DG. Uptake of 2-DG decreased after CSC + AHR antagonist exposure. Glucose transporters (GLUTs) 9a and 12 declined after CSC + AHR antagonist exposure. Synergy between CSC and the AHR antagonist in spermatocytes may disrupt the metabolic profile in vitro. Toxic exposures alter energy homeostasis in early stages of male germ cell development, which could contribute to later effects explaining decreases in sperm motility in smokers." As taken from Omurtag K et al. 2015. Reprod. Sci. 22(1), 94-101. PubMed, 2015 available at: http://www.ncbi.nlm.nih.gov/pubmed/24803506

In a water pipe transfer study, 0.01% of fructose was transferred intact to the smoke (JTI Study Report (s)).

9. Heated/vapor emissions toxicity

No data available to us at this time.

10. Ecotoxicity

10.1. Environmental fate

The Ecological Categorization Results from the Canadian Domestic Substances List state that fructose

is not persistent in the environment

Rational for P	QSAR
Media of concern leading to Categorization	Water
Experimental Biodegradation half-life (days)	Not Available
Predicted Ultimate degradation half-life (days)	15
MITI probability of biodegradation	0.8711
EPI Predicted Ozone reaction half-life (days)	999
EPI Predicted Atmospheric Oxidation half-life (days)	0.1552

Data accessed July 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

The following data are presented in EPISuite:

Henrys Law Constant (25 deg C) [HENRYWIN v3.20]:

Bond Method :	9.72E-015 atm-m3/mole (9.85E-010 Pa-m3/mole)
Group Method:	Incomplete
Henrys LC [via VP/WSol estimate using User-Entered or Estimated values]:	HLC: 4.528E-015 atm-m3/mole (4.588E-010 Pa-m3/mole) VP: 1.91E-008 mm Hg (source: MPBPVP) WS: 1E+006 mg/L (source: WSKOWWIN)

Log Octanol-Air Partition Coefficient (25 deg C) [KOAWIN v1.10]:

Log Kow used:	-1.55 (KowWin est)
Log Kaw used:	-12.401 (HenryWin est)
Log Koa (KOAWIN v1.10 estimate):	10.851
Log Koa (experimental database):	None

Probability of Rapid Biodegradation (BIOWIN v4.10):

Biowin1 (Linear Model):	0.7654

Biowin2 (Non-Linear Model) : Biowin3 (Ultimate Survey Model): Biowin4 (Primary Survey Model) : Biowin5 (MITI Linear Model) : Biowin6 (MITI Non-Linear Model): Biowin7 (Anaerobic Linear Model):	0.4425 3.2201 (weeks) 3.9424 (days) 0.9963 0.8711 0.8000
Ready Biodegradability Prediction:	YES

Hydrocarbon Biodegradation (BioHCwin v1.01):

Structure incompatible with current estimation method!

Sorption to aerosols (25 Dec C)[AEROWIN v1.00]:

Vapor pressure (liquid/subcooled):	3.2E-005 Pa (2.4E-007 mm Hg)
Log Koa (Koawin est):	10.851
Kp (particle/gas partition coef. (m3/ug)): Mackay model: Octanol/air (Koa) model:	0.0938 0.0174

Fraction sorbed to airborne particulates (phi):

Junge-Pankow model:	0.772
Mackay model:	0.882
Octanol/air (Koa) model:	0.582

Atmospheric Oxidation (25 deg C) [AopWin v1.92]:

Hydroxyl Radicals Reaction:

OVERALL OH Rate Constant =	68.9254 E-12 cm3/molecule-sec
Half-Life =	0.155 Days (12-hr day; 1.5E6 OH/cm3)

Half-Life =	1.862 Hrs
Ozone Reaction:	No Ozone Reaction Estimation
Fraction sorbed to airborne particulates (phi): 0.827 (Junge-Pankow, Mackay avg) 0.582 (Koa method) Note: the sorbed fraction may be resistant to atmospheric oxidation	

Soil Adsorption Coefficient (KOCWIN v2.00):

Koc :	10 L/kg (MCI method)
Log Koc:	1.000 (MCI method)
Koc :	0.1426 L/kg (Kow method)
Log Koc:	-0.846 (Kow method)

Aqueous Base/Acid-Catalyzed Hydrolysis (25 deg C) [HYDROWIN v2.00]:

Rate constants can NOT be estimated for this structure!

Volatilization from Water:

Henry LC: 9.72E-015 atm-m3/mole (estimated by Bond SAR Method)

Half-Life from Model River:	8.085E+010 hours (3.369E+009 days)
Half-Life from Model Lake:	8.82E+011 hours (3.675E+010 days)

Removal In Wastewater Treatment:

Total removal:	1.85 percent
Total biodegradation:	0.09 percent
Total sludge adsorption:	1.75 percent
Total to Air:	0.00 percent

(using 10000 hr Bio P,A,S)

Level III Fugacity Model:

	Mass Amount (percent)	Half-Life (hr)	Emissions (kg/hr)
Air	9.82e-007	3.72	1000
Water	30.6	360	1000
Soil	69.4	720	1000
Sediment	0.0688	3.24e+003	0

Persistence Time: 640 hr

10.2. Aquatic toxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that fructose is not inherently toxic to aquatic organisms:

Rational for iT	QSAR
Pivotal value for iT (mg/l)	7900
Toxicity to fathead minnow (LC50 in mg/l) as predicted by Topkat v6.1	7,900
Toxicity to fish (LC50 in mg/l) as predicted by Ecosar v0.99g	290,000
Toxicity to fish (LC50 in mg/l) as predicted by PNN	129,004.65374
Toxicity to daphnia (EC50 in mg/l) as predicted by Topkat v6.1	7,600
Toxicity to fish, daphnia, algae or mysid shrimp (EC50 or LC50 in mg/l) as predicted by Ecosar v0.99g	124,000

Chronic toxicity to daphnia or algae (EC50 in mg/l) as predicted by Ecosar v0.99g	2,640.316
Toxicity to fish (LC50 in mg/l) as predicted by Neutral Organics QSAR in Ecosar v0.99g	2.90E+003

Data accessed July 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 57-48-7:

Values used to Generate ECOSAR Profile

Log Kow: -1.553 (EPISuite Kowwin v1.68 Estimate)

Wat Sol: 7.78E+005 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations Neutral Organics **ECOSAR Class**	**Organism**	**Duration**	**End Pt**	**Predicted mg/L (ppm)**
Neutral Organics :	Fish	96-hr	LC50	2.3e+005
Neutral Organics :	Daphnid	48-hr	LC50	93882.945
Neutral Organics :	Green Algae	96-hr	EC50	18016.305
Neutral Organics :	Fish		ChV	15237.300
Neutral Organics :	Daphnid		ChV	3674.655
Neutral Organics :	Green Algae		ChV	2272.577
Neutral Organics :	Fish (SW)	96-hr	LC50	2.83e+005
Neutral Organics :	Mysid	96-hr	LC50	2.33e+006 *
Neutral Organics :	Fish (SW)		ChV	3508.001
Neutral Organics :	Mysid (SW)		ChV	5.78e+005

Note: * = asterisk designates: Chemical may not be soluble enough to measure this predicted effect. If the effect level exceeds the water solubility by 10X, typically no effects at saturation (NES) are reported.

Record for D-fructose:

Spec. Sci. Name **Spec. Common Name**	**Exp. Type** **Chem. Anal.**	**Media Type** **Loc**	**Resp. Site** **Obs. Dur. (Days)**	**Endpoint** **BCF**	**Trend** **Eff%**	**Effect** **Effect Meas.**	**Conc (Standardized)** **Appl. Rate**	**Stat. Signif.** **Sig. Level**
Fish; Standard Test Species								
Danio rerio	S	FW		NOEC	INC	REP	F 108 mmol/kg	ANOSIG
Zebra Danio	U	LAB	0		~80/	MOTL/		<0.05
Danio rerio	S	FW		NOEC	DEC	REP	F 108 mmol/kg	ANOSIG
Zebra Danio	U	LAB	0.004		>20-<40/	MOTL/		<0.05

As taken from the EPA ECOTOX database.

10.3. Sediment toxicity

No data available to us at this time.

10.4. Terrestrial toxicity

The EPA ECOTOX database provides the following data:

Solanum lycopersicum lycopersicum (tomato): decrease in growth (size) following 1 day of exposure to 2%.

ECOSAR version 1.11 reports the following terrestrial toxicity data for CAS RN 57-48-7:

Values used to Generate ECOSAR Profile

Log Kow : -1.553 (EPISuite Kowwin v1.68 Estimate)

Wat Sol : 7.78E+005 (mg/L, PhysProp DB exp value)

ECOSAR v1.11 Class-specific Estimations: Neutral Organics

ECOSAR Class	Organism	Duration	End Pt	Predicted mg/L (ppm)
Neutral Organics :	Earthworm	14-day	LC50	731.578

“Most haematophagous insect vectors can also use sugar as an energy source; thus their sugar-feeding behaviour influences their longevity and blood-feeding rate and hence their vectorial capacity. Scant information is available on the sugar-feeding behaviour of Culicoides Latreille biting midges (Diptera: Ceratopogonidae), which are vectors of bluetongue and Schmallenberg viruses. The longevity of laboratory-reared Culicoides nubeculosus (Meigen) under fluctuating temperatures (16 and 28 °C) and with access to water or water and blood was on average 6.4 days and 8.9 days, respectively, which was around one third of the lifespan of siblings with access to sugar or sugar and blood (22.2 days and 27.1 days, respectively). Access to honeydew significantly increased the midge's longevity, whereas the provision of extrafloral nectaries had no impact. Females with access to sugar produced a significantly higher number of eggs (65.5 ± 5.2) than their starved sisters (45.4 ± 8.4). More than 80% of field-caught female Culicoides from the two most abundant European groups, Obsoletus (n = 2243) and Pulicaris (n = 805), were fructose-positive. Fructose-positivity was high in all physiological stages and no seasonal variability was noted. The high rate of natural sugar feeding of Culicoides offers opportunities for the development of novel control strategies using toxic sugar baits and for the monitoring of vector-borne diseases using sugar-treated FTA (nucleic acid preservation) cards in the field.” As taken from Kaufmann C et al. 2015. Med. Vet. Entomol. 29(1), 17-25. PubMed, 2017 available at: https://www.ncbi.nlm.nih.gov/pubmed/25155520

10.5. All other relevant types of ecotoxicity

The Ecological Categorization Results from the Canadian Domestic Substances List state that fructose is not bioaccumulative in the environment:

Rational for B	QSAR
Log Kow predicted by KowWin	-1.55
Log BAF T2MTL predicted by Gobas	0.0000540371955876

Log BCF 5% T2LTL predicted by Gobas	0.0005144646215092
Log BCF Max predicted by OASIS	0.949930014724155
Log BCF predicted by BCFWIN	0.5

Data accessed July 2017 on the OECD website: http://webnet.oecd.org/CCRWeb/Search.aspx

The following data are presented in EPISuite:

Bioaccumulation Estimates (BCFBAF v3.01):

Log BCF from regression-based method:	0.500 (BCF = 3.162 L/kg wet-wt)
Log Biotransformation Half-life (HL):	-2.8859 days (HL = 0.001301 days)
Log BCF Arnot-Gobas method (upper trophic):	-0.049 (BCF = 0.8935)
Log BAF Arnot-Gobas method (upper trophic):	-0.049 (BAF = 0.8935)
log Kow used:	-1.55 (estimated)

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13. Last audited

June 2019

I U C L I D

D a t a s e t

Existing Chemical	Substance ID: 57–48–7
CAS No.	57–48–7
EINECS Name	fructose, pure
EINECS No.	200–333–3
Molecular Weight	180
Molecular Formula	C6H12O6

Dataset created by: EUROPEAN COMMISSION – European Chemicals Bureau

This dossier is a compilation based on data reported by the European Chemicals Industry following 'Council Regulation (EEC) No. 793/93 on the Evaluation and Control of the Risks of Existing Substances'. All (non–confidential) information from the single datasets, submitted in the IUCLID/HEDSET format by individual companies, was integrated to create this document.

The data have not undergone any evaluation by the European Commission.

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European Chemicals Bureau

1.0.1 OECD and Company Information

Name: Amway Europe
Street: Ikaroslaan, 2B
Town: 1930 Zaventem
Country: Belgium
Phone: 027209953
Telefax: 027253298

Name: Hydro Chemicals Norge
Street: Fyrstikkalleen 3B, P.O. Box 23 Haugenstua
Town: N–0604 OSLO
Country: Norway
Phone: 47 2243 2400
Telefax: 47 2243 2402
Telex: 71771 nchem n

Name: Roquette Frères
Town: 62136 Lestrem
Country: France
Phone: 33 (0)3 21 63 36 00
Telefax: 33 (0)3 21 63 38 50

Name: Südzucker AG Mannheim/Ochsenfurt
Street: Maximilianstr. 10
Town: 68165 Mannheim
Country: Germany
Phone: 06214210
Telefax: 0621421393
Telex: 463251

1.0.2 Location of Production Site

–

1.0.3 Identity of Recipients

–

1.1 General Substance Information

Substance type: natural substance
Physical status: solid

Substance type: organic
Physical status: solid

Substance type:
Physical status: solid

1.1.1 Spectra

–

1.2 Synonyms

Levulose, D Fructose
Source: Amway Europe Zaventem

levulose; D–fructose
Source: Roquette Frères Lestrem

1.3 Impurities

–

1.4 Additives

–

1.5 Quantity

Quantity 10 000 – 50 000 tonnes

1.6.1 Labelling

–

1.6.2 Classification

–

1.7 Use Pattern

Type: type
Category: Wide dispersive use

Type: industrial
Category: other

Type: use
Category: Food/foodstuff additives

Type: use
Category: Pharmaceuticals

1.7.1 Technology Production/Use

–

1.8 Occupational Exposure Limit Values

Type of limit:
Limit value:
Remark: no exposure limits known
Source: Roquette Frères Lestrem

1.9 Source of Exposure

Remark: Fructose is obtained from glucose in corn syrup by the use of glucose isomerase.
Source: Roquette Frères Lestrem

Remark: one site of manufacture production process: hydrolysis of sucrose, separating of resulting fructose by chromatography crystallization of fructose
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

(1)

1.10.1 Recommendations/Precautionary Measures

-

1.10.2 Emergency Measures

-

1.11 Packaging

-

1.12 Possib. of Rendering Subst. Harmless

-

1.13 Statements Concerning Waste

-

1.14.1 Water Pollution

-

1.14.2 Major Accident Hazards

-

1.14.3 Air Pollution

-

1.15 Additional Remarks

Remark: transportation in vehicles for food only, not relevant for food
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

1.16 Last Literature Search

-

1.17 Reviews

–

1.18 Listings e.g. Chemical Inventories

–

2.1 Melting Point

Value: 100 – 104 degree C
Decomposition: yes
Sublimation: no
Method: OECD Guide–line 102 "Melting Point/Melting Range"
Year: 1981
GLP: no
Remark: Südzucker method based on OECD no. 102
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.2 Boiling Point

–

2.3 Density

Type: density
Value: = 1.59 kg/m3 at 20 degree C
Method: OECD Guide–line 109 "Density of Liquids and Solids"
Year: 1981
GLP: no
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.3.1 Granulometry

–

2.4 Vapour Pressure

–

2.5 Partition Coefficient

–

2.6.1 Water Solubility

Value: ca. 790 g/l at 20 degree C
Qualitative: of very high solubility
pH: ca. 7 at 100 g/l and 20 degree C
Method: OECD Guide–line 105 "Water Solubility"
Year: 1981
GLP: no
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.6.2 Surface Tension

–

2.7 Flash Point

Value:
Type:
Method:
Year:
Remark: not relevant
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.8 Auto Flammability

Value:
Remark: not relevant
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.9 Flammability

–

2.10 Explosive Properties

–

2.11 Oxidizing Properties

Result: no oxidizing properties
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

2.12 Additional Remarks

–

3.1.1 Photodegradation

–

3.1.2 Stability in Water

–

3.1.3 Stability in Soil

Type: **Radiolabel:**
Concentration:
Cation exch. capac.
Microbial biomass:
Method:
Year: **GLP:**
Test substance:
Remark: biodegradable
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

3.2 Monitoring Data (Environment)

–

3.3.1 Transport between Environmental Compartments

–

3.3.2 Distribution

–

3.4 Mode of Degradation in Actual Use

Remark: metabolized
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

(2)

3.5 Biodegradation

Type:
Inoculum:
Method:
Year: **GLP:**
Test substance:
Remark: Biodegradation
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

(3)

3.6 BOD5, COD or BOD5/COD Ratio

B O D 5

Method: other
Year: **GLP:** no
BOD5: = 918 mgO2/l

C O D

Method: other
Year: **GLP:** no
COD: = 1039 mg/g substance

R A T I O B O D 5 / C O D

BOD5/COD: = .884

Remark: BOD5: DIN 38409 part 51
COD: DIN 38409 part 41
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

3.7 Bioaccumulation

Species:
Exposure period:
Concentration:
BCF:
Elimination:
Method:
Year: **GLP:**
Test substance:
Remark: no accumulation
Source: Südzucker AG Mannheim/Ochsenfurt Mannheim

3.8 Additional Remarks

–

AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

–

4.2 Acute Toxicity to Aquatic Invertebrates

–

4.3 Toxicity to Aquatic Plants e.g. Algae

–

4.4 Toxicity to Microorganisms e.g. Bacteria

–

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

–

4.5.2 Chronic Toxicity to Aquatic Invertebrates

–

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Soil Dwelling Organisms

–

4.6.2 Toxicity to Terrestrial Plants

–

4.6.3 Toxicity to other Non–Mamm. Terrestrial Species

–

4.7 Biological Effects Monitoring

–

4.8 Biotransformation and Kinetics

–

4.9 Additional Remarks

–

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity
–

5.1.2 Acute Inhalation Toxicity
–

5.1.3 Acute Dermal Toxicity
–

5.1.4 Acute Toxicity, other Routes
–

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation
–

5.2.2 Eye Irritation
–

5.3 Sensitization
–

5.4 Repeated Dose Toxicity
–

5.5 Genetic Toxicity 'in Vitro'
–

5.6 Genetic Toxicity 'in Vivo'
–

5.7 Carcinogenicity
–

5.8 Toxicity to Reproduction
–

5.9 Developmental Toxicity/Teratogenicity
–

5.10 Other Relevant Information

–

5.11 Experience with Human Exposure

–

6. References

(1) Ullmanns Encyclopedia of Industrial Chemistry, A12, VCH Weinheim 1989

(2) every textbook of Biology

(3) each textbook of Biology

7.1 Risk Assessment

–

Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the evaluation of fructose for labelling purposes

(Request N° EFSA-Q-2005-085)

(adopted on 5 October 2005)

SUMMARY

Fructose is a widespread source of energy in human food. High amounts are contained in many fruits, soft drinks, sweets and confectionery, and some vegetables. Fructose is also a component of sucrose. Sorbitol can be metabolised into fructose in human.

Fructose is not an allergen. However, several adverse reactions to fructose can occur in humans. They result from inborn errors of metabolism. The rare hereditary fructose intolerance (HFI, prevalence 1:23,000 worldwide) and the very rare fructose biphosphatase (FBP'ase) deficiency are severe, potentially life-threatening diseases during infancy. The rare benign fructosuria (excretion of fructose in urine) does not cause adverse reactions. Intestinal fructose malabsorption (loss of fructose in stool) may cause transient abdominal discomfort which can easily be avoided.

Elimination of fructose is the main stay of treatment of HFI and FBP'ase deficiency and has to be instigated after definitive diagnosis. There are no scientifically established and generally accepted tolerable levels for fructose under these conditions.

KEY WORDS

Aldolase B, fructose, fructose biphosphatase deficiency, fructosuria, fructokinase deficiency, GLUT5 transporter, hereditary fructose intolerance, fructose malabsorption, sucrose, sorbitol.

BACKGROUND

Annex IIIa of Directive 2000/13/EC, as amended by Directive 2003/89/EC, establishes a list of ingredients that are known to trigger allergies or intolerances. The aforementioned Directive states that whenever the listed ingredients are used in the production of foodstuffs they must be labelled.

Article 6, paragraph 11 of the same Directive requests that the list in Annex IIIa shall be systematically re-examined and, where necessary, updated on the basis of the most recent scientific knowledge.

Furthermore, paragraph 11 states that Annex IIIa may be amended, in compliance with the procedure referred to in Article 20 (2), on the basis of a scientific opinion of the European Food Safety Authority.

TERMS OF REFERENCE

In accordance with Article 29 (1) (a) of Regulation (EC) N° 178/2002, the European Commission requests the European Food Safety Authority to provide a scientific opinion on the appropriateness for inclusion of fructose and their eventual derived products in the list of food allergens set up in Annex IIIa of Directive 2000/13/EC, in the light of the most recent scientific evidence.

ASSESSMENT

The Panel decided to focus on the evidence basis upon which a decision on the appropriateness of inclusion under the Terms of Reference could be based. The Panel considers the decision whether or not to include fructose a risk management task which is outside the remit of the Panel.

1. INTRODUCTION

Fructose is an important dietary carbohydrate source of energy. It is present in its free form in fruits, together with glucose in the disaccharide sucrose, and as part of oligo- and polysaccharides (inulins, fructans) in many plants. Fructose can also be formed from sorbitol (Rumessen, 1992; van den Berghe, 2000; Chen, 2004). Fructose is present as a commercial sweetener in sweets, confectionery and soft drinks. Mean daily intake of free fructose has been reported to be about 16 g/day. If fructose in sucrose is added, the total fructose exposure in heavy consumers may reach 150 grams daily (Rumessen, 1992; Park and Yetley, 1993).

Intestinal absorption of fructose is accomplished by a transporter called GLUT5 (Wasserman *et al*., 1996). It is metabolised in the liver, kidney and small intestine by the enzymes fructokinase, aldolase B and triokinase. The gluconeogenic pathway including the enzyme fructose-1,6-biphosphatase is also involved in fructose metabolism (van den Berghe, 2000) (see Figure 1).

Fructose is not an allergen. However, different inborn errors of fructose metabolism involving different enzymes exist: 1. Non-severe and 2. Severe ones.

1. Fructose malabsorption is common, but does not lead to severe symptoms and has been questioned to be a disease entity (Rumessen, 1992). Benign fructosuria (i.e. excretion of fructose in urine) is due to deficiency of fructokinase and does not require therapy (van den Berghe, 2000).

2. There are two potentially life-threatening rare inborn errors of fructose metabolism, namely hereditary fructose intolerance (HFI) (Baerlocher *et al*., 1978; Odièvre *et al*., 1978; Ali *et al*., 1998) due to the absence of aldolase B, and fructose-1,6-biphosphatase deficiency (FBP'ase deficiency) (Baker and Winegrad, 1970). Therapy of HFI involves removal of all known dietary sources of fructose, sucrose and sorbitol in natural and processed foods. In FBP'ase deficiency treatment includes limitation of fructose, but no complete avoidance.

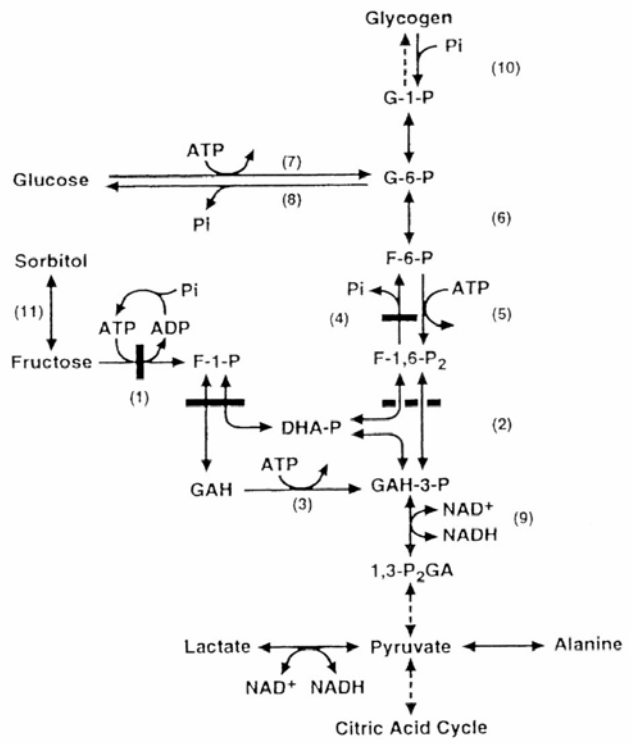

Figure 1. Metabolic pathways of fructose

Fructose is mainly metabolized by a specialized pathway found in the liver, kidney cortex and small intestinal mucosa and composed of fructokinase (*1*), aldolase B (*2*) and triokinase (*3*). Aldolase B also intervenes in the glycolytic-gluconeogenic pathway, which also includes the following enzymes: fructose-1,6-bisphosphatase (*4*), phosphofructokinase (*5*), glucose-6-phosphate isomerase (*6*), glucokinase and hexokinase (*7*), glucose-6-phosphatase (*8*) and glyceraldehyde-3-phosphate dehydrogenase (*9*). Also shown are glycogen phophorylase (*10*) and sorbitol dehydrogenase (*11*). *1,3-P$_2$GA*, 1,3-bisphosphoglycerate; *DHA-P*, dihydroxyacetone phosphate; *F*, fructose; *G*, glucose; *GAH*, glyceraldehyde; *P*, phosphate; *Pi*, inorganic phosphate. The three enzyme defects in fructose metabolism are depicted by *solid bars* across the *arrows*; the diminished activity of aldolase B toward fructose-1,6-bisphosphate in hereditary fructose intolerance is depicted by a *broken bar.*
[Adapted after van den Berghe (2000)]

2. FREQUENCY AND GENETICS

Benign fructosuria (Steinitz and Mizrahy, 1969) is caused by deficiency of fructokinase. Inheritance is autosomal recessive with a prevalence of 1:120,000. The gene encoding fructokinase is located on chromosome 2p23.3. The condition is benign, no treatment is necessary.

Intestinal fructose malabsorption has been found as frequently as in 71% of healthy fructose-challenged children and in 37.5% of healthy fructose-challenged adults (Rumessen, 1992). Fructose malabsorption has been associated to chronic non-specific diarrhoea and abdominal pain in children (Hyams *et al*., 1988; Hoekstra *et al*., 1992; Corpe *et al*., 1999) and adults (Choi *et al*., 2003). However, this condition is not well described and has not been generally accepted as a disease entity.

In addition to this, isolated fructose malabsorption (IFN) has been linked to a deficiency of the GLUT5 transporter (Rumesssen, 1992; Wasserman *et al*., 1996). It is a rare autosomal recessive abnormality. Only single cases have been described (Wasserman *et al.*, 1996).

Hereditary fructose intolerance (HFI) (Tolan, 1995; van den Berghe, 2000) is a rare congenital autosomal recessive disease with a prevalence of 1:23,000. The gene for the defective enzyme aldolase B is located on chromosome 9q22.3. Several mutations have been described. HFI is a severe potentially life-threatening disease.

Fructose biphosphatase deficiency (FBP'ase deficiency) (Baker and Winegrad, 1970; Bührdel *et al*., 1990) is a very rare autosomal recessive disease. Fewer than 100 cases have been reported worldwide in the scientific literature. The gene encoding fructose-1,6-biphosphatase is located on chromosome 9q22.2-22.3. The autosomal recessive disease is severe and life-threatening.

3. DIAGNOSIS AND CLINICAL FEATURES

In benign fructosuria (Steinitz and Mizrahy, 1996; van den Berghe, 2000), ingested fructose cannot be metabolised. Its level is increased in blood and urine where it can be found by a positive test for reducing sugars and by chromatography. There is no adverse reaction and there is no need for any therapy.

Intestinal fructose malabsorption is diagnosed by a pathologic breath hydrogen test after an oral fructose load (Corpe *et al.,* 1999; Hoekstra *et al*., 1993). Fructose malabsorption often presents with unspecific diarrhoea, abdominal pain and bloating. It has been described that the concomitant intake of glucose reduces the symptoms by increasing fructose absorption; however the mechanism is unknown (Wasserman *et al*., 1996). There is no organ damage. The rather non-specific gastrointestinal complaints usually respond well to reduction of the dietary fructose intake (Rumessen, 1992; Born *et al*., 1994; Corpe *et al*., 1999).

In HFI (Baerlocher *et al*., 1978; Odièvre *et al*., 1978; Cox, 1991; Ali *et al*., 1998; Chen, 2004; Van den Berghe, 2000), deficiency of aldolase B leads to liver and kidney accumulation of fructose-1-phosphate which initiates severe toxic symptoms by inhibition of glucose production and depletion of ATP. There are no symptoms as long as the affected subjects do not ingest fructose. Babies do well during breast-feeding but develop symptoms rapidly after introduction of sucrose, fruits and vegetables. In HFI, only infant formulae not containing fructose or sucrose may be used. Symptoms include nausea, vomiting, lethargy and convulsions together with hypoglycaemia. Severe liver disease, failure to thrive and renal dysfunction follow if fructose is not excluded from the diet. Life-threatening symptoms have been described in adults only after intravenous infusions of fructose or sorbitol (Cox, 1993). Some affected subjects may remain undiagnosed for a long time (Burmeister *et al*., 1991). Heterogeneity of HFI is indicated by different mutations of the aldolase B gene and by variability of clinical sensitivity to fructose. Diagnosis is done by assay of aldolase B activity in the liver and by direct DNA analysis. Therapy consists of complete elimination of sucrose, fructose and sorbitol from the diet including food additives and drug preparations. Individual fructose tolerance is variable (see below). Long-term prognosis is good.

Strictly FBP'ase deficiency (Baker and Winegrad, 1970; Bührdel *et al*., 1990; van den Berghe, 2000; Chen, 2004) is not a defect in the fructose pathway but a defect of

gluconeogenesis (compare pathways of fructose, Figure 1). Fifty per cent of patients present as early as in the first week of life with life-threatening acidosis, hypoglycaemia, hyperventilation, convulsions and coma. Metabolic derangement may be rapidly fatal. In infants and small children this presentation is triggered by febrile infections, gastroenteritis and fasting. There is hepatomegaly due to lipid storage. Diagnosis is made by demonstrating the enzyme deficiency in tissue and organ biopsies. Acute treatment consists of glucose infusion and control of acidosis. Fasting should be avoided, particularly during febrile infections. In small children, restriction of fructose, sucrose and sorbitol is also recommended. Long-term prognosis is good once the diagnosis has been made and treatment has been instigated.

4. SUBSTANCES IDENTIFIED TO INDUCE SYMPTOMS IN DISORDERS OF FRUCTOSE METABOLISM

Dietary fructose, sucrose (the disaccharide of glucose and fructose) and sorbitol induce symptoms in susceptible individuals.

5. METHODS OF ANALYSIS

Fructose, sucrose and sorbitol can be analysed in food by enzymatic methods and high-performance liquid chromatography (HPLC) (Kunst *et al*., 1984; Smith *et al*., 1986; Casterline *et al*., 1999; Li *et al*., 2002; Steegmans *et al*., 2004). Limit of detection for fructose is usually 0.1 g/100 mL. This is highly dependent on the complexity of the samples and matrix as well as on extraction methods. Enzymatic and HPLC methods have been standardised (DIN, ISO, AOAC). For specific purposes the limit of detection may be extended to lower sugar concentrations.

6. FRUCTOSE DOSES TRIGGERING CLINICAL MANIFESTATIONS

Benign fructosuria does not require any dietary therapy. For isolated fructose malabsorption dietary control is accomplished empirically and the additional intake of glucose may reduce symptoms.

HFI is a disease entity, where some established knowledge exists about quantitative dietary control and triggering doses (Mock *et al*., 1983; Bell *et al*., 1987; Cox, 1991; van den Berghe, 2000). Dietary therapy is based on food lists (Francis, 1987; van den Berghe, 2000). It has to be started immediately after diagnosis and consists of total exclusion of fructose, sucrose and sorbitol from the diet. One paper advises that short-term (2 days) administration of 4.7 mg of fructose/kg body weight/day was safe and well tolerated among individuals with diagnosed HFI (Barshop *et al*., 2003). Others advise that the total amount of fructose ingested per day should be less than 40 mg/kg body weight/day (Cox, 1991). However, at least in childhood, the intake of fructose should not be determined by subjective tolerance. An insufficient long-term restriction of fructose has been reported to lead to growth retardation (Mock *et al*., 1983). The spectrum of individual fructose tolerance in HFI, however, appears to depend on age (infants being more sensitive than adults) and on the respective gene mutation. While the need for a standardisation of treatment has led to the recommendation that a fructose-free diet should contain less than 1.5 grams of fructose per day (Bell *et al*., 1987), there are no

scientifically established and generally accepted triggering doses of fructose for patients with HFI.

In FBP'ase deficiency, avoidance of dietary fructose and sucrose is only a part of the therapy. In most patients, these substances do not have to be eliminated absolutely. Dietary control is achieved empirically using lists providing fructose, sucrose and sorbitol contents of foods and medications (Francis, 1987; van den Berghe, 2000).

CONCLUSIONS AND RECOMMENDATIONS

Fructose is a widespread source of energy in human food. High amounts are contained in many fruits, soft drinks, sweets and confectionery, and some vegetables. Fructose is also a component of sucrose. Sorbitol can be metabolised into fructose in human.

Fructose is not an allergen. However, several adverse reactions to fructose can occur in humans. They result from inborn errors of metabolism. The rare hereditary fructose intolerance (HFI, prevalence 1:23,000 worldwide) and the very rare fructose biphosphatase (FBP'ase) deficiency are severe, potentially life-threatening diseases during infancy. The rare benign fructosuria (excretion of fructose in urine) does not cause adverse reactions. Intestinal fructose malabsorption (loss of fructose in stool) may cause transient abdominal discomfort which can easily be avoided.

Elimination of fructose is the main stay of treatment of HFI and FBP'ase deficiency and has to be instigated after definitive diagnosis. There are no scientifically established and generally accepted tolerable levels for fructose under these conditions.

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PANEL MEMBERS

Wulf Becker, Francesco Branca, Daniel Brasseur, Jean-Louis Bresson, Albert Flynn, Alan A. Jackson, Pagona Lagiou, Martinus Løvik, Geltrude Mingrone, Bevan Moseley, Andreu Palou, Hildegard Przyrembel, Seppo Salminen, Stephan Strobel, Henk van den Berg, and Hendrik van Loveren.

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SCIENTIFIC OPINION

Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre[1]

EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

This Opinion of the EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) deals with the establishment of Dietary Reference Values for carbohydrates and dietary fibre. Nutritionally, two broad categories of carbohydrates can be differentiated: "glycaemic carbohydrates", i.e. carbohydrates digested and absorbed in the human small intestine, and 'dietary fibre', non-digestible carbohydrates passing to the large intestine. In this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin. The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. The Panel proposes 45 to 60 E% as the reference Intake range for carbohydrates applicable to both adults and children older than one year of age. Although high frequency of intake of sugar-containing foods can increase the risk of dental caries, there are insufficient data to set an upper limit for (added) sugar intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g/day to be adequate for normal laxation in adults. A fibre intake of 2 g/MJ is considered adequate for normal laxation in children from the age of one year. Although there is some experimental evidence that a reduction of the dietary glycaemic index and glycaemic load may have favourable effects on some metabolic risk factors such as serum lipids, the evidence for a role in weight maintenance and prevention of diet-related diseases is inconclusive.

KEY WORDS

Carbohydrates, dietary fibre, sugars, added sugars, glycaemic carbohydrates, oligosaccharides, starch, lignin, glycaemic index, glycaemic load, dietary requirements, blood lipids, lipid profile, glucose tolerance, insulin sensitivity, body weight, type 2 diabetes, blood pressure, cardiovascular disease, coronary heart disease, dental caries, gastrointestinal function, colorectal cancer, mineral absorption

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2 Panel members: Carlo Agostoni, Jean-Louis Bresson, Susan Fairweather-Tait, Albert Flynn, Ines Golly, Hannu Korhonen, Pagona Lagiou, Martinus Løvik, Rosangela Marchelli, Ambroise Martin, Bevan Moseley, Monika Neuhäuser-Berthold, Hildegard Przyrembel, Seppo Salminen, Yolanda Sanz, Sean (J.J.) Strain, Stephan Strobel, Inge Tetens, Daniel Tomé, Hendrik van Loveren and Hans Verhagen.
Correspondence: nda@efsa.europa.eu

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) was asked to deliver a scientific opinion on Population Reference Intakes for the European population, including carbohydrates and dietary fibre.

Nutritionally, two broad categories of carbohydrates can be differentiated: "glycaemic carbohydrates", i.e. carbohydrates digested and absorbed in the human small intestine, and "dietary fibre", non-digestible carbohydrates passing to the large intestine.

The main glycaemic carbohydrates are monosaccharides, disaccharides, malto-oligosaccharides, and starch. In this Opinion the term "sugars" is used to cover monosaccharides and disaccharides. The term "added sugars" refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term "sugars". However, they are partly metabolised and included in "carbohydrates" according to the European legislation.

In this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin, including non-starch polysaccharides (NSP) – cellulose, hemicelluloses, pectins, hydrocolloids (i.e., gums, mucilages, β-glucans), resistant oligosaccharides – fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides, resistant starch – consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches, and lignin associated with the dietary fibre polysaccharides.

Main dietary sources of sugars are fruits, berries, fruit juices, some vegetables, milk and milk products, and foods containing added sucrose and starch hydrolysates (e.g., glucose syrup, high-fructose syrup) such as carbonated beverages and sweets. Main dietary sources of starch are bread and other cereal products, potatoes, tubers and pulses.

Data from dietary surveys show that average carbohydrate intakes in European countries in children and adolescents varied between 43 to 58 E%, and from 38 to 56 E% in adults. Average intakes of sugars varied between 16 to 36 E% in children and adults.

Whole grain cereals, pulses, fruit, vegetables and potatoes are the main sources of dietary fibre. Average dietary fibre intakes varied from 10 to 20 g per day in young children (<10 to 12 years), from 15 to 30 g per day in adolescents, and from 16 to 29 g per day in adults. Average intakes of dietary fibre per MJ ranged from 1.7 to 2.5 g per MJ in (young) children and from 1.8 to 2.9 g per MJ in adults.

Total and glycemic carbohydrates

As energy balance is the ultimate goal, dietary reference values for carbohydrate intake cannot be made without considering other energy delivering macronutrients and will be given as percentage of total energy intake (E%). The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. Generally, an intake of 50 to100 g per day will prevent ketosis. An intake of 130 g per day for both children (>1 year) and adults has been estimated to be sufficient to cover the needs of glucose for the brain. However, these levels of intake are not sufficient to meet energy needs in the context of acceptable intake levels of fat and protein.

Intervention studies provide evidence that high fat (>35 E%), low carbohydrate (<50 E%) diets are associated to adverse short- and long-term effects on body weight, although data are not sufficient to define a Lower Threshold of Intake (LTI) for carbohydrates. Similarly, high carbohydrate diets tend

to induce adverse effects on the blood lipid profile, but there is an insufficient scientific basis for setting a Tolerable Upper Intake Level (UL) for total carbohydrates. The Panel therefore comes to the conclusion that only a Reference Intake range can be given for total carbohydrate intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns).

Based on the above considerations the Panel proposes 45 to 60 E% as the Reference Intake range for carbohydrates. Diets with glycaemic carbohydrate contents of 45 to 60 E%, in combination with reduced intakes of fat and saturated fatty acids (SFA), are compatible with the improvement of metabolic risk factors for chronic disease, as well as with mean carbohydrate intakes observed in some European countries. This intake range applies to both adults and children older than one year of age.

Sugars

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when oral hygiene and fluoride prophylaxis are insufficient. However, available data do not allow the setting of an upper limit for intake of (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by frequency of consumption, oral hygiene, exposure to fluoride, and various other factors.

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for intake of (added) sugars based on their effects on body weight.

Observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake.

Although there is some evidence that high intakes (>20 E%) of sugars may increase serum triglyceride (TG) and cholesterol concentrations, and that >20 to 25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake.

Evidence on the relationship between patterns of consumption of sugar-containing foods and dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines.

The Panel notes that a number of authorities have established upper limits for population average intake or individual intake of added sugars of <10 E% but others have not. Typically, such recommendations reflect a judgement of what level of sugar intake is practically achievable within the context of a nutritionally adequate diet based on known patterns of intake of foods and nutrients in specific populations. It is also noted that the average intake of (added) sugars in some EU Member States exceeds 10 E%, especially in children.

Dietary Fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g per day to be adequate for normal laxation in adults. There is limited evidence to set adequate intakes for children. The Panel considers that the Adequate Intake (AI) for dietary fibre

for children should be based on that for adults with appropriate adjustment for energy intake. A fibre intake of 2 g per MJ is considered adequate for normal laxation in children from the age of one year.

The Panel notes that in adults there is evidence of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g. reduced risk of coronary heart disease and type 2 diabetes and improved weight maintenance. Such evidence should be considered when developing food-based dietary guidelines.

Glycaemic index and glycaemic load

Although there is some experimental evidence that a reduction of the dietary glycaemic index and glycaemic load may have favourable effects on some metabolic risk factors such as serum lipids, the evidence for a role in weight maintenance and prevention of diet-related diseases is inconclusive.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The scientific advice on nutrient intakes is important as the basis of Community action in the field of nutrition, for example such advice has in the past been used as the basis of nutrition labelling. The Scientific Committee for Food (SCF) report on nutrient and energy intakes for the European Community dates from 1993. There is a need to review and if necessary to update these earlier recommendations to ensure that the Community action in the area of nutrition is underpinned by the latest scientific advice.

In 1993, the SCF adopted an opinion on the nutrient and energy intakes for the European Community[4]. The report provided Reference Intakes for energy, certain macronutrients and micronutrients, but it did not include certain substances of physiological importance, for example dietary fibre.

Since then new scientific data have become available for some of the nutrients, and scientific advisory bodies in many Eropean Union Member States and in the United States have reported on recommended dietary intakes. For a number of nutrients these newly established (national) recommendations differ from the reference intakes in the SCF (1993) report. Although there is considerable consensus between these newly derived (national) recommendations, differing opinions remain on some of the recommendations. Therefore, there is a need to review the existing EU Reference Intakes in the light of new scientific evidence, and taking into account the more recently reported national recommendations. There is also a need to include dietary components that were not covered in the SCF opinion of 1993, such as dietary fibre, and to consider whether it might be appropriate to establish reference intakes for other (essential) substances with a physiological effect.

In this context the EFSA is requested to consider the existing Population Reference Intakes for energy, micro- and macronutrients and certain other dietary components, to review and complete the SCF recommendations, in the light of new evidence, and in addition advise on a Population Reference Intake for dietary fibre.

For communication of nutrition and healthy eating messages to the public it is generally more appropriate to express recommendations for the intake of individual nutrients or substances in food-based terms. In this context the EFSA is asked to provide assistance on the translation of nutrient based recommendations for a healthy diet into food based recommendations intended for the population as a whole.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

In accordance with Article 29 (1)(a) and Article 31 of Regulation (EC) No. 178/2002, the Commission requests EFSA to review the existing advice of the Scientific Committee for Food on Population Reference Intakes for energy, nutrients and other substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

In the first instance the EFSA is asked to provide advice on energy, macronutrients and dietary fibre. Specifically advice is requested on the following dietary components:

- Carbohydrates, including sugars;
- Fats, including saturated fatty acids, poly-unsaturated fatty acids and mono-unsaturated fatty acids, *trans* fatty acids;
- Protein;

[4] Scientific Committee for Food, Nutrient and energy intakes for the European Community, Reports of the Scientific Committee for Food 31st series, Office for Official Publication of the European Communities, Luxembourg, 1993.

- Dietary fibre.

Following on from the first part of the task, the EFSA is asked to advise on Population Reference Intakes of micronutrients in the diet and, if considered appropriate, other essential substances with a nutritional or physiological effect in the context of a balanced diet which, when part of an overall healthy lifestyle, contribute to good health through optimal nutrition.

Finally, the EFSA is asked to provide guidance on the translation of nutrient based dietary advice into guidance, intended for the European population as a whole, on the contribution of different foods or categories of foods to an overall diet that would help to maintain good health through optimal nutrition (food-based dietary guidelines).

ASSESSMENT

A draft of this Opinion, agreed by the NDA Panel on 13 March 2009, was published on the EFSA website[5] for public consultation between 5 August and 15 October 2009. The draft Opinion was also discussed at a National Expert Meeting with Member States on Dietary Reference Values held in Barcelona on 7 and 8 September 2009. All the public comments received and comments from Member States that related to the remit of EFSA were assessed and the Opinion has been revised taking relevant comments into consideration. The comments received, a report on the outcome of the public consultation, and the minutes of the meeting with Member States have been published on the EFSA website.

1. Introduction

Carbohydrates are the main source of energy in most human diets. Carbohydrates are defined within European legislation (Directive 90/496/EEC) as "metabolisable carbohydrates and including polyols"[6]. Chemically, dietary fibre is also a carbohydrate (EFSA, 2007; Directive 2008/100/EC[7]).

2. Definition / category

Chemically, carbohydrates include a range of components such as polyhydroxy aldehydes, ketones, alcohols and acids, as well as their derivatives and polymers, e.g. starch and other polysaccharides. The chemical classification of carbohydrates is usually based on molecular size and monomeric composition, three principal groups being sugars (1–2 monomers), oligosaccharides (3–9 monomers) and polysaccharides (10 or more monomers) (FAO/WHO, 1998, see also Table 1). Due to the chemical diversity of carbohydrates, it is only recently that specific methods for analysis of various carbohydrates in foods have become routinely available. Therefore, carbohydrate values on labels and in food tables are often still derived "by difference" (section 2.1.3).

Nutritionally, it is important to differentiate between two broad categories of carbohydrates: those digested and absorbed in the human small intestine, providing carbohydrates to body cells and those passing to the large intestine, forming substrate for the colonic microflora (Asp, 1996; Englyst and Englyst, 2005). A FAO/WHO Expert Consultation on Carbohydrates in Human Nutrition recommended the introduction of the concept "glycaemic carbohydrate", meaning 'providing carbohydrate for metabolism', which corresponds to the previously used term 'available carbohydrates' (FAO/WHO, 1998) and to 'carbohydrates' according to the European legislation. The nondigestible ("unavailable") carbohydrates are commonly referred to as "dietary fibre" (see 2.1.2 for definitions).

[5] http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1211902045161.htm

[6] Council Directive 90/496/EEC of 24 September 1990 on nutrition labelling for foodstuffs. OJ L 276, 6.10.1990, pp. 40–44.

7 Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. OJ L 285, 29.10.2008, pp. 9-12.

Table 1: Main types of carbohydrates (Adapted from Asp, 1996).

Class (DP *)	Sub-group	Components	Monomers	Digestibility**
Sugars (1-2)	Monosaccharides	Glucose		+
		Galactose		+
		Fructose		+
	Disaccharides	Sucrose	Glu, Fru	+
		Lactose	Glu, Gal	+ (–) ***
		Trehalose	Glu	+
		Maltose	Glu	+
Oligosaccharides(3-9)	Malto-oligo-saccharfides	Maltodextrins	Glu	+
	Other oligo-saccharides	α-Galactosides (GOS)	Gal, Glu	–
		Fructo-oligosaccharides (FOS)	Fru, Glu	–
		Polydextrose	Glu	–
		Resistant dextrins	Glu	–
Polyols	Maltitol, sorbitol, xylitol, lactitol			+/–
Polysaccharides (>9)	Starch	Amylose	Glu	+ (–)
		Amylopectin	Glu	+ (–)
		Modified starch	Glu	–
		Resistant starch	Glu	–
		Inulin	Fru	–
	Non-starch poly-saccharides	Cellulose	Glu	–
		Hemicelluloses	Variable	–
		Pectins	Uronic acids	–
		Other hydrocolloids, e.g. gums, mucilages, β-glucans	Variable	–
Related substance		Lignin		–

* DP = Degree of polymerisation
**Denotes digestibility in the small intestine: + digestible, + (–) mainly digestible, +/- partly digestible, – non-digestible
***Lactose is poorly digested by individuals with low intestinal lactase activity
Fru = Fructose, Glu = Glucose, Gal = Galactose

2.1. Categories

2.1.1. Glycaemic carbohydrates

The glycaemic carbohydrates provide carbohydrate to body cells, mainly in the form of glucose. The main glycaemic carbohydrates are (see also Table 1):

- Glucose and fructose (monosaccharides)
- Sucrose and lactose (disaccharides)
- Malto-oligosaccharides
- Starch (polysaccharide)

In this Opinion the term “sugars” covers monosaccharides and disaccharides. In the literature, various terms are used to differentiate between sugars naturally occurring in foods, e.g. “intrinsic” sugars, and sugars and sugar preparations added to foods, e.g. “added” or “extrinsic” sugars” (IoM, 2005; DoH, 1991). In this opinion the term “added sugars” refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing.

Sugar alcohols (polyols) such as sorbitol, xylitol, mannitol, and lactitol, are usually not included in the term "sugars". However, they are partly absorbed and included in "carbohydrates" according to the European legislation.

2.1.2. Dietary fibre

The term "dietary fibre" was originally defined as "that portion of food which is derived from cellular walls of plants which are digested very poorly by human beings" (Trowell, 1972). The recognition that polysaccharides added to foods, notably hydrocolloids, could have effects similar to those originating from plant cell walls led to a redefinition of dietary fibre to include "polysaccharides and lignin that are not digested in the human small intestine" (Trowell et al., 1976). The definition and delimitation of "dietary fibre" has been much debated and related both to physiological considerations and to methods that can be used for dietary fibre analysis in foods (FAO/WHO, 1998; Asp, 1995 and 1996; Englyst and Hudson, 1996; Englyst and Englyst, 2005; Englyst et al., 2007).

Non-starch polysaccharides (NSP) are the main constituents of dietary fibre and include a host of different polymers, highly variable in terms of molecular size and structure, as well as in monomeric composition. Main classes of non-starch polysaccharides are cellulose, hemicelluloses, pectins, and other hydrocolloids. Due to the structural variability, different non-starch polysaccharides may have very different physical-chemical properties, which are of key importance for their physiological effects. For example cellulose is insoluble in water, whereas pectins and hydrocolloids, e.g. guar gum and mucilages, may form highly viscous water solutions. Resistant starch is insoluble and indigestible due to its physical form or enclosure in cellular structures, whereas resistant oligosaccharides are readily soluble in water but do not form viscous solutions. The terms "soluble" and "insoluble" dietary fibre have been used in the literature to differentiate between viscous, soluble types of fibre (e.g. pectins) and insoluble components such as cellulose. The distinction was mainly based on the different physiological effects. However, this differentiation is method-dependent, and solubility does not always predict physiological effects. Therefore, FAO/WHO proposed the distinction between soluble and insoluble fibre should be phased out (FAO/WHO, 1998).

The interest in defining and quantifying dietary fibre in foods lies in the physiological effects that are associated with their consumption, which include decreased intestinal transit time and increased stool bulk, reducing blood total and/or LDL cholesterol concentrations, and reducing post-prandial blood glucose and /or insulin concentrations, among others (AFSSA 2002; NNR, 2004; IoM, 2005; GR, 2006; Mann et al., 2007). These physiological effects of dietary fibre are distinct from those of glycaemic carbohydrates.

In national and international recommendations on dietary fibre intake, the definitions are generally in accordance with and related to analysis with methods approved by the Association of Official Analytical Chemists (AOAC). Definitions differ with respect to some minor components such as fibre of animal origin and some synthetic or isolated fibre constituents (Annex 1).

The U.S. Food and Nutrition Board (FNB) defines "total dietary fiber" as the sum of "dietary fiber", consisting of non-digestible carbohydrates and lignin that are intrinsic and intact in plants, and "functional fiber", consisting of isolated, non-digestible carbohydrate components with demonstrated beneficial physiological effects in humans (IoM, 2005). The rationale behind this differentiation is that there is epidemiological evidence for beneficial effects of foods naturally high in dietary fibre, such as whole-grain cereals, some fruits and vegetables, and that dietary fibre can be regarded as a marker of such foods. The argument that the term "dietary fibre" should be restricted to non-starch polysaccharides of cell wall origin (Englyst and Englyst, 2005; Englyst et al., 2007) has a similar rationale. Consequently, according to the FNB, documentation of the beneficial effects of added, functional fibre is required for inclusion in "total dietary fibre".

The Panel notes that a major problem in making this differentiation in practice is that analytical methods cannot differentiate between "dietary fibre" and "functional fibre" once they occur mixed in a food product,

and similarly NSP from plant cell walls cannot be differentiated from added NSP with similar monomeric composition.

In view of the key importance of small-intestinal digestibility for the nutritional effects of carbohydrates, the Panel considers that dietary fibre should include all non-digestible carbohydrates. This includes non-starch polysaccharides, resistant starch, resistant oligosaccharides with three or more monomeric units and other non-digestible, but quantitatively minor components that are associated with the dietary fibre polysaccharides, especially lignin (Cho et al., 1997; AACC, 2001; AFSSA, 2002; NNR, 2004; GR, 2006). This definition is in accordance with the definition brought to step 8 in the Codex Alimentarius (Codex, 2009) and agreed by the Codex Alimentarius Committee in 2009, although the inclusion of non-digestible carbohydrates with 3 to 9 monomeric residues is so far left to the national authorities. As in the EU definition, beneficial physiological effects have to be demonstrated before addition of natural or synthetic fibre to foods (Annex 1).

The minimum chain length of three monomeric units, degree of polymeration (DP) 3, was set since undigestible oligosaccharides with DP3-9, such as fruco-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) are natural constituents of many foods, quantitatively important in e.g. artichokes and beans, respectively. There is considerable evidence of "fibre-like" effects of these oligosaccharides, such as promoting a presumably "healthy" microflora, short-chain fatty acids (SCFA)-production in the colon, and enhancement of calcium absorption from the colon. Undigestible disaccharides are not prominent constituents of normal foods and not well characterised physiologically. However, if such ingredients will be available in the future and shown to have "fibre-like" effects, there may be reasons to reconsider the DP3 limit.

For the purpose of this Opinion, dietary fibre is defined as non-digestible carbohydrates plus lignin. The Panel considers that the main types of total dietary fibre are:

- Non-starch polysaccharides (NSP) - cellulose, hemicelluloses, pectins, hydrocolloids (i.e. gums, mucilages, β-glucans).
- Resistant oligosaccharides - fructo-oligosaccharides (FOS), galacto-oligosaccharides (GOS), other resistant oligosaccharides.
- Resistant starch - consisting of physically enclosed starch, some types of raw starch granules, retrograded amylose, chemically and/or physically modified starches.
- Lignin associated with the dietary fibre polysaccharides.

Methods of analysis

Current enzymatic gravimetric or enzymatic chemical methods for dietary fibre cover NSP, analytically resistant starch and lignin. However, given that dietary fibre is a mixture of chemically heterogeneous carbohydrate components, several analytical methods are currently required to measure all fractions of dietary fibre. Methods measuring NSP alone (Englyst and Hudson, 1996) give lower estimates than methods for total dietary fibre in foods containing resistant starch, and/or lignin, e.g. whole-grain flour and cereals processed in a way that generates resistant starch. On the other hand, methods determining dietary fibre, including resistant starch, measure the fraction, which includes mainly retrograded amylose, resistant to the enzymes used in the assay. Finally, resistant oligosaccharides and inulin are not included in any of the current methods for total dietary fibre, and therefore need to be measured separately and subsequently added to the total fibre estimate (Cho et al., 1997; Champ et al., 2001 and 2003).

2.1.3. Total carbohydrates

In many food composition tables and in food labelling, carbohydrates are still expressed “by difference”, which means that moisture, protein, fat and ash are analytically determined and the rest named

"carbohydrates". This non-specific procedure includes all kinds of carbohydrates regardless of their physiological and nutritional properties, as well as variable amounts of non-carbohydrate material, e.g. organic acids, lignin and polyphenols (FAO/WHO, 1998; Southgate, 1995; Asp, 1995).

2.2. Metabolism

2.2.1. Glycaemic carbohydrates

The glycaemic carbohydrates provide carbohydrates to body cells, mainly in the form of glucose. In general, only monosaccharides are absorbed in the small intestine. The enzymatic degradation of starch begins by the action of salivary amylase and is continued in the small intestine by pancreatic amylase. The degradation products – mainly maltose and oligosaccharides – are hydrolysed further to glucose by a set of enzymes, "disaccharidases", bound to the brush border membrane of the enterocytes. The same enzymes hydrolyse the dietary disaccharides. Glucose and galactose are absorbed efficiently by a secondary active carrier coupled with sodium (sodium glucose transporter 1, SGLT1), whereas fructose is absorbed by facilitated diffusion that does not involve sodium co-transport (GLUT5). The absorption of monosaccharides is regarded as the rate-limiting step.

Absorbed monosaccharides are transported to the liver and then to the systemic circulation. The cellular uptake is mediated by a number of glucose transporters (GLUT1–4), variously expressed in different tissues. Insulin is a key hormone for the uptake and metabolism of glucose. The plasma insulin concentration increases immediately after ingestion of glycaemic carbohydrates. Unlike glucose, fructose enters body cells without the need for insulin. The metabolism of fructose, therefore, favours lipogenesis more than glucose. In liver cells, fructose is phosphorylated to fructose-1-phosphate that can be converted to fatty acids, providing a route for lipogenesis in addition to that shared with glucose (via glucose/fructose-6-phosphate) (Vasankari and Vasankari, 2006). Both fructose and galactose, the latter arising from hydrolysis of lactose, are also transformed to glucose mainly in the liver.

2.2.2. Glycaemic index and glycaemic load

The concept of glycaemic index (GI) was introduced by Jenkins and co-workers in 1981, in order to rank foods in a standardised way according to their effects on blood glucose levels after a meal. The FAO/WHO Expert Consultation defined GI as the incremental area under the blood glucose response curve during 1.5–3 hours after intake of a 50 g carbohydrate portion of a test food, and expressed as a percentage of the response to the same amount of carbohydrate from a standard food taken by the same subject (FAO/WHO, 1998). Glucose or white bread is used as standard. GI values obtained with the white bread standard are typically about 40% higher than those obtained with the glucose standard which is the generally preferred standard. GI values for about 750 foods have been published (Foster-Powell et al., 2002) and recently updated with additional data to contain 2480 individual food items (Atkinson et al., 2008).

Whereas it was previously assumed that sugars are rapidly absorbed and therefore have a higher GI than polysaccharides (e.g. starch), which are slowly absorbed, a number of food-related factors have been identified to determine the GI. For instance, fructose has a low GI (30 with the white bread reference as 100) and sucrose an intermediate GI, i.e. lower than white bread (Björck et al., 2000). Starchy foods, on the other hand, can have low, intermediate or high GI, depending on their composition (amylose/amylopectin ratio) and physical/chemical state. The swelling and dissolution of starch at wet heat treatment, known as gelatinisation, is particularly important in making starch more readily accessible to digestive enzymes. Physical barriers such as in intact cereal grains, cellular structures in leguminous seeds, parboiled rice and whole fruits, and the protein network in pasta products, are food-related factors lowering the GI. Organic acids (acetic, propionic and lactic acid) decrease the glycaemic response to foods or meals, mainly due to inhibition of gastric emptying (Liljeberg and Björck, 1998). Viscous, soluble types of dietary fibre may also delay gastric emptying, in addition to their inhibitory effect on diffusion and transport in the small intestine (Brown et al., 1999; Jenkins et al., 2000).

In practice, the blood glucose response after a meal is influenced by both the GI and the amount of carbohydrate in a portion of a food. Consequently, the glycaemic load (GL) concept was introduced in 1997 to quantify the glycaemic effect of a portion of food (Salmeron et al., 1997a and 1997b). GL is defined as the amount of glycaemic carbohydrate in a food times the GI of the food/100, and the sum of individual GL values for foods and meals has been used to estimate the glycaemic load of the whole diet.

Studies have shown that the glycaemic response to a meal can be predicted from properly determined GI of the constituent foods (Wolever et al., 2006; Wolever and Jenkins, 1986; Järvi et al., 1999). However, the glycaemic response can also be influenced by the protein and fat content, and by the type and amount of beverage taken with the food (Henry et al., 2006). Flint et al. (2004) found no correlations between predicted postprandial glucose responses based on published GI values for foods and the measured glucose elevations after different breakfast meals containing 50 g available carbohydrates and varying amounts of fat and protein. This indicates that the composition and the size of a meal, i.e. in terms of energy and macronutrient content, are also important determinants for the glycaemic responses. Validated GI values for food products are needed in studies investigating effects of GI.

2.2.3. Dietary fibre

The components included in dietary fibre are by definition resistant to hydrolysis and absorption in the small intestine. They pass the upper gastro-intestinal tract and enter the colon substantially unmodified. Viscous, water-soluble fibre such as β-glucans and pectin can modify blood glucose response and total and LDL-cholesterol concentrations by interfering with digestion and absorption of glycaemic carbohydrates and cholesterol and/or bile acids, respectively. Inhibitory effects on mineral absorption, i.e. of iron, zinc and calcium, have been attributed to fibre-associated complexing compounds, notably phytic acid in cereals and leguminous seeds.

Dietary fibre components are subject to more or less extensive anaerobic fermentation by the colonic microflora. The extent of fermentation is dependent on both substrate and host factors, e.g. molecular structure and physical form of the substrate, bacterial flora and transit time. Less fermentable types of fibre, such as in lignified outer layers of cereal grain, generally have the most prominent faecal bulking effects due to their ability to bind water in the distal colon. Fermentable fibre also contributes to the faecal bulk through increased microbial mass.

Some fermentation products, such as propionic acid and butyrate, may influence also systemic metabolism, i.e. cholesterol synthesis and possibly insulin sensitivity. Fermentable dietary fibre components, including oligosaccharides that are often referred to as “prebiotics”, increase Bifidobacteria and Lactobacilli which produce lactate and short-chain fatty acids such as acetate, propionate and butyrate (Gibson and Roberfroid, 1995). These fatty acids inhibit the fermentation of protein components, which could produce potentially toxic products, especially ammonia and amines. Short-chain fatty acids decrease the pH of the colonic content, which stimulates colonic absorption of minerals, notably calcium, and inhibits formation of potential co-carcinogens from bile acids. Butyrate is a main source of energy for the colonic mucosa and has effects on cell differentiation and apoptosis with possible implications for colon carcinogenesis. Acetate and propionate are absorbed from the colon and thus provide energy to the host (Cummings et al., 2004).

The absorption of fermentation products, i.e. short chain fatty acids, means that dietary fibre contributes to the energy content of the diet, but less than glycaemic carbohydrates. The contribution is variable depending on the extent of fermentation. FAO/WHO (1998) has recommended the use of an average energy factor for dietary fibre, 8 kJ or 2 kcal per g, and this recommendation has been now included in the EU nutrition labelling Directive[8].

[8] Council Directive of 24 September 1990 on nutrition labelling for foodstuffs (90/496/ECC). OJ L 276, 6.10.1990, p.40 ammended by Commission Directive 2008/100/EC of 28 October 2008.

3. Dietary sources and intake data

3.1. Dietary sources

3.1.1. Glycaemic carbohydrates

Main dietary sources of glucose and fructose are fruits, berries, fruit juices and some vegetables. Free galactose is rare in foods, except in fermented and lactase-hydrolysed milk products. Fruits, berries and juices are natural sources of sucrose, although sugar added to foods, carbonated beverages and sweets or in the household usually provides most of the dietary sucrose. More or less completely hydrolysed starch or high fructose syrup, in which about half the glucose is isomerised to fructose, are increasingly used in some countries, to replace sucrose in confectionary and carbonated drinks.

Lactose occurs exclusively in milk and milk products. Human milk has the highest lactose content of all milks, 7 g per 100 g. The lactose content in cow's milk is around 5 g per 100 g. Digestible malto-oligosaccharides originate mainly from partly hydrolysed starch.

Main dietary sources of starch are bread and other cereal products, potatoes, tubers and pulses (FAO/WHO, 1998).

3.1.2. Dietary fibre

Whole grain cereals, pulses, fruit and vegetables and potatoes are the main sources of dietary fibre. Also nuts and seeds contain high concentrations. Cellulose occurs together with hemicelluloses in cereals. The lignified outer layers are the predominant fibre source in whole-grain products. Oats and barley contain high concentrations of a water-soluble, viscous type of polysaccharide, β-glucan. Pectins, a main type of dietary fibre in fruits and vegetables, have similar properties.

3.2. Dietary intake

Typical intakes of carbohydrates and dietary fibre are presented for children and adolescents in 19 countries (Annex 2a and 2b) and for adults in 22 countries in Europe (Annex 3a and 3b). The data refer to individual based food consumption surveys, conducted from 1994 onwards. Most studies comprise national representative population samples. The data were derived from national reports and from a recently published overview (Elmadfa, 2009).

As shown in Annexes 2 and 3, there is a large diversity in the methodology used to assess individual intakes of children, adolescents and adults. Because the different methods apply to different time frames, this inevitably resulted in variance in both the quality and quantity of available data, which make direct comparability difficult. Moreover, age classifications are in general not uniform. Comparability might also be hampered by differences in food composition tables used for the conversion of food consumption data to estimated nutrient intakes (Deharveng et al., 1999). Food consumption data are prone to reporting errors and there might be a varying degree of underreporting in different surveys.

Although these differences may have an impact on the accuracy of between country comparisons and the results should be interpreted with caution, the presented data give a rough overview of the carbohydrate intake in a number of European countries. Most studies reported mean intakes and standard deviations (SD) or mean intakes and intake distributions. In most studies the contribution of carbohydrates to energy intake is based on total energy intake (including the energy from alcohol).

3.2.1. Total carbohydrates

Average carbohydrate intakes in children and adolescents in European countries varied between 41 to 58 E% (Annex 2b). Most of the reported average intakes (82%) were between 47 and 55 E%; approximately 13% were above 55%. Within population ranges varied from 38 to 49 E% (5th percentile) to 63 to 66 E% (95th percentile).

In adults average carbohydrate intakes varied from approximately 38 to 54 E% (Annex 3b). In the various age categories average intake of carbohydrates ranged from approximately 41 to 51 E% (19-34 years), 38 to 49 E% (35 to 64 years) and 40 to 53 E% (65 years and over), respectively. More than half (53%) of the reported mean values were between 45 and 50 E%; Average carbohydrate intakes of 50 E% and higher were achieved in 16% of the adult groups belonging to the age categories 19 to 34 years and 65 years and over. Within population ranges varied from 31 to 34 E% at the lower (5th percentile) to 58 to 61 E% at the upper end (95th percentile) of the distributions. Mean intakes were highest in the Czech Republic and Norway and lowest in Greece and Spain.

As shown in Annexes 2b and 3b, not all countries reported intakes of mono-, disaccharides and polysaccharides. When reported, average intakes of mono- and disaccharides varied between 23 to 36 E% in children and adolescents, with highest intakes in infants, whereas the intake of polysaccharides was between 23 and 25 E%.

In Finnish infants aged 8 to 13 months the reported average intake of sucrose was 3 to 5 E%. This amount increased in children aged 2 to3 years to approximately 10 to 12 E%. In schoolchildren and adolescents average intakes varied between 11 and 25 E%. More than half (56%) of these average intakes were between 10 and 15 E%. Available intake distributions showed that five percent of the children and adolescents had average intakes of 20 E% and above.

In adults the intake of mono and disaccharides varied between 17 to 26 E% and the intake of polysaccharides between 20 to 27 E%.

Average sucrose intake in adults varied from 6 to nearly 14 E%. Average intakes below 11 E% were only observed in the older age categories (35 to 64 years: in 94%; 65 years and over: in 79% of the group). Within population ranges varied from 1 to 4 E% at the lower (5th percentile) to 17 to 25 E% at the upper end (95th percentile) of the distribution.

3.2.2. Dietary fibre

Apart from infants and young children, average dietary fibre intakes varied between 10 to 20 g per day in young children (<10 to 12 years), and from 15 to 33 g per day in adolescents. The highest intakes were observed in German adolescents (Annex 2b). Within population ranges varied from 6 to 8 g per day (5th percentile) to 25 to 46 g per day (95th percentile). Expressed per MJ reported intakes were between 1.7 g per MJ and 2.5 g per MJ. Studies on German children followed from 6 months up to 18 years of age (data not presented) show that the energy adjusted fibre intake was highest at 1 year (3 g per MJ), thereafter declining to about 2.5 g per MJ in preschool- and school-age (Alexy et al., 2006).

In adults average dietary fibre intakes ranged from 15 to 30 g per day. For subjects aged 65 years and over, about 70% of the reported intakes were between 19 and 25 g per day. In the other age categories these percentages were 44% (19-35 years) and 42% (50 to 64 years), respectively. Within population ranges varied from 6 to 9 g per day (5th percentile) to 39-51 g per day (95th percentile). A few countries presented (also) the intake of dietary fibre per MJ. Then daily intakes ranged from 1.6 to 3.6 g per MJ in adults.

4. Overview of dietary reference values and recommendations

A number of national and international organisations have set dietary reference values (DRVs) for carbohydrates (total and/or glycaemic) as well as for dietary fibre (Table 2). Generally, reference intakes are expressed as percent of the total energy intake (E%). For fibre, intakes are expressed in grams per day and/or on an energy basis (per MJ or per 1,000 kcal).

4.1. Glycaemic carbohydrates

According to the Nordic Nutrition Recommendations (NNR, 2004) carbohydrates (including energy from dietary fibre, 8 kJ per g) should provide 50 to 60% of the total energy intake. The population goal is 55 E% from carbohydrates, which should be used for planning purposes. The intake of refined, added sugars should not exceed 10 E%. Although not explicitly stated in the report, it appears that this is a recommendation for individuals. Refined sugars include sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing. The basis for the recommendation on added sugars is to ensure an adequate intake of essential nutrients and dietary fibre, especially in children and older adults with a low energy intake.

The Health Council of the Netherlands (GR, 2001) based their recommendations for digestible carbohydrates on the 97.5 percentile of the endogenous production of glucose. At this level of carbohydrate intake the breakdown of tissue protein is minimal. Using data on average glucose production and assuming a coefficient of variation (CV) of 20%, DRVs were established for the various age and sex groups. The recommendations for glycaemic carbohydrates for adults (19 to 70 years) were calculated to be 272 to 282 g per day, corresponding to about 40 E%. For children and adolescents, recommendations are 45 to 50 E%. No upper limit for digestible carbohydrates is given. No quantitative recommendation was made for (added) sugars. The Netherlands guidelines for healthy eating did not specify recommended values for (added) sugars consistent with adequate nutrition and the prevention of chronic diseases because insufficient scientific evidence was available to support firm conclusions (GR, 2006).

In the Nutritional Recommendations for the French Population (AFSSA, 2001), the Reference Intake for total carbohydrates was set at 50 to 55 E%. It is stated that the reference intake is a consequence of the recommendations for fat (30 to 35 E%) and protein (8 to 10 E%). It is also stated that presently no carbohydrate constituents indispensable for growth and maintenance, which cannot be synthesised by humans, have been identified. Other considerations include e.g. energy density of the diet and effects on serum lipids. A carbohydrate intake above 55 E% is stated to be associated with risk of dyslipidemia (e.g. increased VLDL- and decreased HDL-cholesterol). Various considerations are given with respect to the contribution of MUFA and carbohydrates for reducing cardiovascular risk, treatment of obesity and the metabolic syndrome. Carbohydrates and MUFA should together contribute two-thirds of the total energy intake, with a range for carbohydrates of 40 to 55 E%. No quantitative recommendation was made for (added) sugars.

In the German-Austrian-Swiss recommendations (D-A-CH, 2008), the guiding value ("Richtwert") for carbohydrate intake is at least 50 E%. This value applies to populations and is based on evidence from epidemiological studies, and studies linking a high intake of (saturated) fat with cardiovascular risk factors and other diseases. It is stressed that carbohydrates should be derived from foods rich in starch and dietary fibre, and that intake of refined sugars should be limited. In order to avoid gluconeogenesis from protein (e.g. amino acids) and to inhibit lipolysis at least 25% of the energy should be supplied from carbohydrates. This percentage applies to all ages. No quantitative recommendation was made for (added) sugars.

WHO gives population nutrient intake goals (population average intakes that are judged to be consistent with the maintenance of health in a population) for preventing diet-related chronic diseases (WHO/FAO, 2003). For carbohydrates the population goal is set at 55-75 E%, including dietary fibre. This goal is the percentage of energy available after taking into account the proportion recommended as protein and fat. A recent FAO/WHO Scientific Update on carbohydrate in the human diet proposes that the range is extended

to 50 to 75 E% (Mann et al., 2007). It was proposed that the population average intake of free sugars, defined as "all monosaccharides and disaccharides added to foods, by cook or consumer, plus sugars naturally present in honey, syrups and fruit juice", should not exceed 10 E%. The basis for this goal is that high intakes of free sugars are associated with decreased nutrient density, and risk of weight gain, especially when consumed as beverages.

The UK Committee on Medical Aspects of Food Policy (DoH, 1991) set a dietary reference value (population average intake) for starches and intrinsic and milk sugars of 37 E%. This figure was based on considerations that starch and intrinsic sugars should provide the balance of dietary energy not provided by those nutrients for which the intake should be restricted, i.e. alcohol, protein, fat and non-milk extrinsic sugars. The reference value is applicable to adults and children above 2 years of age. For non-milk extrinsic sugars the population's average intake should not exceed 60 g per day or 10 E%, based mainly on the role of frequent consumption of such sugars in dental caries.

The US Food and Nutrition Board estimated the average requirement of (glycaemic) carbohydrates as 100 g per day for children and adolescents up to 18 years, as well as adults (IoM, 2005), based mainly on data regarding glucose utilisation by the brain. The RDA was set at 130 g per day, assuming a CV of 15%. The RDA corresponds to about 18 and 25 E% in adult males and females, respectively, assuming an energy intake of 2,800 and 2,100 kcal per day, respectively. The US Food and Nutrition Board also set Acceptable Macronutrient Distribution Ranges (AMDR) for total carbohydrates of 45 to 65 E% for individuals. The AMDR are based on evidence indicating a decreased risk for coronary heart disease (CHD) at low intakes of fat and high intakes of carbohydrates, and on evidence for an increased risk of obesity and its complications, including CHD, with high intakes of fat. For added sugars, although there were insufficient data to set a UL (e.g lack of evidence on dose-response for dental caries), a maximal intake level of 25 E% or less from added sugars for individuals was proposed to prevent the displacement of foods that are major sources of essential micronutrients.

Table 2: Recommended dietary intakes for adults.

	USA[a] (IoM, 2005)	Nordic Countries (NNR, 2004)	WHO (2003)	Netherlands (GR, 2001 and 2006)	France, (AFSSA, 2001)	Germany, Austria, Switzerland (D-A-CH, 2008)	Eurodiet (2000)	UK (DoH, 1991)
Protein, E%	10-35	10-20	10-15	8-11	8-10	10-11	-	9
Fat, E%	20-35	25-35	15-30	20-40 20-30/35[b]	30-35	30	< 30	33
Carbohydrates, total, E%	45-65	50-60	55-75	40[c]	50-55	> 50	> 55	47[d]
Sugars, E%	< 25[e]	< 10[e]	< 10[f]	-	-		< 4 occasions per day[g]	<10[h]
Dietary fibre, g/day	w: 25 m: 38	25-35	> 25[i]	32-45	25-30	30	> 25	18[j]
g/MJ	3.4	3		3.4		W: 3 M: 2.4	3	-

(a) AMDR: acceptable macronutrient distribution ranges, applies to individuals. AI for dietary fibre
(b) For subjects with BMI >25 or with undesireable weight gain
(c) RDA for digestible carbohydrates
(d) Intrinsic and milk sugars and starch 37 E%
(e) Refined, added sugars include sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing
(f) Free sugars, defined as all monosaccharides and disaccharides added to foods, plus sugars naturally present in honey, syrups and fruit juice
(g) Corresponds to an intake of < 10 E%
(h) Non-milk extrinsic sugars
(i) Total dietary fibre from wholegrain cereals, fruit and vegetables, 20 g NSP
(j) Refers to non-starch polysaccharides

4.2. Dietary fibre

The Health Council of the Netherlands (GR, 2006) has set guidelines for the intake of dietary fibre mainly based on the importance of dietary fibre for intestinal function and its relationship to risk of coronary heart disease. For children a gradual increase in the intake is recommended from 2.8 g per MJ at 1 to 3 years of age, 3.0 g per MJ at 4 to 8 years of age, 3.2 g per MJ at 9 to 13 years of age to 3.4 g per MJ from 14 years of age. An upper limit is not specified.

In the Nordic Nutrition Recommendations, the recommended intake of dietary fibre is set to 25 to 35 g per day in adults, i.e. approximately 3 g per MJ (NNR, 2004). An Adequate Intake of dietary fibre reduces the risk of constipation and can most likely contribute to protection against colon cancer. No recommendation is given for children due to limited evidence, but it is stated that intake of appropriate amounts of dietary fibre from a variety of foods is important for children as well and that from school age the intake should gradually increase during adolescence to reach the recommended level for adults.

In the WHO report (2003) there is no precise population goal for the intake of total dietary fibre, but at least 25 g per day should be provided from fruit, vegetables and whole-grain foods. This population goal is based on evidence linking high intake of dietary fibre (from fruit, vegetables and whole-grain foods) with decreased risk of e.g. weight gain (convincing), diabetes type 2 (probable), cardiovascular diseases (probable). The food-based recommendation was supported by the recent FAO/WHO Scientific Update on carbohydrate in the human diet (Mann et al., 2007).

In the Nutritional Recommendations for the French Population (AFSSA, 2001), an intake of dietary fibre of above 25 g per day is recommended for maintaining “a healthy colon” and to decrease the risk of colon cancer, with 30 g per day as a preferred level. Also, an increased intake of dietary fibre is stated to be advantageous in conditions such as dyslipidaemia and in diabetes mellitus type 2.

The guiding value for dietary fibre in the German-Austrian-Swiss recommendations is at least 30 g per day, corresponding to about 3 g per MJ for women and 2.4 g per MJ for men, respectively (D-A-CH, 2008). The basis for the value are studies associating increased dietary fibre intake with decreased risk of constipation, diverticulosis, colon cancer, gallstone formation, overweight, hypercholesterolaemia, diabetes mellitus type 2 and atherosclerosis.

The UK Committee on Medical Aspects of Food Policy (DoH, 1991) set a dietary reference value for non-starch polysaccharides (NSP) to 18 g per day, with an individual range of 12 to 24 g per day. The value is based on the effect of non-starch polysaccharides on bowel function and stool weight. The reference value refers to adults and is not applicable to children. An upper limit was set at 32 g per day.

The U.S. Food and Nutrition Board (IoM, 2005) set an Adequate Intake (AI) for total dietary fibre of 3.4 g per MJ (14 g per 1,000 kcal) based on the energy-adjusted median intake associated with the lowest risk of CHD in observational studies. The AI is applicable for all age and sex categories from 1 year of age. The AI corresponds to 25 g per day for women and 38 g per day for men aged 14 to 50 years, respectively.

ESPGHAN (European Society for Pediatric Gastroenterology Hepatology and Nutrition) concluded that by school age an otherwise balanced diet is likely to provide at least 10 g per day of dietary fibre, and that the intake should then gradually increase to reach the recommended level for adults during adolescence. An intake of dietary fibre too high might be a cause of inadequate energy and nutrient density to cover the needs of small children (Aggett et al., 2003).

5. Criteria (endpoints) on which to base the dietary reference values

The amount and type of carbohydrates and dietary fibre in the diet may affect both short-term and long-term metabolic responses such as serum lipids or plasma glucose and insulin concentrations, which can be

regarded as candidate criteria for establishing DRVs. Serum LDL-cholesterol has been causally related to the risk of developing cardiovascular diseases (EFSA, 2004; IoM, 2005), while serum triglycerides, LDL/HDL ratio or total cholesterol/HDL ratio have also been associated with cardiovascular disease (CVD) risk in epidemiological studies (EFSA, 2004; Austin et al., 1998). In addition to cardiovascular diseases, other long-term endpoints for establishing DRVs for both glycaemic carbohydrates and dietary fibre include body weight control, gastrointestinal function, diabetes and some cancers.

The DRVs apply to healthy populations and they are not intended as reference values for the treatment of patients with diseases or conditions like diabetes, obesity, or CVD. However, they apply to healthy subjects with signs of metabolic disturbances like impaired glucose tolerance, elevated blood pressure, serum lipids, etc.

Apart from carbohydrates and dietary fibre, the amount and type of fat and protein in the diet also influence these metabolic factors. As energy balance is the ultimate goal, it is necessary to consider macronutrients in combination and, as a consequence, on an energy basis (percent of energy intake, E%).

5.1. Total glycaemic carbohydrates

Most of the studies addressing the effects of macronutrient manipulation on health outcomes do not allow a precise differentiation between total and glycaemic carbohydrates due to limitations in the food composition data, and thus dietary intake data. However, glycaemic carbohydrates generally contribute 90 to 95% of the total energy derived from carbohydrate intake.

5.1.1. Dietary requirements

Glucose is a preferred energy source for most body cells, and can be stored as glycogen in the liver and in the muscles. The storage capacity is limited, in total to around 500 g for an adult, of which 300 to 400 g can be stored in the muscles. Liver glycogen is essential for liver functions such as detoxification by conjugation with glucuronic acid, and is used to maintain normal blood glucose concentrations between meals. Muscle glycogen is used primarily as a source of energy within the muscles.

Only cells in the central nervous system, red blood cells and some other cells dependent on anaerobic glycolysis have an absolute requirement for glucose (IoM, 2005). The body can synthesise glucose from protein and glycerol. Provided that the diet contains adequate amounts of protein (i.e. amino acids) and glycerol (as e.g. triglycerides) for *de novo* synthesis of glucose, it has been generally assumed that there is no need for dietary carbohydrates (IoM, 2005). Feeding studies in pregnant rats and dogs have, however, shown that diets devoid of carbohydrates can induce still birth, but also high mortality of the offspring and low birth weight (Romsos et al., 1981; Koski et al. 1986: Koski and Hill, 1986).

After a prolonged deficit of glucose, brain cells can adapt partially to utilise fat-derived metabolites, i.e. β-hydroxybutyric acid and acetoacetic acid. A very low carbohydrate diet, providing less than around 50 g per day, however, results in a chronically increased production and plasma concentrations of these acids, referred to as ketosis. Absence of glycogen stores has adverse effects on high-intensity energy production by muscles (Hultman et al., 1999). An intake of 50 to 100 g per day of glycaemic carbohydrates generally prevents ketosis.

In practice, diets totally devoid of carbohydrates have probably not been consumed by any population group during evolution. The diets of Greenland Eskimos and Alaskan Inuits have been reported to contain from 3 to 53 E% carbohydrates; current intakes are >40 E% (Jokelainen, 1965; Bang et al., 1980; Nobmann et al., 2005).

Ketogenic diets with very low carbohydrate contents (from 4 to 5 to 10 E%) have been used as an alternative therapy in children with epilepsy not responding to drug therapy and are a mandatory therapy for some inborn errors of metabolism (e.g. pyruvate dehydrogenase deficiency) (Keene, 2006; Klepper and Voit,

2002; Wexler et al., 1997; Vining, 1999). A number of adverse effects related to such diets have been reported including constipation, nutritional deficiencies and deaths (Wheless, 2001; Papandreou et al., 2006). Severe acidosis is another reported adverse effects of diets very low in carbohydrates consumed by adults who wanted to loose weight (IoM, 2005; Shah and Isley, 2006; Chen et al., 2006a). Methylglyoxal and its by-products accumulate during ketosis and are recognised as a potential cause of blood vessel and tissue damage (Beisswenger et al., 2005).

In conclusion, an intake of 50 to 100 g glycaemic carbohydrates per day is sufficient to avoid ketosis and 130 g per day for both children (> 1 year) and adults is estimated to cover the needs of glucose for the brain (IoM, 2005). However, these levels of intake are not sufficient to meet enegy needs in the context of acceptable intake levels of fat and protein.

5.1.2. Glucose tolerance and insulin sensitivity

The blood glucose concentration is determined by three main factors: the rate of intestinal carbohydrate uptake, the net liver uptake or release (from gluconeogenesis and glycogenolysis), and the peripheral glucose uptake, which is in turn dependent upon the insulin level and the peripheral insulin sensitivity/resistance. With a constant dietary carbohydrate load, there is a range of blood glucose responses between individuals, from low responses with a continuum to what is defined as impaired glucose tolerance (IGT) and type-2 diabetes. Physical activity has the potential to enhance insulin sensitivity and thereby decrease the glycaemic response to a meal (Borghouts and Keizer, 2000; Ivy, 1997).

Some small scale and short-term intervention studies designed to assess the effects of high carbohydrate (50 to 85%E), low fat (<25E%) vs low carbohydrate (8 to 40%E), high fat (>35%) intakes on measures of glucose tolerance or insulin sensitivity suggest that high carbohydrate intakes may improve insulin sensitivity and/or glucose tolerance both in non-diabetic and diabetic subjects (McClenaghan, 2005), although the available data are not consistent (IoM, 2005).

There are no long-term studies published specifically designed to address the impact of macronutrient (i.e. fat/carbohydrate) manipulations on glucose intolerance or insulin sensitivity under isocaloric conditions in adults. *Ad libitum* fat-reduced diets leading to higher carbohydrate intakes (from 46 E% to 55 E%) have been shown to improve glucose tolerance in IGT subjects in the context of significant weight loss (about 3kg) after one year (Swinburn et al., 2001; Mensink et al., 2003). However, no conclusions can be drawn from these studies regarding the effects of carbohydrate consumption itself, independent of weight loss, on glucose tolerance.

The same argument applies to epidemiological studies associating high fat, low carbohydrate intakes, to decreased glucose tolerance or insulin sensitivity. The extent to which those observations are confounded by body weight and/or body fat gain has not been fully elucidated (IoM, 2005).

In conclusion, and although the influence of dietary carbohydrates on glucose tolerance and insulin sensitivity is still unclear, total carbohydrate intakes of 46 to 55 E% appear to be compatible with the maintenance of a normal glucose tolerance and insulin sensitivity in healthy subjects and in subjects with signs of the metabolic syndrome.

5.1.3. Serum lipids

The effects of dietary variation in total carbohydrate intakes on LDL-cholesterol are strongly associated with the type of fat which is replaced by carbohydrates. When saturated fatty acids are kept constant, varying carbohydrate intake as a function of total fat has no effects on LDL-cholesterol concentrations (IoM, 2005).

Data from intervention studies consistently show that increasing carbohydrate intakes (in the range of about 30 to 70 E%) as an inverse function of fat (ranging from 50 to 18 E% as fat) at low intakes of saturated fatty acids (SFA, <10%E) induces a decrease in plasma concentrations of HDL-cholesterol and an increase in the

total/HDL-cholesterol ratio and TG concentrations (Sacks and Katan, 2002; EFSA, 2004; IoM, 2005). This effect is largely attenuated in the lean and physically active (IoM, 2005). Also, TG concentrations are consistently higher when SFA are replaced by carbohydrates rather than by monounsaturated fatty acids (MUFA), particularly in hypertrygliceridaemic subjects (Aro et al., 1998; Mensink et al. 2003; Appel et al. 2005; Berglund et al., 2007; Furtado et al., 2008), and the HDL-cholesterol lowering effect is more pronounced in subjects with higher HDL concentrations at baseline (Obarzanek et al., 2001). However, even if TG concentrations consistently increase with increasing carbohydrate intakes when administered in isocaloric conditions, this effect could be attenuated when carbohydrate-rich diets are consumed *ad libitum*, possibly due to a concomitant reduction in body weight (Rock et al., 2004; Retzlaff et al., 1995; Kasim-Karakas et al., 2000).

In conclusion, the adverse effects of increasing total carbohydrate intakes on the lipid profile provide a basis to set an upper bound of RI for total carbohydrates.

5.1.4. Body weight

The impact of macronutrient manipulation on body weight in the context of weight management in overweight and obese subjects may be different from the effects on prevention of weight gain in leaner persons and may depend on whether diets are administered *ad libitum* or under isocaloric conditions.

In intervention trials tightly controlling energy intake, energy expenditure, weight loss and weight maintenance are a function of energy intake rather than of the macronutrient composition of the diet (Poppitt et al., 2002; IoM, 2005; van Dam and Seidell, 2007; Nordman et al., 2006; Sacks et al., 2009). However, even when an equivalent energy intake is intended, fat-reduced diets (<35 E%) tend to be hypocaloric compared with carbohydrate-reduced diets (<50 E%) and increase long-term compliance with energy restriction, leading to slightly greater weight loss (IoM, 2005; van Dam and Seidell, 2007).

Several randomised intervention studies suggest that fat-reduced (< 25 to 30 E%), moderately high carbohydrate (> 50 E%) diets consumed *ad libitum* have the potential to prevent weight gain in normal weight subjects and produce weight loss in overweight (BMI > 25kg/m^2) individuals as compared to higher fat (> 35 E%), lower carbohydrate (40 to 50 E%) diets (IoM, 2005). However, although very-low carbohydrate diets (< 40 E%) consumed *ad libitum* may have an advantage in terms of weight loss up to 1 year over lower (< 35 E%) and very-low (10 E%) fat diets (van Dam and Seidell, 2007; Gardner et al., 2007; Shai et al., 2008), long-term weight regain tends to be higher and may not offer clear benefits in terms of long-term body weight control (van Dam and Seidell, 2007).

In some long-term (>1 year) intervention studies, dietary modifications with a shift from a habitual Western-type, relatively high fat (35 to 40 E%), moderately low carbohydrate (40 to 50 E%) diets to more carbohydrate-rich (>50 E%), fat-reduced (<30 E%) diets consumed *ad libitum* were reported to be associated with a reduced risk of weight gain or a moderate weight loss in various population groups including normal, overweight and obese subjects (IoM, 2005; Howard et al., 2006a; Lanza et al., 2001).

Results from prospective cohort studies are conflicting with respect to the relationship between carbohydrate intake and weight gain (Halkjær et al., 2006; Gaesser, 2007).

In conclusion, the adverse effects associated with high fat, low carbohydrate diets on short- and long-term body weight control provide a basis to set a lower bound of the RI for total carbohydrates.

5.1.5. Type 2 diabetes mellitus

There are no intervention studies specifically addressing the effect of carbohydrate intake on the risk of developing type-2 diabetes. In two lifestyle, long-term intervention studies including weight loss by reducing fat intake and increasing physical activity, the presumed increase in carbohydrate intake (targeted at 55 E% carbohydrates) was compatible with a lower risk for type 2 diabetes mellitus (Tuomilehto et al.

2001, Knowler et al., 2002; Lindström et al., 2003, 2006a and 2006b). Large-scale observational studies on the effects of carbohydrate intake and risk of diabetes have yielded conflicting results (IoM, 2005).

Several cohort studies have investigated the relationship between intake of total and individual carbohydrates and the risk of developing diabetes type 2 (Murakami et al., 2005; McKeown et al., 2004; Meyer et al., 2000). Generally, no or weak relations between total carbohydrate intake and diabetes risk were observed.

In conclusion, diets providing about 55 E% as carbohydrates are compatible with a lower risk for type 2 diabetes mellitus in the context of concomitant weight loss and physical activity.

5.1.6. Cardiovascular disease

As reviewed (Sacks and Katan, 2002), three dietary intervention studies reducing total fat intake, in particular saturated fat, and increasing the consumption of carbohydrate-rich foods, did not significantly reduce the risk of cardiovascular disease. However, it cannot be excluded that the duration, compliance, and sample sizes may have been insufficient to produce a reduction in coronary events. Also, in the Women's Health Initiative Dietary Modification Trial a dietary intervention that reduced total fat intake and increased intakes of carbohydrates from vegetables, fruits, and grains did not significantly reduce the risk of cardiovascular disease (Howard et al., 2006b).

Data from observational studies, and in particular from the U.S. Nurses Health Study, do not indicate any consistent relationship between total carbohydrate intake and CHD risk (Liu et al., 2000; Oh et al., 2005; Halton et al., 2006). Data from one cohort (Halton et al., 2006) showed that an increased carbohydrate intake was associated with an increased risk of total and haemorrhagic stroke in women with a BMI > 25 kg/m^2 (Oh et al., 2005). Conversely, in two large prospective cohort studies (Trichopoulou et al., 2007; Lagiou et al., 2007), low energy-adjusted carbohydrate intakes, particularly if combined with high protein intakes, were associated with a significantly higher risk of mortality from CVD.

In conclusion, data from intervention and observational studies do not show any consistent relationship between the intake of total or glycaemic carbohydrate intake and the risk of CVD. The ranges of carbohydrate intakes in the studies above vary from 30 to 70 E%.

5.2. Sugars

5.2.1. Nutrient density of diet

Nutrient density is the amount of nutrients in foods per unit of energy. An adequate nutrient density is essential for providing recommended intakes of nutrients, especially in individuals with a low energy intake. There is some evidence that high intakes of added sugars, particularly from low nutrient density foods, might be associated with a decrease in the nutrient density of the diet (‘nutrient dilution’) due to displacement of nutrient rich foods (van Dam and Seidell, 2007). In some EU countries, studies in children and elderly nursing home residents (Lyhne and Ovesen, 1999; Beck and Ovesen, 2002; Alexy et al., 2003a; Øverby et al., 2004; Kranz et al., 2005; Frary et al., 2004) have shown that an intake of >10 to 30 E% of added sugars (mono-, disaccharides and higher saccharides) is associated with a reduced intake of several micronutrients (e.g. calcium, iron, folate, vitamin A) and dietary fibre, especially in children and adults with energy intakes below about 8 MJ per day. Data from the U.S. indicate that nutrient density of the diet among children was negatively correlated to the intake of added sugars in the range of 10 to 25 E% or above, but that clear differences were seen mainly at very high intakes (>25 E%) (IoM, 2005), with some exceptions e.g. calcium in preschool children (Kranz et al., 2005). A systematic review of 15 cross-sectional studies comprising children and adults shows that there are insufficient and conflicting data with respect to the relation between intake of added sugars and nutrient density, with no clear evidence of micronutrient dilution or a threshold for a quantitative amount of added sugar intake for any of the micronutrients

investigated (Rennie and Livingstone, 2007). The observed inverse association of nutrient density or intake with added sugar intake may be partly explained by methodological issues, e.g. different definitions of added sugars, and confounding effects related to differences in energy intake (higher sugar intake as E% in individuals with lower total energy intake and consequently lower nutrient intake). The association between added sugar intake and micronutrient density of the diet is mainly dependent on the intake patterns of the food groups from which added sugars in the diet are derived (Rennie and Livingstone, 2007).

In conclusion, observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake. Evidence on the relationship of foods containing added sugar to micronutrient density of the diet and to micronutrient intake of population groups should be considered when developing food-based dietary guidelines.

5.2.2. Glucose tolerance and insulin sensitivity

Some mainly small, short-term (4 to 6 weeks) studies have investigated the effect of sugar intake on glucose and insulin response comparing individual sugars (sucrose, glucose or fructose) or mixtures of different sugars with starch or "complex carbohydrates". The majority of these used iso-caloric diets aimed at body weight maintenance during the study. Both normal subjects and subjects with impaired insulin responses were included. The amount of sugars in the intervention diets varied from about 3 to10 E% in the "low-sugar" diets to 20 to 30 E% in the high-sugar diets. The studies are summarised in Annex 4. Two of the three studies with sucrose showed increased insulin concentrations at sucrose intakes of 18 and 33 E%, whereas one did not show any difference between diets providing 10 or 25 E% sucrose. One study showed increased glucose concentrations at sucrose intakes of 18 and 33 E%, whereas one did not show any difference between diets providing 10 or 25 E% sucrose.

In conclusion, there are limited, and mainly short-term, data on the effects of high intakes of sugars on glucose and insulin response. Most studies do not find any adverse effects at intakes of predominantly added sugars up to 20 to 25 E%, provided that body weight is maintained.

5.2.3. Serum lipids

A number of mainly small, short-term (2 to 6 weeks) studies have investigated the effect of sugar intake on serum lipids, comparing individual sugars (sucrose, glucose or fructose) or mixtures of different sugars with starch or "complex carbohydrates". The majority of studies used iso-caloric diets aiming at body weight maintenance during the study. Both normal subjects and subjects with impaired insulin responses were included. The amount of sugars in the intervention diets varied from about 3 to 10 E% in "low-sugar" diets to 20 to 30 E% in the high-sugar diets. The studies are summarised in Annex 5. In five of the seven studies, increased sugar intakes led to increases in total and LDL-cholesterol, in four, the TG-concentrations increased, but responses differed according to sex and insulin sensitivity. Effects on HDL-cholesterol were less prominent or not reported. Overall, negative effects were observed at sugar intakes > 20 E%. Only in the study by Hallfrisch et al. (1983) a significant increase in total, LDL-cholesterol and TG was seen at fructose intakes of 7.5 and 15 E%. Effects tended to be more pronounced in subjects with markers of metabolic syndrome, e.g. insulin resistance. Studies also varied with respect to the composition of the basic and intervention diets, which might have influenced the results. For example Black et al. (2006) found that a high-sucrose diet (25 E%) resulted in increases in total and LDL cholesterol by 15% and 24%, respectively, compared to the control diet (10 E% sucrose). The authors hypothesise that the higher level of SFA and lower level of PUFA in the high-sucrose diet could have contributed to the cholesterol-raising effect.

Few long-term studies of the effect of sugars on lipids have been published. In a six-month study by Saris et al. (2000), 316 obese subjects were randomised to three groups, which received either a control diet with 46 E% carbohydrates of which 24 E% was starch and 21 E% sugars, or an intervention diet with 52 to 56 E%

carbohydrates containing mainly starch (33 E% starch, 16 E% sugars) or mainly sugars (sucrose, fructose and lactose, 30 E%). All diets were given *ad libitum*. No significant changes were seen in serum lipids among the groups.

In a sub-study 46 obese subjects with the metabolic syndrome (≥3 risk factors) were randomised to receive either one of three diets *ad libitum*: a control diet with with 29 E% starch and 21 E% sugars, two fat-reduced diets, one with 53 E% carbohydrates, mainly as "complex carbohydrates" (33 E% starch and 18 E% sugars) and one high-sugar diet with 57 E% carbohydrates including 29 E% sugars (sucrose, fructose and lactose) (Poppitt et al., 2002). Thirty-nine subjects completed the study. After six months fasting serum TG was higher in the sugar group than in the "complex-carbohydrate" and control groups, respectively. Weight loss was correlated with a decrease in TG concentrations.

Smith et al. (1996) found that restriction of the intake of added sugars during six months led to reduced TG concentrations in hyper-triglyceridaemic, overweight subjects, even at relatively moderate intakes (from about 12 to 4 E% added sugars). This reduction was partly associated with an (unintentional) weight-loss of <2% of the initial body weight.

In conclusion, a number of small-size controlled, iso-caloric short-term studies (2 to 6 weeks), indicate that high intakes (>20 E%) of sugars, provided predominantly as added sucrose or fructose, may increase serum TG and LDL-cholesterol concentrations, especially in subjects with markers of the metabolic syndrome, e.g. insulin resistance. However, data on dose-response are limited, especially at intakes in the range of 5 to 20 E%, albeit one study found elevated lipid concentrations at a fructose intake of 7.5 E%. Information on the intake of total sugars is lacking in some studies. In long-term intervention studies in which diets were given *ad libitum* (<6 months) changes in blood lipids as result of diets high in sugars (about 30% E) or of sugar restriction (from 12 to 4 E%) are closely related to body weight changes. A number of dietary factors such as fatty acid composition, dietary fibre content and type may modulate the effects. There are insufficient data to set a UL for sugars based on their effects on serum lipids.

5.2.4. Other cardiovascular risk factors

In the study by Marckmann et al. (2000) nonfasting FVIIc (factor VII coagulant activity) was lower on the low-sucrose (2.5 E%) diet compared to the high-sucrose (23 E%) diet.

In a 10-week intervention study, overweight subjects (mean BMI 28kg/m^2) were given 1.3 L sucrose-sweetened or artificially sweetened soft drinks per day while otherwise eating an *ad libitum* habitual diet (Raben et al., 2002). Those subjects consuming the sugar-sweetened soft drink had a sucrose intake of 28 E% and increased their energy intake during the study. At the end of the study blood pressure was increased in this group (SBP +3.8, DBP +4.1 mmHg), while it was decreased among subjects who consumed the artificially sweetened soft drink (SBP –3.1, DBP -1.2 mmHg). Body weight (+1.6 kg) and fat mass (+1.3 kg) increased in the sugar-group, with no significant changes in the control group.

In conclusion, there are insufficient data to set a UL for sugars based on their effects on the risk factors for cardiovascular disease reported in this section.

5.2.5. Body weight

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent (IOM, 2005; van Dam and Seidell, 2007). Either weight loss (Saris et al., 2000) or weight maintenance (Poppitt et al., 2002) has been reported for high carbohydrate (52 to 56 E%), high-sugar (29 to 30 E%) diets as compared to control diets (49 E% as carbohydrates, 21 E% sugars) consumed *ad libitum* for six months. Epidemiological studies do not show a positive correlation between total sugar intake and obesity – rather the opposite (IoM, 2005).

There is some evidence that sugar sweetened beverages do not induce satiety to the same extent as solid forms of carbohydrate, and that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain (van Dam and Seidell, 2007; Mann *et al*., 2007). *Ad libitum* consumption of high sucrose diets (28 E% mainly as beverages) was found to increase body weight and fat mass as compared to lower sucrose diets with artificial sweeteners (Raben et al., 2002). In a systematic review Malik et al. (2006) included 30 studies, mainly in children and adolescents (15 cross-sectional, 10 prospective, and 5 experimental), that investigated the association between sugar-sweetened beverage intake and weight gain. The authors state that large cross-sectional studies and well-powered prospective cohort studies with long periods of follow-up show a positive association between higher intakes of sugar-sweetened beverages and weight gain and obesity in both children and adults. No data on overall effect size were included. Vartanian et al (2007) included 88 studies in a meta-analysis regarding the association between soft drink consumption and body weight. Most studies were cross-sectional (17) and longitudinal (10) and included both children and adults. The overall effect size across studies was 0.08 (expressed as change in BMI and/or body weight) ($p<0.001$). However, results from another meta-analysis of eight prospective studies and two intervention studies among children and adolescents did not show a clear quantitative relationship and suggested that there may be publication bias against studies that do not report statistically significant findings (Forshee et al., 2008). Long-term randomized controlled trials on the effects of sugar sweetened beverages on body weight are lacking (van Dam and Seidell, 2007; Johnson et al., 2009).

Fructose has been suggested to play a specific role in weight gain (Elliot et al., 2002, see 2.2.1). There are, however, few and mainly short-term controlled intervention studies in healthy subjects comparing fructose with other sugars or carbohydrate-sources and these do not allow a conclusion regarding the role of fructose in obesity (Vasankari and Vasankari, 2006).

In conclusion, the evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight. Evidence on the relationship of sugar-sweetened beverages and body weight should be considered when developing food-based dietary guidelines.

5.2.6. Type 2 diabetes

Evidence on the effects of sugar consumption on the risk of developing type 2 diabetes comes primarily from large prospective cohort studies. No (Colditz et al., 1992; Janket et al., 2003) or even inverse (Meyer et al., 2000) associations have been reported for total sugars and/or specific types of sugars and diabetes risk. However, consumption of sugar-sweetened beverages, and particularly if sweetened with glucose or fructose, was found to be positively associated with increased type 2 diabetes risk (Schultze et al., 2004a; Montonen et al., 2007). The available evidence is insufficient to set a UL for sugars based on their effects on type 2 diabetes risk.

5.2.7. Dental caries

Increased risk of dental caries in children is associated with a high frequency (more than about 4 times daily) of intake of cariogenic sugars (mainly sucrose, glucose, and fructose) rather than with the total amount of dietary sugars; the evidence indicates that frequent consumption of sweets and confectionery products and sugar-containing drinks is associated with a higher risk of caries (Moynihan and Petersen, 2004; DoH, 1991; IoM, 2005; Anderson et al., 2009).

Caries develops as tooth substance demineralises upon pH decrease due to fermentation of carbohydrates by tooth-colonising bacteria into different organic acids. Dental caries is an infectious disease, although sucrose and other easily fermentable sugars, e.g. glucose and fructose, play a key role (Navia, 1994; Lingstrom et al., 1997). Foods rich in starch may also contribute, especially when the starch molecule is easily available to

degradation by amylase. The acid production from lactose in dental plaque is normally low, while the fermentation of starch varies greatly and depends on the degree of gelatinisation (Lingstrom et al, 1997). Decreases in pH to well below 5.5 are considered critical for caries development in enamel (the tooth crown). In tooth roots the critical pH for demineralisation is approximately 6.5. In addition to lactic acid, sucrose fermentation produces insoluble extracellular glucose polymers leading to voluminous biofilms that favour colonisation of cariogenic streptococci on the teeth surfaces.

Dental caries prevalence has declined in many European countries during the last decades of the 20th century, but trends vary between countries and age groups (Touger-Decker and van Loveren, 2003; Schulte et al., 2006; Haugejorden and Magne Birkeland, 2006; Demertzi et al., 2006; Stecksen-Blicks et al., 2008; Pitts et al., 2006).

More recently, mainly cross-sectional studies generally find a weak or moderately strong relationship between the intake of sucrose and other sugars and caries prevalence (Burt and Pai, 2001). The impact of fluoride prophylaxis and other lifestyle variables seems to override variations in cariogenic carbohydrate intake in these studies. High intake of sugars has been associated with an increased risk of caries when oral hygiene is simultaneously poor and at a low level of fluoride prophylaxis (Danish Nutrition Council, 2003; Burt and Pai, 2001; Kleemoja-Kujala and Räsänen, 1982). However, results from a Finnish longitudinal study suggest a relationship between the intake of sucrose and sucrose containing foods and caries development during childhood among children with fluoride prophylaxis (Karjalainen et al., 2001; Ruottinen et al., 2004).

Available data do not allow the setting of an UL for sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by various other lifestyle factors (oral hygiene, exposure to fluoride, meal frequency and diet composition), heredity, illness, medication, malnutrition, and flow and composition of saliva.

In conclusion, frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an UL for sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors. Evidence on the relationship of frequency of consumption of sugar-containing foods and dental caries should be considered when developing food-based dietary guidelines.

5.3. Dietary fibre

5.3.1. Dietary requirements

Dietary fibre has not been shown to be an indispensable component of the diet. However, dietary fibre has a major role in bowel function and gastro-intestinal symptoms, such as constipation (IoM, 2005).

5.3.2. Gastrointestinal function

5.3.2.1. Adults

Dietary fibre has a major role in bowel function and gastro-intestinal symptoms, such as constipation, have been linked to low fibre intakes (IoM, 2005). Constipation has been defined as difficulty in passing stools or an incomplete or infrequent passage of hard stools (Longstreth et al, 2006). Constipation occurs in 5 to 18%

of adults in different countries with a greater percent of women and elderly affected and adversely affects the quality of life (Wald et al., 2008). It may also contribute to diverticular disease.

Both observational and experimental data show that dietary fibre is the most important dietary determinant of faecal bulk and transit time (Cummings et al., 1992, Birkett et al., 1997). Dietary fibre from cereals, fruits, and vegetables increases stool weight, which promotes normal laxation in children and adults. In general, the greater the weight of the stool and the more rapid the rate of passage through the colon the better the laxative effect (Birkett et al., 1997).

It has also been demonstrated that different kinds of dietary fibre have different bulking capacity. Dietary fibre in wheat bran and other fibre that is fairly resistant to fermentation in the large bowel has the most pronounced bulking effect (5 to 6 g per g dietary fibre) mainly due to water binding in the distal bowel, whereas more fermentable fibre provide some bulk mainly due to increased bacterial mass (Cummings, 2001).

Although there is no single accepted definition of what constitutes normal laxation, frequency of defaecation is typically about once per day on Western diets (Weaver, 1988). Haack et al. (1998) have indicated that a defecation frequency of about once per day and a transit time in the range of 2 to 3 days may be considered normal laxation. They reported that while increasing intake of fibre (provided by a mixture of fruit, vegetables, and grains) from 16 to 30 g per day increased defaecation frequency from 0.7 to 0.94 times per day, a further increase in fibre intake to 42 g per day had no further effect on defecation frequency, which remained at about once per day. There was no significant change over the range of fibre intakes in gastrointestinal transit time, which remained within the range of 2 to 3 days, or stool moisture, which remained within the range of 70 to 74%. Faecal weight increased from 109 g to 156 g and 195 g at fibre intakes of 16, 30 and 42 g per day, respectively.

Increasing dietary fibre intake from 12 to 45 g per day increased faecal weight from 69 to 184 g per day and reduced transit time from >70 h to 45 h (Stasse-Wolthuis et al., 1978). Mean transit time in UK adults has been reported as 70 h (median 60 h) (Cummings et al., 1992) in a population in which mean stool weight is about 110 g per day, with weights of less than 100 g per day in about 50% of individuals, and dietary fibre intake of about 18 g per day (Cummings et al., 1992). It has been estimated that a dietary fibre intake of 25 g per day is associated with stool weight of about 130 to 150 g per day (Cummings et al., 1992). Birkett et al. (1997) reported that adults who consumed 25 g dietary fibre in their usual diet excreted more than 150 g faeces per day.

Taken together, these data indicate that an intake of 25 g per day of dietary fibre from mixed foods (as AOAC fibre or equivalent) is compatible with an intestinal transit time of about two to three days and a defaecation frequency of 1 per day and a faecal moisture of >70% and may be considered adequate for normal laxation in adults.

5.3.2.2. Children

There is evidence that constipation is a common problem also during childhood (e.g. Loening-Baucke, 1993) and that there is an inverse relationship with dietary fibre intake (Edwards and Parrett, 2003).

There are few data relating intake of dietary fibre to normal laxation in children. Data from long-term intervention and observational studies can, however, give information on fibre intakes that are compatible with adequate growth and development and at the same time provide pre-requisites for good health. Results from the Finnish STRIP-study indicate that a fibre intake corresponding to 2-2.5 g per MJ is compatible with normal growth and development. The fibre intake among German children was already at one year somewhat higher (3 g per MJ) and there are no reports of adverse effects related to the fibre intake.

In conclusion, dietary fibre intake of 2 g per MJ should be adequate for normal laxation in children based on the dietary fibre intake that is considered adequate for normal laxation in adults (25 g, equivalent to 2 to 3 g

per MJ for daily energy intakes of 8 to 12 MJ) and taking into account that energy intake relative to body size in children is higher than in adults.

5.3.3. Glucose tolerance and insulin sensitivity

Few intervention studies have investigated the effects of fibre intake on measures of glucose tolerance or insulin sensitivity.

In the two-year intervention study by Mensink et al. (2003) conducted in subjects with impaired glucose tolerance (IGT), glucose tolerance improved in the intervention group compared to the control group. Dietary fibre intake was 3.1 to 3.3 g per MJ in the intervention group compared to 2.7 g per MJ in the control group.

A number of cohort studies have found that intake of fibre and fibre rich foods such as wholegrain cereals correlated favourably with measures of glucose tolerance or insulin sensitivity. Liese et al. (2005) found that dietary fibre intake was associated positively with insulin sensitivity and inversely with fasting insulin, but not with acute insulin response, in 979 adults with normal or impaired glucose tolerance. In the baseline data of an intervention study by Lau et al. (2005) the intake of dietary fibre was inversely associated with the probability of having insulin resistance (assessed by the homeostasis model assessment of insulin resistance, HOMA-IR) among middle-aged, healthy adults. Similar results were observed in another cross-sectional study on subjects at high risk of type 2 diabetes (relatives of patients with type 2 diabetes) (Ylönen et al., 2003).

In conclusion, increasing intakes of foods rich in dietary fibre are associated with reduced risk of impaired glucose control. Dietary fibre intakes associated with favourable effects are >2.6 g per MJ and about 30 g per day, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.4. Serum lipids

A meta-analysis including 67 intervention studies showed that intakes of 2 to 10 g per day of viscous, soluble fibre (e.g. pectin, oat bran, guar gum, psyllium) were associated with a small, but significant, decrease in total cholesterol (-0.045 mmol/L per gram of dietary fibre) and LDL-cholesterol (-0.057 mmol/L per gram) (Brown et al., 1999). The effects have been confirmed in subsequent studies in both hypercholesterolaemic subjects (Jenkins et al., 2002), and normocholesterolaemic subjects (Berg et al., 2003; Aller et al., 2004), but not in the study by Chen et al. (2006b). Controlled intervention studies have generally shown that fasting TG concentrations are not affected by fibre intake (Queenan et al., 2007; Beer et al., 1995; Behall et al., 2004; Anderson et al., 1995; Braaten et al., 1994; van Horn et al., 1991).

Certain kinds of fibre, especially soluble, viscous types, can, however, reduce post-prandial hyperlipidaemia (Lairon, 2001). These effects are related to diminished cholesterol and/or bile acid absorption (Andersson, 1996) and possibly also to products of colonic fermentation. Effects on lipid metabolism of resistant starch and resistant oligosaccharides demonstrated in experimental animals have so far not been reproduced in man.

In conclusion, viscous types of dietary fibre may contribute to reducing total and LDL-cholesterol concentrations. The effects are limited at amounts usually consumed from foods.

5.3.5. Blood pressure

In a meta-analysis Whelton et al. (2005) evaluated 25 randomised controlled trials with respect to the effect of dietary fibre intake on blood pressure. The difference in fibre intake between intervention and control groups ranged from 3.8 to 12.5 g per day, with a median difference of 10.7 g per day. In eight studies fibre was given as a supplement, otherwise fibre was provided as foods (15 studies, cereal, fruit, fruit/vegetables, cereal/fruit, cereal/vegetables/fruit), pectin (1 study) or guar gum (1 study). Study duration varied from 2 to

26 weeks. Overall, dietary fibre intake was associated with a small, but significant, reduction in diastolic blood pressure (-1.6 mmHg) and a non-significant reduction in systolic BP (-1.1 mmHg). A significant reduction in both systolic and diastolic blood pressure was observed in trials conducted in patients with hypertension (SBP -5.9 mmHg, DBP -4.2 mmHg) and in trials including both normotensive and hypertensive subjects with a duration of the intervention of 8 weeks or longer (SBP -3.1 mmHg, DBP -2.6 mmHg). A further meta-analysis by Streppel et al. (2005) showed similar results. Whether the effect is related to the intake of dietary fibre *per se*, to the consumption of other nutrients in fibre-rich food products with blood-pressure lowering effects, or both, was not addressed.

In conclusion, small, but rather consistent, effects on blood pressure have been observed for diets rich in fibre from e.g. cereals, fruit and vegetables, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.6. Body weight

5.3.6.1. Adults

Reviews of randomised trials have shown weight loss in a majority of studies with no differences between fibre types or between fibre occurring in foods or in supplements (Pereira and Ludwig, 2001; Howarth et al., 2001). This led to the conclusion by the World Health Organisation (WHO) that the evidence for a protective effect against weight gain and obesity of high dietary intake of NSP (dietary fibre) was convincing (WHO/FAO, 2003) and that an intake of at least 25 g total dietary fibre per day from wholegrain cereals, fruit and vegetables would be desireable. Results from seven prospective cohort studies show an inverse relationship between weight gain and baseline intake or change in fibre intake among adults during follow-up periods up to 12 years (Lairon, 2007; Koh-Banerjee et al., 2004). However, Iqbal et al. (2006) found no significant relationship between fibre intake at baseline and subsequent weight change during a 5-year follow-up among 30 to 60 year old men and women.

In the Finnish Diabetes Prevention study an increase in dietary fibre intake was associated with a sustained weight reduction (>5%) (Lindström et al., 2006a). The odds ratio for sustained weight loss at year 3 of the study (1 to 3 year follow up) was 2.04 (95% CI: 1.05 to 3.95) for subjects in the third quartile (3.1 to 3.7 g per MJ) and 2.67 (95%CI: 1.26 to 5.65) for subjects with a fibre intake in the upper quartile (>3.7 g per MJ), compared to subjects in the lowest quartile (< 2.6 g per MJ).

5.3.6.2. Children

In the Finnish intervention study STRIP (Special Turku Coronary Risk Factor Intervention Project) children were given dietary advice from the age of 7 to 8 months and have been followed up to 14 years of age (Niinikoski et al., 2007). These children grew and developed normally (Kaitosaari et al., 2003). Data on fibre intake are available for children up to 7 years of age. Mean intake of fibre varied from 9.2 g per day at 13 months of age to 11.8 g per day at 5 years of age (Lagström et al., 1999). The energy adjusted fibre intake was between 1.9 and 2.5 g per MJ at 13 months of age, 1.8 and 2.3 g per MJ at 3 years of age and 1.7 and 2.4 g per MJ at 5 years of age. There were no differences in body weight or growth in relation to fibre intake. At 7 years of age fibre intake was, depending on dietary pattern, between 12.3 and 15.5 g per day, corresponding to 1.9 and 2.4 g per MJ, respectively (Räsänen et al., 2002).

Studies on children eating mixed diets do not indicate adverse effects on growth due to high fibre intake. On the other hand, there are studies indicating that dietary fibre intake can contribute to lower the risk of obesity (Edwards and Parrett, 2003).

In conclusion, increased intake of dietary fibre, both from naturally fibre-rich foods and added fibre or fibre supplements, has been shown to be related to improved weight maintenance in adults and sustained weight reduction in overweight subjects. Estimated intakes associated with this effect in adults are in the order of

>25 g dietary fibre per day (from wholegrain cereals, fruit and vegetables) and >3.1 g total fibre per MJ. Results of intervention and observational studies in children indicate that a fibre intake corresponding to 2 to 3 g per MJ is compatible with normal growth and development.

5.3.7. Colorectal cancer

The effect of dietary fibre on faecal bulk has been an important parameter in setting recommended dietary intakes for dietary fibre. Intake in adults necessary to obtain a faecal bulk related to a minimal risk of intestinal disorders, particularly colon cancer, has been estimated to be 26-34 g per day (Cummings et al.,1992), 35 to 45 g per day (Spiller and Spiller, 2001) and 32 to 40 g per day (Monro, 2004).

The fermentation of dietary fibre by the colonic microflora has been recognised as important for colonic health and might have systemic metabolic effects through absorption of fermentation products. One of the fermentation products, butyric acid, is of special interest in relation to colon cancer since it is a main source of energy for colonocytes and has effects on cell differentiation, apoptosis and inflammatory processes (Cummings et al., 2004).

A large number of both *in vitro* and *in vivo* studies have provided mechanistic support for protective effects of dietary fibre against colon cancer (e.g., effects on faecal enzymes, secondary co-carcinogenic bile salt metabolites, etc.). Furthermore, there is a relationship between fibre intake and faecal bulk, and further between colon cancer and faecal bulk (Cummings et al., 1992; Birkett et al., 1997). Stool outputs of 150 g per day or more, normally obtained at a dietary fibre intake of at least 25 g per day, have been associated with lower prevalence of colon cancer (Cummings et al., 1992 and 2004).

A number of mainly small-size clinical intervention studies have been performed using polyp recurrence and rectal cell proliferation as surrogate markers for colon cancer (IoM, 2005). A pooled analysis of two larger intervention studies comprising 3,209 subjects with colorectal adenomas showed that increased intakes of fibre were associated with a significantly decreased risk of adenoma recurrence among men, but not in women, after 3 to 4 years (Jacobs et al., 2006). However, the strategy to increase fibre intake differed between studies (wheat bran supplement vs dietary intervention aimed at decreasing fat intake and increasing intake of fibre, fruit and vegetables).

Recent epidemiological studies have, however, given inconsistent results regarding a protective effect of dietary fibre against colorectal cancer (WCRF/AICR, 2007; Otani et al., 2006; Park et al., 2005; Bingham et al., 2003 and 2005). In the EPIC (European Prospective Investigation into Cancer and Nutrition) study comprising more than 0.5 million people from ten different European countries, dietary fibre intake from foods was inversely related to the incidence of large bowel cancer with an adjusted relative risk of 0.75 (95% CI 0.59 to 0.95) for the highest versus the lowest quintile of intake and 0.58 (0.41 to 0.85) after adjustment for more detailed dietary data. The association with colon cancer was strengthened with longer follow-up. However, the association with rectal cancer was no longer significant (Bingham et al., 2005).

In the World Cancer Research Fund/American Institute of Cancer Research (WCRF/AICR) report 16 cohort studies were reviewed and of these, eight could be included in a meta-analysis (WCRF/AICR, 2007). The analysis showed a statistically significant risk reduction of colorectal cancer and the overall relative risk was 0.90 (95% CI: 0.84 to 0.97) for a 10 g per day increase in fibre intake. However, a previous pooled analysis of 13 prospective cohort studies did not show a statistically significant protective effect after adjusting for known risk factors (Park et al., 2005). The WCRF/AICR concluded that foods containing dietary fibre might protect against colorectal cancer, albeit residual confounding in the studies could not be excluded.

In a nested case-control study Peters et al. (2003) found that high intakes of dietary fibre were associated with a lower risk of colorectal adenomas (polyps), especially regarding fibre from grains and cereals and from fruits.

In conclusion, dietary fibre is the most important dietary factor for faecal bulk and regular bowel movements, and might reduce the risk of colon cancer. Previous estimates of fibre intakes in adults necessary to achieve minimal risk of intestinal disorders, particularly colon cancer, range from 26 to 45 g per day. One meta-analysis of prospective cohort studies found a 10% decrease in the risk of colorectal cancer for each 10 g per day increase of dietary fibre intake. However, a consistent relation has not been shown in all cohort studies.

5.3.8. Type 2 diabetes mellitus

In the Finnish Diabetes Prevention Study an increase in dietary fibre intake was associated with a reduced risk of developing diabetes type 2 in subjects with IGT (Lindström et al. 2006b). The adjusted hazards ratios were 0.50 (95% CI: 0.28 to 0.89), 0.71 (0.40 to 1.23) and 0.38 (0.19 to 0.77) for subjects with a fibre intake in the second (2.6 to 3.1 g per MJ per day), third (3.1 to 3.7 g per MJ per day) and upper quartile (>3.7 g per MJ per day), respectively compared to subjects in the reference quartile (<2.6 g per MJ per day). The lowest risk was observed among subjects with both a high fibre (above median, >3.1 g per MJ per day) and low fat (below median, <33.2 E%) intake.

Several prospective cohort studies have investigated the relationship between dietary fibre intake and risk of type 2 diabetes. Most studies have found a decrease in risk with increasing intakes of cereal fibre (Murakami et al., 2005; Krishnan et al., 2007; Schulze et al., 2007) and whole grains (de Munter et al., 2007). Fewer studies have found a significant risk reduction with total dietary fibre intake (Salmerón et al., 1997a and 1997b; Meyer et al., 2000; Montonen et al., 2003). In the latter studies, total dietary fibre intakes of about 25 to 40 g per day have been consistently associated with a significantly lower risk of developing type 2 diabetes compared to fibre intakes of about 12 to 16 g per day.

In conclusion, dietary fibre intakes reported to be associated with a reduced risk for type 2 diabetes are >2.6 g per MJ or about 25 to 30 g per day, although the contribution of dietary fibre *per se* to this effect remains to be established.

5.3.9. Cardiovascular disease

In a meta-analysis of 10 prospective cohort studies an increase in the energy-adjusted fibre intake of 10 g per day was associated with a 14% lower risk of all coronary events (fatal and non-fatal myocardial infarction (MI)) and with a 27% lower risk of coronary death (Pereira et al., 2004). The results also indicate that a reduced relative risk was seen for subjects with an energy-adjusted fibre intake of >24 g per day compared to subjects in the reference category (18 to <21 g per day). The U.S. Food and Nutrition Board used data from three cohort studies regarding coronary heart disease (CHD) as a basis for setting an AI for total dietary fibre (IoM, 2005). The AI (14 g per 1000 kcal, 3.4g per MJ) was derived from the upper quintiles of energy-adjusted intake of dietary fibre.

In conclusion, there is epidemiological evidence for a protective effect of dietary fibre intake >24g per day on cardiovascular disease risk.

5.4. Glycaemic index and glycaemic load

5.4.1. Glucose tolerance and insulin sensitivity

Short- to medium-term (2 weeks to 6 months) intervention studies have shown that diets with reduced GI can improve markers of metabolic control in diabetes type 1 and 2 (Opperman et al., 2004).

A few intervention studies have investigated the role of GI or GL in healthy subjects in relation to the risk of developing IGT or impaired insulin sensitivity, while controlling for dietary fibre intake.

In a 10-week controlled intervention study (Sloth et al., 2004), healthy overweight subjects (BMI: 25 to 30 kg/m^2) were given fat-reduced (22 E%) diets with 57 to 58 E% as total carbohydrates *ad libitum* with either high or reduced GI, but with otherwise similar nutrient composition including dietary fibre content. Both groups lost weight. No significant differences between the groups with respect to metabolic markers such as blood glucose, insulin concentrations or insulin resistance (HOMA-IR) were observed. GI differed by 24 units. Similarly, Philippou et al., (2009) did not observe an effect of low (50) vs high (64) GI diets on insulin sensitivity or beta cell function assessed by HOMA-IR and HOMA-β, respectively, while controlling for the amount of dietary fibre intake (11 vs 13 g per day), whereas Frost et al., (1998) showed a significant increase in insulin sensitivity assessed by a short intravenous glucose tolerance test after consumption of a low GI diet (67 to 71) compared to a high GI diet (87 to 89) both comparable for the amount of dietary fibre (19 vs 18g per day).

In other intervention studies investigating the effects of low and high GI diets consumed *ad libitum* on glucose tolerance or insulin sensitivity, dietary fibre intakes are consistently higher in the low GI diet group and do not generally show an effect of low-GI diets on insulin sensitivity (De Rougemont et al., 2007; Wolever and Mehling, 2002; Wolever and Mehling, 2003; Bouche et al., 2002; Brynes et al., 2003). In an intervention study by Clapp and Lopez, (2007), insulin resistance assessed by the HOMA-IR and the quantitative insulin-sensitivity check (QUICKI) indexes significantly decreased after consumption of a low-GI diet (59) compared to a high GI diet (92) in women. Dietary fibre intake was not reported in the study. In an observational study including 979 adults, Liese et al. (2005) found no relation between GI, GL or carbohydrate intake and measures of insulin sensitivity, insulin secretion, and adiposity in adults with normal or impaired glucose tolerance. Data from the same study showed that average fasting glucose, 2 h plasma glucose after an oral glucose tolerance test and glycated haemoglobin (HbA1c) concentrations were not related to either GI or GL calculated from food frequency questionnaires (and GL adjusted for total energy intake), neither at baseline nor at a 5-year follow-up examination (Mayer-Davis et al., 2006). Also Lau et al. (2005) did not find any association between GI or high GL and insulin resistance assessed by HOMA-IR among 5,675 healthy adults aged 30 to 60 years.

In conclusion, results from observational and mainly short-term intervention studies with controlled diets have given conflicting results with respect to the importance of GI for blood glucose control and insulin sensitivity. The data available do not allow setting a DRV for GI/GL based on this outcome.

5.4.2. Serum lipids

Intervention studies with controlled diets have given conflicting results with respect to effects of GI/GL on serum lipids. This conflict might be due to methodological problems in designing experimental diets that are similar in dietary composition except for GI. In the well-controlled 10-week intervention study by Sloth et al. (2004) in healthy overweight subjects, who received a fat-reduced diet (21 to 23 E% fat, 57 to 58 E% carbohydrates) with either a high- or low-GI, there were no significant differences between the groups with respect to TG and HDL-cholesterol concentrations. However, a 10% reduction in LDL-cholesterol concentrations was obtained in the group that received the low-GI diet. These findings are supported by a meta-analysis of 15 intervention studies comparing effects of low-GI diets with high-GI diets on serum lipids and other risk factors for coronary heart disease (Kelly et al., 2004).

Some cross-sectional epidemiological studies have indicated that diets with a high GI or GL are associated with unfavourable effects on serum lipids (Augustin et al., 2002). For example Liu et al. (2001) found that fasting plasma TG concentration in 185 healthy postmenopausal women was positively related to GL, especially in overweight and obese subjects. GL was also inversely associated with non-fasting HDL-cholesterol concentration. However, there was no association with the energy adjusted total carbohydrate intake. In a cohort study of 355 healthy adults 35 to 65 years old, Oxlund and Heitmann (2006) found that dietary GI was directly related to changes in total and LDL-cholesterol concentrations in men, but not in women, after six years follow-up. No significant relationships were seen for HDL-cholesterol or TG concentrations. Associations were weak and generally confined to some subgroups, e.g. age categories.

In conclusion, effects on serum cholesterol concentrations are largely dependent on the amount and proportion of fatty acids in the diet, but diets with a low GI may contribute to lowering LDL cholesterol. However, the data available are insufficient to set a DRV for GI/GL based on their effects on serum lipids.

5.4.3. Body weight

A systematic review of 6 selected controlled intervention studies including in total 202 overweight or obese subjects showed that weight reduction was about 1 kg greater in subjects allocated to a diet with reduced GI or GL compared to controls (Thomas et al., 2007). Study duration varied between 6 weeks to 6 months. The studies used different designs, e.g. *ad libitum*/energy restriction, low-GI/low-GL or combinations of these factors. Also macronutrient composition varied widely among the studies. The differences in GI between intervention and control diets varied considerably, from 4 to 7 units to 25 to 30 units. In the three studies that used an *ad libitum* design no significant differences in final body weight were found (Bouché et al., 2002; Sloth et al., 2004; Ebbeling et al., 2005). Only one study (Sloth et al. 2004) compared diets with similar nutrient composition, with no significant difference in weight change after 10 weeks. Other short- to medium-term (12 weeks to 4 months) intervention studies in which diets were administered *ad libitum* to mainly overweight and obese subjects have not shown differences in weight change related to GI (Wolever and Mehling, 2002 and 2003; Aston et al., 2008).

Controlled intervention studies of longer duration (1 to 1.5 years) do not show major differences in weight change in overweight/obese subjects related to GI/GL (Ebbeling et al., 2007; Das et al., 2007) or normal/overweight subjects (Sichieri et al., 2007).

Results from cohort studies regarding the relation between GI and/or GL on body weight are equivocal (Gaesser, 2007; McMillan-Price et al., 2006, McMillan-Price and Brand-Miller, 2006; Sloth and Astrup, 2006; Thorsdottir and Birgisdottir, 2005).

In conclusion, available studies do not allow a firm conclusion with respect to effects of diets with different GI and/or GL on body weight, neither to set a DRV for GI/GL based on this outcome. Most studies have been short-term and there are few controlled studies with *ad libitum* food intake. The difference in GI or GL between intervention and control has varied between studies and diets have also differed in other nutritional aspects, e.g. dietary fibre intake and/or energy density. In studies comparing low GL with high GL, macronutrient composition has differed between high and low GL diets, whereas GI may or may not have differed depending on the strategy used for GL manipulation (changes in carbohydrate content, in GI of carbohydrate containing foods, or both).

5.4.4. Type 2 diabetes mellitus

Prospective cohort studies comparing diets with different GI or GL have shown conflicting results with respect to the risk of developing type 2 diabetes (Salmerón et al., 1997a; Salmerón et al., 1997b; Meyer et al., 2000; Hu et al., 2001; Stevens et al., 2002; Hodge et al., 2004; Schulze et al., 2004b; Krishnan et al,. 2007; Mosdøl et al., 2007; Sahyoun et al., 2008; Halton et al., 2008). A meta-analysis including eight studies found an increased risk of diabetes when comparing highest to lowest quintiles of both GI and GL (Barclay et al., 2008). However, the studies by Krishnan et al. (2007), Sahyoun et al. (2008) and Mosdøl et al. (2007), which showed no or an inverse relationship, were not included. Adjustment for dietary factors was limited to dietary fibre intake.

In conclusion, few observational studies indicate that diets with either low GI or low GL might be associated with a decreased risk of developing type 2 diabetes, but data are inconsistent and do not allow setting a DRV for GI/GL on the basis of type 2 diabetes risk.

5.4.5. Cardiovascular disease

Data from the Nurses Health Study show a positive association of GL with risk of cardiovascular events (MI, CHD deaths, Liu et al., 2000; Halton et al., 2006) or stroke in overweight women (Oh et al. 2005). A study among elderly men did not observe any relation between GI and CVD risk (van Dam et al., 2000). GL was not considered in the study.

In a prospective study of 36,246 Swedish men aged 45 to 79 years without diabetes or prior cardiovascular disease, dietary GI and dietary GL were not associated with ischaemic cardiovascular disease or mortality after 6 years follow-up (Levitan et al., 2007a). However, a weak trend for a greater risk of haemorrhagic stroke with increasing GL was observed. In a subsequent study of 4,617 men aged 45 to 79 years with prior cardiovascular disease, dietary GI and GL were not associated with cardiovascular or all-cause mortality after 6 years follow-up (Levitan et al., 2007b). In a recent systematic review of the evidence supporting a causal link between dietary factors and CHD, high GI and high GL were among those showing the strongest associations with increased risk of CHD (Mente et al., 2009).

In conclusion, a few observational studies indicate that diets with high GL might be associated with an increased risk of CVD, but data are inconclusive and do not allow setting a DRV for GI/GL on the basis of CVD risk.

5.4.6. Colorectal cancer

A meta-analysis of 4 case-control and 7 prospective cohort studies found a significantly increased pooled risk of colorectal cancer for the upper compared to the lower quintile of GL (Gnagnarella et al., 2008). There was, however, large heterogeneity between studies and risk estimates were not significant for prospective studies.

In conclusion, the data available do not allow setting a DRV for GI/GL on the basis of colorectal cancer risk.

6. Data on which to base dietary reference values

6.1. Total and glycaemic carbohydrates

Reference values for the intake of glycaemic carbohydrate have to take into account the amount of energy to be provided when reference intakes for protein and fat intake have been met.

The absolute dietary requirement for glycaemic carbohydrates is not precisely known but will depend on the amount of fat and protein ingested. Generally an intake of 50 to 100 g per day will prevent ketosis. An intake of 130 g per day for both children (>1 years) and adults has been estimated to be sufficient to cover the needs of glucose for the brain. This intake corresponds to about 18 and 25 E% in adult males and females assuming an energy intake of 2,800 and 2,100 kcal per day (11,700 and 8,780 kJ per day), respectively. However, these levels of intake are not sufficient to meet energy needs in the context of acceptable intake levels of fat and protein.

Intervention studies provide evidence that high fat, low carbohydrate diets consumed *ad libitum* are associated with an increase in body weight, but data are insufficient to define an LTI for carbohydrates. High carbohydrate diets tend to induce adverse effects on the lipid profile, but there is an insufficient scientific basis for setting an UL for total carbohydrates. The Panel therefore comes to the conclusion that only a Reference Intake Range can be given for total carbohydrate intake, partly based on practical considerations (e.g. current levels of intake, achievable dietary patterns) (see section 3).

Based on the above considerations the Panel proposes 45 to 60 E% as the Reference Intake Range for carbohydrates. Diets with glycaemic carbohydrate contents of 45 to 60 E%, in combination with reduced intakes of fat and SFA, are compatible with the improvement of metabolic risk factors for chronic disease,

as well as with mean carbohydrate intakes observed in some European countries. This intake range applies to both adults and children older than one year of age.

6.2. Sugars

Frequent consumption of sugar-containing foods can increase risk of dental caries, especially when prophylactic measures, e.g. oral hygiene and fluoride prophylaxis, are insufficient. However, available data do not allow the setting of an UL for (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption, and various other factors.

The evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight.

Observed negative associations between added sugar intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to the intake of added sugars *per se*. The available data are not sufficient to set an upper limit for (added) sugar intake.

Most short-term intervention studies on the effects of high intakes of sugars on glucose and insulin response do not find adverse effects at intakes of predominantly added sugars up to 20 to 25 E%, provided that body weight is maintained. Although there is some evidence that high intakes (>20 E%) of sugars may increase serum TG and cholesterol concentrations, and that intakes >20-25 E% might adversely affect glucose and insulin response, the available data are not sufficient to set an upper limit for (added) sugar intake.

Evidence on the relationship of patterns of consumption of sugar-containing foods to dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines.

The Panel notes that some authorities have established upper limits for population average intake or individual intake of added sugars of <10 E% but others have not (see Section 4). Typically, these nutrient recommendations reflect a judgement of what level of sugar intake is practically achievable within the context of a nutritionally adequate diet based on known patterns of intake of foods and nutrients in specific populations. It is also noted that the average intake of (added) sugars in some EU Member States exceed 10 E%, especially in children.

6.3. Dietary fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of about 25 g per day to be adequate for normal laxation in adults.

The Panel notes that there is evidence in adults of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g. reduced risk of coronary heart disease and type 2 diabetes and weight maintenance.

The Panel considers that the AI for dietary fibre for children should be based on that for adults (25 g, equivalent to 2 to 3 g per MJ for daily energy intakes of 8 to 12 MJ) with appropriate adjustment for energy intake. 2 g per MJ is considered to be adequate for normal laxation in children. Table 3 shows AI for fibre in children of different ages based on average energy intakes. Available evidence indicates that a fibre intake corresponding to 2 to 2.5 g per MJ is compatible with normal growth and development in children.

6.4. Glycaemic index and glycaemic load

Although there is some support for a role of GI and GL in the treatment of type-2 diabetes and some evidence suggesting that lowering GI and GL may have favourable effects on some metabolic risk factors such as serum lipids, the evidence regarding their role in the prevention of diet-related diseases is still inconclusive.

CONCLUSIONS

Total and glycaemic carbohydrates

The Panel considers that there is insufficient scientific basis for setting a Lower Threshold Intake (LTI), a Population Reference Intake (PRI), an Adequate Intake (AI) or a Tolerable Upper Intake Level (UL) for total carbohydrates.

Based on the effects of carbohydrates (and fat) intakes on body weight and blood lipids, while taking into account practical considerations (e.g. current levels of intake, achievable dietary patterns), the Panel proposes 45 to 60 E% as the Reference Intake range for carbohydrates. This intake range applies to both adults and children from one year of age.

Sugars

Available data do not allow the setting of a Tolerable Upper Intake Level for total or added sugars, neither an Adequate Intake nor a Reference Intake range.

Dietary fibre

The role of dietary fibre in bowel function was considered the most suitable criterion for establishing an adequate intake. Based on the available evidence on bowel function, the Panel considers dietary fibre intakes of 25 g per day to be adequate for normal laxation in adults. There is limited evidence to set adequate intakes for children. The Panel considers that the AI for dietary fibre for children should be based on that for adults with appropriate adjustment for energy intake. A fibre intake of 2 g per MJ is considered adequate for normal laxation in children from the age of one year.

The Panel notes that there is evidence in adults of benefit to health associated with consumption of diets rich in fibre-containing foods at dietary fibre intakes greater than 25 g per day, e.g reduced risk of coronary heart disease and type 2 diabetes and improved weight maintenance. Such evidence should be considered when developing food-based dietary guidelines.

Glycaemic index and glycaemic load

The evidence regarding the role of the “gylcaemic index” and the “glycaemic load” in prevention of diet-related diseases is still inconclusive.

Table 3. Summary of Dietary Reference Values for carbohydtares and dietary fibre.

Category	Adults	Children
Total carbohydrates, E% (RI)	45-60	45 to 60 (from 1 year of age)
Dietary Fibre, g/day (AI)	25	10 (from 1 to 3 years) 14 (from 4 to 6 years) 16 (from 7 to 10 years) 19 (from 11 to 14 years) 21 (from 15 to 17 years)

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ANNEXES

ANNEX 1 DEFINITIONS OF DIETARY FIBRE IN RECOMMENDATIONS OF FIBRE INTAKE

Source	Definition
Commission Directive 2008/100/EC[9]	For the purposes of this Directive "fibre" means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: - edible carbohydrate polymers naturally occurring in the food as consumed; - edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; - edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence."
SACN, 2008	Material that is resistant to digestion and absorption in the small intestine and that has a demonstrable physiological effect potentially associated with health benefits in the body, such as increasing stool bulk, decreasing intestinal transit time or decreasing post prandial glycaemia. This includes NSP and soluble fibre. Inclusion of other components in the definition would require physiological effects to be demonstrated.
FAO/WHO (Mann et al., 2007)	Intrinsic plant cell wall polysaccharides, NSP. Dietary fibre should reflect the health benefits of a diet rich in fruits, vegetables and whole grains.
GR, 2006	Carbohydrates, compounds analogous to carbohydrates, and lignin and related substances that are not digested or absorbed in the human small intestine. These include: Polysaccharides other than starch, and indigestible oligosaccharides: e.g. cellulose, hemicelluloses such as arabinoxylans, arabinogalactans and xyloglucans, pectin, fructans and some oligosaccharides (inulin, fructo-oligosaccharides, oligofructose), galacto-oligosaccharides and xylo-oligosaccharides, gums and mucilages (for some population groups: lactose) Compounds analogous to carbohydrates: indigestible dextrins (mainly from potatoes and maize), synthetic carbohydrates and their derivatives, polydextrose, methylcellulose, hydroxypropyl methylcellulose, etc. ndigestible starch. Lignin. Substances that occur in products containing lignin or polysaccharides other than starch: wax, cutin, saponins, suberins, tannins.
IoM 2005	Dietary fibre: non-digestible carbohydrates and lignin that are intrinsic and intact in plants, e.g. cellulose, pectin, gums, hemicellulose, b-glucans, and fibres contained in oat and wheat bran, plant carbohydrates that are not recovered by alcohol precipitation (e.g. inulin, oligosaccharides, and fructans), lignin, and some resistant starch. Excluded are non-digestible mono- and disaccharides and polyols, some resistant starch, non-digestible animal carbohydrates. Functional fibre: isolated, non-digestible carbohydrate components that have beneficial physiological effects in humans. May be isolated or extracted using chemical, enzymatic, or aqueous steps. Synthetically manufactured (DP $\geq$3) or naturally occurring isolated oligosaccharides and manufactured resistant starch are included. Naturally occurring polysaccharides or oligosaccharides usually extracted from their plant source that have been modified (e.g. to a shorter polymer length or to a different molecular arrangement) and animal derived non-digestible carbohydrates are included. Excluded are non-digestible mono-

[9] Commission Directive 2008/100/EC of 28 October 2008 amending Council Directive 90/496/EEC on nutrition labelling for foodstuffs as regards recommended daily allowances, energy conversion factors and definitions. OJ L 285, 29.10.2008, pp. 9-12

Source	Definition
	and disaccharides and polyols, some resistant starch, non-digestible animal carbohydrates. Total dietary fibre: sum of Dietary and Functional fibre.
AFSSA, 2001	Dietary constituents not digested by the enzymes of the gastrointestinal tract, mainly plant-derived constituents, e.g. cellulose, hemicellulose, lignin, gums, alginates, carragenans, resistant starch. Minor dietary components such as oligosaccharides (fructo-oligosaccharides, a-galactosides, lactulose), constituents produced by micro-organisms (xanthanes), constituents of crustaceans (e.g. chitine, chitosane) are also included.
D-A-CH, 2008	Dietary fibre comprises those components of vegetable food which are not degraded by physiological enzymes of the human gastrointestinal tract. Dietary fibre, except for lignin, stands for indigestible carbohydrates such as cellulose, hemicellulose, pectin etc. Resistant starch and indigestible oligosaccharides such as oligofructose and oligosaccharides of the raffinose family (raffinose, stachyose, verbascose in pulses) are included.
NNR, 2004	Dietary fibre recommendation refers to dietary fibre naturally occurring in plant foods as measured by AOAC methods for total dietary fibre.
Codex proposal, Alinorm 09/32/26, 2009	Dietary fibre means carbohydrate polymers[1] with ten or more monomeric units[2], which are not hydrolysed by the endogenous enzymes in the small intestine of humans and belong to the following categories: : Edible carbohydrate polymers naturally occurring in the food as consumed, carbohydrate polymers, which have been obtained from food raw material by physical, enzymatic or chemical means and which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities, synthetic carbohydrate polymers which have been shown to have a physiological effect of benefit to health as demonstrated by generally accepted scientific evidence to competent authorities Properties: Dietary fibre generally has properties such as: • Decrease intestinal transit time and increase stools bulk • fermentable by colonic microflora • Reduce blood total and/or LDL cholesterol levels • Reduce post-prandial blood glucose and /or insulin levels. [1] When derived from a plant origin, dietary fibre may include fractions of lignin and/or other compounds when associated with polysaccharides in the plant cell walls and if these compounds are quantified by the AOAC gravimetric analytical method for dietary fibre analysis : Fractions of lignin and the other compounds (proteic fractions, phenolic compounds, waxes, saponins, phytates, cutin, phytosterols, etc.) intimately "associated" with plant polysaccharides are often extracted with the polysaccharides in the AOAC 991.43 method. These substances are included in the definition of fibre insofar as they are actually associated with the poly- or oligo-saccharidic fraction of fibre. However, when extracted or even re-introduced into a food containing non digestible polysaccharides, they cannot be defined as dietary fibre. When combined with polysaccharides, these associated substances may provide additional beneficial effects (pending adoption of Section on Methods of Analysis and Sampling). [2] Decision on whether to include carbohydrates from 3 to 9 monomeric units should be left to national authorities.
DoH,1991	NSP (non alpha-glucan polysaccharides): cellulose, non-cellulose polysaccharides (pectins, glucans, arabinogalactans, arabinoxylans, gums, mucilages, inulin, guar, chitin.

ANNEX 2A POPULATION, METHODS AND PERIOD OF DIETARY ASSESSMENT IN CHILDREN AND ADOLESCENTS IN EUROPEAN COUNTRIES

Country	Population	Dietary method	Year of survey	Reference
AT	Boys and girls aged 7-9 years	3-day record	2007	Elmadfa et al., 2009
	Boys and girls aged 10-14 years	3-day record	2007	Elmadfa et al., 2009
	Boys and girls aged 14-19 years	24-hour recall	2003-2004	Elmadfa et al., 2009
BE	Boys and girls aged 2.5-3 years	3-day record	2002-2003	Huybrechts and DeHenauw, 2007
	Boys and girls aged 4-6.5 years	3-day record	2002-2003	Huybrechts and DeHenauw, 2007
	Boys and girls aged 13-15 years	7-day record	1997	Matthys et al., 2003
	Boys and girls aged 15-18	2x 24-hour recall	2004	De Vriese et al., 2006
CZ	Boys and girls aged 4-6 years	2x 24-hour recall	n.a.[1]	Tláskas, Hrstková.(unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 7-9 years	2x 24-hour recall	n.a.	Tláskas, Hrstková. (unpublished data) (In:Elmadfa, 2009)
DE	Infants aged 12 months	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 18 months	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 2 years	3-day record	1989-2003	Hilbig and Kersting, 2006
	Children aged 3 years	3-day record	1989-2003	Hilbig and Kersting, 2006
	Boys and girls aged 6 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 7-9 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 10-11 years	3-day record	2006	Mensink et al., 2007
	Boys and girls aged 12 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
	Boys and girls aged 13-14 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
	Boys and girls aged 15-17 years	Dietary history (over the last 4 weeks)	2006	Mensink et al.,2007
DK	Boys and girls aged 1-3 years	7-day record	1995	Andersen et al., 1996
	Boys and girls aged 4-5 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 6-9 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 10-13 years	7-day record	2000-2002	Lyhne et al., 2005
	Boys and girls aged 14-17 years	7-day record	2000-2002	Lyhne et al., 2005
FI	Infants aged 8 months	3-day record	1999	Lagstrom, 1999
	Children aged 3 years	4-day record	1999	Lagstrom, 1999
	Children aged 4 years	4 day record	1999	Lagstrom, 1999
	Children aged 4 years	3-day record	2008	Kyttälä et al., 2008
	Children aged 6 years	3-day record	2008	Kyttälä et al., 2008
FR	Boys and girls aged 4-6 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)

	Boys and girls aged 7-9 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
	Boys and girls aged 10-14 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009 (In: Elmadfa, 2009)
GR	Boys and girls aged 4-5 years	3-day record+24-hour recall / 3-day record	2003-2004	Manios et al., 2008
HU	Boys and girls aged 11-14 years	3x 24-hour recall	2005-2006	Biro et al.2007 (In: Elmadfa, 2009)
IE	Boys and girls 5-8 years	7-day record	2003-2004	Irish Universities Nutrition Alliance, National Irish Children's Food Survey. www.iuna.net
	Boys and girls 9-12 years	7-day record	2003-2004	Irish Universities Nutrition, Alliance National Irish Children's Food Survey. www.iuna.net
IT	Boys and girls 4-6 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 7-9 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 10-14 years	7-day record	n.a	D'Amicis, 2000
	Boys and girls 15-18 years	7-day record	n.a	D'Amicis, 2000
NL	Infants aged 9 month	2-day record (independent days)	2002	Boer et al., 2006
	Infants aged 12 monts	2-day record (independent days)	2002	Boer et al., 2006
	Children aged 18 months	2-day record (independent days)	2002	Boer et al., 2006
	Boys and girls aged 2-3 years	2-day record (independent days)	2005-2006	Ocke et al., 2008
	Boys and girls aged 4-6 years	2-day record (independent days)	2005-2006	Ocke et al., 2008
	Boys and girls aged 7-9 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 10-12 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 13-15 years	2-day record	1997-1998	Hulshof et al., 1998
	Boys and girls aged 16-19 years	2-day record	1997-1998	Hulshof et al., 1998
NO	Children aged 2 years	Food Frequency Questionnaire	1998-1999	Lande and Andersen, 2005
	Boys and girls aged 4 years	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 9 years	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 13	4-day record	2000	Øverby and Andersen, 2002
	Boys and girls aged 16-19 years	Food Frequency Questionnaire	1997	Johansson and Solvoll, 1999
PL	Boys and girls aged 4-6 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 7-9 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 10-14 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	24-hour recall	2000	Szponar et al, 2000 (unpublished data) (In: Elmadfa, 2009)
PT	Boys and girls aged 7-9 years	24-hour recall	2000-2002	Moreira et al., 2005
	Boys and girls aged 13 years	24-hour recall	2000-2002	Moreira et al., 2005

SI	Boys and girls aged 14-17 years	Food Frequency Questionnaire	n.a.	Fidler Mis et al. (unpublished data) (In: Elmadfa, 2009)
ES	Boys and girls aged 10-14 years	2x 24-hour recall	2002-2003	Serra Majem and Ribas, 2007; Serra Majem et al., 2006 and 2007 (In: Elmadfa, 2009)
	Boys and girls aged 15-18 years	2x 24-hour recall	2002-2003	Serra Majem and Ribas, 2007; Serra Majem et al., 2006 and 2007 (In: Elmadfa, 2009)
SE	Boys and girls aged 4 years	4-day record	2003	Enghardt-Barbieri et al., 2006
	Boys and girls aged 8-9 years	4-day record	2003	Enghardt-Barbieri et al., 2006
	Boys and girls aged 11-12 years	4-day record	2003	Enghardt-Barbieri et al., 2006
UK	Boys and girls aged 4-6 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 7-10 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 11-14 years	7-day record	1997	Gregory et al., 2000
	Boys and girls aged 15-18 years	7-day record	1997	Gregory et al., 2000

[1]n.a. = not available

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~1-3 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Infants and Toddlers																				
DE	12 mo	432	52.2	6.0																
	18 mo	478	49.8	6.8																
	2	458	49.4	6.6																
	3	427	50.3	6.3																
FI	8 mo	215	58.0	11.0								3.0	2.0		6.7	2.9				
	13 mo	449	54.0	10.0								5.0	3.0		9.0	2.9				
	2	398	50.0	11.0								10.0	4.0		9.7	2.8				
	3	359	51.0	10.0								11.0	5.0		10.9	3.2				
NL	9 mo	333	58.0	4.2	52.6-63.3	36.3	5.5	29.5-43.4										2.4	0.7	1.6-3.2
	12 mo	306	57.4	4.1	52.1-62,7	35.7	4.9	28.6-41.0										2.5	0.5	1.8-3.2
	18 mo	302	57.5	3.9	52.5-62.6	36.3	5.2	29.8-43.0										2.5	0.5	2.9-3.2
NO	2	172	53.3	5.6								11.7	5.8		13.6	5.2				
Pre-school children																				
Males																				
BE	2.5-3	102	54.2	5.2		31.6	5.2		22.6	3.4					14.6	3.4				
DK	1-3	129	51.0									11.0			15.0					
NL	2-3	313	58.0		49-66										13.0		8-19	2.3		1.5-3.2
Females																				
BE	2.5-3	95	52.9	5.4		29.7	5.4		23.0	4.1					13.0	2.8				
DK	1-3	149	50.0									11.0			14.0					
NL	2-3	313	58.0		51-65										12.0		8-17	2.3		1.5-3.1

[1]SE; [2] P2.5-P97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~4-6 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
BE	4-6.5	236	54.2	4.5		31.4	5.2		22.7	3.3					14.6	3.3				
CZ	4-6	641	56.0	5.8																
DE	6	106	53.3	6.2	41.8-63.6										15.7	4.1	9.9-24.1			
DK	4-5	82	50.0	4.1	43.0-56.0							12.0	4.1	6.0-20.0	17	4.6	10.0-24.0	2.2	0.5	1.5-3.1
FI	4	307	53.0									13.7			9.6	3.2				
	6	364	53.0									13.0			11.4	3.7				
FR	4-6	164	49.8	0.8[1]											11.8	0.5[1]				
GR	4-5	356	44.8	6.5																
IT	4-6	21	50.0	5.5											14.9	4.7				
NL	4-6	327	56.8	0.9	48.0-64.0							13.0	2.0		14	3	9.0-20.0	2.2		1.5-3.0
NO	4	206	53.0	5.0								15.0	5.0		12.0	5.0				
PL	4-6	82	57.0	7.9								18.5	5.8		16.8	5.8				
SE	4	302	54.2	4.8	46.5-62.4	28.6						13.8	4.6	7.0-21.6	12.0	3.0	7.0-17.0	1.8	0.5	1.1-2.7
UK	4-6	184	51.6	4.3	43.0-59.4															
Females																				
BE	4-6.5	228	54.9	4.1		31.3	5.1		23.4	3.4					13.9	3.2				
CZ	4-6	446	56.0	5.8											15.3	4.7				
DE	6	102	53.3	5.2	41.8-63.6										15.8	4.7	8.6-24.7			
DK	4-5	116	50.0	4.1	43.0-56.0							12.0	3.9	7.0-20.0	16	4.5	9.0-22.0	2.2	0.5	1.5-3.0
FI	4	307	53.0									13.6			9.4	3.0				
	6	349	53.0									13.8			10.3	3.3				
FR	4-6	162	48.6	0.5[1]											11.5	0.3[1]				
GR	4-5	389	45.2	6.4																
IT	4-6	17	50.3	4.9											15.8	3.9				
NL	4-6	312	57.0	4.0	50.0-64.0										13	3	8.0-17.0	2.0		1.4-2.8
NO	4	185	54.0	6.0								16.0	6.0		12.0	6.0				
PL	4-6	84	55.6	7.5								17.9	6.0		14.6	5.9				
SE	4	288	53.4	5.1	45.4-61.6	28.4						13.7	4.5	6.9-21.2	11.0	3.0	7.0-17.0	1.8	0.4	1.2-2.6
UK	4-6	171	51.4	5.0	42.1-60.5[2]															

[1]SE; [2] P2.5-P97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~7-9 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	7-9	146	51.4	6.8								16.6	7.1		15.0	5.9				
CZ	7-9	940	53.4	6.7																
DE	7-9	321	53.2	6.1	43.6-63.1										17.5	5.3	10.6-26.2			
DK	6-9	174	51.0	3.9	44.0-57.0							13.0	5.2	6.0-22.0	18.0	5.8	11.0-28.0	2.0	0.5	1.3-2.9
FR	7-9	160	49.3	0.5[1]											13.5	0.4[1]				
IE	5-8	145	52.4	4.8	44.5-60.2															
IT	7-9	29													18.5					
NL	7-9	104	52.7	6.6	38.0-63.6	29.6	7.0	18.0-40.5	23.0	4.0	17.0-30.0				17.0	6.0	9.0-25.0	2.0	0.6	1.2-3.0
NO	9	402	54.0	6.0								16.0	6.0		16.0	7.0				
PL	7-9	101	56.3	7.9								17.3	5.6		19.6	6.8				
PT	7-9	1541	48.6	7.8								22.5	7.1		20.2	8.1				
SE	8-9	444	53.0	4.8	45.3-60.5	25.7						12.5	4.3	5.7-20.2	14.0	4.0	8.0-22.0	1.7	0.4	1.2-2.4
UK	7-10	256	52.4	4.1	44.2-60.5															
Females																				
AT	7-9	134	52.2	7.0								18.0	6.9		14.3	4.4				
CZ	7-9	765	53.4	6.7																
DE	7-9	308	54.2	6.7	43.1-66.1										16.8	5.4	10.0-26.2			
DK	6-9	157	51.0	4.3								13.0	4.7	7.0-21.0	17.0	4.5	10.0-25.0	2.1	0.5	1.4-2.9
FR	7-9	14	48.5	0.7[1]											12.2	0.4[1]				
IE	5-8	151	51.5	4.6	43.4-59.6															
IT	7-9	21													15.2	5.0				
NL	7-9	134	52.0	7.3	39.6-63.7	29.5	7.2	18.4-41.5	22.4	4.3	15.7-30.2				15.0	5.0	7.0-23.0	1.9	0.5	1.2-2.8
NO	9	408	55.0	6.0								18.0	6.0		14.0	6.0				
PL	7-9	103	55.5	7.7								16.4	6.2		17.4	6.7				
PT	7-9	1503	48.3	7.9								21.8	7.1		19.4	8.2				
SE	8-9	445	53.3	4.9	44.7-61.1	25.5						12.6	4.2	6.1-19.4	13.0	4.0	8.0-19.0	1.8	0.4	1.2-2.6
UK	7-10	226	51.3	4.3	42.6-59.9[2]															

[1]SE; [2] P2.5-97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~10-14 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	10-14	248	50.8	7.5								17.6	8.2		15.1	6.1				
BE	13-15	74	49.1	4.6		24.3	4.9		24.8	4.4		19.0	5.9					1.8	0.5	
DE	10-11	199	53.2	6.4	43.0-64.5										17.9	6.0	9.0-28.8			
	12	114	52.0	5.4	42.1-60.9										25.3	9.4	12.6-46.1			
	13-14	214	51.7	6.2	43.1-62.5										27.7	12.2	11.8-50.1			
DK	10-13	145	52.0	4.7	44.0-60.0							14.0	5.7	7.0-25.0	18.0	6.3	9.0-28.0	1.9	0.4	1.3-2.6
FR	10-14	160	48.1	0.4[1]											15.2	0.4[1]				
HU	11-14	124	50.3	5.6								11.9	5.6		20.8	5.9				
IE	9-12	148	52.5	5.3	44.6-61.1															
IT	10-14														21.6	7.6				
NL	10-12	112	51.5	6.4	40.5-61.2	27.4	7.2	18.2-35.4	24.0	4.8	16.8-30.5				19.0	6.0	10.0-29.0	2.1	0.6	1.1-3.1
	13-15	137	51.2	5.8	41.8-60.9	27.0	6.2	16.8-37.0	24.1	3.7	17.9-30.1				22.0	7.0	11.0-34.0	2.0	0.5	1.0-3.0
NO	13	590	55.0	7.0								18.0	8.0		16.0	8.0				
PL	10-14	202	53.9	8.0								15.2	5.8		24.6	10.0				
PT	13	987	52.3	5.7								24.2	6.0		25.4	10.3				
SE	11-12	517	52.4	5.6	43.0-61.8	23.4						11.7	5.0	3.9-19.9	13.0	4.0	7.0-21.0	1.7	0.4	1.1-2.5
ES	10-14	66	41.0	4.2								16.1	3.3		18.5	1.6				
UK	11-14	237	51.7	4.6	42.5-59.8															
Females																				
AT	10-14	239	52.1	8.0								16.8	7.3		13.7	4.3				
BE	13-15	89	49.1	5.4		24.3	5.0		24.8	4.6		15.8	5.2					2.0	0.6	
DE	10-11	198	53.1	7.2	43.6-66.4										17.7	5.5	9.3-27.2			
	12	103	52.8	6.6	42.2-65.4										25.0	10.9	11.0-46.5			
	13-14	230	52.7	6.1	44.0-63.3										24.4	8.8	12.6-38.9			
DK	10-13	131	52.0	4.7								14.0	5.0	7.0-23.0	15.0	5.0	8.0-26.0	1.9	0.5	1.3-2.7
FR	10-14	144	48.0	0.4[1]											13.8	0.3[1]				
HU	11-14	111	51.7	5.4								12.5	5.9		20.1	6.7				
IE	9-12	148	52.5	5.3	44.6-61.1															
IT	10-14	47													16.8	3.9				
NL	10-12	124	52.1	6.2	41.2-61.7	28.5	6.2	18.2-38.2	23.6	4.2	16.9-30.1				17.0	6.0	9.0-28.0	2.0	0.6	1.2-3.0
	13-15	117	50.3	6.5	40.2-62.6	26.4	7.2	12.3-37.8	23.8	4.6	17.5-32.7				18.0	6.0	9.0-30.0	2.1	0.7	1.1-3.3
NO	13	515	55.0	6.0								19.0	7.0		14.0	7.0				
PL	10-14	202	54.0	7.7								15.5	6.0		20.9	8.7				

PT	13	1053	52.6	6.2					25.4	7.0		25.2	10.6					
SE	11-12	499	53.2	5.5	44.2-62.3	24.7			12.9	5.0	5.5-22.2	12.0	4.0	6-19		1.8	0.4	1.2-2.5
ES	10-14	53	41.6	3.3					16.0	2.5		17.5	1.1					
UK	11-14	238	51.2	5.2	42.2-62.8[2]													

[1]SE; [2] P2.5-97.5

ANNEX 2B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG CHILDREN AGED ~15-18 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	14->19	1527	46.1	9.9								16.1	8.9		15.6	7.1				
BE	15-18	405	49.5	4.4																
DE	15-17	294	49.6	6.5											26.1	10.7				
DK	14-17	86	50.0	6.1	41-59							13.0	7.2	2-27	19.0	6.0	9-28	1.8	0.5	1.1-2.6
FR	15-18	181	48.7	0.6[1]											16.9	0.8[1]				
IT	15-18	52													23.9	9.1				
NL	16-18	142	49.5	6.6	38.7-59.9	24.9	7.4	14.5-35.7	24.5	4.8	16.5-32.6				24.0	11.0	8-42	2.1	0.7	1.0-3.3
NO	16-19	92	53.7									15.1			26.0					
PL	15-18	174	50.7	7.4								12.5	5.0		32.6	12.5				
SI	15-18	1010	57.0	9.0								11.0	4.0		33.0	21.0				
ES	15-18	61	39.7	4.7								14.8	3.4		18.9	1.8				
UK	15-18	179	50.5	5.4	39.9-60.5															
Females																				
AT	14->19	1422	47.3	10.3								15.7	8.7		13.8	6.0				
BE	15-18	401	50.6	5.4																
DE	15-17	317	52.7	6.4											23.1	8.3				
DK	14-17	117	52.0	5.6	45-63							14.0	6.1	5-23	15.0	5.2	8-24	1.9	0.5	1.2-2.6
FR	15-18	222	48.8	0.7[1]											12.7	0.3[1]				
IT	15-18	47													17.6	4.7				
NL	16-18	129	50.3		37.6-61.1	26.2		12.8-37.5	24.0		16.2-32.9				19.0		8-20	2.1		1.1-3.6
NO	16-19	62	54.6									11.7			21.0	8.0				
PL	15-18	175	54.2	8.5								14.1	5.7		23.0	8.9				
SI	15-18	1214	57.0	8.0								13.0	4.0		27.0	18.0				
ES	15-18	57	38.6	3.7								15.4	2.9		16.2	2.0				
UK	15-18	210	50.6	5.6	39.9-64.0[2]															

[1]SE; [2] P2.5-97.5

ANNEX 3A POPULATION, METHODS AND PERIOD OF DIETARY ASSESSMENT IN ADULTS IN EUROPEAN COUNTRIES.

Country	Population	Dietary method	Year of survey	Reference
AT	Males and females aged 19-64 years	24-hour recall	2007	Elmadfa et al., 2009
	Males and females aged 65 and over	3-day record	2007	Elmadfa et al., 2009
BE	Males and females aged 19-59 years	2x 24-hour recall	2004	De Vriese et al, 2006
	Males and females aged 60-75 years	2x 24-hour recall	2004	De Vriese et al, 2006
	Males and females aged 75+ years	2x 24-hour recall	2004	De Vriese et al, 2006
CZ	Males and females aged 19-64 years	n.a.	n.a.[1]	Cifkova and Skodova, 2004
DE	Males and females aged 35-64 years	24-hour recall	1996-1998	Linseisen et al., 2003
	Males and females aged 19-64 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 19-24 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 25-34 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 35-50 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 51-64 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and females aged 65-80 years	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
	Males and Females aged 65 and over	24-hour recall + Dietary History	2005-2006	Anonymous, 2008
DK	Males and females aged 18-74 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 18-24 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 25-34 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 35-44 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 45-54 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 55-64 years	7-day record	2000-2002	Lyhne et al., 2005
	Males and females aged 65-74 years	7-day record	2000-2002	Lyhne et al., 2005
EE	Males and females aged 19-65 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 19-34 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 35-49 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 50 and over	24-hour recall	1997	Pomerleau et al., 2001
FI	Males and females aged 25-64 years	3-day record	2002	Paturi et al., 2008
	Males and females aged 65-74 years	4-day record	2002	Paturi et al., 2008
FR	Males and females aged 19-64 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009. (In: Elmadfa, 2009)
	Males and females aged 65-75 years	3x 24-hour recall	2006-2007	Castetbon et al. 2009. (In: Elmadfa, 2009)

GR	Males and females aged 19-64 years	FFQ + 24-hour recall in sub group	1994-1999	Greek cohort EPIC study. (In: Elmadfa, 2009)
	Males and females aged 65 and over	FFQ	1994-1999	Greek cohort EPIC study. (In: Elmadfa, 2009)
HU	Males and females aged 11-14 years	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
	Males and females aged 18-59	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
	Males and females aged 60 and over	3-day record	2003-2004	Rodler et al. 2005; Zajkás et al., 2007; Bíró et al., 2007 (In: Elmadfa, 2009)
IE	Males and females 18-64 years	7-day record	1997-1999	Irish Universities Nutrition Alliance, North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 18-35 years	7-day record	1997-1999	Irish Universities Nutrition Alliance, North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 36-50 years	7-day record	1997-1999	Irish Universities Nutrition Alliance ,North/South Ireland Food Consumption Survey. www.iuna.net
	Males and females 51-64 years	7-day record	1997-1999	Irish Universities Nutrition, Alliance North/South Ireland Food Consumption Survey. www.iuna.net
IT	Males and females 19-64 years	7-day record	n.a.	D'Amicis, 2000
	Males and females aged 65 and over	7-day record	n.a.	D'Amicis, 2000
LT	Males and females 19-64 years	24-hour recall	2007	Unpublished data (In: Elmadfa, 2009)
LV	Males and females 19-64 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 19-34 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 35-49 years	24-hour recall	1997	Pomerleau et al., 2001
	Males and females aged 50 and over	24-hour recall	1997	Pomerleau et al., 2001
NL	Males and Females aged 19-64 years	2-day record	1997-1998	Hulshof et al., 1998
	Males and Females aged 65 and over	2-day record	1997-1998	Hulshof et al., 1998
	Males and females aged 19-30 years	2x 24-hour recall	2003	Hulshof and Ocké, 2005
NO	Males and females aged 19-64 years	FFQ	1997	Johansson and Sovoll, 1999
	Males and females aged 65 and over	FFQ	1997	Johansson and Sovoll, 1999
PL	Males and females aged 19-64 years	24-hour recall	2000	Szponar et al., 2000 unpublished data (In: Elmadfa, 2009)
	Males and females aged 65 and over	24-hour recall	2000	Szponar et al., 2000 unpublished data (In: Elmadfa, 2009)
PT	Males and females aged 18+ years	FFQ	n.a.	EPIPorto study (In: Elmadfa, 2009)
	Males and females aged 65 and over	FFQ	n.a.	EPIPorto study (In: Elmadfa, 2009)

RO	Males and females aged 19-64 years	personal interview	2006	National Synthesis 2006 (In: Elmadfa, 2009)
	Males and females aged 65 and over	personal interview	2006	National Synthesis 2006 (In: Elmadfa, 2009)
ES	Males and females aged 18-64 years	24-hour recall	2002-2003	Serra Majem et al., 2007
	Males and females aged 65-75 years	24-hour recall	2002-2003	Serra Majem et al., 2007
SE	Males and females aged 18-74 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 17-24 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 25-34 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 35-44 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 45-54 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 55-64 years	7-day record	1997-1998	Becker and Pearson, 2002
	Males and females aged 65 and over	7-day record	1997-1998	Becker and Pearson, 2002
UK	Males and females aged 19-64 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 19-24 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 25-34 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 35-49 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 50-64 years	7-day record	2000-2001	Henderson et al., 2003
	Males and females aged 65+ years	4-day record	1994-1995	Finch et al., 1998

[1]n.a. = not available

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~19-65 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
AT	19-64	778	42.5	10.5								8.7	6.4		19.5	9.9				
BE	19-59	413	45.2	5.3		24.1	4.8		19.4	5.7										
CZ	19-64	1094	53.9	12.0																
DE	19-64	4912	45.3	8.4																
DK	18-74	1467	43	6.8	35.0-53.0[1]							8.0		3-15[1]	21.0		12.0-32.0[1]			
EE	19-64	900	42.7	14.0																
FI	25-64	730	47.1	8.8								9.7	5.9		24.0	11.0		2.7	1.1	
FR	19-64	852	43.4	0.3[2]											18.7	0.4[2]				
GR	19-64	8365	37.9	5.9																
HU	>18	473	45.0	6.6								7.6	5.2		24.2	6.6				
IE	18-64	662	43.5	6.4	32.6-54.3										23.2	8.5	12.1-38.9			
IT	19-64	660													21.8	6.5				
LT	19-65	849	38.9	9.3								10.8	5.6		20.9	12.4				
LV	19-65	1065	42.2	11.8																
NL	19-64	1836	44.2	7.5																
NO	19-64	1050	51.0	6.0								9.0	6.0		25.0	10.0				
PL	19-64	1106	48.0	8.4								11.0	5.2		29.7	11.4				
PT	19-64	917	47.0	6.8											23.5	9.0				
RO	19-64	177	43.3	9.4																
SE	18-74	589	46	6.0	37.0-56.0							9.0	4.0	3-16	18.0	7.0	9.0-29.0	1.8	0.5	1.1-2.7
ES	18-64	718	40.5			16.6			23.2						19.2					
UK	19-64	833	47.7	6.0	35.9-59.8[4]															
Females																				
AT	19-64	1345	46.0	10.6								10.9	6.6		20.1	9.3				
BE	19-59	460	46.9	6.2		21.4	6.3		24.3	4.1										
CZ	19-64	1094	53.9	12.0												9.0				
DE	19-64	6016	48.7	7.4																
DK	18-74	1684	47	6.6	39.0-55.0[1]							9.0		4-17[1]	19.0		11.0-28.0[1]			
EE	19-65	1115	47.3	12.6																
FI	25-64	846	50.2	8.3								10.5	5.1		21.0	9.0		3.2	1.3	
FR	19-64	1499	44.4	0.2[2]											15.7	0.2[2]				
GR	19-64	12034	39.5	5.4																
HU	>18	706	48.0	5.8								8.6	4.8		21.7	5.6				
IE	18-64	717	46.6	5.6	37.4-56.6										17.4	5.9	9.3-27.5			
IT	19-64	801													18.9	6.1				

LT	19-64	1087	42.9	10.3																			
LV	19-64	1235	44.6	11.9																			
NL	19-64	2112	44.7	7.9																			
NO	19-64	1146	51.0	6.0							9.0	6.0			21.0	8.0							
PL	19-64	1334	51.8	9.1							13.7	6.6			19.7	7.9							
PT	19-64	1472	50.1	5.9											23.7	9.4							
RO	19-64	341	43.6	9.7																			
SE	18-74	626	47	5.0		20.0					9.0	4.0		4-16	16.0	5.0		9-27		2.1	0.5		1.3-3.1
ES	18-64	895	40.7			18.5			21.0						16.9								
UK	19-64	891	48.5	6.7	37.4-61.5[3]																		

[1]P10-P90; [2] SE; [3] P2.5-P97.5

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~19-34 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
DE	19-24	510	46.7	0.35[1]	34.8-60.4										24.6	0.55[1]	10.1-51.4			
	25-34	690	46.1	0.29[1]	34.4-59.4										25.8	0.44[1]	10.5-46.1			
DK	18-25	146	47.0	5.8	39.0-58.0							12	6.5	3-25	19.0	7.3	10.0-32.0	1.8	0.6	1.1-2.9
	25-34	272	46.0	5.4	37.0-55.0							11	5.7	3-20	20.0	6.9	11.0-32.0	1.9	0.5	1.2-2.8
EE	19-34	396	41.8	131																
IE	18-35	253	42.7	6.2	31.9-53.3										22.6	8.5	11.7-38.5			
LV	19-34	337	41.3	11.2																
NL	19-30	352	47.5	4.3	40.4-54.6	23.8	5.2	15.6-32.8							22.7	6.1	13.6-33.4	2.0	0.5	1.2-2.9
SE	17-24	67	49.0	5.0	40.0-57.0							11	5.0	4-21	16.0	7.0	6.0-29.0	1.6	0.4	1.0-2.5
	25-34	128	47.0	6.0	38.0-57.0							10	4.0	4-17	18.0	6.0	9.0-28.0	1.7	0.5	1.1-2.5
UK	19-24	108	49.0	6.3	38.0-63.2															
	25-34	219	47.7	5.8	35.2-58.3															
		Females																		
DE	19-24	510	51.3	0.34[1]											21.7	0.39[3]	8.7-37.1			
	25-34	972	50.0	0.23[1]											24.0	0.31	11.9-41.1			
DK	18-25	213	50.0	5.5	42.0-61.0							13	6.9	4-26	16.0	4.5	9.0-22.0	2.0	0.6	1.2-3.2
	25-34	315	49.0	5.4	40.0-57.0							12	5.8	4-23	17.0	4.5	10.0-25.0	2.1	0.7	1.2-3.3
EE	19-34	459	46.4	13.0																
IE	18-35	269	46.6	5.1	39.0-55.7										16.1	5.1	8.8-25.7			
LV	19-34	342	44.3	12.1																
NL	19-30	398	49.3	5.5	40.5-58.4	25.6	6.1	16.3-36.3							17.0	4.5	10.2-25.0	2.2	0.6	1.3-3.3
SE	17-24	70	50.0	6.0	39.0-61.0							11	4.0	5-18	15.0	5.0	7.0-24.0	1.9	0.5	1.2-2.9
	25-34	132	48.0	5.0	40.0-56.0							10	4.0	5-16	15.0	4.0	9.0-23.0	1.9	0.5	1.2-2.8
UK	19-24	104	49.1	8.3	36.5-62.7[2]															
	25-34	210	48.7	5.8	36.6-61.9[2]															

[1]SE; [2] P2.5-P97.5

ANNEX 3B INTAKE OF CARBOHYDRATES AND DIETARY FIBRE AMONG ADULTS AGED ~35-64 YEARS IN EU COUNTRIES.

Country	Age yrs	N	Total Carbohydrates (E%)			Mono- and Disaccharides (E%)			Polysaccharides (E%)			Sucrose (E%)			Dietary Fibre (g)			Dietary Fibre (g/MJ)		
			mean	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95	*mean*	*SD*	P5 - P95
Males																				
DE	35-64[1]	1013	40.2	9.9		17.6			22.4			8.8	6.0		21.8	11.5				
	35-64[2]	1032	38.5	9.4		17.4			20.8			8.9	5.7		21.9	9.2				
	35-50	2079	45.1	0.17[3]	32.0-58.0										27.3	0.26[3]	11.9-48.2			
	51-64	1633	44.4	0.19[3]	32.4-57.2										27.4	0.28[3]	12.4-48.2			
DK	35-44	330	43.0	5.6	34.0-53.0							8.0	4.8	2-18	22.0	7.6	11-34	2.0	0.5	1.3-2.9
	45-54	312	42.0	6.6	31.0-53.0							7.0	4.4	1-15	22.0	9.0	10-39	2.2	0.6	1.3-3.4
	55-64	242	42.0	6.9	31.0-53.0							6.0	4.1	1-14	21.0	8.3	10-37	2.2	0.7	1.2-3.4
EE	35-49	319	43.6	15.5																
	50+	185	43.1	13.0																
IE	36-50	236	43.3	6.3	32.2-53.9										23.6	8.1	12.8-38.8			
	51-64	173	45.2	6.8	32.9-55.9										17.3	7.1	9.1-31.5			
LV	35-49	372	42.6	11.8																
	50+	356	43.2	12.4																
SE	35-44	143	45.0	5.0	38.0-54.0							8.0	4.0	3-14	18.0	7.0	9.0-30.0	1.8	0.4	1.2-2.5
	45-54	18	46.0	6.0	36.0-56.0							8.0	4.0	3-16	19.0	7.0	9.0-34.0	1.9	0.6	1.2-2.8
	55-64	68	47.0	5.0	39.0-55.0							7.0	4.0	3-14	18.0	5.0	12.0-29.0	2.0	0.4	1.5-2.8
UK	35-49	253	47.5	5.9	36.0-59.9[4]															
	50-64	253	47.4	6.2	35.6-59.5[4]															
Females																				
DE	35-64[1]	1078	43.0	10.2		21.5			21.1			10.9	6.6		19.6	9.0				
	35-64[2]	898	43.9	10.6		23.3			20.1			12.1	7.3		19.4	8.2				
	35-50	2694	48.0	0.14											24.7	0.19	11.5-42.9			
	51-64	1840	47.7	0.18											26.1	0.24	12.7-44.2			
EE	35-49	376	47.4	12.4																
	50+	280	48.5	12.3																
DK	35-44	359	47.0	5.9	38.0-57.0							9.0	5.4	3-18	15.0	5.0	8.0-26.0	2.3	0.7	1.4-3.5
	45-54	370	46.0	6.1	36.0-56.0							7.0	4.1	2-15	15.0	5.2	8.0-24.0	2.5	0.6	1.6-3.6
	55-64	263	46.0	6.5	35.0-56.0							8.0	4.1	2-14	16.0	5.6	9.0-27.0	2.6	0.7	1.6-3.9
IE	36-50	286	44.7	6.1	33.9-54.1										18.2	5.3	10.7-28.5			

	51-64	162	47.5	6.2	37.3-57.6							18.2	6.2	8.8-28.2			
LV	35-49	396	43.4	11.7													
	50+	496	45.7	12.0													
SE	35-44	132	46.0	6.0	37.0-54.0				9.0	3.0	4-14	16.0	4.0	9.0-22.0	2.0	0.5	1.4-2.9
	45-54	153	47.0	5.0	39.0-55.0				8.0	3.0	4-14	17.0	5.0	10.0-27.0	2.2	0.5	1.4-3.3
	55-64	81	48.0	5.0	40.0-57.0				8.0	4.0	4-13	18.0	5.0	10.0-28.0	2.4	0.5	1.7-3.5
UK	35-49	318	48.6	6.8	35.3-62.4[4]												
	50-64	259	48.1	6.7	32.2-59.4[4]												

[1]Cohort Heidelberg; [2]Cohort Potsdam; [3]SE ; [4] P2.5-P97.5;

ANNEX 4 EFFECTS OF SUGAR INTAKE ON GLUCOSE AND INSULIN RESPONSE IN CONTROLLED INTERVENTION STUDIES IN ADULTS.

Study	Study design	Subjects	Total fat, E%	CHO, E%	Sugars, E%	Results
Reiser et al. 1979	6-week cross-over, iso-energetic	10 men, 9 women, 35-55 y	42	43	30 E% sucrose or wheat starch	Fasting serum glucose and insulin higher on sucrose diet
Reiser et al 1981	6-week cross-over, iso-energetic	24 adults with impaired insulin response	42	44	5, 18 or 33 E% sucrose or starch	Fasting and postprandial insulin higher on 18 or 33 E% sucrose diets
Swanson et al. 1992	4-week cross-over, iso-energetic	14 healthy adults	32-34	51-52	20 E% as fructose or starch (< 3 E% fructose)	No sign. differences between the diets in mean values of hemoglobin A1c, serum glycosylated albumin, fasting plasma glucose, peak postprandial plasma glucose, or integrated plasma glucose response.
Bantle et al., 2000	6-week cross-over, iso-energetic	12 men, 12 women (BMI <32)	30	55	17 E% fructose or glucose. Total sugars (glucose, fructose, sucrose and lactose) about 21 E% in both diets	Lower postprandial plasma glucose serum insulin, and daylong insulin concentrations on fructose diet
Black et al. 2006	6 wk cross-over, iso-energetic	13 W/M 33 y, BMI: 26.6	33	55	10 or 25 E% sucrose	No difference in glucose serum insulin or peripheral insulin resistance

ANNEX 5 EFFECTS OF SUGAR INTAKE ON SERUM LIPIDS IN CONTROLLED INTERVENTION STUDIES IN ADULTS

Study	Study design	Subjects	Total fat (E%)	CHO (E%)	Sugars (E%)	Results
Short-term						
Hallfrisch et al., 1983	5-week cross-over	12 men hyper-insulinemic 12 with normal insulin response			0, 7.5, and 15 E% fructose replacing starch	Total and LDL-chol +5-7% (sign) in both groups on 7.5 E% and 15 E% fructose diets. Fasting TG +30% and +61%(sign) on 7.5 and 15 E% fructose diets, respectively
Reiser et al. 1989	5-week cross-over	10 hyper-insulemic and 11 normal men	36	51	20 E% as either added fructose or high-amylose cornstarch	Fasting TG (+46%), total chol (+11%) in the hyperinsulinemics, and +20% and +8%, respectively, in normal men on the fructose diet. LDL-chol (+12%) in the normal men on the fructose diet
Swanson et al., 1992	4-week cross-over	14 healthy subjects	30	55	20 E% added fructose or starch	No difference in fasting serum TG. Total and LDL chol +9% and +11% (sign), respectively, on fructose diet
Bantle et al. 2000	6-week cross-over	12 men and 12 women BMI < 32			17 E% as fructose or glucose, Total sugars 21E%	Increased fasting and daylong TG in men, but not in women, on fructose diet. Total and LDL-chol higher on day 28, but not at the end of the study period on the fructose diet.
Marckmann et al. 2000	2 wk cross-over *ad libitum*.	20 W, 21-52 y BMI:	28-29	59	2.5 or 23 E% sucrose	Fasting and nonfasting TG, total and LDL-chol lower on the low-sucrose diet.
Black et al. 2006	6 wk cross-over, iso-energetic	13 W/M 33 y, BMI: 26.6			10 or 25 E% sucrose	Total and LDL chol +15% and +24%, respectively on high sucrose diet. HDL-chol and TG unchanged.
Erkkila et al. 2007	8 wk	34			Increased sucrose intake from 7-9 to 15 E% (40 g/day)	No significant effects on lipid concentrations
Long-term						
Saris et al. 2000	6-month parallel, *ad libitum*	316 obese			Control: 46 E% CHO (21 E% sugars) Intervention diets: 52–56 E% CHO, starch (16 E% sugars) or high-sugar (sucrose, fructose and lactose, 30 E%).	No significant changes were seen in serum lipids among the groups
Poppitt et al., 2002	6 months	46 obese subjects with the metabolic syndrome			3 diets, "complex carbohydrate", 21 or 29 E% sugars (as sucrose, fructose and lactose)	Fasting TG higher in 29 E% sugar group than in the "complex-carbohydrate" and 21 E% sugar diet. Weight loss correlated with a decrease in TG concentrations.

GLOSSARY / ABBREVIATIONS

Added sugars	Term used to describe sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing
AFSSA	Agence Française de Sécurité Sanitaire des Aliments
AICR	American Institute of Cancer Research
AI	Adequate Intake
AMDR	Acceptable Macronutrient Distribution Ranges
AOAC	Association of Official Analytical Chemists
BMI	Body Mass Index
CHD	Coronary Heart Disease
CV	Coefficient of Variation
DMFT	Decayed, Missing and Filled Teeth in the permanent teeth
dmft	Decayed, missing and filled teeth in the primary teeth
CVD	Cardiovascular Disease
DoH	Department of Health
DP	Degree of Polymerisation
DRVs	Dietary Reference Values
EC	European Commission
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
EU	European Union
FAO	Food and Agriculture Organisation of the United Nations
FFQ	Food Frequency Questionnaire
FNB	U.S. Food and Nutrition Board
FOS	Fructo-oligosaccharides
GI	Glycaemic Index

GL	Glycaemic Load
GLUT	Glucose Transporter
GOS	Galacto-oligosaccharides
HDL-cholesterol	High Density Lipoprotein-cholesterol
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HOMA-ß	Homeostasis Model Assessment of ß-cell function
IGT	Impaired Glucose Tolerance
IoM	U.S. Institute of Medicine of the National Academy of Sciences
LDL-cholesterol	Low Density Lipoprotein-cholesterol
LTI	Lower Threshold Intake
Metabolic syndrome	Cluster of cardiovascular risk factors including clinical measures such as waist circumferences, blood pressure, triglycerides, high-density lipoproteins, blood glucose, and insulin sensitivity. Usually three criteria are needed for the diagnosis of Metabolic Syndrome
MI	Myocardial Infarction
MUFA	Monounsaturated Fatty Acids
NSP	Non-Starch Polysaccharides
NNR	Nordic Nutrition Recommendations
PRI	Population Reference Intake
QUICKI	Quantitative Insulin-Sensitivity Check Index
RI	Reference Intake ranges for macronutrients
SCF	Scientific Committee on Food
SCFA	Short-Chain Fatty Acids
SD	Standard Deviation
SFA	Saturated Fatty Acids
SGLT 1	Sodium Glucose Transporter 1
STRIP	Special Turku Coronary Risk Factor Intervention Project
Sugars	Term conventionally used to describe mono- and disaccharides
Sugar	Term used to describe sucrose
TG	Triglycerides

UL	Tolerable Upper Intake Level
US	United States
VLDL	Very low Density Lipoproteins
WCFR	World Cancer Research Fund
WHO	World Health Organization

European Food Safety Authority
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EFSA will provide scientific advice on the daily intake of added sugar in food by early 2020. The Authority aims to establish a science-based cut-off value for daily exposure to added sugars from all sources which is not associated with adverse health effects. The work will be carried out following a request from Denmark, Finland, Iceland, Norway and Sweden.

Added sugars from all sources comprise sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup, and other sugar preparations consumed as such or added during food preparation and manufacturing.

The adverse health effects under consideration will include body weight, glucose intolerance and insulin sensitivity, type-2-diabetes, cardiovascular risk factors, as well as dental caries. In its assessment, EFSA will look at the general healthy population, including children, adolescents, adults and the elderly.

The advice will guide Member States when establishing recommendations for the consumption of added sugars and in planning food-based dietary guidelines.

Sweden is coordinating the request to EFSA on behalf of the five Nordic countries. Annica Sohlström, the Director General of the Swedish National Food Agency, said: "We welcome EFSA's acceptance of the mandate which reflects the need to scientifically evaluate the links between added sugar and health at a European level."

What is going to happen next?

EFSA will establish an ad-hoc working group with expertise in dietary exposure, epidemiology, human nutrition, diet-related chronic diseases and dentistry. The five Nordic countries that initiated this mandate will be invited to the working group as observers.

EFSA will use its established methodology to develop a protocol on how to carry out the assessment. Known as Prometheus – PROmoting METHods for Evidence Use in Scientific assessments – the method shows how EFSA selects evidence, how this evidence contributes to the risk assessment and how EFSA reports on the entire process and it results.

In line with its commitment to openness and transparency, EFSA will engage with stakeholders throughout the assessment process. It will hold two public consultations, inviting feedback on the draft protocol in the first half of 2018 and on the draft opinion in late 2019, which will also involve a face-to-face meeting with stakeholders.

Background

In 2010, EFSA published its Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre, which also included sugar. At this time, the available evidence was insufficient to set an upper limit for the daily intake of total or added sugars. New scientific evidence has come to light since then. There has also been growing public interest in the impact of the consumption of sugar-containing foods and beverages on human health.

- Mandate page in the Register of Questions [1]

Media contacts:

EFSA Media Relations Office [2]
Tel. +39 0521 036 149
E-mail: Press@efsa.europa.eu [3]

STATEMENT OF EFSA

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Protocol for the scientific opinion on the Tolerable Upper Intake Level of dietary sugars

European Food Safety Authority (EFSA)

Abstract

In June 2016, EFSA received a mandate from the national food competent authorities of five European countries (Denmark, Finland, Iceland, Norway and Sweden) to provide a dietary reference value (DRV) for sugars, with particular attention to added sugars. A draft protocol was developed with the aim of defining as much as possible beforehand the strategy that will be applied for collecting data, appraising the relevant evidence, and analysing and integrating the evidence in order to draw conclusions that will form the basis for the Scientific Opinion on sugars. As EFSA wished to seek advice from stakeholders on this draft protocol, the NDA Panel endorsed it for public consultation on 12 December 2017. The consultation was open from 9 January to 4 March 2018. A technical meeting with stakeholders was held in Brussels on 13 February 2018, during the consultation period. After consultation with stakeholders and the mandate requestors, EFSA interprets this mandate as a request to provide scientific advice on an Tolerable Upper Intake Level (UL) for (total/added/free) sugars, i.e. the maximum level of total chronic daily intake of sugars (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. The assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose) taken through the oral route. The health outcomes of interest relate to the development of metabolic diseases and dental caries. The final version of the protocol was endorsed by the EFSA Panel on Dietetic Products, Nutrition and Allergies on 28 June 2018.

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Keywords: sugars, tolerable upper intake level, adverse effects, metabolic diseases, dental caries, protocol

Requestors: National Food Agency, Sweden; Finnish Food Safety Authority, EVIRA, Finland; National Food Institute (DTU), Denmark; Norwegian Scientific Committee for Food Safety, Norway; Icelandic Food and Veterinary Authority, Iceland

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Correspondence: nda@efsa.europa.eu

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1. Introduction and scope of the protocol

This document outlines the protocol for the Scientific Opinion on the Tolerable Upper Intake Level of dietary sugars of the EFSA Panel on Nutrition, Dietetic Products and Allergies (NDA Panel), supported by the ad-hoc Working Group (WG) on sugars. This draft protocol has been developed with the aim of defining as much as possible beforehand the strategy that will be applied for collecting data (i.e. which data to use for the assessment and how to identify and select them), appraising the relevant evidence, and analysing and integrating the evidence in order to draw conclusions that will form the basis for the Scientific Opinion.

The protocol has been developed following the principles and process illustrated in the EFSA PROMETHEUS project (PROmoting METHods for Evidence Use in Scientific assessments) (EFSA, 2015a).

A draft of this protocol was open for public consultation from 9 January to 4 March 2018. The public consultation included a technical meeting with stakeholders held in Brussels on 13 February 2018. The draft protocol has been amended in view of the comments received. All comments received have been addressed and published in a technical report (EFSA, 2018).

2. Background and rationale of the mandate

In June 2016, the national food competent authorities of five European countries (Denmark, Finland, Iceland, Norway and Sweden) sent a request to the European Food Safety Authority (EFSA) in order to provide a dietary reference value (DRV) for sugars, with particular attention to added sugars, on the basis of most recent scientific evidence. After discussing the mandate at its plenary meeting on 22–23 September 2016, the NDA Panel asked for some clarifications to the requestors, particularly regarding the type of DRV to be established, the exposure of interest, the target population and the health outcomes to be considered. In February 2017, the requestors clarified that they were interested in a science-based cut-off value for a daily exposure to added sugars from all sources (i.e. sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup and other isolated sugar preparations used as such or added during food preparation and manufacturing) which is not associated with adverse health effects. The target population for the assessment was defined as the general healthy population, including children, adolescents, adults and the elderly. The requestors also clarified that the request relates to an update of the EFSA's Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre (EFSA NDA Panel, 2010a) in relation to the effects of added sugars on nutrient density, glucose tolerance and insulin sensitivity, serum lipids, other cardiovascular risk factors (blood pressure), body weight, type 2 diabetes and dental caries in adults and children.

In the EFSA's 2010 opinion, the term 'added sugars' referred to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing.

With regard to the effects of added sugar intake, the NDA Panel reached the following conclusions on the outcomes assessed:

- Micronutrient density of the diet: observed negative associations between added sugars intake and micronutrient density of the diet are mainly related to patterns of intake of the foods from which added sugars in the diet are derived rather than to the intake of added sugars per se. The available data are not sufficient to set an upper limit for (added) sugars intake.
- Glucose and insulin response: there are limited, and mainly short-term, data on the effects of high intakes of sugars on glucose and insulin response. Most studies do not find any adverse effects at intakes of predominantly added sugars up to 20–25% of total energy (E%), provided that body weight is maintained.
- Serum lipids: although there is some evidence that high intakes (> 20 E%) of sugars may increase serum triglycerides and cholesterol concentrations, the available data are not sufficient to set an upper limit for (added) sugar intake.
- Body weight: the evidence relating high intake of sugars (mainly as added sugars), compared to high intakes of starch, to weight gain is inconsistent for solid foods. However, there is some evidence that high intakes of sugars in the form of sugar-sweetened beverages (SSBs) might contribute to weight gain. The available evidence is insufficient to set an upper limit for sugars based on their effects on body weight.

- Type 2 diabetes: controversial findings on the association between total sugars and/or specific types of sugars and diabetes risk were reported in large prospective cohort studies. However positive associations were found between SSBs and increased type 2 diabetes risk. The available evidence was found insufficient to set a Tolerable Upper Level of Intake (UL) for sugars based on their effects on type 2 diabetes risk.
- Dental caries: available data do not allow the setting of a UL for (added) sugars on the basis of a risk reduction for dental caries, as caries development related to consumption of sucrose and other cariogenic carbohydrates does not depend only on the amount of sugar consumed, but it is also influenced by oral hygiene, exposure to fluoride, frequency of consumption and various other factors.

The NDA Panel concluded that the available data did not allow the setting of a UL for total or added sugars, neither an Adequate Intake (AI) nor a Reference Intake range (RI). However evidence on the relationship between patterns of consumption of sugar-containing foods and dental caries, weight gain and micronutrient intake should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines (FBDG).

3. Terms of reference as provided by the mandate requestor

The request is for scientific assistance in line with Regulation (EC) No 178/2002 in assessing a DRV for added sugars, which would benefit risk managers and substantially support their work with dietary guidelines and nutrient recommendations if they could base their advices on an up-to-date assessment by EFSA.

To this end, EFSA has been requested to update its Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre published in 2010 (EFSA NDA Panel, 2010a), on the basis of the most recent scientific evidence, in order to derive a science-based cut-off value for a daily exposure to added sugars which is not associated with adverse health effects.

The mandate requestor clarified that the intake of interest is added sugars from all sources, i.e. sucrose, fructose, glucose, starch hydrolysates such as glucose syrup, high-fructose syrup and other isolated sugar preparations used as such or added during food preparation and manufacturing. The health outcomes of interest are those already addressed in the EFSA 2010 opinion, i.e. micronutrient density of the diet, glucose tolerance and insulin sensitivity, serum lipids, other cardiovascular risk factors (blood pressure), body weight, type 2 diabetes and dental caries in adults and children.

To address this mandate, EFSA is requested to consider published reports from national and international bodies/authorities addressing the health effects of added sugars, as well as systematic reviews and meta-analysis published since 2010 on this topic.

4. Background information

To address this mandate EFSA is requested to consider, as background information and sources of data, published reports from national and international authorities/bodies addressing the health effects of sugars, as well as systematic reviews and meta-analysis published since 2010 on this topic.

An overview of the most recent existing DRVs and recommendations issued by other national and international authorities/bodies can be found in Appendix A. These publications will be used as sources of individual studies meeting the inclusion criteria for the present assessment through the scrutiny of their reference list.

A scoping literature search for systematic reviews and meta-analysis addressing the health effects of sugars or any of its dietary sources published in English since 2009 has also been performed. The list of the references identified and their main characteristics (e.g. exposure and endpoints of interest) can be found in Appendix B. These systematic reviews and meta-analysis will be used in two ways:

a) As sources of individual studies meeting the inclusion criteria for the present assessment through the scrutiny of their reference list;
b) As starting point for the literature searches to be carried out in the context of this assessment, whenever appropriate (see Section 9.2).

Data on the most recent national recommendations on sugars consumption in European countries will also be gathered through a questionnaire to National Competent Authorities (Appendix C).

5. Interpretation of the Terms of Reference

5.1. Definition of the exposure

Different terms and definitions have been used by researches and risk managers for dietary sugars. Among these:

i) *Sugars (total),* which are glycaemic carbohydrates composed of 1–2 monomers found in food. The main types of sugars found in mixed diets are the monosaccharides glucose, fructose and galactose, and the disaccharides sucrose, lactose, maltose and trehalose (FAO/WHO, 1998; EFSA NDA Panel, 2010a).

ii) *Added sugars*, which include sugars (mono- and disaccharides) used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table. This definition was first applied in the 2000 US Dietary Guidelines for Americans (USDA/HHS, 2000), and then by the IoM (2005), EFSA (EFSA NDA Panel, 2010a) and some European countries (Nordic Council of Ministers, 2014).

iii) *Free sugars*, which include monosaccharides (glucose, fructose and galactose) and disaccharides (sucrose, lactose, maltose and trehalose) added to foods by the manufacturer, cook, or consumer plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates. This term has been used by the World Health Organization (WHO, 2003, 2015).

iv) *Non-milk extrinsic (NME) sugars*, defined as sugars not located within the cellular structure of a food, such as those found in fruit juice, honey and syrups, and those added to processed foods, excluding lactose in milk. The term originated from the UK Department of Health (1989) as opposed to *intrinsic sugars*, which are those located within the cellular structure of a food (e.g. naturally found in fruits and vegetables). On its assessment of 2015, the UK Scientific Advisory Committee on Nutrition (SACN) (2015) adopted the WHO definition of *free sugars* to provide recommendations on sugar intakes.

This assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose) taken through the oral route only. Sugar alcohols (polyols), other substances used as sugar replacers and other mono- or disaccharides present in the diet in marginal amounts, are not included in the term 'sugars' for the purpose of this assessment.

To allow comparability with previous assessments done by EFSA and by other bodies, total sugars, added sugars and free sugars as defined above will be addressed in this protocol.

5.2. Objectives of the risk assessment

This mandate is a request for EFSA to update its Scientific Opinion on Dietary Reference Values for carbohydrates and dietary fibre published in 2010 (EFSA NDA Panel, 2010a) with respect to (added) sugars, and therefore interpreted as a request in the context of establishing ULs for nutrients for the general European population. The principles for the application of risk assessment to nutrients in general, and for deriving ULs in particular, have been described elsewhere (SCF/EFSA NDA Panel, 2006; EFSA NDA Panel, 2010b).

EFSA interprets this mandate as a request to provide scientific advice on an UL for (total/added/free) sugars, i.e. the maximum level of total chronic daily intake of sugars (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. 'Tolerable intake' in this context connotes what is physiologically tolerable and is a scientific judgement as determined by assessment of risk, i.e. the probability of an adverse effect occurring at some specified level of exposure.

ULs may be derived for various life stage groups in the population. The UL is not a recommended level of intake. If there are no, or insufficient, data on which to base the establishment of a UL, as it was the case in 2010 for total or added sugars (EFSA NDA Panel, 2010a), an indication should be given on the highest level of chronic daily intake (from all sources) where there is reasonable confidence in data on the absence of adverse effects (i.e. a science-based cut-off value for a daily exposure which is not associated with adverse health effects). If there are no, or insufficient, data on which to base the establishment of a UL or a cut-off value for (total/added/free) sugars from all sources because the evidence available relates to one or few sources only, or to a particular type of sugar (e.g. fructose, glucose, sucrose), and the extrapolation of the results to (total/added/free) sugars from all sources is found to be unjustified, scientific advice could be provided on quantitative

intakes in relation to one or few sugar sources only, and/or in relation to one type of sugar only (e.g. fructose, glucose, sucrose) (Figure 1).

The characterisation of the risk (SCF/EFSA NDA Panel, 2006) includes a description of the scientific uncertainties associated with the UL estimates in order to indicate the degree of scientific confidence that can be placed in these estimates. It will also include an estimate of intake for population groups as well as an indication of the margin between actual intakes and the UL, and an indication of circumstances, if any, in which risk is likely to arise. If a UL for (total/added/free) sugars cannot be established, other values could be used instead to characterise the risk (Figure 1).

It is out of the scope of this assessment to address possible beneficial health effects of sugars or of particular dietary sources of sugars.

The outcome of the assessment is expected to assist Member States and health professionals in establishing nutrient goals for populations and recommendations for individuals, and when developing FBDG.

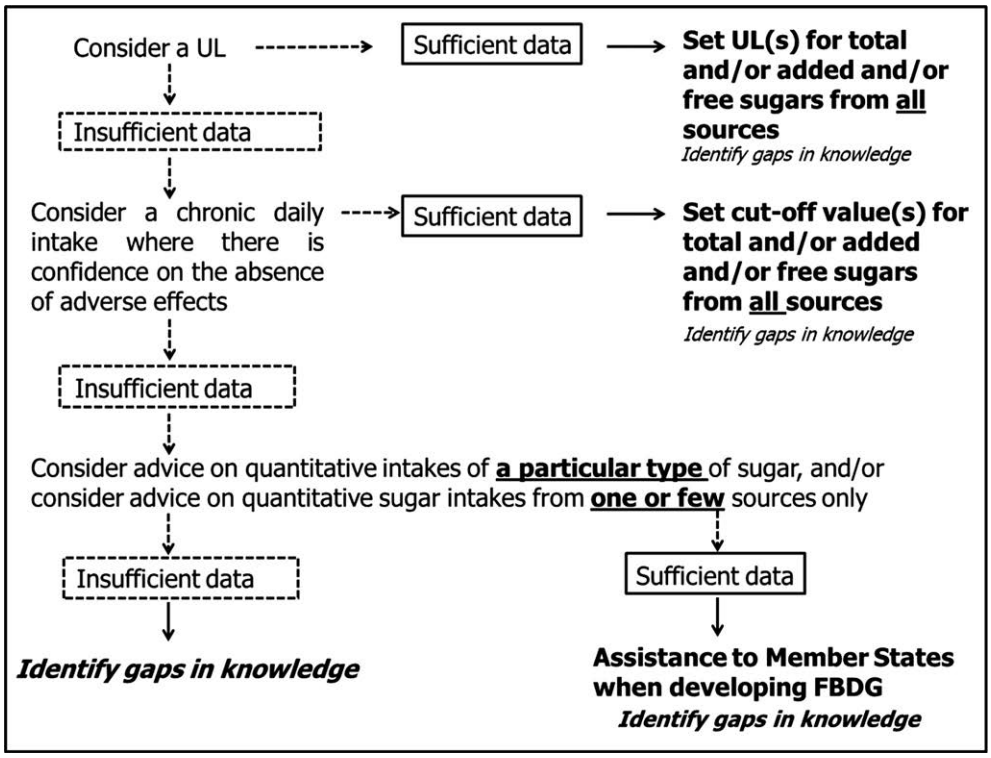


Figure 1: Step-wise process to provide scientific advice on total/added/free sugars

5.3. Target population

Adverse effects of nutrients are influenced by physiological changes and common conditions associated with growth and maturation that occur during an individual's lifespan. Therefore, where necessary, and to the extent possible, ULs are derived for each separate life-stage group, e.g. infants, children, adults, the elderly and women during pregnancy or lactation. Even within relatively homogenous life-stage groups, there is a range of sensitivities to adverse effects, e.g. sensitivity is influenced by body weight and lean body mass.

The derivation of ULs for the general population, divided into various life-stage groups, accounts for normally expected variability in sensitivity due to e.g. ethnicity, dietary habits, nutritional status, physical activity level (PAL) and metabolic risk profile. This includes individuals within the general population at risk of chronic disease (e.g. with excess body weight, elevated blood pressure, blood glucose and/or blood lipids but not on pharmacological treatment for the condition). It excludes, however, subpopulations with distinct vulnerabilities due to genetic predisposition or other considerations (e.g. individuals with inborn errors of carbohydrate metabolism) because including these would result in ULs which are significantly lower than are needed to protect most people against adverse effects of high intakes. Subpopulations needing special protection (e.g. individuals on

pharmacological treatment for obesity, diabetes, hypertension, dyslipidaemia, chronic liver or renal diseases) are better served through the use of public health screening, health care providers, or other individualised strategies.

The following groups will be considered *a priori*:

- Infants ≥ 4 to < 12 months
- Toddlers (young children) ≥ 1 to < 3 years
- Other children ≥ 3 to < 10 years
- Adolescents ≥ 10 to < 18 years
- Adults ≥ 18 to < 65 years
- Elderly adults ≥ 65 years
- Pregnant women

The age ranges may be modified by the NDA Panel depending on the available data, e.g. children may be further categorised according to the type of dentition (primary – milk or secondary – permanent) in relation to dental caries.

For this assessment, it is anticipated that lactating women will be considered together with other women in child-bearing age.

Infants < 4 months of age will be excluded from the assessment on the assumption that they are exclusively fed with breast milk or breast milk substitutes (EFSA NDA Panel, 2009).

5.4. Adverse effects and endpoints

The assessment will focus on possible adverse effects of sugars intake on endpoints selected based on the scope of the mandate, on previous assessments done by other bodies (Appendix A), on the systematic reviews available (Appendix B) and on the comments received through the public consultation (EFSA, 2018).

Both disease endpoints and other endpoints will be addressed. Disease endpoints are considered to be the most direct, or applicable, to the assessment. Other endpoints are relevant but less direct, and can include upstream indicators, risk factors, intermediate endpoints or measures related to disease endpoints.

The disease endpoints of interest are:

a) Incidence/severity of dental caries
b) Incidence of metabolic diseases:
   i) Obesity,
   ii) Type 2 diabetes mellitus (T2DM) and gestational diabetes mellitus (GDM),
   iii) Hypertension,
   iv) Dyslipidaemia,
   v) Cardiovascular diseases (CVDs),
   vi) Non-alcoholic fatty liver disease and non-alcoholic steatohepatitis (NAFLD and NASH),
   vii) Gout.

Other endpoints of interest are:

a) Measures of body fatness and body fat distribution; birth weight-related endpoints,
b) Ectopic fat deposition (e.g. muscle, liver),
c) Measures of glucose homoeostasis,
d) Blood pressure,
e) Blood lipids,
f) Uric acid,

The outcome variables that will be investigated in relation to the above-mentioned endpoints are depicted in Section 9.1.

6. Identification of the assessment subquestions

The identification of the assessment subquestions follows the principles of risk assessment for nutrients (SCF/EFSA NDA Panel, 2006). The assessment subquestions are illustrated in Table 1.

For **hazard identification**, human studies provide the most relevant data and, when they are of sufficient quality and extent, are given the greatest weight. Owing to the wealth of human studies

available on sugars intake and that extrapolation from animal models to humans is subject to considerable uncertainties, the Panel considers that evidence for adverse effects of sugars on humans should be obtained from human studies (**subquestions 4 and 5**).

Decisions on whether structural or functional alterations observed in human studies represent adverse effects or changes of little or self-limiting biological importance will be based on scientific judgement and will be taken on a case-by-case basis. In this context, it will be important to determine:

a) the causal significance of an exposure–effect association indicated by observational studies, primarily for disease endpoints. Criteria for judging this include demonstration of a temporal relationship, consistency, strength of association, a dose–response relationship (a biological gradient), specificity, biological plausibility and coherence; and
b) the clinical significance of an intake–effect relationship indicated by intervention studies primarily on other (than disease) endpoints. For energy-containing macronutrients, such as sugars, it will be important to determine how such relationships relate to energy-wise comparable intakes of other energy-containing macronutrients which are reasonably expected to replace the macronutrient under assessment in the diet. For sugars, the Panel considers that other glycaemic carbohydrates (e.g. starch) are the most appropriate comparison (EFSA NDA Panel, 2010a). Isocaloric comparisons with other macronutrients, however, may provide additional information which could be considered in the assessment.

Relevant experimental data (animal models, *in vitro* data) and knowledge of the molecular or cellular events underlying the adverse effect (mode of action) can assist in dealing with problems of data interpretation (**subquestion 6**).

Hazard characterisation includes a dose–response assessment which addresses the relationship between (total/added/free) sugars intake (dose) and adverse effect (in terms of intake and severity). Only human studies will be considered for that purpose (**subquestions 4 and 5**). The selection of the most appropriate or critical data set(s) for deriving the UL will be made on the basis of data quality, taking into account the related uncertainties and the possibility to derive quantitative estimates. Data on bioavailability (digestion, absorption and metabolism) will be considered to explain any apparent differences in dose response between different types, forms and sources of sugars (**subquestion 3**). Critical data set(s) should document the route of exposure and magnitude and duration of intake, and the intake that does not produce adverse effects as well as the intake which produces adverse effects. If more than one data set is found to be suitable and the UL that can be derived from each of them differs (e.g. for different disease endpoints), scientific advice will be provided for each of them separately. If there are no, or insufficient, data on which to base the establishment of a UL, an indication will be given on the highest level of intake of (total/added/free) sugars where there is reasonable confidence in data on the absence of adverse effects (Figure 1).

Risk characterisation includes a description of the scientific uncertainties associated with the UL estimates (or alternative estimates as the case may be; Figure 1) to indicate the degree of scientific confidence that can be placed in these estimates. Where data are available, it also includes an estimate of intake (from occurrence data and food consumption data; **subquestions 1 and 2**) for population groups as well as an indication of the margin between actual intakes and the UL, and an indication of circumstances, if any, in which risk is likely to arise.

Table 1: Assessment subquestions to be answered

Number	Subquestion
1	What are the levels of (total/added/free) sugars in foods and beverages in Europe?
2	What is the distribution of intakes of (total/added/free) sugars from all dietary sources (and by food source) by population group?
3	What are the digestion, absorption and metabolism of different types of sugars from different sources in humans?
4	What is the relationship between the intake of (total/added/free) sugars and metabolic diseases (disease endpoints and other endpoints) in the target population?
5	What is the relationship between the intake of (total/added/free sugars) and dental caries in the target population?
6	Which could be the potential mode(s) of action underlying the adverse effects (if any) of (total/added/free) sugars intake?

7. Methods to answer subquestions 1 and 2

7.1. Levels of sugars in foods and beverages in Europe

7.1.1. Development of a food composition database for total sugars

Since only total sugars are subject to mandatory labelling in Europe and there are no analytical methods to distinguish between added/free sugars and other sugars present in foods, available data on total sugars will be used as starting point to estimate the levels of added and free sugars in foods and beverages. To that end, a food composition database for total sugars will be developed first.

Data on total sugars will be extracted from the EFSA's food composition database, which was compiled as a deliverable of the procurement project 'Updated food composition database for nutrient intake' (Roe et al., 2013). The aim of the project was to provide EFSA with an updated food composition database covering approximately 1,750 food entries in the EFSA FoodEx2 classification system[1] with additional FoodEx2 facet descriptors, and to expand the data set to include harmonised information on the most common composite recipes of European countries and harmonised information on food supplements. In case no country-specific data were available for certain food codes, data compilers borrowed compatible data from other countries and/or from similar foods.

The EFSA's food composition database contains raw data for energy, macro- and micronutrients from national food composition databases up to 2012 provided by fourteen national food database compiler organisations. Within the EFSA's food composition database, 12 countries provided data on total sugars covering about 1290 FoodEx2 codes. The number of values for total sugars available for a given FoodEx2 code is variable.

For the purpose of this Scientific Opinion, a single European food composition database for total sugars will be developed from the information available in the national food composition databases. To that end, an outlier analysis will be performed to identify any value which deviates from the others for a given food code, which could be either wrongly attributed values (data cleaning) or values describing real variability in total sugars content. For food codes for which no outliers can be identified, the mean will be taken as a unique value.

The outlier assessment will prioritise foods with a high content of total sugars and foods largely consumed by one or more population subgroups. The expected variability in the total sugars content will decrease as the FoodEx2 level increases. Such variability will inform decisions regarding the level of FoodEx2 at which values for total sugars need to be attributed, together with data on the frequency of consumption gathered from the EFSA food consumption database. In this context, the Mintel Global New Products Database (GNPD),[2] an online database which monitors product introductions in consumer markets of packaged goods worldwide, will be used to check if the variability detected in the EFSA's food composition database regarding the total sugars content of manufactured foods reflects real variability of products in the market, and also to decide whether more than one scenario needs to be considered for risk characterisation regarding one or more food groups, and at which FoodEx2 level (i.e. whether two extreme values (lowest and highest), rather than a mean value, will need to be assigned to a food code to evaluate the impact of this variability on total sugars intake) (Figure 2).

A similar process has been undertaken to build the EFSA's food composition database for a number of vitamins and minerals in order to support EFSA's scientific opinions on DRVs for nutrients.

7.1.2. Development of food composition databases for added and free sugars

A European food composition database for added sugars in foods and beverages will be developed taking into account the 10-step methodology described by Louie et al. (2015) for added sugars and adapted by FSANZ[3] to determine the amount of added sugars in foods in the AUSNUT 2010–2013 food nutrient database. Although the definition of added sugars (a component of free sugars) in the

[1] https://www.efsa.europa.eu/en/data/data-standardisation

[2] The Mintel GNPD contains information on over two million food and beverage products, of which more than 800,000 are or have been available on the European food market. Mintel started covering European Union's food markets in 1996. Twenty out of the 28 EU member countries and Norway are present in the Mintel GNPD. The database provides the compulsory ingredient information presented in the labelling of products and the nutritional facts when available on the labels, which provide information about the use of sugars as ingredients and about the total sugar content of foods. http://www.mintel.com/global-new-products-database

[3] http://www.foodstandards.gov.au/science/monitoringnutrients/ausnut/foodnutrient/Pages/Determining-the-amount-of-added-sugars-and-free-sugars-in-foods-listed-in-the-AUSNUT-201113-dataset.aspx

Australian code includes maltodextrin and similar products, a decision was made by FSANZ not to capture these ingredients in the data set of added sugars (and therefore neither in the data set of free sugars) to maintain consistency with the definition of sugars used in nutrition labelling and with international food composition database practice where total sugars have been defined as being only mono- and di-saccharides. The same approach will be followed by EFSA for the same reasons.

The food composition database for added sugars will be developed for all FoodEx2 codes for which a consumption has been reported in the EFSA Comprehensive Food Consumption Database (see Section 7.2.1) in combination with the relevant FoodEx2 facet descriptors included in the EFSA FoodEx2 classification system (e.g. sugar free facet), using as starting point the food composition database for total sugars (Section 7.1.1). The step-wise methodology described by Kibblewhite et al. (2017) will be adapted to develop the food composition database for free sugars by applying the definition given in Section 5.1 of this protocol as strictly as possible. Databases for added and free sugars will be developed in close consultation with the mandate requestor (Figure 2). All the steps of the process will be thoroughly documented.

7.2. Estimates of intake of total, added and free sugars from all dietary sources

Estimates of intake of total, added and free sugars from all dietary sources will be obtained using data from the EFSA Comprehensive Food Consumption Database in combination with the food composition databases for total, added and free sugars (Sections 7.1.1 and 7.1.2).

7.2.1. The EFSA Comprehensive Food Consumption Database

Food consumption data from the EFSA Comprehensive Food Consumption Database (hereinafter referred as Comprehensive Database) will be used in order to assess the intake of free sugars. The Comprehensive Database provides a compilation of existing national information on food consumption at individual level. It was first established in 2010 (EFSA, 2011; Huybrechts et al., 2011; Merten et al., 2011). The latest version of the Comprehensive Database, updated on 26 April 2018, contains results from 61 different dietary surveys carried out in 25 different Member States.

Within the dietary surveys, subjects are classified in different age groups as follows:

1) Infants: 1–11 months old
2) Toddlers: ≥ 1 year to < 3 years old
3) Other children: ≥ 3 years to < 10 years old
4) Adolescents: ≥ 10 years to < 18 years old
5) Adults: ≥ 18 years to < 65 years old
6) Elderly: ≥ 65 years to < 75 years old
7) Very elderly: ≥ 75 years old

Data from infants 4 to 11 months old only will be considered in this assessment.

Overall, the Comprehensive Database is the most complete and detailed collection of food consumption data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from three to 7 days per subject. Surveys with only one observation day per subject, or which used food frequency questionnaires (FFQ) for data collection, were excluded. Owing to the differences in the methods used for data collection, direct country-to-country comparisons can be misleading. Detailed information on the different dietary surveys included in the Comprehensive Database is shown on the EFSA website,[4] including the number of subjects and days available for each age group. If new food consumption surveys become available during the assessment and up to December 2018, the most recent survey for a given country and age group will be used.

The linking between the foods consumed and the food composition databases for total, added and free sugars (Sections 7.1.1 and 7.1.2) will be done through the FoodEx2 system (EFSA, 2015b).

7.2.2. Sugars intake calculation

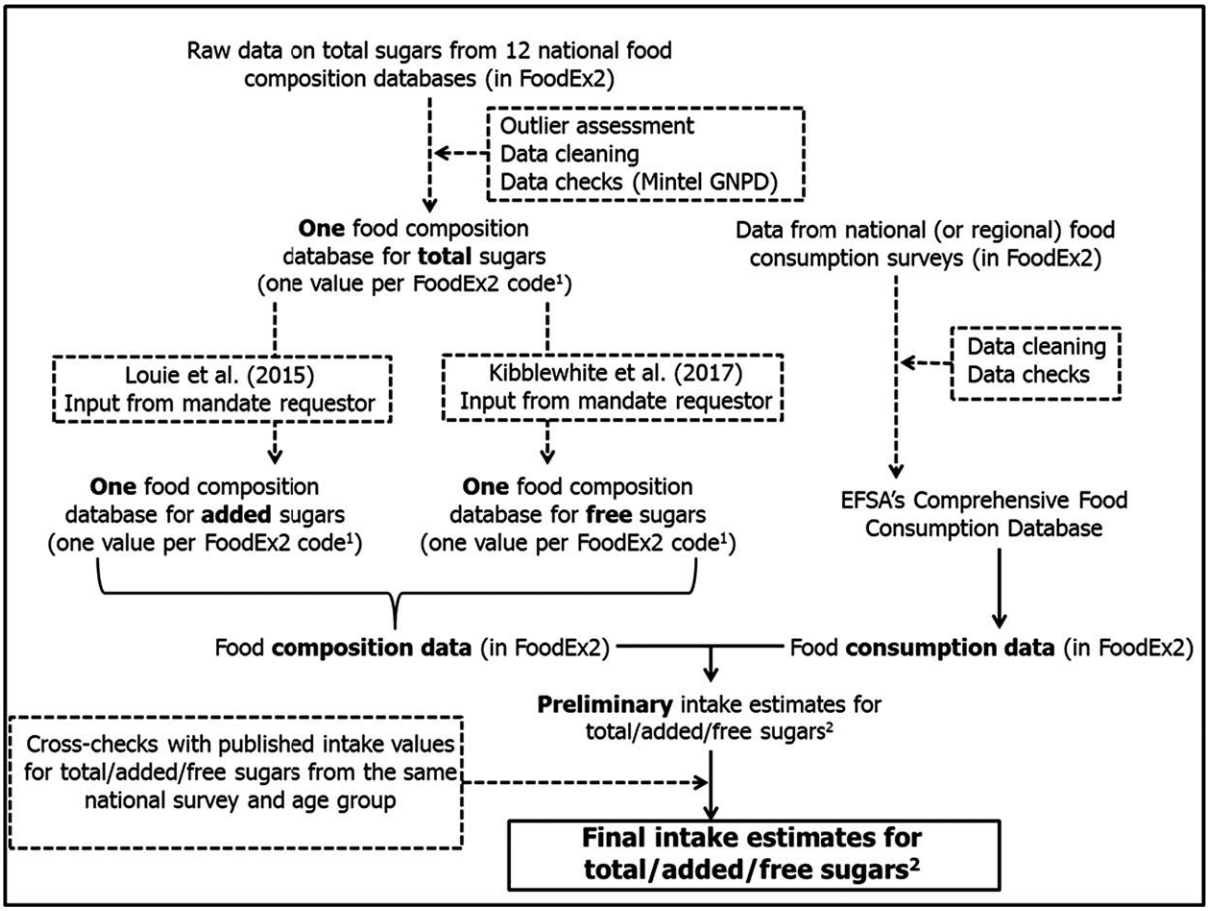
The intake of total/added/free sugars (in grams per day) will be calculated at individual level by multiplying the average daily consumption for each food or food group with the corresponding

[4] http://www.efsa.europa.eu/en/food-consumption/comprehensive-database

concentration of total/added/free sugars, summing up the respective intakes throughout the diet. The intake of total/added/free sugars will also be expressed as percentage of total energy intake (%E) and as percentage of non-alcohol energy intake (non-alcohol E%). In line with the Guidance of EFSA for the Use of the EFSA Comprehensive European Food Consumption Database in Exposure Assessment (EFSA, 2011), chronic total/added/free sugars intake calculations will be performed only for subjects with at least two reporting days. In this context, chronic total/added/free sugars intake refers to the arithmetic mean of all reporting days available for the same subject. The intake will be modelled using the SAS software (SAS Enterprise Guide 5.1, 2013). The mean as well as the 5th, 50th and 95th percentiles of intake will be derived for each survey and age group (and sex group, if appropriate), respectively. Data on the contribution of different food groups to (total/added/free) sugars intake by survey and age group will also be presented.

Different intake scenarios could be considered in the intake calculation process, especially if more than one value for total/added/free sugars is assigned to one or more FoodEx2 codes in the food composition database (Figure 2).

To evaluate the accuracy of the results obtained, these will be compared with published intake values for total/added/free sugars from the same survey data set and age group, whenever available. These data will be retrieved through a questionnaire to the National Competent Authorities of European countries (Appendix C).



Legend to Figure 2: 1) Two values for a given FoodEx2 code may be assigned when both the observed variability in sugars content and the frequency of consumption are high, so that different intake scenarios could be considered; 2) In grams per day and as E% per country and population group.

Figure 2: Steps that will be followed to estimate intakes of total, added and free sugars

8. Method to answer subquestion 3

In order to address the digestion, absorption and metabolism of different types of free sugars from different food matrices in humans, background information will be gathered by the WG experts and EFSA staff through a narrative review. Recent textbooks, authoritative reviews and research papers retrieved through searches in bibliographic databases, and selected on the basis of their relevance, will be used as sources of information.

9. Methods to answer subquestions 4 and 5

Subquestions 4 and 5 will be answered by performing systematic reviews and, possibly, dose–response meta-analyses if the available data allow doing so. The conceptual framework for the systematic reviews on sugars intake in relation to disease and other endpoints is shown in Figure 3.

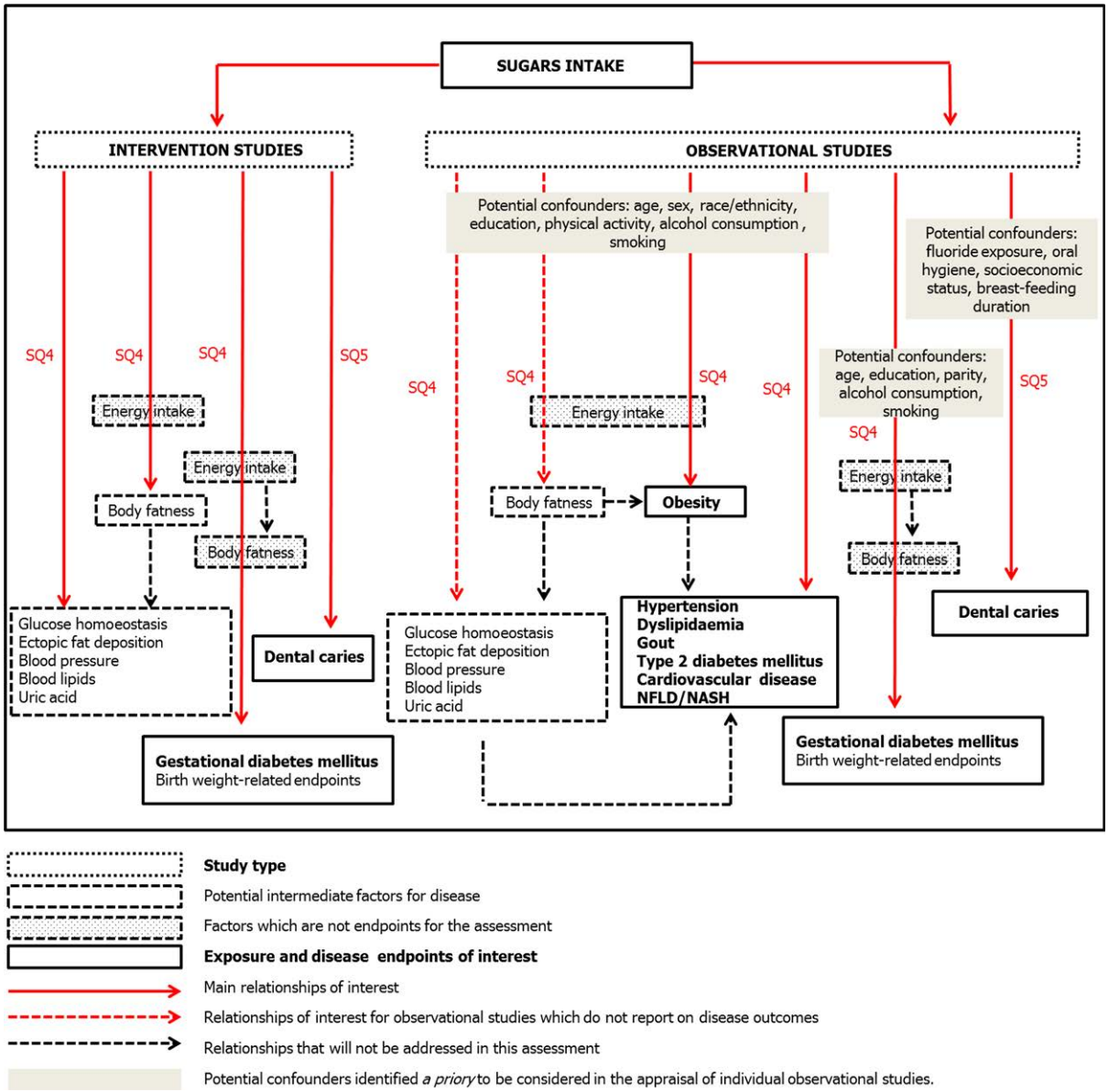


Figure 3: Conceptual framework for the systematic reviews on sugars intake in relation to disease endpoints and other endpoints

9.1. Eligibility criteria for study selection

The selection of human studies relevant to subquestions 4 and 5 will be performed using the eligibility criteria described in Table 2.

For subquestion 4, the minimum study duration for the inclusion of intervention studies has been selected by considering the time generally required for the stabilisation of the endpoints assessed following a nutritional intervention. The minimum study duration for the inclusion of observational studies for subquestion 4, and for the inclusion of intervention and observational studies for subquestion 5, is based on the minimum time estimated to be needed for the disease to develop in individuals free of the disease at baseline (expert judgement).

Regarding the study location, no limits are applied. It is acknowledged, however, that the background diet may affect the relationship between the intake of sugars and the endpoints being addressed, and that major differences in the background diet may limit the extrapolation of the results obtained outside Europe to the European population. This aspect will be considered when synthesising the evidence (Section 9.6).

Table 2: Eligibility criteria for human studies to address subquestions 4 and 5

INTERVENTION STUDIES		
Study design	In	Randomised controlled trials Non-randomised, comparative studies of interventions[a]
	Out	Single-arm intervention studies with no control group
Study duration	In	Depending on the endpoints addressed, as follows: Measures of body fatness ≥ 6 weeks Ectopic fat deposition ≥ 2 weeks Measures of glucose homoeostasis: ≥ 1 week to ≥ 12 weeks[b] Blood pressure, blood lipids and uric acid ≥ 4 weeks Dental caries: ≥ 1 year for primary dentition and ≥ 18 months for permanent dentition Pregnancy endpoints: any duration
	Out	Studies of shorter duration
Study location	In	Any location
Population	In	Adults (≥ 18 years) and children (4 months to < 18 years) from the general population, including overweight or obese subjects, subjects at risk of disease (e.g. with impaired glucose tolerance, impaired fasting glucose, NAFLD), and subjects with one or more features of the metabolic syndrome which are not on pharmacological treatment during the intervention
	Out	Studies targeting individuals with a disease (except for obesity), either untreated or under pharmacological/surgical treatment for the disease, or individuals on a therapeutic diet, including weight-loss diets Studies in individuals under physical training programs (e.g. athletes, military), except for dental caries
Intervention/control	In	Studies aiming at: – isocaloric exchanges of sugars with other glycaemic carbohydrates (e.g. starch) – isocaloric exchanges of sugars with macronutrients other than glycaemic carbohydrates (e.g. protein, fat) – isocaloric exchanges of different types of sugars (e.g. fructose vs glucose) – replacing sugar-containing foods with sugar-reduced or sugar-free foods (either under controlled or free-living conditions) – a quantitative change (increase, decrease) in sugars intake from one or more sources vs usual diet or no advice (either under controlled or free-living conditions)
	Out	Studies aiming at: – a quantitative change in sugars intake in the context of energy-restricted diets aiming at weight loss – studies not providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. studies reporting only on the frequency of consumption of one or more dietary sources of sugars with unknown sugars content)

		– interventions targeting simultaneous changes in dietary/lifestyle factors other than the amount of sugars intake from one or more sources relative to the control group (e.g. advice on teeth brushing or physical exercise provided to the intervention or control group only)
Endpoints of interest	In	*Body fatness and ectopic fat deposition* – Measured body weight, BMI, waist circumference, sagittal diameter – Body fat measured by neutron activation analysis (NAA), imaging techniques (DXA, MRI, CT), hydrostatic weighing, or air displacement plethysmography. – VAT assessed by imaging techniques (CT, MRI) – Ectopic fat deposition in muscle or the liver assessed by CT, MRI, magnetic resonance spectroscopy (MRS) or in biopsies *Glucose homoeostasis* – Dynamic indices of insulin sensitivity and/or beta-cell function calculated from measures of plasma glucose, serum insulin and C-peptide (when available) during clamp tests (hyperinsulinaemic-euglycaemic, hyperglycaemic), frequently sampled intravenous glucose tolerance tests (FSIGT), standard oral glucose tolerance test (OGTT), the continuous infusion of glucose with model assessment (CIGMA), or insulin suppression tests – Fasting plasma glucose, fasting serum insulin and static indices of insulin sensitivity and/or beta-cell function thereof (e.g. HOMA, QUICKI) – Indices of blood glucose control (HbA1c, fructosamine) *Blood pressure* – SBP and DBP (point ambulatory or home BP, 24-h BP) *Blood lipids* – total-c, LDL-c, HDL-c, VLDL-c, TG, apoB100, apoA1 and ratios thereof *Liver* – NAFLD/NASH activity scores as defined by the authors *Uric acid* – Uric acid concentrations in blood *Dental caries* – Indices of dental caries measured by a trained observer *Pregnancy endpoints* – Birth-weight related endpoints (e.g. birth weight, small for gestational age, large for gestational age) as defined by the authors – Incidence of GDM as defined by the authors
	Out	– Self-reported body weight, BMI, waist circumference, sagittal diameter – Body composition assessed by BIA or skinfold thickness – Dental caries self-reported or reported by parents; other endpoints (e.g. amount of dental plaque; plaque pH) – Studies not including at least one of the endpoints listed above
Language	In	Full-text document in English
	Out	Articles with the full text in another language
Publication year	In	Up to March 2018
Publication type	In	Primary research studies (i.e. studies generating new data) reported in full-text articles Primary research studies reported in letters to editors if the information provided is sufficient to allow a scientific evaluation of the results Systematic reviews and meta-analyses[(c)]

	Out	Narrative reviews, expert opinions, editorials and letters to editors not reporting on primary data Meetings' abstracts and posters Conference proceedings PhD theses Grey literature
OBSERVATIONAL STUDIES		
Study design	In	Prospective, longitudinal, observational (prospective cohort and nested case-control) studies
	Out	Retrospective case–control studies Cross-sectional studies Ecological studies Case studies/case series
Study duration	In	≥ 18 months for dental caries for permanent dentition ≥ 1 year follow-up for all other disease endpoints, including dental caries for primary dentition Pregnancy endpoints: any duration
	Out	Studies with a shorter follow-up
Study location	In	Any location
Population	In	Adults (≥ 18 years) and children (4 months to < 18 years) from the general population Studies targeting individuals at risk of disease (e.g. with impaired glucose tolerance, impaired fasting glucose, the metabolic syndrome, overweight, NAFLD) not on pharmacological treatment for the condition Studies in which prevalent cases of the disease endpoint of interest at baseline were excluded for data analysis
	Out	Studies targeting individuals with a disease (except for obesity), either untreated or under dietary or pharmacological/surgical treatment for the disease Studies in which prevalent cases of the disease endpoint of interest at baseline were not excluded for data analysis
Exposure	In	– Studies comparing different levels of sugars intake from one or more sources – Studies providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. in absolute amounts, as %E, as non-alcohol %E) *Eating conditions*: *ad libitum* *Method to quantify sugars intake (one or more of the following)*: – 24-h urinary excretion of fructose and sucrose – Food records (at least 2 reporting days) – Diet recalls (at least 2 reporting days) – Dietary history – FFQs – Dietary interview in combination with one or more of the above
	Out	– Studies not providing sufficient information to allow quantitative estimates of sugars intake, whether total or from one or more dietary sources (e.g. studies reporting only on the frequency of consumption of one or more dietary sources of sugars with unknown sugar content) *Eating conditions*: under dietary controlled conditions prior to the dietary intake assessment *Method to assess intake of sugars*: – Any other method
Endpoints of interest	In	*Disease endpoints*: – Incidence of obesity as defined by the authors – Incidence of T2DM as defined by the authors – Incidence of hypertension as defined by the authors

		– Incidence of dyslipidaemia as defined by the authors – Incidence of stroke [haemorrhagic (intracerebral, subarachnoid) and/or ischaemic; fatal and/or non-fatal] – Incidence of coronary heart disease (fatal and/or non-fatal) – Incidence of myocardial infarction (fatal and/or non-fatal) – Incidence of congestive heart failure – Incidence of cardiac death – Incidence of fatal and/or non-fatal cardiovascular events (composite endpoint) – Other endpoints of fatal and/or non-fatal cardiovascular events as defined by the authors – Incidence of NAFLD or NASH as defined by the authors – Incidence of non-alcoholic liver fibrosis/cirrhosis/liver failure as defined by the authors – Incidence of gout – Indices of dental caries measured by a trained observer *Other endpoints*: – Incidence of overweight/high waist circumference as defined by the authors – Incidence of impaired fasting glucose – Incidence of hyperuricaemia – Endpoints as defined above for human intervention studies – Self-reported body weight and related endpoints – Body fat assessed by skinfold thickness or BIA *Pregnancy endpoints*: – As defined above for human intervention studies
	Out	– Self-reported waist circumference – Overall mortality – Dental caries self-reported or reported by parents; other endpoints (e.g. amount of dental plaque; plaque pH) – Studies not reporting at least one of the endpoints listed above
Language	In	Full-text document in English
	Out	Articles with the full text in another language
Publication year	In	Up to March 2018
Publication type	In	Primary research studies (i.e. studies generating new data) reported in full-text articles Primary research studies reported in letters to editors if the information provided is sufficient to allow a scientific evaluation of the results Systematic reviews and meta-analyses[(c)]
	Out	Narrative reviews, expert opinions, editorials and letters to editors not reporting on primary data Meetings' abstracts and posters Conference proceedings PhD theses Grey literature

NAFLD: non-alcoholic fatty liver diseases; DXA: dual-energy X-ray absorptiometry; MRI: magnetic resonance imaging; CT: computed tomography; VAT: visceral adipose tissue; HOMA: homeostasis model assessment; QUICKI: Quantitative insulin sensitivity check index; SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; VLDL-c: very low-density lipoprotein cholesterol; GDM: gestational diabetes mellitus; BMI: body mass index; BIA: Bioelectrical impedance analysis; FFQ: Food frequency questionnaire; NASH: non-alcoholic steatohepatitis.

(a): Prospective studies that compare the effects of two or more interventions which did not use randomisation to allocate individuals or clusters to the comparison groups.

(b): For dynamic indices of insulin sensitivity and/or beta-cell function $\geq$ 1 week; for static indices and fructosamine $\geq$ 4 weeks; for HbA1c $\geq$ 12 weeks.

(c): Systematic reviews, including meta-analyses, on this topic that will be identified during the process of literature screening will be collected for the purpose of reviewing the reference list but will not be considered to contribute to the final number of studies considered eligible unless they also contain original data.

9.2. Literature searches for studies meeting the eligibility criteria

For subquestions 4 and 5, an extensive literature search will be performed in bibliographic databases. Sources of grey literature and databases of theses/dissertations will not be searched.

The bibliographic databases listed in Table 3 will be searched in order to identify relevant studies.

Table 3: Bibliographic databases to be searched for relevant studies

Database	Platform	Types of studies
Cochrane Library. Cochrane Central Register of Controlled Trials (CENTRAL)	Wiley	Intervention studies
Cochrane Library. Cochrane Database of Systematic Reviews (CDSR)	Wiley	Systematic reviews
Cochrane Library. Database of Abstracts of Reviews of Effects	Wiley	Systematic reviews
Embase	Elsevier	Systematic reviews, intervention studies, observational studies
PubMed	NLM	Systematic reviews, intervention studies, observational studies
Scopus	Elsevier	Systematic reviews, intervention studies, observational studies

For subquestion 4, literature searches will be performed by type of endpoint. Previous systematic reviews with similar review questions, similar or broader inclusion criteria and appropriate search strategies were identified during the scoping searches (see Appendix B). Therefore, date limits will be applied to the searches for subquestion 4 (by endpoint) and subquestion 5 using these systematic reviews as starting point whenever possible (Table 4). Studies published before these dates will be retrieved by hand-searching the reference lists of the systematic reviews (Appendix B) and from existing reports by other authorities/bodies (Appendix A). No retrospective date limits will be applied for endpoints for which no existing systematic review can be taken as starting point.

Table 4: Date limits applied to the searches and systematic reviews used as sources of relevant studies

Subquestion	Endpoints	Date limit	Systematic review
4	Adipose tissue	Intervention and observational studies: December 2011	Te Morenga et al. (2013)
4	Blood pressure	Interventions: August 2013	Te Morenga et al. (2014)
		Observational studies: no date limit	
4	Blood lipids	Interventions: August 2013	Te Morenga et al. (2014)
		Observational studies: no date limit	
4	All other endpoints	Intervention and observational studies: no retrospective date limit	
5	Dental caries	Intervention and observational studies: November 2011	Moynihan and Kelly (2014)

Date limits might be changed should new systematic reviews on the topic be identified which are considered to adequately cover the relevant literature. Existing systematic reviews (Appendix B) with narrower inclusion criteria regarding either the exposure (e.g. limited to SSBs) or the study duration (e.g. (Sonestedt et al., 2012; SACN, 2015)) will be hand searched.

The search terms that will be used for the exposure and the various endpoints of interest are depicted in Appendix D. The specific search strategies for each database will be developed at a later stage. The performance of the search strings will be tested against the reference lists of the systematic reviews shown in Appendix B.

The output from the searched databases, including all indexed fields per hit (e.g. title, authors, abstract), will be exported into separate Endnote® files, allowing a count of the individual hits per database. All the studies included in the above-mentioned systematic reviews will be added to specific Endnote® libraries. Files will then be combined and duplicate records will be removed.

The files obtained will be transferred into DistillerSR® Web-Based Systematic Review Software (Evidence Partners, Ottawa, Canada) for the selection procedure (see Section 9.3).

9.3. Study selection process

The whole selection process will be performed with DistillerSR®. Studies to be included in the review will be selected using a two-step selection procedure:

1) **Screening of title and abstract** to identify potentially relevant studies that will be included for full-text screening, applying the selection criteria described in Section 9.1. If the information contained in the title or abstract is not relevant to the research objectives, the article will not be selected for full-text assessment. During the screening process, studies will be categorised into two groups corresponding to the two subquestions that are the objectives of these systematic reviews.
   This step will be conducted by WG experts and/or EFSA staff in duplicate, and by using machine learning techniques where/when appropriate. The results of the use of machine learning will be evaluated by a reviewer and documented. If the title and/or abstract do not mention the endpoints of interest for the assessment, but the words 'adverse effects' or 'side effects' are mentioned, the paper will be included to check if these effects have any relation to the endpoints of interest. If there are doubts or divergences which cannot be resolved between the two reviewers, the full article will be screened.

2) **Screening of full text** to assess if the article is relevant to the risk assessment.
   This step will be conducted by WG experts and/or EFSA staff, in duplicate, for the references retrieved. Possible divergences will be discussed by the whole WG on sugars, in case these would highlight the need for amendments to the inclusion/exclusion criteria. The content of the full text will be checked against the inclusion and exclusion criteria established in the protocol.

Possible divergences or doubt for inclusion of domain-specific articles will be discussed together with the relevant expert from the WG, also in case these would highlight the need for amendments to the inclusion/exclusion criteria.

Articles reporting solely on digestion, absorption or metabolism (i.e. without reporting on the endpoints of interest), or reporting only on endpoints other than those listed in Section 9.1, will not be included in this research subquestions but will be flagged for subquestions 3 and 6, where appropriate.

Screeners will be trained using written documentation on study eligibility. Eligibility criteria will be pilot tested on a subset of records, and refined if prone to misinterpretation. The results of the different phases of the study selection process will be reported in a flowchart as recommended in the PRISMA statement on preferred reporting items for systematic reviews and meta-analyses (Moher et al., 2009).

9.4. Data extraction from included studies

Data will be extracted from the studies using predefined forms that comprise data on the characteristics of the studies (e.g. study design), their key-elements (e.g. population, intervention/exposure, comparator, outcomes (endpoints), setting and duration), results, aspects related to the internal validity of the studies (e.g. confounders, randomisation) and funding source.

Studies for which the information provided in the publication(s) does not allow a full scientific evaluation by the WG experts (e.g. studies with missing or ambiguous information) will be excluded at this step. The reasons for exclusion will be clearly indicated.

The data will be extracted in the original units of measurement, which will be subsequently harmonised to allow data analysis. The authors may be contacted to retrieve additional data if needed.

Regarding the intervention/exposure, data on the food source(s) of sugars, on the type of sugars consumed (e.g. fructose, glucose, sucrose) and on the frequency of sugars consumption will be extracted from the included studies whenever available. For observational studies, data on disease endpoints (dichotomous variables, i.e. incidence of hypertension, incidence of type 2 diabetes) will be extracted first whenever available. Data on other endpoints (continuous variables, e.g. blood pressure, fasting glucose) will only be extracted if data on corresponding disease endpoints are not reported (Figure 3).

If a full-text document reports on more than one study, the individual studies will be identified at this step to allow for data extraction at individual study level. If a single study is reported in more than one publication, different publications reporting on the same study (e.g. on different outcomes

(endpoints), at different time points) will be identified at this step. Data will be extracted for the last observation available for each endpoint (corresponding to the longest intervention or follow-up) for data analysis. Exceptions to this rule will be duly justified (e.g. on the basis of attrition and sample size, compliance with the intervention, other factors).

Clear instructions for extracting data will be developed. The data extraction forms will be created in DistillerSR® (Evidence Partners, Ottawa, Canada) and/or Excel, and pilot tested on a subset of studies. The piloting will also be used to identify sources of contextual (i.e. related to the key elements of the studies) heterogeneity. The forms and instructions will be refined if needed.

Data will be extracted from each individual study by one EFSA staff or one WG expert. In the piloting phase, extracted data will be validated by another EFSA staff or WG expert, in order to identify sources of possible errors. The data extraction will be then conducted by one EFSA staff/WG expert. Data quality checks will be performed for each study (Section 9.6).

9.5. Appraisal of the internal validity of the included studies

The risk of bias (RoB) of a given study in relation to a specific outcome (endpoint) refers to the risk of systematic errors in the design, recruitment, data collection or analysis that results in a mistaken estimation of the true effect of the exposure on the outcome.

The internal validity or RoB of each individual study included in the assessment will be appraised using a customised version of the OHAT/NTP RoB tool, which is suitable for both intervention and observational studies.[5] This tool was developed based on guidance from the Agency for Healthcare Research and Quality (Viswanathan et al., 2012), the Cochrane risk-of-bias tool for non-randomised studies of interventions (Sterne et al., 2014), the Cochrane Handbook (Higgins and Green, 2011), CLARITY Group at McMaster University (CLARITY, 2013) and other sources. The OHAT/NTP RoB tool was developed to provide a parallel approach to the evaluation of the RoB in the context of hazard identification for human risk assessment of chemicals, and to facilitate consideration of RoB across evidence streams (i.e. human, animal and mechanistic studies) with common terms and categories for RoB rating. For this assessment, the use of the tool will be limited to the aspects relevant to intervention and prospective observational studies in humans.

For each study, the appraisal will be done at outcome level, because for the same study the design and conduct may affect the RoB differently depending on the endpoints measured. Each study will be appraised by two independent experts from the WG ('the reviewers'). Possible discrepancies will be discussed by the whole WG. If upon further discussion the WG cannot reach an agreement on a RoB rating for a particular domain, the more conservative judgment (the highest RoB) will be selected.

The OHAT/NTP RoB tool outlines 10 RoB questions, grouped in 6 bias domains (selection, confounding, performance, attrition/exclusion, detection and selective reporting) - plus 'other sources of bias' -, which help identify the practices that may introduce bias (Table 5). Each RoB question addresses aspects relevant to specific study designs, i.e. 8 questions apply to intervention studies and 7 questions apply to prospective observational (cohort and nested case–control) studies (Table 5). Reviewers are required to answer RoB questions by applying a 4-level rating scale (Figure 4).

The RoB questions and rating instructions provided in the tool will be tailored to the specific subquestions illustrated in this protocol.

Table 5: Extracted from OHAT/NTP RoB tool (source: OHAT Handbook – 9 January 2015)[(a)]

Bias Domains and Questions	Controlled intervention*	Observational
Selection Bias		
1. Was administered dose or exposure level adequately randomized?	X	
2. Was allocation to study groups adequately concealed?	X	
3. Did selection of study participants result in appropriate comparison groups?		X
Confounding Bias		
4. Did the study design or analysis account for important confounding and modifying variables?		X

[5] https://ntp.niehs.nih.gov/ntp/ohat/pubs/riskofbiastool_508.pdf

Bias Domains and Questions	Controlled intervention*	Observational
Performance Bias		
5. Were the research personnel and human subjects blinded to the study group during the study?	X	
Attrition/Exclusion Bias		
6. Were outcome data complete without attrition or exclusion from analysis?	X	X
Detection Bias		
7. Can we be confident in the exposure characterization?	X	X
8. Can we be confident in the outcome assessment?	X	X
Selective Reporting Bias		
9. Were all measured endpoints reported?	X	X
Other Sources of Bias		
10. Were there no other potential threats to internal validity (e.g., statistical methods were appropriate and researchers adhered to the study protocol)?	X	X

*: Includes studies in humans with a controlled exposure including randomised controlled trials and non-randomised intervention studies.
(a): https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf

++	**Definitely Low** risk of bias	There is direct evidence of low risk-of-bias practices (May include specific examples of relevant low risk-of-bias practices)
+	**Probably Low** risk of bias	There is indirect evidence of low risk-of bias practices OR it is deemed that deviations of low risk-of bias practices for these criteria during the study would not appreciably bias results, including consideration of direction and magnitude of bias
-/NR	**Probably High** risk of bias	There is indirect evidence of high risk-of-bias practices OR there is insufficient information (e.g. not reported or "NR") provided about relevant risk-of bias practices
--	**Definitely High** risk of bias	There is direct evidence of high risk-of-bias practices (May include specific examples of relevant high risk-of-bias practices)

Figure 4: Answer format for the RoB questions (source: OHAT/NTP RoB tool)[5]

The OHAT/NTP RoB tool encourages judging the direction of bias, when possible. Empirical evidence about the direction of bias is discussed for each of the RoB questions. If there is no clear rationale for judging the likely direction of bias, reviewers are invited to simply outline the evidence and not to attempt a guess. This approach will be followed.

Once customised, the tool will be created in the review management software DistillerSR® to allow web-based appraisal of the studies.

Specific elements identified *a priori* and that will be considered in the assessment of confounding and biases related to the exposure and outcome characterisation are discussed below.

9.5.1. Consideration of potential confounders

When assessing the RoB of studies concerned with causality (e.g. which investigate the effect of an exposure on disease), confounding should be addressed regardless of the study design. When present, confounding may lead to a biased estimate of the effect of exposure on disease, either closer to the null (resulting in underestimation of the exposure effect) or departing from the null, and can even reverse the apparent direction of the effect.

There are several requirements for a factor to actually act as a confounder, as described by McNamee (McNamee, 2003) and illustrated below. The factor must:

1) be a cause of the disease, or a surrogate measure of the cause, in unexposed people; factors satisfying this condition are called 'risk factors'; and

2) be correlated, positively or negatively, with exposure in the study populations. If the study population is classified into exposed and unexposed groups, this means that the factor has a different distribution (prevalence) in the two groups; and
3) not be affected by the exposure. When a factor is an intermediate factor in the relationship between the exposure and the disease, it is affected by both the exposure and the disease, or shares a common cause with the disease which is in turn affected by the exposure, such factor cannot be considered a confounder.

Whereas criteria 1 and 3 can be judged based on current (expert) knowledge of external empirical evidence (e.g. prior research), criteria 2 can only be judged from internal evidence (i.e. evidence from the study under evaluation with respect to the RoB). Therefore, whether potential confounders identified *a priori* on the basis on current knowledge can actually confound the association between exposure and disease in a particular study by making the study groups not comparable in terms of disease risk is to be addressed on a case-by-case basis, in the context of each particular study.

The OHAT/NTP RoB tool does not include a separate question for confounding in human intervention studies because randomisation and allocation concealment should adequately address the issue of confounding. It recognises, however, that in some cases appropriate procedures for randomisation and allocation concealment may fail in accounting for confounding. For example, in the context of this assessment, confounding could be a concern if there are important differences among study groups in baseline characteristics which are risk factors for the outcome (endpoint) but not affected by the exposure (e.g. age, sex, PAL). In accordance with the OHAT/NTP guidance, for intervention studies where confounding is strongly suspected despite the fact that randomisation and allocation concealment are rated at 'probably low' or 'definitely low risk of bias', confounding will be addressed under 'other potential threats to internal validity' (OHAT/NTP, 2015).

In observational studies, potential confounding can be reduced by good design and appropriate statistical analysis (control for measured confounders), although it is acknowledged that the problem of confounding cannot be resolved fully in non-randomised designs. The aim of controlling for confounders in observational studies is to allow comparability of the study groups for the risk of disease which is not affected by the exposure, so that any observed difference in risk among groups can be attributed to differences in the exposure of interest.

Being sugars an energy-containing nutrient, an increase in energy intake and/or an increase in body fatness could be considered intermediate factors in the causal pathway between high sugars intake and adverse effects related to glucose homoeostasis, ectopic fat deposition, blood pressure, blood lipids and uric acid, which in turn could mediate the effect of high sugars intake on the incidence of hypertension, dyslipidaemia, gout, T2DM mellitus, CVD and NFLD/NASH (Figure 3). An increase in energy intake and/or an increase in body fatness could also be considered intermediate factors in the causal pathway between high sugars intake and adverse pregnancy outcomes (endpoints). All these intermediate factors could potentially be affected by the exposure and therefore do not meet the criteria to be considered as confounders (McNamee, 2003), but rather as a potential source of over-adjustment bias. The extent to which an effect of sugars intake on the endpoints of interest (except obesity and dental caries) may be mediated by changes in energy intake and/or body fatness will be explored in the synthesis of the evidence (Section 9.6). In addition, there are dietary factors known to be risk factors for one or more of the above-mentioned diseases, such the intake of sodium, potassium, saturated and *trans* fatty acids, or dietary fibre. However, these may or may not be associated with both the exposure and the endpoint within a given study, and therefore, they will be not considered as potential confounders *a priori*, but rather in the context of individual studies.

Based on recent publications, the Panel identified *a priori* an indicative list of potential factors that could confound the relationship between the intake of sugars and measures of body fatness, ectopic fat deposition, glucose homoeostasis, blood lipids, blood pressure and uric acid, and the relationship between the intake of sugars and incidence of overweight/obesity, hypertension, dyslipidaemia, gout, T2DM, cardiovascular disease and liver disease: age, sex, race/ethnicity, education (or education of the parents for studies in children), smoking habits, physical activity, alcohol consumption.

The Panel also identified *a priori* an indicative list of potential factors that could confound the relationship between the intake of sugars and dental caries: fluoride exposure (e.g. water fluoride, use of fluoride toothpaste, supplements), oral hygiene practices, socioeconomic status and breast feeding duration for studies on young children. Frequency of sugars consumption has been highly correlated to the amount of sugars consumed in observational studies and it is likely to be affected by the exposure, so that it will not be considered as a potential confounder in observational studies in relation to dental caries.

As for pregnancy outcomes, the Panel identified *a priori* the following factors that could potentially confound the relationship between the intake of sugars and birthweight-related endpoints or the incidence of GDM: parity, maternal age, education, alcohol consumption and smoking.

When assessing RoB in observational studies, the reviewers will consider, for each study, whether these factors can confound the association on a case-by-case basis. Additional confounders may be identified by the reviewers. The reviewers will consider whether the confounding variables were measured reliably and consistently within each study and whether the design and/or the data analysis adequately accounted for potential confounding (e.g. multivariable analysis, stratification).

9.5.2. Confidence in the exposure characterisation

The exposure of interest for the assessment is the daily intake of total/added/free sugars from all dietary sources. It is acknowledged however, that few of the available individual studies investigating the health effects of dietary sugars may have used the definition of added or free sugars as described in this protocol (Section 5.1) to characterise the intervention/exposure. In this context, the confidence in the exposure characterisation that will be assessed in relation to the RoB of individual studies refers to the confidence on the quantitative estimates for the sugar fraction that is being investigated in the study, and not the extent to which the exposure investigated on each study reflects the intake of total/added/free sugars from all dietary sources as defined in this protocol. The latter aspect will be discussed when integrating and weighing the evidence in light of the identified uncertainties to provide scientific advice on total/added/free sugars (see Figure 1 and Section 12).

In assessing RoB, reviewers will consider the risk of errors in the estimate of sugar intake for individuals and related risks of misclassification of individuals according to their exposure. The accuracy of sugar intake estimates may be affected by (i) the method (or combination of methods) used to assess the sugar fraction of interest for the study (e.g. 24-h urinary excretion of fructose and sucrose vs dietary records vs diet recalls vs FFQs; specificity of FFQs for the exposure of interest; validation; number of days recorded); (ii) the accuracy of 24-h urine collections and the accuracy of reporting dietary intakes (e.g. self vs dietitian assisted compilation of FFQs); (iii) systematic changes in habitual diet prior to the intake assessment. The reviewers will consider the resulting risk of misclassification in appraising the studies.

9.5.3. Confidence in the outcome assessment

Confidence in the outcome (endpoint) requires valid, reliable and sensitive methods to assess the outcome applied consistently across groups (OHAT/NTP, 2015). Outcome misclassification or measurement error may be unrelated to the exposure (non-differential) or related to the exposure (differential).

Factors that will be considered by the reviewers while assessing bias in relation to the outcome assessment include: (1) the objectivity of the outcome assessment, (2) the consistency in measurement of endpoints, and (3) the blinding of the outcome assessors (for knowledge of the exposure) (OHAT/NTP, 2015).

9.5.4. Summarising the internal validity of each individual study

Each study will be reported using a tabular summary form which will include the key elements of the study and a summary of the results of the critical appraisal.

When all the studies have been summarised in this way, the WG will consider whether and how to combine the scores from the RoB questions at the level of individual studies. The WG may consider using an algorithm to combine the scores in a weighted or unweighted manner: if so, the rationale for the chosen algorithm will be documented. Alternatively, the RoB scores may be kept separate for each RoB question and taken into account in the synthesis of evidence. The results of the RoB assessment will be taken into account in the weight of evidence assessment and uncertainty analysis (Sections 11 and 12).

9.6. Synthesis of the evidence

Data from included individual studies will be considered separately for each of study design and for each endpoint to derive single lines of evidence (Figure 3).

Information on inclusion criteria, RoB assessment and endpoints as extracted from the individual studies will be summarised in evidence tables. Data quality checks will be performed for each study. For each variable, the proportion of missing observations will be assessed; range checks will be carried out for all included variables to ensure that all values are reasonable; categorical variables will be

tabulated and key variables will be cross-tabulated to check for internal consistency. For intervention studies, results from intention-to-treat analyses will be preferred over per-protocol analyses if both are reported. In the case of missing data, flexible and transparent strategies will be pursued, such as requesting missing data from the authors, re-doing the analysis or placing the original results in adequate context according to the feasibility and adequacy of these approaches on a per-study basis. Effect estimates such as relative risks and odds ratios for dichotomous variables for disease endpoints, and differences in means for continuous variables for other endpoints along with measures of their statistical precision (usually 95% confidence intervals) will be extracted from the studies and reported in the assessment.

Whenever data allow for a meaningful quantitative synthesis of the evidence, effect estimates from intervention studies (for all endpoints; by study design) and observational studies (for disease endpoints) will be pooled separately and assessed through meta-analysis and dose–response meta-analysis, using fixed- and random-effects models as appropriate (Orsini et al., 2012; Crippa and Orsini, 2016; Discacciati et al., 2017). Dose–response meta-analysis is a statistical technique that aims to characterise the smooth and gradual change in non-linear responses along the range of a quantitative exposure using aggregated data from several studies. Within intervention studies, effect estimates will be pooled separately by study design (e.g. isocaloric exchange of sugars with other macronutrients, changes in sugars intake under free-living conditions). For observational studies, the suitability of unadjusted and adjusted models will be considered case-by-case for data analysis depending on the level of control for potential confounders and the risk of over-adjustment bias.

In addition to the meta-analyses stratified by study design, subgroup/stratified analyses will be performed according to age, gender, changes in body fatness (except for obesity and dental caries), type of dentition (for dental caries), type of sugar/food source/frequency of consumption, country or continent, and other factors suspected or known to modify the association between sugars intake and the endpoints assessed. Subgroup and sensitivity analyses will also be performed according to the RoB of the included studies, the degree of control for confounding, the methodology and quality of the exposure assessment, duration of follow-up for both the intervention and the observational studies, and possibly by the source of study funding. If needed, sensitivity analyses will be performed to evaluate the robustness of the findings and the possible influence of different biases on the summary pooled effect estimates (Arah et al., 2008; Rothman et al., 2012; Corbin et al., 2017). Influence analysis will be carried out by examining whether removal of single studies influences the results of the meta-analyses, and the reasons underlying such an influence on the effect estimates, if any (Rothman et al., 2012).

Statistical heterogeneity across study-specific findings will be taken into account in the statistical model and evaluated by visual inspection of forest plots and the I2 statistic (Higgins and Thompson, 2002; Higgins et al., 2003), and an attempt will be made to identify its sources.

The possibility of publication bias will be investigated using one or more of the following approaches: (a) visual inspection of funnel plots to investigate the association between study size and effect size (Altman, 1986); (b) Egger's regression test (Egger et al., 1997; Sterne and Egger, 2005); and (c) trim-and-fill analysis (Duval and Tweedie, 2004; Rothstein et al., 2005) following the approach of Peters Jaime et al. (2007).

If there are studies that are relevant but cannot be included in meta-analyses (e.g. due to differences in study design), their contribution to the assessment will be integrated with the results of the meta-analysis by a weight of evidence approach (Section 11). If none of the relevant studies for an effect are suitable for meta-analysis, evidence synthesis for that effect will be performed by a weight of evidence approach (Section 11).

9.7. Plans for updating the literature searches and dealing with newly available evidence

The literature searches performed as detailed above (Section 9.2) will be repeated approximately 3 months before the planned date of endorsement of the draft opinion by the Panel. Databases and keywords will be those of the original searches. Date limits will be defined based on the cut-off date of the preceding searches. The papers retrieved by these additional searches will be screened for relevance applying the same criteria.

Relevant studies will be reviewed by the WG experts and their contribution to the assessment will be integrated (with the results of a meta-analysis or otherwise) by a weight of evidence approach (Section 11), but will not be considered for inclusion in any meta-analysis.

10. Methods to answer subquestion 6

In order to address the mode(s) of action for possible adverse health effects of (total/added/free) sugars identified in subquestions 4 and 5, background information will be gathered by the WG experts and EFSA staff through a narrative review. Recent textbooks, authoritative reviews and research papers retrieved through searches in bibliographic databases, and selected on the basis of their relevance, will be used as sources of information. Articles reporting solely on digestion, absorption or metabolism that are identified/retrieved during the screening of the full text in the context of the systematic reviews described in Section 9 will also be considered, where appropriate. The mode of action by which sugars can contribute to the development of dental caries (subquestion 5), however, is considered to be well known.

11. Methods for integrating and weighing the evidence to set a UL for sugars

Integration of evidence will be performed at a number of levels and by different methods, according to what is appropriate given the available evidence (Figure 5). For subquestions 4 and 5, the following integration steps may be needed:

- Where appropriate, different studies (by study design) for the same endpoint will be combined by meta-analysis.
- Results from meta-analysis will be integrated with evidence from other relevant studies on the same endpoint (if there are any that could not be included in the meta-analysis) by a weight of evidence approach.
- For endpoints where meta-analysis is not feasible, relevant studies will be integrated by a weight of evidence approach.
- Where appropriate, results for different endpoints of the same type (e.g. disease and other endpoints relating to the same chronic disease) may be integrated by a weight of evidence approach.

The outcome of subquestions 4 and 5 will be integrated with the endpoints of subquestions 3 and 6 by a weight of evidence approach. The results of this will form the Panel's conclusions on the levels of intake for total/added/free sugars (UL or otherwise; Figure 1): this may result in more than one level of intake, depending on whether there is material variation between sugar fraction, effects and/or population groups.

The results of subquestion 1 (levels of total/added/free sugars in foods and beverages) will be integrated by calculation with food consumption data to address subquestion 2, resulting in intake assessments for the European population.

Risk characterisation will be performed by comparing results of the intake assessment (from subquestions 1 and 2) with the levels of intake (UL or otherwise; Figure 1) for total/added/free sugars (from subquestions 3, 4, 5 and 6) (Figure 5).

In several of the steps described above, integration will be performed by a weight of evidence approach using expert judgement. The methods will vary, depending on the evidence to be integrated and the specific considerations involved. In each case, the principles of EFSA's guidance on weight of evidence will be applied (EFSA Scientific Committee, 2017). Evidence will be organised into lines of evidence, where helpful. Relevance, reliability (including the RoB evaluations described in earlier sections) and consistency will be taken into account when weighing the evidence. Formal (EFSA, 2014) or semi-formal (EFSA Scientific Committee, 2018) methods for expert knowledge elicitation (EKE) will be used where appropriate. Detailed protocols will be established for each stage of the weight of evidence process before it is performed. Each stage of the process will be documented, including the reasons for any deviations from the protocol.

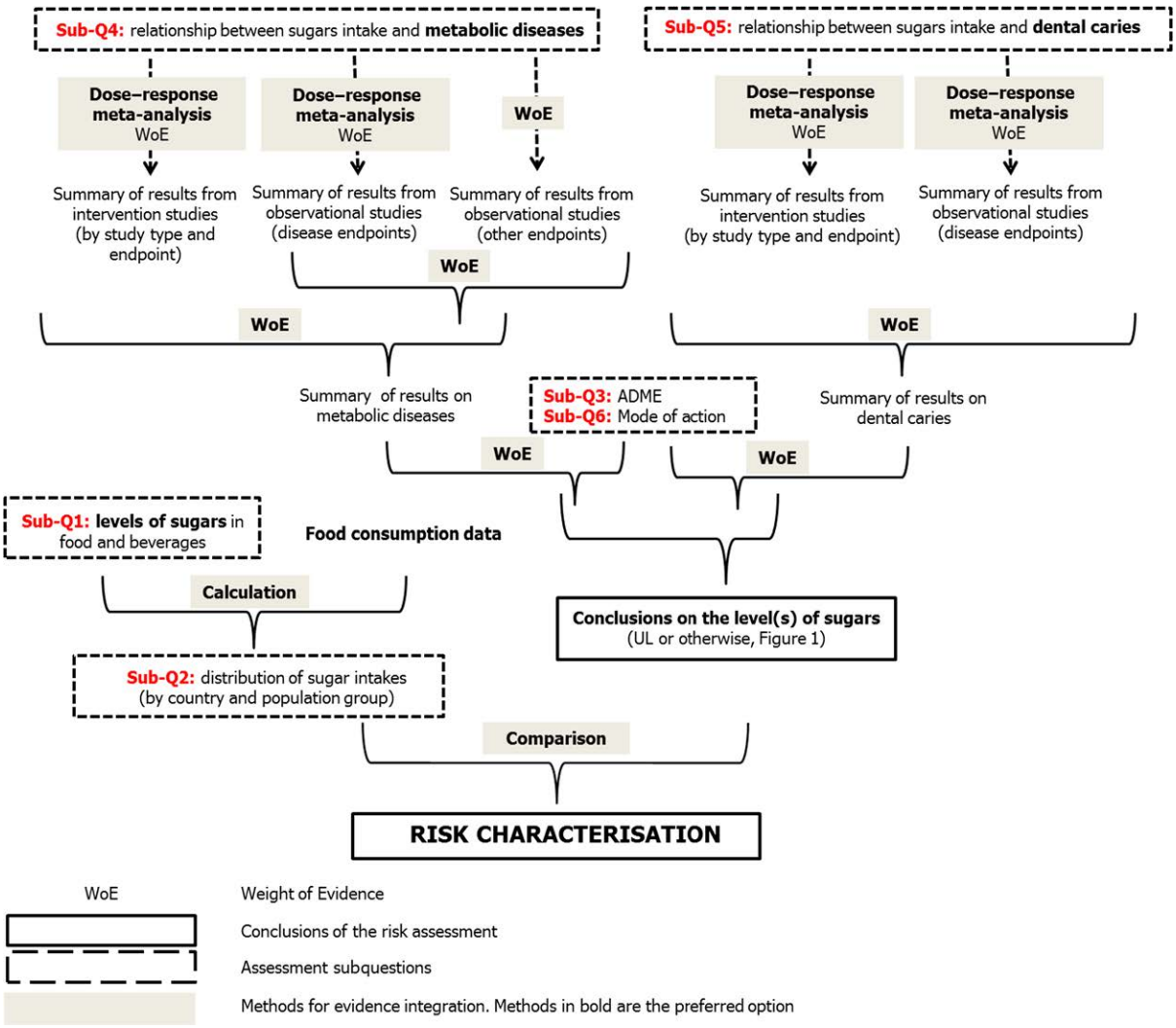


Figure 5: Conceptual framework for evidence integration

12. Evaluating the uncertainty in the body of evidence

Uncertainties in the estimates of total/added/free sugar intake in European countries may arise from inaccuracies in mapping food consumption data according to the FoodEx2 classification, from analytical errors or from errors in estimating the levels of total sugars in the national food composition tables, from errors in attributing levels of added/free sugars to foods from their content of total sugars, and from replacing missing values by values of similar food groups in the added/free sugars intake estimation process. These uncertainties may, in principle, result in both too high and too low estimates of total/added/free sugars intake.

For disease and other endpoints, once the individual studies are appraised for internal validity and after synthesising the evidence for each endpoint, line of evidence (i.e. intervention studies separately from observational studies; intervention studies by design) and subquestion, the uncertainties in the body of evidence will be identified, including factors such as the consistency of results, the precision of effect/association estimates and/or dose–response models, the internal and external validity (directness, generalisability, applicability) of the included studies, and gaps in knowledge.

Uncertainty analysis will be performed following approaches recommended by EFSA (EFSA Scientific Committee et al., 2018) for case-specific assessments. Uncertainty affecting each subquestion will be identified, and taken into account when evaluating the overall uncertainty for the main endpoints of the assessment: UL for the intake of total/added/free sugars (or otherwise; Figure 1) and risk characterisation. The overall uncertainty will be evaluated by expert judgement using either formal or semi-formal EKE methods (EFSA, 2014; EFSA Scientific Committee, 2018). For the UL of total/added/

free sugars (or otherwise; Figure 1), the weight of evidence (Section 11) and uncertainty analysis may be addressed together in a single EKE procedure. Detailed protocols cannot be specified in advance, but will be established for each stage of the uncertainty analysis before it is performed. Each stage of the process will be documented, including the reasons for any deviations from the protocol.

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Abbreviations

AHA	American Heart Association
AI	adequate intake
ANSES	French Agency for Food, Environmental and Occupational Health & Safety
AUSNUT	Australian food and nutrient database
BIA	bioelectrical impedance analysis
BMI	body mass index
BP	blood pressure
CHD	coronary heart disease
CIGMA	Continuous infusion of glucose with model assessment
CT	computed tomography
CVD	cardiovascular disease
DRV	dietary reference value
DBP	diastolic blood pressure
DXA	dual-energy X-ray absorptiometry
EKE	expert knowledge elicitation

ESPGHAN	European Society for Paediatric Gastroenterology, Hepatology and Nutrition
FBDG	food-based dietary guidelines
FFQ	food frequency questionnaire
FoodEx2	Standardised food classification and description system developed by EFSA
FSANZ	Food Standards Australia New Zealand
FSIGT	Frequently sampled intravenous glucose tolerance tests
GNPD	Global New Products Database
GNS	German Nutrition Society
HDL-c	high-density lipoprotein cholesterol
HFCS	high-fructose corn syrups
HOMA	homeostasis model assessment
IoM	Institute of Medicine
LDL-c	low-density lipoprotein cholesterol
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver diseases
NAA	neutron activation analysis
NASH	non-alcoholic steatohepatitis
NDA	EFSA Panel on Nutrition, Dietetic Products and Allergies
NME	non-milk extrinsic
NTP	National toxicology program
OGTT	oral glucose tolerance test
OHAT	Office of health assessment and translation
PAL	physical activity level
PC	prospective cohort studies
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMETHEUS	PROmoting METHods for Evidence Use in Scientific assessments
QUICKI	Quantitative insulin sensitivity check index
RCT	randomised controlled trial
RI	Reference Intake range
RoB	risk of bias
SBP	systolic blood pressure
SACN	UK Scientific Advisory Committee on Nutrition
SSB	sugar-sweetened beverage
SSD	sugar-sweetened soft drinks
T2DM	type 2 diabetes mellitus
TG	triglycerides
Total-c	total cholesterol
UL	Tolerable upper level of intake
VAT	visceral adipose tissue
VLDL-c	very low-density lipoprotein cholesterol
WG	Working Group
WHO	World Health Organization

Appendix A – Overview of dietary reference values and recommendations

In 2010, in the context of setting dietary reference values for carbohydrates and dietary fibre, the European Food safety Authority (EFSA NDA Panel, 2010a) concluded that the available data **did not allow the setting of a UL for total or added sugars**, neither an Adequate Intake (AI) nor a Reference Intake range (RI). However, evidence on the relationship between patterns of consumption of sugar-containing foods and **dental caries**, **weight gain** and **micronutrient intake** should be considered when establishing nutrient goals for populations and recommendations for individuals and when developing food-based dietary guidelines (FBDG).

The evidence-based Guideline of the German Nutrition Society (GNS) on carbohydrate intake and prevention of nutrition-related diseases (Hauner et al., 2012) recommended reducing the consumption of SSBs, but did **not provide a quantitative limit for sugar intake** or any components of this. The basis for this recommendation was probable evidence that high consumption of SSBs increases the risk of obesity and type 2 diabetes in adults, and the high consumption of SSBs particularly among adolescents and young adults in Germany.

The Nordic Nutrition Recommendations (Nordic Council of Ministers, 2014) limited the intake of **added sugars** (sucrose, fructose and starch hydrolysates) to **< 10% of the total energy intake** for the general population to ensure adequate intakes of micronutrients and dietary fibre (**micronutrient density of the diet**), which was found particularly important for children and persons with a low energy intake. It was also recommended to limit the consumption of SSBs because associated with an increased **risk of type 2 diabetes** and **excess weight gain**, and to avoid frequent consumption of sugar-containing foods to reduce the **risk of dental caries**.

The UK Scientific Advisory Committee on Nutrition (SACN, 2015) recommended that the average population intake of **free sugars** (all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and unsweetened fruit juices) should not exceed **5% of total energy intake** for age groups from 2 years upwards. Evidence from intervention studies showing that increasing sugars intake **increases energy intake** in individuals consuming an *ad libitum* diet and that SSBs are linked to **weight gain** in children and adolescents, and evidence from prospective cohort studies showing that the consumption of sugars is associated with increased **risk of dental caries** and intake of SSBs are associated with an **increased risk of type 2 diabetes** mellitus were at the basis of this recommendation.

The French Agency for Food, Environmental and Occupational Health & Safety (ANSES) issued an opinion on the establishment of recommendations on sugar intake (ANSES, 2016). The opinion focused on the metabolic effects of sugars in food, whether naturally present or added, and their involvement in the development of chronic diseases (metabolic diseases, cancer and cardiovascular diseases). ANSES set an upper intake limit for **total sugars** of **100 g/day** for the adult healthy population, which excludes lactose and galactose naturally present in milk and dairy products. The upper intake limit was calculated from the minimum daily consumption of fructose (50 g) for which a significant increase in **blood concentrations of triglycerides** was observed in intervention studies, and considering that an intake of 50 g of fructose corresponds to an intake of 100 g of sucrose.

The Institute of Medicine of the US National Academy of Sciences (IoM, 2005) concluded that there was **insufficient evidence to set a UL for added sugars** (sugars and syrups that are added to foods during processing or preparation). However, a maximal intake level of **≤ 25% of total energy intake** was suggested to prevent the displacement of foods that are major sources of essential micronutrients (**micronutrient density of the diet**).

The 2015–2020 Dietary Guidelines for Americans (HHS/USDA, 2015) recommended that individuals aged 2 years and older should derive **< 10% of total energy intake** from **added sugars** in order to achieve healthy eating patterns within calorie limits (**micronutrient density of the diet**). This recommendation was based on food pattern modelling and national data on intakes of calories from added sugars.

The World Health Organization (WHO) appraised the evidence available on the effects of **free sugars** on the risk of non-communicable diseases in adults and children, with a particular focus on **weight gain** and **dental caries** (WHO, 2015). The WHO recommended reducing the intake of free sugars to **< 10% of total energy intake** in both adults and children, with a **conditional** recommendation to reduce it further to **< 5% of total energy intake**.

Finally, two professional associations have issued recommendations on sugar intake for children only (up to 18 years of age).

The American Heart Association (AHA) reviewed the scientific evidence on the cardiovascular health effects of **added sugars** in **children** (Vos et al., 2016). Strong evidence was found to support an association between the intake of added sugars and **increased cardiovascular disease risk** in children through **increased energy intake**, **increased adiposity** and **dyslipidaemia**. AHA provided recommendations that **added sugars** (all sugars used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table) should be consumed up to a **maximum amount** of **25 g per day** by **children > 2 years** of age and **avoided** by **children < 2 years** of age.

In 2017, the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee on Nutrition (Fidler et al., 2017), reviewed the scientific evidence on the relationship between sugars intake and: (a) the development of sweet taste or flavour preference, and (b) health outcomes. The Committee concluded that a preference for sweet taste is driven by interplay of many factors and that the association between high consumption of SSBs in early and late childhood could not be demonstrated to be causal. Building on the conclusions reached by WHO (2015), SACN (2015) and the AHA (Vos et al., 2016) regarding the effect of sugars intake and different health outcomes in paediatric populations, the Committee recommended that intakes of **free sugars** should be reduced and minimised with a desirable **upper limit of < 5% energy intake in children and adolescents** aged 2–18 years. This represents 15–28 g of free sugars for girls and 16–37 g for boys. Intakes should be **even lower in infants and toddlers < 2 years**.

A tabulated overview of these recommendations is given in Table A.1.

Table A.1: Summary of existing recommendations on sugar intake

Guideline	Target population	Sugar fraction	Recommendation	Basis (endpoint)	Other endpoints assessed	Review method
EFSA NDA Panel (2010a)	General population	Added sugars	Consider when setting FBDGs	Dental caries Body weight Micronutrient density	Glucose homoeostasis, risk of T2DM, blood lipids, blood pressure, CVD risk	Narrative
German Nutrition Society (2012)	General population	SSBs	Limit consumption	Obesity Risk of T2DM	BP/hypertension, metabolic syndrome, CHD risk, cancer	Systematic
Nordic Council of Ministers (2014)	General population	Added sugars	< 10E%	Micronutrient density	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), glucose homoeostasis, blood lipids, blood pressure, CVD risk, uric acid	Systematic
Health Council of the Netherlands (2015)	General population	SSBs	Limit consumption	Obesity Risk of T2DM	–	Systematic
SACN (2015)	General population (>2 years)	Free sugars	≤ 5E%	Energy intake	Dental caries (frequency of intake), weight gain and risk of T2DM (SSBs), blood lipids, blood pressure, CHD, glucose homoeostasis	Systematic
ANSES (2016)	Adults	Total sugars	100 g/day	Fasting triglycerides	Weight gain, glucose homoeostasis, blood lipids, intrahepatic lipids and risk of NAFLD, uric acid, blood pressure	Systematic
IoM (2005)	General population	Added sugars	< 25E%	Micronutrient density	CHD risk, energy intake, body weight, blood lipids, cancer	Narrative
HHS/USDA (2015)	General population	Added sugars	< 10E%	Micronutrient density	–	Food pattern modelling and national data on added sugars intake
WHO (2015)	General population	Free sugars	< 10E% < 5E% conditional	Body weight Dental caries	–	Systematic

Guideline	Target population	Sugar fraction	Recommendation	Basis (endpoint)	Other endpoints assessed	Review method
American Heart Association (2016)	Children	Added sugars	25 g/day ≥ 2 years Avoided < 2 years	Energy intake Adiposity Dyslipidaemia CVD risk	Micronutrient density, blood pressure, risk of NAFLD, glucose homoeostasis, risk of T2DM	Narrative
ESPGHAN (2017)	Children	Free sugars	≤ 5E% ≥ 2 years (lower for < 2 years)	Dental caries Weight gain (SSBs) CVD and T2DM (fructose)	Preference for sweet taste	Narrative/systematic

FBDG: food-based dietary guidelines; T2DM: type 2 diabetes mellitus; CVD: cardiovascular disease; SSB: sugar-sweetened beverage; CHD: coronary heart disease; NAFLD: non-alcoholic fatty liver diseases.

Appendix B – Systematic reviews and meta-analysis on the relationship between added/free sugars and their sources and surrogate/disease endpoints

A scoping literature search was performed to identify systematic reviews and meta-analysis published in English since 2009 addressing the health effects of added sugars/non-milk extrinsic sugars/free sugars or any of its dietary sources.

The full list of references identified is reported in Table B.1 together with the specific exposure and outcome(s) of interest. All reviews for which the exposure of interest was added, free or total sugars from all dietary sources are presented in Table B.2. Based on the inclusion criteria identified for subquestions 5 and 6 (see Section 9.1), reviews having the same or wider inclusion criteria were used as a basis to update or build new literature searches (see Section 9.2).

Table B.1: Overview of systematic reviews and meta-analysis published since 2009 on the relationship between sugars and their sources and endpoints of interest

Reference	Endpoint (population subgroup)	Exposure
Anderson et al. (2009)	Caries (adults and children)	Sucrose including sucrose-based carbonated soft drinks, baked goods, sweets and table sugar, as added to other foods and drinks
Auerbach et al. (2017)	Obesity (children)	Fruit juice
Avery et al. (2015)	Obesity (children)	SSBs
Bucher Della Torre et al. (2016)	Obesity (children)	SSBs
Chiavaroli et al. (2015)	Blood lipids (adults and children)	Fructose
Chiu et al. (2014)	NAFLD (adults)	Fructose
Chung et al. (2014)	Liver health (adults)	Fructose
Crowe-White et al. (2016)	Obesity (children)	Fruit juice
Fattore et al. (2017)	Blood lipids, blood pressure (adults)	Free sugars (fructose, sucrose and glucose)
Gibson (2008)	Obesity (adults and children)	Sugar-sweetened soft drinks (SSD)
Gibson et al. (2013)	Blood lipids, blood pressure, glucose metabolism (adults)	Sucrose
Greenwood et al. (2014)	Risk of T2DM	SSD
Huang et al. (2014)	Risk of CVD	SSBs
Imamura et al. (2015)	Risk of T2DM	SSB, fruit juice
Jayalath et al. (2014)	Blood pressure	Fructose-containing sugar (high-fructose corn syrup, sucrose and fructose)
Jayalath et al. (2015)	Blood pressure	SSBs containing free or bound fructose
Kaiser et al. (2013)	Obesity (adults and children)	SSBs
Kelishadi et al. (2014)	Blood lipids, blood pressure, glucose metabolism	Fructose

Reference	Endpoint (population subgroup)	Exposure
Keller and Bucher Della Torre (2015)	Blood lipids, Blood pressure, glucose metabolism, risk of CVD	SSBs
Kim and Je (2016)	Blood pressure	SSBs and artificially sweetened beverages
Ma et al. (2016)	Obesity (adults and children)	Fructose, glucose, SSB, HFCS
Malik et al. (2010)	Risk of T2DM	SSBs
Malik et al. (2013)	Obesity (adults and children)	SSBs
Malik et al. (2014)	Blood pressure	SSBs
Mattes et al. (2011)	Obesity (adults and children)	SSBs
Moynihan and Kelly (2014)	Dental caries (adults and children)	Total sugars, free sugars, added sugars, sucrose, NME sugars
Perez-Morales et al. (2013)	Obesity (children)	SSBs
SACN (2015)	Blood lipids, blood pressure, dental caries, glucose metabolism, obesity, risk of CVD, risk of T2DM	Total carbohydrate, sugars reported as a nutrient (fructose, sucrose, lactose, glucose), table sugar and other extrinsic sugars (syrups), food format (solid vs. liquid, which includes SSBs)
Singh et al. (2011)	Risk of gout (adults)	SSBs, fructose
Sonestedt et al. (2012)	Blood lipids, blood pressure, glucose metabolism, risk of CVD, risk of T2DM	SSBs, sugars, sucrose and fructose
Te Morenga et al. (2013)	Obesity (adults and children)	Free sugars
Te Morenga et al. (2014)	Blood lipids, blood pressure	Sugar (sucrose) or free sugars
David Wang et al. (2014)	Blood lipids	Fructose
Wang et al. (2014)	Glucose metabolism (adults)	Fruit juice
Xi et al. (2014)	Risk of T2DM	Fruit juice
Xi et al. (2015)	Blood pressure, risk of CVD	SSBs
Zheng et al. (2015)	Obesity (adults and children)	SSBs

SSB: sugar-sweetened beverage; NAFLD: non-alcoholic fatty liver diseases; T2D: type 2 diabetes mellitus; CVD: cardiovascular disease; HFCS: high-fructose corn syrup.

Table B.2: Selected systematic reviews with research question partially or completely overlapping with the present work

Systematic review	Subquestion 4					Subquestion 5
	Fattore et al. (2017)[(a)]	**SACN (2015)**	**Sonestedt et al. (2012)**	**Te Morenga et al. (2013)**	**Te Morenga et al. (2014)**	**Moynihan and Kelly (2014)**
Endpoints	BP, blood lipids and body weight	All	Glucose metabolism, BP, blood lipids, risk of T2D, risk of CVD	Obesity	BP, blood lipids	Caries
Databases	PubMed/MEDLINE, EMBASE, Cochrane Library Hand search	Medline MEDLINE In-Process & Other Non-Indexed Citations Embase CAB Abstracts ISI Web of Science BIOSIS The Cochrane Library Hand search	PubMed SveMed+	OVID Medline, Embase PubMed, CiNAHL, Scopus, Web of Science	OVID Medline, Embase PubMed, CiNAHL, Scopus, Web of Science Grey literature Hand search	MEDLINE, EMBASE, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, LILACS, CNKI, Wanfang South African Department of Health databases
Search dates	Up to 22 October 2015	1990–December 2010	January 2000–October 2010Update November 2010–December 2011	Up to December 2011	1960–August 2013	1950–November 2011
Language limit	English	English	English or a Nordic language	English	English	No limit
Study type	Intervention studies	RCT PC	RCT PC	RCT PC	RCT	Intervention, cohort, population or cross-sectional
Study duration	≥ 2 weeks	RCT > 6 weeks (≥ 3 days for energy intake/satiety) PC > 3 years	RCT ≥ 4 weeks (drop out ≤ 50%) PC ≥ 4 years	RT ≥ 2 weeks PC ≥ 1 year	RCT≥ 2 weeks	
Population	Adults	Adults/children	Adults/children	Adults/children	Adults/children	Adults/children

BP: blood pressure; T2D: type 2 diabetes; CVD: cardiovascular disease; RCT: randomised controlled trial; PC: prospective cohort studies.
(a): Not used as primary source of information as the endpoints were assessed under isocaloric conditions.

Appendix C – Questionnaire to National Competent Authorities of European countries

NAME: ☐

COUNTRY: ☐

ALIGFFILIGLIATION: ☐

E MAIL: ☐

DATE: ☐

- EFSA has been requested to provide a scientific opinion on the Tolerable Upper Intake Level of dietary sugars. The assessment concerns the main types of sugars (mono- and disaccharides) found in mixed diets (i.e. glucose, fructose, galactose, sucrose, lactose, maltose and trehalose). Sugar alcohols (polyols), other substances used as sugar replacers, and other mono- or disaccharides present in the diet in marginal amounts, are not included in the term "sugars" for the purpose of this assessment. In this context, EFSA will address:
  - **Total sugars:** i.e. the monosaccharides glucose, fructose, and galactose, and the disaccharides sucrose, lactose, maltose and trehalose present in foods;
  - **Added sugars:** sugars used as ingredients in processed and prepared foods and sugars eaten separately or added to foods at the table; and
  - **Free sugars:** added sugars plus sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates.

In the context, EFSA would like, through this questionnaire, to gather information on the following:

a) Data available from national food consumption surveys (or regional food consumption surveys e.g. targeting specific population groups such as infants or pregnant women) on the intake of **total/added/free sugars**;
b) National food composition data on **total, added and free sugars** if available, together with a description of the methods used to estimate **added** and **free sugars** in foods;
c) National dietary recommendations on the amount of sugars (whether total, added or free) to be consumed in the diet, if available.

To answer this questionnaire, please tick the relevant boxes. If you have doubts or queries in relation to the compilation of this questionnaire, please contact us at: nda@efsa.europa.eu

1) Please specify the last national food consumption survey(s) carried out in your country, indicating the year (or time frame) in which the survey was conducted, the method used for data collection (food diaries, food records, 24-h dietary recalls, other), the number of days in which data was collected for each subject, and the age group(s) included in the survey. Regional food consumption surveys should only be indicated if they targeted specially infants (up to 12 months of age) or pregnant women. Food consumption surveys using food–frequency questionnaires for data collection should not be included.

Name of survey	Type of survey (National/regional)	Year (range)	Method for data collection	Number of days/subject	Age groups included

Add as many rows as needed.

2) Please indicate whether there are publications available (in any language) on the intake of total sugars, added sugars, and/or free sugars in your country at national level (i.e. from the most recent national food consumption surveys that you have listed in question 1):

Author/year	Total sugars	Added sugars	Free sugars
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no
	☐ yes ☐ no	☐ yes ☐ no	☐ yes ☐ no

Add as many rows as needed.

Please provide the full list of references, as well as the full text if available to you ☐

Note: *Should you wish to share data not published yet but soon to be (i.e. on a confidential basis/embargo until publication), please specify the foreseen/assumed date of publication, and note that only data published by March 2019 will be accepted. Please also specify if you have anything against sharing unpublished data with the Experts of the Working Group on Sugar, for the purposes of this risk assessment.*

If NO publications are available on the intake of total sugars, added sugars, and/or free sugars in your country at national level → please go directly to question 5.

3) The national food composition database used to calculate the intake of total sugars, added sugars, and/or free sugars in the afore-mentioned publications contains data on:

Total sugars ☐ yes ☐ no

Glucose ☐ yes ☐ no

Fructose ☐ yes ☐ no

Sucrose ☐ yes ☐ no

Lactose ☐ yes ☐ no

Other sugars ☐ yes ☐ no. If yes, please specify ☐

Please provide the link to a website where the database can be downloaded from or a contact address/details of the person(s) responsible for the maintenance/update of the database ☐

4) Your national food composition database contains information on:

Added sugars ☐ yes ☐ no

Free sugars ☐ yes ☐ no

If the answer to any of the above is yes, please specify in detail the methodology that has been used to estimate the content of added sugars and/or free sugars in foods ☐

5) Does your country have some type of national recommendations on the amount of sugars to be consumed in the diet?

☐ yes ☐ no

If the answer is no → please go directly to question 9.

6) Please specify the national dietary recommendations to limit the amount of sugars consumed in the diet available in your country, indicating the type of recommendation (e.g. in the context of setting dietary reference values for nutrients, in the context of developing Food–Based Dietary Guidelines (FBDG)) and who was involved in their development (government bodies, scientific societies, industry, non-profit organisations, other), and the year in which the recommendation was issued. Please provide a link to the full text of the recommendation, if available. If more than one national recommendation is available in your country (e.g. established by different bodies; FBDGs targeting only specific age groups), use as many rows as needed.

a) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

b) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

c) National dietary recommendation: ☐ Body/organisation ☐ Year ☐

7) National recommendations on the consumption of dietary sugars (total, added and/or free) are directed to:

Please note that age ranges below are only indicative

☐ General population

☐ Old adults (>65 years)

☐ Adults (18-65 years)

☐ Adolescents (14-18 years)

☐ Others ☐

☐ Infants (up to 12 mo)

☐ Young children (1-3 years)

☐ Pre-school children (3-6 Years)

☐ Schoolchildren (6-14 years)

☐ Pregnant women

☐ Lactating women

8) Which diet-related health problems were considered when developing recommendations for the intake of total, added and/or free sugars consumption in your country? Please tick as many as needed:

☐ Cardiovascular diseases

☐ Dyslipidaemia

☐ Hypertension

☐ Diabetes

☐ Nutrient deficiencies

☐ Cognitive impairement

☐ Dental caries

☐ Overweight/obesity

☐ Cancer

☐ Adverse pregnancy outcomes

☐ Others (please specify) ☐

☐ None

9) Please add any general or specific comment, you might have: ☐

We kindly ask you to send back the filled survey by e-mail to: nda@efsa.europa.eu

Thank you very much for your participation in this survey

Appendix D – Exposure and endpoints search terms for subquestions 4 and 5

Subject index terms, where available, will be combined with free-text terms. Free-text terms will be searched in title and abstract fields in Embase and PubMed; and in title, abstract and keyword fields in the Cochrane Library databases and Scopus.

Exposure

Free sugars		
Subject index terms[6]		Free-text terms[7] (Cochrane Library, Embase, PubMed, Scopus)
MeSH (Cochrane Library, PubMed)	Emtree (Embase)	
"Monosaccharides"[Mesh:noexp]	'sugar intake'/exp	Sugar* OR Sucrose* OR Fructose* OR Galactose* OR Lactose* OR Trehalose* OR Maltose* OR Glucose* + dieta* OR diete* OR diet OR diets OR intake* OR consum* OR feed* OR food OR foods OR supplement*
"Glucose"[Mesh:noexp]	'glucose intake'/exp	
"Fructose"[Mesh]	'fructose intake'/exp	
"Galactose"[Mesh]	'lactose intake'/exp	
"Disaccharides"[Mesh:noexp]	'sugar'/exp	Disaccharide*
"Sucrose"[Mesh:noexp]	'monosaccharide'/de	Di saccharide*
"Lactose"[Mesh]	'glucose'/exp	Monosaccharide*
"Trehalose"[Mesh]	'fructose'/exp	Mono saccharide*
"Maltose"[Mesh]	'galactose'/exp	Simple carbohydrate*
"Dietary Sugars"[Mesh]	'disaccharide'/de	Refined carbohydrate*
"Dietary Sucrose"[Mesh]	'sucrose'/exp	Syrup*
"High Fructose Corn Syrup"[Mesh]	'maltose'/exp	Honey
"Honey"[Mesh]	'lactose'/exp	Candy
"Molasses"[Mesh]	'trehalose'/exp	Candies
"Carbonated Beverages"[Mesh]	'syrup'/exp	Sweet
"Energy Drinks"[Mesh]	'honey'/exp	Sweets
"Fruit and Vegetable Juices"[Mesh]	'molasses'/exp	Sweetened
"Beverages/adverse effects"[Mesh]	'sweetened beverage'/exp	Pastr*
"Candy"[Mesh]	'soft drink'/exp	Confection*
"Chocolate"[Mesh]	'energy drink'/exp	Patisserie
	'sports drink'/exp	Soft + drink* OR beverage*
	'fruit and vegetable juice'/exp	Softdrink*
	'carbonated beverage'/exp	Fizzy + drink* OR beverage*
	'confectionary'/de	Carbonated + drink* OR beverage*
	'sugar confectionary'/exp	Soda + drink* OR beverage
		Energy + drink* OR beverage*
		Sports + drink* OR beverage*
		SSBs OR SSDs + beverage OR drink
		SSB OR SSD + beverage OR drink
		Juice*
		Smoothie*

[6] [Mesh] indicates that the MeSH term will be exploded, including in the search the terms below in the MesH hierarchy if available. [Mesh:noexp] indicates that the MesH term will not be exploded, the terms below in the MeSH hierarchy will not be searched. /exp indicates that the Emtree term will be exploded, including in the search terms below in the Emtree hierarchy if available. /de indicates that the Emtree term will not be exploded, the terms below in the Emtree hierarchy will not be searched.

[7] Asterisk symbol '*' indicates truncation. Plus sign '+' indicates the search terms will be linked with the Boolean operator AND.

Endpoints

Adipose tissue		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Adipose Tissue"[Mesh:noexp] "Abdominal Fat"[Mesh:noexp] "Intra-Abdominal Fat"[Mesh] "Subcutaneous Fat, Abdominal"[Mesh] "Subcutaneous Fat"[Mesh] "Body Weights and Measures"[Mesh:noexp] "Body Fat Distribution"[Mesh] "Adiposity"[Mesh] "Body Mass Index"[Mesh] "Body Size"[Mesh:noexp] "Body Weight"[Mesh: noexp] "Body Weight Changes"[Mesh] "Weight Gain"[Mesh] "Weight Loss"[Mesh]Overweight[Mesh] Obesity[Mesh:noexp] "Obesity, Morbid"[Mesh] "Pediatric Obesity"[Mesh] "Obesity, Abdominal"[Mesh] "Sagittal Abdominal Diameter"[Mesh] "Waist Circumference"[Mesh] "Body Composition"[Mesh] "Body Constitution"[Mesh:noexp]	'adipose tissue'/de 'abdominal fat'/exp 'abdominal subcutaneous fat'/exp 'intraabdominal fat'/exp 'body fat'/exp 'body fat distribution'/exp 'fat pad'/exp 'weight, mass and size'/de 'body weight'/de 'lean body weight'/exp 'body weight change'/exp 'body weight fluctuation'/exp 'body weight gain'/de 'body weight loss'/exp 'body weight variation'/exp'obesity'/exp 'body mass'/exp 'body size'/exp 'sagittal abdominal diameter'/exp 'waist circumference'/exp 'body composition'/de 'body distribution'/exp 'body constitution'/exp	Adipos* Fat pad Fat pads Body fat* Fatty tissue* Body size Abdominal fat Intra-abdominal fat Intraabdominal fat Fat distribut* Ectopic fat Waist circumference* Abdominal diameter Obese* Obesi* Obeso* Overweight* Weight + gain OR loss OR chang* OR reduc* OR maint* OR watch* OR variation OR control* OR Body OR lean Body mass Bmi Body composition* Body constitution*

Glucose homeostasis		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Diabetes Mellitus, Type 2"[Mesh]"Hyperinsulinism"[Mesh:noExp] "Insulin Resistance"[Mesh] "Metabolic Syndrome"[Mesh] "Blood Glucose"[Mesh] "Insulin/blood"[Mesh] "Hyperglycemia"[Mesh] "Glucose Intolerance"[Mesh] "Carbohydrate Metabolism"[Mesh] "Glycated Hemoglobin A"[Mesh] "Fructosamine"[Mesh] "Metabolic Diseases"[Mesh:NoExp]	'non insulin dependent diabetes mellitus'/exp'hyperinsulinism'/exp 'hyperinsulinemia'/exp 'insulin resistance'/exp 'metabolic syndrome X'/exp 'glucose blood level'/exp 'insulin'/exp AND 'blood'/exp 'hyperglycemia'/exp 'glucose intolerance'/exp 'hemoglobin A1c'/exp 'fructosamine'/exp 'fructosamine blood level'/exp 'metabolic disorder'/de	Diabet* + type 2 OR type II OR type2 OR typeii Late OR adult* OR matur* OR slow* OR stabl* + onset + diabetesNon-insulin-depend* + diabetes Noninsulin depend* + diabetes Hyperinsulinism Hyperinsulinemia Insulin + resistan* OR sensitivity OR tolerance OR intolerance OR control OR fasting Metabolic syndrome Glucose + tolerance OR intolerance OR fasting OR blood Hyperglycemia* Glycated OR Glycosylated + Hemoglobin OR haemoglobin Hemoglobin A Haemoglobin A Hemoglobin A1c Haemoglobin A1C Hemoglobin Aic Haemoglobin AiC HbA1c HbA(1c) HbA1 HbA 1c Hb A1c Hb a 1c Fructosamine + blood OR serum OR plasma

Cardiovascular system		
Subject index terms		**Free-text terms (Cochrane Library, Embase, PubMed, Scopus)**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	
"Cardiovascular Diseases"[Mesh] "Stroke"[Mesh] "Hypertension"[Mesh] "Prehypertension"[Mesh] "Atherosclerosis"[Mesh] "Ischemic Attack, Transient"[Mesh] "Heart Diseases"[Mesh] "Myocardial Ischemia"[Mesh] "Angina Pectoris"[Mesh] "Acute Coronary Syndrome"[Mesh] "Myocardial Infarction"[Mesh] "Non-ST Elevated Myocardial Infarction"[Mesh] "ST Elevation Myocardial Infarction"[Mesh] "Coronary Disease"[Mesh] "Cardiovascular System"[Mesh] "Blood Pressure"[Mesh] "Cholesterol"[Mesh:noExp] "Cholesterol, HDL"[Mesh] "Cholesterol, LDL"[Mesh] "Cholesterol, VLDL"[Mesh] "Dyslipidemias"[Mesh:noExp] "Hyperlipidemias"[Mesh:noExp] "Hypercholesterolemia"[Mesh] "Hyperlipoproteinemias"[Mesh] "Lipids "[Mesh] "Triglycerides"[Mesh] "Lipoproteins"[Mesh:NoExp] "Apolipoproteins"[Mesh]	'cardiovascular disease'/exp 'coronary artery disease'/exp 'cerebrovascular accident'/exp 'atherosclerosis'/exp 'transient ischemic attack'/exp 'heart infarction'/exp 'non ST segment elevation myocardial infarction'/exp 'ST segment elevation myocardial infarction'/exp 'acute coronary syndrome'/exp 'abnormal blood pressure'/de 'hypertension'/exp 'prehypertension'/exp 'heart disease'/exp 'angina pectoris'/exp 'heart death'/exp 'congestive heart failure'/exp 'cardiovascular system'/exp 'blood pressure'/exp 'cholesterol'/de 'cholesterol ester'/exp 'high density lipoprotein cholesterol'/exp 'low density lipoprotein cholesterol'/exp 'very low density lipoprotein cholesterol'/exp 'cholesterol metabolism'/exp 'disorders of cholesterol metabolism'/exp 'dyslipidemia'/exp 'hyperlipidemia'/exp 'hypercholesterolemia'/exp 'hypertriglyceridemia'/exp 'lipid blood level'/exp 'cholesterol blood level'/exp 'triacylglycerol blood level'/exp 'triacylglycerol'/exp	CV disease* CVD CVDs CHD CHDs Cardiovascular OR coronary OR heart OR cardiac + disease* OR disorder* OR event* OR risk* OR complication* OR outcome* OR morbidit* OR mortalit* OR death* OR failure* Stroke* Cerebrovascular accident* Apoplex* Acute coronary syndrome Angina* Stenocardia Heart muscle OR cardiac muscle OR myocardial OR myocardium OR cardiac OR coronary OR heart OR transient OR cardiomyophath* + ischemi* OR ischaem* Myocardial infarct* Heart attack* STEMI NSTEMI Blood pressure Arterial pressure Diastolic Systolic Blood pressure Prehypertens* Hypertens* Atherosclero* LDL-C HDL-C Cholesterol Hypercholesterol* Hypertriglycer*

	'lipoprotein'/de 'apolipoprotein B100'/exp 'apolipoprotein A1'/exp	Dyslipidemi* Dyslipoproteinemi* Hyperlipidemia* Hyperlipemi* Lipidemi* Lipemi* Hyperlipoprotein* Lipid Lipids Lipoprotein* Triglycerid* triacylglycerol Fasting TG Apolipoprotein* ApoB100 ApoB Apo B Apo B100 ApoA1 ApoA ApoAi Apo A Apo A1 Apo Ai

Liver function		
Subject index terms		**Free-text terms**
MeSH (Cochrane Library, Pubmed)	**Emtree (Embase)**	**(Cochrane Library, Embase, PubMed, Scopus)**
"Fatty Liver"[Mesh:noexp] "Non-alcoholic Fatty Liver Disease"[Mesh] "Liver Cirrhosis"[Mesh:NoExp] "Liver Failure"[Mesh]	'liver fat'/exp 'fatty liver'/de 'nonalcoholic fatty liver'/exp 'liver cirrhosis'/exp 'liver fibrosis'/exp 'liver failure'/exp	Fatty liver NAFLD Steatohepatiti* Steatohepatiti* NASH Steatos* Fat liver accumul* Cirrhos* OR Fibros* OR failure* OR insufficienc* + liver OR Hepatic

Dental caries		
Subject index terms		Free-text terms
MeSH (Cochrane Library, Pubmed)	Emtree (Embase)	(Cochrane Library, Embase, PubMed, Scopus)
"Oral health"[Mesh] "Dental Caries"[Mesh] "Cariogenic Agents"[Mesh] "DMF Index"[Mesh] "Diet, Cariogenic"[Mesh]	'dental health'/exp 'dental caries'/exp 'cariogenic agent'/exp 'cariogenic diet'/exp 'caries assessment'/exp 'DMF index'/exp 'DMFS index'/exp 'DMFT index'/exp	Oral health Dental health Caries Carious Cariogen* Dental OR teeth OR tooth OR root + decay* OR white spot* OR cavit* DMF DMFT DMFS DFT DEFT DEFS

Pregnancy outcomes		
Subject index terms		Free-text terms
MeSH (Cochrane Library, Pubmed)	Emtree (Embase)	(Cochrane Library, Embase, PubMed, Scopus)
"Birth Weight"[Mesh] "Fetal Macrosomia"[Mesh] "Fetal Weight"[Mesh] "Gestational Age"[Mesh] "Infant, Low Birth Weight"[Mesh] "Infant, Small for Gestational Age"[Mesh] "Infant, Very Low Birth Weight"[Mesh] "Infant, Extremely Low Birth Weight"[Mesh] "Diabetes, Gestational"[Mesh] "Pregnancy in Diabetics"[Mesh]	'birth weight'/exp 'high birth weight'/exp 'low birth weight'/exp 'small for date infant'/exp 'very low birth weight'/exp 'extremely low birth weight'/exp 'fetus weight'/exp 'gestational age'/exp 'tall stature'/de 'macrosomia'/exp 'large for gestational age'/exp 'pregnancy diabetes mellitus'/exp	Weight + birth OR newborn* OR new born* OR nenonat* OR fetal OR foetal OR fetus OR foetus Size + birth OR newborn* OR new born* OR neonat* OR fetal OR foetal OR fetus OR foetus Macrosomia* Macrosomatia* SGA OR LGA + infant* OR neonat* OR newborn* OR child* OR baby OR babies Gestational age OR gestation age OR gestational time OR gestation time "small for date" OR "large for date" + infant* OR neonat* OR newborn* OR child OR children OR baby OR babies Gestational OR pregnant* OR pregnanc* + diabetes DGM

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

9 October 2017
EMA/CHMP/460886/2014
Committee for Human Medicinal Products (CHMP)

Information for the package leaflet regarding fructose and sorbitol used as excipients in medicinal products for human use

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30 Churchill Place ● Canary Wharf ● London E14 5EU ● United Kingdom
Telephone +44 (0)20 3660 6000 **Facsimile** +44 (0)20 3660 5555
Send a question via our website www.ema.europa.eu/contact

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Information for the package leaflet regarding fructose and sorbitol used as excipients in medicinal products for human use

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Executive summary

This document has been written in the context of the revision of the Annex of the European Commission Guideline on 'Excipients in the labelling and package leaflet of medicinal products for human use' [2, 9].

Fructose and sorbitol are used as excipients in a variety of oral (tablets, capsules, suspensions) and topical formulations (creams, emulsions). Sorbitol (and rarely fructose) is also used as a protein/peptide stabiliser in some medicinal products for parenteral use such as blood products (immunoglobulins) and vaccines.

Since sorbitol is rapidly converted in vivo to fructose by sorbitol dehydrogenase in the liver, systemic safety concerns regarding fructose also apply to sorbitol. Therefore, both substances are combined in one review. In addition, the systemic concerns related to fructose may also apply to other sugars that are metabolised to fructose (e.g. maltitol).

Both fructose and sorbitol are already included in the Annex of the current Guideline with a threshold zero triggering a warning about the risk in case of intolerance to some sugars. Although patients with the rare genetic disorder of hereditary fructose intolerance (HFI) are likely to take into account this warning, there is a concern that it is insufficient for patients who are not aware of their underlying HFI, are unconscious due to an acute medical condition, or are too young for the diagnosis to have been made.

Patients with HFI develop a natural defence mechanism against fructose and sorbitol by vomiting any food containing either of the two substances. Since this defence mechanism is bypassed as soon as fructose or sorbitol is delivered intravenously, a much higher risk is associated with solutions for parenteral (especially intravenous) use.

During the centralised procedure of the intravenous immunoglobulin Flebogamma DIF which contains a high content of sorbitol as an excipient (amount sorbitol administered per single dose: 0.2–2 g/kg) and is indicated for use in children it became obvious that the warning in the Annex of the guideline on Excipients dated 2003 [9] was insufficient.

It is proposed to strengthen the warning for medicinal products containing fructose/sorbitol and given intravenously with regard to the risk for very young children or unconscious patients. This warning provides potential signs of HFI in babies and alerts parents and HCPs that administration in children below 2 years of age with undiagnosed HFI may be fatal. Additional recommendations include the need for a detailed history with regard to HFI symptoms in each patient prior to therapy and the consideration of a contraindication of such medicinal products in children < 2 years of age. It is recognised that the latter has to be judged on a case by case basis, since there might be exceptional cases in which the medicine should still be applicable if necessary (e.g. life-threatening diseases with no alternative treatments).

However, some vaccines and other parenteral biologicals (e.g. monoclonal antibodies) contain very low amounts of sorbitol as an excipient (8–16 mg per dose). They have been administered for a long time without any known incidence of severe events due to HFI, in particular vaccines recommended for children below 2 years of age (MMR, VZV). Therefore the warning for those products should differ from the warning for products administered intravenously.This is why a threshold above zero was derived for oral and parenteral (other than intravenous (IV)) products. By applying this threshold the current misleading warning in the package leaflet of vaccines will not be obligatory any longer.

There is no scientifically established and generally accepted safe dose for patients with HFI. Nevertheless, a threshold dose of 5 mg/kg/day was derived for oral and parenteral (other than IV) products as a limit above which a warning in the PIL is deemed necessary and useful. An oral, subcutaneous or intramuscular dose of 5 mg/kg/day is expected to result in a systemic exposure level (C_{max} and AUC) not higher than that reached after an oral dietary intake of 10 mg/kg/day, which is the lowest level of restriction for the oral fructose intake in patients with HFI. This dose level corresponds to 1/8 of the internationally recommended upper level of oral fructose intake in HFI of 40 mg/kg/day and corresponds to 1/50 of the challenge dose of intravenous fructose (0.25 g/kg) applied in the fructose tolerance test which has been used in the past for diagnosis of HFI. This conservative approach was chosen in view of the potentially fatal condition and the fact that the intake from a medicinal product will always be additive to the dietary intake.

Thus, for all oral and parenteral (except IV) products below the threshold of 5 mg/kg/day it is proposed to declare the sorbitol content but not to include a warning. Above this threshold the warning remains as it was before for all administration routes.

A comprehensive review of the gastrointestinal effects of fructose and sorbitol has not been undertaken at this time. The current threshold of 10 g sorbitol for oral products triggering a warning on gastrointestinal discomfort and laxative effects is still considered valid but it is amended to be expressed in mg/kg so it is more appropriate for medicinal products use in children.

A new reference to the potential effects of sorbitol on the oral bioavailability of other drugs administered concomitantly is given in the comments section.

The current reference regarding diabetes has been deleted, since the small amounts of fructose (or sorbitol) in medicines are not expected to affect the glycaemia in patients with diabetes mellitus.

Updated information in the package leaflet

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Fructose	Oral, parenteral	Zero	This medicine contains x mg fructose in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	The additive effect of concomitantly administered products containing fructose (or sorbitol) and dietary intake of fructose (or sorbitol) should be taken into account.
	Oral	Zero	*[If the medicine is in contact with teeth (e.g. oral liquids, lozenges or chewable tablets) and is intended for long term use:]* Fructose may damage teeth.	Oral products used frequently or over a long period of time, e.g. for two weeks or longer.
	Intravenous (IV)	Zero	If you (or your child) have hereditary fructose intolerance (HFI), a rare genetic disorder, you (or your child) must not receive this medicine. Patients with HFI cannot break down fructose in this medicine, which may cause serious side effects. You must tell your doctor before receiving this medicine if you (or your child) have HFI or if your child can no longer take sweet foods or drinks because they feel sick, vomit or get unpleasant effects such as bloating, stomach cramps or diarrhoea.	Patients with hereditary fructose intolerance (HFI) must not be given this medicine unless strictly necessary. Babies and young children (below 2 years of age) may not yet be diagnosed with hereditary fructose intolerance (HFI). Medicines (containing fructose) given intravenously may be life-threatening and must be contraindicated in this population unless there is an overwhelming clinical need and no alternatives are available. A detailed history with regard to HFI symptoms has to be taken of each patient prior to being given this medicinal product.

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
	Oral, parenteral (other than IV)	5 mg/kg/day	If your doctor has told you that you (or your child) have an intolerance to some sugars or if you have been diagnosed with hereditary fructose intolerance (HFI), a rare genetic disorder in which a person cannot break down fructose, talk to your doctor before you (or your child) take or receive this medicine.	Patients with hereditary fructose intolerance (HFI) should not take/be given this medicinal product.

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sorbitol (E 420)	Oral, parenteral	Zero	This medicine contains x mg sorbitol in each <dosage unit><unit volume> <which is equivalent to x mg/<weight><volume>>.	The additive effect of concomitantly administered products containing sorbitol (or fructose) and dietary intake of sorbitol (or fructose) should be taken into account. The content of sorbitol in medicinal products for oral use may affect the bioavailability of other medicinal products for oral use administered concomitantly.
	Intravenous (IV)	Zero	Sorbitol is a source of fructose. If you (or your child) have hereditary fructose intolerance (HFI), a rare genetic disorder, you (or your child) must not receive this medicine. Patients with HFI cannot break down fructose, which may cause serious side effects. You must tell your doctor before receiving this	Patients with hereditary fructose intolerance (HFI) must not be given this medicine unless strictly necessary. Babies and young children (below 2 years of age) may not yet be diagnosed with hereditary fructose intolerance (HFI). Medicines (containing sorbitol/fructose) given

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
			medicine if you (or your child) have HFI or if your child can no longer take sweet foods or drinks because they feel sick, vomit or get unpleasant effects such as bloating, stomach cramps or diarrhoea.	intravenously may be life-threatening and should be contraindicated in this population unless there is an overwhelming clinical need and no alternatives are available. A detailed history with regard to HFI symptoms has to be taken of each patient prior to being given this medicinal product.
	Oral, parenteral (other than IV)	5 mg/kg/day	Sorbitol is a source of fructose. If your doctor has told you that you (or your child) have an intolerance to some sugars or if you have been diagnosed with hereditary fructose intolerance (HFI), a rare genetic disorder in which a person cannot break down fructose, talk to your doctor before you (or your child) take or receive this medicine.	Patients with hereditary fructose intolerance (HFI) should not take/be given this medicinal product.
	Oral	140 mg/kg/day	Sorbitol may cause gastrointestinal discomfort and mild laxative effect.	

Scientific background

1. Characteristics

1.1. Category (function)

Fructose is a monosaccharide.

Sorbitol is a monosaccharide alcohol.

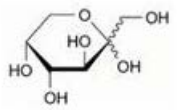
1.2. Physico-chemical Properties

Fructose *(Ph.eur. 01/2016:0188)*

D-*arabino*-Hex-2-ulopyranose (Levulose)

$C_6H_{12}O_6$

Fructosum



M_r 180.2

Appearance: white or almost white, crystalline powder. It has a very sweet taste.

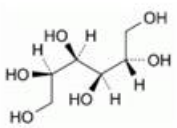
Solubility: very soluble in water, soluble in ethanol (96 per cent).

Sorbitol (Ph.eur. 04/2016:0435):

D-Glucitol (D-sorbitol)

$C_6H_{14}O_6$

Sorbitolum



M_r 182.2

Appearance: white or almost white, crystalline powder.

Solubility: very soluble in water, practically insoluble in ethanol (96 per cent). It shows polymorphism.

1.3. Use in medicinal products

Functions in medicinal products formulations

Fructose and sorbitol are used as excipients in their capacity as sweeteners, flavour constituents, modifier of viscosity, humectant, emollient/plasticisers and protein stabiliser in some medicinal products for parenteral use.

Both sugars are used in high concentrations as active pharmaceutical ingredients in parenteral nutrition formulations (energy source), as laxatives and osmotic diuretics, but this is out of the scope of this document.

Fructose / Sorbitol content in medicinal products for parenteral use

Sorbitol is included in protein/peptide formulations for parenteral use such as intravenous immunoglobulins (IVIGs), monoclonal antibodies, growth factors (e.g. filgrastim) and vaccines (e.g. measles, varicella, yellow fever).

An inquiry about all authorised medicinal products for parenteral use containing sorbitol or fructose as an excipient (centrally in EU and nationally in Germany, France and other MS) revealed that the amount of sorbitol per dose varied considerably between the products. The highest content is found in the intravenous immunoglobulin Flebogamma DIF (50 mg/ml) which corresponds to an amount of 0.2–2 g sorbitol per single dose per kg bodyweight. This would result in a total exposure of 2–20 g/dose in a 10-kg child. The sorbitol content in other biological medicinal products for parenteral use e.g. monoclonal antibodies, growth factors and vaccines is markedly lower, resulting in a total exposure of 2–80 mg/dose. The parenteral vaccines comprise measles and varizella zoster vaccines and combinations thereof (MMR, MMRV), and the yellow fever vaccine Stamaril®. These products contain sorbitol at 8–16 mg/dose and are authorised in children > 9 months. Thus, the parenteral sorbitol dose per kg bodyweight will not exceed 2.4 mg/kg (assumption: lower 3rd centile of weight-for-age in girls at 9 months: 6.6 kg; WHO Multicentre Growth Reference Study Group, 2006).

With regard to fructose, one parenteral product was identified which is an intravenous antimycotic lipopeptide (anidulafungin) formulation (Ecalta®) and contains 204 mg/dose of fructose.

The administration route is mainly subcutaneous and intravenous. All products contain a warning statement that fulfils the requirement of the current Guideline with regard to HFI; however, it is questionable whether the wording for vaccines is meaningful/ appropriate with respect to the age of the vaccinated subjects.

Fructose / Sorbitol content in medicinal products for oral use

In oral use sorbitol can be found in various liquid medicines such as antiallergics (e.g. desloratadine), antiemetics (e.g. ondansetron) and some antibiotics. It is also a common excipient in many types of tablets, e.g. orodispersible tablets. It can also be found in one oral vaccine preparation (Rotarix®).

Fructose is less often used in oral medicines, but can be found in products such as the antivirals Kaletra® (oral liquid) or Isentress® (chewing tablets).

Authorised medicinal products for oral use may contain sorbitol / fructose in the range 2–90% per dosing unit (tablet, millilitre) [20]. Typical products are solutions and suspensions of antibiotics and antitussives, of which some are authorised from birth. The highest sorbitol and fructose exposures are in the range 400–500 mg/kg.

Rotarix® reconstituted suspension, an oral vaccine authorised from birth, contains 13.5 mg/dose sorbitol which normally equals less than 10 mg/kg in a neonate.

2. Pharmaco-toxicological data

An animal model of fructose intolerance in rats exists since 1974 [18].

3. Pharmacokinetics (in humans)

3.1. Absorption

Fructose is the main sugar found in fruit, vegetables and honey. Free fructose, ingested orally either as monosaccharide or enzymatically cleaved from sucrose, is absorbed directly by the intestine. It is transported through the intestinal epithelium by GLUT-5 (passive) and GLUT-2 (active) transporters [4].

Studies indicate that fructose absorption is dose-dependent and facilitated by the simultaneous ingestion of glucose [12]. Saturation of absorptive capacity is reached with doses of about 0.5 g/kg body weight, above which a significant proportion of subjects show signs of fructose malabsorption [19]. Plasma fructose concentration time courses are available from 19 healthy subjects after oral intake of 50 g fructose [22], and from 8 healthy adults after a test meal of 0.5 g/kg fructose [21]. Both showed a peak in plasma fructose after 30 min (t_{max}).

Sorbitol is incompletely absorbed at a rate much slower than that of fructose, and absorption seems dose and concentration dependent. When sorbitol is given as a single oral dose, the intestinal absorptive capacity has been estimated to be no more than 2–10 g; furthermore, it has been suggested that, when given together, sorbitol and fructose compete for absorption [14].

There are no data on rate and extent of absorption (i.e. bioavailability) of fructose or sorbitol when administered subcutaneously or intramuscularly, e.g. after administration as excipients in vaccines or other biologicals.

3.2. Metabolism

Fructose is transported into the liver by the GLUT2 transporter. Uptake of fructose by the liver is not regulated by insulin. (However, insulin is capable of increasing the abundance and functional activity of GLUT5 in skeletal muscle.) The first step in the metabolism of fructose is the phosphorylation of fructose to fructose 1-phosphate by fructokinase, thus trapping fructose for metabolism in the liver. Fructose 1-phosphate then undergoes hydrolysis by fructose 1,6-bisphosphate aldolase (aldolase B) to form DHAP and glyceraldehydes. This enzyme is defective in hereditary fructose intolerance, HFI (see chapter on clinical safety data).

Sorbitol is rapidly converted to fructose by sorbitol dehydrogenase in the liver. Together with aldose reductase, it provides a way for the body to produce fructose from glucose without using ATP. Sorbitol dehydrogenase uses NAD+ as a cofactor; its reaction is sorbitol + NAD+ --> fructose + NADH + H+. A zinc ion is also involved in catalysis. The organs that use it most frequently include the liver and seminal vesicle.

4. Clinical safety data

4.1. Hereditary Fructose Intolerance (HFI)

Background

HFI is a hereditary autosomal recessive deficit in fructose 1,6-bisphosphate aldolase (aldolase B), the main enzyme responsible for hepatic metabolism of fructose. It is expressed in the liver, small intestine, and proximal renal tubule where it facilitates assimilation of dietary fructose by catalysing

the cleavage of fructose-1-phosphate [1]. A lack of functional aldolase B leads to an accumulation of toxic fructose 1-phosphate in liver cells, ultimately resulting in hepatic decompensation.

Patients with HFI develop a natural defence mechanism against fructose and sorbitol by vomiting any food containing either of the two substances. This defence mechanism is bypassed as soon as fructose or sorbitol is delivered intravenously. Therefore, a much higher risk is associated with solutions for parenteral use.

The worldwide incidence of HFI at birth is estimated at 1:20.000–1:30 000. The prevalence of HFI in adults is unknown.

HFI has to be distinguished from DFI (dietary fructose intolerance, fructose malabsorption), which is an uncomfortable but benign condition. It occurs when the amount of fructose consumed is more than the small intestines can absorb. Unabsorbed fructose acts as an osmotic load which can cause diarrhoea and other gastrointestinal symptoms [10].

Diagnosis

Clinical

The disorder is not apparent until the infant is weaned and fed with formula, juice, fruits, or baby foods or honey that contain fructose. In young children a spontaneous aversion for fructose develops and the onset of symptoms may be delayed when parents scrupulously respect the child's tastes for food and do not impose fructose-containing products rejected by the child.

Biopsy and testing

A formal HFI diagnosis can be made by demonstrating deficient (<10%) fructose 1 phosphate aldolase activity in tissue biopsy specimens (liver biopsy preferred). Detection can also be made by a fructose intolerance test (0.5 g/kg of an IV 30% fructose solution). This test induces within 30 min. hypoglycemia, hypophosphoremia, and hyperurecemia with signs of hepatocellular insufficiency. However, this test may be dangerous, and many clinicians prefer to perform a hepatic biopsy right away.

Nowadays, for diagnosis of HFI the molecular analysis of the Aldolase B gene is the preferred method.

Genetic

The ALDOB gene (on Chr. 9) of the enzyme fructose 1 phosphate aldolase (or aldolase B) has been sequenced and a direct diagnosis is possible by using DNA prepared from nucleated blood cells. Four identical aldolase B enzymes need to form a tetramer to work. More than 50 mutations in the ALDOB gene have been found to cause HFI. Most of these mutations replace single amino acids in the aldolase B enzyme and result in the production of an enzyme with reduced function. Three specific mutations account for 95% of patients of European origin. A mutation found in approximately half of people with HFI replaces the amino acid alanine with the amino acid proline at position 149 in the enzyme (written as Ala149Pro or A149P). This mutation alters the 3-dimensional shape of the enzyme making it difficult for the aldolase B enzymes to form tetramers and thus metabolise fructose.

Symptoms

Clinical symptoms usually include vomiting, anorexia, gastro-intestinal disorders, apathy, height and weight retardation and hepatomegaly. Forced ingestion of a large amount of fructose may lead to acute hepatic decompensation, with major hepatocellular insufficiency, haemorrhagic syndrome and

jaundice. The reason for the severe adverse events with infusion of fructose solution, including death, is not hypoglycaemia but the intracellular accumulation of fructose 1-phosphate which is highly toxic.

Biochemical signs are postprandial hypoglycaemia, hypophosphatemia, hyperlactacidemia and hyperuricaemia. Prolongation of coagulation time, increased hepatic enzymes and increased bilirubin are also often present. Methionine and plasma tyrosin are often elevated. A proximal and distal renal tubulopathy is frequent.

Treatment

The treatment consists in complete withdrawal of fructose, sorbitol and saccharose, which can lead to resolution of hepatic disorders. In case of suspected fructose intolerance during infusion of fructose or sorbitol, infusion has to be stopped immediately, normal glycaemia has to be re-established and organ function has to be stabilised by intensive care procedures.

Safety data

If fructose (or sorbitol) containing infusions are given in hospitals to children not yet diagnosed with HFI, acute hepatic and renal insufficiency can develop, followed by death within a few days.

Babies and young children represent the population with the highest risk, as HFI may not yet have been diagnosed, but inadvertent administration of parenteral fructose (sorbitol) solutions can also have life-threatening consequences in adult HFI patients.

On 2nd March 2001 a "Benefit-risk evaluation of fructose and sorbitol-containing solutions for parenteral use" was performed by Daniel Brasseur and Xavier Kurz following the death of an 18-month-old child who had been administered a fructose-containing solution (IV) [11]. In the literature up to 1993 twenty-three (23) cases were reported, whereof 17 were fatal (73%). Not all 17 case reports had information on dosing. However, three adult patients died after receiving ~ 25–50 g of sorbitol (in one case an infusion over 10 h). The report concludes: "An excessive amount of fructose or sorbitol is not necessary for a fatal outcome". Death typically occurred within 3–10 days after the first fructose or sorbitol administration as a consequence of severe renal and liver failure.

Although this assessment was done with regard to the fructose and sorbitol-containing solutions for parenteral nutrition in which the sugars act as active substances and are present in high concentrations, the conclusions of the assessment had an impact on the EU authorisation procedure for Flebogamma DIF, a sorbitol-containing intravenous immunoglobulin.

Flebogamma DIF (Grifols) solutions for infusion contain 50 mg sorbitol/ml as an excipient and 50 or 100 mg IgG/ml as the active substance. According to the recommended dosing, a child with e.g. Kawasaki's disease weighing 10 kg would require 20 g IgG which would translate to 20 g of intravenous sorbitol. This is in the order of magnitude of the fatal doses for HFI adults mentioned above.

Thus, in the updated SmPC for Flebogamma DIF children under 2 years of age were excluded from the indications and a statement was added: "*In babies and young children (aged 0–2 years) hereditary fructose intolerance (HFI) may not yet be diagnosed and may be fatal, thus, they must not receive this medicinal product.*"

Furthermore, the following statement was added under Section 4.4:

> *Sorbitol*
>
> *Each ml of this medicinal product contains 50 mg of sorbitol. Patients with rare hereditary problems of fructose intolerance must not take this medicine.*

In persons more than 2 years old with HFI, a spontaneous aversion for fructose-containing foods develops and may be combined with the onset of symptoms (vomiting, gastro-intestinal disorders, apathy, height and weight retardation). Therefore a detailed history with regard to HFI symptoms has to be taken of each patient prior to receiving Flebogamma DIF.

In case of inadvertent administration and suspicion of fructose intolerance the infusion has to be stopped immediately, normal glycaemia has to be re-established and organ function has to be stabilized by means of intensive care.

Interferences with determination of blood glucose levels are not expected.

4.2. Gastrointestinal effects of oral use

The current version of the guideline acknowledges the potential laxative effect of oral sorbitol and includes a threshold of 10 g triggering a warning that the product "may have a mild laxative effect". Although this limit might be suitable for adults, it is probably less relevant for children of low age and weight. It is also known that children can be more sensitive to this effect compared to adults, although the variability may be high [13]. Furthermore, the gastrointestinal effects are not limited to laxation but also include other signs of GI discomfort (e.g. bloating, cramps).

4.3. Potential pharmacokinetic interaction

In addition, it has been described in the literature that some liquid oral formulations containing sorbitol may affect the bioavailability of other drugs administered concomitantly. For example, lamivudine bioavailability is affected by sorbitol contained in the oral solution of abacavir (340 mg sorbitol/ml) and the oral suspension of nevirapine (162 mg sorbitol/ml) [8]. Also risperidone bioavailability (i.e. C_{max} and AUC) is affected in some subjects when sorbitol amounts as low as 7.5 and 60 mg are given [17].

4.4. Diabetes

Fructose in medicines represents a small fraction of fructose intake from the diet (less than 10%) in terms of calorific value. Furthermore, it is not expected to affect the glycaemia in patients with diabetes mellitus. Therefore, adding such information in the package leaflet is not considered to be relevant. A similar rationale applies to sorbitol.

5. Safety information relevant for the package leaflet

5.1. Hereditary Fructose Intolerance (HFI)

Summary of concerns

Although the risk of administration of products containing fructose or sorbitol to patients with HFI has been reduced by the current warning statement, there is a concern that this warning is insufficient for the patients in whom HFI has not yet been diagnosed and particularly the small children. This concern triggered the rewording of the Flebogamma DIF product information (see above). Therefore, in the revised guideline, a new strengthened and more detailed warning was derived from this rewording.

In parenteral products (see 1.3.) the total sorbitol exposure in adults varies by a factor of 70,000 (e.g. Scintimun®: 2 mg/dose versus Flebogamma DIF: 140 g/dose), while monoclonal antibodies, growth factors and vaccines contain very low amounts of sorbitol (2–80 mg/dose). Particularly in the case of vaccines, which are applied only once (or twice) in lifetime and are administered subcutaneously (or

intramuscularly), the absorption of sorbitol is much slower than in the IV administration; therefore it is questionable whether a special warning with regard to HFI is appropriate.

The parenteral vaccines containing sorbitol are administered in children > 9 months. At that age HFI is more likely to have been diagnosed, but if the child is not yet weaned this might still not be the case. However, the maximum parenteral sorbitol dose per kg bodyweight is estimated to be 2.4 mg/kg (see 1.3). Considering that proposed new strengthened warning should alert parents and HCPs that administration may be fatal in children < 2 years of age, this is not appropriate for these vaccines recommended at the age of < 2 years. Moreover, a warning does not enhance the decision-making process for parents or healthcare professionals as children are too young to be diagnosed. Furthermore, these vaccines have been administered for a long time without any incidence of severe events due to fructose intolerance.

Then, the next question would be whether a threshold can be identified below which oral and parenteral (other than IV) administered fructose/sorbitol is tolerated by an HFI patient at different ages or whether medicinal products for children < 2 years should be absolutely fructose/sorbitol-free.

Non-toxic dose

Since the molecular weight of fructose and sorbitol is quite similar (see 1.2), in all calculations below they are regarded as equivalent.

Fatal parenteral administrations of fructose and sorbitol cause acute hepatorenal failure associated with bleeding. Adult patients have died after receiving 25 g of sorbitol intravenously [11].

In the fructose tolerance test which has been used in the past for diagnosis of HFI, patients are challenged IV with fructose 0.25 g/kg body weight. As a result glycogenolysis is inhibited and gluconeogenesis is impaired [5]. Diagnosis by a fructose tolerance test leads to the same acute symptoms as HFI and can be life threatening. It can be deduced that this dose can definitely be regarded as toxic and should therefore be above a potential threshold.

Patients with HFI are treated by strict fructose-free diet. Although the precise daily limit of oral fructose is unknown a restriction to less than 40 mg/kg/day is recommended [11]. Mock et al. demonstrated in two unrelated boys with HFI (5.3 and 3.8 years of age) with isolated growth retardation in infancy that a more stringent restriction of dietary fructose to less than 40 mg fructose per kg body weight daily lead to an increase in growth velocity to the 75th and 97th percentile, respectively [16]. When the restriction of dietary fructose was relaxed (from 10 to 250 mg/kg/day), neither of the boys had symptoms, hypoglycaemia, or evidence of hepatic or renal dysfunction. However, both had sustained hyperuricaemia and hyperuricosuria and increases in the plasma concentration and urinary excretion of magnesium [5]. The authors do not exclude that chronic ingestion of even trace amounts of these sugars lead to long-term effects.

The Scientific Panel on Dietetic Products, Nutrition and Allergies of EFSA published in 2005 an opinion paper on a request from the Commission related to the evaluation of fructose for labelling purposes [6]. They stated that the spectrum of individual fructose tolerance in HFI appears to depend on age (infants being more sensitive than adults) and on the respective gene mutation and that there are no scientifically established and generally accepted triggering doses for patients with HFI.

However, a publication by Barshop et al. 2003 indicates that short-term (2 days) dietary intake of 4.7 mg of fructose/kg/day was safe and well tolerated among individuals with diagnosed HFI [3]. Five subjects with HFI (ages 14–52 years; 4 males, 1 female) participated in a prospective, non-randomised open challenge with FOS at 6 g/m^2/d for 2 days. A female infant (5 months) with resolved neonatal hepatitis was also studied. Diet records were maintained for the 48-hr period and analysed

for dietary fructose. Tolerance was assessed through evaluation of serum AST, ALT, GGT, glucose, bilirubin, uric acid, phosphorus, and electrolytes, upon initiation and at 12-hour intervals during the challenge. Blood chemistry values were within normal ranges and did not change appreciably during the study period, except for two patients with slight elevations of uric acid. One subject reported gastric discomfort on day 2 of the FOS challenge. These data suggest that approximately 4.7 mg fructose/kg bw/day for 2 days is safe and well tolerated among individuals with diagnosed HFI.

Conclusion and recommendation / Derivation of a threshold

There is no scientifically established and generally accepted safe dose for patients with HFI. However, an internationally accepted safety recommendation restricts the oral fructose intake in HFI below 40 mg/kg/day. The most stringent restriction of the oral fructose intake in HFI described in the literature is 10 mg/kg/day [5]. This level was chosen for the calculation of a threshold. This conservative approach was chosen as the intake from a medicinal product will always be additive to the dietary intake.

At this recommended level of oral intake (10–40 mg/kg) the absorption capacity of the intestines is probably not reached (0.5 g/kg BW, see PK section 3.1), hence 100% bioavailability can be assumed.

After subcutaneous or intramuscular administration of vaccines systemic bioavailability of sorbitol is considered kinetically comparable to the oral administration with regard to the velocity and amount of absorption of fructose/sorbitol. However, since there are no PK data on subcutaneous (s.c.) or intramuscular (i.m.) absorption of fructose/sorbitol available, it cannot be excluded that absorption from these routes might be even faster than the oral absorption and C_{max} reached in plasma after subcutaneous administration might be higher than after an oral application of the same dose/kg. Therefore, the orally “tolerable” dose of 10 mg/kg/day was divided by a factor of 2 to be safely applicable also for the s.c./i.m. route. Thus, a threshold dose of 5 mg/kg/day for oral and parenteral (other than IV) products was chosen which is expected to result in a systemic exposure level not higher than that reached after an oral dietary fructose intake of 10 mg/kg/day. This threshold is not regarded as “safe daily dose” but as limit above which a detailed warning in the PIL is deemed necessary and useful.

This threshold would be in line with the observation of the well tolerated oral dose of 4.7 mg/kg/day observed in the small challenge study by Barshop et al. [3] which included one child of 5 months of age.

Furthermore, the oral rotavirus vaccine Rotarix® is administered twice to newborns from 6 weeks up to 16 weeks of age, when HFI is probably not yet diagnosed. It contains 13.5 mg/dose which equals to 4.5–6.8 mg/kg in a 2–3 kg newborn. There are no indications that this level gave rise to adverse events or even death related to HFI.

Therefore, in the revised annex of the guideline, for all oral and parenteral (except IV) products below this threshold, the declaration of the sorbitol content without a warning is considered appropriate. Above this threshold the warning remains as it was before as it is regarded as sufficient for the safe use of orally administered drugs, while a new intravenous threshold zero is introduced, triggering the inclusion of a more detailed warning and a recommendation for interdicting the use in children below 2 years of age. It is recognised that the latter has to be judged on a case by case basis, since there might be exceptional cases in which the medicine should still be applicable if necessary (e.g. life-threatening diseases with no alternative treatments).

5.2. Laxative and gastrointestinal effects

When taken orally in larger amounts sorbitol (and to a less extent fructose) can also cause laxative effects and gastrointestinal discomfort. Sorbitol is often used in formulations intended for children and infants and young children might be more sensitive to this effect [14].

The current (adult) threshold of 10 g of sorbitol triggering a warning on potential laxative effect is considered correct but it is proposed to express it in mg/kg so it is more appropriate for medicinal products used in children. Therefore a weight adjusted threshold of 140 mg/kg (equivalent of 10 g/70 kg) is proposed for all patients.

5.3. Potential interaction

There is some recent evidence in the literature that orally administered sorbitol can affect the bioavailability of other concomitant oral drugs (see 4.3). A sorbitol amount as low as 7.5 mg is accused, corresponding to 0.107 mg/kg body weight in a 70 kg adult. Therefore, a statement pointing out the potential effects of sorbitol on the bioavailability of other drugs administered concomitantly was added in the comments section of sorbitol which refers to all routes of administration at threshold zero.

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Annex 1 - Information in the package leaflet as per 2003 Guideline [9]

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Fructose	Oral Parenteral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product	SPC proposal for section 4.3: Patients with rare hereditary problems of fructose intolerance should not take this medicine.
		5 g	Contains x g fructose per dose. This should be taken into account in patients with diabetes mellitus.	
	Oral liquids, lozenges and chewable tablets	Zero	May be harmful to the teeth	Information to be included only when the medicinal product may be intended for chronic use, e.g. for two weeks or more.

Name	Route of Administration	Threshold	Information for the Package Leaflet	Comments
Sorbitol E420	Oral Parenteral	Zero	If you have been told by your doctor that you have an intolerance to some sugars, contact your doctor before taking this medicinal product.	SPC proposal: Patients with rare hereditary problems of fructose intolerance should not take this medicine.
	Oral	10 g	May have a mild laxative effect Calorific value 2.6 kcal/g sorbitol	

Safety Assessment of Monosaccharides, Disaccharides, and Related Ingredients as Used in Cosmetics

Status:	Final Report
Release Date:	April 4, 2014
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The 2014 Cosmetic Ingredient Review Expert Panel members are: Chairman, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A. Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D., Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is Lillian J. Gill, D.P.A. This safety assessment was prepared by Monice M. Fiume, Assistant Director/Senior Scientific Analyst and Bart Heldreth, Ph.D., Chemist.

© Cosmetic Ingredient Review
1620 L Street, NW, Suite 1200 ◊ Washington, DC 20036 ◊ ph 202.331.0651 ◊ fax 202.331.0088 ◊
cirinfo@cir-safety.org

ABSTRACT

The Expert Panel assessed the safety of 25 monosaccharides, disaccharides, and related ingredients, and concluded these ingredients are safe as used in cosmetics. Many of these ingredients are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts; seven of the ingredients are listed by the FDA as GRAS food substances. The most commonly reported cosmetic function is as a skin conditioning agent; other commonly-reported functions are use as a humectant or as a flavoring agent. The Panel reviewed the animal and clinical data included in this assessment, acknowledged that the oral safety of many of these ingredients has been well established, and found it appropriate to extrapolate the existing information to conclude on the safety of all the monosaccharides, disaccharides, and related ingredients.

INTRODUCTION

This report addresses the safety of the following 25 monosaccharides, disaccharides, and related ingredients as used in cosmetic formulations:

Calcium Gluconate[#]
Fructose[#]
Fucose
Galactose
Galactosyl Fructose
Galacturonic Acid
Gluconic Acid
Glucose[#]
Isomalt[##]
Kefiran
Lactitol[##]
Lactose[##]
Lactulose
Maltose
Mannose
Melibiose
Potassium Gluconate[#]
Rhamnose
Ribose
Sodium Gluconate[#]
Sucralose[#]
Sucrose[#]
Trehalose[##]
Xylobiose
Xylose

[#]generally recognized as safe (GRAS) food additive or approved direct food additive
[##]listed in the Food Chemical Codex

The monosaccharides, disaccharides, and related ingredients have a number of reported functions in cosmetics, and the most common use is as a skin conditioning agent (Table 1).[1] Other commonly-reported functions are use as a humectant or as a flavoring agent.

Most of these ingredients included in this safety assessment are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts. Several are listed by the Food and Drug Administration (FDA) as GRAS food additives[2] or direct food additives, and/or are listed in the *Food Chemicals Codex*[3] as used in foods; for these ingredients, the focus of this assessment will be on dermal effects, primarily dermal irritation and sensitization. This approach is supported by the fact that some of these ingredients, namely fructose, galactose, glucose, lactose, sodium gluconate, and sucrose, are listed in Annex IV of the European Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH),[4] which "sets out substances that are exempted from the registration, evaluation and downstream user provisions of REACH as sufficient information is known about these substances that they are considered to cause minimum risk because of their intrinsic properties."[5]

For those ingredients that are not identified as common dietary substances, i.e., fucose, galactosyl fructose, galacturonic acid, kefiran, lactulose, mannose, melibiose, and xylobiose, a search for oral toxicity data was performed. Very limited published data were found.

CHEMISTRY

Definition

A monosaccharide is a carbohydrate that cannot be decomposed to a simpler carbohydrate by hydrolysis, and is often called a simple sugar.[6] A disaccharide is a carbohydrate that yields two monosaccharides upon hydrolysis. Many of these ingredients exist in equilibrium between an open chain form and one or more ring forms resulting in a hemiacetal or hemiketal linkage involving the aldehyde (aldose) or ketone (ketose) moiety of the open chain form, with two possible stereochemical configurations (Figure 1). The resulting stereoisomers are called anomers and the stereocenter is referred to as the anomeric carbon.

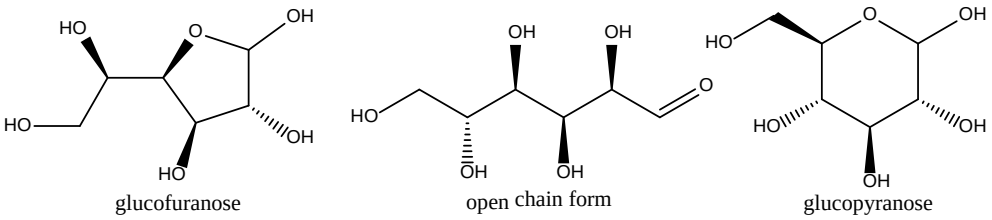


Figure 1. Structural forms of D-glucose (stereoisomer found in natural sources) that exist in equilibrium.

The definition and structure of each ingredient included in this report is provided in Table 1.

Chemical and Physical Properties

Due to the high degree of substitution with hydroxyl groups, the mono- and disaccharides are very hydrophilic and readily dissolve in aqueous solvent systems. These sugars have molecular weights ranging from 142 to 391 Daltons, and are solids at room-temperature, with many having multiple known crystalline forms (Table 2[3,7-29]).

Natural Occurrence and Methods of Manufacture

The manufacture of the majority of these monosaccharides, disaccharides, and related ingredients is accomplished by extraction from plant sources (Table 3). For instance, the sugar industry processes sugar cane and sugar beets to obtain sucrose.[30] Sugar cane contains 70% water; 14% fiber; 13.3% saccharose (about 10 to 15% sucrose), and 2.7% soluble impurities. Sugar cane is extracted with water, clarified to remove mud, evaporated to prepare syrup, crystallized to separate the liquor, and centrifuged to separate molasses from the crystals. Sugar crystals are then dried and may be further refined before bagging for shipment. Sugar beet (water, 75%; sugar, 17%) processing differs in the washing, preparation, and extraction. After washing, the beet is sliced and extracted with water. Sugar refining involves removal of impurities and decolorization. The steps generally followed include affination (mingling and centrifugation), melting, clarification, decolorization (with activated carbon, ion exchange resins, etc.), evaporation, crystallization, and finishing.

Constituents/Impurities

Purity and composition specifications are available for the food and pharmaceutical uses of many of these ingredients (Table 4).

USE

Cosmetic

The ingredients included in this safety assessment have a variety of functions in cosmetics. The most common function is as a skin conditioning agent; many also are reported to function as flavoring agents (Table 1).

The FDA collects information from manufacturers on the use of individual ingredients in cosmetics as a function of cosmetic product category in its Voluntary Cosmetic Registration Program (VCRP). VCRP data obtained from the FDA in 2014[31] and data received in response to a survey of the maximum reported use concentration by category conducted by the Personal Care Products Council (Council) in 2013[32] indicate that 22 of the 25 ingredients included in this safety assessment are used in cosmetic formulations.

According to the VCRP data, sucrose has the greatest number of reported uses, 738, followed by trehalose with 474 uses and glucose with 425 uses (Table 5).[31] A concentration of use survey conducted by the Council found that the use of these mono- and disaccharides varies widely by ingredient and use-type.[32] Glucose has the highest reported use concentration in a leave-on product; it is reported to be used at 91% in "other" non-coloring hair preparations. It is also used at 97.8% in an ingestible oral hygiene product. Sucrose has the next highest reported use concentration; it is used at up to 58% in leave-on formulations (i.e., in other skin care preparations) and 65% in rinse-off products (i.e., in other personal cleanliness products). However, most of the ingredients are used at less than 1% in leave-on products.

The three ingredients not reported to be used are galactose, galacturonic acid, and lactulose (Table 6).

VCRP data indicate that glucose, lactose, sodium gluconate, sucrose, and trehalose are used in baby products; however concentration of use data for baby products were not reported by industry. Some of the ingredients are used in products that could be incidentally, or are purposely, ingested (e.g., 97.8% glucose in an ingestible oral hygiene product), and some are used near the eye area or mucous membranes (e.g., 2% sucrose in eye lotion and 65% in personal cleanliness products, respectively). Additionally, some of these ingredients are used in cosmetic sprays and powders that could possibly be inhaled (e.g., glucose is used at 1% in a spray body and hand preparation). In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of

droplets/particles <10 µm compared with pump sprays.[33,34] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and bronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[35,36]

All of the ingredients included in this safety assessment are listed in the European Union inventory of cosmetic ingredients.[37]

Non-Cosmetic

Several of the ingredients have specific GRAS food and direct food additive uses:

- Calcium gluconate: GRAS designation; a direct food additive that meets the specifications of the *Foods Chemical Codex*; it is used as a firming agent, formulation aid, sequestrant, and texturizer at levels not to exceed current good manufacturing practices (GMP); GMP result in a maximum level, as served, of 1.75% for baked goods; 0.4% for dairy product analogs; 4.5% for gelatins and puddings; and 0.01% for sugar substitutes (21CFR184.1199)
- Fructose: a direct food additive; in high fructose corn syrup (containing approximately 42 or 55% fructose); high fructose corn syrup must conform to the identity and specifications listed in the monograph entitled "High-Fructose Corn Syrup" in the *Food Chemicals Codex*, with no limitation other than current GMP (21CFR184.1866)
- Glucose: GRAS direct food additive (D-glucose) meeting the specifications of the *Foods Chemical Codex*; it is used in foods with no limitation other than current GMP (21CFR184.1857)
- Potassium gluconate: GRAS designation; does not have a CFR citation.[2] The Select Committee on GRAS Substances (SCOGS) concluded there is no evidence in the available information on potassium gluconate that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or might reasonably be expected in the future.[38]
- Sodium gluconate: GRAS designation; as a sequestrant in food, with no limitation other than current GMP (21CFR182.6757)
- Sucralose: direct food additive as a multipurpose additive that meets the specifications of the *Foods Chemical Codex*; it is used as a sweetener in foods generally, in accordance with current GMP in an amount not to exceed that reasonably required to accomplish the intended effect (21CFR172.831)
- Sucrose: GRAS designation; a direct food additive that must be of a purity suitable for its intended use, with no limitation other than current GMP (21CFR184.1854)

Lactulose is an approved drug used to treat constipation.[39] A general list of non-cosmetic uses, including food uses that are not affirmed as GRAS or those that are inactive ingredients in approved drugs, are listed in Table 7.[3,9,10,12,26,39-41] Table 8 provides a listing of those ingredients that are nutritive and non-nutritive sweeteners.[3,14,42,43]

In Europe, the following are listed in REACH Annex IV: fructose; galactose; glucose; lactose; sodium gluconate; sucrose.[4] Substances included in Annex IV are exempted from registration (as well as downstream user requirements and evaluation) for all their possible uses irrespective of the tonnage in which they are manufactured or imported (currently or in the future).

TOXICOKINETICS

Although many of the ingredients included in this safety assessment are food ingredients, they are not all processed by the body in the same manner (see Tables 8 and 9). Some are nutrients, which are absorbed intact in the small intestines and then metabolized in the body to serve as sources of energy, and others are not (Table 8). For example, glucose[44] and potassium gluconate,[45,46] are rapidly absorbed in the small intestine (Table 9[9,12,23,27,38,44-61]). In contrast, isomalt is absorbed only to a limited extent,[12] and lactitol,[12] lactulose,[52] and sucralose,[23,55,57] are not absorbed in the gut. Trehalose can be metabolized by trehalase in the gut to produce glucose, which can then be readily absorbed. Some of these ingredients (e.g., gluconic acid, potassium gluconate, and sodium gluconate) are important intermediates in carbohydrate metabolism; gluconic acid is a normal metabolic product of glucose oxidation, and the amounts produced endogenously are much greater than what is consumed.[9] Because the absorption, distribution, metabolism, and elimination of most of the ingredients included in this safety assessment have been reviewed to evaluate their use as common dietary substances, only summary information is provided in this report.

Dermal Penetration

In Vitro

Glucose

The permeability coefficient for glucose was determined *in vitro* using full thickness mouse skin and the dermis of nude mice.[62] Unlabeled glucose, 0.01 M, was first used on both sides of the skin to saturate the sorptive capacity of the cell system. A concentration of 3.3×10^{-6} M D-[1,3-^{14}C]glucose, supplied as a sterile aq. solution containing 3% alcohol, was placed in the donor cell. After 6 h, the permeability coefficient of glucose was 9.5×10^{-5} cm/h through full-thickness skin

and 0.29 cm/h through the dermis. The permeation rate continued to increase as a function of time; the researchers stated that physical and chemical deterioration of the barrier phase seemed to be responsible for the increase in permeation.

In Vivo

Glucose

The transdermal penetration of glucose through Rhesus monkey skin was measured using optical coherence tomography (OCT).[63] The hair on the right hind leg of four anesthetized monkeys was shaved, a probe holder was taped to the shaved skin, and 0.2 ml of 20% concentrated glucose in distilled water was applied topically through the hole in the probe holder during the course of imaging. The skin was imaged using OCT for 8 min prior to application of the glucose, and then for 2 h after application. The diffusion process was monitored in a 140 µm thick region 210 µm below the dermis region. The mean permeability rate of 20% glucose was calculated to be $(4.41 \pm 0.28) \times 10^{-6}$ cm/sec.

TOXICOLOGICAL STUDIES

Most of the ingredients included in this assessment are found in foods, and the daily exposure from that food use would result in a much larger systemic dose than that resulting from use in cosmetic products. Numerous studies and reviews have been published about the safety of dietary exposure to mono- and disaccharides. Examples of these reviews include the "Evaluation of the Health Aspects of Sucrose as a Food Ingredient",[49] and "Evaluation of the Health Aspects of Sodium, Potassium Magnesium, and Zinc Gluconates as Food Ingredients".[50] Also, many of the ingredients included in this report are used as inactive ingredients in approved drugs that are administered via numerous routes. Consequently, systemic toxicity is not addressed further in this report for those ingredients that are GRAS food substances, direct food additives, or identified in the *Food Chemicals Codex* as used in foods. The focus of the safe use of those mono- and disaccharides as cosmetic ingredients is on the potential for irritation and sensitization. When available, dermal toxicity, ocular irritation, and genotoxicity studies are included.

For the ingredients that are not identified as common dietary substances, i.e., the monosaccharides fucose, galacturonic acid, and mannose and the disaccharides galactosyl fructose, kefiran, lactulose, melibiose, and xylobiose, a search for oral toxicity data was performed. However, very little published data were found.

Single Dose (Acute) Toxicity

Dermal

Lactitol

The dermal LD_{50} of lactitol in rabbits is >4500 mg/kg bw.[20]

Oral

Lactulose

The oral LD_{50} of lactulose is 48.8 ml/kg in mice and >30 ml/kg in rats.[21]

Repeated Dose Toxicity

Oral

Lactulose

Groups of eight male albino rats were fed a diet containing 0.0, 0.5, 1.0, 2.0, or 5.0% (equivalent to 0.0, 1.1, 2.2, 4.0, and 11.3 g/kg bw/day, respectively) of a 50% lactulose syrup for 21 weeks.[64] None of the animals died during the study, and no signs of general toxicity were observed. Mild diarrhea was reported for animals fed >2.2 g/kg bw/day of the test material; diarrhea subsides with 3-5 h of feeding. Feed consumption was not statistically significantly affected at any dose level. The organ weights were similar for treated and control animals. A statistically significant increase in cecal weights in the 2 and 5% groups was considered an adaptive reaction. No toxicologically-significant changes in hematology, clinical chemistry, or urinalysis parameters were reported.

Ocular Irritation

In Vitro

Gluconic Acid

The ocular irritation potential of a 50% aq. solution of gluconic acid was evaluated *in vitro* in enucleated rabbit eyes.[9] The test material was applied to four eyes and observed over a period of 4 h following application. Slight corneal swelling and slight permeability of the superficial epithelial cells were not considered to be of any toxicological significance.

Isomalt

A battery of *in vitro* tests were performed to determine the ocular irritation potential of isomalt; based on the overall results of each test included in the battery, isomalt was classified as a non-irritant. A neutral red uptake (NRU) assay was performed in human keratinocytes, and the cytotoxicity of undiluted isomalt to the cells was measured after 24-h exposure.[65] Two experiments were performed. Undiluted isomalt was classified as a non-irritant in this *in vitro* test.

A red blood cell lysis and denaturation (RBC) assay, comprised of two range-finding and denaturation assays and two lysis assays, was performed in calf red blood cells.[66] Concentrations of ≤100,000 mg/l isomalt were tested. Isomalt did not induce hemolysis or protein denaturation, and was classified as a non-irritant. Based on the lack of induction of hemolysis, the predicted *in vivo* ocular irritation potential corresponded to a modified maximum average score of 0.

The third test in the battery was the hen's egg test on the chorioallantoic membrane (HET-CAM) in which isomalt was tested undiluted according to the endpoint assessment and at concentrations of 10 and 50% (w/w) in water according to the reaction-time method.[67] Each aspect of the experiment was performed twice. According to COLIPA (now, Cosmetics Europe) classifications, undiluted isomalt was classified as a slight irritant when tested undiluted in the endpoint assessment; the 10% and 50% concentrations were classified as non-irritant using the reaction-time method.

In Vivo – Non-Human

Gluconic Acid

A 50% aq. solution of gluconic acid was not irritating to rabbit eyes.[9] A 50% solution of gluconic acid (pH 1.8; 0.1 ml) was instilled into the conjunctival sac of one eye in nine New Zealand white rabbits; the contralateral eye served as an untreated control. The eyes of three animals were rinsed after 2 sec, and of another three animals after 4 sec; the eyes of the remaining three animals were not rinsed. The eyes were examined for irritation 1, 24, 48, and 72 h and 7 days after instillation. Slight redness and conjunctival swelling were observed initially; however, no signs of irritation were observed after 72 h.

Lactitol

Lactitol was not irritating to rabbit eyes.[20] The study was performed according to the Organisation for Economic Co-operation and Development (OECD) Guideline 405.[68] No other details were provided.

In Vivo – Human

Lactose

A face and neck formulation containing 2.48% lactose did not produce irritation or hypersensitivity in a 4-wk safety-in use ophthalmological evaluation.[69] Thirty-one subjects participated in the study.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

According to the package insert for the prescription drug lactulose, in studies of mice, rats, and rabbits, doses of lactulose solution up to 6 or 12 ml/kg/day produced no deleterious effects on breeding, conception, or parturition.[21] (Details were not provided.)

GENOTOXICITY

The genotoxicity of a number of the mono- and disaccharides has been evaluated in *in vitro* and *in vivo* studies. The results of these studies are overwhelmingly negative (Table 10[9,20,27,53,70-75]).

CARCINOGENICITY

According to the package insert for the prescription drug lactulose, administration of lactulose solution in the diet of mice for 18 mos in concentrations of 3 and 10% (v/w) did not produce any evidence of carcinogenicity.[21] (Details were not provided.)

IRRITATION AND SENSITIZATION

Dermal Irritation/Sensitization

Dermal irritation and sensitization studies are summarized in Table 11. In non-human studies, a 50% aq. solution of gluconic acid was not a dermal irritant[9] and lactitol was not an irritant or sensitizer in rabbits.[20] In human repeated insult patch tests (HRIPTs), formulations containing 10% rhamnose,[76] up to 8% glucose,[77,78] 5% mannose,[79] 2.48% lactose,[69] and less than 1% isomalt,[80] kefiran,[69] lactitol,[69] sucralose,[81] and xylobiose[82] were not irritants or sensitizers. A formulation containing 10% rhamnose did induce a significant irritation reaction in one subject,[76] and irritation was observed in 16% of the subjects during induction in an HRIPT of a rinse-off hair product containing 29% sucrose (tested as a 50% dilution); no sensitization reactions were reported for this product.[83]

OCCUPATIONAL EXPOSURE LIMITS

Sucrose

The National Institute for Occupational Safety and Health (NIOSH) recommended exposure limit (REL) for sucrose is a time-weighted average (TWA) of 10 mg/m^3 (total exposure) and TWA of 5 mg/m^3 (respiratory exposure).[25] The Occupational Safety and Health Administration (OSHA) permissible exposure limit (PEL) is a TWA of 15 mg/m^3 (total) and TWA of 5 mg/m^3 (respiratory). The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit value (TLV) is 10 mg/m^3 as TWA; it is in category A4, not classifiable as a human carcinogen.

SUMMARY

This report addresses the safety of 25 monosaccharides, disaccharides, and related ingredients as used in cosmetics. Many of these ingredients are GRAS food substances, direct food additives, or common dietary sugars, dietary sugar replacements, or very closely related analogs; for these ingredients, the focus of this review was on dermal irritation and sensitization. For the ingredients that are not identified as dietary substances, oral toxicity data were searched.

The monosaccharides, disaccharides, and related ingredients are reported to have a number of functions in cosmetics, and the most common function is as a skin conditioning agent; use as a humectant or flavoring agent was also common. According to VCRP data obtained from the FDA and concentration of use data obtained by the Council, 22 of the 25 ingredients reviewed in this assessment are reported to be in use. Sucrose has the greatest number of reported uses, 738, and glucose has the highest reported use concentration, 97.8% in an ingested breath freshener and 91% in "other" hair coloring products. The number of uses and maximum concentration of use varies widely by ingredient and type of use; most of the ingredients are used in leave-on products at less than 1%. Non-cosmetic uses include food use and use as inactive ingredients in approved drugs.

Although many of the ingredients included in this safety assessment are food ingredients, they are not all processed by the body in the same manner; some (e.g., glucose) are sources of energy and others (e.g., sucralose) are not. Also, absorption is not the same for each of these ingredients; some are absorbed in the intestines (e.g., glucose and potassium gluconate), while others are not absorbed in the gut (e.g., lactitol and sucralose).

In vitro, the permeability coefficient of glucose was 9.5×10^{-5} cm/h through full thickness nude mouse skin and 0.29 cm/h through the dermis (only) of nude mouse skin. *In vivo* in Rhesus monkeys, using OCT, the mean permeability rate of 20% glucose was calculated to be $(4.41 \pm 0.28) \times 10^{-6}$ cm/sec.

Lactulose fed to rats at concentrations of up to 5.0% of 50% lactulose syrup for 21 weeks did not result in toxicity. Mild diarrhea was reported for animals fed >2.2 g/kg bw/day of the test material; diarrhea subsides with 3-5 h of feeding. Doses of up to 12 ml/kg/day of lactulose solution produced no deleterious effects on breeding, conception, or parturition in mice, rats, or rabbits. No evidence of carcinogenicity was observed in mice with dosing of up to 10% lactulose solution in the diet for 18 mos.

A battery of *in vitro* tests were performed to determine the ocular irritation potential of isomalt; based on the results, isomalt was classified as a non-irritant. Gluconic acid, as a 50% aq. solution, and lactitol, concentration not specified, were not irritating to rabbit eyes. A face and neck formulation containing 2.48% lactose did not produce irritation or hypersensitivity in a 4-wk safety-in use ophthalmological evaluation

In non-human studies, a 50% aq. solution of gluconic acid was not a dermal irritant and lactitol was not an irritant or sensitizer in rabbits. In human repeated insult patch tests (HRIPTs), formulations containing 10% rhamnose, 8% glucose, 5% mannose, 2.48% lactose, and less than 1% isomalt, kefiran, lactitol, sucralose, and xylobiose were not irritants or sensitizers. A formulation containing 10% rhamnose did induce a significant irritation reaction in one subject, and irritation was observed in 16% of the subjects during induction in a HRIPT of a product containing 29% sucrose (that was a rinse-off hair product tested as a 50% dilution); no sensitization reactions were reported for this product.

Lactitol, sodium gluconate, sucralose, sucrose and trehalose were not genotoxic *in vitro*. Additionally, the genotoxic potential of sodium gluconate, sucralose, and trehalose was evaluated *in vivo*; again negative results were obtained.

DISCUSSION

The Panel reviewed this safety assessment of monosaccharides, disaccharides, and related ingredients. Most of these ingredients are common dietary sugars, dietary sugar replacements, or very closely related analogs and salts. Several are GRAS food additives, direct food additives, listed in the *Food Chemicals Codex* as used in foods, and/or listed in REACH Annex IV. Because the oral safety of these ingredients has been well-documented, systemic toxicity is not a concern of the Panel.

Some of the ingredients, however, are not GRAS food substances or direct food additives; even so, these ingredients are either listed in the *Food Chemicals Codex* as having a function in foods, listed in the Everything Added to Foods in the United States (EAFUS) inventory, and/or listed as an inactive ingredient in oral drugs. Moreover, the leave-on use concentrations of these ingredients are typically less than 1%. Therefore, the Panel stated that although oral toxicity data are very limited and reproductive toxicity data are mostly absent, the systemic toxicity of these ingredients was not a concern because of the low concentrations of use and their limited systemic exposure from dermal application.

The Panel commented that sucrose is used at high concentrations in some products that come in contact with mucous membranes (i.e., 65% in personal cleanliness products). The Panel noted that sucrose is a GRAS food substance, and therefore, the Panel was not concerned about this reported use. Additionally, the Panel observed that glucose is reported to be used at 97.8% in an ingestible oral hygiene product, but recognized that glucose is a GRAS direct food additive with no limitations other than following current good manufacturing practice. However, if an ingredient that does not have GRAS

food additive status was used at concentrations such as these with similar exposure-types, the Panel would most likely want data substantiating the safety of that use, such as metabolism after oral administration.

The Panel discussed a human repeated insult patch test of a hair product that contained 29% sucrose, diluted to 50%, that reported irritation during induction. The Panel concluded that the irritation reported was likely attributable to a surfactant effect, and was not due to sucrose itself. The Panel acknowledged that sucrose and glucose are used in cosmetics at relatively high concentrations, and that data from irritation and sensitization studies at maximum use concentrations of these ingredients are lacking; however, based on the clinical experience of the Panel, there is little concern that these ingredients are irritants or sensitizers.

Because some of the ingredients included in this safety assessment can be used in products that may be aerosolized, the Panel discussed the issue of incidental inhalation exposure. Most of the use concentrations of the ingredients used in cosmetic products that may be aerosolized are less than 1% (e.g., glucose is used at 1% in a spray body and hand preparation). In the absence of inhalation data, the Panel noted that 95% – 99% of droplets/particles produced in cosmetic aerosols would not be respirable to any appreciable amount. The Panel acknowledged that the potential for inhalation toxicity is not limited to respirable droplets/particles deposited in the lungs, but because of the small actual exposure in the breathing zone and the concentrations at which the ingredients are used, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects.

Finally, because many of these ingredients are obtained from plant sources, the Expert Panel expressed concern regarding pesticide residues and heavy metals that may be present. They stressed that the cosmetics industry should continue to use the necessary procedures to limit these impurities in the ingredient before blending into cosmetic formulation.

CONCLUSION

The CIR Expert Panel concluded that the following 25 monosaccharides, disaccharides, and related ingredients are safe in the present practices of use and concentration in cosmetics described in this safety assessment:

calcium gluconate
fructose
fucose
galactose*
galactosyl fructose
galacturonic acid*
gluconic acid
glucose
isomalt
kefiran
lactitol
lactose
lactulose*
maltose
mannose
melibiose
potassium gluconate
rhamnose
ribose
sodium gluconate
sucralose
sucrose
trehalose
xylobiose
xylose

**Not reported to be in current use. Were ingredients in this group not in current use to be used in the future, the expectation is that they would be used in product categories and at concentrations comparable to others in this group.*

TABLES

Table 1. Definitions, Structures, and Reported Functions

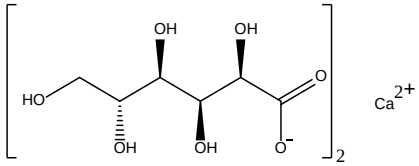
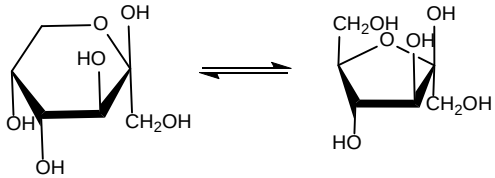
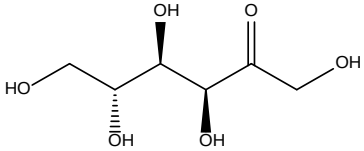
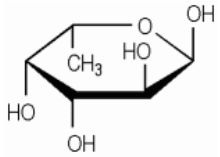
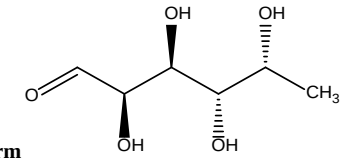
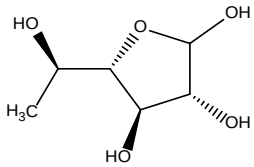
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Calcium Gluconate 299-28-5	the calcium salt of gluconic acid		chelating agent; skin-conditioning agent - miscellaneous
Fructose 30237-26-4 57-48-7 (D-)	a sugar which occurs in fruit and honey; *fructose exists in solution primarily as two cyclized forms in equilibrium, namely fructopyranose and fructofuranose.*	 ***** open chain form that exists between the furanose and pyranose forms** 	flavoring agent; humectants skin-conditioning agent - humectant
Fucose 2438-80-4 (L-) 3615-37-0 (D-)	the organic compound that conforms to the formula provided; *fucose is a deoxyhexose that is present in a wide variety of organisms; unlike most sugars, fucose occurs in nature as the L-form and lacks a hydroxyl group on the carbon at the 6-position (C-6).*	  ***** open chain form**  ***** furanose form**	skin-conditioning agent - miscellaneous

Table 1. Definitions, Structures, and Reported Functions

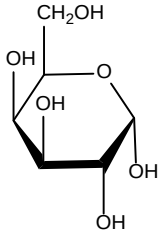
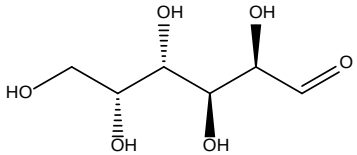
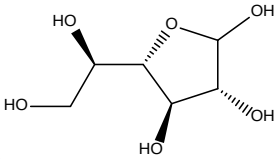
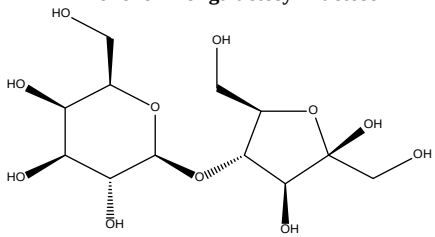
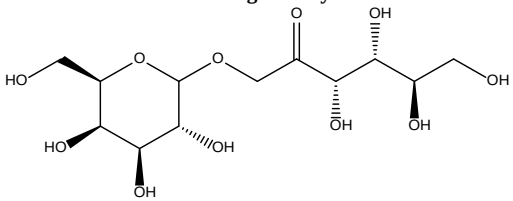
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Galactose 59-23-4	the sugar that conforms to the formula provided; *galactose is the C4 epimer of glucose*	 ***** open chain form**   ***** furanose form**	skin-conditioning agent - miscellaneous
Galactosyl Fructose 110312-93-1	a disaccharide consisting of galactose and fructose	***** one form of galactosyl fructose**  ***** one form of galactosyl fructose** 	skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

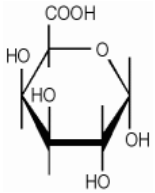
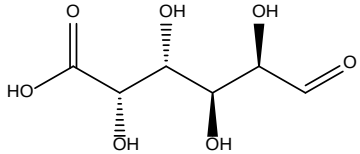
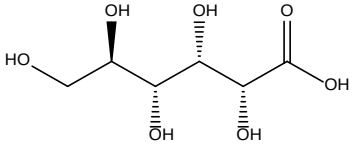
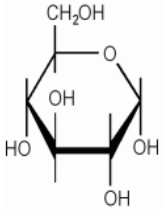
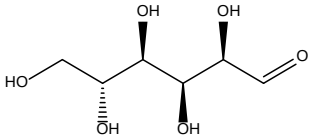
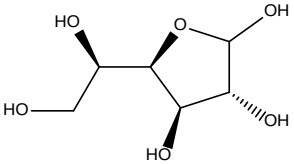
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Galacturonic Acid 14982-50-4 (DL-) 552-12-5 (D-) 685-73-4 (D-)	the organic compound that conforms to the formula provided; *galacturonic acid is the c-6 oxidation product of galactose*	  *** open chain form	chelating agent; skin-conditioning agent - humectant; pH adjuster
Gluconic Acid 133-42-6; 526-95-4	the organic compound that conforms to the formula provided; *gluconic acid is the C1 oxidation product of glucose*		chelating agent; fragrance ingredient
Glucose 50-99-7 (D-) 58367-01-4 (DL-) 5996-10-1 (DL-) 8029-43-4	a sugar that is generally obtained by the hydrolysis of starch	  *** open chain form  *** furanose form	flavoring agent; humectants; skin-conditioning agent-humectant; skin-conditioning agent – miscellaneous

Table 1. Definitions, Structures, and Reported Functions

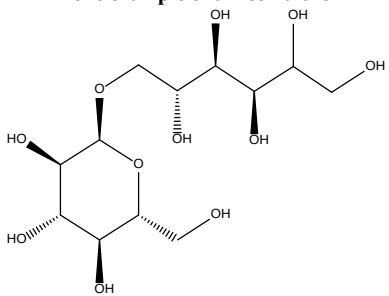
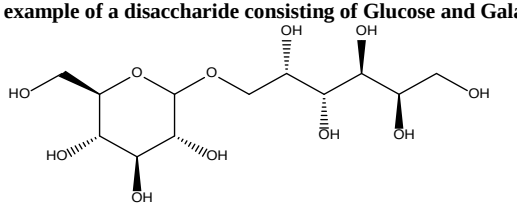
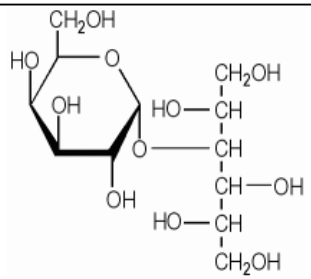
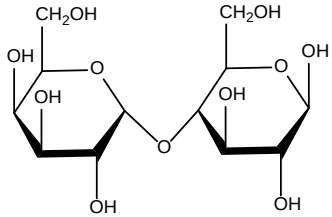
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Isomalt 64519-82-0	a mixture of polysaccharides produced by the enzymatic rearrangement of sucrose; it consists chiefly of 1-O-α-D-glucopyranosyl-D-mannitol dihydrate and 6-O-α-D-glucopyranosyl-d-sorbitol	***** one example of an isomalt form** 	anticaking agent; bulking agent; flavoring agent
Kefiran 86753-15-3	a disaccharide consisting of glucose and galactose	***** one example of a disaccharide consisting of Glucose and Galactose** 	skin-conditioning agent - humectant
Lactitol 585-86-4	a disaccharide polyol obtained by the controlled hydrogenation of lactose		flavoring agent; humectant; skin-conditioning agent - humectant
Lactose 63-42-3	the disaccharide that conforms to the formula provided; *lactose is the disaccharide (β1 →4) galactosyl-glucose*		skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

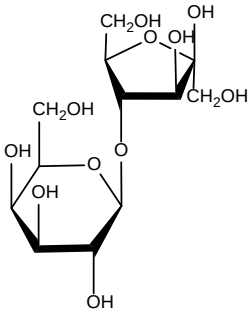
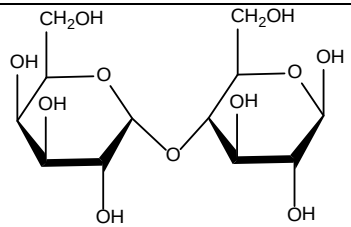
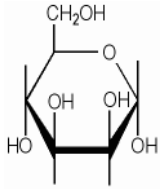
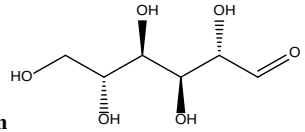
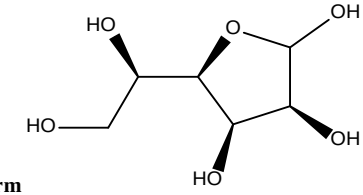
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Lactulose 4618-18-2	the disaccharide that conforms to the formula provided; *lactulose is the disaccharide (β1 →3) galactopyranosyl-fructo-furanose*		skin-conditioning agent - humectant
Maltose 16984-36-4; 69-79-4	the sugar that conforms to the formula provided; *maltose is the disaccharide α(1 →4) glucosyl-glucose*		flavoring agent; humectant; skin-conditioning agent - humectant
Mannose 3458-28-4	the sugar that conforms to the formula provided; *mannose is the C2 epimer of glucose*	  *** **open chain form**  *** **furanose form**	humectant; skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

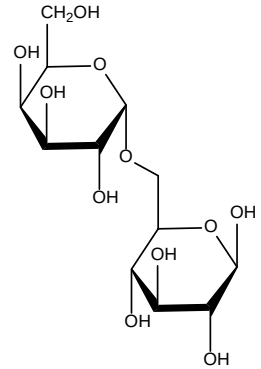
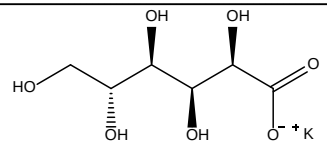
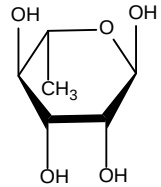
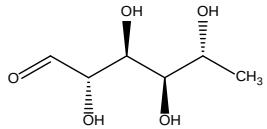
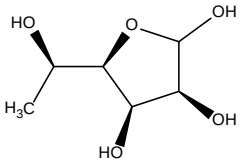
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Melibiose 5340-95-4; 585-99-9	the carbohydrate that conforms to the formula provided; *melibiose is the disaccharide α(1→6) galactosyl-glucose*		skin-conditioning agent – humectant
Potassium Gluconate 299-27-4	the potassium salt of gluconic acid		chelating agent; skin-protectant
Rhamnose 10030-85-0 3615-41-6 (L-)	the organic compound that conforms to the formula provided; *unlike most naturally abundant sugars, rhamnose occurs in nature as the L form and lacks a hydroxyl group on the carbon at the 6-position (C6)*	  ***** open chain form**  ***** furanose form**	flavoring agent; fragrance ingredient

Table 1. Definitions, Structures, and Reported Functions

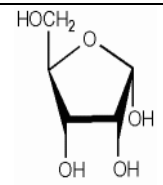
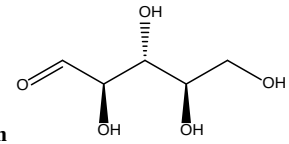
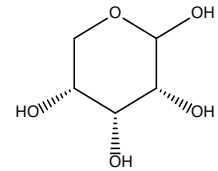
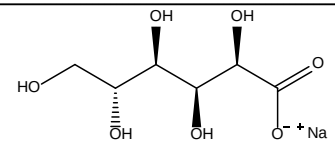
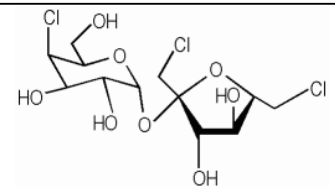
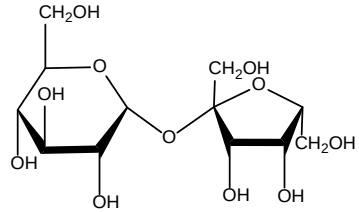
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Ribose 50-69-1	the sugar that conforms to the formula provided; *ribose is an aldopentose*	 *** open chain form  *** pyranose form 	humectant; skin-conditioning agent - humectant
Sodium Gluconate 14906-97-9 527-07-1	the sodium salt of gluconic acid		chelating agent; skin-conditioning agent - miscellaneous
Sucralose 56038-13-2	the organic compound that conforms to the formula provided; *sucralose is a selectively tri-chlorinated analog of sucrose (1,6-fructo- and 4-galacto-chlorinated)*		flavoring agent
Sucrose 57-50-1	the disaccharide that conforms to the formula provided; *sucrose is the disaccharide α(1→4) glucosyl-fructose*		flavoring agent; humectant

Table 1. Definitions, Structures, and Reported Functions

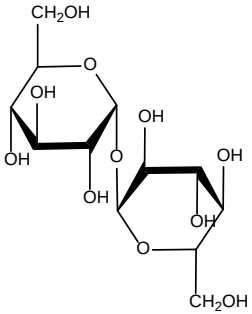
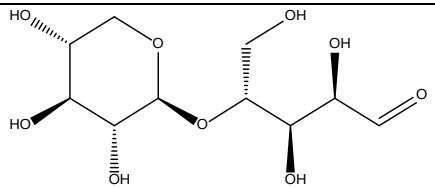
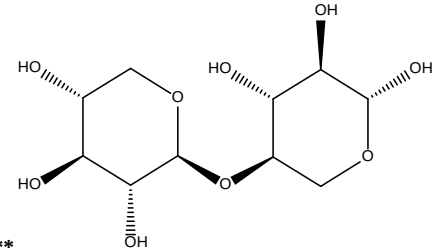
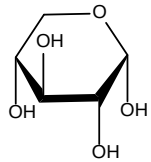
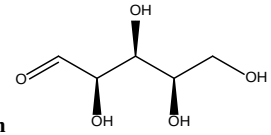
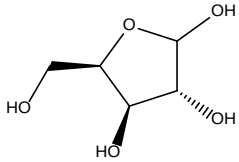
Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Trehalose 99-20-7; 6138-23-4	the disaccharide that conforms to the formula provided; *trehalose is the disaccharide α(1 →1) glucosyl-glucose*		flavoring agent; humectant
Xylobiose 6860-47-5	a disaccharide consisting of two xylose units with β-1 to β-4 linkage	 ***  ***	skin-conditioning agent - humectant

Table 1. Definitions, Structures, and Reported Functions

Ingredient (CAS No.)	Definition[1]*	Structure[1]***	Reported Function(s)[1]
Xylose 58-86-6	the sugar that conforms to the formula provided; *xylose is an aldopentose*	 *** open chain form  *** furanose form 	flavoring agent; fragrance ingredient; humectant; skin-conditioning agent - humectant

*The italicized text represents additions made by CIR staff.
Structures preceded with asterisks (***) have been added by CIR staff

Table 2. Chemical and Physical Properties

Property	Description	Reference
	Calcium Gluconate	
physical characteristics	odorless, white, crystalline granules or powder	7
molecular weight	430.4	8
melting point	120°C	9
solubility	soluble in water; insoluble in ethanol	7
density	0.30-0.65 g/cm³ (bulk density)	9
log P_{ow}	-7.51 (estimated)	9
	Fructose	
physical characteristics	D-: orthorhombic, bisphenoidal prisms from alcohol DL-: needles from methanol white crystals or powder	10 11
molecular weight	180.16	10
particle size distribution	crystalline fructose: 170-450 µm powdered fructose: 25-40 µm	12
melting point	D-: decomposes at 103-105°C DL-: 129-130°C	10
solubility	D-: freely soluble in water; slightly soluble in cold and freely soluble in hot acetone; soluble in methanol, ethanol, pyridine, ethylamine, and methylamine; insoluble in ether	3,10
specific optical rotation (α^{20}_{D})	D-: shows mutarotation; -132° to -92°	10
density	1.59 kg/m^3 (20°C)	13
pK_a	D-: 12.06 (18°C)	10
specific gravity (d^{16}_{4})	DL-: 1.665	10
	Fucose	
physical characteristics	D-, α-form: needles from alcohol; sweet taste L-, α-form: minute needles from absolute alcohol	10
molecular weight	164.16	10
melting point	D-, α-form: 144°C L-, α-form: 140°C	10
solubility	D-, α-form: soluble in water; moderately soluble in alcohol L-, α-form: soluble in water and alcohol	10
specific optical rotation (α^{19}_{D})	D-, α-form: shows mutarotation; +127.0° (7 min) ⟶+89.4° (31 min) ⟶+77.2° (71 min) ⟶ +76.0° (final value 146 min)	10
specific optical rotation (α^{20}_{D})	L-, α-form: shows mutarotation, -124.1° (10 min) ⟶-108.0° (20 min) ⟶-91.5° (36 min) ⟶-78.6° (70 min) ⟶-75.6° (final value, 24 hrs)	10
	Galactose	
physical characteristics	α-form: prisms from water or ethanol β-form: crystals monohydrate: prisms from water	10
molecular weight	180.16	10
melting point	α-form: 167°C β-form: 167°C monohydrate: 118-120°C	10
solubility	α-form: freely soluble in hot water; soluble in pyridine; slightly soluble in alcohol	10
specific optical rotation (α_{D})	α-form: +150.7° ⟶ +80.2° (water) β-form: +52.8° ⟶ +80.2° (water) D-, α-form: (α^{20}_{D}): +78.0° to 81.5°	10 14
	Galactosyl Fructose	
molecular weight	342.30 (predicted)	15
boiling point	780.1 ± 60°C (at 760 Torr; predicted)	15
log P	-2.810 ± 0.846 (at 25°C; predicted)	15
	Galacturonic Acid	
physical characteristics	α-form: monohydrate, needles	10
molecular weight	194.14	10
melting point	α-form: 159°C β-form: 166°C	10
solubility	α-form: soluble in water; slightly soluble in hot alcohol; practically insoluble in ether	10
specific optical rotation	α-form, (α^{20}_{D}): +98.0° ⟶ +50.9° (water) β-form, (α_{D}): +27° ⟶ +55.6° (water)	10
	Gluconic Acid	
physical characteristics	crystals; mild acid taste white crystalline powder anhydrous: commercial form is a 50% aq. solution, which is a colorless to brownish liquid.	16 17 9,17
molecular weight	196.16	16

Table 2. Chemical and Physical Properties

Property	Description	Reference
melting point	131°C	16
solubility	freely soluble in water; slightly soluble in alcohol; insoluble in ether and most other organic solvents	16
stability	in aq. solutions, the acid is partially transformed into an equilibrium mixture with γ - and δ-gluconolactones	16
	reacts with strong oxidants	17
	on combustion, forms carbon monoxide	17
specific optical rotation (α^{20}_{D})	-16.7°	16
density	1.23 g/cm^3	17
log P_{ow}	-1.87 (estimated)	17
pK_a	12.06 (18°C)	16
	Glucose	
physical characteristics	α-form monohydrate: crystals from water α-form anhydrous: crystals from hot ethanol or water β-form: crystals from hot water and ethanol, from diluted acetic acid, or from pyridine	10
	white D-glucose: powder with sweet taste	18
molecular weight	180.16	10
melting point	α-form monohydrate: 83°C α-form anhydrous: 146°C β-form: 148-155°C	10
solubility	α-form anhydrous: soluble in hot glacial acetic acid, pyridine, aniline; very sparingly soluble in absolute alcohol, ether, acetone	10
log P_{ow}	D-glucose: -3.3	18
specific optical rotation	α-form monohydrate, (α^{20}_{D}): +102.0° ⟶ +47.9°C (water) α-form anhydrous, (α^{20}_{D}): +112.2° ⟶ +52.7°C (water) β-form, (α^{20}_{D}): +18.7° ⟶ +52.7° (water)	10
stability	D-glucose reacts violently with strong oxidants	18
	Isomalt	
physical characteristics	white crystalline, odorless, slightly hydroscopic substance	3,19
molecular weight	380.32	3
boiling point	788.5 ± 60°C (at 760 Torr; predicted)	15
solubility	soluble in water; very slightly soluble in ethanol	3,19
log P	-2.810 ± 0.846 (at 25°C; predicted)	15
pK_a	12.89 ± 0.70 (25°C) (predicted)	15
	Lactitol	
physical characteristics	crystals from absolute ethanol; strongly hygroscopic monohydrate: white, sweet, odorless crystalline solid; non-hygroscopic dihydrate: white, sweet, odorless crystalline powder	10
molecular weight	344.31 (anhydrous); 362.37 (monohydrate)	3,10
melting point	146°C monohydrate: 94-97°C dihydrate: 75°C (food-grade)	10
partition coefficient	< -3 (20°C)	20
solubility	soluble in water, dimethyl sulfoxide, *N*,*N*-dimethylformamide; slightly soluble in ethanol, ether	10
specific optical rotation	(α^{23}_{D}): +14° monohydrate, (α^{22}_{D}): +12.3° dihydrate, (α^{25}_{D}): +13.5 – 15.0°	10
	Lactose	
physical characteristics	α-lactose monohydrate: monoclinic sphenoidal crystals from water; faintly sweet taste; readily absorbs odors	10
molecular weight	342.30	10
particle size distribution	varies by grade	12
melting point	α-lactose monohydrate: 201-202°C	10
solubility	α-lactose monohydrate: practically insoluble in alcohol; insoluble in chloroform, ether	10
specific optical rotation	α-lactose monohydrate, (α^{20}_{D}): shows mutarotation; +92.6° ⟶+83.5° (10 min.) ⟶+69° (50 min) ⟶+52.3° (22 h) β-lactose, (α^{25}_{D}): +34° (3 min) ⟶+39° (6 min) ⟶+46° (1 hr) ⟶+52.3° (22 h)	10
K_a (16.5°C)	α-lactose monohydrate: 6.0 x 10^{-13}	10
	Lactulose	
physical characteristics	hexagonal clustered plates from methanol	10
molecular weight	342.30 (anhydrous); 360.32 (monohydrate)	3,10
melting point	168-171°C	10
solubility	freely soluble in water	21
specific optical rotation (α^{22}_{D})	shows mutarotation; constant value after 24 h, -51.5°	10

Table 2. Chemical and Physical Properties

Property	Description	Reference
	Maltose	
physical characteristics	monohydrate: crystals from water or diluted alcohol	10
molecular weight	342.30	10
melting point	monohydrate: 102-103°C	10
solubility	α-lactose monohydrate: practically insoluble in alcohol; insoluble in chloroform, ether	10
pH	anhydrous: 3.7-4.7; monohydrate: 4.5-5.5	14
specific optical rotation (α^{20}_{D})	monohydrate: shows mutarotation; +111.7° ⟶+130.4°	10
pK_a (21°C)	monohydrate: 12.05	10
	Mannose	
physical characteristics	α-form: crystals from methanol β-form: orthorhombic, bisphenoidal needles from alcohol or acetic acid; sweet taste with bitter aftertaste	10
molecular weight	180.16	10
melting point	α-form: 133°C β-form: decomposes at 132°C	10
specific optical rotation	α-form, (α_{D}): +29.3° ⟶ +14.2° (water) β-form, (α^{20}_{D}): -17.0° ⟶ +14.2° (water)	10
	Melibiose	
physical characteristics	dihydrate: monoclinic crystals from water of diluted alcohol	10
molecular weight	342.30	10
dihydrate	α-form: 84-85°C	10
specific optical rotation (α^{20}_{D})	dihydrate:: +111.7° ⟶ +129.5°	10
	Potassium Gluconate	
physical characteristics	yellowish-white crystals; mild, slightly saline, taste	16
molecular weight	234.25 (anhydrous); 252.26 (monohydrate)	3,16
melting point	decomposes at 180°C	16
solubility	freely soluble in water and glycerin; practically insoluble in alcohol, ether, benzene, and chloroform	3,16
log P_{ow}	-5.99 (estimated)	9
pH	7.5-8.5 (aq. solution)	16
stability	stable in air	16
specific optical rotation (α^{20}_{D})	-16.7°	16
density	0.80 g/cm^3 (20°C; bulk density)	9
	Rhamnose	
physical characteristics	α-form: monohydrate, holohedric rods from water; hemihedric monoclinic columns from alcohol; very sweet taste β-form: needles; hygroscopic	10
molecular weight	164.16	10
melting point	α-form: 82-92°C; sublimes at 105°C and 2 mm Hg β-form: 122-126°C	10
specific optical rotation	α-form, (α^{20}D): shows mutarotation; -7.7° ⟶ +8.9° β-form, (α^{20}_{D}): -17.0° ⟶ +31.5°	10
specific gravity (d^{20}_{4})	1.4708	10
stability	α-form: loses water of crystallization upon heating, and partially changes to the β-modification β-form: changes into crystals of the α-modification upon exposure to moist air	10
	Ribose	
physical characteristics	plates from absolute alcohol	10
molecular weight	150.13	10
melting point	87°C	10
solubility	soluble in water, slightly soluble in alcohol	10
specific optical rotation (α^{20}_{D})	final, shows complex mutarotation; -25°	10
	Sodium Gluconate	
physical characteristics	white crystals white to tan, granular to fine, crystalline powder technical grade may have a pleasant odor	22 3 16
molecular weight	218.14	16
melting point	170-175°C; decomposes at 196-198°C	22
solubility	soluble in water; sparingly soluble in alcohol; insoluble in ether	16
log P_{ow}	-5.99 (estimated)	22
density	1.8 g/cm^3	22
	Sucralose	
physical characteristics	anhydrous crystalline form: orthorhombic needle-like crystals; intensely sweet taste	10
molecular weight	397.63	10

Table 2. Chemical and Physical Properties

Property	Description	Reference
particle size distribution	90% <12 µm	12
solubility	soluble in water	23
octanol/water partition coefficient	-0.51 ($\log_{10}$ P)	12
specific optical rotation (α_D)	+68.2°	10
	(α^{20}_D): +84.0° to +87.5°, calculated on the anhydrous basis	3
	Sucrose	
physical characteristics	monoclinic sphenoidal crystals, crystalline masses, blocks, or powder; sweet taste	10
	hard, white, odorless crystals, lumps, or powder; may have a characteristic caramel odor when heated	24
molecular weight	342.30	10
melting point	decomposes at 160-186°C	10
solubility	moderately soluble in glycerol, pyridine; practically insoluble in dehydrated alcohol	10
log P_{ow}	-3.67	25
specific optical rotation	(α^{20}_D): +65.9° to +66.7°	3
	(α^{25}_D): +66.47 to +66.49°	10
pK_a	12.62	10
specific gravity (d^{25}_4)	1.587	10
stability	stable in air hydrolyzed to glucose and fructose by diluted acids and by invertase	10
	Trehalose	
physical characteristics	orthorhombic, bisphenoidal crystals for diluted alcohol; sweet taste	10
	typically found in the dihydrate form; characterized by low hygroscopicity	26,27
molecular weight	342.30	10
melting point	the dihydrate melts at 97°C; additional heat drives off the water of crystallization until it resolidifies at 130°C; the anhydrous then melts at 210°C	27
solubility	very soluble in water, formamide, and dimethyl sulfoxide; soluble hot alcohol; slightly soluble to insoluble in ether	3,10
stability	very stable and chemically unreactive; does not dissociate into two reducing monosaccharidic constituents unless exposed to extreme hydrolytic conditions or to the actions of trehalase	28
specific optical rotation (α^{20}_D)	+178°	10
	Xylobiose	
molecular weight	282.24 (predicted)	15
boiling point	604 ± 55°C (at 760 Torr; predicted)	15
log P	-2.900 ± 0.852 (at 25°C; predicted)	15
pK_a	12.40 ± 0.20 (25°C) (predicted)	15
	Xylose	
physical characteristics	monoclinic needles or prisms; very sweet taste	10
	white, odorless, crystal or crystalline powder with a sweet taste	29
molecular weight	150.13	10
melting point	144-145°C	10
solubility	soluble in glycerol, pyridine, hot alcohol	10
specific optical rotation (α^{20}_D)	shows mutarotation; +92° ⟶+18.6° (16 hrs)	10
pK_a (18°C)	12.14	10
specific gravity (d^{20}_4)	1.525	10

Table 3. Natural Occurrence and /or Methods of Preparation

Ingredient	Natural Occurrence and/or Method of Preparation	Reference
Fructose	- occurs in many fruits and in honey	10
	- prepared by adding absolute alcohol to the syrup obtained from the acid hydrolysis of inulin; prepared from dextrose; prepared from sucrose by enzymatic conversion - obtained from glucose in corn syrup by the use of glucose isomerase	3,13
Fucose	D-: obtained from glucosides found in various species of Convolvulaceae	10
	L-: occurs in seaweed - *Ascophyllum nodosum*, (*Fucus nodosus*), *Fucus vesiculosu*., *F. serratus*, *F. virsoides*, *Fucaceae* - and in gum tragacanth L-: a common component of many N- and O-linked glycans and glycolipids produced by mammals	48
Galactose	- constituent of many oligo- and polysaccharides in pectins, gums, and mucilages; isolation in the processing of the red algae, *Porphyra umbilicalis*	10
	- a product of lactose metabolism	14
Galacturonic Acid	obtained by hydrolysis of pectin where it is present as polygalacturonic acid	10
Gluconic Acid	- prepared by oxidation of glucose; produced commercially using *Aspergillus niger*, *A. fumaricus*, *Aerobacter aceti*, *Penicillium chrysogenum*, or other *Penicillia*	16,50

Table 3. Natural Occurrence and /or Methods of Preparation

Ingredient	Natural Occurrence and/or Method of Preparation	Reference
Glucose	- produced by the complete hydrolysis of corn starch with safe and suitable acids or enzymes, followed by refinement and crystallization from the resulting hydrolysate - occurs naturally and in the free state in fruits and other parts of plants; combined in glucosides, in disaccharides and oligosaccharides, in the cellulose and starch of polysaccharides, and in glycogen; manufactured on a large scale from starch; below 50°C, α-D-glucose hydrate is the stable crystalline form, above 50°C, the anhydrous form is obtained, and at higher temperatures, β-D-glucose is formed - normal human blood contains 0.08-0.1%	21CFR184.1857 10
Isomalt	produced from food-grade sucrose in a two-stage process: beet sugar is converted by enzymatic transglucosidation into isomaltulose, which undergoes catalytical hydrogenation to produce isomalt	12
Lactitol	prepared by the hydrogenation of lactose	10
Lactose	- present in the milk of mammals: human, 6.7%: cow, 4.5% - by-product of the cheese industry, produced from whey - β-lactose: obtained by crystallizing concentrated solutions of α-lactose above 93.5°C	10
Lactulose	- synthetic disaccharide composed of galactose and fructose - can be produced from agricultural by-products and from lactose	10 64
Maltose	obtained in 80% yield by enzymatic (diastase) degradation of starch	10
Mannose	α-form prepared by treating ivory nut shavings with H_2SO_4	10
Melibiose	prepared from raffinose by fermentation with top yeast, which removes the fructose	10
Potassium Gluconate	prepared by the reaction of potassium hydroxide or carbonate with gluconic acid	45
Rhamnose	- occurs free in poison sumac; combined in the form of glycosides of many plants; isolated from the walls of gram-negative bacteria α-form: obtained by crystallization from water or ethyl alcohol β-form: prepared by heating α-rhamnose monohydrate on a steam bath	10
Ribose	prepared by hydrolysis of yeast-nucleic acid; obtained from glucose, nucleosides, D-erythrose, and L-glutamic acid; obtained by the reduction of D-ribonic acid	10
Sucralose	- chlorinated derivative of sucrose - synthesized by selective chlorination of sucrose at three of the primary hydroxyl groups - can be synthetized by the reaction of sucrose (or an acetate) with thionyl chloride	10 55 12
Sucrose	- obtained from sugar cane and sugar beet: sugar cane (*Saccharum officinarum*) contains 10-15% sucrose, sugar beet (*Beta vulgaris*) contains 10-17% sucrose - sucrose is obtained by crystallization from sugar cane or sugar beet juice that has been extracted by pressing or diffusion, then clarified and evaporated - most abundant carbohydrate in the sap of land plants	10 21CFR184.1854 49
Trehalose	- found in fungi, bacteria, yeasts, and insects; isolated from the ergot of rye; isolated from yeast - produced from starch using the enzymes maltooligosyl-trehalose synthase and maltooligosyl-trehalose trehalohydrolase	10 27
Xylose	- widely distributed in plant materials, especially wood (maple, cherry), in straw, and in hulls; not found in the free state – is found in the form of xylan, a polysaccharide consisting of D-xylose units occurring in association with cellulose; also occurs as part of glycosides; can be isolated from corn cobs - produced industrially by hydrolysis of extracts from cotton seed shells, press residue of sugarcane and beech tree chips	10 29

Table 4. Purity specifications

Ingredient	Purity Specifications
Fructose	food use: NMT 0.018% chloride; NMT 0.1 mg/kg lead; NMT 0.5% glucose; NMT 0.1% hydroxymethylfurfural, calculated on the dried ash and free-ash basis; NMT 0.5% loss on drying; NMT 0.5% residue on ignition (sulfated ash)[3] USP: NMT 1 ppm arsenic;; NMT 5 ppm heavy metals: NMT 0.5% loss on drying; NMT 0.5% residue on ignition[14]
Galactose	USP: NMT 1.0% water; NMT 0.1% residue on ignition[14]
Isomalt	food use: NMT 7% water; NMT 0.05% sulfated ash; NMT 3% D-mannitol; NMT 6% D-sorbitol; NMT 0.3% reducing sugars; NMT 2 mg/kg nickel; NMT 1 mg/kg lead[19]; - NMT 1 mg/kg lead; NMT 2 mg/kg nickel; NMT 3% mannitol and NMT 6% sorbitol; NMT 0.3% cuprous oxide (as glucose); NMT 7.0% water; NMT 0.05% residue on ignition (sulfated ash)[3] USP: NMT 7% water; NMT 1 µg/g nickel; NMT 10 µg/g heavy metals; NMT 0.3% reducing sugars[14]
Lactitol	food use: NMT 1 mg/kg lead; NMT 1 mg/kg nickel; NMT 4.0% other hydrogenated saccharides (polyols); NMT 5% water; NMT 0.3% cuprous oxide residue; NMT 0.1% residue on ignition (sulfated ash)[3] USP: 4.5-5.5% water, monohydrate, or 10.5%, dihydrate; NMT 0.5% water, anhydrate; NMT 0.5% residue on ignition[14]
Lactose	food use: NMT 0.5 mg/kg arsenic; NMT 0.5 mg/kg lead; NMT 0.3% residue on ignition (sulfated ash)[3]; loss on drying: not less than 4.5% and NMT 5.5%, monohydrate and spray-dried mixture; NMT 1.0%, anhydrous[3] USP: water: NMT 1.0%, anhydrous, 4.5-6.5%, monohydrate; heavy metals: 5 µg/g, anhydrous and monohydrate; loss on drying: NMT 0.5%, anhydrous and monohydrate; residue on ignition: NMT 0.1%, anhydrous and monohydrate[14]
Maltose	USP: water: NMT 1.5%, anhydrous, 4.5-5.5%, monohydrate; NMT 5 µg/g heavy metals; NMT 0.05% residue on ignition[14]

Table 4. Purity specifications

Ingredient	Purity Specifications
Potassium Gluconate	food use: NMT 1% calculated as D-glucose;[84] NMT 2 mg/kg lead;[3,84] NMT 1.0% reducing substances; NMT 3.0% (anhydrous) and 6.0-7.5% (monohydrate) loss on drying[3] USP: NMT 0.002% heavy metals; NMT 1.0% reducing substances; loss on drying: NMT 3.0%, anhydrous, and 6.0-7.5%, monohydrate [14]
Sodium Gluconate	food use: NMT 2 mg/kg lead; NMT 0.5% reducing substances, calculated as D-glucose [3] USP: NMT 0.001% lead; NMT 0.002% heavy metals; NMT 0.5% reducing substances[14]
Sucralose	food use: NMT 1 mg/kg lead; NMT 2.0% water; NMT 0.1% methanol; NMT 0.7% residue on ignition (sulfated ash)[3] USP: NMT 2.0% water; NMT 0.001% heavy metals; NMT 0.7% residue on ignition[14]
Sucrose	food use: NMT 1 mg/kg arsenic; NMT 0.1 mg/kg lead; NMT 0.1% invert sugars; NMT 0.15% residue on ignition (sulfated ash); NMT 0.1% loss on drying[3] USP: NMT 5 ppm heavy metals; NMT 0.05% residue on ignition[14]
Trehalose	food use: NMT 0.1 mg/kg lead; NMT 11.0% water; NMT 0.05% residue on ignition (sulfated ash)[3]
Xylose	USP: NMT 5 ppm iron; NMT 0.001% heavy metals; NMT 0.1% loss on drying; NMT 0.5% residue on ignition[14]

Abbreviation: NMT – not more than

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]
	Calcium Gluconate		**Fructose**		**Fucose**	
Totals*	**68**	**0.0000075-1**	**172**	**0.0001-20**	**3**	**NR**
Duration of Use						
Leave-On	*50*	*0.0000075-1*	*144*	*0.0002-2*	*3*	*NR*
Rinse Off	*18*	*0.0000075-0.1*	*28*	*0.0001-20*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	4	0.0000075-.05	10	0.002-0.075	1	NR
Incidental Ingestion	NR	0.00006-0.5	1	NR	NR	NR
Incidental Inhalation-Spray	21[a]; 13[b]	spray: 0.0006-0.1 0.0000075-0.01[a]	1; 62[a]; 50[b]	0.23; aerosol: 0.0002 0.08-2[a]	2[b]	NR
Incidental Inhalation-Powder	2; 13[b]	0.2	50[b]	0.002	2[b]	NR
Dermal Contact	61	0.0000075-1	153	0.0003-20	3	NR
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	7	0.008-0.1	18	0.0001-0.1	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	4	0.00006-0.5	4	0.0015-0.002	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

	Galactosyl Fructose		**Gluconic Acid**		**Glucose**	
Totals*	**1**	**NR**	**2**	**0.0001-0.18**	**425**	**0.00003-97.8**
Duration of Use						
Leave-On	*1*	*NR*	*2*	*0.0001-0.18*	*276*	*0.0001-91*
Rinse Off	*NR*	*NR*	*NR*	*NR*	*140*	*0.00003-97.8*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*9*	*19*
Exposure Type						
Eye Area	NR	NR	NR	NR	28	0.0001-0.48
Incidental Ingestion	NR	NR	NR	NR	1	0.059-97.8 (97.8 is an ingested breath freshener)
Incidental Inhalation-Spray	1[b]	NR	2[a]	NR	1; 38[a]; 101[b]	0.24; spray: 1 0.0045-2.9[a]
Incidental Inhalation-Powder	1[b]	NR	NR	NR	3[c]; 101[b]	NR
Dermal Contact	1	NR	2	0.001-0.18	319	0.0001-84
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	36	0.00003-91
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	1	0.0004
Mucous Membrane	NR	NR	4	NR	29	0.00063-97.8 (97.8 is an ingested breath freshener)
Baby Products	NR	NR	NR	NR	4	NR

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]
	Isomalt		**Kefiran**		**Lactitol**	
Totals*	**12**	**0.19-7.77**	**2**	**0.1**	**9**	**0.15-0.2**
Duration of Use						
Leave-On	*11*	*0.19*	*2*	*0.1*	*NR*	*NR*
Rinse Off	*1*	*7.77*	*NR*	*NR*	*9*	*0.15-0.2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	2	NR	NR	NR	NR	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	2[a]; 5[b]	NR	2[b]	NR	NR	NR
Incidental Inhalation-Powder	5[b]	NR	2[b]	NR	NR	NR
Dermal Contact	10	0.19	2	0.1	4	0.15-0.2
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	NR	NR	5	NR
Hair-Coloring	NR	7.77	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	1	0.2
Baby Products	NR	NR	NR	NR	NR	NR

	Lactose		**Maltose**		**Mannose**	
Totals*	**77**	**0.0005-9.4**	**3**	**0.3-0.5**	**5**	**5**
Duration of Use						
Leave-On	*28*	*0.0005-6*	*3*	*0.3-0.5*	*5*	*5*
Rinse Off	*48*	*0.038-9.4*	*NR*	*0.5*	*NR*	*NR*
Diluted for (Bath) Use	*1*	*8*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	8	NR	1	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	4[a]; 10[b]	0.0005[a]; 6[b]	NR	NR	4[a]	NR
Incidental Inhalation-Powder	10[b]	6[b]	NR	NR	NR	NR
Dermal Contact	70	0.001-6	1	0.3-0.5	5	5
Deodorant (underarm)	NR	aerosol: 0.038 not spray: 0.075-0.25	NR	NR	NR	NR
Hair - Non-Coloring	3	0.0005-9.4	NR	NR	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	3	0.3	1	NR	NR	NR
Mucous Membrane	33	0.038-8 diluted use product:	NR	NR	NR	NR
Baby Products	1	NR	NR	NR	NR	NR

	Melibiose		**Potassium Gluconate**		**Rhamnose**	
Totals*	**2**	**0.1-0.25**	**8**	**0.002-0.1**	**7**	**5-10**
Duration of Use						
Leave-On	*2*	*0.1-0.25*	*7*	*0.002-0.1*	*7*	*5-10*
Rinse Off	*NR*	*NR*	*1*	*NR*	*NR*	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	1	0.1	1	NR	1	NR
Incidental Ingestion	NR	NR	NR	NR	NR	NR
Incidental Inhalation-Spray	NR	NR	1[a]; 3[b]	0.05[a]	4[a]	NR
Incidental Inhalation-Powder	NR	NR	3[b]	NR	NR	NR
Dermal Contact	2	0.1-0.25	7	0.002-0.1	7	5-10
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	1	0.05	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	NR	NR	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR

Table 5. Frequency and concentration of use according to duration and type of exposure

	# of Uses[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]	*# of Uses*[31]	*Max. Conc. of Use (%)*[32]
	Ribose		**Sodium Gluconate**		**Sucralose**	
Totals*	**13**	**0.05**	**168**	**0.0000075-12**	**84**	**0.012-1.2**
Duration of Use						
Leave-On	*11*	*0.05*	*78*	*0.0000075-12*	*39*	*0.2-0.6*
Rinse Off	*2*	*NR*	*90*	*0.0000075-0.8*	*45*	*0.012-1.2*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	NR	NR	5	0.0000075-0.2	1	NR
Incidental Ingestion	NR	NR	NR	0.00006-0.75	68	0.012-1.2
Incidental Inhalation-Spray	7[a]; 2[b]	NR	29[a]; 27[b]	spray: 0.0006 0.0000075-0.6[a]	3[a]	0.012-0.95[a]
Incidental Inhalation-Powder	2[b]	NR	27[b]	NR	NR	NR
Dermal Contact	13	0.05	104	0.0000075-5	16	0.5-0.6
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	NR	NR	61	0.2-12	NR	NR
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	NR	NR	19	0.00006-0.8	68	0.012-1.2
Baby Products	NR	NR	1	NR	NR	NR

	Sucrose		**Trehalose**		**Xylobiose**	
Totals*	**738**	**0.001-65**	**474**	**0.0001-2**	**2**	**0.0075-0.15**
Duration of Use						
Leave-On	*423*	*0.001-58*	*356*	*0.00055-2*	*2*	*0.075-0.15*
Rinse Off	*303*	*0.001-65*	*118*	*0.0001-1*	*NR*	*0.0075-0.05*
Diluted for (Bath) Use	*12*	*1-52*	*NR*	*NR*	*NR*	*NR*
Exposure Type						
Eye Area	57	0.0035-2	47	0.02-1.1	NR	NR
Incidental Ingestion	4	9-45	3	0.005-0.1	NR	NR
Incidental Inhalation-Spray	4; 157[a]; 84[b]	0.002; spray: 1; 0.002-2[a]	4; 163[a]; 88[b]	0.002-1[a]	1[a]; 1[b]	0.091[a]
Incidental Inhalation-Powder	4; 84[b]	NR	1; 88[b]; 1[c]	0.12	1[b]	NR
Dermal Contact	672	0.001-65	376	0.00055-2	2	0.0075-0.15
Deodorant (underarm)	NR	aerosol: 0.004 not spray: 0.005-0.009	NR	NR	NR	NR
Hair - Non-Coloring	53	0.001-10.5	91	0.0001-1	NR	0.091
Hair-Coloring	5	NR	NR	NR	NR	NR
Nail	2	13.6	1	1	NR	NR
Mucous Membrane	205	0.001-65	11	0.005-0.1	NR	0.0075
Baby Products	1	NR	1	NR	NR	NR

	Xylose			
Totals*	**75**	**0.1-1**		
Duration of Use				
Leave-On	*68*	*0.1-0.11*		
Rinse Off	*7*	*0.1-1*		
Diluted for (Bath) Use	*NR*	*NR*		
Exposure Type				
Eye Area	1	NR		
Incidental Ingestion	NR	NR		
Incidental Inhalation-Spray	4; 10[a]; 13[b]	pump spray: 0.11		
Incidental Inhalation-Powder	13[b]	NR		
Dermal Contact	28	NR		
Deodorant (underarm)	NR	NR		
Hair - Non-Coloring	47	0.1-0.11		
Hair-Coloring	NR	1		
Nail	NR	NR		
Mucous Membrane	NR	NR		
Baby Products	NR	NR		

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses
a Includes products that can be sprays, but it is not known whether the reported uses are sprays
b Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories
c Includes products that can be powders, but it is not known whether the reported uses are powders
NR – not reported

Table 6. Ingredients Not Reported to be Used
Galactose
Galacturonic Acid
Lactulose

Table 7. Examples of non-cosmetic uses

Ingredient	Use	Reference
Calcium Gluconate	- a direct food additive used as a firming agent, formulation aid, sequestrant, and texturizer - used as mineral supplements in pharmaceutical injection solutions - GRAS in animal feed	21CFR184.1199 9 21CFR582.1199; 21CFR582.6199
Fructose	- listed in the *United States Pharmacopeia* (USP)*/National Formulary* (NF) - inactive ingredient for approved drugs; used in oral, intravenous, and rectal drugs - can function as a dissolution enhancer, flavoring agent, sweetening agent, and tablet diluent in pharmaceuticals, is used tablets, syrups, and solutions as a flavoring and sweetening agent	3 14 40 12
Galactose	- listed in the USP/NF - inactive ingredient for approved drugs; used in oral and rectal products	14 40
Gluconic Acid	industrial cleaning; metal surface treatment; textile bleach stabilizer; aluminum processing ; chelating agent in dispersive cements, cleaning products, pharmaceuticals, and food stuff; sequestering agent in dispersive building materials	9
Glucose	- in sweeteners and table syrups, with specifications defined in the CFR - in a glucose/glycine/electrolyte in animal drugs, feeds, and related products - listed in the USP/NF as a liquid - approved as an inactive ingredient for approved drugs; used in oral products	21CFR168.110, 111, 120, 121 21CFR520.550 14 40
Isomalt	- listed in the *Foods Chemicals Codex* as a texturizer, formulation aid, surface finishing agent, stabilizer, thickener - listed in the USP/NF - inactive ingredient for approved drugs; used in oral products - can function as a coating agent, granulation aid, medicated confectionary base, sweetening agent, or tablet and capsule diluent in pharmaceuticals; a non-cariogenic excipient used in tablets or capsules, coatings, sachets, and effervescent tablets; often used in buccal applications	3 14 40 12
Lactitol	- listed in the *Foods Chemicals Codex* as a humectant, stabilizer - listed in the USP/NF - inactive ingredient for approved drugs; used in oral products (the monohydrate) - can function as a sweetening agent, tablet and capsule diluent, and therapeutic agent in pharmaceuticals; used as a non-cariogenic replacement for sucrose, a diluent in solid dosage forms, and therapeutically in the treatment of encephalopathy and as a laxative	3 14 40 12
Lactose	- in sweeteners and table syrups, with specifications defined in the CFR - used as a nutrient in the preparation of modified milk and food for infants and convalescents (predominantly the α-form, but also the β-form) - listed in the *Foods Chemicals Codex* as a processing aid, humectant (anhydrous form), texturizer - inactive ingredient for approved drugs; used in transdermal, oral, sublingual, buccal, inhalation, subcutaneous, vaginal, intravenous, intramuscular, and rectal drugs - in pharmaceuticals, lactose, anhydrous can function as a directly compressible tablet excipient, dry powder inhaler carrier, lyophilization aid, tablet and capsule diluent, tablet and capsule filler; widely used in direct compression tableting applications and as a tablet and capsule filler and binder, and it can be used in i.v. injections - lactose, monohydrate can function as a dry powder inhaler carrier, lyophilization aid, tablet binder, tablet and capsule diluent, tablet and capsule filler; is widely used as a filler and diluent in tablets and capsules - lactose, inhalation can function as a diluent and as a dry powder inhaler carrier; it is widely used as a carrier, diluent, and flow aid in dry powder formulations, and when of suitable particle size, it can be used to prepare soft pellets of dry powder inhaler formulations - lactose, spray-dried can function as a directly compressible tablet excipient, tablet and capsule diluent, tablet and capsule filler; widely used as a binder, filler-binder, and flow aid in direct compression tableting	21CFR168.122 10 3 40 12
Lactulose	- listed in the USP/NF as a concentrate - an approved drug used to treat constipation; used in oral and rectal products	14 39
Maltose	-listed in the Everything Added to Food in the United States (EAFUS) inventory - listed in the USP/NF - inactive ingredient for approved drugs; used in oral drugs (the anhydrous form) - can function as a sweetening agent and tablet excipient in pharmaceuticals	41 14 40 12
Mannose	inactive ingredient for approved drugs; used in oral drugs (D-mannose)	40
Potassium Gluconate	- listed in the *Foods Chemicals Codex* as a sequestrant - listed in the USP/NF	3 14
Rhamnose	listed in the EAFUS inventory	41
Ribose	listed in the EAFUS inventory	41
Sodium Gluconate	- GRAS as a sequestrant in animal drugs, feeds, and related products, with no limitation other than current GMP - listed in the *Foods Chemicals Codex* as sequestrant - listed in the USP/NF - inactive ingredient for approved drugs; used in oral products	21CFR582.6757 3 14 40
Sucralose	- listed in the *Foods Chemicals Codex* as a flavor enhancer - listed in the USP/NF - inactive ingredient for approved drugs; used in oral, sublingual, and buccal drugs - can function as a sweetening agent in pharmaceuticals	3 14 40 12

Table 7. Examples of non-cosmetic uses

Ingredient	Use	Reference
Sucrose	- as the starting material in the fermentative production of ethanol, butanol, glycerol, citric acid, and levulinic acid	10
	- listed in the *Foods Chemicals Codex* as a formulation and texturizing aid	3
	- inactive ingredient for approved drugs; used in topical, oral, sublingual, buccal, subcutaneous, intravenous, and rectal drugs	40
	- functions as a confectionary base, coating agent, granulation aid, suspending agent, sweetening agent, tablet binder, tablet and capsule diluent, tablet filler, therapeutic agent, and viscosity increasing agent in pharmaceuticals; widely used in oral formulations	12
Trehalose	- listed in the *Foods Chemicals Codex* as a humectant, stabilizer, thickener, texturizer	3
	- used as an excipient in a few monoclonal antibody products	26
	- can function as a color adjuvant, flavor enhancer, freeze-drying agent, humectant, stabilizing agent, sweetening agent, table diluent, and thickening agent in pharmaceuticals; used for the lyoprotection of therapeutic proteins	12
Xylose	- listed in the EAFUS inventory	41
	- listed in the USP/NF	14

Table 8. Nutritive and non-nutritive sweeteners and food additives

Nutritive[3,14,42,85]	Non-Nutritive[3,43]
fructose	lactitol
galactose	sucralose
glucose	xylose
isomalt	
lactose	
maltose	
potassium gluconate	
sodium gluconate	
sucrose	
trehalose	

Table 9. Summary metabolism data

Ingredient (GRAS foods are noted)	Metabolism Data	Reference
	Absorbed and Metabolized (Nutritive)	
Calcium Gluconate (GRAS)	calcium and the gluconate anion are common constituents of food and are metabolized by the normal metabolic processes in man	47
Fucose	L-fucose is a common component of many *N*- and *O*-linked glycans and glycolipids produced by mammalian cells	48
Fructose (GRAS)	- metabolism of fructose occurs mainly in the liver; it is converted partially to dextrose and to lactic and pyruvic acid; further metabolism occurs by a variety of metabolic pathways	12
	- serum fructose levels were higher in adult humans fed sucrose than when fed a mixture of glucose and fructose; release of fructose by hydrolysis of sucrose within the brush border may facilitate absorption of fructose; also the furanose ring structure of fructose as released may be more readily absorbed than the equilibrium mixture of pyranose and furanose forms attained after being in solution for some time	49
Galactose	actively absorbed from the gut; converted in the liver through the Leloir pathway to yield glucose-6-phosphate	85-87
Gluconic Acid	a normal metabolic product of glucose oxidation, is an important intermediate in carbohydrate metabolism in mammals; contributes to the synthesis of nicotinamide-adenine dinucleotide phosphate (NADPH), and it leads to the formation of ribose-5-phosphate; the amount produced endogenously is many times greater than the largest amounts likely to be consumed from food; the daily production of gluconate from endogenous sources is about 450 mg/kg for a 60 kg person	9,38,50
Glucose (GRAS)	rapidly absorbed from the small intestine, principally by an active mechanism	44
Potassium Gluconate (GRAS)	- important intermediate in carbohydrate metabolism	9
	- readily absorbed in the intestine, the potassium ion ionize almost immediately to potassium and gluconic acid; with parental administration, a significant portion (60-85%) is excreted unchanged in the urine	45,46
Ribose	rapidly and extensively metabolized; converted to glucose via the pentose phosphate pathway in the liver and other tissues	88
Sodium Gluconate (GRAS)	important intermediate in carbohydrate metabolism	9

Table 9. Summary metabolism data

Ingredient (GRAS foods are noted)	Metabolism Data	Reference
Sucrose	- known to be a relatively efficient source of energy; rapidly metabolizable for utilization and storage - hydrolyzed in the small intestine by sucrose to yield dextrose and fructose, which are then absorbed - there is evidence that sucrose can be absorbed unchanged to a small extent, particularly at high dietary level; nearly all ingested sucrose is absorbed as glucose and fructose, its metabolism is essentially that of these two monosaccharides - excreted unchanged in the urine when administered intravenously	51 12 49 12
	Metabolized in the small intestines	
Lactose	broken down in the gut by lactase to produce glucose and galactose	85
Maltose	broken down in the gut by maltase to yield two glucose molecules	85
Trehalose	- rapidly metabolized in the gut to glucose by trehalase - metabolism is essentially identical to that of other disaccharides that are consumed as part of the human diet	12 27,53
	Not Absorbed (or Limited Absorption)	
Isomalt	hydrolysis and absorption in the small intestine is limited because the glycoside linkage between the mannitol or sorbitol moiety and the glucose moiety is very stable; the majority of isomalt is fermented in the large intestine (nutritive)	12
Lactitol	not absorbed in the small intestine; broken down by microflora in the large intestine (non-nutritive)	12
Lactulose	-not readily absorbed from the intestine in humans; not hydrolyzed by intestinal disaccharidases; <1% of a 5 g dose given orally was recovered in the urine - reaches the large intestine essentially unchanged, where it is metabolized by bacteria with the formation of low molecular weight acids	52 21
Mannose	little disposition of glycogen in the liver following oral ingestion; transport across the liver is approximately 1/10 that of glucose, suggesting diffusion; significant amounts excreted in the urine following oral administration; no significant reabsorption by the kidney	54
Sucralose (GRAS)	- highly water-soluble, not lipophilic, and does not bioaccumulate; the major portion of an oral dose of sucralose is unabsorbed and excreted unchanged in the feces of rats, mice, rabbits, dog, and man; only two minor metabolites were detected following oral dosing in the mouse, rat, and man, and only one urinary metabolite was found in the rabbit and the dog - not metabolized or used for energy in mammalian systems	23,55-59,89 60
	Limited Absorption/Not Metabolized	
Xylose	- D-xylose is passively absorbed in rats; in rats and man, oral absorption was incomplete (about 70% absorbed) and xylose was eliminated primarily unchanged in the urine	61

Table 10. Genotoxicity studies

Test Article	Concentration/Vehicle	Procedure	Test System	Results	Reference
		IN VITRO			
Calcium Gluconate	12.5, 25 and 50 µg/ml	Ames test; with and without metabolic activation	*Salmonella typhimurium* strains TA1535, TA1537, TA1538	negative	70
Calcium Gluconate	7.5, 15 and 30 µg/ml	with and without metabolic activation	*Saccharomyces cerevisiae* strain D4	negative	70
Lactitol	not provided	reverse mutation assay; details not provided	*S. typhimurium* (stains not specified)	negative	20
Lactitol	not provided	mammalian gene mutation assay; details not provided	human lymphocytes	negative	20
Sodium Gluconate	0.006, 0.012, and 0.024 µg/ml	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*S. typhimurium* strains TA1535, TA1537, TA1538	negative	71
Sodium Gluconate	12.5, 25, and 50 µg/ml	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*Saccharomyces cerevisiae* strain D4	negative	71
Sucralose	0.16-10 mg/plate; distilled water was the vehicle	Ames test, with and without metabolic activation; appropriate positive and negative controls were used	*S. typhimurium* strains TA1535, TA1537, TA1538, TA98, TA100	negative	72
Sucralose	0.16-10 mg/plate; distilled water was the vehicle	DNA damage test; appropriate positive and negative controls were used	*Escherichia coli* strains W3110 and P3478	negative	72
Sucralose	≤10 mg/ml; distilled water was the vehicle	mouse lymphoma assay, with and without metabolic activation; appropriate positive and negative controls were used	L5178Y TK +/- mouse lymphoma cells	originally classified as equivocal results; redefined as negative using revised criteria	72
Sucralose	8, 40, and 200 µg/ml; distilled water was the vehicle	human peripheral lymphocyte assay, without metabolic activation; appropriate positive and negative controls were used	human lymphocytes	negative	72
Sucrose	156-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	73
Sucrose	156-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	74
Sucrose	1311-5000 µg/ml	mouse lymphoma assay, with and without metabolic activation; appropriate controls were used	L5178Y mouse lymphoma cells	negative	75
Trehalose	312.5-5000 µg/plate	Ames test, with and without metabolic activation; appropriate controls were used	*S. typhimurium* strains TA1535, TA1537, TA98, and TA100; *E. coli* strain WP2 uvrA	negative	27
Trehalose	to 312, 1250, or 5000 µg/ml	chromosomal aberration assay, with and without metabolic activation; appropriate controls were used	Chinese hamster ovary cells	negative	27
		IN VIVO			
Sodium Gluconate	0, 2.5, 5, or 10 g/kg in physiological saline	chromosomal aberration assay; mice were given a single oral 1 ml dose	mouse bone marrow cells; C57BL male mice, 3/group	not clastogenic; all animals of the 5 and 10 g/kg groups died	9
Sodium Gluconate	0, 1.25,or 2.5 g/kg in physiological saline	chromosomal aberration assay; mice were dosed orally with 1 ml, 1x/day for 4 days	mouse bone marrow cells; C57BL male mice, 2 (control and low dose) or 3 (high dose)/group	not clastogenic; 1 animal of each test group died	9
Sucralose	0.5, 1, and 2 g/kg bw in distilled water	chromosomal aberration assay; rats were dosed by gavage daily for 5 days; aberrations were evaluated 6 h after the final dose	rat bone marrow cells; male and female Sprague-Dawley rats, 5/group	negative; no mortality	72
Sucralose	2 or 10 g/kg bw in distilled water	micronucleus test; 5 male and 5 female CD-1 COBS Swiss mice were dosed twice by gavage in 24 h; micronuclei were evaluated after 6 h, the study was preliminary and was not Good Laboratory Practices (GLP)-compliant	male and female CD-1 COBS Swiss mice; 5/sex/group	negative	72
Sucralose	1 or 5 g/kg bw in distilled water	micronucleus test; mice were given a single dose by gavage, and micronuclei were evaluated 24, 48,or 72 h after dosing	female CD-1 Swiss mice; 5/sex/group	negative	72
Trehalose	1250, 2500, or 5000 mg/kg	micronucleus test; mice were dosed intraperitoneally and then killed 1 or 2 days after dosing; cyclophosphamide was used as the positive control.	male and female mice; 5/group	negative	27

Table 10. Genotoxicity studies

Test Article	Concentration/Vehicle	Procedure	Test System	Results	Reference
Trehalose	1.25, 2.5, and 5 g/kg in distilled water	micronucleus test; mice were dosed by gavage for 3 days and killed on day 4	male mice; 10/group	negative; no mortality	[53]

Table 11. Irritation and Sensitization Studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
			NON-HUMAN		
Gluconic Acid	50% aq. solution 0.5 ml	6 rabbits/group	- 1 sq. in. occlusive patch was applied for 4 h - test sites of one group was abraded - test sites were scored after 24, 48, and 72 h	- slight erythema observed during the initial observation; it is not clear if this is only for abraded skin - no signs of irritation at 72 h	[9]
Lactitol	not specified	rabbits; no./group not specified	- study was performed according to the OECD Guidelines 404 and 406, respectively.[90,91] (No other details were provided)	- not an irritant or sensitizer	[20]
			HUMAN		
hair styling cream containing **0.08% glucose**	applied neat	100 subjects	HRIPT induction: the test material was applied neat under semi-occlusive patches; 9 applications were made over a 3-wk period; the first patch was applied for 48 h, and the remainder for 24 h challenge: the patch was applied after a 2-wk non-treatment period to a previously untreated site; the test sites were scored 48 and 96 h after application.	not an irritant or a sensitizer	.[78]
a leave-in hair product containing **8% glucose**	applied neat 0.2 ml	208 subjects	HRIPT; 24-h, 2 cm^2, semi-occlusive patches were used	not a sensitizer 1% of subjects had a "+" reaction during induction	[77]
mixture containing **isomalt**	final applied concentration of isomalt is 0.94% 20 µl	49 subjects	- single insult patch test; test material was applied to the ventral forearm using Finn Chambers, and the test site was scored 15 min, 24 h, and 48 h after patch removal - SDS (not defined) was used as a positive control - water was the negative control	not an irritant; no reactions to the test formulation were observed	[80]
face and neck product containing **0.1% kefiran**	applied neat	100 subjects	HRIPT using semi-occlusive patches	not an irritant or sensitizer	[69]
paste mask and mud pack containing **0.15% lactitol**	applied neat	28 subjects	4-wk in-use dermal study with open applications	not an irritant	[69]
paste mask and mud pack containing **0.15% lactitol**	applied neat	110 subjects	HRIPT using semi-occlusive patches	not an irritant or sensitizer	[69]
face and neck product containing **2.48% lactose**	applied neat	114 subjects	HRIPT using occlusive patches	not an irritant or sensitizer	[69]

Table 11. Irritation and Sensitization Studies

Test Article	Concentration/Dose	Test Population	Procedure	Results	Reference
leave-on facial product containing **5% mannose**	applied neat	103 subjects	HRIPT with 48-72 h occlusive induction patches and a 48-h challenge patch - distilled water was used as a negative control.	not an irritant or a sensitizer	79
leave-on formulation containing **10% rhamnose**	applied neat	106 subjects	HRIPT using 48-72 h occlusive patches - distilled water was used as a negative control.	- not a sensitizer - irritation reaction consisting of severe to mild erythema, bulla, coloration, fissuring, and scabbing was observed in one subject	76
lip balm formulation containing **0.6% sucralose**	applied neat	50 subjects	modified Draize HRIPT; similar to that described previously, with the exceptions that all the induction patches were applied for 24 h, the challenge patch was applied for 24 h, and the challenge sites scored 24 and 48 h after application	not an irritant or sensitizer	81
rinse-off hair product containing **29% sucrose**	diluted to 50% in distilled water 0.02 ml over 50 mm^2	102 subjects	HRIPT using 48-72 h occlusive patches for induction, and a 48-h patch at challenge	- not an irritant or sensitizer - mean irritation index of <0.25; 16% of the subjects presented with score ≥2 reactions during induction	83
eye cream formulation containing **0.1% xylobiose**	applied neat	56 subjects	HRIPT using 24-h occlusive patches	not an irritant or sensitizer	82

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SAFETY DATA SHEET

Version 4.8
Revision Date 03/26/2018
Print Date 07/28/2018

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name	:	D-(−)-Fructose
Product Number	:	PHR1002
Brand	:	Sigma-Aldrich
CAS-No.	:	57-48-7

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses	:	Laboratory chemicals, Synthesis of substances

1.3 Details of the supplier of the safety data sheet

Company	:	Sigma-Aldrich 3050 Spruce Street SAINT LOUIS MO 63103 USA
Telephone	:	+1 800-325-5832
Fax	:	+1 800-325-5052

1.4 Emergency telephone number

Emergency Phone #	:	+1-703-527-3887 (CHEMTREC)

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture.

2.2 GHS Label elements, including precautionary statements

Not a hazardous substance or mixture.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms	:	D-Levulose Fruit sugar
Formula	:	$C_6H_{12}O_6$
Molecular weight	:	180.16 g/mol
CAS-No.	:	57-48-7
EC-No.	:	200-333-3

No components need to be disclosed according to the applicable regulations.

4. FIRST AID MEASURES

4.1 Description of first aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
No data available

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Avoid dust formation. Avoid breathing vapours, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions
No special environmental precautions required.

6.3 Methods and materials for containment and cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place.

Store at Room Temperature.
Storage class (TRGS 510): 13: Non Combustible Solids

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
General industrial hygiene practice.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
No special environmental precautions required.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: crystalline Colour: colourless
b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	5.0 - 7 at 18 g/l at 25 °C (77 °F)
e)	Melting point/freezing point	119 - 122 °C (246 - 252 °F)

f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	No data available
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	No data available
n)	Water solubility	18 g/l at 20 °C (68 °F) - completely soluble
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Hazardous decomposition products formed under fire conditions. - Carbon oxides
Other decomposition products - No data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
Inhalation: No data available

Dermal: No data available

LD50 Intravenous - Rabbit - 15 g/kg

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is on OSHA's list of regulated carcinogens.

Reproductive toxicity
No data available

No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: LS7120000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

No data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

SARA 302 Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components
No components are subject to the Massachusetts Right to Know Act.

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Fructose	57-48-7	

New Jersey Right To Know Components

	CAS-No.	Revision Date
Fructose	57-48-7	

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

Further information
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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information
Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

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