WHAT LEVEL OF ADDED OR FREE SUGAR IS COMMENSURATE WITH GOOD HEALTH OUTCOMES?

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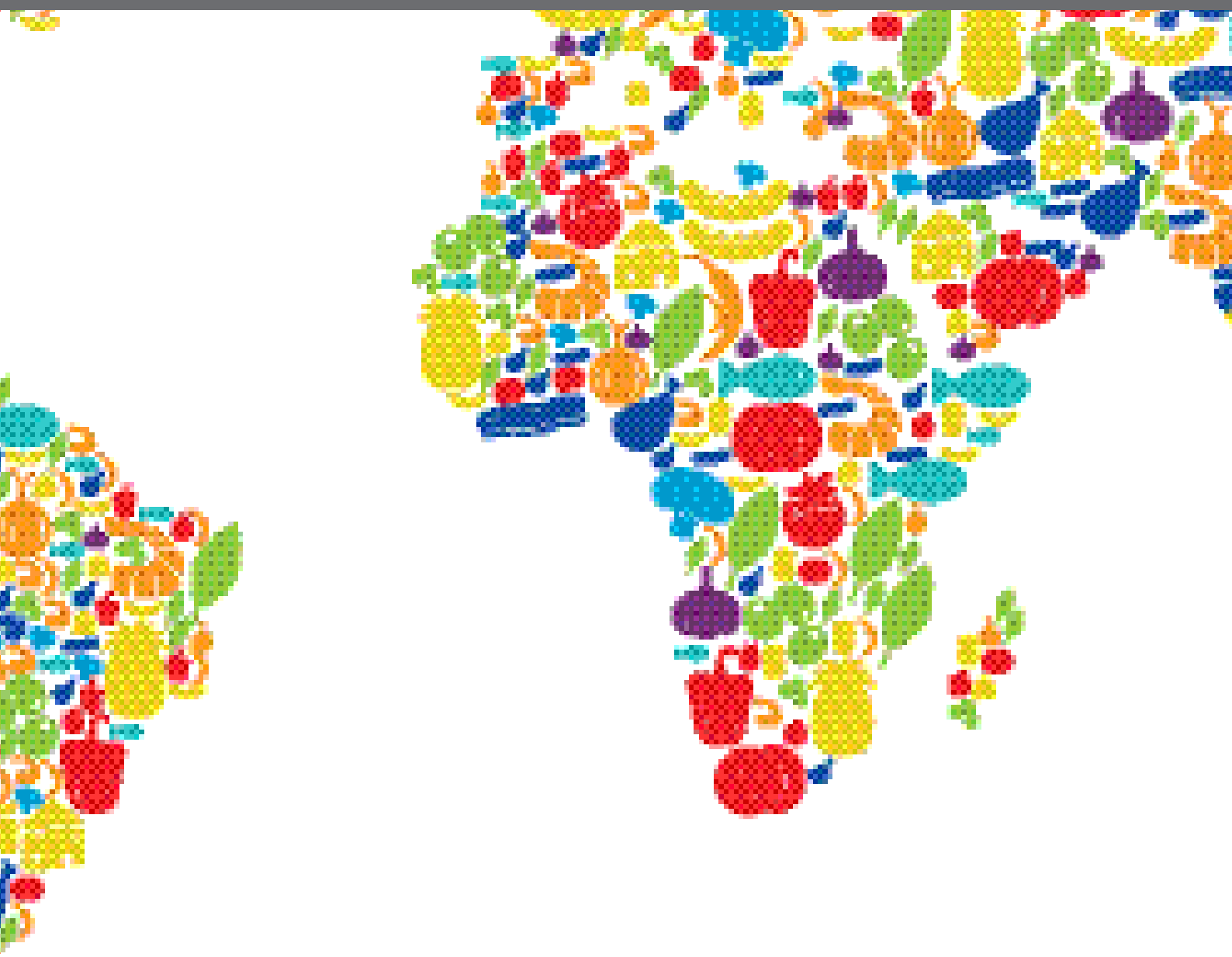

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WHAT LEVEL OF ADDED OR FREE SUGAR IS COMMENSURATE WITH GOOD HEALTH OUTCOMES?

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EDITORIAL
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Editorial: What Level of Added or Free Sugar Is Commensurate With Good Health Outcomes?

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Keywords: total sugars, added sugars, free sugars, sugar-sweetened beverages, chronic disease, artificial sweeteners

Editorial: on the Research Topic

What Level of Added or Free Sugar Is Commensurate with Good Health Outcomes?

Is there a sweet spot for added sugars? There is consensus that any source of excess calories will contribute to weight gain and metabolic disease, but there is still debate on the level of added or free sugars which is commensurate with both good health and enjoyment of food. While guidelines for the range of energy (%E) as carbohydrate, fat, and protein have widened, the reverse is true of added sugar. In previous decades, health authorities agreed that 10%E was an appropriate upper cut-off, even if strong evidence was lacking. Nonetheless, since 2015, there have been moves to reduce that cut-point to 5%E (1, 2). In the eyes of many, eating as little sugar as possible is ideal.

I have concerns about limiting added (or free sugars) to <5%E. This perspective is informed by knowledge of food science and technology, human evolution, and the role that sweetness plays in encouraging the consumption of healthy foods (e.g., wholegrains). The paradox of falling consumption of added sugars with increasing prevalence of overweight and obesity is now evident in many developed countries (3, 4). In Australia, peak intake of micronutrients is observed within the range 5–15%E from free sugars (5). But of greater concern is the potential of unanticipated and undesirable consequences of health advice on added sugars and sugar-sweetened beverages (SSB). These include the increased incidence of restrictive eating disorders such as orthorexia nervosa (6) and an increase in alcohol consumption and deaths due to alcohol-related disease (7). In Australia, the consumers who avoid SSB, drink twice as many calories in the form of alcoholic beverages as the highest consumers of SSB (8). We should also recall that the history of nutrition science is replete with examples of where we got it wrong, including the "great protein fiasco" (9) and low-fat diets (10).

In this special issue of *Frontiers in Nutrition*, we hoped that the "sweet spot" (the highest level associated with no effect or harm) could be defined with a greater level of certainty. In a well-designed 4-week randomized controlled trial conducted by Te Morenga et al., overweight adults ($n = 48$) randomized to consuming 1,800 kJ of SSB (~100 g added sugar, equivalent to ~1,000 mL of SSB or ~20%E) showed no changes in weight, blood pressure or other cardiometabolic factors compared with those assigned to consuming fruit with a similar energy content (97 g naturally-occurring sugars). However, men (but not women) showed an increase in uricemia, a risk factor for gout. Clearly, further studies in vulnerable groups of similar design and longer duration are needed.

Some studies directly addressed the question of safe levels of intake. In a Swedish population ($n = 22,877$), during a mean follow-up of nearly 20 years, >20%E as added sugar was associated with increased coronary events (HR = 1.39) and stroke risk (HR = 1.31) compared to 7.5–10%E as added sugar (Janzi et al.). Surprisingly, participants with the *lowest* intake (<5%E) had the *highest* risk of atrial fibrillation and aortic stenosis. This result is difficult to explain but emphasizes

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the complexity of nutrition and observational studies. In a well-characterized older cohort of Australians (n = 1,713), changes in the %E from added sugars were not associated over time with changes in body weight, regardless of source—beverage or non-beverage (Moshtaghian et al.). Earlier work in this cohort found those consuming <5%E as added sugar, were higher consumers of alcohol (11).

Added sugars are used in tiny amounts (∼1–2% w/w) to enhance flavors and improve palatability. If this encourages excessive energy intake, then the same must be said of salt, herbs, spices, and soy sauce, all of them in use for thousands of years. In large amounts, the physicochemical, technological, and functional characteristics of sugars influence human metabolism as reviewed by Brouns. Sugars also influence dental health. The effect of sugars on oral health was the conditional reason that WHO recommended consumption of free sugars to below 5% of total energy (2). But Brouns reminds us that frequency, contact time, and rapidly digestible starches and acidic foods like wine and fruit also affect dental health. Reducing the amount of added sugars from SSB will not be effective if starchy snacks and sticky confectionery are consumed instead (3).

Determining the extent to which added sugars contribute to disease in various populations is challenging because it is difficult to accurately measure intakes. Biomarkers of sugar intake may therefore be helpful although this does not distinguish added sugars from sugars in fruit and vegetables. Te Morenga's second paper (Te Morenga et al.) found that the sum of urinary [sucrose + fructose] was weakly but significantly correlated (r = 0.23) with intakes of total sugars and with added sugars from SSB (n = 0.26). Interestingly, they found a higher correlation (r = 0.40) with the C-13 carbon isotope ratio of alanine. Similarly, in the DONALD study (n = 254 adolescents), Della Della Corte et al. backed up dietary records with measurement of fructose and the sum of [fructose + sucrose] in two complete 24-h urine collections. They found no prospective associations between adolescent intake of fructose, sucrose, glucose, added, free, and total sugar with adult insulin sensitivity as measured by HOMA2-%S. Indeed, higher fructose in urine was associated with *improved* insulin sensitivity in females (but not males).

An underlying assumption of recommendations to reduce SSBs, is that water will take their place. In the US population (NHANES, n = 22,716), Drewnoski's group reported that SSB consumption had declined by ∼20% in volume between 2011 and 2016 (Vieux et al.), whereas plain and bottled water increased by just ∼10%. The opposing time trends were not uniform—lower income and minority groups consumed more bottled water and relatively little tap water. In this context, changes in intake of alcoholic beverages and other sources of energy (e.g., chocolate) must be explored.

Pang et al. attracted the highest number of views with their review of the current state of knowledge on artificial sweeteners, reminding us that they are not all the same, with different chemical structures, absorption, and metabolic effects. Despite many being in use for 50 years, there are still very few long-term studies to show that substituting sugars and SSB with non-caloric alternatives is of benefit. And finally, an updated meta-analysis and systematic review by Zafar et al. confirmed that chronic consumption of fructose is neither more beneficial nor harmful than sucrose or glucose for glycemia and other metabolic outcomes.

Taken together, this collection of 11 papers provides evidence that a diet containing >20% added sugars may have adverse effects, but so too, a diet containing <5% added sugar. At worst, such a restrictive diet can create food fear or an unhealthy relationship with food and alcohol, especially for women and girls. As the Swedish study found (Janzi et al.), the sweet spot may therefore lie somewhere between 7.5 and 10%E as added sugars. Many will agree that public health interventions and food taxes to prevent obesity and related diseases should promote the quality of the overall diet, not a singular focus on reducing sugar and SSB intakes.

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The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. Scientific Advisory Committee on Nutrition. *Carbohydrates and Health*. London: The Stationery Office Ltd (2015).
2. World Health Organisation. *Guideline: Sugars Intake for Adults and Children*. Geneva: World Health Organisation (2015).
3. Brand-Miller JC, Barclay AW. Declining consumption of added sugars and sugar-sweetened beverages in Australia: a challenge for obesity prevention. *Am J Clin Nutr*. (2017) 105:854–63. doi: 10.3945/ajcn.116.145318
4. Rippe JM, Angelopoulos TJ. Added sugars and risk factors for obesity, diabetes and heart disease. *Int J Obesity*. (2016) 40:S22–7. doi: 10.1038/ijo.2016.10
5. Mok A, Ahmad R, Rangan A, Louie JCY. Intake of free sugars and micronutrient dilution in Australian adults. *Am J Clin Nutr*. (2018) 107:94–104. doi: 10.1093/ajcn/nqx008
6. Zickgraf HF, Ellis JM, Essayli JH. Disentangling orthorexia nervosa from healthy eating and other eating disorder symptoms: relationships with clinical impairment, comorbidity, and self-reported food

choices. *Appetite*. (2019) 134:40–9. doi: 10.1016/j.appet.2018.12.006
7. Chikritzhs T, Allsop SJ, Moodie AR, Hall WD. Per capita alcohol consumption in Australia: will the real trend please step forward? *Med J Austral*. (2010) 193:594–7. doi: 10.5694/j.1326-5377.2010.tb04069.x
8. Wong THT, Buyken AE, Brand-Miller JC, Louie JCY. Is there a soft drink vs. alcohol seesaw? A cross-sectional analysis of dietary data in the Australian Health Survey 2011–12. *Euro J Nutr*. (2020) 59:2357–67. doi: 10.1007/s00394-019-02084-4
9. Mclaren D. The great protein fiasco. *Lancet*. (1974) 304:93–6. doi: 10.1016/S0140-6736(74)91649-3
10. Ludwig DS. Lowering the bar on the low-fat diet. *JAMA*. (2016) 316:2087–8. doi: 10.1001/jama.2016.15473
11. Moshtaghian H, Louie JCY, Charlton KE, Probst YC, Gopinath B, Mitchell P, et al. Added sugar intake that exceeds current recommendations is associated with nutrient dilution in older Australians. *Nutrition*. (2016) 32:937–42. doi: 10.1016/j.nut.2016.02.004

Conflict of Interest: JB-M is President of the Glycemic Index Foundation and overseas a glycemic index testing service at the University of Sydney. She receives royalties from the University of Sydney and popular books about nutrition and health.

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REVIEW
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Saccharide Characteristics and Their Potential Health Effects in Perspective

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To understand the effects of saccharides on our metabolism and health, we need a clear understanding of what they are, how they differ, and why some types are deemed "less healthy" and others "better for health." There are various ways to look at this topic. Firstly, saccharides can be classified according to their degree of polymerization (DP). This classification is useful when qualitative or quantitative analysis and calculation of intakes are required or for food-labeling definitions. However, it does not account for the fact that saccharides with a similar DP can differ in molecular composition, which will influence digestion, absorption, and metabolism. Secondly, another approach widely used in the biomedical and nutritional sciences is therefore a physiological classification, which addresses the rate and degree of digestibility and absorption, the glycemic response, and the metabolic fate. The individual health status also plays a role in this respect. An active, lean person will have a metabolic response that differs from an inactive person with overweight and insulin resistance. However, this approach will not give a complete answer either because the characteristics of the matrix/meal in which these carbohydrates (CHOs) are present will also influence the responses of our body. Thirdly, one can also rank CHOs by comparing their functional/technological properties, such as relative sweetness, viscosity, and solubility. Understanding CHO characteristics and related physiological responses will help understand health and disease implications. Therefore, a brief outline of different carbohydrate classifications is presented. This outline will be placed in the context of potential overall effects after consumption. The answer to the question whether we should we eat less of certain sugars depends on the angle from which you look at this matter; for example, do you address this question from a single molecular characteristic point of view or from a meal quality perspective? Looking at one particular CHO characteristic will almost always lead to a different conclusion (e.g., the labeling of fructose as toxic) than evaluating from a "total perspective" (fructose has adverse effects in certain conditions). Examples are given to help understand this matter for the benefit of justified dietary/food-based recommendations.

Keywords: saccharide-characteristics, carbohydrate-classification, added sugars, free sugars, sugar-functionality, glycemic index, sugars and health

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INTRODUCTION

Sugars and other carbohydrates (CHOs) have many characteristics, ranging from molecular composition to functional, physiological, and biochemical behavior. As any individual characteristic of a given CHO can influence its physiological properties, it should be viewed in the context of all other characteristics. For example, different sugars can be similar with respect to their monomer composition but may differ in the bonds between these constituents. Ingesting sucrose, which delivers the monomers glucose and fructose for absorption, can lead to different gastrointestinal and post-absorptive effects compared with ingesting glucose or fructose as a single source. In a solid, liquid, or viscous matrix, the same sugars will show different physiological responses. For this reason, it needs to be acknowledged that looking at one particular CHO characteristic will almost always lead to a different conclusion about potential health effects than looking from a "total" perspective as regards the effect of the carbohydrate in a certain meal/pattern and lifestyle. A consequence may be that misinterpretations and misconceptions are being created by interpreting the effects of saccharides on health in a strongly reductionistic way (1–4). In this light, the reader will be provided with condensed information on specific compositional characteristics of CHOs, especially sugars, which have physiological and metabolic effects. The individual characteristics will be discussed in the context of what they mean for the potential overall effects on health and disease, and why food authorities are shifting to more qualitative food-based guidelines (5–8).

CHEMICAL CLASSIFICATION OF SACCHARIDES AND ITS MEANING

Saccharides can be ranked according to the characteristics of their molecular composition. This ranking includes individual monomers (monosaccharides) and the number of bonds. For example, sucrose is comprised of two monomers, glucose and fructose, which are linked by an α1,2 glycosidic bond, having a degree of polymerisation (DP) of 2. Chemical classifications that are commonly used by nutrition and food safety authorities (9) are as follows:

1) Sugars (monosaccharides and disaccharides, DP 1–2)
2) Oligosaccharides (DP 3–9)
3) Polysaccharides (DP $\geq$ 10).

Within these categories, dietary CHOs can be further subclassified as presented in **Table 1** below.

Dietary CHOs can be further subclassified as presented in **Table 1** below.

TABLE 1 | Chemical classification of carbohydrates (9–11) *Maltodextrins are an industrially hydrolyzed starch product.

Classification	Sub-group	Examples
Sugars (DP 1-2)	• Monosaccharides • Disaccharides • Sugars alcohols/polyols	• Glucose, fructose galactose, mannose, arabinose, xylose, erythrose, and others. • Sucrose, isomaltulose, lactose, maltose, trehalose, and others. • Sorbitol, mannitol, lactitol, xylitol, erythritol
Oligosaccharides (DP 3-9)	• Maltodextrins* (Malto-oligosaccharides) • Non-digestible oligosaccharides • Starch	• *Contain: glucose, maltose gluco-oligosaccharides • Raffinose, stachyose, fructo-oligosaccharides (FOS), arabino-oligosaccharides (AXOS), and others. • Amylose, amylopectin, and modified starches.
Polysaccharides (DP >9)	• Non-starch polysaccharides (NSP) • Resistant starch (RS)	• Pectin, cellulose, hemicellulose, hydrocolloids (Arabic gum, guar gum, others). • RS type 1,2,3, and 4

During hydrolysis, a mixture of gluco-oligosaccharides, maltose, and glucose is formed. The quantity of glucose and maltose present in "maltodextrins" depends on the extent of hydrolysis (rate x time).

Same Degree of Polymerization but Different Effects

When present in disaccharides, the bonds of the composing monomers (α or β glycosidic bond) can differ, which will affect the rate of digestion and absorption. In **Table 2**, the chemical classifications and molecular characteristics of selected CHOs (types, bonds) are given, along with some selected characteristics of digestion, absorption, distribution, and metabolic fate.

To explain how CHOs with a similar monomer composition can differ in their degree of digestion and absorption, we will give two examples: [1] sucrose and isomaltulose, and [2] amylose and amylopectin starch.

1) The disaccharides sucrose and isomaltulose are both composed of the two monomers glucose and fructose. However, the linkage between the two monomers differs. Sucrose has an α-1,2 bond, whereas isomaltulose has an α-1,6 bond (see **Figure 1**). Due to its more stable α-1,6 glycosidic bond, hydrolysis by small intestinal disaccharidases is slow. In human small intestinal mucosa homogenates as an enzyme source, the hydrolysis rate was 26–45% compared with sucrose (21). The result is a lower glycemic and insulinemic response (22), and consequently a reduced rate of metabolism (23).
2) One may wonder why the example of amylose and amylopectin starch is being discussed alongside sugars. The reason for including the example of starch is that sugars deliver their constituent monomers to the intestinal cells for absorption as a digestive fate. In the light of the generally accepted definition that sugars are all CHOs with a DP

Abbreviations: AGEs, Advanced glycosylation end-products; CHO, carbohydrate; CHOs: carbohydrates; DP, degree of polymerization; GI, glycemic index; MRPs, Maillard reaction products; SCFAs, short chain fatty acids; SSBs, sugar sweetened beverages.

TABLE 2 | Chemical and physiological characteristics of sugars and other glycemic carbohydrates.

CHO	Type	Digestive enzyme	In gut lumen	Enterocyte uptake	In blood	Possible metabolic fate options	GI
Glucose	Monosaccharide	-	Glucose	-	Glucose	Used as fuel, stored as glycogen and/or converted to other metabolites	100
Fructose	Monosaccharide	-	Fructose	-	Lactate, glucose, fructose	Partially converted to lactic acid and glucose, used as fuel or stored as glycogen, and fatty acids used as fuel or triacylglycerol stored as lipid	19
Sucrose	Disaccharide: glucose -fructose, α1-2 bond	Sucrase	Glucose, fructose	Glucose, fructose	glucose, lactate, fructose	see glucose and fructose above	65
Isomaltulose	Disaccharide: glucose -fructose, α1-6 bond	Isomaltase	Glucose, fructose	Glucose, fructose		See fate of glucose and fructose above	32
Galactose	Monosaccharide	-	Galactose	-	Galactose	Liver conversion to glucose, see fate of glucose above	25
Lactose	Disaccharide: glucose -galactose, α1-4 bond	Lactase	Glucose, galactose	Glucose, galactose	Glucose, galactose	See fate of glucose and galactose above	45
Honey	Glucose 30.3%, fructose 38.4%, sucrose 1.3%	Sucrase	Glucose, fructose	Glucose, fructose	Glucose, lactate, fructose	See glucose and fructose above	50
Maple syrup	Sucrose 98%, glucose 1%, fructose 1%	Sucrase	Glucose, fructose	Glucose, fructose	Glucose, lactate, fructose	See glucose and fructose above	54
HFCS 55	Fructose 55%, glucose, 43% gluco-oligo saccharides 3%	α-Dextrinase	Glucose, fructose	Glucose, fructose	Glucose, lactate, fructose	See glucose and fructose above	58
Starch	Glucose polymers: amylopectin α1-4 and α1-6 bonds. Amylose α1-4 bonds	Amylase from saliva, pancreas	Maltose, glucose	Maltose, glucose	Glucose	See fate of glucose above	40–110*
Maltodextrins	Glucose polymer, α1-4 glycosidic bonds	α -Dextrinase	Glucose, maltose	Maltose, glucose	Glucose	See glucose above	110
Maltose	Disaccharide: glucose-glucose, α1-4 glycosidic bond	Maltase	Glucose	Glucose	Glucose	See glucose above	105
Trehalose	Disaccharide: glucose-glucose, α1-1 glycosidic bond	Trehalase	Glucose	Glucose	Glucose	See glucose above	70
Sorbitol*	Sugar alcohol	-	Sorbitol	-	Sorbitol	Liver conversion to fructose and glucose, see above	4

*For a review of fructose, see Tappy and Lê (12). For a review of lactose and galactose, see University of Waterloo (13). One example of a low-caloric/low-glycemic sugar replacer is given. In the gut, sorbitol, a sugar-alcohol, is slowly absorbed (25–80% of the consumed dose) by facilitated diffusion. Absorbed sorbitol passes the liver, where it is converted to fructose and glucose (14). The unabsorbed fraction is transported to the large bowel, where it is fermented. When sorbitol is consumed in high doses, potential side effects can occur as a result of osmotic water shifts from blood into the gut, resulting in rumbling, loose stools, or diarrhea (extensive details about polyols can be found in Livesey (14), Ghosh and Sudha (15), Rice et al. (16). For a review of low- and non-caloric/non-glycemic sweeteners compared with caloric sweeteners, see Rogers et al. (17). *The glycemic index of starchy foods varies according to the molecular content of amylose, amylopectin, fiber, presence of protein, and characteristics of the food matrix, resulting in a range of reported values. For extensive glycemic index data [see (18)], International Tables of Glycemic Index and Glycemic Load Values, the online University of Sydney searchable data GI; http://www.glycemicindex.com/foodSearch.php. For further extensive details, see Queen Mary University London (19), Nomenclature of Carbohydrates (Available online at: https://www.qmul.ac.uk/sbcs/iupac/2carb/00n01.html#0121) and nomenclature of sugar alcohols (20).*

of 1–2, it becomes clear that both "sugar" and "starch" deliver "sugars" to the intestinal cells for absorption. In terms of metabolic responses, especially when comparing "sugars" with "starches," it is good to have a clear comparative view. Plant starch generally contains 20–30% by weight of amylose and 70–80% by weight of amylopectin. Amylose (**Figure 2A**) contains linear chains of approximately 300–3,000 glucose monomers in length, connected by α-1,4 bonds. In amylopectin, there is also a linear basic structure in which glucose monomers are linked by α-1,4 bonds (**Figures 2B,C**), but there are side branches along this linear base initiated with α-1,6 bonds. This situation results in a molecule with many branching endpoints and a more open structure in which digestion enzymes can act, compared with the more closed linear helix formation of amylose. The digestive enzyme α-amylase is responsible for the breakdown of the starch into dextrins (maltotriose, DP3) and maltose (DP2), which are in turn digested by epithelial maltase, resulting in glucose monomers. It is often suggested that the amylose content is the most important factor in determining the rate of digestion and absorption as well as the related glycemic response, but recent research shows that the picture is more complex (25). It appears that the interaction between the molecular and granular structure (helix formation, number of pores, size of the molecule, amylopectin sidechain length distribution and crystalline structure, the latter two being the most important) causes the variation in the rate of digestion across botanical sources (25). The latter leads to relatively rapid digestion and a significant blood glucose response. The potential of starch to affect the blood glucose response, expressed as a

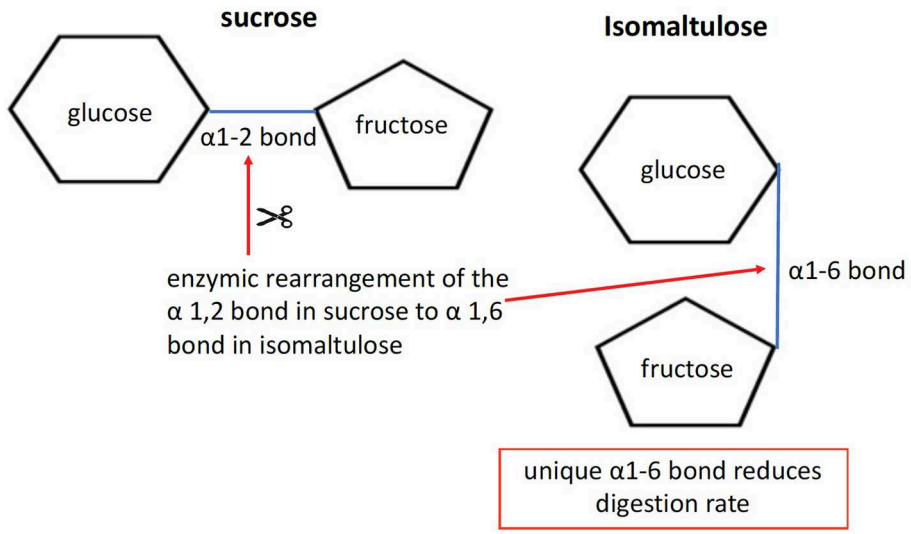

FIGURE 1 | Molecular structure of isomaltulose. By using the microbial enzyme "glucosyl transferase" for rearranging the bond structure from α1-2 in sucrose, as base substrate, to α1-6, isomaltulose is formed.

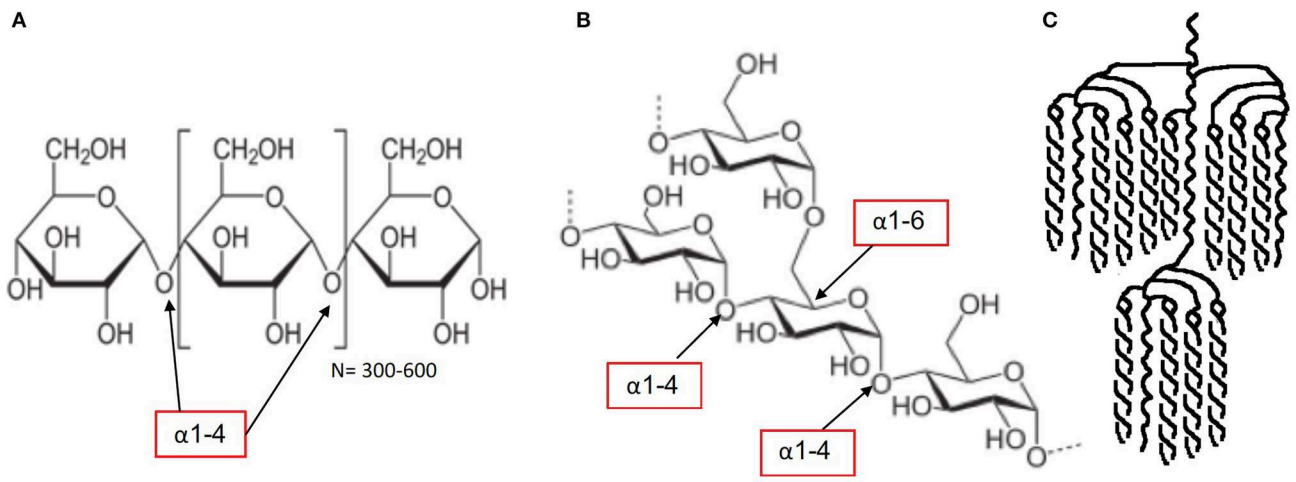

FIGURE 2 | Molecular structure starch: amylose starch **(A)**, amylopectin starch **(B,C)** [figures source (24): starch, retrieved Jan 2020].

glycemic index (GI) value, can therefore vary considerably depending on the content of amylopectin and amylose (26–28). Interestingly, despite only small differences in amylose content, *in vitro* cumulative starch hydrolysis shows that wheat starch is more rapidly digested than potato starch (being the most resistant starch), with corn, high-amylose corn, and pea starch having intermediate values (25).

Accordingly, rapidly digestible (available) CHOs, slowly digestible (available) CHOs, and non-digestible (non-available) CHOs (dietary fibers) can be ranked (18, 29–31). Along similar lines, digestible starch (glycemic) and resistant starch (not digested, non-glycemic) are both polysaccharides composed of glucose monomers and are both present in starchy foods, but they differ strongly in bioavailability. As a result, there is a wide range of GI values for different varieties of rice, cereals, potatoes and derived products, ranging from relatively low to high GI values (18). For this reason, one cannot establish a generic GI value for starchy foods. These aspects are important to understand for situations in which a rapid or sustained delivery of glucose to the circulation and tissues is required (e.g., sports nutrition or compensation of insulin dosage-induced hypoglycemia in diabetes patients), or generally to be avoided (type 2 diabetes).

In infant nutrition, sports nutrition and sometimes in clinical nutrition, maltodextrins resulting from industrial enzymic starch degradation are used, having a mixed content of glucose oligosaccharides, maltose, and glucose. It is often suggested that these maltodextrins are complex CHOs which result in a low and sustained glycemic response. However, there are no data

to support this suggestion. In fact, the enzymic digestion of maltodextrins appears to take place at a high rate, which is also reflected by comparable post-ingestion insulin responses as well as oxidation rates during exercise compared with glucose [(32); **Table 3**].

GLYCEMIC INDEX CLASSIFICATION AND ITS MEANING

The potential of CHOs to raise the level of blood glucose is often expressed as a glycemic index (GI) value. A high value refers to a strong elevation of blood glucose and is often seen as less healthy, whereas a low value is often seen as beneficial. When determining the GI value, glucose usually serves as the reference food with a glycemic index of 100. A food portion containing an amount of 50 g of available CHOs is ingested and the area under the blood glucose response curve is divided by the area resulting from the ingestion of 50 g of glucose. Full details on this matter can be found in Brouns et al. (34).

TABLE 3 | The glycemic index value of the plain carbohydrate tested vs. glucose as reference-control.

Glucose	GI−100
French baguette	GI−95
French fries	GI−75
Fructose, mean of three studies	GI−15
Macaroni, white boiled, mean of three studies	GI−50
Potato boiled, mean of seven studies	GI−53
Ripe banana, mean of nine studies	GI−48
Sourdough rye bread	GI−53
Spaghetti, white boiled. Mean of eight studies	GI−41
Sweet potato	GI−61
Sucrose	GI−67
White rice, mean of eight studies	GI−59
White wheat bread, mean of seven studies	GI−70
Whole grain rye bread, mean of four studies	GI−58

Data Source: Atkinson et al. (18) and University of Sidney (33) online searchable data GI, International Tables of Glycemic Index and Glycemic Load Values.

Table 3 gives some examples of the glycemic index values of foods. It is important to understand that the glycemic index value in isolation cannot fully explain the physiological impact of CHO-based foods and beverages on health and disease. For example, the ingestion of 5 grams of glucose will not induce measurable hyperglycemia, despite its high GI value of 100. However, the ingestion of 50 grams will increase blood glucose very significantly. Thus, any GI value should be interpreted in the light of the quantity ingested. For this reason, the concept of the "glycemic load" of CHO-containing meals has been defined as a relevant approach. In addition, it needs to be noted that the GI value of any food prepared using these CHOs as a meal component is highly influenced by other factors that affect the rate of ingestion as well as the subsequent transit, digestion and absorption, see **Figure 3**. Examples are the content of enzyme inhibitors (e.g., α-amylase inhibitors) present in the CHO source, the overall macronutrient composition (quantity and type of CHO, fat, protein), the content and characteristics of dietary fibers (e.g., soluble, viscous, insoluble, bulking), the level of processing (e.g., level of refinement, such as the separation of bran and germ during milling, resulting in "refined" white flour), as well as the matrix effects (e.g., liquid vs. solid, starch in a compact elastic spaghetti structure vs. starch in a well-cooked soft potato). In the case of drinks, energy content and osmolality are factors which can significantly affect the gastric emptying rate as well as the related supply to the gut for absorption, depending on the concentration (35).

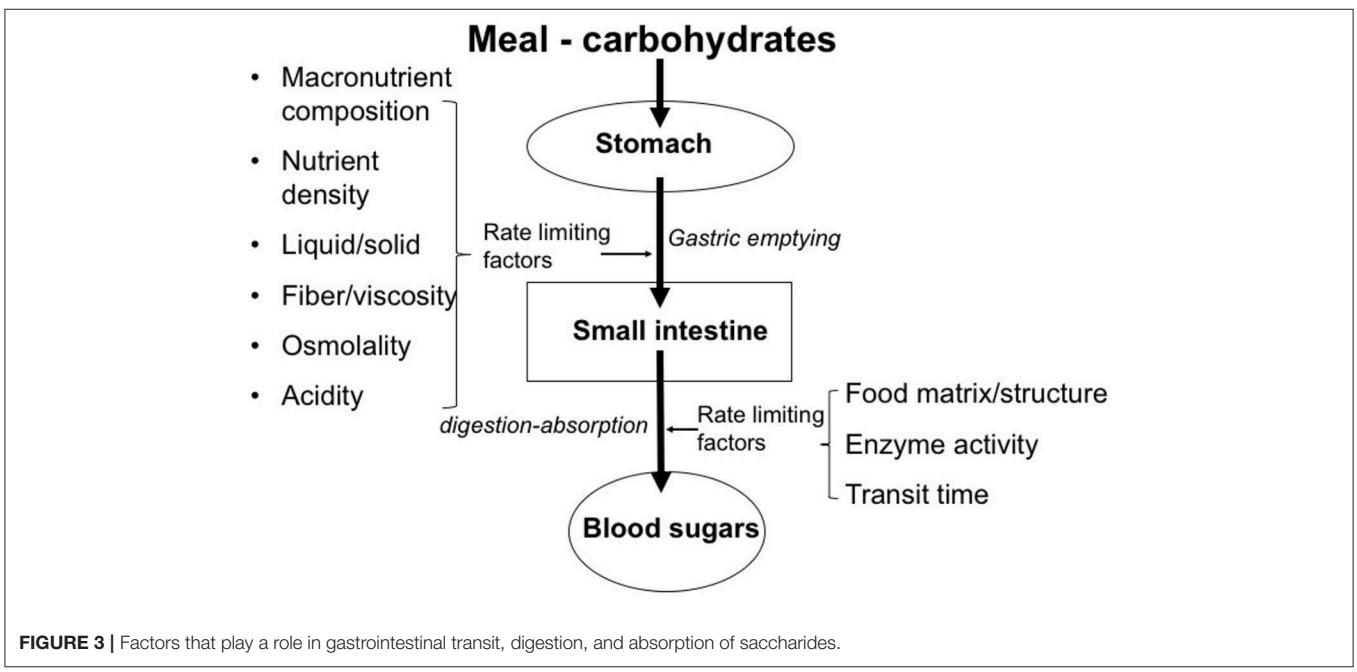

FIGURE 3 | Factors that play a role in gastrointestinal transit, digestion, and absorption of saccharides.

There is still one other point that needs to be addressed, especially related to sugars. The GI value of fructose (27) is very low and that of sucrose (36) is moderate. Thus, in terms of the viewpoint that a low to moderate GI is beneficial for health, one might conclude that fructose and sucrose are preferable for health to starches that have a much higher GI value. Based on current knowledge, this point is hard to substantiate. The suggestion that fructose is a single cause of non-alcoholic fatty liver disease driven by its dietary intake cannot be justified either based on data from excessive consumption (37).

The view that sugars added to beverages are a causal factor for obesity and diabetes is well-documented, because they cause a positive energy balance. However, in the case of sugars added to solid foods such as confectionery, this causal association has not been shown (2, 38–40). Data showing that two thirds of added sugars are being consumed in solid foods and only one third in beverages (41) raise questions about other factors that may play a role in addition to sugar (2, 3). From the above, it is clear that a focus on single CHO types, single CHO characteristics, or consumption in isolation as a single supply source has limited generalizability, especially when one wants to understand the overall effects of the diet containing these CHOs on postprandial appetite regulation, glycemia, lipidemia, low-grade inflammatory potential and possible health outcomes (42, 43). Moreover, the physiological status of the person in question also plays a significant role in how the human body manages the metabolism of saccharides. Elite endurance athletes such as professional cyclists ingest large amounts of refined carbohydrates, to a large extent in beverages, to maintain a high glucose availability for the benefit of delaying fatigue and maintaining a high-performance capacity. They burn the calories ingested, even when these exceed 6,500 kcal/day for 21 days (44), and accordingly do not become develop overweight. Based on these and other observations, their metabolism of the carbohydrates and the interrelationship with lipid metabolism will be quite different from that of inactive overweight individuals who are insulin-resistant or who suffer from type 2 diabetes when they consume large quantities of sugar-sweetened beverages [e.g., (45, 46)]. In this respect, it is obvious that specific food-based dietary guidelines are required for certain population subgroups.

LEGAL AND WHO DEFINITION OF "ADDED SUGARS" AND "FREE SUGARS"

With respect to the classification and labeling of food and beverages, one should note that the term "sugars" on the food label generally stands for *"monosaccharides and disaccharides."* In this respect, glucose and fructose are both simple sugars, but they behave very differently with regard to their metabolic effects. The hormonal responses that they induce (glucose is a significant driver of glycemia and insulin secretion, while fructose only has very minor effects on glycemia and insulin) and their metabolic fate, which includes the conversion to other intermediates such as organic acids (in particular lactic acid) as well as fatty acids, their use as fuel and their possible storage as glycogen or lipids differ. For this reason, it is important to have a basic understanding of the flow: type of carbohydrate → molecular characteristics → physiological aspects (digestion, absorption, and metabolic fate) → effects on health.

To give an example, oral glucose appears in blood as glucose and drives glycemia in a 1:1 ratio depending on the dose. Fructose, however, behaves differently because of its conversion to other metabolites and because of its very low insulinemic response (47). Although glucose and fructose are very often compared as monomers in metabolic studies, it needs to be addressed that humans usually do not consume fructose in isolation but almost always in combination with glucose, as it is present in sucrose- and HFCS-sweetened beverages, fruit juices, fruit syrups (see **Figure 5**), and fruits. Accordingly, the interpretation of data derived from studies in which fructose was supplied as monomer in high amounts should be seen in the light that this does not represent to normal human consumption situation. Concerns that all fructose from consumed SSBs and fruit juices goes straight to the liver where it is all converted to lipid are not supported by evidence. In contrast, most fructose is converted to non-lipid substrates.

Recently Jang et al. (48) (**Figure 4**) performed double labeling studies allowing for quantitatively tracing the metabolic fate of fructose vs. glucose after supply to the mice. These researchers gave fructose together with glucose at 1:1 ratio, as normally is the case in human consumption of fructose containing saccharide sources. It needs to be noticed though, that for this work in mice, oral gavage by which the test dose was directly given into the stomach, was used. Using this procedure a large amount of fructose reaches the small intestine with much faster kinetics than typical human fructose consumption. However, while mouse metabolism is ∼10× faster than humans, rendering the faster fructose dose to metabolic rate ratio similar between the species (Jang, 2020 personal communication). Using this procedure, it was shown that a large fraction of the fructose absorbed in the small intestine is converted to glucose and organic acids within the enterocytes to such an extent that only very little fructose spills over to the liver. Thus, instead of the common perception that the liver is the prime fructose clearing-organ, it appears that small intestine fulfills this role. In case an acute high-dose of fructose saturates intestinal absorption and metabolic conversion capacity, a fraction on non-absorbed fructose partly passes from the small intestine to the colon, to be subsequently fermented by the microbiota giving rise to short chain fatty acids, mostly acetate, which will be absorbed and passed on the liver. The fraction of fructose that escapes metabolic conversion by the enterocytes also passes on the liver. Both acetate and fructose entering the liver can serve as a substrate for de novo triacylglycerol synthesis. The latter, however, remains relatively small, even in a situation of acute very high doses of fructose. Studies using stable isotopes in humans (1, 52) showed that the 3–6 h after ingestion high doses of fructose only a small percentage (<1%- max 3%) was converted to fatty acids. Thus, previous human work is in line with the new insights obtained by Jang et al. (48). Future studies in humans need to verify how much fructose, at real-life intake

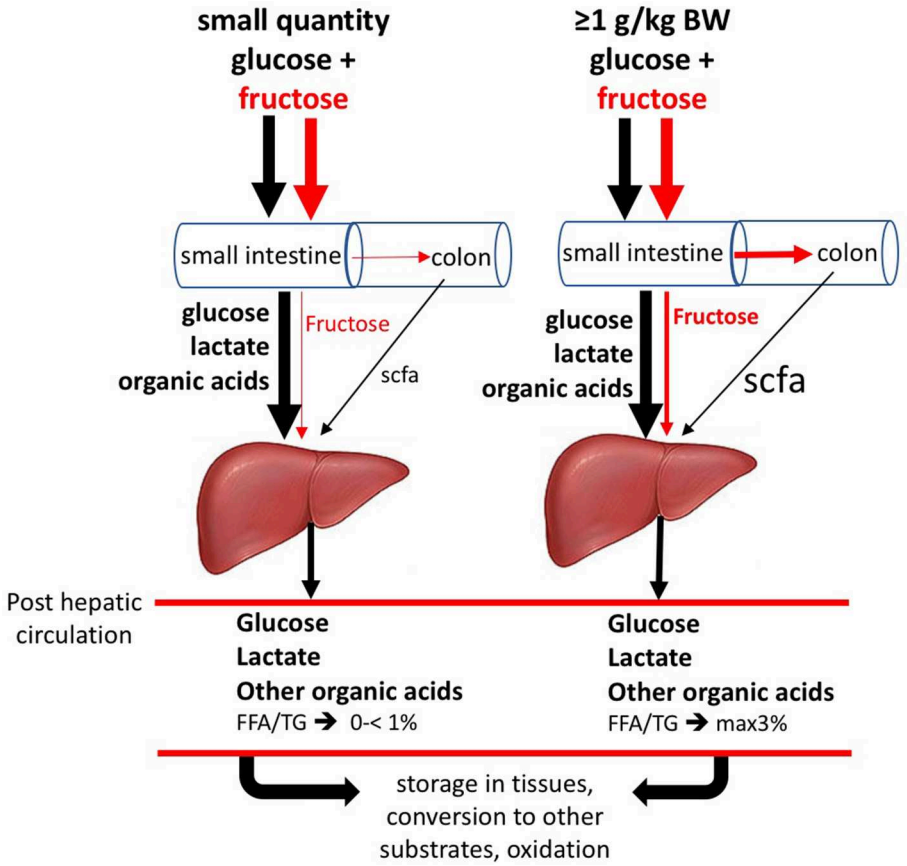

FIGURE 4 | Metabolic fate of oral fructose. When ingesting small doses of fructose (F) and glucose together, as in human nutrition, most absorbed F is converted to glucose, lactic acid and other organic acids within the enterocytes, which appear in the portal vein supplying the liver. The amount of F passing to the liver after small oral doses is negligible. Glucose largely passes the liver and enters the blood circulation to be available to all tissues. Lactate will favorably be converted into liver glycogen. Non-converted lactate will pass on to the blood circulation. After ingesting acute large doses (≥1 g/kg body weight, equivalent to >1 liter of sugar-sweetened beverage/juice), F partly escapes its own slow absorption process and will pass on to the colon, where it may cause osmotic fluid shifts potentially leading to laxation and will be fermented by the microbiota leading to the formation of short-chain fatty acids, mostly acetate, which will be absorbed and pass on to the liver with portal blood. In this situation, the absorbed but non-converted fraction of F will serve as substrate for *de novo* fatty acids synthesis, along with the acetate coming from the colon. As a result of the above, F enters the circulation only in very small quantities. (Based on data from (12, 48–53)]. Figure based on data from Jang et al. (48) and Zhao et al. (53).

levels (concerning dose-time interrelationships generally much lower than experimental supply levels), really passes on the liver and the colon and what the conversion rate is to liver fat. To put this in perspective, early human studies, using the ileostomy model or breath hydrogen as marker of malabsorption, showed very clearly that fructose ingested as monomer at doses of >25 g induces malabsorption. However, when co-ingested with glucose (such as isomaltulose or sucrose)— even up to acute doses of up to 100 g sucrose (equivalent to about 1 l of SSB or fruit juice)— this is not the case (54–59). Since humans seldom consume fructose in isolation, this is an important point to consider. In addition, it needs to be addressed what other factors, apart from fructose contribute to the novo lipogenesis. In very recent work, it was shown that fructose fermentation derived acetate contributes to liver lipogenesis (53). Concerning the latter, a range of well-fermentable dietary fibers give rise to a significant amount of SCFA the cecum and colon, most importantly acetate, propionate, and butyrate, generally in a molar range of 70:20:10%, respectively. Individuals who consume relatively high amounts of dietary fiber such as fruit fibers and fructans (inulin) generally suffer less from being overweight. Why would fiber derived acetate, compared to fructose derived acetate, not or differently contribute to fatty liver? Is there a protecting role from propionate? (60). And, Why do physically active lean individuals, who consume substantial amounts of sugar, not suffer from an overweight and fatty liver, whereas most overweight individuals do? Is excess calories/positive energy balance the prime driving factor?

Natural and Refined Sugars: Do They Differ?

The metabolism of isolated monosaccharides and disaccharides (glucose, fructose, and sucrose/table sugar) is basically similar to that present in natural sources which contain mixtures of these

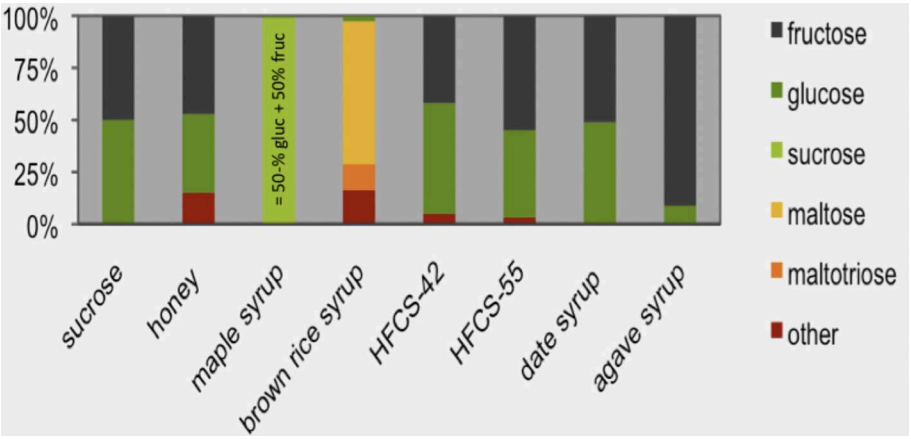

FIGURE 5 | Sugars in syrups. The sugar monomer content of sucrose (sucrose water content is subtracted from the total mass and this value is set at 100%) is compared with high-fructose corn syrup (HFCS, containing either 42 or 55% fructose) and other types of syrups. Maple syrup consists almost entirely of sucrose [source: Andrea et al. (61)].

sugars, such as in fruits or fruit-derived syrups. Because of their molecular similarity and related physiological responses, sugars naturally present in honey, fruit-derived syrups (**Figure 5**) and fruit juices have recently been proposed by the WHO (62) to be of similar nature as "commonly added sugars". This approach has led to a new, mutually inclusive category of "free sugars" and to questions about the scientific basis of the term "free sugar". For example, why are sugars in 100% fruit juice "free sugar" and the same sugars naturally present in the fruit not? Why is milk sugar naturally present in milk not considered to be a free sugar? In this respect, fruit juices have been classified in many epidemiological studies together with sugar-sweetened beverages (SSBs) as one category of "sugar-sweetened beverages". Such a pooling of beverages and related observational data has resulted in the conclusion that fruit juices, similar to sucrose-sweetened drinks, are a cause of obesity. This outcome has led to international recommendations for reducing the consumption of "free sugar." **Table 4** gives an overview of definitions for "added sugars" used by various health authorities, as recently reviewed by Buyken et al. (63).

Based on the molecular similarity of sugars, the pooling of juices and SSBs is understandable. However, data from intervention studies do not support this assumption. Murphy et al. (66) evaluated the effects of 100% fruit juice and measures of glucose control as well as insulin sensitivity in a systematic review and meta-analysis of randomized controlled trials. In this research, clinical trials were eligible for inclusion if the following criteria were met: [1] the trial was randomized and conducted in human subjects; [2] the trial was a controlled intervention providing 100% fruit juice and a control beverage (e.g., sugar/carbohydrate or energy-matched beverage, water or no beverage); [3] the fruit juice consumed was identified as 100% fruit juice; [4] subjects consumed 100% fruit juice for a minimum of 2 weeks; [5] outcome data for at least one measure of glucose control or insulin sensitivity were reported; and [6] reported outcomes included change from baseline values or baseline and endpoint values with error terms. It was concluded that the repeated intake of 100% fruit juice does not have a significant effect on glycemic control or measures of insulin resistance, which is consistent with findings from some observational studies in which the consumption of 100% fruit juice was studied separately from SSBs and in which lifestyle factors were also taken into account (2, 67). One reason may be that juice contains a wide variety of micronutrients and plant-bioactive substances from the original fruit, which may be "protective" (68). Another reason may be that individuals who decide to consume 100% juice instead of SSBs also make other healthy lifestyle decisions. As a result, 100% juice consumers usually have

TABLE 4 | Definitions of "added sugars" and their use in governmental reports [Source: Buyken et al. (63)].

"Sugars" are generally defined as "mono- and disaccharides." Accordingly, "added sugars" is mostly considered to be "added mono- and disaccharides."
• WHO report (62): introduced the term "free sugars" as "all monosaccharides and di-saccharides added to foods by the manufacturer, cook, or consumer, plus sugars naturally present in honey, syrups, and fruit juices."
• US: United States Food and Drug Administration (US-FDA)-(64) and United States Department of Agriculture: Added Sugars are all sugars that are either added during the processing of foods, or are packaged as such, and these include sugars (free, mono- and disaccharides), syrups, naturally occurring sugars that are isolated from a whole food and concentrated so that sugar is the primary component (e.g., fruit juice concentrates), and other caloric sweeteners.
• UK: SACN report (39) adopted the term "free sugars from WHO," which now replaces the terms "added sugars" and "non- milk extrinsic sugars" (NMES) used previously. "Free sugars' comprises all monosaccharides* and disaccharides* added to foods by the manufacturer, cook or consumer, plus sugars naturally present in honey, syrups and unsweetened fruit juices. Under this definition, it includes lactose (the sugar in milk), when naturally present in milk and milk products, and the sugars contained within the cellular structure of foods (particularly fruits and vegetables) are excluded."
• EU: EFSA report (65): added sugars are "mono- and disaccharides and starch hydrolysates (e.g., glucose syrup, fructose syrup, maltodextrins) added during food preparation and manufacturing."

TABLE 5 | Some physicochemical, technological, and functional characteristics that are important for food design and food processing.

- Sweetness
- Solubility
- Viscosity
- Reducing power
- Crystallinity
- Glass transition temperature
- Cooling effect (mouth)
- Melting temperature
- Freezing behavior

a more favorable body mass index [(69, 70); BMI], while the quality of the daily diet also appears to be better, as has been observed in both children and adults (68, 71). Very recently, Khan et al. (4) challenged the classification of juices in the same box as soda, since their consumption is associated with different health effects. This example also shows that looking at a single sugar type or sugar characteristic in isolation is not meaningful and may lead to wrong interpretations with respect to health.

Although the metabolism of CHO molecules naturally present in food or isolated (such as plain table sugar) is basically identical, it is important to understand as well that the food matrix can play a significant role in the rate of intake, digestion and absorption. The effects of sucrose added to a beverage (rapid gastric emptying and small intestinal absorption) will lead to a rapid increase in blood glucose and insulin, which differs from effects in a solid matrix such as confectionery (lower rate of digestion and absorption as well as a less rapid increase in blood glucose and insulin). As a consequence of its rapid gastrointestinal transit, sucrose in a beverage induces less satiation compared with sucrose in a solid food. This "incomplete sensing" drives "unnoticed" calorie intake, a positive energy balance and obesity, when happening frequently (72).

PHYSICOCHEMICAL, TECHNOLOGICAL, AND FUNCTIONAL CHARACTERISTICS INFLUENCE SUGARS METABOLISM

Sugars can also be listed according to their physicochemical, technological, and functional characteristics, which are important for food design and food processing (see **Table 5**). These characteristics can also affect the responses in our body. Two examples will be given here:

A) The relative sweetness of sugars (**Table 6**) plays an essential role when sweetening foods and beverages. The lowest amount of a sugar needed to realize a certain sweetness is determined by the highest relative sweetness. Most used for sweetening is sucrose, the reason why the sweetness of sucrose is set at 100%. To replace sucrose (sweetness = 100) in a drink with glucose (in a concentration of 10% of its relative sweetness ≅ 70), 30% more glucose is required to achieve the same degree of sweetness. As a consequence, the beverage will contain more calories! To replace 100 g of sucrose in a beverage with fructose (relative sweetness of fructose at higher concentrations ≅ 150), 33% less sugar is required. As a consequence, the drink will contain less calories but also a high level of fructose, which may cause gastrointestinal distress/diarrhea and unfavorable metabolic effects.

B) Glucose, fructose, galactose, lactose and maltose are reducing sugars. Sucrose and trehalose are non-reducing sugars. During the cooking/baking/roasting of food at high temperatures, reducing sugars react with amino acids in a Maillard reaction. This "browning reaction," such as when baking meat or bread or roasting coffee, affects the taste and flavor. For this reason, selective use can be made of reducing sugars to obtain the desired browning and flavor (74). There is a wide range of Maillard reaction products (MRPs) known to influence digestive physiology, gut microbiota and metabolism, which are also suspected of triggering an immune reaction to and the allergenic potential of proteins (75). Overheating leads to the formation of advanced glycosylation end products (AGEs), which are thought to influence inflammation and possibly insulin resistance, whereas acrylamide (a product resulting from a reaction of a reducing sugar with the amino acid asparagine) is a known carcinogen. This information has prompted strategies to limit the formation of harmful MRPs. For example, limiting sugars as well as the asparagine content of potato and cereal products before thermal processing by measures such as selecting potato varieties with a low content of reducing sugars may help reduce acrylamide. Targeted potato storage temperatures such as storage below 8°C causes an increase in reducing sugar content and higher amounts of acrylamide. Modifying heat-processing conditions (time, temperature) and applying appropriate preheating treatments, such as soaking or blanching, can also help impact on the level of reducing sugars and thereby reduce the formation of MRPs (76, 77).

TABLE 6 | Relative sweetness of sugars.

Sugar	Relative sweetness
Monosaccharides	
Fructose	115–180*
Glucose	50–70*
Galactose	54
Disaccharides	
Sucrose (gluc+fruc)	100
Maltose (gluc+gluc)	30–50*
Lactose (gluc+galac)	15–40*
Isomaltulose (gluc+fruc)	50
Trehalose (gluc+gluc)	45
HFCS–(gluc + 55% fruc)	>100
HFCS–(gluc + 42% fruc)	100

**Degree of sweetness is influenced by concentration and higher at higher concentrations. Gluc, glucose; fruc, fructose; galac, galactose [Source: Clemens (73)].*

SUGARS AND ORAL HEALTH

Recently, the WHO (62) recommended to reduce the consumption of free sugars to preferably below 5% of the total energy intake as a conditional *recommendation for both adults and children, the most important reason being the detrimental effects on oral health, despite the fact that the evidence was judged to be of a very low quality (62). [*Conditional recommendations are made when there is less certainty "about the balance between the benefits and harms or disadvantages of implementing a recommendation." This means that "policy-making will require substantial debate and involvement of various stakeholders" for translating them into action (78)].

This evidence was based on data derived by experts as published in various reviews (79–82). Detrimental effects of sugars on oral health occur along two main routes: Firstly, this can be in the form of demineralization of enamel and dentine caused by acid, resulting from saccharolytic fermentation of sugars by oral microbiota; these monosaccharides and disaccharides include glucose derived from starch degradation by salivary amylase; Secondly, detrimental effects can result from exposure to food acids added to sugar-sweetened or light drinks, or acids naturally present such as in citrus juices, resulting in a low drink PH. These food-acids will directly erode the enamel and dentine without intervention of the oral microbiota In normal conditions, the acid present in the food/drink or formed by the microbiota is buffered over time and hence neutralized by saliva. In addition, saliva at neutral pH is supersaturated for calcium and phosphate, enabling the repair of the acid-induced demineralization (83). Acids derived from sugars can cause net demineralization when frequently taken and this is more detrimental if salivary buffer capacity is exceeded when saliva production is low or absent. Examples of the latter are athletes during intensive exercise when saliva production is inhibited and persons suffering from a low or absent salivary flow as a result of cancer radiation treatment, autoimmune diseases, (multiple) medications or physiologically by ageing.

Many studies have been performed to define the *in vivo* (in situ) cariogenic and erosive effects of sugars and acids on tooth mineral by the application of small intra-oral blocks of dentine or enamel or by using standardized solution enamel-rinsing essays *in vitro*. In the latter, the effects of remineralization can also be studied in detail. Depending on the frequency and dose, sucrose, glucose, fructose, lactose or starch may all result in demineralization (84–88). It appears that the molecular composition of sugars plays a role in the degree of fermentability by oral microbiota. For example, sucrose is composed of glucose and fructose, has an α-1,2 bond and is more rapidly fermented, and this lead to a critical lowering of plaque pH than isomaltulose, which is composed of the same monomers but which has an α-1,6 bond. Along similar lines, starch which is rapidly degradable by amylase and which leads to a higher glucose availability appears to be more cariogenic than slowly digestible starch which contains a higher fraction of amylose (89). It needs to be considered in this respect that a sticky food-matrix will increase tooth surface contact exposure time, thus enhance detrimental effects on tooth mineral. Sucrose is known to be most potent in causing cariogenicity, which raises questions; since the effects appear to be more potent than the effects of its composing monomers glucose and fructose.

Recently, it was hypothesized that an oral microbiota imbalance due to frequent sucrose exposure may be a causal factor driving sucrose to be more harmful because sucrose exposure disrupted the homeostasis between acid-producing and alkali-producing bacteria (90). Because the oral microbial composition and metabolism changed significantly with sucrose exposure, while no significant difference was detected after lactose and glucose exposure, the authors claim that these findings indicate that the cariogenicity of sugars is closely related to their effects on the oral microecology.

Acidified drinks containing substantial amounts of sucrose are of particular concern (91, 92), because they do not only cause caries but also dental erosion. Even acidified drinks with low sugar contents or without sugars making use of non-sugar sweeteners are erosive because of the acids present therein result (93). Despite the primary focus on the role of sugars in causing caries, it should be noted that the process of dental erosion and caries initiation is multifactorial (36, 94).

In particular, the effects of sucrose appear to be of great concern during childhood, given the fact that SSBs intake significantly increases the caries burden in 10-year-olds with attenuated effects in 15-year-olds-age groups that are known to be the highest consumers of free sugars. To prevent caries, SSBs consumption should therefore be reduced, especially in children and adolescents (95). Of great concern are a simultaneous combination of high sipping frequency and low PH beverage and sugar concentration, especially in young children, leading to early childhood caries. Giving very young children sugary drinks in a sipping bottle will lead to continuous small quantities flushing especially of the front teeth. This process will be even more detrimental if the child falls asleep, resulting in a low salivary flow and the reduction of the salivary-buffering effect (96, 97). There is no doubt about the fact that sugar and food acids are not the only factors of importance. Poor oral hygiene, use of fluoride, appropriate salivary flow, presence of calcium in the drink/food, type of food acid used (94), consumption pattern and bottle or breastfeeding (97) play a role in the etiology of caries. In addition, the frequency of exposure may be more relevant than the quantity. Van Loveren (98) addressed the question of which sugar-reducing strategy is the best for caries prevention. To answer this question, the following aspects should be addressed: the shape of the dose-response association between sugar intake and caries, the influence of fluoridated toothpaste on the association of sugar intake and caries, as well as the relative contribution of frequency and amount of sugar intake to caries levels. The author argues that when fluoride is appropriately used, the relation between sugar consumption and caries is very low or absent. The high correlation between amount and frequency hampers the decision on which of the two is more important. Reducing the amount without reducing the frequency does not seem to be an effective approach to prevent caries.

CONCLUDING REMARKS

All rapid fermentable sugars give rise to acid production by microbiota present in the oral cavity which, dependent on frequency of exposure, salivary buffer capacity, presence of calcium for remineralization and oral hygiene status will impact on erosive potential and cariogenicity. All digestible CHOs deliver "sugars" as monosaccharides to the gut epithelium for absorption. Post-absorption, the metabolism of these monomers is basically identical and independent of the original source. However, the way in which CHOs have been processed (natural, low-processed vs. refined/highly processed, and heat-exposed), the matrix in which these CHOs are present (e.g., liquid, solid, viscous, and non-viscous), the co-presence of other nutrients (e.g., proteins, polyphenols, vitamins, minerals, and plant-bioactive substances) in the natural CHO source/matrix vs. their absence in refined CHOs and the dose ingested all play a role in the overall effects in the human body. Looking at one particular CHO characteristic will almost always lead to a different conclusion, such as that fructose is toxic (99) than evaluating from a "total perspective"; fructose is only toxic at excessive exposure levels that do not mimic human consumption (1, 3). It appears that mutual and interactive effects exceed the sum of the individual characteristics, while they also determine the effects on health and disease. For this reason, an increased focus on the overall effects and quality of carbohydrate sources and meals for food-based guidelines rather than individual component-based recommendations is desired.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

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REFERENCES

1. van Buul VJ, Tappy L, Brouns FJ. Misconceptions about fructose-containing sugars and their role in the obesity epidemic. *Nutr Res Rev*. (2014) 27:119–30. doi: 10.1017/S0954422414000067
2. Sievenpiper JL, Tappy L, Brouns F. Fructose as a driver of diabetes: an incomplete view of the evidence. *Mayo Clin Proc*. (2015) 90:984–8. doi: 10.1016/j.mayocp.2015.04.017
3. Khan TA, Sievenpiper JL. Controversies about sugars: results from systematic reviews meta-analyses on obesity, cardiometabolic disease diabetes. *Eur J Nutr*. (2016) 55(Suppl. 2):25–43. doi: 10.1007/s00394-016-1345-3
4. Khan TA, Chiavaroli L, Zurbau A, Sievenpiper JL. A lack of consideration of a dose-response relationship can lead to erroneous conclusions regarding 100% fruit juice and the risk of cardiometabolic disease. *Eur J Clin Nutr*. (2019) 73:1556–60. doi: 10.1038/s41430-019-0514-x
5. Brink E, van Rossum C, Postma-Smeets A, Stafleu A, Wolvers D, van Dooren C, et al. Development of healthy and sustainable food-based dietary guidelines for the Netherlands. *Public Health Nutr*. (2019) 22:2419–35. doi: 10.1017/S1368980019001435
6. Sirichakwal PP, Sranacharoenpong K, Tontisirin K. Food based dietary guidelines (FBDGs) development and promotion in Thailand. *Asia Pac J Clin Nutr*. (2011) 20:477–83. doi: 10.6133/apjcn.2011.20.3.19
7. EU Science HUB. (2019) Food based dietary guidelines in Europe. Available online at: https://ec.europa.eu/jrc/en/health-knowledge-gateway/promotion-prevention/nutrition/food-based-dietary-guidelines
8. European Food Safety Authority (EFSA). Scientific opinion on establishing food-based dietary guidelines. *EFSA J*. (2010) 8:1460. doi: 10.2903/j.efsa.2010.1460
9. FAO/WHO. *Carbohydrates in Human Nutrition: Report of a Joint FAO/WHO Expert Consultation*, (1998). Available online at: http://www.fao.org/3/W8079E/W8079E00.htm (accessed March 1, 2020).
10. Champ M, Langkilde AM, Brouns F, Kettlitz B, le Bail Collet Y. Advances in dietary fibre characterisation. 1. Definition of dietary fibre, physiological relevance, health benefits and analytical aspects. *Nutr. Res. Rev*. (2003) 16:71–82. doi: 10.1079/NRR200364
11. Champ M, Langkilde AM, Brouns F, Kettlitz B, le Bail-Collet Y. Advances in dietary fibre characterisation. 2. Consumption, chemistry, physiology and measurement of resistant starch; implications for health and food labelling. *Nutr Res Rev*. (2003) 16:143–61. doi: 10.1079/NRR200254
12. Tappy L, Lê KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev*. (2010) 90:23–46. doi: 10.1152/physrev.00019.2009
13. University-of-Waterloo. *Lactose-Galactose Web Info*. Available online at: http://watcut.uwaterloo.ca/webnotes/Metabolism/OtherSugars.html (accessed March 1, 2020).
14. Livesey G. Health potential of polyols as sugar replacers, with emphasis on low glycaemic properties. *Nutr Res Rev*. (2003) 16:163–91. doi: 10.1079/NRR200371
15. Ghosh S, Sudha ML. A review on polyols: new frontiers for health-based bakery products. *Int J Food Sci Nutr*. (2012) 63:372–9. doi: 10.3109/09637486.2011.627846
16. Rice T, Zannini E, Arendt E, Coffey A, Rice T, Zannini E. Coffey AA review of polyols - biotechnological production, food applications, regulation, labeling and health effects. *Crit Rev Food Sci Nutr*. (2019) 1–18. doi: 10.1080/10408398.2019.1625859
17. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int J Obes (Lond)*. (2016) 40:381–94. doi: 10.1038/ijo.2015.177
18. Atkinson FS, Foster-Powell K, Brand-Miller JC. International tables of glycemic index and glycemic load values. *Diabetes Care*. (2008) 31:2281–3. doi: 10.2337/dc08-1239
19. Queen-Mary-University-London. *Nomenclature of Carbohydrates*. Available online at: https://www.qmul.ac.uk/sbcs/iupac/class/carbo.html (accessed March 1, 2020).
20. Grembecka M. "Sugar alcohols." In: *Encyclopedia of food chemistry*. Elsevier (2019) 265–75. doi: 10.1016/B978-0-08-100596-5.21625-9
21. Lina BA, Jonker D, Kozianowski G Isomaltulose (Palatinose): a review of biological and toxicological studies. *Food Chem Toxicol*. (2002) 40:1375–81. doi: 10.1016/S0278-6915(02)00105-9
22. van Can JG, Ijzerman TH, van Loon LJ, Brouns F, Blaak EE. Reduced glycaemic and insulinaemic responses following isomaltulose ingestion: implications for postprandial substrate use. *Br J Nutr*. (2009) 102:1408–13. doi: 10.1017/S0007114509990687

23. Achten J, Jentjens RL, Brouns F, Jeukendrup AE. Exogenous oxidation of isomaltulose is lower than that of sucrose during exercise in men. *J Nutr*. (2007) 137:1143–8. doi: 10.1093/jn/137.5.1143
24. Wikipedia. *Starch*. Available online at: Available online at: https://en.wikipedia.org/wiki/Starch (accessed March 1, 2020).
25. Martens BMJ, Gerrits WJJ, Bruininx EMAM, Schols HA. Amylopectin structure crystallinity explains variation in digestion kinetics of starches across botanic sources in an in vitro pig mode$l. *J Anim Sci Biotechnol*. (2018) 91:2–13. doi: 10.1186/s40104-018-0303-8
26. Lin L, Zhang Q, Zhang L, Wei C. Evaluation of the molecular structural parameters of normal rice starch and their relationships with its thermal and digestion properties. *Molecules*. (2017) 22:1526. doi: 10.3390/molecules22091526
27. Zenel AM, Stewart ML. High Amylose White Rice Reduces Post-Prandial Glycemic Response but Not Appetite in Humans. *Nutrients*. (2015) 7:5362–74. doi: 10.3390/nu7075225
28. Luhovyy BL, Mollard RC, Yurchenko S, Nunez MF, Berengut S, Liu TT, et al. The effects of whole grain high-amylose maize flour as a source of resistant starch on blood glucose, satiety, food intake in young men. *J Food Sci*. (2014) 79:H2550–6. doi: 10.1111/1750-3841.12690
29. Jenkins DJ, Wolever TM, Taylor RH, Barker H, Fielden H, Baldwin JM, et al. Glycemic index of foods: a physiological basis for carbohydrate exchange. *Am J Clin Nutr*. (1981) 34:362–6. doi: 10.1093/ajcn/34.3.362
30. Englyst HN, Kingman *SM*, Cummings JH. Classification measurement of nutritionally important starch fractions. *Eur J Clin Nutr*. (1992) 46 (Suppl. 2):S33–50.
31. Englyst KN, Liu S, Englyst HN. Nutritional characterization measurement of dietary carbohydrates. *Eur J Clin Nutr*. (2007) 61(Suppl. 1):S19–39. doi: 10.1038/sj.ejcn.1602937
32. Hofman DL, van Buul VJ, Brouns FJ. Nutrition, health, and regulatory aspects of digestible maltodextrins. *Crit Rev Food Sci Nutr*. (2016) 56:2091–100. doi: 10.1080/10408398.2014.940415
33. University-of-Sidney. *University of Sidney Searchable Data Base GI*. Availabe online at: http://www.glycemicindex.com/foodSearch.php (accessed March 1, 2020).
34. Brouns F, Bjorck I, Frayn KN, Gibbs AL, Lang V, Slama G, et al. Glycaemic index methodology. *Nutr Res Rev*. (2005) 18:145–71. doi: 10.1079/NRR2005100
35. Brouns F. Gastric emptying as a regulatory factor in fluid uptake. *Int J Sports Med*. (1998) 19(Suppl. 2):S125–8. doi: 10.1055/s-2007-971976
36. Touger-Decker R, van Loveren C. Sugars and dental caries. *Am J Clin Nutr*. (2003) 78(suppl):881S—92S. doi: 10.1093/ajcn/78.4.881S
37. Ter Horst KW, Serlie MJ. (2017) Fructose Consumption, Lipogenesis, and Non-Alcoholic Fatty Liver Disease. *Nutrients*. 9(9). doi: 10.3390/nu9090981
38. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomized controlled trials and cohort studies. *Br Med J*. (2012) 345:e7492. doi: 10.1136/bmj.e7492
39. SACN-UK. *Carbohydrates and Health*. (2015). Available online at: https://www.gov.uk/government/publications/sacn-carbohydrates-and-health-report (accessed March 1, 2020).
40. Gasser CE, Mensah FK, Russell M, Dunn SE, Wake M. Confectionery consumption overweight, obesity, related outcomes in children adolescents: a systematic review meta-analysis. *Am J Clin Nutr*. (2016) 103:1344–56. doi: 10.3945/ajcn.115.119883
41. Ervin RB. *Consumption of Added Sugars Among, U.S. Adults, 2005–2010*. US dept Health Human Services NCHS data brief (2013). Available online at: https://www.cdc.gov/nchs/data/databriefs/db122.pdf (accessed March 1, 2020).
42. Delzenne N, Blundell J, Brouns F, Cunningham K, De Graaf K, Erkner A, et al. Gastrointestinal targets of appetite regulation in humans. *Obes Rev*. (2010) 11:234–50. doi: 10.1111/j.1467-789X.2009.00707.x
43. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors low-grade inflammation in relation to overweight obesity. *Br J Nutr*. (2011) 106(Suppl. 3):S5–78. doi: 10.1017/S0007114511005460
44. Saris WH, van Erp-Baart MA, Brouns F, Westerterp KR, ten Hoor F. Study on food intake energy expenditure during extreme sustained exercise: the Tour de France. *J Sports Med*. (1989) 10(Suppl. 1):26–31. doi: 10.1055/s-2007-1024951
45. Elia M, Stubbs RJ, Henry CJK. Differences in fat, carbohydrate, and protein metabolism between lean and obese subjects undergoing total starvation. *Obesity Res*. (1999) 7:597–604. doi: 10.1002/j.1550-8528.1999.tb00720.x
46. Clamp L, Hehir AP, Lambert EV, Beglinger C, Goedecke JH. Lean and obese dietary phenotypes: Differences in energy and substrate metabolism and appetite. *Br J Nutr*. (2015) 114:1724–33. doi: 10.1017/S0007114515003402
47. Lee BM, Wolever TM. Effect of glucose, sucrose fructose on plasma glucose insulin responses in normal humans: comparison with white bread. *Eur J Clin Nutr*. (1998) 52:924–28. doi: 10.1038/sj.ejcn.1600666
48. Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab*. (2018) 27:351–61 e3. doi: 10.1016/j.cmet.2017.12.016
49. Acheson KJ, Schutz Y, Bessard T, Anantharaman K, Flatt JP, Jéquier E. Glycogen storage capacity and de novo lipogenesis during massive carbohydrate overfeeding in man. *Am J Clin Nutr*. (1988) 48:240–7. doi: 10.1093/ajcn/48.2.240
50. Hellerstein MK, Schwarz JM, Neese RA. Regulation of hepatic de novo lipogenesis in humans. *Annu Rev Nutr*. (1996) 16:523–57. doi: 10.1146/annurev.nu.16.070196.002515
51. Silva JCP, Marques C, Martins FO, Viegas I, Tavares L, Macedo MP, et al. Determining contribution exogenous glucose and fructose to de novo fatty acid and glycerol synthesis in liver and adipose tissue. *Metab Eng*. (2019) 56:69–76. doi: 10.1016/j.ymben.2019.08.018
52. Sun SZ, Empie MW. Fructose metabolism in humans - what isotopic tracer studies tell us. *Nutr Metab (Lond)*. (2012) 9:89. doi: 10.1186/1743-7075-9-89
53. Zhao S, Jang C, Liu J, Uehara K, Gilbert M, Izzo L, et al. Dietary fructose feeds hepatic lipogenesis via microbiota-derived acetate. *Nature*. (2020) 579:586–91. doi: 10.1038/s41586-020-2101-7fRefe
54. Gibson PR, Newnham E, Barrett JS, Shepherd SJ, Muir JG. Review article: fructose malabsorption and the bigger picture. *Aliment Pharmacol Ther*. (2007) 25:349–63. doi: 10.1111/j.1365-2036.2006.03186.x
55. Normèn L, Lærke HN, Jensen BB, Langkilde AM, Andersson H. Small-bowel absorption of D-tagatose and related effects on carbohydrate digestibility: an ileostomy study. *Am J Clin Nutr*. (2001) 73:105—10 doi: 10.1093/ajcn/73.1.105
56. Ladas SD, Grammenos I, Tassios PS, Raptis SA. Coincidental malabsorption of lactose, fructose, and sorbitol ingested at low doses is not common in normal adults. *Dig Dis Sci*. (2000) 45:2357–62. doi: 10.1023/A:1005634824020
57. Rumessen JJ, Gudmand-Hoyer E. Absorption capacity of fructose in healthy adults. *Comparison with sucrose and its constituent monosaccharides Gut*. (1986) 27:1161–8. doi: 10.1136/gut.27.10.1161
58. Kneepkens CMF. What happens to fructose in the gut? *Scand J Gastroenterol*. (1989) 24(Suppl. 171):1–8. doi: 10.3109/00365528909091365
59. Holub I, Gostner A, Theis S, Nosek L, Kudlich T, Melcher R, et al. Novel findings on the metabolic effects of the low glycaemic carbohydrate isomaltulose (Palatinose). *Br J Nutr*. (2010) 103:1730–7. doi: 10.1017/S0007114509993874
60. Chambers ES, Byrne CS, Rugyendo A, Morrison DJ, Preston T, Tedford C, et al. The effects of dietary supplementation with inulin and inulin-propionate ester on hepatic steatosis in adults with non-alcoholic fatty liver disease. *Diabetes Obes Metab*. (2019) 21:372–6. doi: 10.1111/dom.13500
61. Andrea M, van de Heuvel I, Brouns F. Fruit syrups: sweet concentrated sources. *World Food Ingredients*. (2016) 44—46.
62. WHO. *Guideline: Sugars Intake for Adults and Children*. (2015). Available online at: https://apps.who.int/iris/bitstream/handle/10665/149782/9789241549028_eng.pdf (accessed March 1, 2020).
63. Buyken AE, Mela DJ, Dussort P, Johnson IT, Macdonald IA, Stowell JD, et al. Dietary carbohydrates: a review of international recommendations and the methods used to derive them. *Eur J Clin Nutr*. (2018) 72:1625–43. doi: 10.1038/s41430-017-0035-4
64. US-FDA- 2014. *Additional Information about High-Intensity Sweeteners Permitted for Use in Food in the United States*. Available online at: https://www.fda.gov/food/food-additives-petitions/additional-information-about-high-intensity-sweeteners-permitted-use-food-united-states (accessed March 1, 2020).
65. EFSA. Scientific opinion on dietary reference values for carbohydrates and dietary fibre. *EFSA J*. (2010) 8:1462. doi: 10.2903/j.efsa.2010.1462
66. Murphy MM, Barrett EC, Bresnahan KA, Barraj LM. 100 % Fruit juice and measures of glucose control and insulin sensitivity: a systematic review

and meta-analysis of randomised controlled trials. *J Nutr Sci.* (2017) 6:e59. doi: 10.1017/jns.2017.63
67. Wang B, Liu K, Mi M, Wang J. Effect of fruit juice on glucose control and insulin sensitivity in adults: a meta-analysis of 12 randomized controlled trials. *PLoS ONE.* (2014) 9:e95323. doi: 10.1371/journal.pone.0095323
68. Yang M, Lee SG, Wang Y, Lloyd B, Chung SJ, Song WO, et al. Orange juice, a marker of diet quality, contributes to essential micronutrient and antioxidant intakes in the United States population. *J Nutr Educ Behav*. (2013) 45:340–8. doi: 10.1016/j.jneb.2012.07.005
69. Wang Y, Lloyd B, Yang M, Davis CG, Lee S-G, Lee W, et al. Impact of orange juice consumption on macronutrient and energy intakes and body composition in the US population. *Public Health Nutr.* (2012) 15, 2220–27. doi: 10.1017/S1368980012000742
70. Auerbach BJ, Wolf FM, Hikida A, Vallila-Buchman P, Littman A, Thompson D, et al. Fruit juice and change in BMI: a meta-analysis. *Pediatrics.* (2017) 139:1–12. doi: 10.1542/peds.2016-2454
71. O'Neil CE, Nicklas TA, Zanovec M, Fulgoni VL, III. Diet quality is positively associated with 100% fruit juice consumption in children adults in the United States: NHANES 2003-2006. *Nutr J.* (2011) 10:17. doi: 10.1186/1475-2891-10-17
72. Pan A, Hu FB. Effects of carbohydrates on satiety: differences between liquid and solid food. *Curr Opinion Clin Nutr Metab Care*. (2011) 14:385–90 doi: 10.1097/MCO.0b013e328346df36
73. Clemens RA. Functionality of sugars in foods and health. *Compreh Rev Food Sci Food Safety.* (2016) 15:433–70. doi: 10.1111/1541-4337.12194
74. Lund MN, Ray CA. Control of maillard reactions in foods: strategies and chemical mechanisms. *J Agric Food Chem.* (2017) 65:4537–52. doi: 10.1021/acs.jafc.7b00882
75. Teodorowicz M, van Neerven J, Savelkoul H. Food Processing: the influence of the maillard reaction on immunogenicity and allergenicity of food proteins. *Nutrients*. (2017) 9:835. doi: 10.3390/nu9080835
76. Delgado-Andrade C, Fogliano V. Dietary advanced glycosylation end-products (dAGEs) and melanoidins formed through the maillard reaction: physiological consequences of their intake. *Annu Rev Food Sci Technol.* (2018) 9:271–91. doi: 10.1146/annurev-food-030117-012441
77. Rifai L, Saleh FA. A review on acrylamide in food: occurrence, toxicity, and mitigation strategies. *Int J Toxicol.* (2020) 39, 93–102. doi: 10.1177/1091581820902405
78. Blas E, Koller T, Magar V, Thomas R, Vogel J, Abou-Setta A, et al. WHO library cataloguing-in-publication data WHO handbook for guideline development. *Peer Rev Med Heal Organ World Heal Organ*, (2018) 1. Available online at: www.who.int/about/licensing/copyright_form/en/index.html
79. Moynihan PJ, Kelly SA. Effect on caries of restricting sugars intake. *J Dent Res.* (2014) 93:8–18. doi: 10.1177/0022034513508954
80. Moynihan PJ. Sugars and dental caries: evidence for setting a recommended threshold for intake. *Adv.* (2016) 7:149–56. doi: 10.3945/an.115.009365
81. Sheiham A, James WP. A reappraisal of the quantitative relationship between sugar intake and dental caries: the need for new criteria for developing goals for sugar intake. *BMC Public Health*. (2014) 14:863. doi: 10.1186/1471-2458-14-863
82. Breda J, Jewell J, Keller A. The importance of the world health organization sugar guidelines for dental health and obesity prevention. *Caries Res*. (2019) 53:149–152. doi: 10.1159/000491556
83. García-Godoy F, Hicks MJ. Maintaining the integrity of the enamel surface: the role of dental biofilm, saliva and preventive agents in enamel demineralization and remineralization. *J Am Dental Assoc.* (2008) 139:25S−34S. doi: 10.14219/jada.archive.2008.0352
84. Lingström P, Birkhed D, Ruben J, Arends J. Effect of frequent consumption of starchy food items on enamel and dentin demineralization and on plaque pH in situ. *J Dent Res.* (1994) 73:652–60. doi: 10.1177/00220345940730031101
85. Cury JA, Rebelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CP. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. *Caries Res.* (2000) 34:491–7. doi: 10.1159/000016629
86. Aires CP, Tabchoury CP, Del Bel Cury AA, Cury JA. Effect of a lactose-containing sweetener on root dentine demineralization in situ. *Caries Res.* (2002) 36:167–9. doi: 10.1159/000059331
87. Aires CP, Del Bel Cury AA, Tenuta LM, Klein MI, Koo H, Duarte S, et al. Effect of starch and sucrose on dental biofilm formation and on root dentine demineralization. *Caries Res.* (2008) 42:380–6. doi: 10.1159/000154783
88. Vale GC, Tabchoury CP, Arthur RA, Del Bel Cury AA, Paes Leme AF, Cury JA. Temporal relationship between sucrose-associated changes in dental biofilm composition and enamel demineralization. *Caries Res.* (2007) 41:406–12. doi: 10.1159/000105764
89. Halvorsrud K, Lewney J, Craig D, Moynihan PJ. Effects of starch on oral health: systematic review to inform WHO guideline. *J Dent Res.* (2019) 98:46–53. doi: 10.1177/0022034518788283
90. Du Q, Fu M, Zhou, Y. Cao Y, Guo T, Zhou Z, et al. Sucrose promotes caries progression by disrupting the microecological balance in oral biofilms: an *in vitro* study. *Sci Rep.* (2020) 10:2961. doi: 10.1038/s41598-020-59733-6
91. Cheng R, Yang H, Shao MY, Hu T, Zhou XD. Dental erosion and severe tooth decay related to soft drinks: a case report and literature review. *J Zhejiang Univ Sci B.* (2009) 10:395–9. doi: 10.1631/jzus.B0820245
92. Sheiham A, James WP. Diet and dental caries: the pivotal role of free sugars reemphasized. *J Dent Res.* (2015) 94:1341–7. doi: 10.1177/0022034515590377
93. Owens BM, Kitchens M. The erosive potential of soft drinks on enamel surface substrate: an in vitro scanning electron microscopy investigation. *J Contemp Dent Pract.* (2007) 8:11–20. doi: 10.5005/jcdp-8-7-11
94. Tahmassebi JF, Duggal MS, Malik-Kotru G, Curzon ME. Soft drinks and dental health: A review of the current literature. *J Dent.* (2006) 34:2–11. doi: 10.1016/j.jdent.2004.11.006
95. Pitchika V, Standl M, Harris C, Thiering E, Hickel R, Heinrich J, et al. Association of sugar-sweetened drinks with caries in 10- and 15-year-olds. *BMC Oral Health.* (2020) 20:81 doi: 10.1186/s12903-020-01068-9
96. Baghlaf K, Muirhead V, Moynihan P, Weston-Price S, Pine C. Free sugars consumption around bedtime and dental caries in children: a systematic review. *JDR Clin Trans Res.* (2018) 3:118–29. doi: 10.1177/2380084417749215
97. Avila WM, Pordeus IA, Paiva SM, Martins CC. Breast and bottle feeding as risk factors for dental caries: a systematic review and meta- analysis. *PLoS ONE.* (2015) 10:e0142922. doi: 10.1371/journal.pone.0142922
98. Van Loveren C. Sugar restriction for caries prevention: amount and frequency. Which is more important? *Caries Res*. (2019) 53:168–75. doi: 10.1159/000489571
99. Lustig RH, Schmidt LA, Brindis CD. Public health: The toxic truth about sugar. *Nature.* (2012) 482:27–9. doi: 10.1038/482027a

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Opposing Consumption Trends for Sugar-Sweetened Beverages and Plain Drinking Water: Analyses of NHANES 2011–16 Data

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Background: Choosing water in place of sugar-sweetened beverages (SSB) can reduce added sugars while maintaining adequate hydration. The present goal was to examine 2011–16 time trends in SSB vs. water consumption across US population subgroups.

Methods: Dietary intake data for 22,716 persons aged >4 years came from two 24-h dietary recalls in successive cycles of the National Health and Examination Survey (NHANES 2011–16). Water intakes (in mL/d) from plain water (tap and bottled) and from beverages (SSB and not-SSB) were the principal outcome variables. Intakes were analyzed by age group, income to poverty ratio (IPR), and race/ethnicity. Time trends by demographics were also examined.

Results: SSB and water intakes followed distinct social gradients. Most SSB was consumed by Non-Hispanic Black and lower-income groups. Most tap water was consumed by Non-Hispanic White and higher-income groups. During 2011–16, water from SSB declined from 322 to 262 mL/d ($p < 0.005$), whereas plain water increased (1,011–1,144 mL/d) ($p < 0.05$). Groups aged <30 years reduced SSB consumption ($p < 0.0001$) but it was groups aged >30 years that increased drinking water ($p < 0.001$). Non-Hispanic White groups reduced SSB and increased tap water consumption. Non-Hispanic Black and lower income groups reduced SSB and increased bottled water, not tap.

Conclusion: The opposing time trends in SSB and water consumption were not uniform across age groups or sociodemographic strata. Only the non-Hispanic White population reduced SSB and showed a corresponding increase in tap water. Lower-income and minority groups consumed relatively little plain drinking water from the tap.

Keywords: water tap, water bottled, sugar-sweetened beverages, NHANES 2011–2016, hydration, time trends

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INTRODUCTION

Choosing plain drinking water in place of sugar-sweetened beverages (SSB) is one way to maintain hydration while reducing added sugars (1, 2). Recent analyses of the three cycles of the National Health and Nutrition Examination Survey (NHANES 2011–2016) pointed to an overall decline in the consumption of sugar sweetened beverages (SSB),

a finding consistent with prior reports (3, 4). This decline was offset, in part, by a corresponding increase in consumption of plain drinking water (5). Hydration was not affected, since water intakes in mL from all sources: drinking water, caloric and non-caloric beverages, and moisture from foods remained constant (5). However, given documented differences in SSB and water consumption patterns by age and demographic groups, the looked-for increases in the consumption of plain water (2) may not have occurred equally across all population strata.

First, consumption patterns for SSB and water follow very distinct socio-demographic gradients (6). SSB consumption is highest among younger adults (aged <30 years), lower income groups, and the Hispanic and non-Hispanic Black population (4, 6, 7). By contrast, plain water consumption is higher among the non-Hispanic White population and higher income groups (5–7). A social gradient also applies to tap water: its consumption was higher among groups with higher education and incomes as well as the non-Hispanic White population (5, 6).

Dietary advice to choose plain water in place of SSB may not be effective, if the beverage behaviors and consumption patterns differ across population subgroups. For example, most SSB are consumed by teenagers and young adults (5, 7) and many interventions have focused on that age group. The Healthy, Hunger-Free Kids Act of 2010 (8) required schools in the National School Lunch Program (9) to make free drinking water available during meal times (10). Schools were to allow students to have water bottles in class and to provide hydration stations (11). Based on NHANES 2011–16 data, SSB consumption has in fact declined nationally, especially among teenagers and young adults (3, 5). However, it is unclear whether this decline was accompanied by a corresponding increase in plain drinking water, tap, or bottled. Furthermore, recent data suggest that children and teenagers consume SSB and water at different times of day (12).

More SSBs are consumed by lower income and minority groups (5–7). Soda taxes were intended to reduce SSB consumption among those populations as a means to combat obesity, diabetes, and other health related problems (13). Since then, soda taxes have been credited with reducing SSB sales in selected jurisdictions, but have not been widely implemented (14). It is not clear whether the SSB were replaced with more nutrient-dense beverages or with plain drinking water. The equivalent of a 10% SSB tax led to a nonsignificant 1.9% increase in total untaxed beverage consumption (e.g., water) (14). No data on any postulated health benefits of SSB reduction on a population level are as yet available (15).

The present analyses were based on three cycles of the nationally representative NHANES 2011–2016 dietary intakes database for the US population (age ≥ 4 years) (16). The goal was to compare time trends in water and SSB consumption by age, income, and race/ethnicity. It is important to know whether the stated objectives of the US public health policies regarding replacing SSB with drinking water are being achieved across all racial/ethnic groups and socioeconomic strata.

METHODS

NHANES 2011–16 Participant Characteristics

NHANES participants were stratified by age, race/ethnicity, and income. For primary analyses age was stratified into two categories (4–30 and ≥31 years) as SSB consumption tends to be higher among the younger age groups as compared to the older age groups. Additional analyses examined beverage consumption for more precise age groups: 4–8, 9–13, 14–18, 19–30, 31–50, 51–70 years, and >70 years. These age groups generally correspond to the age groups used by the IOM. Race/ethnicity was defined as: non-Hispanic White, non-Hispanic Black, Mexican American, other Hispanic, and other/mixed race. Family income-to-poverty ratio (IPR) is the ratio of family income to the federal poverty threshold; the cut-points for IPR were <1, 1–1.99, 2–3.49, and ≥3.5.

NHANES 2011–16 Dietary Intakes

Consumption data for drinking water, beverages, and foods came from three cycles of the nationally representative NHANES, corresponding to years 2011–12, 2013–2014, and 2015–2016 (16). The three NHANES cycles provided a nationally representative sample of 22,716 age ≥ 4 years.

The NHANES 24-h recall uses a multi-pass method, where respondents reported the types and amounts of all food and beverages consumed in the preceding 24 h from midnight to midnight (17). The multi-pass method was conducted by a trained interviewer using a computerized interface (18). Respondents first identified a quick list of foods and beverages consumed. The time and occasion for each food item was also obtained. A more detailed cycle then recorded the amounts consumed, followed by a final probe for any often-forgotten foods (beverages, condiments). Day one interviews were conducted by trained dietary interviewers in a mobile examination center. Day two interviews were conducted by telephone some days later (19).

For children 4–5 years, dietary recall was completed entirely by a proxy respondent (i.e., parent or guardian with knowledge of the child's diet) (17). Proxy assisted interviews were conducted with children 6–11 years of age. Adolescents 12–19 years were the primary source of dietary recall data but could be assisted by an adult who had knowledge of their diet.

We used a combination of the 1-day value and the 2-day mean to make use of all available dietary data. About 90% of people had two recalls. This method included all NHANES participants, even those without a second recall. Water consumers were defined as those NHANES participants who were drinking water on day 1, 2, or both.

Water Intakes From Water and Other Beverages

Plain drinking water included tap and bottled. Other beverages were classified as sugar sweetened beverages (SSB) and non-sugar sweetened beverages (non-SSB). Sugar sweetened beverages included regular soda, fruit drinks, sports drinks, energy drinks, presweetened ready-to-drink tea, and sweetened ready-to-drink coffee. Non-SSB included unsweetened milk and milk beverages,

milk substitutes, fruit juice, diet soda, hot tea/coffee, alcoholic beverages, enhanced water, and supplemental beverages. These analyses were for water from water and SSB and non-SSB only. For example, milk consumed with cereal (i.e., not as a beverage) was not assigned to a beverage category.

The NHANES 24-h recalls for each participant provided information on the amount in grams of each food and beverage consumed (16). The present results were for mL of water derived from water and from selected beverages and not for the volume of the beverages themselves (which may not be 100% water).

IRB and Ethical Approvals

Approvals for the conducts of the NHANES surveys had been obtained by the National Center for Health Statistics (NCHS) (20). Adult participants provided written informed consent. For children, parental/ guardian written informed consent was obtained. Children and adolescents ≥ 12 years of age provided additional written consent. All NHANES data are publicly available on the NCHS and USDA websites (16). Following University of Washington (UW) policies, analyses of public data do not involve "human subjects" and their use does not require an IRB review or an exempt determination. Such data may be used and analyzed without any involvement of the Human Subjects Division or the UW Institutional Review Board.

Statistical Analyses

The survey-weighted mean intakes of water from SSB, other beverages (non-SSB), and drinking water in mL/day were evaluated overall and by age group, family income-to-poverty ratio, and race/ethnicity for each NHANES cycle from 2011 to 2016. First, trends in sources of hydration were compared between NHANES cycles in adults and children together and separately. For each source of hydration, a regression analysis for sample survey data was performed with water intakes from water and from beverages as dependant variable and NHANES cycles as ordinal independent one. For some analyses, water was split into tap and bottled. Tests of NHANES cycle effect over intake as well as tests for linear trend were reported. In order to assess whether previously observed trends remained in some specific strata of population, analysis was redone after stratification of the sample by detailed age classes, income to poverty ratio, and race/ethnicity. Survey-weighted means and corresponding standard errors were reported. All analyses accounted for the complex survey design of NHANES and captured nationally representative dietary behaviors of the US population between 2011 and 2016. All analyses were conducted using SAS software, version 9.4 (SAS Institute Inc., Cary NC, USA) by using SURVEYREG and SURVEYMEANS procedures, and an α level of 5% was used for all statistical tests.

TABLE 1 | Time trends in water intakes (mL/day) from beverages including SSB and from plain drinking water, tap, and bottled (mean, standard error).

	NHANES cycle				
	2011–12	**2013–14**	**2015–16**	***p*-value**	***p*-trend**
		All >4 years	*N* = 22,716		
Beverages + water	2,108 (45)	2,077 (44)	2,114 (46)	0.8197	0.92
Beverages	1,097 (31)	1,038 (32)	970 (21)	**0.0046**	**0.0014**
SSB	322 (12)	283 (14)	262 (13)	**0.0055**	**0.0017**
Water	1,011 (33)	1,039 (30)	1,144 (38)	**0.0297**	**0.0108**
		Age 4–30 years	*N* = 10,701		
Beverages + water	1,747 (44)	1,758 (48)	1,710 (62)	0.8248	0.6303
Beverages	865 (16)	827 (32)	708 (16)	**<0.0001**	**<0.0001**
SSB	393 (13)	352 (17)	279 (16)	**<0.0001**	**<0.0001**
Water	882 (44)	930 (37)	1,001 (57)	0.2653	0.1055
		Age > 30 years	*N* = 12,015		
Beverages + water	2,336 (59)	2,275 (50)	2,364 (46)	0.4248	0.7108
Beverages	1,243 (37)	1,169 (34)	1,131 (30)	0.0753	**0.0241**
SSB	276 (19)	238 (16)	251 (14)	0.3269	0.2877
Water	1,092 (38)	1,106 (35)	1,232 (38)	**0.0195**	**0.0114**
		Females	*N* = 11,510		
Beverages + water	1,907(41)	1,917 (37)	1,937 (43)	0.8798	0.6201
Beverages	924 (24)	875 (27)	785 (23)	**0.0005**	**0.0001**
SSB	251 (13)	217 (14)	204 (17)	0.0829	**0.0397**
Water	982 (30)	1,042 (28)	1,152 (40)	**0.0059**	**0.0014**
		Males	*N* = 11,206		
Beverages + water	2,314 (67)	2,240 (52)	2,299(58)	0.6268	0.8673
Beverages	1,274 (50)	1,205 (39)	1,163 (34)	0.1962	0.0736
SSB	394 (12)	349 (17)	322 (17)	**0.0059**	**0.0023**
Water	1,040 (49)	1,035 (36)	1,136 (28)	0.1382	0.1367

RESULTS

Time Trends in SSB and Water Consumption 2011–2016

Table 1 shows water intakes from water and other beverages for each NHANES cycle from 2011 to 2016. There were no significant differences in water intakes from beverages and drinking water combined between 2011 and 2016. The total amount of water was around 2,100 mL/d, evenly split between beverages and plain drinking water, tap, and bottled. No significant time trends in total water intakes were observed for the entire sample or by specific age groups.

For the total sample, there was a significant decline in water from beverages (−11.6%; $p = 0.005$) that was driven by a

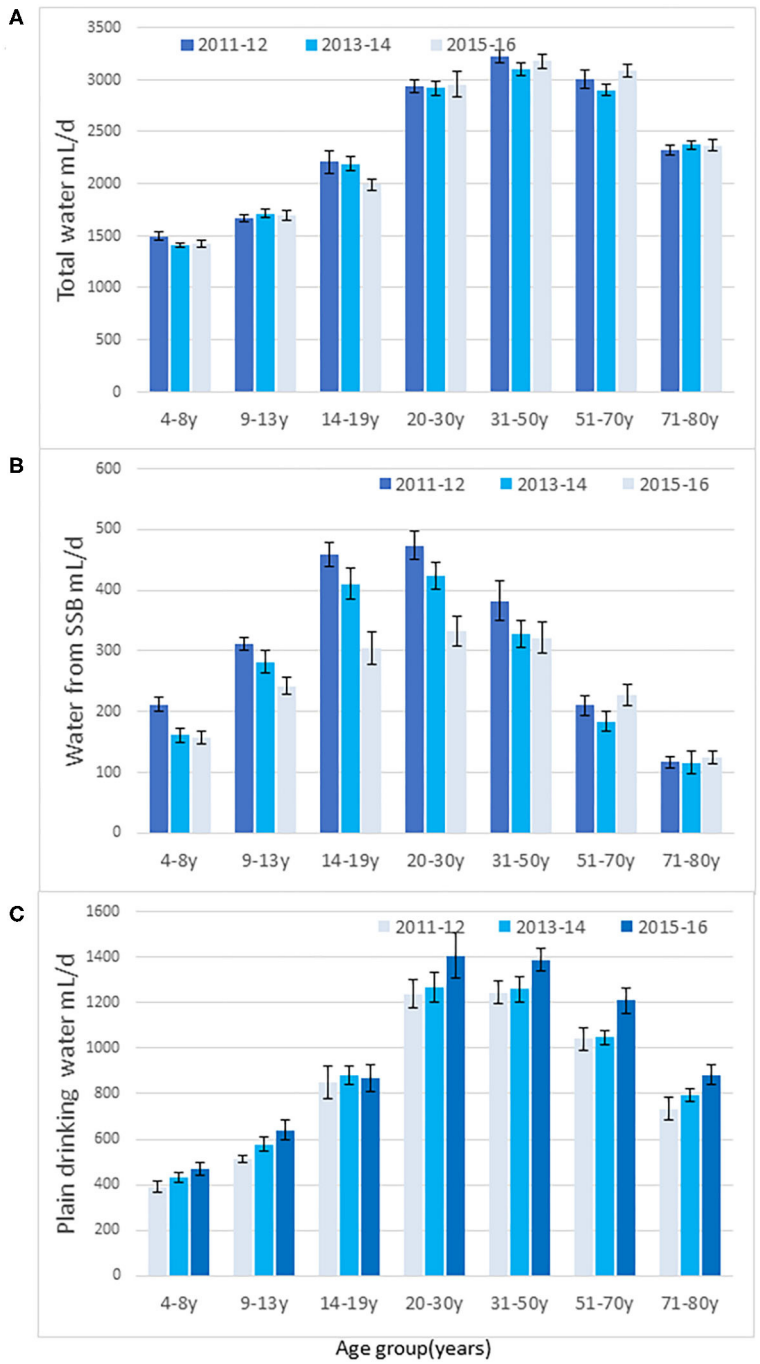

FIGURE 1 | Time trends in total water intakes (mL/d) from water, beverages, and water from foods **(A)**, water from SSB **(B)**, and drinking water **(C)** by age group for each NHANES cycle. Data are means and SEM.

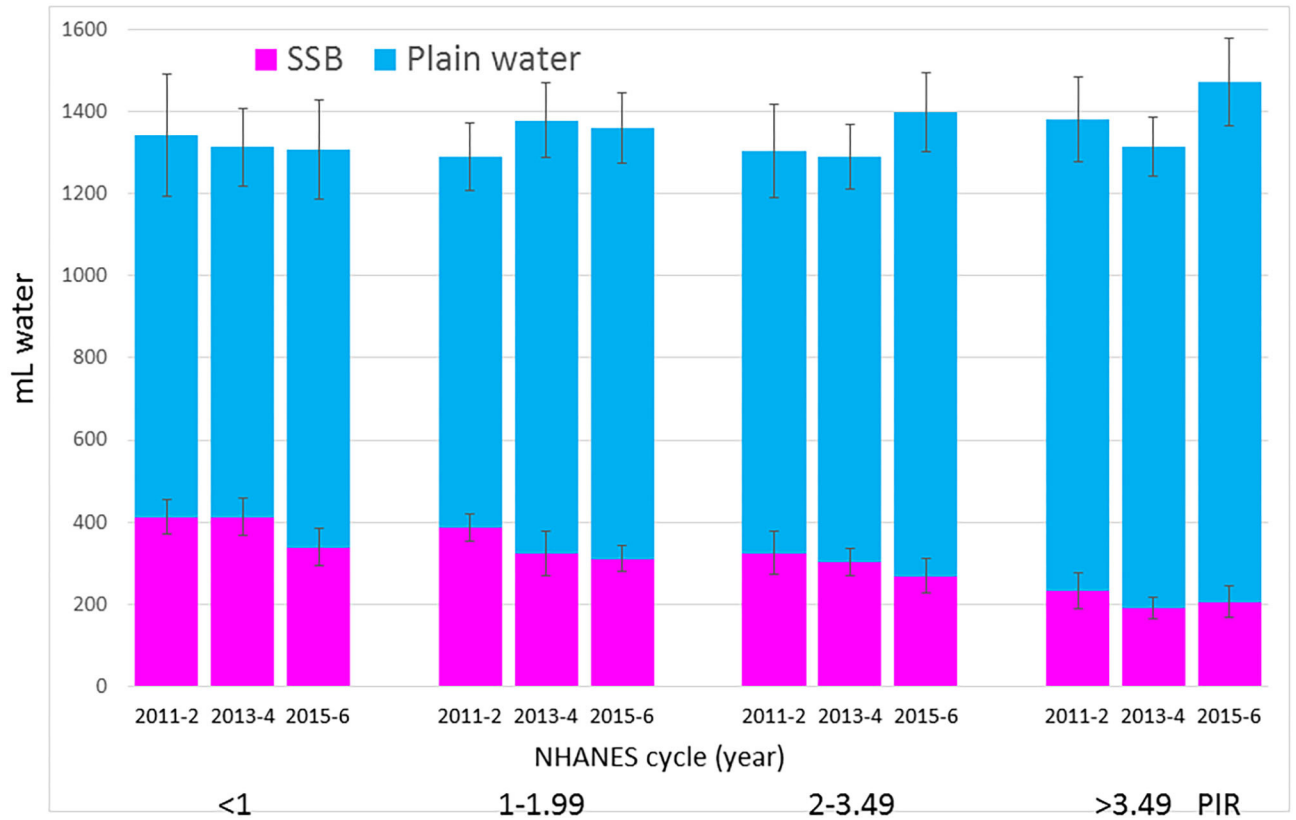

FIGURE 2 | Time trends in water from SSB and drinking water (tap and bottled) by IPR and NHANES cycle. Data are means and SEM.

significant reduction in SSB (−18.6%; $p = 0.0055$). For the 4–30 years group, the reduction in beverages was significant (−18.2%; $p < 0.0001$) and the reductions in SSB (−29%; $p < 0.0001$) and not-SSB ($p < 0.0001$) were significant as well. The increases in plain water intakes was significant for the total sample ($p < 0.05$) and for adults >30 years ($p < 0.05$), but not for the 4–30 years age group. It appears that SSB intakes declined among people 4–30 years whereas plain water intakes increased among people >30 years.

Table 1 also shows time trends for SSB and water by sex. Reduced beverage and SSB intakes were accompanied by increased water intakes ($p < 0.005$) among females. No corresponding increase in water consumption paralleled SSB reduction among males.

Time Trends for SSB and Water by Age Group

Figure 1 shows time trends for SSB and water by more finely differentiated age groups. First, as shown in **Figure 1A** total water intakes (beverages and plain drinking water) increased with age, peaked through the 31–50 years age groups, and then declined. There was no significant effect of the NHANES cycle.

Figure 1B shows that the decline in SSB was most pronounced and significant among persons aged <30 years. The decline in SSB was not significant among persons >30 years. The biggest decline (−33%) was observed among teenagers (ages 14–19 years), consistent with other reports (3, 5). However, the expected replacement of SSB with plain water in the 14–19 years age group was not observed. Rather, **Figure 1C** shows that increases in water consumption were more pronounced among adults over the age of 30 years (trend analyses $p < 0.05$). The increases in water intakes were significant for the 9–13 years ($p < 0.05$) and for the 51–70 years age group ($p < 0.05$).

Time Trends for SSB and Water by IPR

Figure 2 shows trends for SSB and water by family income. First, the income gradient for SSB was obtained across all NHANES cycles. Lower income groups were also the ones that reduced SSB the most ($p < 0.03$). **Figure 2** also shows that the opposing income gradient for plain water also held across all NHANES cycles; higher intakes of plain drinking water were observed among the higher income groups. Those groups also showed the highest increases in water intakes.

Figure 3 shows the increase in tap water for the higher IPR groups. For lower IPR groups water from the tap did not increase while also showing that the lower IPR groups had a substantial and significant increase in the consumption of bottled water. It appears that the significant reduction in water from SSB among lower IPR groups was accompanied by a marked increase (144

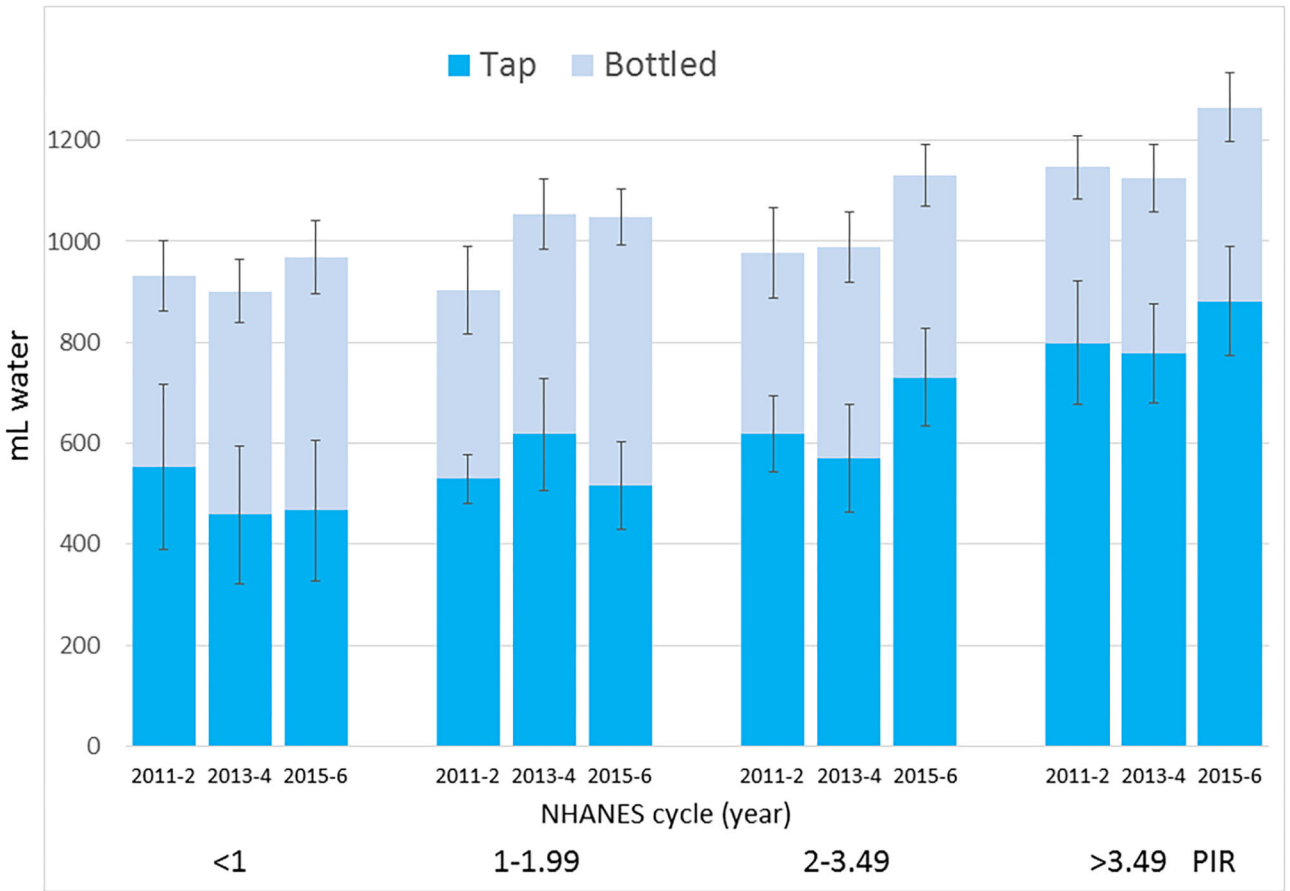

FIGURE 3 | Time trends in tap water and bottled water intakes by IPR and NHANES cycle. Data are means and SEM.

mL/d in the IPR 1–1.99 group) in bottled water but not in tap water.

Water From SSB and Plain Water by Race/Ethnicity

Figure 4A shows the race/ethnicity gradient in SSB consumption. Non-Hispanic Black and Mexican American groups consumed most SSB. A significant decline in SSB was observed among non-Hispanic White ($p < 0.05$) and non-Hispanic Black groups ($p < 0.01$). No significant decline in SSB was observed for other racial/ethnic groups.

Figure 4B shows the opposing social gradient for tap water consumption. Tap water intakes were highest for non-Hispanic White and lowest for non-Hispanic Black and for Mexican American groups, whose consumption was below 400 mL/d. Analyses of whether the SSB were being replaced by plain water, tap, or bottled, pointed to some weak trends. The increase in tap water intakes was almost significant among the non-Hispanic White group (p for trend =0.057) but not in any other group.

Figure 4C shows that the non-Hispanic White group consumed the least bottled water (300 mL/d). Bottled water intakes were significantly higher among the non-Hispanic Black, Mexican American, and other Hispanic groups. Bottled water intakes increased among the non-Hispanic Black population (p for trend <0.05) but not in any other group.

The *Y*-axes of **Figure 4** are shown on the same scale to demonstrate the profound social gradients in the consumption of tap water as opposed to bottled water. Intakes of tap water among the non-Hispanic White group were higher than for the non-Hispanic Black, Mexican American, and other Hispanic groups. Conversely, intakes of bottled water among the non-Hispanic White group were lower than for the non-Hispanic Black, Mexican American, and other Hispanic groups.

DISCUSSION

Replacing caloric SSB with plain and non-caloric drinking water has been a priority area for public health nutrition (2). The goal of Dietary Guidelines for Americans, soda taxes, and numerous school-based initiatives is to make plain drinking water the beverage of choice (1).

The present analyses of the 2011–16 NHANES dataset confirm that the consumption of SSB in the US continues to drop (3, 5). Conversely, the consumption of plain

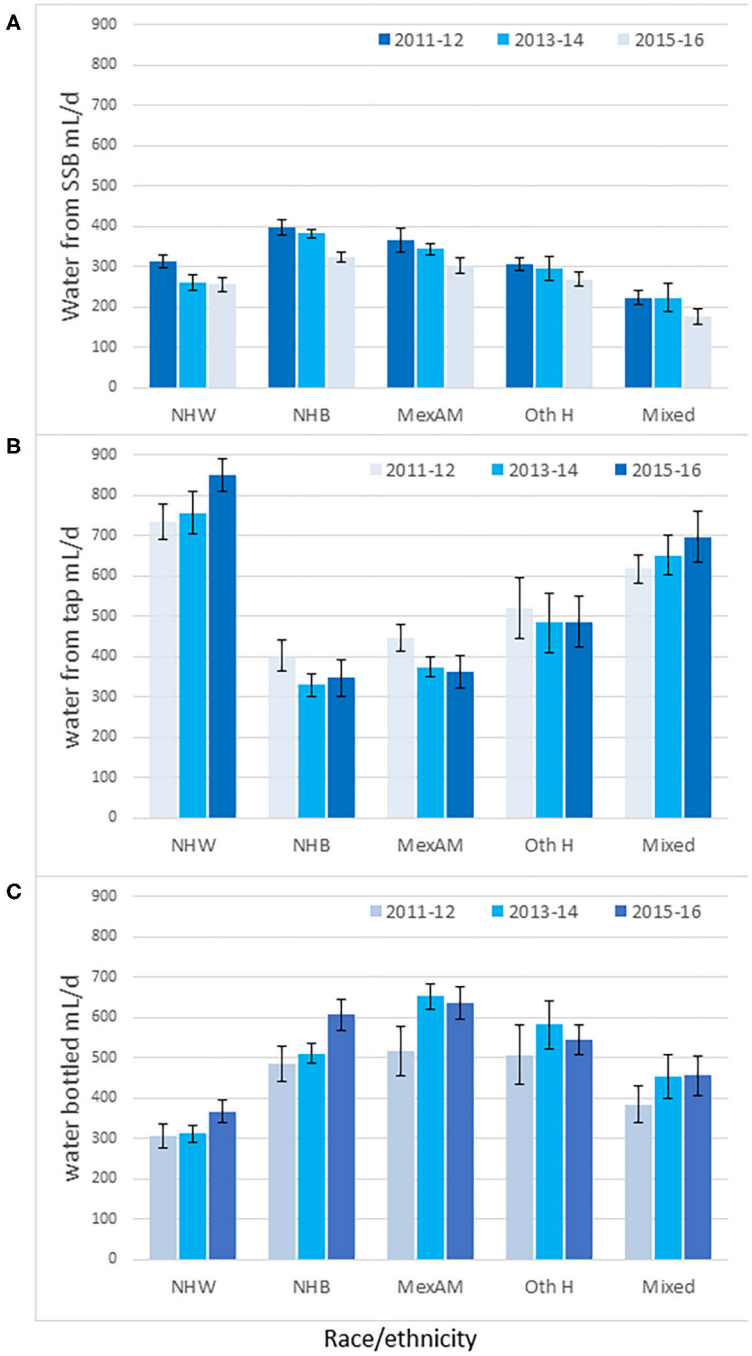

FIGURE 4 | Time trends in water intakes (mL/d) from SSB **(A)**, tap **(B)** and bottled water **(C)** by race/ethnicity and NHANES cycle. Non-Hispanic White (NHW), Non-Hispanic Black (NHB), Mexican American (MexAm), other Hispanic (Oth H). Data are means and SEM.

drinking water is on the rise. However, the patterns of substitution were very different by age group, income, and race/ethnicity (21, 22).

Dietary advice to choose water *in place* of SSB may not be effective if the two beverages are normally consumed in different places, at different times of day (12) or at different eating occasions, or if habitual consumption patterns vary by age group, income, or race/ethnicity (5). SSB consumption and water intakes in the NHANES sample followed opposing income gradients. First: lower IPR groups consumed most SSB and least water; higher IPR groups consumed less SSB and more water. The non-Hispanic White population consumed least SSB, less bottled water than the other groups, and by far the most water from the tap.

The groups with the greatest reduction in SSB were not the same ones that increased the consumption of plain water. For example, the reduction in SSB was associated with younger age groups (<30 year) while the increase in plain water was associated with older age groups (>30 year). Despite federal regulations and the encouragement from schools, there was no evidence that a reduction in SSB consumption among teenagers was accompanied by a corresponding increase in plain drinking water. Clearly, the anticipated increase in plain water consumption has not been uniform across population groups.

The reduction in SSB consumption was strongest among the highest consumers, namely lower income groups. That is of interest because lower-income groups may be particularly sensitive to SSB taxes. However, those groups did not show a corresponding increase in tap water intakes. Lower IPR groups did reduce SSB and one such group (IPR 1–1.99) increased the consumption of bottled water, not tap water. Similarly, the non-Hispanic Black group, another high intake group also reduced SSB intakes and increased bottled water intakes, not tap.

There was one group that showed a decline in SSB and a corresponding increase in tap water consumption. Those were the non-Hispanic White group, who had the lowest intakes of SSB and the highest intakes of tap water. In that group, the decline in SSB was offset by an increase in tap water.

The trend away from tap water among lower income groups is troubling. It was the higher IPR groups that consumed more municipal tap water, whereas lower IPR groups consumed more bottled water. These observations are consistent with previous reports that non-Hispanic White and higher income groups consumed most tap water (6); Mexican Americans drank the most bottled water and the least tap water (5, 6).

This could be due to the "Flint effect," that is the perception that tap water is safe to drink only in affluent neighborhoods (22, 23). One paper (23) notes that the mistrust of tap water was one reason for SSB consumption. The odds of consuming ≥1 SSB/d among Hispanic respondents who mistrusted their local tap water was twice that of those who did not (23). As the quality of tap water in lower income areas becomes problematic (22, 24), the consumption of bottled water is on the rise among lower income groups and the non-Hispanic Black group.

Many initiatives have focused on tap water describing it as "the perfect, no-cost, no-calorie beverage, and it comes right out of the kitchen tap" (25). Providing tap water to children is another initiative (26, 27).

Making water the national beverage of choice (DGAs) is a strategy that needs to be more sensitive to the quality of the local water supply and to community resources, wants, and needs.

The present analyses had limitations. First, the NHANES data are based on self-report and are subject to random and systematic reporting errors. A 24-h recall may systematically underestimate water and other beverage intake, especially outside of meals since it is very difficult for individuals to remember exactly how much tap water they had outside of meals. The present estimates, based on a combination of day 1 and 2 dietary recalls may have been affected by differences in data collection procedures across the 2 days. Fluid-specific records, used in smaller scale studies, may provide higher quality data. The use of proxy respondents for children ages 4–5 years and proxy assisted interviews for children 6–11 make the collection of accurate data especially challenging. The two days of dietary recalls used different methods to collect the data, which may affect the estimates of water consumption. However, the NHANES has the advantage of being based on a large, nationally representative population sample. The NHANES dataset forms the basis for dietary surveillance in the US.

CONCLUSION

Reduced intakes of SSB among non-Hispanic White groups and among females were accompanied by a parallel increase in plain water intakes. Less consistent trends were observed among other population subgroups. Non-Hispanic Black and lower income groups consumed more bottled water. Non-Hispanic While and higher-income groups consumed more plain water from the tap. Successful implementation of Dietary Guidelines to choose water over SSB may depend on population beverage habits. Further research is needed to understand how these changes are being made and whether further interventions may be necessary.

DATA AVAILABILITY STATEMENT

Data used in the study is publicly available through the NHANES database (available at https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by https://www.cdc.gov/nchs/nhanes/about_nhanes.htm (accessed October 9, 2019). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

All authors (FV, MM, CR, PB, and AD) conceptualized study design, formulated analytical questions, and contributed to the manuscript preparation. CR created the dataset, while FV and MM performed the principal analyses. AD acted as lead writer of the paper. All authors (FV, MM, CR, PB, and AD) reviewed and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.587123/full#supplementary-material

REFERENCES

1. U.S. Department of Health Human Services U.S. Department of Agriculture. *Dietary Guidelines for Americans 2015-2020. 8th ed*. Washington, DC: U.S. Government Printing Office (2015).
2. Vargas-Garcia EJ, Evans CEL, Prestwich A, Sykes-Muskett BJ, Hooson J, Cade JE. Interventions to reduce consumption of sugar-sweetened beverages or increase water intake: evidence from a systematic review and meta-analysis. *Obes Rev*. (2017) 18:1350–63. doi: 10.1111/obr.12580
3. Bleich SN, Vercammen KA, Koma JW, Li Z. Trends in beverage consumption among children and adults, 2003-2014. *Obesity*. (2018) 26:432–41. doi: 10.1002/oby.22056
4. Rosinger A, Herrick K, Gahche J, Park S. *Sugar-Sweetened Beverage Consumption among U.S. Youth, 2011-2014*. NCHS Data Brief, No. 271. Hyattsville, MD: National Center for Health Statistics (2017).
5. Vieux F, Maillot M, Rehm CD, Barrios P, Drewnowski A. Trends in tap and bottled water consumption among children and adults in the United States: analyses of NHANES 2011-16 data. *Nutr J*. (2020) 19:10. doi: 10.1186/s12937-020-0523-6
6. Drewnowski A, Rehm CD, Constant F. Water and beverage consumption among adults in the United States: cross-sectional study using data from NHANES 2005-2010. *BMC Public Health*. (2013) 13:1068. doi: 10.1186/1471-2458-13-1068
7. Drewnowski A, Rehm CD, Constant F. Water and beverage consumption among children age 4-13y in the United States: analyses of 2005-2010 NHANES data. *Nutr J*. (2013) 12:1. doi: 10.1186/1475-2891-12-85
8. USDA Food and Nutrition Service. *Healthy Hunger-Free Kids Act*. (2013). Available online at: https://www.fns.usda.gov/school-meals/healthy-hunger-free-kids-act (accessed November 19, 2019).
9. USDA Food and Nutrition Service. *National School Lunch Program*. Available online at: https://www.fns.usda.gov/nslp (accessed November 19, 2019).
10. USDA Food and Nutrition Service. *School Breakfast Program*. Available online at: https://www.fns.usda.gov/sbp/school-breakfast-program (accessed November 19, 2019).
11. Centers for Disease Control and Prevention. *Water Access*. (2019). Available online at: https://www.cdc.gov/healthyschools/npao/wateraccess.htm/ (accessed November 19, 2019).
12. Vieux F, Maillot M, Rehm CD, Barrios P, Drewnowski A. The timing of water and beverage consumption during the day among children and adults in the United States: analyses of NHANES 2011-2016 data. *Nutrients*. (2019) 11:2707. doi: 10.3390/nu11112707
13. Ross J, Lozano-Rojas F. *Are Sugar-Sweetened Beverage Taxes Regressive? Evidence from Household Retail Purchases*. Tax Foundation (2018). Available online at: https://taxfoundation.org/soda-taxes-regressive/ (accessed April 6, 2020).
14. Teng AM, Jones AC, Mizdrak A, Signal L, Genç M, Wilson N. Impact of sugar-sweetened beverage taxes on purchases and dietary intake: systematic review and meta-analysis. *Obes Rev*. (2019) 20:1187–204. doi: 10.1111/obr.12868
15. Kaiser KA, Shikany JM, Keating KD, Allison DB. Will reducing sugar-sweetened beverage consumption reduce obesity? Evidence supporting conjecture is strong, but evidence when testing effect is weak. *Obes Rev*. (2013) 14:620–33. doi: 10.1111/obr.12048
16. Centers for Disease Control Prevention. *National Center for Health Statistics (NHANES) – About the National Health Nutrition Examination Survey*. CDC (2017). Available online at: https://www.cdc.gov/nchs/nhanes/about_nhanes.htm (accessed October 9, 2019).
17. Centers for Disease Control and Prevention. *National Health and Nutrition Examination Survey Questionnaire: Dietary Interview Component*. (2011). Available online at: https://www.cdc.gov/nchs/data/nhanes/nhanes_11_12/dietary_post_recall_qs_2011.pdf (accessed October 9, 2019).
18. Centers for Disease Control and Prevention. *MEC In-Person Dietary Interviewers Procedures Manual*. (2002). Available online at: https://www.cdc.gov/nchs/data/nhanes/nhanes_03_04/DIETARY_MEC.pdf
19. Centers for Disease Control and Prevention. *National Health and Nutrition Examination Survey Questionnaire: Phone Follow-Up Dietary Interviewer Procedures Manual*. (2010). Available online at: https://wwwn.cdc.gov/nchs/data/nhanes/2009-2010/manuals/phone_follow_up_dietary_procedures_manual_mar_2010.pdf (accessed October 9, 2019).
20. Centers for Disease Control Prevention. *NHANES - NCHS Research Ethics Review Board Approval*. (2017). Available online at: https://www.cdc.gov/nchs/nhanes/irba98.htm%0D (accessed October 9, 2019).
21. Rosinger AY, Herrick KA, Wutich AY, Yoder JS, Ogden CL. Disparities in plain, tap and bottled water consumption among US adults: National Health and Nutrition Examination Survey (NHANES) 2007-2014. *Public Health Nutr*. (2018) 21:1455–64. doi: 10.1017/S1368980017004050
22. Brooks CJ, Gortmaker SL, Long MW, Cradock AL, Kenney EL. Racial/ethnic and socioeconomic disparities in hydration status among US adults and the role of tap water and other beverage intake. *Am J Public Health*. (2017) 107:1387–94. doi: 10.2105/AJPH.2017.303923
23. Onufrak SJ, Park S, Sharkey JR, Sherry B. The relationship of perceptions of tap water safety with intake of sugar-sweetened beverages and plain water among US adults. *Public Health Nutr*. (2014) 17:179–85. doi: 10.1017/S1368980012004600
24. Gostin LO. Politics and public health: the flint drinking water crisis. *Hastings Cent Rep*. (2016) 46:5–6. doi: 10.1002/hast.598
25. Beckerman J. *Drink to Your Health, With Water*. Providence Health Plan. Available online at: https://healthplans.providence.org/fittogether/find-your-fit/healthy-eating/beverages/drink-to-your-health-with-water (accessed September 28, 2020).
26. Shamah-Levy T, García-Chávez C, Rodríguez-Ramírez S. Association between plain water and sugar-sweetened beverages and total energy intake among mexican school-age children. *Nutrients*. (2016) 8:710. doi: 10.3390/nu8120710
27. Lawman HG, Lofton X, Grossman S, Root M, Perez M, Tasian G, et al. A randomized trial of a multi-level intervention to increase water access and appeal in community recreation centers. *Contemp Clin Trials*. (2019) 79:14–20. doi: 10.1016/j.cct.2019.02.003

Conflict of Interest: FV and MM are employees of MS-Nutrition, a start-up. PB and CR are employed by PepsiCo Inc. AD has received contracts, consulting fees and honoraria from entities both public and private with an interest in nutrient profiling of beverages and in beverage consumption, including manufacturers and distributors of both SSB and bottled water such as PepsiCo, Nestlé, and Danone. The views expressed in this work are those of the authors and do not necessarily reflect the position or policy of PepsiCo Inc.

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ORIGINAL RESEARCH
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Associations Between Added Sugar Intake and Risk of Four Different Cardiovascular Diseases in a Swedish Population-Based Prospective Cohort Study

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Aims: Although diet is one of the main modifiable risk factors of cardiovascular disease, few studies have investigated the association between added sugar intake and cardiovascular disease risk. This study aims to investigate the associations between intake of total added sugar, different sugar-sweetened foods and beverages, and the risks of stroke, coronary events, atrial fibrillation and aortic stenosis.

Methods: The study population consists of 25,877 individuals from the Malmö Diet and Cancer Study, a Swedish population-based prospective cohort. Dietary data were collected using a modified diet history method. National registers were used for outcome ascertainment.

Results: During the mean follow-up of 19.5 years, there were 2,580 stroke cases, 2,840 coronary events, 4,241 atrial fibrillation cases, and 669 aortic stenosis cases. Added sugar intakes above 20 energy percentage were associated with increased risk of coronary events compared to the lowest intake category (HR: 1.39; 95% CI: 1.09–1.78), and increased stroke risk compared to intakes between 7.5 and 10 energy percentage (HR: 1.31; 95% CI: 1.03 and 1.66). Subjects in the lowest intake group for added sugar had the highest risk of atrial fibrillation and aortic stenosis. More than 8 servings/week of sugar-sweetened beverages were associated with increased stroke risk, while ≤2 servings/week of treats were associated with the highest risks of stroke, coronary events and atrial fibrillation.

Conclusion: The results indicate that the associations between different added sugar sources and cardiovascular diseases vary. These findings emphasize the complexity of the studied associations and the importance of considering different added sugar sources when investigating health outcomes.

Keywords: cardiovascular diseases, added sugar intake, dietary sugar, sugar-sweetened beverages, cardiometabolic risk factors

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INTRODUCTION

Cardiovascular disease (CVD) is responsible for one-third of all global deaths annually (1). Although the etiologies of CVDs vary, atherosclerosis is a common underlying mechanism, and there are many shared risk factors including hypertension, diabetes, hyperlipidemia, congenital heart defects and various lifestyle factors. In addition to the many shared risk factors, the presence of one CVD often increases the risk of developing another, which leads to high levels of comorbidity among individuals with CVD (2).

A poor diet is one of the main modifiable risk factors for CVD (2). Although added sugar consumption has been linked to various cardiometabolic risk markers (3, 4), the long-term effects and associations with incident CVD risk are not yet fully understood. Further, a majority of studies have primarily focused on the intake of sugar-sweetened beverages (SSBs) (3, 5), rather than the totality of added sugars. This is important because SSBs on average account for only 14% of added sugar intake in Sweden (6) and 23% in the US (7). The evidence regarding an association between SSB consumption and CVD risk is currently inconclusive. A systematic review by Keller et al. found that the evidence for an association between SSBs and vascular events was inconsistent, as two of three studies found an association with stroke and two of four studies found an association with coronary heart disease (8). Another systematic review reported inconclusive evidence regarding the association between sugar intake and SSBs and cardiovascular outcomes due to the low number of studies available (9).

It is hypothesized that SSB consumption could adversely impact health as a consequence of liquid calories causing insufficient satiety signaling and thereby promoting weight gain, a prominent risk factor for many CVDs (10). Another hypothesis is the distinct metabolism of fructose, one of the monosaccharides forming the commonly used added sugar sucrose. Some of the ingested fructose is metabolized into lipids in the liver, which is theorized to result in elevated triglyceride synthesis and increased hepatic lipid content, ultimately increasing the risk of CVD (11). Since different sources of added sugar may vary in composition, energy density, and absorption, it is important to distinguish between different sources of added sugar when studying their associations with health outcomes.

This study aims to investigate the associations between the intake of added sugar, as well as sugar-sweetened foods and beverages, and the risks of four different CVDs: stroke, coronary events, atrial fibrillation, and aortic stenosis.

Abbreviations: ApoA-1, Apolipoprotein A-1; ApoB, Apolipoprotein B; BMI, Body Mass Index; CI, Confidence interval; CVD, Cardiovascular disease; E%, percentage of energy intake; HR, Hazard ratio; ICD-9, International Classification of Diseases 9; IQR, Interquartile range; SD, standard deviation; SSB, Sugar-sweetened beverage.

MATERIALS AND METHODS

Study Population

The study population is derived from the Malmö Diet and Cancer Study: a prospective population-based cohort study in southern Sweden. Recruitment was carried out through mailed invitation letters and distribution of invitations in public areas between 1991 and 1996. All men born between 1923 and 1945 and women born between 1923 and 1950 in Malmö were invited to participate, with the only exclusion criteria being insufficient Swedish language skills and mental incapacity. Of 68,905 individuals eligible for participation, 28,098 (40.8%) completed the baseline examinations. We excluded individuals with a history of aortic stenosis ($n = 57$), atrial fibrillation ($n = 286$), stroke ($n = 300$), coronary events ($n = 544$), and diabetes mellitus ($n = 1{,}244$) at baseline, resulting in a study sample of 25,877 individuals. A total of 139 individuals (81 women and 59 men) were lost to follow-up, with the primary reason being permanent emigration.

Ethical approval for the Malmö Diet and Cancer Study has been granted by the Regional Ethical Review Board in Lund, Sweden (LU/90-51), and written informed consent was obtained from all participants prior to participation.

Data Collection

Baseline data collection was carried out using questionnaires covering medication, medical history, socioeconomic factors and current lifestyle, such as leisure-time physical activity (metabolic equivalent of task hours/week based on the duration and intensity of 17 different activities) (12), smoking status (never, current, or former smoker) and educational level according to Swedish educational degrees (<9, 9 years, upper secondary school, and university with or without a degree). Information about alcohol consumption was retrieved from the questionnaire as well as the 7-day food diary; zero-consumers indicated no consumption during the past year. The rest of the participants were divided into sex-specific quintiles based on their alcohol consumption reported in the 7-day food diary. Participants who reported drastic dietary changes prior to baseline examinations were identified through the self-administered questionnaires (13), and potential energy misreporters were identified using Black's revised Goldberg method (14) based on their estimated energy expenditure. Misreporting was defined as having an energy intake to basal metabolic rate ratio outside of the 95% confidence interval (CI) of the physical activity level (PAL). The procedure has been described in more detail previously (15).

Anthropometric measurements including weight, height, waist circumference, and body fat percentage were collected by registered nurses. Further, blood pressure was measured in all participants, and hypertension was determined according to the American Heart Association and the National Heart, Lung, and Blood Institute's definitions, which is a systolic blood pressure of ≥130 mmHg, diastolic blood pressure of ≥85 mm Hg, or use of antihypertensive drugs (16). Non-fasting blood samples were also collected, from which the concentrations of apolipoprotein B (ApoB) and apolipoprotein A-1 (ApoA-1) were determined using

a Siemens BNII immunonephelometric analyzer during the year 2013 (Siemens, Newark, DE, USA) (17).

Dietary Assessment

Dietary data were collected using a modified diet history method that included a 7-day food diary covering cooked meals, cold beverages and dietary supplements as well as a 168-item diet history questionnaire covering the general meal pattern as well as the frequency and portion-size of non-cooked meals during the preceding 12 months. In addition, a 60-min (until September 1st, 1994) or 45-min (after September 1st, 1994) diet history interview was conducted to collect information about serving sizes and cooking methods of the foods recorded in the food diary. The participants' dietary intakes were estimated by adding up the reported intakes of the modified diet history method. The collected data were then converted into daily nutrient and energy intakes using the Malmö Diet and Cancer Study Food and Nutrient Database, originating from a database by the Swedish National Food Agency. A version of the modified diet history method was validated against an 18-day weighted food record, demonstrating a moderately strong correlation for sucrose, the most common added sugar in Sweden, with Pearson's correlation coefficients of 0.74 for women and 0.60 for men (18).

Added Sugar Variables

The participants' added sugar intakes were estimated based on the collected dietary data. The estimated sucrose and monosaccharide contents of the participants' reported intake of fruits and berries, vegetables, and juice were subtracted from their total intake of sucrose and monosaccharides to obtain an estimation of their added sugar intakes, which includes honey and syrup intake. This process has been explained in detail previously (19). Added sugar intake was then divided into six categories with focus on the current and suggested nutritional recommendations (20, 21) of 10 E% and 5 E%, respectively, as well as to allow extreme intakes to be studied. The categories were created according to percentage of non-alcoholic energy intake (E%) as follows: <5 E%, 5–7.5 E%, 7.5–10 E%, 10–15 E%, 15–20 E%, and >20 E% (19).

Treats included pastries, sweets, chocolate, and ice cream, while toppings included table sugar, syrups, honey and jams. Treats are generally considered more energy dense than toppings, as they tend to have higher fat contents, whereas toppings tend to have larger proportions of energy from sugar. As previous studies have shown that liquid sugar is metabolized differently and has different health outcomes from those associated with solid sugar (11) an SSB category was also created by combining the intake of carbonated and noncarbonated sweetened drinks and fruit drinks, but excluding the intake of pure fruit juice.

The consumed amounts of treats, toppings and SSBs were recoded from grams/day to servings/week based on average serving sizes according to the Swedish National Food Agency's food database and information from manufacturers (22). The consumption of treats was classified as ≤2, >2–5, >5–8, >8–14, and >14 servings/week; topping consumption was classified as ≤2, >2–7, >7–14, >14–28, and >28 servings/week; and SSB intake was classified as ≤1, >1–3, >3–5, >5–8, and >8 servings/week (19).

Endpoint Ascertainment

The participants were followed until diagnosis of the studied outcomes, death, emigration from Sweden or the end of the follow-up period (December 31st, 2016). Endpoints were ascertained using the Swedish National Inpatient Register and the Cause of Death Register, according to the International Classification of Diseases 9th revision (ICD-9) and the corresponding codes in the ICD-10. These registers include all residents of Sweden, and there was therefore no loss to follow-up during registry linkage.

The studied endpoints were stroke (ICD-9 codes 430, 431, 434, 436), coronary events (ICD-9 codes 410–414), atrial fibrillation (ICD-9 code 427 or code 4,273 in the Cause of Death Register), and aortic stenosis (ICD-9 code 424.1). Incident coronary events were defined as a diagnosis of myocardial infarction, other forms of ischemic heart disease or angina pectoris. Incident stroke was defined as a diagnosis of subarachnoid or intracerebral hemorrhage, occlusion of cerebral arteries or other acute cerebrovascular disease. Atrial fibrillation was defined as a diagnosis of either atrial fibrillation or flutter events. Aortic stenosis was defined as a diagnosis of aortic valve disorders. The Swedish National Inpatient Register has previously been shown to have high diagnostic validity, with positive predictive values over 90% for the studied outcomes (23, 24).

Statistical Analyses

All analyses were conducted using IBM SPSS Statistics (version 24; IBM corporation, Armonk, NY, USA). $P < 0.05$ denoted statistical significance. Population characteristics were analyzed across the added sugar intake categories, using the chi-square test for categorical variables, and a univariate general linear model for continuous variables. Normally distributed continuous variables are expressed as means and standard deviations (SDs), while skewed continuous variables are expressed as medians and interquartile ranges (IQRs).

Cox hazards regression models with follow-up as time scale were used to study the associations between the intakes of added sugar, SSBs, treats and toppings and the risks of incident stroke, coronary events, atrial fibrillation, and aortic stenosis. The results are presented as hazard ratios (HRs) with 95% CI. The lowest intake category was generally used as reference, but wherever a U-shaped trend was observed, the intake category with the lowest risk was used as reference. The associations between the studied exposures and outcomes were investigated using four different models. The basic model was adjusted for age (years), sex, season of dietary assessment (spring, summer, autumn, winter), diet assessment method (45 or 60-min diet history interview), and total energy intake (kilocalories/day). The second model was further adjusted for the following lifestyle factors as categorical variables: smoking status, educational level, leisure-time physical activity, and alcohol consumption. The main model further included additional adjustment for body mass index (BMI) (kg/m^2) categories and dietary factors, including intakes of processed meat (g/day), coffee (g/day), saturated fatty acids (E%), and fiber density (g/1,000 kilocalories). Adjustment for BMI separately without the dietary covariates was also tested, as it was suspected to be a particularly prominent confounder. The final model was further adjusted for potential mediators

TABLE 1 | Associations between intake of added sugar and risk of incident stroke, coronary events, atrial fibrillation and aortic stenosis.

	Added sugar intake categories						
	<5 E% (*n* = 2,354)	5–7.5 E% (*n* = 5,027)	7.5–10 E% (*n* = 6,709)	10–15 E% (*n* = 8,735)	15–20 E% (*n* = 2,377)	>20 E% (*n* = 675)	*P*-trend
Stroke							
Cases/person-years	220/45,382	459/99,027	665/132,501	896/16,9957	251/45,522	89/12,129	
Basic model	1	0.84 (0.71–0.99)	0.84 (0.72–0.98)	0.85 (0.73–0.98)	0.88 (0.73–1.06)	1.28 (1.00–1.64)	0.62
Main model	1	0.87 (0.74–1.03)	0.88 (0.76–1.04)	0.87 (0.75–1.02)	0.89 (0.73–1.08)	1.16 (0.89–1.51)	0.39
Basic model[a]	1.19 (1.02–1.39)	1.00 (0.89–1.12)	1	1.00 (0.91–1.11)	1.05 (0.91–1.21)	1.52 (1.22–1.90)	–
Main model[a]	1.13 (0.97–1.33)	0.99 (0.87–1.11)	1	0.99 (0.89–1.10)	1.00 (0.86–1.17)	1.31 (1.03–1.66)	–
Coronary events							
Cases/person-years	216/45,758	526/98,903	712/132,271	1,000/169,782	271/45,356	115/12,192	
Basic model	1	1.01 (0.86–1.19)	0.96 (0.82–1.11)	1.01 (0.87–1.17)	1.01 (0.84–1.21)	1.73 (1.37–2.17)	0.02
Main model	1	1.02 (0.87–1.20)	0.99 (0.84–1.15)	1.00 (0.86–1.17)	0.92 (0.76–1.11)	1.39 (1.09–1.78)	0.47
Atrial fibrillation							
Cases/person-years	365/44,955	795/97,087	1,140/129,803	1,434/167,028	403/44,768	104/12,168	
Basic model	1	0.88 (0.78–1.00)	0.87 (0.77–0.98)	0.81 (0.72–0.91)	0.85 (0.74–0.99)	0.89 (0.71–1.10)	0.09
Main model	1	0.90 (0.80–1.03)	0.90 (0.80–1.02)	0.85 (0.75–0.96)	0.90 (0.78–1.05)	0.91 (0.72–1.15)	0.40
Aortic stenosis							
Cases/person-years	59/46,590	126/101,045	161/135,715	250/173,9587	53/46,720	20/12,632	
Basic model	1	0.85 (0.63–1.16)	0.74 (0.55–1.00)	0.86 (0.65–1.15)	0.68 (0.47–0.99)	1.05 (0.63–1.75)	0.46
Main model	1	0.86 (0.63–1.17)	0.76 (0.56–1.03)	0.89 (0.66–1.20)	0.69 (0.47–1.02)	0.97 (0.57–1.66)	0.84

[a]*Analysis of added sugar was carried out twice using different reference categories for stroke (<5 and 7.5–10 E%) due to the U-shaped trend. E%, Energy percentage.*
The associations were determined using multivariable Cox proportional hazards regression model and are expressed as hazard ratio with a 95% confidence interval and P-value for the linear trend. The basic model was adjusted for age, sex, season of dietary assessment, diet method, and energy intake. The main model was adjusted for age, sex, season of dietary assessment, diet method, energy intake, smoking status, educational level, leisure-time physical activity, alcohol consumption, BMI, and dietary habits including intake of processed meat, coffee, saturated fatty acids, and fiber density.

including the ApoB/ApoA-1 ratio, hypertension, and the use of lipid-lowering medications.

The proportional hazards assumptions were tested by plotting the partial residual plots for each variable against time using a scatterplot to see whether the hazards were proportional over time. To attain proportionality, all models were stratified by sex, as it was the most inconsistent covariate over time. A sensitivity analysis was conducted using the main model by excluding potential energy misreporters and individuals who had reported drastic diet changes prior to baseline assessments. In order to account for comorbidities, an additional sensitivity analysis was conducted by studying solely the first reported diagnosis for each participant. In this analysis, subjects who had experienced an incident event of another CVD prior to diagnosis of the specific outcome of interest in the analysis were excluded. In addition, participants with a prior incidence of diabetes mellitus (ICD-9 codes 150.0–150.9 or ICD-10 codes E10–E14) were also excluded in the comorbidity sensitivity analysis. The two sensitivity analyses were conducted both separately and combined.

RESULTS

Population Characteristics

The study population consisted of 25,877 individuals aged 45–74 years (mean age of 57.8 years, 62.4% female). The mean added sugar intake was 10.1 E%, and the mean BMI was 25.6 kg/m^2. During a mean follow-up of 19.5 years there were 2,580 stroke cases, 2,840 coronary events, 4,241 atrial fibrillation cases and 669 aortic stenosis cases. Individuals with high added sugar intake were more frequently male, older, and with lower BMI than individuals with low added sugar intake. Lower added sugar consumers tended to be overrepresented when it came to potential energy misreporting (primarily underreporting) and prior drastic diet changes, while they were generally more physically active and had a higher education level than higher added sugar consumers (**Supplementary Table 1**).

Added Sugar and CVD Risk

In the main model, no linear associations were found between added sugar intake and the studied outcomes. However, a U-shaped trend was observed for added sugar intake and risk of incident stroke; consumers in the 7.5–10 E% group had the lowest risk, while increased risks were observed among the lowest (HR: 1.14; 95% CI: 0.97–1.34) and highest (HR: 1.31; 95% CI: 1.03–1.66) intake groups. For coronary events, an increased risk was observed for added sugar intakes above 20 E% compared to the lowest intake group (HR: 1.39; 95% CI: 1.09–1.78) (**Table 1**).

The lowest added sugar intake group had the highest risk of atrial fibrillation, with the lowest risk found among intakes of 10–15 E% (HR: 0.85; 95% CI: 0.75–0.96). Similarly, a borderline significant decreased risk of incident aortic stenosis was observed among intakes of 7.5–10 E% (HR: 0.75; 95% CI: 0.56–1.03) and

15–20 E% (HR: 0.69; 95% CI: 0.47–1.02) compared to the lowest intake group (**Table 1**). None of the associations were changed when the main model was further adjusted for the potential mediators ApoB/ApoA-1 ratio, hypertension, and the use of lipid-lowering medications (**Supplementary Tables 2–5**).

Sugar-Sweetened Foods and Beverages and CVD Risk

The lowest intake group of treats (≤2 servings/week) was found to have the highest risks of stroke, coronary events, atrial fibrillation, and aortic stenosis. No associations were found between intake of toppings and any of the studied outcomes (**Figure 1**).

For SSBs, an increased risk of stroke was observed in the highest intake group (>8 servings/week) compared to the lowest intake group (<1 serving/week) (HR: 1.19; 95% CI: 1.01–1.40). No associations were found between the consumption of SSBs and incident coronary events, atrial fibrillation, or aortic stenosis (**Figure 1**).

Sensitivity Analyses

When excluding potential energy misreporters and diet changers, the association between added sugar intake and stroke was strengthened, as an increased risk was observed in the highest intake group compared to the lowest intake group (HR:1.57; 95% CI: 1.12–2.19) (**Table 2**), as well as a positive linear trend (*P*-trend: 0.04). When further excluding incidence of the other diagnoses prior to diagnosis of each studied outcome, the association with stroke was additionally strengthened (HR: 1.70; 95% CI: 1.15–2.51; **Table 3**). Similarly, the association between added sugar and aortic stenosis was strengthened in the combined sensitivity analysis, in which a decreased risk was observed for intakes between 15 and 20 E% compared to the lowest intake (HR: 0.50; 95% CI: 0.26–0.97), and a borderline significant negative trend was found (*P*-trend: 0.07; **Table 3**). The association between added sugar intake and coronary events was attenuated when excluding potential energy misreporters and diet changers (**Table 2**).

For treats, a majority of the associations were attenuated in the combined sensitivity analysis, though a tendency of the highest risk being found in the lowest intake group remained for stroke, coronary events, and aortic stenosis (**Table 3**). The association between topping intake and stroke was strengthened in the combined sensitivity analysis, in which an increased risk of stroke was observed in the highest intake group (HR: 1.39; 95% CI: 1.05–1.84). Similarly, the association for SSB intake was strengthened, as a positive trend (*P*-trend: <0.01) as well as an increased risk of stroke in the highest intake group (HR: 1.30; 95% CI: 1.03–1.65) was observed (**Table 3**).

DISCUSSION

We found that high added sugar intakes (>20 E%) were associated with increased risks of incident stroke and coronary events. A high intake of SSBs (>8 servings/week) could potentially explain part of the association with stroke. Contrary to our hypothesis, the lowest added sugar intake group (<5 E%) was indicated to have the highest risk of atrial fibrillation and aortic stenosis, and consumption of treats was negatively associated with risks of stroke, coronary events and atrial fibrillation.

Previous studies that have investigated the association between added sugar and incident CVD risk are lacking; however, there are a few studies that have examined the association with CVD mortality. Results from the NIH-AARP Diet and Health Study did not find an association between added sugar intake and risk of CVD mortality (25), although a tendency of a U-shaped association was observed. A U-shaped association has previously also been reported between added sugar intake and CVD mortality in the Malmö Diet and Cancer Study, with the highest risks being observed at ≥20 E% and second highest at <5 E (19). In a prospective cohort study of 11,733 US adults, added sugar was found to be linearly associated with CVD mortality when studying intakes between <10 and >25 E%, as intakes of >25 E% were associated with a 2.75-fold higher CVD mortality risk compared with <10 E% (5). The discrepancies between the study results could be explained by different consumption patterns, or that intakes below 5 E% were not studied separately in the latter study.

We observed a 19% increased risk of stroke in the highest intake group (>8 servings/week) of SSBs compared to the lowest intake group, while no associations were found for the other outcomes. The results from a Japanese prospective cohort study indicated that SSB consumption was associated with increased ischemic stroke risk, particularly among females, while no association was found with overall stroke or coronary events (26). It is therefore possible that the associations found in our study would have been even stronger if ischemic stroke cases were analyzed separately, though this hypothesis has yet to be tested. In contrast, the Male Health Professional Follow-up Study indicated a significant 20% increased risk of incident coronary heart disease among the highest quartile of SSB consumers compared to the lowest quartile, while no association with incident stroke was reported (27). The Nurses' Health Study, however, found associations between SSBs and both coronary heart disease and stroke (28, 29). Our study is, to the best of our knowledge, the first to investigate the association between the intake of SSBs and atrial fibrillation and aortic stenosis. As no differences were found when further adjusting the main model for the ApoB/ApoA-1 ratio, hypertension and use of lipid-lowering medication in our study, they are likely not strong mediating factors of the observed associations with added sugar, though a mediation analysis is required to confirm this. Previous results from the Malmö Diet and Cancer Study have shown a weak association between intake of sugar-sweetened foods and drinks and ApoB/ApoA (17). SSB consumption has been shown to adversely affect fasting blood glucose levels, inflammatory markers, and various blood lipids (30). Results from the Malmö Diet and Cancer Study have previously indicated an association between SSBs and circulating triglycerides (19); thus, it is possible that increased triglycerides could partly mediate the observed association between the intake of SSBs and the risk of incident stroke. Further, higher micronutrient dilution has been observed with very high free sugar intakes (>25 E%), as well as with

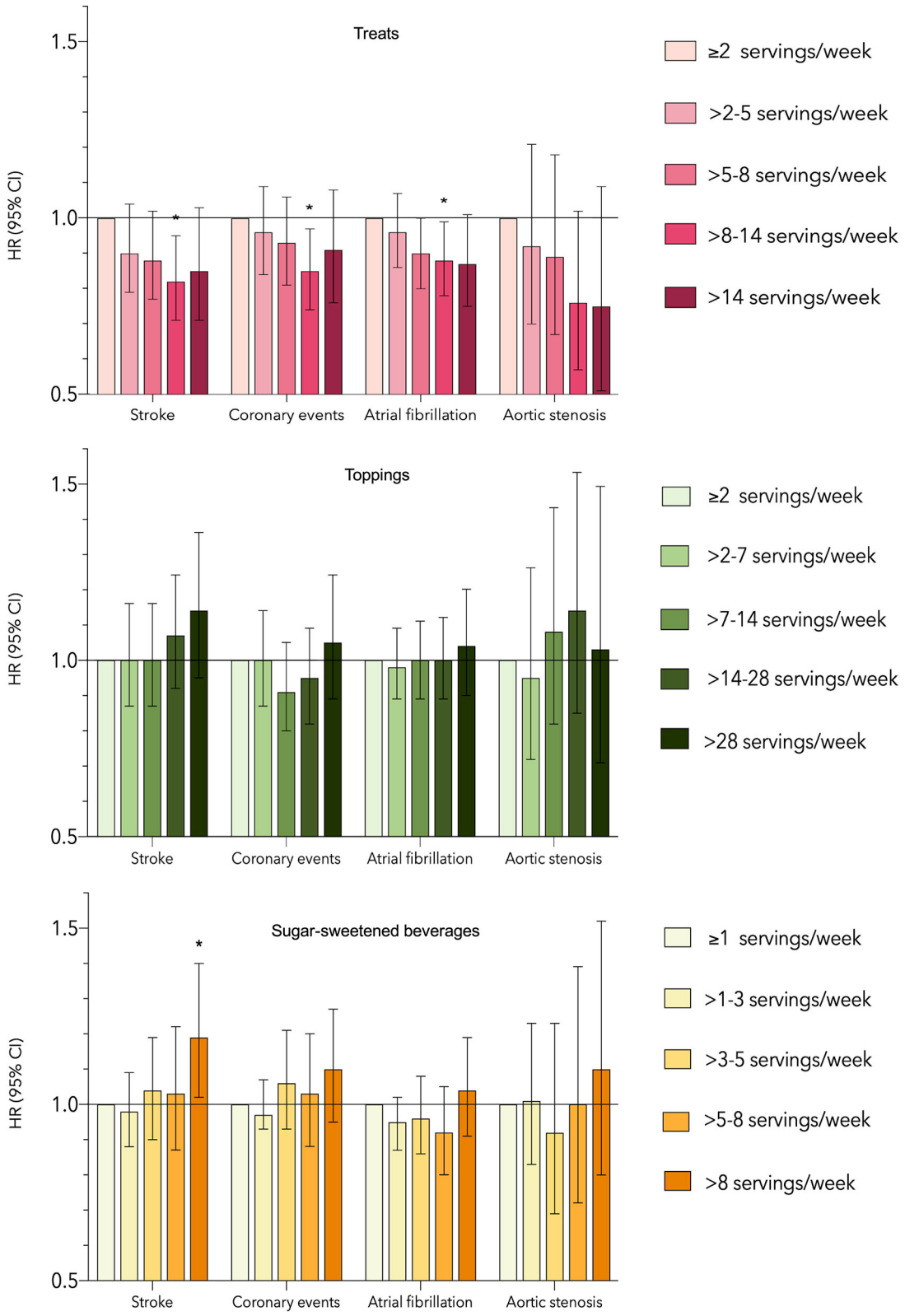

FIGURE 1 | Associations between intake of treats, toppings, sugar-sweetened beverages, and risk of incident stroke, coronary events, atrial fibrillation and aortic stenosis using the main model. *Statistically significant association. HR, Hazard ratio; CI, Confidence interval.

TABLE 2 | Sensitivity analysis excluding energy misreporters and participants who had made drastic dietary changes prior to baseline examinations, after which 16,781 participants remained.

		Stroke		Coronary events		Atrial fibrillation		Aortic stenosis	
Intake	***n***	**Cases/PY**	**HR (95% CI)**	**Cases/PY**	**HR (95% CI)**	**Cases/PY**	**HR (95% CI)**	**Cases/PY**	**HR (95% CI)**
Added sugar, E%									
<5	1,246	107/23,760	1	115/23,899	1	190/23,488	1	33/24,293	1
5–7.5	3,092	293/60,381	1.01 (0.81–1.27)	336/60,330	1.07 (0.87–1.33)	473/59,322	0.88 (0.75–1.05)	74/61,662	0.77 (0.51–1.17)
7.5–10	4,455	438/87,454	0.97 (0.78–1.21)	469/87,325	0.98 (0.80–1.20)	759/85,698	0.91 (0.77–1.07)	106/89,656	0.70 (0.47–1.04)
10–15	5,978	604/116,375	0.95 (0.76–1.18)	674/116,298	0.95 (0.78–1.17)	978/114,228	0.84 (0.71–0.99)	166/119,044	0.79 (0.53–1.17)
15–20	1,597	182/30,403	1.09 (0.84–1.41)	185/30,346	0.91 (0.71–1.16)	288/29,883	0.97 (0.80–1.18)	29/31,331	0.53 (0.31–0.89)
>20	413	65/7,417	1.57 (1.12–2.19)	67/7,460	1.28 (0.94–1.76)	63/7,535	0.86 (0.64–1.16)	11/7,787	0.77 (0.37–1.58)
P-trend			0.04		0.66		0.87		0.20
Treats, servings/week									
≤2	1,496	158/27,352	1	180/27,502	1	226/27,180	1	35/28,175	1
>2–5	4,236	408/82,251	0.85 (0.71–1.02)	453/82,303	0.92 (0.77–1.09)	667/80,850	0.97 (0.83–1.13)	107/84,070	0.96 (0.65–1.42)
>5–8	4,590	452/89,914	0.82 (0.68–0.99)	496/89,827	0.91 (0.76–1.09)	728/88,482	0.92 (0.79–1.07)	120/92,039	0.94 (0.63–1.38)
>8–14	4,869	505/95,240	0.80 (0.66–0.96)	526/95,233	0.82 (0.68–0.97)	846/93,307	0.92 (0.79–1.07)	119/97,639	0.77 (0.52–1.15)
>14	1,590	166/31,032	0.80 (0.63–1.01)	191/30,793	0.85 (0.68–1.06)	284/30,333	0.93 (0.77–1.12)	38/31,847	0.74 (0.45–1.21)
P-trend			0.12		0.01		0.61		0.08
Toppings, servings/week									
≤2	1,783	146/35,137	1	174/35,013	1	252/34,585	1	36/35,799	1
>2–7	4,237	381/83,694	0.99 (0.82–1.20)	386/83,971	0.91 (0.76–1.09)	622/82,515	0.96 (0.83–1.12)	90/85,626	0.97 (0.65–1.44)
>7–14	4,699	448/91,414	0.98 (0.81–1.18)	462/91,538	0.86 (0.72–1.03)	782/89,599	1.00 (0.87–1.16)	130/93,327	1.22 (0.83–1.79)
>14–28	4,216	476/81,319	1.06 (0.87–1.29)	526/81,030	0.90 (0.75–1.098)	763/79,466	0.98 (0.84–1.14)	119/83,542	1.17 (0.78–1.74)
>28	1,846	238/34,225	1.30 (1.04–1.63)	298/34,105	1.03 (0.84–1.27)	332/33,988	1.02 (0.85–1.22)	44/35,477	1.09 (0.68–1.77)
P-trend			<0.01		0.34		0.82		0.76
Sugar-sweetened beverages, servings/week									
≤1	9,609	968/185,693	1	1,041/185,809	1	1,608/182,216	1	243/190,184	1
>1–3	3,595	332/71,326	0.95 (0.84–1.08)	370/71,146	0.98 (0.87–1.11)	555/70,123	0.94 (0.86–1.04)	86/72,854	0.97 (0.76–1.25)
>3–5	1,524	146/29,797	0.95 (0.80–1.14)	170/29,672	0.99 (0.84–1.17)	246/29,191	0.98 (0.85–1.12)	40/30,446	1.02 (0.72–1.44)
>5–8	1,053	115/20,430	1.07 (0.87–1.30)	130/20,466	1.04 (0.86–1.25)	171/20,260	0.97 (0.82–1.13)	26/21,096	1.00 (0.66–1.50)
>8	1,000	128/18,542	1.24 (1.02–1.51)	135/18,565	1.03 (0.86–1.25)	171/18,362	1.01 (0.86–1.20)	24/19,191	0.94 (0.61–1.47)
P-trend			0.02		0.72		0.91		0.75

E%, Energy percentage. PY, Person-years. HR, Hazard ratio. The associations were determined using multivariable Cox proportional hazards regression model and are expressed as HR with a 95% confidence interval and P-value for the linear trend. All analyses were carried out with adjustment according to the main model: age, sex, season of dietary assessment, diet method, energy intake, smoking status, educational level, leisure-time physical activity, alcohol consumption, body mass index, and dietary habits including intake of processed meat, coffee, saturated fatty acids, and fiber density.

very low intakes (< 5 E%) (31), which could help explain the increased risks observed at both ends of the added sugar intake spectra the main results of our study. Significant inverse linear associations between added sugar and micronutrient intake have however been reported in the Malmö Diet and Cancer study (32), which is consistent with the results of the sensitivity analyses for stroke in this study. In our study, exclusion of participants who had reported prior drastic diet changes, and potential energy misreporters, resulted in an attenuation of the increased risks of stroke found at added sugar <5 E%. This indicates the role of dietary measurement error for the observed increased risks in the lowest intake category prior to sensitivity analyses.

The sensitivity analyses strengthened certain associations while attenuating others, ultimately emphasizing that dietary risk factors may vary between CVDs. In the sensitivity analysis where solely the first reported diagnosis of the included outcomes for each participant was studied, the association between stroke and added sugar was slightly strengthened, and the negative association between aortic stenosis and added sugar was strengthened. Thus, it is possible that not taking comorbidities into account could have steered the associations toward the null. This tendency might be due to the different etiologies of the studied diseases, highlighting the importance of taking comorbidity into consideration. For example, aortic stenosis has previously not been associated with dietary factors otherwise strongly associated with many cardiovascular diseases, such as dietary fiber intake or dietary patterns recommended for CVD prevention (33).

Diets commonly recommended to decrease the risk of CVD include the Mediterranean diet and The Dietary Approaches to Stop Hypertension (DASH) (34, 35). Both

TABLE 3 | Sensitivity analysis excluding diet changers, energy misreporters and individuals with prior incidence of other diagnoses (aortic stenosis, atrial fibrillation, stroke, coronary events or diabetes) for each particular outcome.

	Stroke		Coronary events		Atrial fibrillation		Aortic stenosis	
Intake	**n/Cases/PY**	**HR (95% CI)**	**n/Cases/PY**	**HR (95% CI)**	**n/Cases/PY**	**HR (95% CI)**	**n/Cases/PY**	**HR (95% CI)**
Added sugar, E%								
<5	897/79/16,723	1	867/77/16,374	1	955/136/17,862	1	840/22/16,183	1
5–7.5	2,158/204/41,212	0.98 (0.75–1.28)	2,138/242/41,078	1.13 (0.87–1.46)	2,297/343/43,653	0.94 (0.77–1.15)	2,009/54/39,546	0.88 (0.53–1.45)
7.5–10	3,091/392/59,891	0.94 (0.73–1.21)	3,049/326/58,896	1.02 (0.79–1.31)	3,349/561/63,995	0.98 (0.81–1.19)	2,858/69/57,113	0.69 (0.42–1.13)
10–15	4,174/421/80,383	0.90 (0.69–1.16)	4,137/479/79,858	0.96 (0.75–1.24)	4,452/697/84,944	0.86 (0.71–1.04)	3,854/101/76,795	0.70 (0.43–1.14)
15–20	1,103/132/20,934	1.08 (0.80–1.46)	1,086/138/20,370	0.99 (0.74–1.34)	1,181/209/21,924	1.02 (0.81–1.29)	990/18/19,388	0.50 (0.26–0.97)
>20	288/49/5,012	1.70 (1.15–2.51)	276/43/4,863	1.33 (0.89–1.98)	279/40/5,047	0.95 (0.66–1.38)	245/6/4,561	0.80 (0.31–2.07)
P-trend		0.05		0.78		0.73		0.07
Treats, servings/week								
≤2	1,064/112/18,968	1	1,035/126/18,619	1	1,105/152/19,854	1	978/26/18,144	1
>2–5	2,990/293/57,219	0.94 (0.75–1.17)	2,945/325/56,720	1.02 (0.82–1.25)	3,175/474/60,401	1.03 (0.86–1.24)	2,767/69/54,691	0.86 (0.54–1.37)
>5–8	3,258/317/63,057	0.88 (0.71–1.10)	3,220/352/62,303	0.97 (0.78–1.20)	3,473/533/66,584	0.99 (0.83–1.19)	3,016/73/60,236	0.78 (0.49–1.24)
>8–14	3,348/351/64,483	0.86 (0.68–1.07)	3,309/367/63,910	0.86 (0.69–1.06)	3,613/616/68,899	1.00 (0.83–1.21)	3,076/80/61,429	0.70 (0.44–1.13)
>14	1,051/114/20,127	0.88 (0.66–1.17)	1,044/135/19,887	0.93 (0.72–1.21)	1,147/211/21,688	1.09 (0.87–1.37)	959/22/19,087	0.62 (0.34–1.15)
P-trend		0.47		0.05		0.55		0.14
Toppings, servings/week								
≤2	1,289/92/25,032	1	1,290/125/25,126	1	1,378/180/26,594	1	1,221/23/24,338	1
>2–7	3,008/272/58,634	1.14 (0.90–1.45)	2,940/255/57,855	0.86 (0.69–1.07)	3,199/461/61,920	1.02 (0.86–1.21)	2,800/62/56,270	1.09 (0.67–1.79)
>7–14	3,323/326/63,710	1.12 (0.89–1.43)	3,259/339/62,762	0.88 (0.71–1.09)	3,581/585/68,068	1.05 (0.88–1.24)	3,078/87/60,949	1.25 (0.77–2.03)
>14–28	2,838/324/53,901	1.16 (0.91–1.49)	2,817/377/53,243	0.90 (0.73–1.12)	3,059/545/57,274	1.01 (0.84–1.21)	2,587/70/50,944	1.12 (0.67–1.85)
>28	1,253/173/22,578	1.39 (1.05–1.84)	1,247/209/22,452	0.94 (0.74–1.21)	1,296/215/23,570	0.98 (0.79–1.22)	1,110/28/21,084	1.11 (0.60–2.04)
P-trend		0.04		0.96		0.51		0.40
SSBs, servings/week								
≤1	6,751/671/128,328	1	6,654/734/126,912	1	7,270/1,190/136,921	1	6,233/153/122,511	1
>1–3	2,539/241/49,787	0.99 (0.85–1.14)	2,509/267/49,212	0.99 (0.86–1.14)	2,690/390/52,510	0.90 (0.80–1.01)	2,361/61/47,824	1.09 (0.80–1.47)
>3–5	1,048/105/20,183	1.03 (0.83–1.27)	1,041/123/20,063	1.04 (0.86–1.27)	1,118/175/21,189	0.98 (0.83–1.15)	969/27/19,276	1.13 (0.74–1.73)
>5–8	711/82/13,695	1.10 (0.87–1.39)	696/87/13,427	0.96 (0.77–1.21)	744/115/14,316	0.89 (0.73–1.08)	645/16/12,932	1.02 (0.60–1.72)
>8	662/88/11,862	1.30 (1.03–1.65)	653/94/11,824	1.08 (0.86–1.35)	691/116/12,491	1.01 (0.83–1.23)	588/13/11,041	0.98 (0.54–1.76)
P-trend		<0.01		0.92		0.90		0.72

E%, Energy percentage. PY, Person-years. HR, Hazard ratio. SSBs, Sugar-sweetened beverages. The associations were determined using multivariable Cox proportional hazards regression model and are expressed as HR with a 95% confidence interval and P-value for the linear trend. All analyses were carried out with adjustment for age, sex, season of dietary assessment, diet method, energy intake, smoking status, educational level, leisure-time physical activity, alcohol consumption, body mass index, and dietary habits including intake of processed meat, coffee, saturated fatty acids, and fiber density.

of the mentioned diets emphasize intake of unsaturated fats, lean meats and high-quality carbohydrates such as fruits and vegetables combined with limited intake of saturated fat, cholesterol and salt. Only the DASH diet explicitly recommends restricting added sugar intake, though generally, Mediterranean style diets tend to be low in added sugar as well (34, 35). As the associations between added sugar intake and CVD incidence are not yet fully known, we believe that the results of this study provide an important contribution to the future development of dietary guidelines for CVD prevention.

A major strength of this study was the large sample size, which allowed for rigorous sensitivity analyses. However, as the number of aortic stenosis cases was very low in the highest intake category, and especially in sensitivity analyses, an even larger study sample would have been beneficial for studying this outcome. Additional strengths of the Malmö Diet and Cancer Study include the comprehensive dietary assessment, as well as the ability to exclude diet changers and potential energy misreporters. Although the added sugar intakes in this study are based on estimations, the estimated intakes correspond well with those reported in national health surveys (6).

To isolate the studied diet-outcome associations, we adjusted for many confounders using a pre-specified model based on existing literature, though some residual confounding may exist. For example, reliable data on sodium intake, which national surveys have reported to exceed the recommended intakes (6, 36), and trans-fatty acid (TFA) intake were not available from the Malmö Diet and Cancer Study. Thus, they were not adjusted for despite them being established risk factors of CVD (1). Although the national average intake of TFA is ∼0.5 E% (6), which is below the Food and Agriculture Organization of the United Nations recommended upper limit of 1 E% (37), it cannot be ruled out that the participants' intakes of trans-fatty acid intake or sodium have affected the results. Due to the nature of observational studies regarding risk of confounding, randomized controlled trials are ultimately required to be able to draw any conclusions about causality.

Baseline data were collected between 1991 and 1996, and lifestyle and dietary patterns may therefore differ from those of today's population. For example, the consumption of chocolate and confections has increased drastically between 1990 and 2017, and the consumption of SSBs has more than doubled during the same time period (38). Additionally, the consumption patterns of added sugar and sugar-sweetened foods and beverages may also differ between countries and age groups, further affecting the generalizability of the study results.

Dietary recommendations regarding added sugar intake vary globally, but the Nordic Nutrition Recommendations state that added sugar intake should not exceed 10 E%, with the basis mainly being prevention of caries, overweight, secondary diseases and micronutrient dilution (20, 32). The World Health Organization has a similar recommendation for free sugars, recommending it should be kept below 10 E%, and also suggest further reducing the intake to below 5 E% (21). According to a national survey from year 2010 to 2011, the average intake of added sugar in Sweden was estimated to be 9.6 E%, with ∼40% of the Swedish population failing to achieve the national recommendation (6). The findings of this study do not support a reduction of the upper recommended limit of 10 E% to 5 E%, but a general reduction of added sugar intake and SSB consumption in the population could be beneficial for prevention of stroke and coronary events.

CONCLUSION

The results of this study indicate that the associations vary, both between different CVDs and different sources of added sugar. Added sugar intakes of >20 E% were associated with increased risks of incident stroke and coronary events, while the lowest intake category of added sugar was indicated to have the highest risk of atrial fibrillation and aortic stenosis. High intakes of SSBs (>8 servings/week) were associated with increased stroke risk, while consumption of treats was negatively associated with risks of stroke, coronary events and atrial fibrillation. The findings indicate that a general reduction of added sugar and SSB consumption in the population could be beneficial for prevention of stroke and coronary events.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of ethical and legal restrictions. Requests to access the datasets should be directed to the Chair of the Steering Committee for the Malmö cohorts, see instructions at https://www.malmo-kohorter.lu.se/english.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Regional Ethical Review Board in Lund, Sweden (LU/90-51). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

The study was devised by ES, and the analysis plan was constructed by SJ, ES, and SR. SJ conducted the analyses and drafted the manuscript, which was then critically revised by ES, SR, EG-P, and LJ. All authors helped in interpretation of the results, gave final approval, and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.603653/full#supplementary-material

REFERENCES

1. Mendis S, Puska P, Norrving B. *Global Atlas on Cardiovascular Disease Prevention and Control*. Geneva: World Health Organization (2011).
2. Kendir C, van den Akker M, Vos R, Metsemakers J. Cardiovascular disease patients have increased risk for comorbidity: a cross-sectional study in the Netherlands. *Eur J Gen Pract.* (2018) 24:45–50. doi: 10.1080/13814788.2017.1398318
3. Rippe JM, Angelopoulos TJ. Sugars, obesity, and cardiovascular disease: results from recent randomized control trials. *Eur J Nutr.* (2016) 45–53. doi: 10.1007/s00394-016-1257-2
4. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am J Clin Nutr.* (2014) 100:65–79. doi: 10.3945/ajcn.113.081521
5. Yang Q, Zhang Z, Gregg EW, Flanders WD, Merritt R, Hu FB. Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA Intern Med.* (2014) 174:516–24. doi: 10.1001/jamainternmed.2013.13563
6. Amcoff E, Edberg A, Barbieri HnE, Lindroos AK, Nälsén C, Pearson M, et al. *Riksmaten - Vuxna 2010-11: Livsmedels- och Näringsintag Bland Vuxna i Sverige (Food and Nutrient Intake among Adults in Sweden)*. Uppsala: Swedish National Food Agency (2012).
7. Bailey RL, Fulgoni VL, Cowan AE, Gaine PC. Sources of added sugars in young children, adolescents, and adults with low and high intakes of added sugars. *Nutrients.* (2018) 10:102. doi: 10.3390/nu10010102
8. Keller A, Heitmann BL, Olsen N. Sugar-sweetened beverages, vascular risk factors and events: a systematic literature review. *Public Health Nutr.* (2015) 18:1145–54. doi: 10.1017/S1368980014002122
9. Sonestedt E, Overby NC, Laaksonen DE, Birgisdottir BE. Does high sugar consumption exacerbate cardiometabolic risk factors and increase the risk of type 2 diabetes and cardiovascular disease? *Food Nutr Res.* (2012) 56:19104. doi: 10.3402/fnr.v56i0.19104
10. Khan TA, Sievenpiper JL. Controversies about sugars: results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. *Eur J Nutr.* (2016) 55:25–43. doi: 10.1007/s00394-016-1345-3
11. Malik VS, Hu FB. Fructose and cardiometabolic health: what the evidence from sugar-sweetened beverages tells us. *J Am Coll Cardiol.* (2015) 66:1615–24. doi: 10.1016/j.jacc.2015.08.025
12. Mutie PM, Drake I, Ericson U, Teleka S, Schulz CA, Stocks T, et al. Different domains of self-reported physical activity and risk of type 2 diabetes in a population-based Swedish cohort: the Malmo diet and Cancer study. *BMC Public Health.* (2020) 20:261. doi: 10.1186/s12889-020-8344-2
13. Sonestedt E, Wirfalt E, Gullberg B, Berglund G. Past food habit change is related to obesity, lifestyle and socio-economic factors in the Malmo Diet and Cancer Cohort. *Public Health Nutr.* (2005) 8:876–85. doi: 10.1079/PHN2005736
14. Black AE. Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord.* (2000) 24:1119–30. doi: 10.1038/sj.ijo.0801376
15. Mattisson I, Wirfalt E, Aronsson CA, Wallstrom P, Sonestedt E, Gullberg B, et al. Misreporting of energy: prevalence, characteristics of misreporters and influence on observed risk estimates in the Malmo Diet and Cancer cohort. *Br J Nutr.* (2005) 94:832–42. doi: 10.1079/BJN20051573
16. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation.* (2005) 112:2735–52. doi: 10.1161/CIRCULATIONAHA.105.169404
17. Frondelius K, Borg M, Ericson U, Borné Y, Melander O, Sonestedt E. Lifestyle and dietary determinants of serum apolipoprotein A1 and apolipoprotein B concentrations: cross-sectional analyses within a Swedish cohort of 24,984 individuals. *Nutrients.* (2017) 9:211. doi: 10.3390/nu9030211
18. Riboli E, Elmståhl S, Saracci R, Gullberg B, Lindgärde F. The Malmö Food Study: validity of two dietary assessment methods for measuring nutrient intake. *Int J Epidemiol.* (1997) 26(Suppl 1):S161–73.
19. Ramne S, Alves Dias J, González-Padilla E, Olsson K, Lindahl B, Engström G, et al. Association between added sugar intake and mortality is nonlinear and dependent on sugar source in 2 Swedish population-based prospective cohorts. *Am J Clin Nutr.* (2019) 109:411–23. doi: 10.1093/ajcn/nqy268
20. Nordic Council of Ministers. *Nordic Nutrition Recommendations 2012: Integrating Physical Activity and Nutrition*. 5th ed. Copenhagen: Nordic Council of Ministers (2014).
21. World Health Organization. *Guideline: Sugars Intake for Adults and Children*. Geneva: World Health Organization (2015).
22. Wirfalt E, Mattisson I, Johansson U, Gullberg B, Wallstrom P, Berglund G. A methodological report from the Malmo Diet and Cancer study: development and evaluation of altered routines in dietary data processing. *Nutr J.* (2002) 1:3. doi: 10.1186/1475-2891-1-3
23. Ludvigsson JF, Andersson E, Ekbom A, Feychting M, Kim J-L, Reuterwall C, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health.* (2011) 11:450. doi: 10.1186/1471-2458-11-450
24. Smith JG, Newton-Cheh C, Almgren P, Struck J, Morgenthaler NG, Bergmann A, et al. Assessment of conventional cardiovascular risk factors and multiple biomarkers for the prediction of incident heart failure and atrial fibrillation. *J Am Coll Cardiol.* (2010) 56:1712–9. doi: 10.1016/j.jacc.2010.05.049
25. Tasevska N, Park Y, Jiao L, Hollenbeck A, Subar AF, Potischman N. Sugars and risk of mortality in the NIH-AARP Diet and Health Study. *Am J Clin Nutr.* (2014) 99:1077–88. doi: 10.3945/ajcn.113.069369
26. Eshak ES, Iso H, Kokubo Y, Saito I, Yamagishi K, Inoue M, et al. Soft drink intake in relation to incident ischemic heart disease, stroke, and stroke subtypes in Japanese men and women: the Japan Public Health Centre-based study cohort I. *Am J Clin Nutr.* (2012) 96:1390–7. doi: 10.3945/ajcn.112.037903
27. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. *Circulation.* (2012) 125:1735–41, S1. doi: 10.1161/CIRCULATIONAHA.111.067017
28. Fung TT, Malik V, Rexrode KM, Manson JE, Willett WC, Hu FB. Sweetened beverage consumption and risk of coronary heart disease in women. *Am J Clin Nutr.* (2009) 89:1037–42. doi: 10.3945/ajcn.2008.27140
29. Bernstein AM, de Koning L, Flint AJ, Rexrode KM, Willett WC. Soda consumption and the risk of stroke in men and women. *Am J Clin Nutr.* (2012) 95:1190–9. doi: 10.3945/ajcn.111.030205

30. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. *Am J Clin Nutr.* (2011) 94:479–85. doi: 10.3945/ajcn.111.013540
31. Mok A, Ahmad R, Rangan A, Louie JCY. Intake of free sugars and micronutrient dilution in Australian adults. *Am J Clin Nutr.* (2018) 107:94–104. doi: 10.1093/ajcn/nqx008
32. Gonzalez-Padilla E, Dias JA, Ramne S, Olsson K, Nalsen C, Sonestedt E. Association between added sugar intake and micronutrient dilution: a cross-sectional study in two adult Swedish populations. *Nutr Metab (Lond).* (2020) 17:15. doi: 10.1186/s12986-020-0428-6
33. Larsson SC, Wolk A, Back M. Dietary patterns, food groups, and incidence of aortic valve stenosis: a prospective cohort study. *Int J Cardiol.* (2019) 283:184–18. 2018/11/13. doi: 10.1016/j.ijcard.2018.11.007
34. Rees K, Takeda A, Martin N, Ellis L, Wijesekara D, Vepa A, et al. Mediterranean-style diet for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev.* (2019) 3:CD009825. doi: 10.1002/14651858.CD009825.pub3
35. Chiavaroli L, Viguiliouk E, Nishi SK, Blanco Mejia S, Rahelic D, Kahleova H, et al. DASH dietary pattern and cardiometabolic outcomes: an umbrella review of systematic reviews and meta-analyses. *Nutrients.* (2019) 11:338. doi: 10.3390/nu11020338
36. Becker W, Pearson M. *Riksmaten 1997-98: Kostvanor Och Näringsintag i SVERIGE : Metod-och Resultatanalys.* Uppsala: Livsmedelsverket (2002).
37. Food and Agriculture Organization of the United Nations. Fats and fatty acids in human nutrition. Report of an expert consultation. *FAO Food Nutr Pap.* (2010) 91:1–166.
38. Swedish Board of Agriculture. *Food Consumption and Nutritive Values, Data Up to 2017*. Swedish Board of Agriculture (2018).

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REVIEW
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The Impact of Artificial Sweeteners on Body Weight Control and Glucose Homeostasis

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A poor diet is one of the leading causes for non-communicable diseases. Due to the increasing prevalence of overweight and obesity, there is a strong focus on dietary overconsumption and energy restriction. Many strategies focus on improving energy balance to achieve successful weight loss. One of the strategies to lower energy intake is refraining from sugars and replacing them with artificial sweeteners, which maintain the palatability without ingesting calories. Nevertheless, the safety and health benefits of artificial sweeteners consumption remain a topic of debate within the scientific community and society at large. Notably, artificial sweeteners are metabolized differently from each other due to their different properties. Therefore, the difference in metabolic fate of artificial sweeteners may underlie conflicting findings that have been reported related to their effects on body weight control, glucose homeostasis, and underlying biological mechanisms. Thus, extrapolation of the metabolic effects of a single artificial sweetener to all artificial sweeteners is not appropriate. Although many rodent studies have assessed the metabolic effects of artificial sweeteners, long-term studies in humans are scarce. The majority of clinical studies performed thus far report no significant effects or beneficial effects of artificial sweeteners on body weight and glycemic control, but it should be emphasized that the study duration of most studies was limited. Clearly, further well-controlled, long-term human studies investigating the effects of different artificial sweeteners and their impact on gut microbiota, body weight regulation and glucose homeostasis, as well as the underlying mechanisms, are warranted.

Keywords: artificial sweeteners, obesity, type 2 diabetes mellitus, insulin resistance, gut microbiota

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INTRODUCTION

Diet is among the most important health influencers. Along with globalization and economic growth, a shift in dietary habits has occurred since 1970 (1, 2). Energy intake has increased along with the consumption of animal fat and energy-dense foods, while fiber intake has decreased (2). This dietary shift contributes to the rise of non-communicable diseases, including obesity, type 2 diabetes mellitus (T2DM), cardiovascular disease, and cancer (3–5). A poor diet was found to be the leading risk factor of death and third leading risk factor for disability-adjusted life-years loss in the United States (6). Globally, 11 million deaths and 255 million disability-adjusted life-years were attributable to dietary risk factors in 2017 (7). Due to the increasing trends in overweight and obesity, there is a strong focus on dietary overconsumption and energy restriction. In 2016, there were more than 1.9 billion overweight adults and 650 million obese adults, representing a

global prevalence of 13% (8). Beside adults, the prevalence of childhood obesity has also increased dramatically worldwide. Over 340 million children and adolescents (5–19 year of age) were overweight or obese in 2016 (8).

However, obesity and its associated metabolic disorders, including T2DM, cardiovascular disease, and fatty liver disease, are preventable. Many strategies exist to achieve successful weight loss by improving dietary habits and energy balance. However, even more challenging than achieving weight loss is the maintenance of body weight after weight loss (9). The intake of sugar contributes to the overall energy density of diets, thereby promoting obesity (10, 11). In particular the consumption of sugar-sweetened beverages has been associated with cardiometabolic complications, driven by an increased energy intake and obesity (12). Therefore, one common approach to improve energy balance is to refrain from sugars by replacing them with artificial sweeteners. Although the World Health Organization (WHO) recommends free sugar intake of <10% of total energy intake, preferably <5% of total energy intake as a conditional recommendation, a large proportion of the European population appears to exceed this threshold, especially children (13). For instance, 81% of the Dutch population does not fulfill this recommendation as the intake of free sugars equals ∼14% of total energy intake in the Netherlands (14).

As artificial sweeteners offer a sweeter taste without calories, the replacement of sugars with these sweeteners seems promising in reducing sugar and energy intake. Meta-analyses of Randomized Controlled Trials (RCTs) have shown that daily energy intake (after 4 or 10 weeks) and sugar intake (after 4 weeks) were lower in healthy, overweight, and obese individuals receiving artificial sweeteners as a replacements of sugars in the diet (15). Sweeteners are classified as natural sweeteners and artificial sweeteners. Artificial sweeteners are further classified as nutritive and non-nutritive sweeteners, depending on whether they contain calories. The nutritive sweeteners include the monosaccharide polyols (e.g., xylitol, mannitol, and sorbitol) and the disaccharide polyols (e.g., lactitol and maltitol). The non-nutritive sweeteners, known as artificial sweeteners, include substances from different chemical classes that are 30–13,000 times sweeter than sucrose (16). Artificial sweeteners are metabolized differently and have different properties, including sweetness intensity, persistence of sweet taste, coating of the teeth, and aftertaste effects (15). Therefore, each sweetener is unique and may affect the perceived taste or use in food applications differently (17). Sweetener consumption is highly prevalent in both adults and children and is expected to increase even more in the near future. In the United states, ∼25% of children and >41% of adults consumed artificial sweeteners in 2009–2012, representing a 200% increase in consumption in children and a 54% increase among adults compared to data from 1999 to 2000 (18). Between these decades, a rise in food products containing artificial sweeteners occurred with more than 6,000 new products launched in the United states alone (19). Currently, six different artificial sweeteners are approved by the Food and Drug Administration (FDA) as food additives in the United States, including saccharin, sucralose, aspartame, advantame, acesulfame-potassium, and neotame (20). Furthermore, thaumatin, steviol glycosides, obtained from the leaves of *Stevia* plant, and *Luo Han Guo* fruit extracts have been granted the Generally Recognized as Safe (GRAS) status by the FDA (20, 21). In the European Union, the range of approved artificial sweeteners is broader, as cyclamate, aspartame-acesulfame salt, and neohesperidin dihydrochalcone are also approved by the EU Scientific Committee on Food (22–24). Other artificial sweeteners have not been assessed yet or are declared as unsafe for usage.

Despite the fact that many national authorities have recognized artificial sweeteners as safe and well-tolerated, a lot of controversies about the effects of sweeteners on human health still exist. Whereas, some longitudinal cohort studies show an association between artificial sweeteners consumption and reduced risk of T2DM, overweight and obesity, other observational studies have yielded opposite findings (25–28). Furthermore, longitudinal cohort studies found a positive association between the consumption of artificial sweeteners and the risk of hypertension, stroke, and cardiovascular events (29). Thus, although the use of artificial sweeteners seem promising in assisting weight loss, artificial sweeteners have been linked to a variety of health concerns, including obesity and its related cardiometabolic disturbances (29–31). Importantly, however, it cannot be excluded that the associations found in these observational and prospective cohort studies studies are largely explained by an increase in artificial sweetener intake to compensate for an unhealthy diet or lifestyle in general (reverse causation). The safety and health benefits of artificial sweeteners consumption remain controversial. Considering the rising prevalence of obesity and T2DM along with the increased consumption of artificial sweeteners, it is important to clarify their health benefits and/or harms (18, 32, 33). Therefore, the physiological health effects of artificial sweeteners should be elucidated.

In this review, we provide an overview of the physiological effects of artificial sweeteners on body weight control and glucose homeostasis. Furthermore, the pharmacokinetics of the commonly used artificial sweeteners will be addressed to identify the controversies of the existing evidence surrounding their use. Subsequently, effects of artificial sweeteners on body weight and glycemic control will be discussed.

METHODS

Ample data is available on the effects of artificial sweeteners on body weight and glucose homeostasis. Nevertheless, fewer studies are available reporting the effects of specific artificial sweeteners. A review of the literature was conducted using PubMed databases in the period January–April 2020. The following search terms were used for artificial sweeteners: "artificial sweeteners" OR "non-caloric sweeteners" OR "non-nutritive sweeteners" OR "aspartame" OR "sucralose" OR "acesulfame potassium" OR "acesulfame-K" OR "steviol glycoside" OR "rebaudioside" OR "saccharin." Different combinations of search terms were used, with and without the artificial sweetener search term, including pharmacokinetics (MeSH terms), body

weight (MeSH terms), adiposity (MeSH terms), caloric intake (MeSH terms), sweet taste receptors (MeSH terms), gut-brain axis (MeSH terms), adipogenesis (MeSH terms), microbiota (MeSH terms), short chain fatty acids (MeSH terms), free fatty acid receptors (MeSH terms), energy expenditure (MeSH terms), glucose homeostasis (MeSH terms), insulin secretion (MeSH terms), and inflammation (MeSH terms). Articles written in English language were included. No data restrictions were applied. Reference lists of relevant systematic reviews were screened to identify further relevant citations. Human studies were mainly selected for this review to address the effect of artificial sweeteners on parameters related to body weight or adiposity and glucose homeostasis. In case of limited or lacking human data, rodent studies and *in vitro* studies were also considered. Studies in healthy adults as well as adults living with overweight, obesity or diabetes were included. RCTs (including weight-loss studies), prospective cohort studies, cross-sectional studies, and meta-analyses were included in the literature search. Studies included the use of artificial sweeteners solely, without carbohydrate or caloric content modification, unless specified otherwise. Studies with children (≤18 years), pregnant women, or individuals with acute or chronic diseases other than obesity and diabetes were excluded. Furthermore, studies that did not specify the type of artificial sweetener were excluded. We have identified 5 meta-analyses of RCTs or RCTs studying the effects of specific artificial sweeteners on adiposity and 20 meta-analyses of RCTs or RCTs studying the effects of specific artificial sweeteners on glucose homeostasis as indicated in **Tables 1**, **2**, respectively. Retrieved papers were first screened by title and subsequently by abstract based on the criteria. Full papers were reviewed in case the abstract was insufficient to determine the eligibility. Endnote X8 was used for the management of articles and citations. In total, 164 publications were identified that matched these criteria.

PHARMACOKINETICS

To determine safety of artificial sweeteners the FDA considers probable intake, cumulative effects from all uses, and toxicological data in animals. The European Food Safety Authority (EFSA) evaluates and confirms that the intake of artificial sweeteners, within the acceptable daily intake (ADI), does not cause cancer or other health-related problems, and are therefore safe for human consumption (56, 57). Although authorities consider artificial sweeteners as safe as they do not pose any health-related problems, when consumed within the ADI, no specific safety claims have been made about the effects of sweeteners on non-communicable diseases, such as obesity and T2DM. Despite the fact that several artificial sweeteners are tested for pharmacological and toxicological aspects, the concerns about the effects of unmetabolized compounds on non-communicable diseases still exist. Artificial sweeteners have distinct structures and are metabolized differently as some but not all are digested or fermented (**Figure 1**). The most common artificial sweeteners such as acesulfame potassium, saccharin, aspartame, sucralose, and steviol glycoside will be discussed in the present review.

Acesulfame Potassium

Acesulfame potassium (acesulfame-K) (6-Methyl-1,2,3-oxathiazin-4(3H)-one 2,2-dioxide), belonging into the oxathiazinodioxide class of chemicals, is a white crystalline powder and is ∼200 times sweeter compared to sucrose (58, 59). Due to the higher intensity and the longer persistence of the sweetness, acesulfame-K is used in a wide range of products, mainly soft drinks. Although this sweetener contains potassium, its intake does not influence systemic potassium levels (60). Acesulfame-K is not metabolized by the body (61). Following ingestion, acesulfame-K is completely absorbed into the systemic circulation and distributed (58, 62) (**Figure 1**). The absorption of acesulfame-K is very rapid, thereby making it unlikely that it will reach the lower gastrointestinal (GI) tract to impact the gut microbiota upon administration of a normal ADI-dosage (63). Within 24 h after ingestion, acesulfame-K is primarily excreted via the kidneys into the urine (>99%), with <1% excreted in feces (58, 62).

Saccharin

Saccharin (1,1-dioxo-1,2-benzothiazol-3-one) is available in three different forms: in acid form, or bound to sodium or calcium (64). The most common form is sodium salt due to its high solubility and stability. Saccharin is ∼300 times sweeter than sucrose (62, 64). Similarly to acesulfame-K, saccharin is not metabolized by the body (65). Therefore, the FDA considers saccharin as safe (20). After ingestion of saccharin, ∼85–95% is absorbed and bound to plasma proteins to be distributed via blood (58) (**Figure 1**). Thereupon, the saccharin is excreted in the urine, while the remaining 5–15% passes through the GI-tract entirely to be eliminated in the feces unchanged (58, 66). Therefore, a fraction of saccharin that is not immediately absorbed is able to affect the gut microbiota composition (58).

Aspartame

Aspartame ((3S)-3-amino-4-[[(2S)-1-methoxy-1-oxo-3-phenylpropan-2-yl]amino]-4-oxobutanoic acid) is approximately 200 times sweeter than sucrose (58). In contrast to other artificial sweeteners, aspartame contains 4 calories per gram. Nevertheless, due to the sweetening intensity, only a small amount of aspartame is used in products to achieve sweetness. Therefore, few calories are derived from aspartame in sweetener products. Upon ingestion, aspartame is broken down in the small intestine by esterases and peptidases to aspartic acid, phenylalanine, and methanol (16, 67) (**Figure 1**). Only the hydrolyzed components are absorbed into the circulation and metabolized following their normal metabolic pathways (68). Methanol is metabolized in the liver, while aspartate acid and phenylalanine enter the free amino acid pool. Thereupon, the components are taken up by peripheral tissues, utilized for protein synthesis and metabolism, and excreted. Aspartame does not accumulate in the body as it is rapidly digested (57). Neither aspartame nor its components reach the colon. Therefore, aspartame is not able to affect the gut microbiota (58, 69).

TABLE 1 | Characteristics of human studies investigating the effect of specific artificial sweeteners on body weight or adiposity.

References	Study type	Duration	Participants	Dosage artificial sweetener	Comparator	Adiposity measure	Statistical significance
Aspartame							
(34)	Meta-analysis	Acute−16 weeks	Obese, T2DM	162 mg, *ad libitum*, or 500 ml beverage	Sucrose or water	Body weight	N.S.
(34)	Meta-analysis	Acute	Obese, T2DM	162 mg or 500 ml beverage	Sucrose	Body weight	N.S.
Steviol glycoside							
(35)	Meta-analysis	90 days−2 years	Healthy, T1DM, T2DM	3.75–1,500 mg/day	Placebo (talcum, maize starch or unspecified)	BMI	N.S.
Saccharin							
(36)	RCT	12 weeks	Overweight, obese	1.25–1.75 L/daily	Sucrose	Body weight	N.S.
Sucralose							
(37)	RCT	7 days	Healthy	780 mg/day	Placebo (calcium carbonate)	Body weight	N.S.
(38)	RCT	14 days	Healthy	36 mg/day in commercial sachets	Control group	Body weight and BMI	N.S.

RCT, Randomized Controlled Trial; T2DM, Type 2 Diabetes Mellitus; T1DM, Type 1 Diabetes Mellitus; BMI, body mass index; N.S, non-significant.

Sucralose

Sucralose (2R,3R,4R,5R,6R)-2-[(2R,3S,4S,5S)-2,5-bis (chloromethyl)-3,4-dihydroxyoxolan-2-yl]oxy-5-chloro-6-(hydroxymethyl)oxane-3,4-diol) is very similar to sucrose in structure. However, the three hydroxyl groups attached to the sucrose molecule are replaced by chlorine atoms, thereby changing the confirmation of the molecule, to form sucralose (58). Thus, glycosidic enzymes are unable to recognize and digest sucralose. Although sucralose is made from sugar, it provides no calories as it is not digested in the body (16, 70). Sucralose is 600 times sweeter compared to sucrose. Most of the sucralose passes through the GI tract entirely to be directly eliminated in the feces, whereas a small amount (11–27%) is absorbed and is directed toward the kidneys to be excreted in the urine (71) (**Figure 1**). Nevertheless, sucralose was found to be non-nutritive to bacteria and resistant to fermentation, while affecting microbiota through bacteriostatic effects (72).

Steviol Glycoside

Steviol glycosides (13-Hydroxykaur-16-en-18-oic acid) are the chemical compounds responsible for the sweet taste and can be found on the leaves of the South American plant *Stevia rebaudiana* (73). Steviol glycosides are ∼100 to 300 times sweeter compared to sucrose (73). Steviol glycosides cannot be hydrolyzed by the digestive enzymes and acids present in the upper GI tract (58, 74). Nevertheless, the microbiota in the colon, primarily Bacteroides, is able to degrade steviol glycosides (75). Therefore, steviol glycosides are able to modulate the gut microbiota as they encounter it directly. Steviol glycoside is degraded by cleavage of the glycoside linkage, thereby forming steviol, steviolbioside, and glucose (76–78) (**Figure 1**). In turn, steviolbioside will be converted to steviol (78). The formed glucose is either utilized by colonic bacteria or absorbed, metabolized, and excreted into the expired air as carbon dioxide and water, while steviol is absorbed and enters the liver via the portal vein (79, 80). Nonetheless, the entry of steviol into the portal vein is slow due to the slow metabolization by the colonic bacteria, depending on the species (81). In the liver, steviol is glucoronidated and excreted into the urine (82, 83).

Body Weight and Adiposity

An increased body weight and adiposity develop under conditions of a positive energy balance. The regulation of energy balance is a complex process that involves homeostatic regulation of energy intake and energy expenditure. Although artificial sweeteners are as sweet or even sweeter than natural sugars, the caloric content and the metabolism routes are different. Therefore, it is likely that artificial sweeteners may affect energy balance, and thus body weight, differently compared to natural sugars via underlying physiological processes comprising the gut microbiota, the reward-system, and adipogenesis (**Figure 2**). Considering the increase in the prevalence of overweight and obesity and the rising interest in losing weight, preventing weight gain and maintaining weight loss, it is important to elucidate the effects of artificial sweeteners on body weight control. Meta-analysis, based on RCTs, showed that there is no significant difference in body weight change between overweight and lean individuals consuming artificial sweeteners compared to those receiving sugars or cellulose as placebo for <6 months (15). Furthermore, Azad et al. (29) reported no significant effects of artificial sweeteners on weight change compared to sugar or water in people living with obesity, based on meta-analysis of long-term RCTs (≥6 months). Interestingly, however, other meta-analysis of RCTs (4 weeks to 40 months) showed that the intake of artificial sweeteners resulted in reduced body weight in overweight and lean individuals compared to sugar or water (84). Notably, however, this meta-analysis included 4 out of 12 intervention studies carried out in the context of a weight loss

TABLE 2 | Characteristics of human studies investigating the effect of specific artificial sweeteners on glucose homeostasis.

References	Study type	Duration	Participants	Dosage artificial sweetener	Comparator	Measure of glucose homeostasis	Statistical significance
Aspartame							
(39)	RCT	Acute	Healthy	169 mg	Water	Glucose levels	N.S
(40)	RCT	Acute	Obese	500 ml beverage	Water	Glucose levels	N.S.
(43)	RCT	Acute	T2DM	400 mg in beverage	Unsweetened flavored beverage	Glucose levels	N.S.
(44)	RCT	Acute	Healthy, overweight	250 mg	Water	Glucose levels	N.S.
(45)	RCT	Acute	Healthy	400 mg	Placebo (corn flour)	Glucose levels	N.S.
(46)	RCT	Acute	Healthy, T2DM	72 mg	Water	Glucose levels	N.S.
(47)	RCT	2 weeks	Healthy	425 mg/day	-	Glucose levels, HbA1c	N.S.
(48)	RCT	2 weeks	Diabetic (not specified)	125 mg/day	-	Glucose levels	N.S.
(49)	RCT	6 weeks	T2DM	163 mg/day	Sucrose	Glucose levels, HbA1c	N.S.
(50)	RCT	18 weeks	T1DM, T2DM	270 mg/day	Placebo (corn starch)	Glucose levels, HbA1c	N.S.
Steviol glycoside							
(35)	Meta-analysis	3–6 months	Healthy, T1DM, T2DM	3.75–1,500 mg/day	Placebo (talcum, starch or unspecified)	HbA1c	N.S.
(35)	Meta-analysis	3–24 months	Healthy, T1DM, T2DM	3.75–1,500 mg/day	Placebo (talcum, starch or unspecified)	Glucose levels	N.S.
Saccharin							
(43)	RCT	Acute	Healthy, T1DM, T2DM	135 mg in beverage	Unsweetened flavored beverage	Glucose levels	N.S.
Acesulfame-K							
(39)	RCT	Acute	Healthy	220 mg	Water	Glucose levels	N.S.
Sucralose							
(39)	RCT	Acute	Healthy	62 mg	Water	Glucose levels	N.S.
(51)	RCT	Acute	Healthy	60 mg	Glucose	Glucose levels	N.S.
(52)	RCT	Acute	Healthy	50 ml beverage	Water	Glucose levels	N.S.
(53)	RCT	Acute	Healthy	80 mg infusion	Saline infusion	Glucose levels	N.S.
(42)	RCT	Acute	Healthy	960 mg infusion	Saline infusion	Glucose levels	N.S.
(46)	RCT	Acute	Healthy, T2DM	24 mg	Water	Glucose levels	N.S.
(54)	RCT	10 days	Healthy	60 mg in beverage	-	Insulin sensitivity	N.S.
(54)	RCT	10 days	Healthy	60 mg + maltodextrin	-	Insulin sensitivity	↓, $P < 0.043$
(47)	RCT	2 weeks	Healthy	0.136 mg/day	-	Insulin sensitivity	N.S.
(38)	RCT	2 weeks	Healthy	36 mg/day + maltodextrin/ dextrose	Control group	Insulin sensitivity	−17.7%, $P < 0.04$
(55)	RCT	13 weeks	T2DM	667 mg/day	Placebo (cellulose)	HbA1c	N.S.

Acesulfame-K, acesulfame potassium; RCT, Randomized Controlled Trial; T2DM, Type 2 Diabetes Mellitus; T1DM, Type 1 Diabetes Mellitus; HbA1c, glycated hemoglobin; N.S, non-significant.

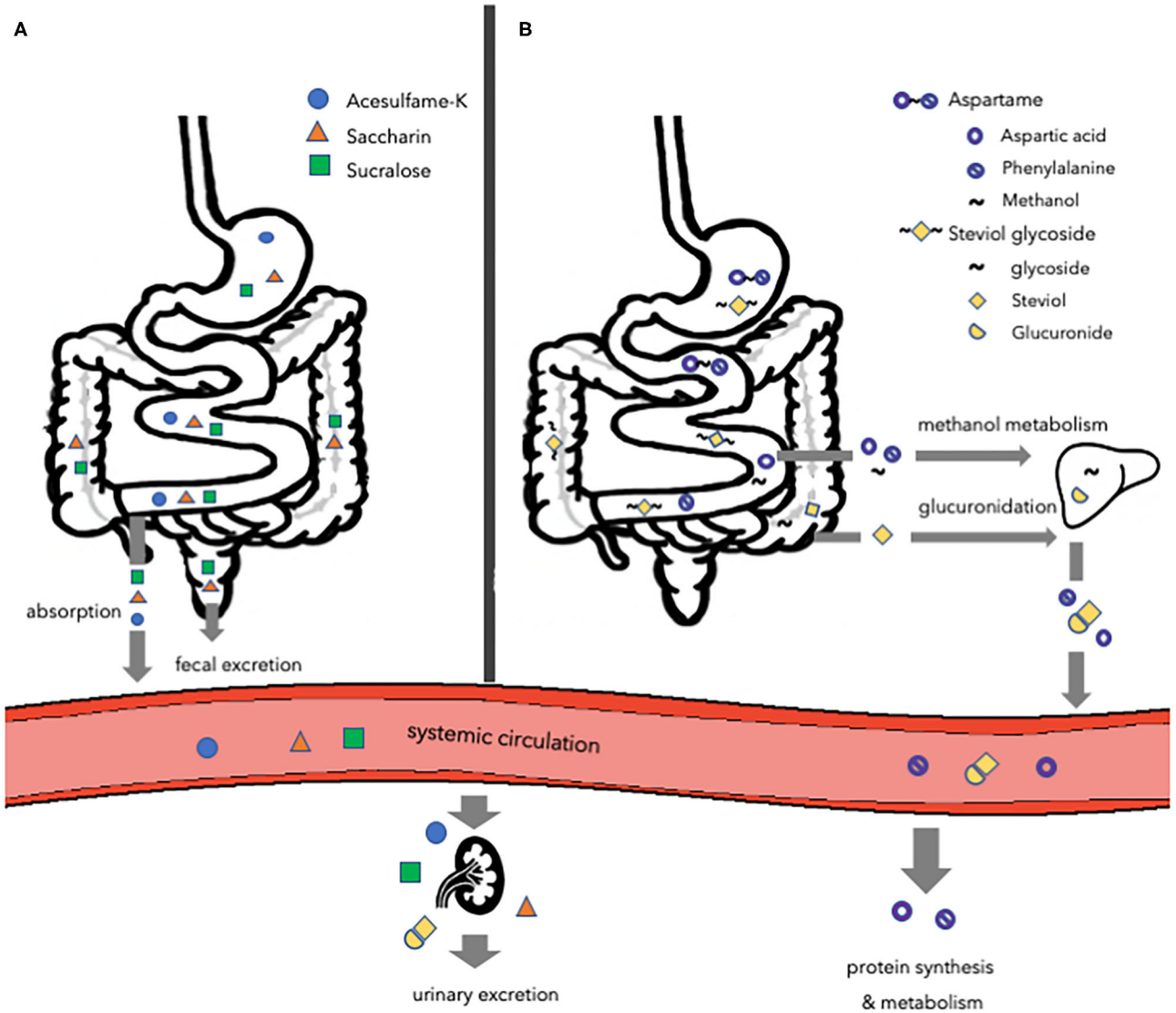

FIGURE 1 | Overview of the major routes of absorption, digestion, metabolism, and excretion of different types of artificial sweeteners. **(A)** Acesulfame-K, saccharin, and sucralose. Acesulfame-K is completely absorbed into the systemic circulation to be excreted in the urine via the kidneys. The majority of saccharin is absorbed and distributed, while the remaining amount passes the gastrointestinal tract to be eliminated in the feces. Most of the sucralose passes the gastrointestinal tract to be eliminated in the feces, while a small amount is directed toward the kidneys to be excreted in the urine. **(B)** Aspartame and steviol glycoside. Aspartame is digested in the small intestine and the hydrolyzed components are absorbed and metabolized following their normal metabolic pathways. Steviol glycoside is fermented by the gut microbiota to form steviol, which is absorbed into the liver and excreted in the urine. Acesulfame-K, acesulfame potassium.

program (84). Nevertheless, these findings strongly suggest that artificial sweeteners may have neutral or beneficial effects on long-term body weight control.

Considering specific types of artificial sweeteners, meta-analyses, based on RCTs, showed no effect of aspartame consumption on body weight compared to sugar or water in individuals with either obesity or T2DM (34) (**Table 1**). Only studies wherein aspartame was evaluated alone were included in the meta-analyses to clarify the specific effects of aspartame without interference of results obtained due to the consumption of other sweeteners. However, large heterogeneity was found due to different treatment patterns for aspartame and sugar or water. Similarly, meta-analysis, based on RCTs, showed no effect of steviol glycoside consumption on BMI compared to talcum, maize starch, or unspecified matching placebo in healthy individuals and patients with diabetes (35). Additionally, subgroup analyses showed no significant effect of steviol glycoside on BMI in either healthy individuals and patients with diabetes. The results indicate that these artificial sweeteners do not affect body weight. However, the effects of acesulfame-K and saccharin can still be debated, as there is no consistent evidence, and meta-analyses are lacking. More

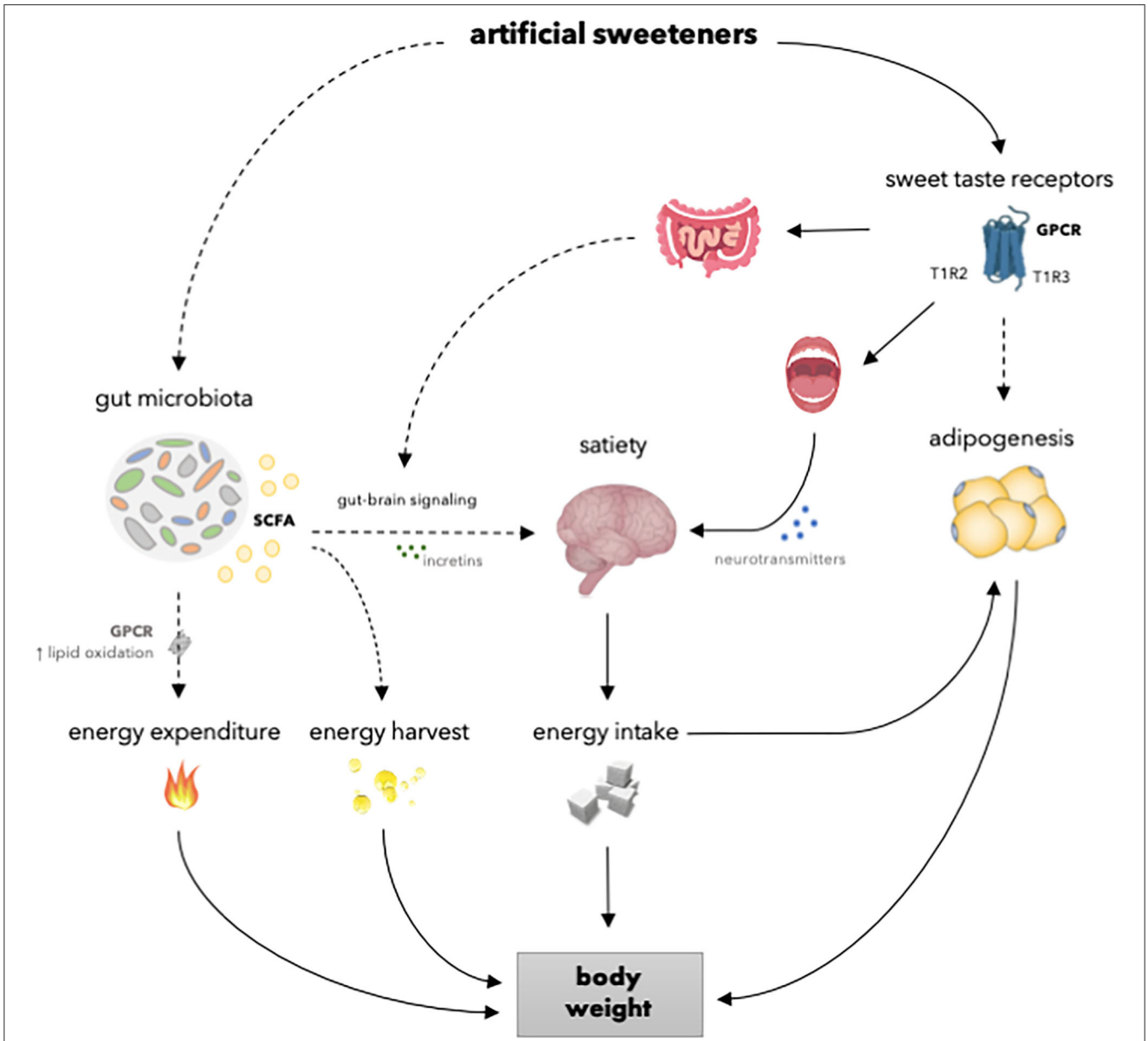

FIGURE 2 | Overview of the mechanisms of how artificial sweeteners may affect physiological processes involved in body weight regulation. Artificial sweeteners interact with T1R-family of sweet-taste receptors in the oral cavity and gastrointestinal tract, thereby able to affect satiety and, in turn, energy intake and body weight. However, *in vivo* studies have shown no effect of artificial sweeteners on the secretion of incretins. Furthermore, several artificial sweeteners may reach the adipose tissue to interact with T1R-family of sweet-taste receptors and affect adipogenesis and, in turn, body weight. Moreover, several artificial sweeteners are able to induce gut microbiota alterations. Thereupon, SCFA production is enhanced. It can be speculated that SCFA may, in turn, increase energy expenditure due to enhanced lipid oxidation and affect satiety by modulating gut-brain signaling via incretins. Dashed lines indicate that the effect is dependent on type of artificial sweetener and/or that results are inconsistent or hypothetical. SCFA, short chain fatty acids; GPCR; G-protein coupled receptor; T1R2, taste receptor type 1 member 2; T1R3, taste receptor type 1 member 3.

specifically, one study that used the ADI-dosage for human consumption (15 mg/kg/day) showed no effect on body weight in mice after 8 weeks of acesulfame-K consumption, while another study shows an increase in body weight by exceeding the ADI more than 2-fold (37.5 mg/kg/day) after 4 weeks in mice (85, 86). Furthermore, saccharin consumption was found to increase body weight in mice compared to water, sucrose or glucose, whereas other studies in rodents have shown reduced or unchanged body weight compared to mice receiving water, glucose, fructose or sucrose (87–94). However, the absorption of saccharin is lower in rodents compared to humans due to a relative higher stomach pH in rodents (92). Furthermore,

differences in perception of sweetness for individual artificial sweeteners exist between different rodent species and strains (95). Therefore, perception and post-ingestive responses of rodents might differ from humans. Nevertheless, human data on the effect of acesulfame-K on body weight is currently lacking. Moreover, human data on the effect of saccharin on body weight is scarce with only one study showing no significant effects on body weight after 12 weeks of saccharin consumption compared to sucrose in overweight and obese individuals (36). Moreover, sucralose consumption has been reported to have no effect on body weight in mice compared to water, and in human studies compared to placebo (calcium carbonate) or control (no-intervention) (37, 38, 85, 88, 96). Notably, contradictory results from rodent studies for the effect on body weight exist only for acesulfame-K and saccharin, which are largely or entirely absorbed in their intact form, thereby being able to reach the peripheral tissues. Consistently, rodent and human studies found no effect of sucralose on body weight as only a small amount is absorbed in its intact form, thereby reaching the microbiota in a larger amount compared to acesulfame-K and saccharin (37, 38, 85, 88, 96). As artificial sweeteners have different metabolic fates, differences in physiological effects affecting energy balance and adiposity should be elucidated.

THE INTERACTION BETWEEN ARTIFICIAL SWEETENERS, REWARD, AND ADIPOSITY

Reward

As artificial sweeteners contain no or low amounts of calories, one might expect that these sweeteners may contribute to lower energy intake and thus body weight reduction. Nevertheless, controversies exist whether artificial sweeteners affect appetite, hunger, and eating behavior, and if these effects are beneficial or not. One driving aspect in eating behavior is the reward of food. The reward system plays an important role in regulating energy intake, and can be divided into sensory and post-ingestive reward (19, 97). After ingestion of either natural sugars or artificial sweeteners, gustatory information is perceived by sweet taste receptors, which are heterotrimeric G-protein coupled receptors (GPR) consisting of two subunits, namely taste receptor type 1 member 2 (T1R2) and 3 (T1R3) (98, 99). The sweet taste receptors are located in taste buds in the oral cavity and outside the oral cavity, including the intestine and pancreatic β-cells (100). The binding sites of sweet taste receptors are different for artificial sweeteners and natural sugars (101). Upon interaction of sweet compounds to the sweet receptor T1R2/T1R3, the heterotrimeric G protein, α-gustducin, is activated (102). As a result, the subunits Gβγ are dissociated and can interact with phospholipase Cβ2 (PLC-β2), which in turn increases production of inositol 1,4,5-triphosphate and diacylglycerol (103). Consequently, the transient receptor potential cation channel subfamily M member 5 is activated, thereby increasing intracellular calcium and neurotransmitter release (104–106). As artificial sweeteners and natural sugars bind differently to the sweet taste receptors, the gustatory branch is activated differently as well (19, 101). Thereupon, artificial sweeteners may generate weaker signals that are sent to areas involved in reward and satisfaction, as consistently demonstrated by using functional Magnetic Resonance Imaging (fMRI) in several randomized cross-over trials (107, 108).

Likewise, the ingestion of artificial sweeteners induces a signaling cascade outside of the oral cavity. Within the GI tract, sweet taste receptors are primarily located on enteroendocrine L- and K-cells (104). The signal transduction pathway is similar as in cells present in the oral cavity. Upon ligand binding of natural sugars to sweet taste receptors, enteroendocrine L-cells secrete glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), whereas K-cells secrete glucose-dependent insulinotropic peptide (GIP) (100). These hormones are able to cross the semi-permeable blood-brain barrier, thereby reaching the hypothalamus and affecting food intake by reducing appetite and increasing satiety (41). However, artificial sweeteners may not be potent secretagogues for GLP-1, PYY, and GIP to the same extent *in vivo* as natural sugars, since the secretion is nutrient-dependent (39, 109, 110). For instance, aspartame is digested and absorbed before reaching the lower GI tract to bind to the sweet taste receptors. Acesulfame-K, sucralose, steviol glycoside, and saccharin pass through the lower GI tract to be absorbed, digested or eliminated directly. Consistently, mice studies and human crossover trials in lean and obese individuals have shown no significant effects of artificial sweeteners on incretin secretion (39, 40, 42, 51–53, 111, 112). In addition to the lack of an effect on incretin secretion, two human crossover studies showed no effect on appetite upon sucralose or aspartame-sweetened diet coke consumption in healthy and obese individuals (40, 52). Furthermore, randomized cross-over trials showed weaker reward and satisfaction signals upon aspartame or sucralose ingestion in healthy individuals, thereby suggesting that caloric intake is required in evoking a hypothalamic response (107, 108). Therefore, it has been suggested that artificial sweeteners do not activate the food reward pathways in the same way as natural sugars. The elimination of the post-ingestive reward holds true for non-caloric artificial sweeteners, whereas the intake of artificial sweeteners in the presence of carbohydrates may elicit post-ingestive incretin responses, as demonstrated using sucralose-sweetened beverages (54). Based on the above, it can be postulated that artificial sweeteners solely offer less reward compared to natural sugars, although it should be emphasized that the differences in reward response has not been shown in the context of a whole-meal approach or diets, where sugar was replaced by artificial sweeteners.

Energy Intake

The lack in complete satisfaction may drive the assumption that artificial sweeteners fuel food seeking behavior, thereby contributing to increased or no differences in energy intake. However, less satisfaction does not necessarily translate into compensatory (excess) energy intake (113–116). RCTs have shown that the reduced caloric intake by replacing natural sugars with artificial sweeteners is not completely compensated (117, 118). As a result, energy intake after the use of artificial sweeteners is still lower compared to natural sugars, even after putative compensatory energy intake. Therefore, the

compensatory energy intake does not seem to pose a threat to weight gain and may aid in weight loss (maintenance). Furthermore, meta-analysis of acute RCTs ($\leq$1 day) showed that artificial sweeteners decrease energy intake in comparison to caloric sweeteners in overweight and lean individuals, whereas no difference was found in comparison with water (84). In a meta-analysis of long-term RCTs (4 weeks to 40 months), artificial sweeteners were found to decrease energy intake compared to caloric sweeteners or water (84). Similarly, a meta-analysis including RCTs with a study duration of 4–10 weeks showed reduced energy and sugar intake in lean and overweight individuals consuming artificial sweeteners compared to those receiving sugar (15). Taken together, these findings suggest that compensatory energy intake during consumption of artificial sweeteners does not seem to occur in the short- and long-term, or at least does not completely compensate for the reduced caloric intake compared to sugar intake.

Adipogenesis

Sweet taste receptors are expressed in many organs, including adipose tissue (119). Not all artificial sweeteners will reach the adipose tissue as some are not absorbed into the systemic circulation. The sweet taste-sensing receptor in adipose tissue differs in comparison to the receptors in sweet taste buds or in the GI tract. In adipocytes, the expression of T1R3 was found to be higher than T1R2, suggesting that a higher percentage of T1R3 is present as a homomer (120). Nevertheless, increased adipogenesis and reduced lipolysis were found, independent of T1R2 and T1R3, upon *in vitro* stimulation of adipocytes with saccharin (119). It has been suggested that saccharin act on a protein kinase A-mediated mechanism downstream of cyclic adenosine monophosphate (cAMP). Consequently, hormone sensitive lipase (HSL) phosphorylation is reduced by regulating HSL phosphatase, thereby inhibiting lipolysis (119). Likewise, acesulfame-K was found to stimulate adipogenesis (119). However, the active concentrations of saccharin and acesulfame- K in adipocytes (4.5 mM) were higher than expected to be observed in humans as bolus oral doses of maximum daily intake of saccharin, for instance, results in peak plasma concentrations of $\sim$75 μm (119). Similarly, other *in vitro* studies in human mesenchymal stem cells showed increased fat accumulation and upregulation of genes involved in adipogenesis upon stimulation with a higher sucralose concentration (0.45 or 4.5 mM) (121). Notably, as discussed earlier, contradictory results regarding body weight were found for acesulfame-K and saccharin. Thus, since these artificial sweeteners are largely or entirely absorbed, it could be argued that they reach the adipose tissue and may impact adipogenesis. Nevertheless, Masubuchi et al. (120) showed reduced adipogenesis in *3T3-L1* cells upon stimulation with saccharin or sucralose (20 mM) by activation of adenylate cyclase-cAMP signaling pathway. Along with cAMP-dependent pro-adipogenic signals, cAMP-independent anti-adipogenic signals are generated, which may dominate the formal signal to inhibit adipogenesis (120). Hence, studies investigating the role of artificial sweeteners and peripheral sweet taste receptors are scarce, and existing *in vitro* studies examining the effects of artificial sweeteners on adipogenesis provide inconsistent results (119–121).

THE INTERACTION BETWEEN ARTIFICIAL SWEETENERS, GUT MICROBIOTA, AND ENERGY BALANCE

Alterations in Gut Microbiota

Gut microbiota and the produced microbial fermentation products are key to many aspects of human health (122). Besides the involvement of fermenting indigestible food components, gut microbiota seems closely linked to metabolism, energy balance, and the immune system (123). An important modifying factor influencing the composition of the microbiota, and thereby the overall health, is diet (124). Artificial sweeteners may alter the gut microbiota composition, evidenced by increased gut microbiota dysbiosis and an increased Firmicutes:Bacteroidetes ratio in a cross-sectional study with morbidly obese individuals (125). Moreover, another cross-sectional study showed no association between aspartame or acesulfame-K consumption and bacteria abundance profiles or predicted gene function (126). However, bacterial diversity differed between aspartame or acesulfame-K consumers and non-consumers (126). Furthermore, Suez et al. (89) demonstrated that artificial sweeteners are able to induce glucose intolerance in mice and distinct human subsets by altering the gut microbiome. Supplementation of saccharin (5 mg/kg/d) for 1 week induced an elevated glycemic response after an oral glucose load, which was associated with microbiome alterations in a small group of study participants clustered as "responders" ($n = 4$), while no response was found in the other individuals ("non-responders", $n = 3$) (89). The poor glycemic response in the "responders" was replicated in mice upon fecal transplantation. Similarly to the above mentioned cross-sectional study (126), the microbiome composition between the "responders" and "non-responders" were already distinct prior to saccharin exposure, thereby suggesting that humans feature an unique response to artificial sweeteners and that the gut microbiome may serve as a predictor for the susceptibility (89). Nevertheless, in the latter study there was no placebo group in the short-term intervention study and the number of individuals was small, indicating that replication of these findings is required. Overall, human trials investigating the effect of artificial sweeteners on gut microbiota are scarce.

Regarding rodent studies, an increased Firmicutes:Bacteroidetes ratio, resembling that of obese individuals, was found in mice after 11 weeks of saccharin consumption (89). Consistently, modulation of the gut microbiota was found in other rodent studies upon saccharin consumption, as a minor fraction of saccharin is not absorbed and will concentrate in the colon (96, 127). Besides saccharin, sucralose was consistently found to affect microbiota in mice as it accumulates in the colon (85, 88, 96). However, contradictory results regarding the effect of acesulfame-K on gut microbiota composition have also been found in rodents (85, 86). This discrepancy is at least partly explained by the difference of administered dosage. More specifically, one study that used

the ADI-dosage for human consumption (15 mg/kg/day) showed no effect on microbiota composition in mice after 8 weeks consumption, while another study that applied a dosage that exceeds the ADI more than 2-fold (37.5 mg/kg/day), showed an increase in Bacteroides and Firmicutes after 4 weeks consumption in mice (69, 85, 86). Since the absorption of acesulfame-K is very rapid, it is unlikely that it will reach the lower GI tract upon administration of a normal ADI-dosage (63). Regarding other artificial sweeteners, aspartame does not affect the gut microbiota, since it is digested and broken down into residual components before entering the lower GI tract (58). Whereas, steviol glycoside encounters the microbiota directly in order to be fermented. Controversial results exist between *in vivo* and *in vitro* studies using human feces as well as *E.coli* cell lines. *In vitro* fermentation studies using human feces showed no effect of steviol glycoside on microbiota composition (75, 128). Other *in vitro* studies using *E.coli* cell lines showed selective growth inhibition upon steviol glycoside stimulation, or little or no effect on bacterial growth (96, 129). Nevertheless, the consumption of steviol glycoside (2–3 mg/kg) was found to alter gut microbiota composition in mice after 9 weeks (130).

As gut microbiota is closely linked to many aspects of health, changes in microbiota composition may lead to negative alterations in metabolic homeostasis. Suez et al. (89) showed an increase in the glycan degradation pathway, along with an increased Firmicutes:Bacteroidetes ratio, in mice after 11 weeks of saccharin consumption. As a result, glycans are fermented to form short chain fatty acids (SCFA), including acetate and propionate (89, 131). In addition, sucralose was found to increase cecal propionate levels in mice after 8 weeks of consumption (132). In contrast, acesulfame-K consumption did not affect SCFA levels in mice following 8 weeks of consumption upon normal ADI-dosage (85). Furthermore, steviol glycoside was found to increase SCFA after 9 weeks of steviol glycoside consumption in rodents and in studies using an *in vitro* model of the human colon (GIS1) (130, 133). The increase in SCFA levels may be an indicator of enhanced energy harvest, as the capacity to extract energy has been suggested to be increased as result of artificial sweetener consumption. Butyrate, particularly, serves as an energy supply for ∼60–70% for colonocytes and gut epithelial cells (134, 135). Whereas, acetate mainly contributes to lipogenesis in the cytosol of hepatocytes and adipocytes or can be oxidized in skeletal muscle (136, 137). In addition, propionate serves as a precursor for gluconeogenesis, lipogenesis, and protein synthesis (89, 138, 139). However, the significance of energy harvest in humans is still unclear, and increased SCFA concentrations have merely been associated with beneficial health effects in humans (140).

Gut-Brain Signaling

In the small intestine, propionate is able to bind to GPR43 and GPR41, free fatty acid receptors (FFAR) 2 and 3, respectively, in the enteroendocrine L-cells (141). Upon binding to the receptors, the secretion of GLP-1 and PYY is stimulated (142). Mice lacking FFAR2 or FFAR3 were found to have reduced SCFA-triggered GLP-1 secretion *in vitro* and *in vivo* (143). Furthermore, we have recently performed a double-blind, crossover study, showing increased PYY concentration upon acute colonic administration of mixtures of acetate, propionate, and butyrate in overweight or obese men (144). Therefore, it is tempting to speculate that artificial sweeteners, that are able to modulate gut microbiota, are able to affect gut-brain signaling, via increased SCFA production. Besides gut-brain signaling, SCFA are found to affect appetite regulation and leptin secretion, as described more extensively elsewhere (140). Nevertheless, human studies investigating the effect of artificial sweeteners on hunger-satiety cycle, via SCFA, are currently lacking.

Energy Expenditure

Besides affecting the hunger-satiety cycle, SCFA may modulate body weight control by influencing energy expenditure. Our recently performed double-blind, crossover study, showed increased lipid oxidation, and thus energy expenditure, upon acute colonic infusions of SCFA in overweight or obese men (144). Consistently, mice studies have shown increased lipid oxidation by increasing sympathetic activity in brown adipose tissue, via gut-neural signaling, upon SCFA administration (145–147). However, the relevance of brown adipose tissue in body weight regulation in humans seems less evident, as it may only contribute to a very minor extent to energy expenditure (148). Acetate and butyrate were found to enhance lipid oxidation in mice studies and *in vitro* studies using bovine hepatocytes, possibly mediated via GPR41 and GPR43 (140, 141, 149–152). Nevertheless, *in vivo* studies found no effect on energy expenditure in mice after 40 weeks of acesulfame-K exposure or 5 weeks of saccharin exposure (89, 153). Similar to findings in liver, SCFA were found to enhance lipid oxidation in skeletal muscle as shown in rodents and C2C12 myotubes (154–156). However, human data regarding the effects of SCFA on tissue metabolism are currently lacking. Moreover, human evidence of the effects of artificial sweeteners on microbiota alterations, and subsequently SCFA production, are very limited. Thus, although it is tempting to speculate that artificial sweeteners may affect energy expenditure through altered SCFA production in the gut, further studies are needed to investigate this.

Importantly, the putative beneficial effects of the intake of artificial sweeteners, by SCFA production, are mainly based on studies in rodents. Furthermore, no difference in energy expenditure, using ventilated-hood and 24 h whole body indirect calorimetry, was found upon sucralose consumption in acute studies and long-term (10 weeks) RCTs, whereas lipid oxidation was enhanced and carbohydrate oxidation was decreased compared to sucrose in normal weight and overweight individuals (157, 158). Moreover, no changes in energy expenditure, estimated based on accelerometry, were observed upon saccharin-, aspartame-, sucralose-, or steviol glycoside-sweetened beverage consumption for 12 weeks compared to sucrose in overweight or obese individuals (36). These findings may imply that a reduction in energy intake rather than an increase in energy expenditure may contribute to the beneficial effects of sucralose on body weight control.

GLUCOSE HOMEOSTASIS

Besides potentially affecting body weight control, artificial sweeteners may also affect glycemic control, since glucose absorption may be reduced upon replacement of available carbohydrates. However, this does not necessarily translate into an improved glucose homeostasis, since alterations in intestinal glucose transport and absorption, insulin resistance, and reduced insulin secretory capacity by artificial sweeteners may contribute to impaired glucose homeostasis (**Figure 3**). However, the results of systemic reviews and meta-analysis that have been performed to investigate the relationship between artificial sweetener intake and glucose homeostasis or risk of T2DM are controversial. Daher et al. (159) reported that the majority of systemic reviews and meta-analysis, based on RCTs or prospective cohort studies in healthy individuals yielded no conclusive evidence that artificial sweeteners increase the risk for T2DM. Other intervention studies in healthy individuals and patients with diabetes showed no significant effect of artificial sweeteners on glucose homeostasis (glucose and insulin levels) (159). On the other hand, systematic reviews and meta-analysis, based on prospective cohort studies in healthy individuals, showed a positive association between artificial sweetener intake and the incidence of T2DM, independent of adiposity (although attenuated after adjustment for BMI) (159). However, the evidence for a relationship between artificial sweeteners and T2DM is based on prospective cohort studies using only baseline exposure and may be caused by reverse causation. Hence, evidence from systematic and meta-analysis does not consistently show that artificial sweeteners reduce the risk of T2DM in humans.

Considering specific types of artificial sweeteners, glucose homeostasis seems to be unaffected by aspartame and steviol glycoside. No significant effect on glucose levels and glycated hemoglobin (HbA1c) levels were found after acute or long-term aspartame consumption (39, 40, 43–50) (**Table 2**). Similarly, a meta-analysis of long-term RCTs showed no effect of steviol glycoside on glucose levels and HbA1c levels in healthy individuals and patients with diabetes (35). Regarding other artificial sweeteners, glucose levels were not found to be affected by acute saccharin consumption in healthy individuals and patients with diabetes, and acute acesulfame-K consumption in healthy individuals (39, 43). In addition, mice studies found no effect on glucose tolerance upon acesulfame-K consumption (153). Nevertheless, data from rodent studies on saccharin consumption remain controversial, as one study showed an increase in glucose tolerance after 11 weeks of commercial saccharin added to drinking water, whereas another study found no effect after 7 weeks of pure saccharin added to drinking water (89, 160). However, the discrepancies may be explained by differences in caloric content of the drinking water, as the study showing increased glucose tolerance used a commercial sweetener (Sucrazit), consisting out of 95% glucose and 5% saccharin, whereas the other study showing no effect used pure saccharin (89, 160). More specifically, one study that used the ADI-dosage for human consumption (15 mg/kg/day) showed no effect on body weight in mice after 8 weeks of acesulfame-K consumption, while another study shows the opposite by exceeding the ADI more than 2-fold (37.5 mg/kg/day) after 4 weeks in mice (85, 86). Furthermore, glucose and HbA1c levels were not affected by acute or long-term sucralose consumption in healthy individuals and patients with diabetes (39, 42, 46, 51–53, 55). Remarkedly, short-term sucralose consumption alone showed no effect on insulin sensitivity in healthy individuals, whereas sucralose-sweetened beverages, containing carbohydrates, or sucralose sachets added to carbohydrate-containing beverages or meals, decreased insulin sensitivity in healthy individuals (38, 47, 54). Therefore, it has been suggested that sucralose may impair glucose metabolism only when co-ingested with carbohydrates. The role of artificial sweeteners in enhancing intestinal glucose absorption, thereby perturbating glucose homeostasis in the presence of carbohydrate content, can be speculated (as discussed below). The discrepancies of the effects of artificial sweeteners on glucose homeostasis may be explained by the difference in types of artificial sweeteners and the intake of artificial sweeteners solely or in combination with carbohydrates. Nevertheless, more human studies are needed to confirm these findings, and assess whether these putative effects on glucose homeostasis can be translated to a situation where artificial sweeteners are consumed as part of the diet with other dietary components.

Intestinal Glucose Absorption

The GI tract plays a major role in the regulation of glucose homeostasis. As artificial sweeteners may impact gut microbiota and function, they are able to alter intestinal glucose absorption and thus postprandial glucose levels. Upon ingestion of carbohydrates, glucose is largely absorbed across the enterocytes of the intestinal wall via sodium-glucose cotransporter-1 (SGLT1) on the apical membrane and the passive glucose transporter 2 (GLUT2) on the basolateral membrane (106). The sweet taste receptors located in the GI tract serve as glucose sensors to adapt dietary glucose concentrations (161). Upon binding of glucose to the sweet taste receptors, the secretion of GLP-1, GLP-2, and GIP is enhanced, which in turn increases the expression of GLUT2 (162, 163). However, artificial sweeteners alone seem not able to elicit the same effects as natural sugars *in vivo* due to lack of caloric content, as discussed earlier. Nevertheless, SGLT1 was found to be upregulated by sucralose, acesulfame-K, and saccharin in wild-type mice, but not in mice lacking T1R3 or α-gustducin (161). This was not found for aspartame, as mice do not sense it as sweet (161). In addition, sucralose, acesulfame-K, and saccharin were found to increase GLUT2 insertion into the apical membrane, thereby increasing the rate of intestinal glucose absorption in mice (164). Nevertheless, a cross-over study of intraduodenal infusion of sucralose (960 mg) in healthy individuals showed no difference in intestinal glucose absorption compared to saline infusion in combination with glucose (53). Additionally, intraduodenal infusion of sucralose (80 and 800 mg) was not found to stimulate GIP release compared to saline infusion in combination with glucose in healthy individuals (42). Notably, however, the measurement of intestinal glucose absorption in the latter study is less sensitive compared to the methodology applied in the rodent studies, as intestinal glucose absorption rate is indirectly measured by

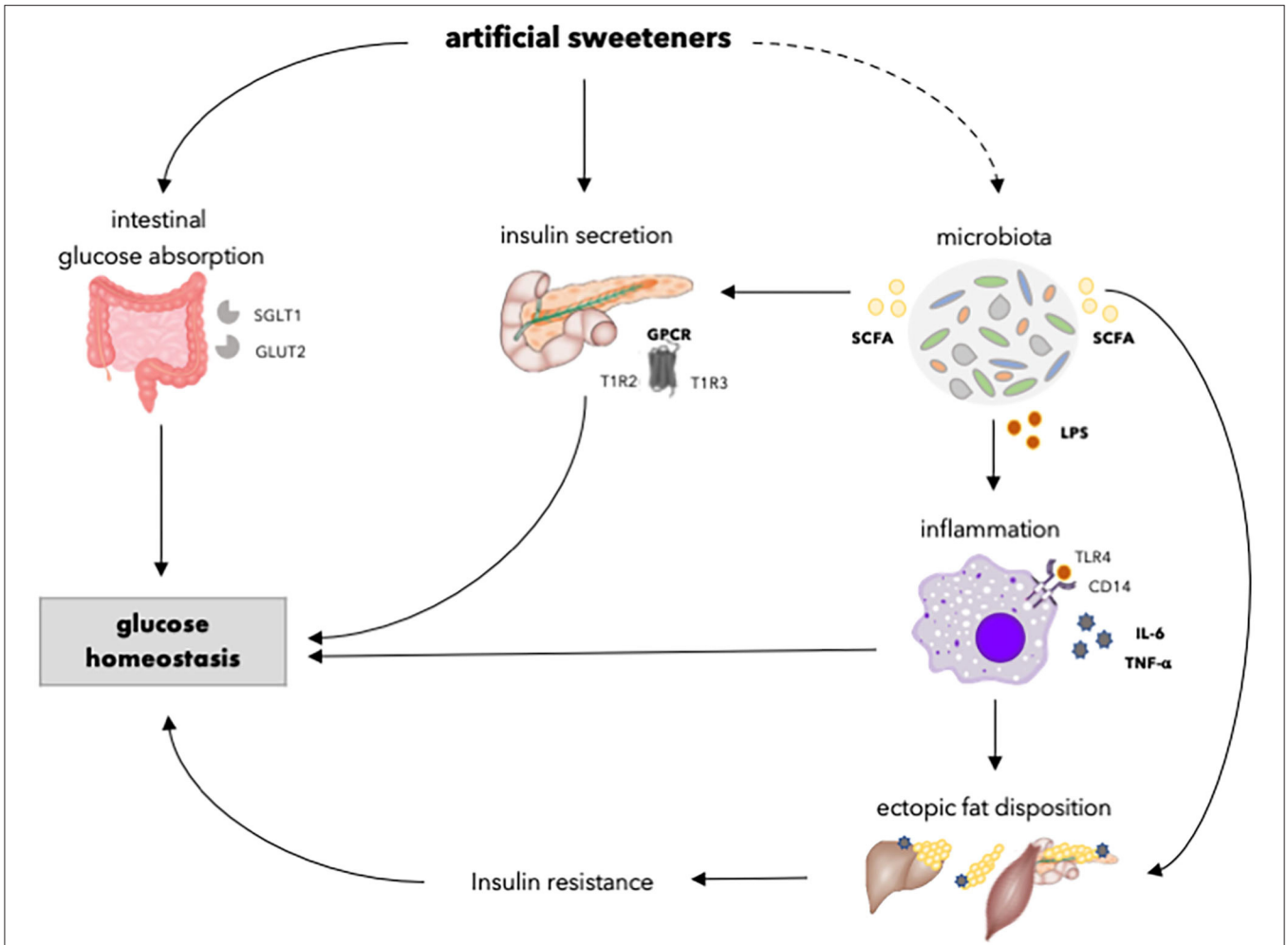

FIGURE 3 | Overview of the effects of artificial sweeteners on physiological processes involved in glucose homeostasis. Artificial sweeteners may enhance intestinal glucose absorption by upregulating SGLT1 and GLUT2. Furthermore, artificial sweeteners affect insulin secretory capacity by interacting with GPCR. Moreover, the artificial sweetener-induced gut microbiota dysbiosis, in turn, may affect insulin secretion via the enhancement of SCFA. Upon dysbiosis, LPS levels may increase, and endotoxemia and chronic inflammation occurs, which might affect ectopic fat accumulation and insulin resistance. Dashed lines indicate that the effect is dependent on type of artificial sweetener. SGLT1, sodium glucose transporter 1; GLUT2, glucose transporter 2; GPCR, G-protein coupled receptor; T1R2, taste receptor type 1 member 2; T1R3, taste receptor type 1 member 3; SCFA, short chain fatty acids; LPS, lipopolysaccharide; TLR4, toll-like receptor 4; CD14, cluster of differentiation antigen 14.

adding a non-metabolizable glucose analog to the intestinal perfusate (106). To date, no significant effects of artificial sweeteners on intestinal glucose absorption have been reported in humans.

Insulin Secretion

The intake of nutrients is associated with a large set of sensory cues that enables the human body to prepare for metabolic digestion and utilization. Exposure to sweet-tasting sugars, even before ingestion, triggers physiological responses related to the release of insulin or incretin in order to reduce blood glucose levels. However, artificial sweeteners are not able to prepare the GI tract for digestion and utilization of nutrients as well as sugars (107, 165). Smeets and colleagues (107) have shown in a randomized crossover study in healthy individuals that there was no cephalic insulin response upon tasting of aspartame, while an early rise in insulin concentration was found when tasting glucose. Likewise, no cephalic response upon sucralose has been reported in a randomized crossover study in healthy individuals (52). Furthermore, while natural sugars are able to stimulate the secretion of incretins, thereby stimulating β-cells to secrete insulin, artificial sweeteners do not directly induce incretin secretion as this appears nutrient-dependent (39, 109, 110, 166). Moreover, insulin secretion is stimulated upon the interaction of both natural sugars and artificial sweeteners with sweet-taste receptors in pancreatic β-cells by initiating a signal transduction pathway via Ca^{2+} and cAMP-dependent mechanism (167). Taken together, this may suggest that artificial sweeteners stimulate insulin secretion less compared to natural sugars.

In agreement with this, the majority of acute and short-term (7–12 days) RCTs showed no significant effect of sucralose consumption or intravenous infusion on circulating insulin levels compared to water, glucose, sucrose, placebo (calcium carbonate), or saline infusion as control in healthy individuals (36, 37, 42, 51, 52, 168). Only three studies reported opposite findings, of which two studies found increased insulin levels after acute (48 mg) or long-term (4 weeks, 200 mg/day) sucralose consumption compared to water or placebo (unspecified) in obese or healthy individuals (169–171). The reasons for these discrepant findings are not clear but may be related to differences in study population or duration of the intervention. Moreover, Sylvetsky et al. (171) showed increased insulin levels after acute intake of a diet-beverage including sucralose, acesulfame-K, and aspartame compared to carbonated water (seltzer) in healthy individuals. Nevertheless, no differences in insulin levels were found upon water with sucralose consumption compared to water consumption alone, thereby indicating that the taste associated with diet soda or other ingredients may affect the insulin secretion. Furthermore, acute and longer-term (12–16 weeks) studies showed no effect of saccharin, acesulfame-K, steviol glycoside, and aspartame consumption on insulin levels in healthy, diabetic, overweight, or obese individuals (36, 39, 40, 43–45, 48, 172–174). Taken together, the available human data suggests that artificial sweeteners do not significantly affect insulin levels.

Insulin Resistance

Insulin resistance is a major factor in the pathophysiology of T2DM, of which the pathogenesis involves the accumulation of ectopic fat and the activation of innate immune pathways, thereby interfering with insulin signaling and action (175). The artificial sweetener-induced gut microbiota dysbiosis has been linked to metabolic endotoxemia and the development of an inflammatory state, at least in rodents (89, 127, 176). Suez et al. (89) showed an altered host metabolism by downstream effects of microbiota in mice upon saccharin intake. The authors found enriched microbial pathways, associated with metabolic syndrome, in mice, including lipopolysaccharide (LPS) synthesis, which is a breakdown product of the outer membrane of Gram-negative bacteria (89, 177). Microbiota dysbiosis is considered to be related to the loss of gut mucosal integrity as the expression of tight junction proteins is reduced, among other mechanisms (176, 178). Therefore, LPS may translocate from the gut into the portal or systemic circulation, thereby able to stimulate the activation of pro-inflammatory macrophages and the secretion of pro-inflammatory cytokines (127, 177, 179–181). Other studies showed disrupted intestinal epithelial barrier *in vitro* using Caco-2 cells upon saccharin stimulation, whereas aspartame, acesulfame-K, and sucralose did not alter intestinal permeability (176). Similarly to the study of Suez et al. (89), other rodent studies showed increased LPS concentration, and subsequently enhanced inflammation, in mice upon saccharin consumption by interfering with the gut microbiota (127, 176). Regarding other artificial sweeteners, the intake of acesulfame-K (exceeding the ADI-dosage for humans by more than twice) or sucralose was found to enhance inflammation in mice, whereas steviol glycoside was found to reduce inflammation by attenuating LPS-induced pro-inflammatory cytokine production in Caco-2 cells and by regulating TLR2 and cytokine expression in *S. aureus*-infected mouse mammary gland (86, 182–184). This indicates that steviol glycoside possess anti-inflammatory properties, whereas saccharin, acesulfame-K, and sucralose may increase inflammation in rodent studies and *in vitro*. The resulting endotoxins and inflammatory cytokines are able to infiltrate peripheral tissues and release TNFα, IL-1β, and IL-6, which may interfere with insulin signaling and insulin-stimulated glucose uptake (185–187). Furthermore, inflammatory molecules may inhibit adipogenesis by constraining the hyperplastic expandability of adipose tissue (188). As a result, adipocyte turnover and adipose tissue expansion is reduced, leading to lipid overflow and fat accumulation in non-adipose tissues. This ectopic fat, as well as the accumulation of bioactive lipid metabolites, may disturb cellular function, ultimately contributing to insulin resistance and a reduced β-cell function, as described more extensively elsewhere (189).

Besides an enrichment of LPS synthesis, Suez et al. (89) showed an increase in SCFA production, through alterations in gut microbiota composition, in mice upon saccharin consumption. The authors suggested that the enhanced SCFA may serve as an energy source for the host or signaling molecules or substrates for gluconeogenesis, *de novo* lipogenesis and cholesterol synthesis (89). Counterintuitively, SCFA have most often been associated with positive health effects (140). SCFA were found to counteract LPS-induced inflammation by reducing pro-inflammatory cytokines and enhancing anti-inflammatory cytokines in murine macrophages (190). Furthermore, *in vitro* studies have found an attenuation of lipolysis upon SCFA stimulation in *3T3-L1* adipocytes, thereby reducing plasma free fatty acids (191–194). Likewise, rodent studies have demonstrated that SCFA may reduce intracellular lipid accumulation, thereby alleviating oxidative stress (195–197). In addition, as mentioned before, SCFA may affect energy metabolism, for instance via the enhancement of lipid oxidation in human studies (143). Repeatedly, artificial sweeteners have been found to increase lipid oxidation compared to sucrose in acute and long-term (10 weeks) RCTs in normal and/or overweight individuals (157, 158). Chern et al. (158) suggested that the difference in metabolism between sucralose and sucrose is attributed to the distinct carbohydrate content and the fact that sucrose is able to initiate carbohydrate-specific physiological responses, including the secretion of insulin and GLP-1. Taken together, it can be speculated that artificial sweeteners, to some extent, play a protective role in adiposity and insulin resistance by counteracting the LPS-induced inflammation and subsequent impairment of insulin signaling. However, it remains to be investigated whether the findings of Suez et al. (89) in mice are translatable to humans regarding the metabolic consequences of artificial sweetener-induced microbiota alterations. Furthermore, human evidence of the effects of artificial sweeteners on inflammation is currently lacking.

CONCLUSION AND PERSPECTIVES

The scope of this review was to review the physiological effects of artificial sweeteners on body weight control and glucose homeostasis, and to identify the controversies of the existing evidence between different artificial sweeteners surrounding their use. Although artificial sweeteners maintain the same palatability as natural sugars, the metabolic routes are different. Therefore, artificial sweeteners affect body weight and glucose homeostasis differently compared to natural sugars via underlying physiological processes comprising the gut microbiota, reward-system, adipogenesis, insulin secretory capacity, intestinal glucose absorption, and insulin resistance. The gut microbiota, in particular, may play a major role in the physiological effects of artificial sweeteners on body weight regulation and glucose homeostasis. There is mechanistic evidence that artificial sweeteners may induce gut microbiota dysbiosis, by altering the gut microbiota composition and function. Although different physiological processes are involved in the effect of artificial sweeteners on metabolic health, meta-analyses of RCTs or RCTs and prospective cohort studies suggest that artificial sweeteners may have a neutral effect on body weight and glycemic control, respectively, or may have a beneficial effect on long-term body weight regulation. Even though the majority of human studies report no significant effects of artificial sweeteners on body weight and glycemic control, it should be emphasized that the study duration of most studies was limited. Furthermore, unlike rodent studies, long-term studies investigating the underlying physiological effects body weight control on metabolic health of artificial sweeteners in humans are scarce and therefore warranted. Currently, within the European H2020 project SWEET (www.sweetproject.eu), a human multicenter study is ongoing which aims to investigate the use of artificial sweeteners within the context of a healthy lifestyle on body weight maintenance after weight loss and on metabolic health risk. Notably, artificial sweeteners are metabolized differently and may not all elicit the same metabolic effect as, for instance, components may affect the gut microbiota composition directly and others are easily digested and absorbed. Not all studies investigating the effects of artificial sweeteners on body weight control and glucose homeostasis take into account the different metabolic pathways of distinct artificial sweeteners. Therefore, human data on the effects of distinct artificial sweeteners are limited or lacking. The difference in metabolic fate of artificial sweeteners may underlie conflicting findings that have been reported related to their effects on body weight control, glucose homeostasis, and underlying biological mechanisms. Therefore, extrapolation of the metabolic effects of a single artificial sweetener to all artificial sweeteners is not appropriate.

In this regard, future studies should consider the metabolic pathways of different artificial sweeteners. Further (long-term) human research investigating the underlying physiological pathways of different artificial sweeteners on microbiota alterations and its related metabolic pathway is warranted to evaluate the potential impact of their use on body weight control and glucose homeostasis. Ultimately, it would be interesting to elucidate the impact of initial microbiota composition as a predictor for the response to artificial sweeteners in humans.

AUTHOR CONTRIBUTIONS

MP drafted and edited the manuscript. GG and EB conceptualized and reviewed the manuscript. All authors approved the final version of the manuscript to be published.

REFERENCES

1. Hu F. Obesity and mortality: watch your waist, not just your weight. *Arch Intern Med.* (2007) 167:875–6. doi: 10.1001/archinte.167.9.875
2. Popkin B, Adair L, Ng S. Now and then: the global nutrition transition: the pandemic of obesity in developing countries. *Nutr Rev.* (2012) 70:3–21. doi: 10.1111/j.1753-4887.2011.00456.x
3. Grosso G, Bella F, Godos J, Sciacca S, Del Rio D, Ray S, et al. Possible role of diet in cancer: systematic review and multiple meta-analyses of dietary patterns, lifestyle factors, and cancer risk. *Nutr Rev.* (2017) 75:405–19. doi: 10.1093/nutrit/nux012
4. Pan A, Lin X, Hemler E, Hu F. Diet and cardiovascular disease: advances and challenges in population-based studies. *Cell Metab.* (2018) 27:489–96. doi: 10.1016/j.cmet.2018.02.017
5. Ley S, Hamdy O, Mohan V, Hu F. Prevention and management of type 2 diabetes: dietary components and nutritional strategies. *Lancet.* (2014) 383:1999–2007. doi: 10.1016/S0140-6736(14)60613-9
6. US Burden of Disease Collaborators, Mokdad A, Ballestros K, Echko M, Glenn S, Olsen H, et al. The state of US Health, 1990-2016: burden of diseases, injuries, and risk factors among US states. *JAMA.* (2018) 319:1444–72. doi: 10.1001/jama.2018.0158
7. GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990–2017: a systematic analysis for the Global burden of disease study 2017. *Lancet*. (2019) 393:1958–72. doi: 10.1016/S0140-6736(19)30041-8
8. World Health Organization. *Noncommunicable Diseases Country Profiles 2018*. Geneva (2018).
9. van Baak M, Mariman E. Mechanisms of weight regain after weight loss — the role of adipose tissue. *Nat Rev Endocrinol.* (2019) 15:274–87. doi: 10.1038/s41574-018-0148-4
10. Khan T, Sievenpiper J. Controversies about sugars: results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. *Eur J Nutr.* (2016) 55(Suppl. 2):25–43. doi: 10.1007/s00394-016-1345-3
11. Blaak E. Carbohydrate quantity and quality and cardio-metabolic risk. *Curr Opin Clin Nutr Metab Care.* (2016) 19:289–93. doi: 10.1097/MCO.0000000000000290
12. Richelsen B. Sugar-sweetened beverages and cardio-metabolic disease risks. *Curr Opin Clin Nutr Metab Care.* (2013) 16:478–84. doi: 10.1097/MCO.0b013e328361c53e
13. Azaïs-Braesco V, Sluik D, Maillot M, Kok F,Moreno LA. A review of total & added sugar intakes and dietary sources in Europe. *Nutr J.* (2017) 16:6. doi: 10.1186/s12937-016-0225-2
14. Sluik D, van Lee L, Engelen A, Feskens E. Total, free, and added sugar consumption and adherence to guidelines: the dutch national food consumption survey 2007–2010. *Nutrients.* (2016) 8:70. doi: 10.3390/nu8020070
15. Toews I, Lohner S, Küllenberg de Gaudry D, Sommer H, Meerpohl J. Association between intake of non-sugar sweeteners and health

outcomes: systematic review and meta-analyses of randomised and non-randomised controlled trials and observational studies. *BMJ.* (2019) 15:l156. doi: 10.1136/bmj.l156
16. Whitehouse C, Boullata J, McCauley L. The potential toxicity of artificial sweeteners. *AAOHN J.* (2008) 56:251–9. doi: 10.3928/08910162-20080601-02
17. Fitch C, Keim K, Dietetics. AoNa. Position of the academy of nutrition and dietetics: use of nutritive and nonnutritive sweeteners. *J Acad Nutr Diet.* (2012) 112:739–58. doi: 10.1016/j.jand.2012.03.009
18. Sylvetsky A, Jin Y, Clark E, Welsh J, Rother K, Talegawkar S. Consumption of low-calorie sweeteners among children and adults in the United States. *J Acad Nutr Diet.* (2017) 117:441–8. doi: 10.1016/j.jand.2016.11.004
19. Yang Q. Gain weight by "going diet?" Artificial sweeteners and the neurobiology of sugar cravings: neuroscience 2010. *Yale J Biol Med.* (2010) 83:101–8.
20. FDA. *US Food and Drug Administration. High-Intensity Sweeteners*. (2014). Available online at: https://www.fda.gov/food/food-additives-petitions/high-intensity-sweeteners (accessed January 8, 2020).
21. FDA. *Agency Response Letter GRAS* Notice No. GRN 000738 [THAUMATIN sweetener and food flavor modifier] (2018).
22. Mortensen A. Sweeteners permitted in the European Union: safety aspects. *Scand J Food Nutr.* (2006) 50:104–16. doi: 10.1080/17482970600982719
23. *Revised Opinion of the Scientific Committee on Food on Cyclamic Acid and its Sodium and Calcium Salts (expressed on 9 March 2000).* Scientific Committee on Food (2000). Available online at: https://ec.europa.eu/food/sites/food/files/safety/docs/sci-com_scf_out53_en.pdf (accessed January 10, 2020).
24. Food Standards Agency. *Current EU Approved Additives and their E Numbers*. (2016). Available online at: https://www.food.gov.uk/business-guidance/approved-additives-and-e-numbers (accessed January 10, 2020).
25. Greenwood D, Threapleton D, Evans C, Cleghorn C, Nykjaer C, Woodhead C, et al. Association between sugar-sweetened and artificially sweetened soft drinks and type 2 diabetes: systematic review and dose-response meta-analysis of prospective studies. *Br J Nutr.* (2014) 112:725–34. doi: 10.1017/S0007114514001329
26. Fowler S, Williams K, Resendez R, Hunt K, Hazuda H, Stern M. Fueling the obesity epidemic? Artificially sweetened beverage use and long-term weight gain. *Obesity.* (2008) 16:1894–900. doi: 10.1038/oby.2008.284
27. Nettleton J, Lutsey P, Wang Y, Lima J, Michos E, Jacobs DJ. Diet soda intake and risk of incident metabolic syndrome and type 2 diabetes in the multi-ethnic study of atherosclerosis (MESA). *Diabetes Care.* (2009) 32:688–94. doi: 10.2337/dc08-1799
28. Sakurai M, Nakamura K, Miura K. Sugar-sweetened beverage and diet soda consumption and the 7-year risk for type 2 diabetes mellitus in middle- aged Japanese men. *Eur J Nutr.* (2014) 53:251–8. doi: 10.1007/s00394-013-0523-9
29. Azad M, Abou-Setta A, Chauhan B, Rabbani R, Lys J, Copstein L, et al. Nonnutritive sweeteners and cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials and prospective cohort studies. *CMAJ.* (2017) 189:E929–39. doi: 10.1503/cmaj.161390
30. Fowler S. Low-calorie sweetener use and energy balance: results from experimental studies in animals, and large-scale prospective studies in humans. *Physiol Behav.* (2016) 164(Pt. B):517–23. doi: 10.1016/j.physbeh.2016.04.047
31. Laverty A, Magee L, Monteiro C, Saxena S, Millett C. Sugar and artificially sweetened beverage consumption and adiposity changes: National longitudinal study. *Int J Behav Nutr Phys Act.* (2015) 12:137. doi: 10.1186/s12966-015-0297-y
32. Purohit V, Mishra S. The truth about artificial sweeteners – Are they good for diabetics? *Indian Heart J.* (2018) 70:197–9. doi: 10.1016/j.ihj.2018.01.020
33. Swithers S. Artificial sweeteners produce the counterintuitive effect of inducing metabolic derangements. *Trends Endocrinol Metab.* (2013) 24:431–41. doi: 10.1016/j.tem.2013.05.005
34. Santos N, de Araujo L, de Luca Canto G, Guerra E, Coelho M, Borin M. Metabolic effects of aspartame in adulthood: a systematic review and meta-analysis of randomized clinical trials. *Crit Rev Food Sci Nutr.* (2018) 58:2068–81. doi: 10.1080/10408398.2017.1304358
35. Bundgaard Anker CC, Rafiq S, Jeppesen PB. Effect of steviol glycosides on human health with emphasis on type 2 diabetic biomarkers: a systematic review and meta-analysis of randomized controlled trials. *Nutrients.* (2019) 11:1965. doi: 10.3390/nu11091965
36. Higgins K, Mattes R. A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *Am J Clin Nutr.* (2019) 109:1288–301. doi: 10.1093/ajcn/nqy381
37. Thomson P, Santibañez R, Aguirre C, Galgani J, Garrido D. Short-term impact of sucralose consumption on the metabolic response and gut microbiome of healthy adults. *Br J Nutr.* (2019) 122:856–62. doi: 10.1017/S0007114519001570
38. Romo-Romo A, Aguilar-Salinas C, Brito-Córdova G, Gómez-Díaz R. Sucralose decreases insulin sensitivity in healthy subjects: a randomized controlled trial. *Am J Clin Nutr.* (2018) 108:485–91. doi: 10.1093/ajcn/nqy152
39. Steinert R, Frey F, Töpfer A, Drewe J, Beglinger C. Effects of carbohydrate sugars and artificial sweeteners on appetite and the secretion of gastrointestinal satiety peptides. *Br J Nutr.* (2011) 105:1320–8. doi: 10.1017/S000711451000512X
40. Maersk M, Belza A, Holst J, Fenger-Gron M, Pedersen S, Astrup A, et al. Satiety scores and satiety hormone response after sucrose-sweetened soft drink compared with isocaloric semi-skimmed milk and with non-caloric soft drink: a controlled trial. *Eur J Clin Nutr.* (2012) 66:523–9. doi: 10.1038/ejcn.2011.223
41. Heijboer A, Pijl H, Van den Hoek A, Havekes L, Romijn J, Corssmit E. Gut-brain axis: regulation of glucose metabolism. *Neuroendocrinology.* (2006) 18:883–94. doi: 10.1111/j.1365-2826.2006.01492.x
42. Ma J, Bellon M, Wishart J, Young R, Blackshaw L, Jones K, et al. Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. *Am J Physiol Gastrointest Liver Physiol.* (2009) 296:G735–9. doi: 10.1152/ajpgi.90708.2008
43. Horwitz D, McLane M, Kobe P. Response to single dose of aspartame or saccharin by NIDDM patients. *Diabetes Care.* (1988) 11:230–4. doi: 10.2337/diacare.11.3.230
44. Rodin J. Comparative effects of fructose, aspartame, glucose, and water preloads on calorie and macronutrient intake. *Am J Clin Nutr.* (1990) 51:428–35. doi: 10.1093/ajcn/51.3.428
45. Hall W, Millward D, Rogers P, Morgan L. Physiological mechanisms mediating aspartame-induced satiety. *Physiol Behav.* (2003) 78:557–62. doi: 10.1016/s0031-9384(03)00034-9
46. Temizkan S, Deyneli O, Yasar M. Sucralose enhances GLP-1 release and lowers blood glucose in the presence of carbohydrate in healthy subjects but not in patients with type 2 diabetes. *Eur J Clin Nutr.* (2015) 69:162–6. doi: 10.1038/ejcn.2014.208
47. Ahmad S, Friel J, MacKay D. The effect of the artificial sweeteners on glucose metabolism in healthy adults: a randomized double-blinded crossover clinical trial. *Appl Physiol Nutr Metab.* (2019) 45:606–12. doi: 10.1139/apnm-2019-0359
48. Okuno G, Kawakami F, Tako H, Kashihara T, Shibamoto S, Yamazaki T. Glucose tolerance, blood lipid, insulin and glucagon concentration after single or continuous administration of aspartame in diabetics. *Diabetes Res Clin Pract.* (1986) 2:23–7. doi: 10.1016/s0168-8227(86)80025-0
49. Colagiuri S, Miller J, Edwards R. Metabolic effects of adding sucrose and aspartame to the diet of subjects with noninsulin-dependent diabetes mellitus. *Am J Clin Nutr.* (1989) 50:474–8. doi: 10.1093/ajcn/50.3.474
50. Nehrling J, Kobe P, McLane M, Olson R, Kamath S, Horwitz D. Aspartame use by persons with diabetes. *Diabetes Care.* (1985) 8:415–7. doi: 10.2337/diacare.8.5.415
51. Wu T, Zhao B, Bound M, Checklin H, Bellon M, Little T, et al. Effects of different sweet preloads on incretin hormone secretion, gastric emptying, and postprandial glycemia in healthy humans. *Am J Clin Nutr.* (2012) 95:78–83. doi: 10.3945/ajcn.111.021543
52. Ford H, Peters V, Martin N, Sleeth M, Ghatei M, Frost G, et al. Effects of oral ingestion of sucralose on gut hormone response and appetite in healthy normal-weight subjects. *Eur J Clin Nutr.* (2011) 65:508–13. doi: 10.1038/ejcn.2010.291
53. Ma J, Chang J, Checklin H, Young R, Jones K, Horowitz M, et al. Effect of the artificial sweetener, sucralose, on small intestinal glucose

absorption in healthy human subjects. *Br J Nutr.* (2010) 104:803–6. doi: 10.1017/S0007114510001327
54. Dalenberg J, Patel B, Denis R. Short-term consumption of sucralose with, but not without, carbohydrate impairs neural and metabolic sensitivity to sugar in humans. *Cell Metab.* (2020) 31:493–502.e7. doi: 10.1016/j.cmet.2020.01.014
55. Grotz V, Henry R, McGill J, Prince M, Shamoon H, Trout J, et al. Lack of effect of sucralose on glucose homeostasis in subjects with type 2 diabetes. *J Acad Nutr Diet.* (2003) 103:1607–12. doi: 10.1016/j.jada.2003.09.021
56. European Food Safety Authority. Outcome of the public consultation on a draft protocol for the assessment of hazard identification characterisation of sweeteners. *EFSA J.* (2020) 17:1803E. doi: 10.2903/sp.efsa.2020
57. European Food Safety Authority. Scientific opinion on the re-evaluation of aspartame (E 951) as a food additive. *EFSA J.* (2013) 11:3496. doi: 10.2903/j.efsa.2013.3496
58. Magnuson B, Carakostas M, Moore N, Poulos S, Renwick A. Biological fate of low-calorie sweeteners. *Nutr Rev.* (2016) 74:670–89. doi: 10.1093/nutrit/nuw032
59. von Rymon Lipinski G. The new intense sweetener acesulfame K. *Food Chem.* (1985) 16:259–69. doi: 10.1016/0308-8146(85)90120-7
60. American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care.* (2010) 33(Suppl. 1):S62–9. doi: 10.2337/dc14-S081
61. Joint FAO/WHO Expert Committee on Food Additives. *555: Acesulfame Potassium.* Food Additives Series. Geneva: World Health Organization (1983).
62. Renwick A. The metabolism of intense sweeteners. *Xenobiotica.* (1986) 16:1057–71. doi: 10.3109/00498258609038983
63. von Rymon Lipinski G, Klug C, Acesulfame K. In: O'Brien NL, editor. *Alternative Sweeteners*. 4th ed. Boca Raton, FL: CRC Press (2012). p. 13–30.
64. Pearson R. Saccharin. In: O'Brien Nabors L e, editor. *Alternative Sweeteners*. 4th ed. Boca Raton, FL: CRC Press (2012). p. 147–66.
65. Renwick A. The disposition of saccharin in animals and man—a review. *Food Chem Toxicol.* (1985) 23:429–35. doi: 10.1016/0278-6915(85)90136-x
66. Sweatman T, Renwick A. The tissue distribution and pharmacokinetics of saccharin in the rat. *Toxicol Appl Pharmacol.* (1980) 55:18–31. doi: 10.1016/0041-008x(80)90215-x
67. Butchko H, Stargel W, Comer C. Aspartame: review of safety. *Regul Toxicol Pharmacol.* (2002) 35:S1–93. doi: 10.1006/rtph.2002.1542
68. Butchko H, Stargel W, Comer C, Mayhew D, Andress S. Aspartame. In: O'Brien NL, editor. *Alternative Sweeteners*. Boca Raton, FL: CRC Press (2012). p. 41–61.
69. Ruiz-Ojeda FJ, Plaza-Díaz J, Sáez-Lara MJ, Gil, A. Effects of sweeteners on the gut microbiota: a review of experimental studies and clinical trials. *Adv Nutr.* (2019) 10:S31–48. doi: 10.1093/advances/nmy037
70. Chattopadhyay S, Raychaudhuri U, Chakraborty R. Artificial sweeteners – a review. *J Food Sci Technol.* (2014) 51:611–21. doi: 10.1007/s13197-011-0571-1
71. Knight I. The development and applications of sucralose, a new high-intensity sweetener. *Can J Physiol Pharmacol.* (1994) 72:435–9. doi: 10.1139/y94-063
72. Omran A, Ahearn G, Bowers D, Swenson J, Coughlin C. Metabolic effects of sucralose on environmental bacteria. *J Toxicol.* (2013) 2013:372986. doi: 10.1155/2013/372986
73. Cardello H, Da Silva M, Damasio M. Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. *Plant Foods Hum Nutr.* (1999) 54:119–29. doi: 10.1023/a:1008134420339
74. Hutapea A, Toskulkao C, Buddhasukh D, Wilairat P, Glinsukon T. Digestion of stevioside, a natural sweetener, by various digestive enzymes. *J Clin Biochem Nutr.* (1997) 23:177–86. doi: 10.3164/jcbn.23.177
75. Gardana C, Simonetti P, Canzi E, Zanchi R, Pietta P. Metabolims of stevioside and rebaudioside A from Stevia rebaudiana extracts by human microflora. *J Agric Food Chem.* (2003) 51:6618–22. doi: 10.1021/jf0303619
76. Wingard R, Brown J, Enderlin F. Intestinal degradation and absorption of the glycosidic sweeteners stevioside and rebaudioside A. *Experientia.* (1980) 36:519–20. doi: 10.1007/BF01965774
77. Yokoyama H, Mori K, Emoto M, Araki T, Teramura M, Mochizuki K, et al. Non-oxidative glucose disposal is reduced in type 2 diabetes, but can be restored by aerobic exercise. *Diabetes Obes Metab.* (2008) 10:400–7. doi: 10.1111/j.1463-1326.2007.00716.x
78. Purkayastha S, Pugh G, Lynch B. *In vitro* metabolism of rebaudioside B, D, and M under anaerobic conditions: comparison with rebaudioside A. *Regul Toxicol Pharmacol.* (2014) 68:259–68. doi: 10.1016/j.yrtph.2013.12.004
79. Kinghorn A, Wu C, Soejarto D. Stevioside. In: O'Brien NL, editor. *Alternative Sweeteners*. 4th ed. Boca Raton, FL: CRC Press (2012).
80. Carakostas M, Curry L, Boileau A. Overview: the history, technical function and safety of rebaudioside A, a naturally occurring steviol glycoside, for use in food and beverages. *Food Chem Toxicol.* (2008) 46(Suppl. 7):S1–10. doi: 10.1016/j.fct.2008.05.003
81. Koyama E, Kitazawa K, Ohori Y. *In vitro* metabolism of the glycosidic sweeteners, stevia mixture and enzymatically modified stevia in human intestinal microflora. *Food Chem Toxicol.* (2003) 41:359–74. doi: 10.1016/s0278-6915(02)00235-1
82. Wheeler A, Boileau A, Winkler P, Compton J, Prakash I, Jiang X, et al. Pharmacokinetics of rebaudioside A and stevioside after single oral doses in healthy men. *Food Chem Toxicol.* (2008) 46(Suppl. 7):S54–60. doi: 10.1016/j.fct.2008.04.041
83. Roberts A, Renwick A. Comparative toxicokinetics and metabolism of rebaudioside A, stevioside, and steviol in rats. *Food Chem Toxicol.* (2008) 46(Suppl. 7):S31–9. doi: 10.1016/j.fct.2008.05.006
84. Rogers P, Hogenkamp P, Graaf de C, Higgs S, Lluch A, Ness A, et al. Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int J Obes.* (2016) 40:381–94. doi: 10.1038/ijo.2015.177
85. Uebanso T, Ohnishi A, Kitayama R, Yoshimoto A, Nakahashi M, Shimohata T, et al. Effects of low-dose non-caloric sweetener consumption on gut microbiota in mice. *Nutrients.* (2017) 9:560. doi: 10.3390/nu9060560
86. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS ONE.* (2017) 12:e0178426. doi: 10.1371/journal.pone.0178426
87. Alkafafy M-S, Ibrahim Z, Ahmed M, El-Shazly S. Impact of aspartame and saccharin on the rat liver: biochemical, molecular, and histological approach. *Int J Immunopathol Pharmacol.* (2015) 28:247–55. doi: 10.1177/0394632015586134
88. Glendinning J, Hart S, Lee H, Maleh J, Ortiz G, Ryu Y, et al. Low-calorie sweeteners cause only limited metabolic effects in mice. *Am J Physiol Regul Integr Comp Physiol.* (2020) 318:R70–80. doi: 10.1152/ajpregu.00245.2019
89. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss C, Maza O, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature.* (2014) 514:181–6. doi: 10.1038/nature13793
90. Zhao X, Yan J, Chen K, Song L, Sun B, Wei X. Effects of saccharin supplementation on body weight, sweet receptor mRNA expression and appetite signals regulation in post-weanling rats. *Peptides.* (2018) 107:32–8. doi: 10.1016/j.peptides.2018.07.006
91. Feijó F, Ballard C, Foletto K, Batista B, Neves A, Ribeiro M, et al. Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult wistar rats, at similar total caloric intake levels. *Appetite.* (2013) 60:203–7. doi: 10.1016/j.appet.2012.10.009
92. Azeez O, Alkass S, Persike D. Long-term saccharin consumption and increased risk of obesity, diabetes, hepatic dysfunction, and renal impairment in rats. *Medicina.* (2019) 55:681. doi: 10.3390/medicina55100681
93. Swithers S, Davidson T. A role for sweet taste: calorie predictive relations in energy regulation by rats. *Behav Neurosci.* (2008) 122:161–73. doi: 10.1037/0735-7044.122.1.161
94. Foletto K, Melo Batista B, Neves A. Sweet taste of saccharin induces weight gain without increasing caloric intake, not related to insulin-resistance in wistar rats. *Appetite.* (2016) 96:604–10. doi: 10.1016/j.appet.2015.11.003
95. Morahan H, Leenaars C, Boakes R, Rooney K. Metabolic and behavioural effects of prenatal exposure to non-nutritive sweeteners: a systematic review and meta-analysis of rodent models. *Physiol Behav.* (2020) 213:112696. doi: 10.1016/j.physbeh.2019.112696

96. Wang QP, Browman D, Herzog H, Neely GG. Non-nutritive sweeteners possess a bacteriostatic effect and alter gut microbiota in mice. *PLoS ONE.* (2018) 13:e0199080. doi: 10.1371/journal.pone.0199080
97. Avena N, Rada P, Hoebel B. Evidence for sugar addiction: behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neurosci Biobehav Rev.* (2008) 32:20–39. doi: 10.1016/j.neubiorev.2007.04.019
98. Fernstrom J, Munger S, Sclafani A, de Araujo I, Roberts A, Molinary S. Mechanisms for sweetness. *J Nutr.* (2012) 142:1134S−41S. doi: 10.3945/jn.111.149567
99. Laffitte A, Neiers F, Briand L. Functional roles of the sweet taste receptor in oral and extraoral tissues. *Curr Opin Clin Nutr Metab Care.* (2014) 17:379–85. doi: 10.1097/MCO.0000000000000058
100. Kojima I, Nakagawa Y. The role of the sweet taste receptor in enteroendocrine cells and pancreatic β-cells. *Diabetes Metab J.* (2011) 35:451–7. doi: 10.4093/dmj.2011.35.5.451
101. Cui M, Jiang P, Maillet E, Max M, Margolskee R, Osman R. The heterodimeric sweet taste receptor has multiple potential ligand binding sites. *Curr Pharm Des.* (2006) 12:4591–600. doi: 10.2174/138161206779010350
102. McLaughlin S, McKinnon P, Margolskee R. Gustducin is a taste-cell-specific G protein closely related to the transducins. *Nature.* (1992) 357:563–9. doi: 10.1038/357563a0
103. Zhang Y, Hoon M, Chandrashekar J, Mueller K, Cook B, Wu D, et al. Coding of sweet, bitter, and umami tastes: different receptor cells sharing similar signaling pathways. *Cell.* (2003) 112:293–301. doi: 10.1016/s0092-8674(03)00071-0
104. Liauchonak I, Qorri B, Dawoud F, Riat Y, Szewczu M. Non-nutritive sweeteners and their implications on the development of metabolic syndrome. *Nutrients.* (2019) 11:644. doi: 10.3390/nu11030644
105. Chandrashekar J, Hoon M, Ryba N, Zuker C. The receptors and cells for mammalian taste. *Nature.* (2006) 444:288–94. doi: 10.1038/nature05401
106. Brown R, Rother K. Non-nutritive sweeteners and their role in the gastrointestinal tract. *J Clin Endocrinol Metab.* (2012) 97:2597–605. doi: 10.1210/jc.2012-1475
107. Smeets P, de Graaf C, Stafleu C, van Osch M, van der Grond J. Functional magnetic resonance imaging of human hypothalamic responses to sweet taste and calories. *Am J Clin Nutr.* (2005) 82:1011–6. doi: 10.1093/ajcn/82.5.1011
108. van Opstal A, Kaal I, van den Berg-Huysmans A, Hoeksma M, Blonk C, Pijl H, et al. Dietary sugars and non-caloric sweeteners elicit different homeostatic and hedonic responses in the brain. *Nutrition.* (2019) 60:80–6. doi: 10.1016/j.nut.2018.09.004
109. Holst J. On the physiology of GIP and GLP-1. *Horm Metab Res.* (2004) 36:747–54. doi: 10.1055/s-2004-826158
110. Han P, Bagenna B, Fu M. The sweet taste signalling pathways in the oral cavity and the gastrointestinal tract affect human appetite and food intake: a review. *Int J Food Sci Nutr.* (2019) 70:125–35. doi: 10.1080/09637486.2018.1492522
111. Fujita Y, Wideman R, Speck M, Asadi A, King D, Webber T, et al. Incretin release from gut is acutely enhanced by sugar but not by sweeteners *in vivo*. *Am J Physiol Endocrinol Metab.* (2009) 269:E473–9. doi: 10.1152/ajpendo.90636.2008
112. Swithers S, Laboy A, Clark K, Cooper S, Davidson T. Experience with the high-intensity sweetener saccharin impairs glucose homeostasis and GLP-1 release in rats. *Behav Brain Res.* (2012) 233:1–14. doi: 10.1016/j.bbr.2012.04.024
113. Lavin J, French S, Read N. The effect of sucrose- and aspartame-sweetened drinks on energy intake, hunger and food choice of female, moderately restrained eaters. *Int J Obes Relat Metab Disord.* (1997) 21:37–42. doi: 10.1038/sj.ijo.0800360
114. Holt S, Sandona N, Brand-Miller J. The effects of sugar-free vs sugar-rich beverages on feelings of fullness and subsequent food intake. *Int J Food Sci Nutr.* (2000) 51:59–71. doi: 10.1080/096374800100912
115. Gatenby S, Aaron J, Jack V, Mela D. Extended use of foods modified in fat and sugar content: nutritional implications in a free-living female population. *Am J Clin Nutr.* (1997) 65:1867–73. doi: 10.1093/ajcn/65.6.1867
116. Anton S, Martin C, Han H, Coulon S, Cefalu W, Geiselman P, et al. Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite.* (2010) 55:37–43. doi: 10.1016/j.appet.2010.03.009
117. Porikos K, Hesser M, van Itallie T. Caloric regulation in normal-weight men maintained on a palatable diet of conventional foods. *Physiol Behav.* (1982) 29:293–300. doi: 10.1016/0031-9384(82)90018-x
118. Naismith D, Rhodes C. Adjustment in energy intake following the covert removal of sugar from the diet. *J Hum Nutr Diet.* (1995) 8:167–75. doi: 10.1111/j.1365-277X.1995.tb00309.x
119. Simon B, Parlee S, Learman B, Mori H, Scheller E, Cawthorn W, et al. Artificial sweeteners stimulate adipogenesis and suppress lipolysis independently of sweet taste receptors. *J Biol Chem.* (2013) 288:32475–89. doi: 10.1074/jbc.M113.514034
120. Masubuchi Y, Nakagawa Y, Ma J, Sasaki T, Kitamura T, Yamamoto Y, et al. A novel regulatory function of sweet taste-sensing receptor in adipogenic differentiation of 3T3-L1 cells. *PLoS ONE.* (2013) 8:e54500. doi: 10.1371/journal.pone.0054500
121. Sen S, Rouphael C, Houston S. Abstract P029: sucralose promotes increase in fat accumulation in human mesenchymal stem cells. *Circulation.* (2015) 131:AP029.
122. Canfora E, Meex R, Venema K, Blaak E. Gut microbial metabolites in obesity, NAFLD and T2DM. *Nat Rev Endocrinol.* (2019) 15:261–73. doi: 10.1038/s41574-019-0156-z
123. Jandhyala S, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol.* (2015) 21:8787–803. doi: 10.3748/wjg.v21.i29.8787
124. Turnbaugh P, Bäckhed F, Fulton L, Gordon J. Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. *Cell Host Microbe.* (2008) 3:213–23. doi: 10.1016/j.chom.2008.02.015
125. Farup P, Lydersen S, Valeur J. Are nonnutritive sweeteners obesogenic? Associations between diet, faecal microbiota, and short-chain fatty acids in morbidly obese subjects. *J Obes.* (2019) 2019:4608315. doi: 10.1155/2019/4608315
126. Frankenfeld C, Sikaroodi M, Lamb E, Shoemaker S, Gillevet P. High-intensity sweetener consumption and gut microbiome content and predicted gene function in a cross-sectional study of adults in the United States. *Ann Epidemiol.* (2015) 25:736–42.e4. doi: 10.1016/j.annepidem.2015.06.083
127. Bian X, Tu P, Chi L, Gao B, Ru H, Lu K. Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chem Toxicol.* (2017) 107(Pt B):530–9. doi: 10.1016/j.fct.2017.04.045
128. Mahalak K, Firrman J, Tomasula P, Nuñez A, Lee J, Bittinger K, et al. Impact of steviol glycosides and erythritol on the human and cebus apella gut microbiome *J Agric Food Chem.* (2020) 68:13093–101. doi: 10.1021/acs.jafc.9b06181
129. Li S, Chen T, Dong S, Xiong Y, Wei H, Xu F. The effects of rebaudioside a on microbial diversity in mouse intestine. *J Agric Food Chem.* (2014) 51:6618–22. doi: 10.3136/fstr.20.459
130. Nettleton JE, Klancic T, Schick A, Choo AC, Shearer J, Borgland SL, et al. Low-Dose Stevia (Rebaudioside A) consumption perturbs gut microbiota and the mesolimbic dopamine reward system. *Nutrients.* (2019) 11:1248. doi: 10.3390/nu11061248
131. Koropatkin N, Cameron E, Martens E. How glycan metabolism shapes the human gut microbiota. *Nature Rev Microbiol.* (2012) 10:323–35. doi: 10.1038/nrmicro2746
132. Steensels S, Cools L, Avau B, vancleef L, Farré R, Verbeke K, et al. Supplementation of oligofructose, but not sucralose, decreases high-fat diet induced body weight gain in mice independent of gustducin-mediated gut hormone release. *Mol Nutr Food Res.* (2017) 61. doi: 10.1002/mnfr.201600716
133. Vamanu E, Pelinescu D, Gatea F, Sârbu I. Altered *in vitro* metabolomic response of the human microbiota to sweeteners. *Genes.* (2019) 10:535. doi: 10.3390/genes10070535
134. Scheppach W. Effects of short chain fatty acids on gut morphology and function. *Gut.* (1994) 35(Suppl. 1):S35–8. doi: 10.1136/gut.35.1_suppl.s35
135. Schwiertz A, Taras D, Schäfer K, Beijer S, Bos N, Donus C, et al. Microbiota and SCFA in lean and overweight healthy subjects. *Obesity.* (2010) 18:190–5. doi: 10.1038/oby.2009.167

136. den Besten G, Lange K, Havinga R, van Dijk T, Gerding A, van Eunen K, et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol.* (2013) 305:G900–10. doi: 10.1152/ajpgi.00265.2013
137. Chambers E, Preston T, Frost G, Morrison D. Role of gut microbiota-generated short-chain fatty acids in metabolic and cardiovascular health. *Curr Nutr Rep.* (2018) 7:198–206. doi: 10.1007/s13668-018-0248-8
138. Wolever T, Spadafora P, Eshuis H. Interaction between colonic acetate and propionate in humans. *Am J Clin Nutr.* (1991) 53:681–7. doi: 10.1093/ajcn/53.3.681
139. Turnbaugh P, Ley R, Mahowald M, Magrini V, Mardis E, Gordon J. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature.* (2006) 444:1027–31. doi: 10.1038/nature05414
140. Canfora E, Jocken J, Blaak E. Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol.* (2015) 11:577–91. doi: 10.1038/nrendo.2015.128
141. Brown A, Goldsworthy S, Barnes A, Eilert M, Tcheang L, Daniels D, et al. The Orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. *J Biol Chem.* (2003) 278:11312–9. doi: 10.1074/jbc.M211609200
142. Karaki S, Mitsui R, Hayashi H, Kato I, Sugiya H, Iwanaga T, et al. Short-chain fatty acid receptor, GPR43, is expressed by enteroendocrine cells and mucosal mast cells in rat intestine. *Cell Tissue Res.* (2006) 324:353–60 doi: 10.1007/s00441-005-0140-x
143. Tolhurst G, Heffron H, Lam Y, Parker H, Habib A, Diakogiannaki E, et al. Short-Chain fatty acids stimulate glucagon-like peptide-1 secretion via the g-protein–coupled receptor FFAR2. *Diabetes.* (2012) 61:364–71. doi: 10.2337/db11-1019
144. Canfora E, van der Beek C, Jocken J, Goossens G, Holst J, Olde Damink S, et al. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep.* (2017) 7:2360. doi: 10.1038/s41598-017-02546-x
145. Li Z, Yi C, Katiraei S. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut.* (2018) 67:1269–79. doi: 10.1136/gutjnl-2017-314050
146. Kimura I, Inoue D, Maeda T, Hara T, Ichimura A, Miyauchi S, et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci USA.* (2011) 108:8030–5. doi: 10.1073/pnas.1016088108
147. Murakami Y, Ojima-Kato T, Saburi W, Mori H, Matsui H, Tanabe S, et al. Supplemental epilactose prevents metabolic disorders through uncoupling protein-1 induction in the skeletal muscle of mice fed high-fat diets. *Br J Nutr.* (2015) 114:1774–83. doi: 10.1017/S0007114515003505
148. Boon M, van Marken Lichtenbelt W. Brown adipose tissue: a human perspective. *Handb Exp Pharmacol.* (2016) 233:301-19. doi: 10.1007/164_2015_11
149. Li X, Chen H, Guan Y, Li X, Lei L, Liu J, et al. Acetic acid activates the AMP-activated protein kinase signaling pathway to regulate lipid metabolism in bovine hepatocytes. *PLoS ONE.* (2013) 8:e67880. doi: 10.1371/journal.pone.0067880
150. Kondo T, Kishi M, Fushimi T, Kaga T. Acetic acid upregulates the expression of genes for fatty acid oxidation enzymes in liver to suppress body fat accumulation. *J Agric Food Chem.* (2009) 57:5982–6. doi: 10.1021/jf900470c
151. Zhang B, Zhou G, Li C. AMPK: an emerging drug target for diabetes and the metabolic syndrome. *Cell Metab.* (2009) 9:407–16. doi: 10.1016/j.cmet.2009.03.012
152. Bonini J, Anderson S, Steiner D. Molecular cloning and tissue expression of a novel orphan G protein-coupled receptor from rat lung. *Biochem Biophys Res Comm.* (1997) 234:190–3. doi: 10.1006/bbrc.1997.6591
153. Cong W, Wang R, Cai H, Daimon C, Scheibye-Knudsen M, Bohr V, et al. Long-term artificial sweetener acesulfame potassium treatment alters neurometabolic functions in C57BL/6J mice. *PLoS ONE.* (2013) 8:e70257. doi: 10.1371/journal.pone.0070257
154. Yamashita H, Maruta H, Jozuka M, Kimura R, Iwabuchi H, Yamato M, et al. Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem.* (2009) 73:570–6. doi: 10.1271/bbb.80634
155. Gao Z, Yin J, Zhang J, Ward R, Martin R, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes.* (2009) 58:1509–17. doi: 10.2337/db08-1637
156. Jäger S, Handschin C, St-Pierre J, Spiegelman B. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci USA.* (2007) 104:12017–22. doi: 10.1073/pnas.0705070104
157. Sørensen L, Vasilaras T, Astrup A, Raben A. Sucrose compared with artificial sweeteners: a clinical intervention study of effects on energy intake, appetite, and energy expenditure after 10 wk of supplementation in overweight subjects. *Am J Clin Nutr.* (2014) 100:36–45. doi: 10.3945/ajcn.113.081554
158. Chern C, Tan S. Energy expenditure, carbohydrate oxidation and appetitive responses to sucrose or sucralose in humans: a pilot study. *Nutrients.* (2019) 11:1782. doi: 10.3390/nu11081782
159. Daher M, Matta J, Abdel Nour A. Non-nutritive sweeteners and type 2 diabetes:Should we ring the bell? *Diabetes Res Clin Pract.* (2019) 155:107786. doi: 10.1016/j.diabres.2019.107786
160. Leibowitz A, Bier A, Gilboa M, Peleg E, Barshack I, Grossman, E. Saccharin increases fasting blood glucose but not liver insulin resistance in comparison to a high fructose-fed rat model. *Nutrients.* (2018) 10:341. doi: 10.3390/nu10030341
161. Margolskee R, Dyer J, Kokrashvili Z, Salmon K, Ilegems E, Daly K, et al. T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proc Natl Acad Sci USA.* (2007) 104:15075–80. doi: 10.1073/pnas.0706678104
162. Shirazi-Beechey S, Moran A, Batchelor D, Daly K, Al-Rammahi M. Glucose sensing and signalling; regulation of intestinal glucose transport. *Proc Nutr Soc.* (2011) 70:185–93. doi: 10.1017/S0029665111000103
163. Kellett G, Brot-Laroche E, Mace O, Leturque A. Sugar absorption in the intestine: the role of GLUT2. *Annu Rev Nutr.* (2008) 28:35–54. doi: 10.1146/annurev.nutr.28.061807.155518
164. Mace O, Affleck J, Patel N, Kellett G. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol.* (2007) 582(Pt 1):379–92. doi: 10.1113/jphysiol.2007.130906
165. Berthoud H, Bereiter D, Trimble E, Siegel E, Jeanrenaud B. Cephalic phase, reflex insulin secretion. Neuroanatomical and physiological characterization. *Diabetologia.* (1981) 20:393–401.
166. Suzuki K, Jayasena C, Bloom S. Obesity and appetite control. *Exp Diabetes Res.* (2012) 2012:824305. doi: 10.1155/2012/824305
167. Nakagawa Y, Nagasawa M, Yamada S, Hara A, Mogami H, Nikolaev V, et al. Sweet taste receptor expressed in pancreatic b-cells activates the calcium and cyclic AMP signaling systems and stimulates insulin secretion. *PLoS ONE.* (2009) 4:e5106. doi: 10.1371/journal.pone.0005106
168. Brown A, Bohan B, MM, Onken K, Beitz D. Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women. *Nutr Res.* (2011) 31:882–8. doi: 10.1016/j.nutres.2011.10.004
169. Pepino M, Tiemann C, Patterson B, Wice B, Klein S. Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care.* (2013) 36:2530–5. doi: 10.2337/dc12-2221
170. Lertrit A, Srimachai S, Saetung S, Chanprasertyothin S, Chailurkit L, Areevut C, et al. Effects of sucralose on insulin and glucagon-like peptide-1 secretion in healthy subjects: a randomized, double-blind, placebo-controlled trial. *Nutrition.* (2018) 55–56:125–130. doi: 10.1016/j.nut.2018.04.001
171. Sylvetsky AC, Brown RJ, Blau JE, Walter M, Rother KI. Hormonal responses to non-nutritive sweeteners in water and diet soda. *Nutr Metab.* (2016) 13:71. doi: 10.1186/s12986-016-0129-3
172. Härtel B, Schneider B, Bier A. The influence of sweetener solutions on the secretion of insulin and blood glucose level. *Ernährungsunschau.* (1993) 40:152–5.
173. Barriocanal L, Palacios M, Benitez G, Benitez S, Jimenez J, Jimenez N, et al. Apparent lack of pharmacological effect of steviol glycosides used as sweeteners in humans. A pilot study of repeated exposures in some normotensive and hypotensive individuals and in type 1 and type 2 diabetics. *Regul Toxicol Pharmacol.* (2008) 51:37–41. doi: 10.1016/j.yrtph.2008.02.006

174. Maki K, Curry L, Reeves M, Toth P, McKenney J, Farmer M, et al. Chronic consumption of rebaudioside A, a steviol glycoside, in men and women with type 2 diabetes mellitus. *Food Chem Toxicol.* (2008) 47:S47–53. doi: 10.1016/j.fct.2008.05.007
175. Samuel V, Shulman G. Integrating mechanisms for insulin resistance: common threads and missing links. *Cell.* (2012) 148:852–71. doi: 10.1016/j.cell.2012.02.017
176. Santos P, Caria C, Gotardo E, Ribeiro M, Pedrazzoli J, Gambero A. Artificial sweetener saccharin disrupts intestinal epithelial cells' barrier function *in vitro*. *Food Funct.* (2018) 9:3815–22. doi: 10.1039/c8fo00883c
177. André P, Laugerette F, Féart, C. Metabolic endotoxemia: a potential underlying mechanism of the relationship between dietary fat intake and risk for cognitive impairments in humans? *Nutrients.* (2019) 11:1887. doi: 10.3390/nu11081887
178. Leung C, Rivera L, Furness J, Angus P. The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol.* (2016) 13:412–25. doi: 10.1038/nrgastro.2016.85
179. de La Serre C, Ellis C, Lee J, Hartman A, Rutledge J, Raybould H. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. *Am J Physiol Gastrointest Liver Physiol.* (2010) 299:G440–8. doi: 10.1152/ajpgi.00098.2010
180. Wright S, Ramos R, Tobias P, Ulevitch R, Mathison J. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science.* (1990) 249:1431–3. doi: 10.1126/science.1698311
181. Bleau C, Karelis A, St-Pierre D, Lamontagne L. Crosstalk between intestinal microbiota, adipose tissue and skeletal muscle as an early event in systemic low-grade inflammation and the development of obesity and diabetes. *Diabetes Metab Res Rev.* (2015) 31:545–61. doi: 10.1002/dmrr.2617
182. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. Gut microbiome response to sucralose and its potential role in inducing liver inflammation in mice. *Front Physiol.* (2017) 7:487. doi: 10.3389/fphys.2017.00487
183. Boonkaewwan C, Burodom A. Anti-inflammatory and immunomodulatory activities of stevioside and steviol on colonic epithelial cells. *J Sci Food Agric.* (2013) 93:3820–5. doi: 10.1002/jsfa.6287
184. Wang T, Guo M, Song X, Zhang Z, Jiang H, Wang W, et al. Stevioside plays an anti-inflammatory role by regulating the NF-κB and MAPK pathways in S. aureus-infected mouse mammary glands. *Inflammation.* (2014) 37:1837–46. doi: 10.1007/s10753-014-9915-0
185. Stienstra R, Tack C, Kanneganti T, Joosten L, Netea M. The inflammasome puts obesity in the danger zone. *Cell Metab.* (2012) 15:10–8. doi: 10.1016/j.cmet.2011.10.011
186. Rotter V, Nagaev I, Smith U. Interleukin-6 (IL-6) induces insulin resistance in 3T3-L1 adipocytes and is, like IL-8 and tumor necrosis factor. *J Biol Chem.* (2003) 278:45777–84. doi: 10.1074/jbc.M301977200
187. Jager J, Grémeaux T, Cormont M, Le Marchand-Brustel Y, Tanti J. Interleukin-1beta-induced insulin resistance in adipocytes through down-regulation of insulin receptor substrate-1 expression. *Endocrinology.* (2007) 148:241–51. doi: 10.1210/en.2006-0692
188. Jiang N, Li Y, Shu T, Wang J. Cytokines and inflammation in adipogenesis: an updated review. *Front Med.* (2019) 13:314–29. doi: 10.1007/s11684-018-0625-0
189. Stinkens R, Goossens G, Jocken J, Blaak E. Targeting fatty acid metabolism to improve glucose metabolism. *Obes Rev.* (2015) 16:715–57. doi: 10.1111/obr.12298
190. Liu T, Li J, Liu Y, Xiao N, Suo H, Xie K, et al. Short-chain fatty acids suppress lipopolysaccharide-induced production of nitric oxide and proinflammatory cytokines through inhibition of NF-κB pathway in RAW264.7 cells. *Inflammation.* (2012) 35:1676–84 doi: 10.1007/s10753-012-9484-z
191. Ge H, Li X, Weiszmann J, Wang P, Baribault H, Chen J, et al. Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology.* (2008) 149:4519–26. doi: 10.1210/en.2008-0059
192. Aberdein N, Schweizer M, Ball D. Sodium acetate decreases phosphorylation of hormone sensitive lipase in isoproterenol-stimulated 3T3-L1 mature adipocytes. *Adipocyte.* (2014) 3:121–5. doi: 10.4161/adip.27936
193. Carmen G, Víctor S. Signalling mechanisms regulating lipolysis. *Cell Signal.* (2006) 18:401–8. doi: 10.1016/j.cellsig.2005.08.009
194. den Besten G, van Eunen K, Groen A, Venema K, Reijngoud D, Bakker B. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res.* (2013) 54:2325–40. doi: 10.1194/jlr.R036012
195. den Besten G, Bleeker A, Gerding A, van Eunen K, Havinga R, van Dijk T, et al. Short-chain fatty acids protect against high-fat diet-induced obesity via a PPARγ-dependent switch from lipogenesis to fat oxidation. *Diabetes.* (2015) 64:2398–408. doi: 10.2337/db14-1213
196. Mollica M, Raso G, Cavaliere G, Trinchese G, Filippo C, Aceto S, et al. Butyrate regulates liver mitochondrial function, efficiency, and dynamics in insulin-resistant obese mice. *Diabetes.* (2017) 66:1405–18. doi: 10.2337/db16-0924
197. Sahuri-Arisoylu M, Brody L, Parkinson J, Parkes H, Navaratnam N, Miller A, et al. Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int J Obes.* (2016) 40:955–63. doi: 10.1038/ijo.2016.23

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The Prospective Association of Dietary Sugar Intake in Adolescence With Risk Markers of Type 2 Diabetes in Young Adulthood

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Purpose: To examine the prospective relevance of dietary sugar intake (based on dietary data as well as urinary excretion data) in adolescent years for insulin sensitivity and biomarkers of inflammation in young adulthood.

Methods: Overall 254 participants of the DONALD study who had at least two 3-day weighed dietary records for calculating intakes of fructose, glucose, sucrose, total, free, added sugars, total sugars from sugar-sweetened beverages (SSB), juice, and sweets/sugar or at least two complete 24 h urine samples ($n = 221$) for calculating sugar excretion (urinary fructose and urinary fructose + sucrose) in adolescence (females: 9–15 years, males: 10–16 years) and a fasting blood sample in adulthood (18–36 years), were included in multivariable linear regression analyses assessing their prospective associations with adult homeostasis model assessment insulin sensitivity (HOMA2-%S) and a pro-inflammatory score (based on CRP, IL-6, IL-18, leptin, chemerin, adiponectin).

Results: On the dietary intake level, no prospective associations were observed between adolescent fructose, sucrose, glucose, added, free, total sugar, or total sugar from SSB, juice or sweets/sugar intake and adult HOMA2-%S ($p > 0.01$). On the urinary level, however, higher excreted fructose levels were associated with improved adult HOMA2-%S ($p = 0.008$) among females only. No associations were observed between dietary or urinary sugars and the adult pro-inflammatory score ($p > 0.01$).

Conclusion: The present study did not provide support that dietary sugar consumed in adolescence is associated with adult insulin sensitivity. The one potential exception was the moderate dietary consumption of fructose, which showed a beneficial association with adult fasting insulin and insulin sensitivity.

Keywords: dietary sugar, urinary sugar, insulin sensitivity, fasting insulin, systemic inflammation

INTRODUCTION

It has been proposed that dietary sugar intake plays a causal role in the development of type 2 diabetes (T2D) (1–4), yet data on this topic are conflicting (5, 6). Due to its unregulated uptake and hepatic metabolism, the fructose component of high-sugar foods has been singled out as a key promotor of adverse cardiometabolic health outcomes when consumed in high amounts (7, 8). High intake levels of fructose administered in such intervention and acute studies do not however represent common intake patterns consumed habitually over time. In addition, dietary fructose that occurs naturally in whole fruits and vegetables provides only modest amounts of fructose combined with phytochemicals and fiber (9, 10), therefore amounts as well as types/sources of ingested fructose are of importance when considering its relation to risk factors of T2D (11). Dietary fructose elicits lower insulin secretion as compared to dietary glucose (12–14), and there is some evidence indicating that fructose intake/substitution can beneficially affect blood glucose levels (15, 16). Clarifications from prospective studies concerning the role of dietary fructose and other sugar types in the development of insulin sensitivity are needed.

It has additionally been postulated that dietary sugar intake leads to increased inflammatory processes in humans. While some evidence from human intervention trials points toward pro-inflammatory effects of sucrose and fructose vs. glucose (17, 18), our previous systematic review and meta-analysis of human intervention trials based on limited evidence found that dietary fructose does not contribute more to subclinical inflammation than other dietary sugars (19). Observational studies link the consumption of SSB to increased chronic inflammation (1, 20–22), yet it is unclear whether a modest and habitual sugar intake in adolescence is associated with later development of systemic inflammation.

Adolescents generally consume more added sugars (mainly as soft drinks) than other age groups (23, 24). Adolescence is also characterized by substantial hormonal, metabolic, and lifestyle changes, which is why this developmental stage is considered a critical period for later metabolic diseases (25). Dietary assessment methods are prone to measurement errors (26) and sugars are among the nutrients that are frequently underreported (27, 28) especially by adolescents who may be susceptible to socially desired reporting. Therefore, dietary biomarkers of 24 h urinary sucrose and urinary fructose have been introduced (29, 30), potentially allowing for greater accuracy in determining the impact dietary sugar intake during adolescence could have on adult metabolic health.

This analysis examined the prospective association between the intake of dietary sugar in adolescent years and the target outcomes of T2D risk factors (insulin sensitivity, fasting insulin, and systemic inflammation) measured in adulthood. By using a comprehensive approach, tests were performed on the basis of chemical sugar types (fructose, glucose, sucrose), sugar use (total sugar, added sugar, free sugar), and sugar sources [total sugars from sugar-sweetened beverage (SSB), juice, sweets/sugar] as well as urinary sugar excretion levels. This unique approach allows for a comprehensive investigation into how various forms of sugar measured on the self-reported dietary level as well as the biomarker level are related to risk factors for T2D.

MATERIALS AND METHODS

Study Population

The present analysis was based on data from the DOrtmund Nutritional and Anthropometric Longitudinally Designed Study (DONALD Study), an open-ended and ongoing study conducted in Dortmund, Germany. In this cohort, approximately 35–40 healthy infants are recruited per year and first examined at the ages of 3 or 6 months. Each child returns for 3 more visits in the first year, 2 in the second year, and then once annually until adulthood. Between infancy and adulthood, detailed information on diet, metabolism, growth, and development are collected. This study began collecting this data in 1985. Components of the annual assessment and interview include anthropometric assessments, medical investigations, weighed 3-day dietary records and 24 h urine samples (from age 3–4 years onwards). Parental examinations (anthropometric measurements, lifestyle interviews) take place every 4 years. All examinations are performed with parental and later on, participants' written consent. Since 2005, participants are invited for follow-up in adulthood including fasting blood draw. The study has been previously described in more detail (31), and was approved by the Ethics Committee of the University of Bonn (Germany) according to the guidelines of the Declaration of Helsinki.

Study Sample

At the time of this analysis, 397 participants had provided a fasting blood sample in adulthood (18–39 years) for the measurement of type 2 diabetes risk markers. Additionally, participants fulfilled the eligibility criteria of being singletons, born at term (37 to <43 gestation weeks) with normal birthweight. To estimate habitual intake of dietary sugars during adolescence (females: 9–15 years, males: 10–16 years), participants additionally had to have provided at least two 3-day weighed dietary records in the period of adolescence (with >50% plausible records) (32) ($n = 277$) or at least two complete 24 h urine samples in adolescent years ($n = 246$) for the measurement of excreted fructose and sucrose, validated biomarkers of sugar intake (29, 33). The plausibility of dietary records was estimated by calculating the ratio between reported total energy intake and estimated basal metabolic rate (estimated according to age- and sex-specific equations of Schofield) (34). To identify energy underreporting, pediatric cutoffs from Sichert-Hellert et al. were used (32). Underreporters were not excluded from the analyses, as this procedure only identifies underreported energy intake, but no selective underreporting of food groups or sugar intake. Instead a sensitivity analysis excluding energy underreporters was performed. Anthropometric measurements from adolescence and adulthood as well as information on relevant covariates and outcome variables were required, resulting in analysis populations of 254 participants for the dietary intake sample and 221 participants for the HOMA-%S biomarker sample (see **Tables 1**, **2**) (with $n = 220$ providing both dietary and biomarker data). The inflammatory score sample

population differed slightly ($n = 253$ in dietary sample, and $n = 219$ in the biomarker sample). Participants with fasting glucose concentrations above the threshold (>2.5 mmol/L) for calculating HOMA2-%S were included in the analysis ($n = 254$).

Dietary Assessment

Dietary intake data of the participants are collected annually by 3-day weighed dietary records under the professional direction of a dietician. All consumed foods as well as leftovers were weighed to the nearest gram or alternatively are recorded semi-quantitatively if weighing was not possible. The calculation of energy and nutrient intakes that are based on dietary records is carried out by using the in-house food database called LEBTAB, which is continuously updated (31). The composition of staple foods is based on the German food composition tables BLS 3.02. Energy and nutrient contents of commercial food products, i.e., processed foods and ready-to-eat-meals were estimated by recipe simulation using labeled ingredients and nutrient contents. In this analysis, we calculated the intake of added, free, and total sugar, as well as fructose (defined as simple fructose + one-half of sucrose), glucose and sucrose. Total sugar was defined as the sum of all mono- and disaccharides in foods. Added sugar was defined as sugars added to foods during processing or home preparation (including honey, molasses, fruit juice concentrate, brown sugar, corn sweetener, sucrose, lactose, glucose, high-fructose corn syrup, and malt syrup). Because free sugar was not included in LEBTAB, we expanded the definition from the World Health Organization (WHO) of free sugar as suggested by the Scientific Advisory Committee on Nutrition (SACN) (25, 36) who states that "food subject to blending, pulping, or macerating which breaks down the cellular structure should also be considered as containing free sugars." Therefore, sugars from juices, juice spritzers and smoothies were also considered to be free sugars in our study. Further, SSB were defined as sweetened fruit juice drinks and nectars, soft drinks/sodas, sweetened teas and waters, instant beverages (except dairy drinks), and sweetened sports drinks. Juices were defined as fruits and vegetable juices, juice spritzers, and smoothies. The sugar/sweets food group was defined as sugars and other sweeteners (including syrups), sweet spreads, sweets (candies) and marshmallows, chocolate and bars, ice cream, jelly, desserts, sweet sauces, and sweet baking ingredients. Individual dietary sugar intakes were averaged over the three recorded days. Habitual intake was described by calculating an individual mean from all available records during adolescence (2–7 records per person, mean = 6).

Anthropometric Measurements

Anthropometric measurements were taken by trained nurses according to standard procedures. Standing height was measured to the nearest 0.1 cm (digital stadiometer: Harpenden Ltd., Crymych, UK) and body weight to the nearest 0.1 kg (electronic scale: Seca 753E, Seca Weighing and Measuring Systems, Hamburg, Germany). From these measurements, BMI SD scores (sex- and age-specifically standardized according to German references) (37) and overweight during adolescence were defined and calculated according to the International Obesity Task Force (35). Waist circumference was measured at the midpoint between the lower rib and iliac crest to the nearest 0.1 cm. Average coefficients of variation were obtained from annual quality checks for biceps, triceps, subscapular, and supra-iliacal skinfolds.

Collection and Analysis of 24 h Urine Samples

Participants are requested to collect 24 h urine annually according to standardized instructions. The participants were asked to void their bladders upon getting up in the morning and this micturition was completely discarded. This sets the start of the collection which ends with voiding the bladder in the next morning. All micturitions from the 24 h sampling period were collected in provided Extran-cleaned (Extran, MA03, Merck Darmstadt, Germany) preservative-free 1 L plastic containers and stored immediately at ≤-12°C. After transport to the study center the samples were stored at −22°C until thawed for analysis. Completeness of 24 h urine collections was determined by measuring creatinine excretions assessed photometrically by the kinetic Jaffé procedure on a creatinine analyzer (Beckman-2; Beckman Instruments) (38). Participants are asked to collect a 24 h urine on the last day of the 3-day dietary record, but this is not always the case and some persons do not provide 24 h urines during some of the years.

Urinary fructose and sucrose excretions were measured in the laboratory of the Department of Food and Nutritional Sciences at the University of Reading using LC-MS and quantified using stable-isotope labeled internal standards ($^{13}C_{12}$-sucrose and $^{13}C_6$-fructose, Sigma Aldrich, Gillingham, UK). After shipping on dry ice, urine samples were stored at −80°C until analysis and thawed at 4°C. Samples were separated by HPLC and detected by tandem mass spectrometry using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK). The concentration range was 0.1–500 μmol/L (Fructose: 0.02–90.1 mg/L; sucrose: 0.03–171.2 mg/L). To calculate daily excretions concentrations were converted to mg/d by using the molar mass of fructose or sucrose and multiplied with the 24 h urine volume (39).

Collection of Blood Parameters

Venous blood samples were drawn after an overnight fast, centrifuged at 4°C and stored at −80°C. The following blood analytes were measured at the German Diabetes Center: plasma high-sensitivity C-reactive protein (hsCRP) using the Roche/Hitachi Cobas c311 analyzer (Roche diagnostics, Mannheim, Germany), plasma high-sensitivity interleukin (IL)-6 with the Human IL-6 Quantikine HS, plasma adiponectin with the Human Total Adiponectin/Acrp30 Quantikine ELISA and serum leptin with the Leptin Quantikine ELISA kits all from R&D Systems (Wiesbaden, Germany), serum IL-18 with the Human IL-18 ELISA kit from MBL (Nagoya, Japan), and plasma chemerin with the Human Chemerin ELISA kit from BioVendor (Brno, Czech Republic). Plasma concentrations of insulin were analyzed at the Laboratory for Translational Hormone Analytics of the University of Giessen using an immunoradiometric assay (IRMA, DRG Diagnostics, Marburg, Germany) and the updated HOMA2-%S, a measurement of insulin sensitivity. HOMA2-%S

TABLE 1 | Baseline characteristics of DONALD participants in adolescence (males: 10–16 years, females: 9–15 years): anthropometry, dietary and urinary data as well as early life and socioeconomic factors.

	Dietary sample			Urinary sample		
	n	**M (*n* = 124)**	**F (*n* = 130)**	***n***	**M (*n* = 109)**	**F (*n* = 112)**
Age (years)	254	13.0 (13.0, 13.1)	12.0 (11.9, 12.0)	221	13.0 (13.0, 13.0)	12.0 (12.0, 12.0)
Anthropometric data						
BMI-SD score	254	−0.18 ± 0.77	−0.23 ± 0.92	221	−0.16 ± 0.80	−0.22 ± 0.93
BMI (kg/m^2)	254	18.8 (17.7, 20.2)	17.8 (16.5, 20.1)	221	19.1 (17.7, 20.3)	17.9 (16.5, 20.3)
Body fat (%)	254	14.8 (11.6, 18.6)	19.6 (16.8, 24.9)	221	15.2 (11.6, 18.8)	19.6 (16.9, 25.3)
Overweight (%)[a]	254	22.6	22.3	221	25.7	22.3
Dietary data						
Total energy (MJ/d)	254	9.0 (8.1, 10.2)	7.1 (6.6, 8.1)	221	9.0 (8.3, 10.2)	7.2 (6.6, 8.1)
Fat (%E)	254	35.3 ± 3.8	36.1 ± 3.5	221	34.9 ± 3.4	36.2 ± 3.5
Protein (%E)	254	13.2 ± 1.3	12.9 ± 1.7	221	13.2 ± 1.3	12.9 ± 1.7
Fiber (g/MJ)	254	2.4 (2.1, 2.7)	2.5 (2.2, 2.8)	221	2.4 (2.2, 2.8)	2.5 (2.1, 2.8)
Carbohydrate (%E)	254	51.3 ± 3.8	51.1 ± 4.3	221	51.5 ± 3.9	51.1 ± 4.4
Total sugar (%E)	254	26.8 ± 5.0	27.1 ± 5.0	221	27.0 ± 5.1	27.0 ± 4.9
Added sugar (%E)	254	14.3 ± 4.3	14.1 ± 4.7	221	14.2 ± 4.4	14.1 ± 4.7
Free sugar (%E)	254	18.2 ± 4.6	17.7 ± 5.0	221	18.4 ± 4.6	17.6 ± 5.0
Sucrose (%E)	254	14.4 ± 3.8	14.6 ± 3.9	221	14.4 ± 3.8	14.5 ± 3.8
Fructose (%E)	254	11.3 ± 2.6	11.4 ± 2.5	221	11.4 ± 2.6	11.3 ± 2.4
Glucose (%E)	254	11.5 ± 2.5	11.8 ± 2.7	221	11.4 ± 2.5	11.8 ± 2.7
Sources of total sugar						
Juice (%E)	254	4.0 ± 3.5	3.6 ± 2.9	221	4.3 ± 3.6	3.5 ± 2.9
SSB (%E)	254	4.5 ± 3.9	3.9 ± 3.7	221	4.4 ± 3.8	4.0 ± 3.5
Fruits and vegetables (%E)	254	3.2 ± 1.8	4.3 ± 1.9	221	3.2 ± 1.8	4.3 ± 1.9
Sweet breads/cakes (%E)	254	1.2 ± 0.8	1.4 ± 0.8	221	1.3 ± 0.8	1.4 ± 0.9
Sweets/sugar (%E)	254	6.2 ± 2.5	7.0 ± 2.7	221	6.0 ± 2.4	6.9 ± 2.6
Sweetened cereals (%E)	254	1.0 ± 0.9	0.7 ± 0.6	221	1.1 ± 1.1	0.7 ± 0.7
Dairy sugars (%E)	254	5.3 ± 2.5	4.8 ± 2.2	221	5.5 ± 1.5	4.8 ± 1.0
Urinary data						
Urinary fructose (mg/d)				221	22.3 (14.5, 32.3)	21.2 (13.4, 32.3)
Fructose+sucrose (mg/d)				221	52.7 (37.2, 79.0)	46.3 (34.4, 68.2)
Creatinine (mmol/L)				221	9.5 (6.7, 11.5)	7.6 (6.0, 10.0)
Urea (mmol/L)				221	323 (255, 416)	272 (216, 349)
Urine Volume (L/d)				221	0.9 (0.7, 1.3)	1.0 (0.7, 1.2)
Early life/socioeconomic data						
Birth weight (g)	254	3500 (3150, 3845)	3405 (3100, 3700)	221	3550 (3180, 3850)	3400 (3100, 3655)
Gestational age (week)	254	40 (39, 41)	39 (38, 41)	221	40 (39, 41)	40 (39, 41)
Gestational weight gain (kg)	254	12.0 (9.5, 14.5)	12.0 (9.0, 15.0)	221	12 (10, 15)	12 (10, 15)
Maternal age at birth (year)	254	30.7 (28.3, 33.7)	30.0 (27.8, 32.7)	221	30.8 (28.3, 33.6)	29.7 (27.7, 32.6)
Full breastfeeding >2 weeks (%)	254	74	73	221	75	76
Paternal education ≥12 y (%)	254	65	57	221	64	57
Any smokers in household (%)	254	27	37	221	28	37

Values are means ± SD, medians (25th, 75th percentile) or relative frequencies. BMI, body mass index; %E = percentage of total energy intake; DONALD Dortmund Nutritional and Anthropometric Longitudinally Designed; Pubertal age: mean age at pubertal data collection (mean of multiple time points). [a]*Defined according to age- and sex-specific cut points of the International Obesity Task Force (1, 35); Dietary fructose intake is defined to be free fructose plus 50% of sucrose. Dietary glucose intake is defined to be free glucose plus 50% of sucrose.*

TABLE 2 | Follow-up data on DONALD participants in early adulthood (18–36 years): anthropometric and lifestyle, dietary and blood data.

	Dietary sample			Urinary sample		
	n	**M (*n* = 124)**	**F (*n* = 130)**	***n***	**M (*n* = 109)**	**F (*n* = 112)**
Adult age (years)	254	20.5 (18.1, 23.0)	21.3 (18.1, 24.2)	221	19.0 (18.1, 23.0)	21.3 (18.1, 24.2)
Anthropometric data						
BMI (kg/m^2)	253	22.7 (21.1, 25.6)	21.9 (20.5, 24.1)	221	22.7 (21.0, 25.6)	21.9 (20.5, 24.1)
Body fat (%)	253	17.2 (13.4, 22.2)	30.4 (27.2, 33.3)	221	17.4 (13.3, 21.9)	30.5 (27.0, 33.2)
Current smoking (%)	235	36.8	32.7	202	32.3	28.4
Physical activity level[a]	252	1.2 (1.1, 1.4)	1.2 (1.1, 1.2)	220	1.2 (1.1, 1.4)	1.2 (1.1, 1.3)
Alcohol intake (g/d)	228	1.3 (0.01, 12.5)	0.2 (0.0, 2.9)	203	1.4 (0.1, 11.6)	0.3 (0.1, 3.0)
Dietary data						
Total energy (MJ/d)	229	10.6 (9.3, 12.5)	7.9 (6.6, 8.8)	203	10.5 (9.3, 12.4)	8.0 (6.7, 9.0)
Added sugar (%E)	229	13.3 ± 6.8	12.7 ± 7.4	203	13.4 ± 7.4	12.8 ± 7.2
Protein (%E)	229	14.3 ± 3.8	13.5 ± 2.6	203	14.5 ± 3.9	13.4 ± 2.2
Carbohydrates (%E)	229	48.6 ± 6.7	51.0 ± 6.4	203	48.8 ± 7.0	51.0 ± 6.1
Fat (%E)	229	36.0 ± 5.0	34.6 ± 4.7	203	36.3 ± 5.1	34.9 ± 5.9
Fiber (g/MJ)	229	2.4 (2.0, 2.9)	2.2 (1.9, 2.7)	203	2.2 (1.9, 2.7)	2.5 (2.2, 3.0)
Blood data						
Fasting blood glucose (mmol/L)	254	5.5 (5.1, 5.8)	5.2 (4.9, 5.4)	221	5.5 (5.1, 5.8)	5.2 (4.9, 5.4)
Insulin (pmol/L)	254	64.1 (52.7, 85.5)	71.4 (55.4, 88.2)	221	64.0 (52.1, 85.8)	72.9 (57.3, 89.4)
HOMA2-%S	254	81.7 (61.7, 100.4)	73.5 (960.5, 94.2)	221	81.8 (60.9, 100.6)	73.0 (60.5, 93.9)
hsCRP (mg/L)	250	0.5 (0.3, 1.1)	1.2 (0.6, 2.6)	217	0.5 (0.3, 1.3)	1.3 (0.6, 2.7)
IL-6 (pg/mL)	250	0.7 (0.5, 1.0)	0.7 (0.5, 1.0)	217	0.7 (0.5, 1.0)	0.7 (0.5, 1.0)
IL-18 (pg/mL)	250	252 (204, 308)	246 (209, 306)	217	249 (204, 303)	247 (207, 306)
Chemerin (ng/mL)	250	141 (123, 160)	165 (150, 184)	217	141 (123, 159)	165 (150, 183)
Leptin (ng/mL)	250	2.4 (1.2, 5.0)	11.6 (7.8, 18.0)	217	2.3 (1.1, 5.1)	11.7 (7.8, 18.2)
Adiponectin (μg/mL)	250	6.2 (4.5, 9.2)	8.7 (6.5, 12.5)	217	6.4 (4.7, 9.2)	8.7 (6.4, 12.9)
Inflammatory score	250	−0.13 (−0.37, 0.28)	−0.07 (−0.38, 0.37)	217	−0.15 (−0.37, 0.26)	−0.06 (−0.38, 0.38)

Values are means ± SD, medians (25th, 75th percentile) or relative frequencies. BMI, body mass index; %E = percentage of total energy intake; DONALD Dortmund Nutritional and Anthropometric Longitudinally Designed; HOMA2-%S updated homeostasis model assessment of insulin sensitivity, hsCRP high-sensitivity C-reactive protein. [a]Based on energy expenditure levels.

was calculated by using the HOMA2 calculator (40). It is a reciprocal of HOMA2-IR (insulin resistance) and is a function of glucose metabolism driven by the action of insulin.

To examine the association of dietary sugar on chronic low-grade inflammation in the DONALD Study, the pro-inflammatory markers CRP, IL-6, IL-18, chemerin, and leptin and the anti-inflammatory adipose tissue hormone adiponectin were considered. These biomarkers of subclinical inflammation were selected because they are the most commonly measured inflammation-related biomarkers in clinical and epidemiologic studies with established associations with cardiometabolic diseases (41–45).

A pro-inflammatory score, assumed to be more predictive of inflammation than single markers (43), was obtained as follows: (1) standardization of each inflammatory parameter (hsCRP, IL-6, IL-18, chemerin, leptin, adiponectin) by sex (mean = 0, *SD* = 1), (2) assignment of a minus sign to the anti-inflammatory parameter adiponectin to align its impact with the pro-inflammatory parameters, and (3) averaging all. This index has been used in previous publications (46, 47).

Assessment of Further Covariates

Additional covariates were assessed either at the child's admission into the study or at follow-up visits. Characteristics of birth were retrieved from the "Mutterpass" (a German standardized pregnancy and birth document). Child's parents were interviewed in order to collect familial information, disease history, socioeconomic status and other anthropometrical and medical examinations. Smoking status, high paternal educational status (≥12 years of schooling), and physical activity of the participants was also assessed by questionnaires.

Statistical Analysis

Characteristics of the study population are presented as mean ± SD or median (25th, 75th percentile) for continuous variables and as absolute (relative) frequencies for categorical variables (see **Tables 1, 2**).

To achieve normal distribution in outcome variables we used $\log_e$ or square root transformations. Before calculating the individual means from available records or urines during adolescence, dietary variables were energy-adjusted by the

residual method and standardized by age group and sex to account for age- and sex-dependent intake differences. Urinary excretion variables were also standardized by age group and sex but were not energy-adjusted so as to keep the dietary and urinary analyses separate, thereby avoiding the mixing of potential errors from dietary record assessments with biomarker measurements, as they are differently biased.

Prospective associations between dietary sugar intake (total sugar, added sugar, free sugar, sucrose, fructose, glucose, total sugar from SSB, juice, and sweets/sugar) or sugar excretion (fructose excretion, sucrose excretion, sum of both) during adolescence and risk markers of type 2 diabetes or inflammation in early adulthood were analyzed by multivariable linear regression models, using the transformed variables. Formal interaction analyses indicated a trend in sex-interactions for insulin sensitivity and excreted fructose biomarker level ($P_{interaction} = 0.06$); therefore, sex-stratified analyses were performed for all outcomes on both the dietary and the biomarker level in order to allow comparability.

Initial regression models (model A) included the predictors sugar intake (total, free, added, sucrose, fructose, or glucose) or urinary biomarkers (fructose or sum of both) as well as age at time of blood draw. Adjusted models (model B) were constructed by individual examination of potential influencing covariates and hierarchical inclusion (16) of those which substantially modified the predictor–outcome associations (≥10%) or significantly predicted the outcome. Potential confounding covariates considered in the hierarchical approach were (1) *early life factors* [birth weight (g), gestational age (week), maternal age at birth (year), full breastfeeding ≥ 4 months (yes/no), and gestational weight gain (kg)], (2) *socioeconomic factors and parental health status* [smokers in the household (yes/ no), paternal school education ≥12 years (yes/no), parental overweight (BMI ≥25 kg/m^2 yes/no) and parental history of diabetes (yes/no)], (3) *predictor-specific adolescent data* [BMI, BMI-SD score, percent body fat, age, energy- and fructose-adjusted flavonoid intake and glycemic index, and energy-adjusted fiber intake in models with the dietary predictors sugar intake]. For biomarker analyses, urinary variables [24 h-creatinine excretion (mmol/d), 24 h-urea excretion (mmol/d), urine volume (L/d), excreted hippuric acid (mmol/d)] were also considered. In conditional models (model C) we additionally included adult body fat (%) to examine whether observed associations were independent of adult body composition. To retain comparability of results, models were adjusted identically for closely related outcomes (parameters of insulin sensitivity (fasting insulin, HOMA2-%S) and separately for the pro-inflammatory score) and the building of the models was done for the primary exposures, i.e., dietary fructose or excreted fructose and then used for analyses of the secondary exposures, i.e., free sugar, total sugar, etc. Results from regression analyses are presented as adjusted least-square means (95% CI) by tertiles of the respective predictor with *p*-values from models with the predictors as continuous variables.

Our main analyses did not include nutritional factors that provide energy so as to avoid presenting estimates that partially reflect the substitution of specific sugars for other macronutrients. Additional models were run that explicitly assess the effect of a substitution of various dietary sugar fractions for non-sugar carbohydrates, i.e., total carbohydrates (g) minus all mono- and disaccharides (g). To simulate substitution effects, total energy and the energy-bearing nutrients to be held constant (fats, plant/animal protein and sugar-containing carbohydrates) were included in the models (48). All results from substitution analyses are presented in **Supplementary Material** for fully adjusted models.

As mentioned in the methods section, adolescents are susceptible to underreporting energy intake, therefore records were checked for energy underreporting. The number of records in which energy levels were underreported was 209 (12.6%). These were collected from 109 participants, and were excluded for sensitivity analyses; i.e., sensitivity analyses were based on 1,446 records from 277 participants.

Additional sensitivity analyses in subsamples of participants who had provided the following data were performed in dietary/urinary models: (a) levels of adult physical activity (low/medium/high; $n = 252/218$), (b) adult alcohol consumption (g/d; $n = 229/203$), (c) adult smoking (no, yes, earlier; $n = 235/202$).

The SAS statistical software package version 9.2 (SAS Institute Inc., Cary, NC) was used for all statistical analyses. To account for potential multiple testing, $p < 0.01$ were considered to indicate statistical significance, $p < 0.05$ were considered to indicate a trend.

RESULTS

Characteristics of the participants at baseline and at follow-up are presented in **Tables 1**, **2**, respectively. The median follow-up times between the mean age during adolescence and adulthood were 9.0 years in the dietary sample and 8.6 years in the urinary sample. Participants were characterized by an above-average socioeconomic status as measured by the high percentage of participants' fathers with an education level >12 years. Tertiles of fructose, sucrose, glucose, total sugar, free sugar and added sugar intakes as well as the urinary sugars are shown in **Tables 3**–**5**. For results on sources of sugar (total sugars from SSB, juice and sweets/sugar; see **Supplementary Table 4**).

Adolescent Sugar Intake and Adult Insulin and Insulin Sensitivity

Intakes of dietary fructose, glucose or sucrose in adolescence were not independently associated with adult HOMA2-S% or insulin levels (all $p > 0.01$, **Table 3**). Similarly, there were no independent associations between total, free, or added sugar as well as total sugar intakes from SSB, juice, and sweets/sugar in adolescence and adult HOMA2-S% or insulin levels (all $p > 0.01$, **Table 4**).

On the biomarker level, a higher adolescent excretion of urinary fructose was associated with lower fasting insulin and higher adult insulin sensitivity among females ($p = 0.007$ and $p = 0.008$, respectively, **Table 5**, model C; **Figure 1**). Among males, sugar excretion levels were not associated with adult insulin sensitivity markers.

TABLE 3 | Sex-stratified prospective associations of total dietary fructose, sucrose, and glucose intake during adolescence with markers of insulin sensitivity in early adulthood [$n = 254$: (124 males, 130 females)].

	Tertiles of fructose intake				Tertiles of glucose intake				Tertiles of sucrose intake			
Females	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Dietary sugar (g/d)[a]	36 (32; 43)	47 (44; 53)	63 (56; 70)		39 (32; 43)	50 (43; 58)	65 (58; 70)		46 (39; 55)	60 (53; 69)	83 (70; 89)	
Insulin (pmol/L)												
Model A	79.3 (69.5; 89.2)	80.3 (70.1; 90.5)	72.6 (62.7; 82.5)	**0.03**	85.5 (75.9; 95.2)	78.0 (67.9; 88.1)	68.7 (59.0; 78.3)	**0.05**	82.8 (73.0; 92.5)	80.1 (70.3; 89.9)	68.8 (58.7; 78.8)	**0.03**
Model B	76.3 (66.7; 85.9)	79.9 (70.0; 89.8)	72.4 (62.8; 82.0)	0.11	81.5 (71.4; 91.6)	76.8 (66.6; 87.0)	70.8 (61.0; 80.6)	0.25	77.9 (67.5; 88.2)	79.0 (69.3; 88.7)	71.5 (61.4; 81.6)	0.20
Model C (conditional)	76.6 (66.9; 86.3)	79.2 (69.2; 89.2)	72.7 (63.0; 82.4)	0.17	80.9 (70.9; 90.9)	77.5 (67.4; 87.6)	70.7 (61.0; 80.4)	0.36	78.7 (68.5; 89.0)	79.0 (69.5; 88.6)	70.7 (60.6; 80.7)	0.20
HOMA2-%S												
Model A	71.8 (65.0; 79.5)	71.7 (64.6; 79.6)	79.1 (71.5; 87.6)	**0.04**	67.8 (61.4; 74.9)	73.7 (66.5; 81.8)	81.6 (73.9; 90.1)	0.06	68.8 (62.3; 76.0)	73.7 (66.7; 81.5)	80.9 (73.0; 89.7)	**0.03**
Model B	74.2 (67.3; 81.8)	72.5 (65.6; 80.2)	78.9 (71.5; 87.0)	0.13	68.8 (62.3; 76.0)	73.7 (66.7; 81.5)	81.0 (73.0; 89.7)	0.28	72.7 (65.4; 80.7)	74.6 (67.6; 82.2)	78.3 (70.7; 86.8)	0.25
Model C (conditional)	78.1 (69.1; 88.3)	76.2 (67.8; 85.6)	86.6 (77.1; 97.2)	0.20	71.5 (64.6; 79.1)	74.1 (66.9; 82.1)	79.6 (72.2; 87.8)	0.39	72.0 (64.9; 79.8)	74.5 (67.7; 82.1)	79.1 (71.4; 87.5)	0.24
Males	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Dietary sugar (g/d)[a]	47 (41; 50)	62 (52; 68)	79 (71; 89)		48 (38; 55)	61 (55; 72)	76 (67; 87)		58 (46; 69)	73 (62; 90)	99 (86; 114)	
Insulin (pmol/L)												
Model A	70.9 (60.9; 80.9)	78.3 (68.1; 88.4)	63.7 (53.8; 73.6)	0.95	65.4 (55.1; 76.9)	79.9 (68.8; 91.1)	71.8 (60.8; 82.8)	0.99	73.0 (62.5; 83.6)	72.8 (62.9; 82.6)	66.8 (56.6; 77.0)	0.41
Model B	71.0 (60.1; 81.9)	79.1 (68.7; 89.5)	64.2 (53.9; 74.5)	0.99	68.6 (57.5; 79.7)	72.5 (62.1; 83.0)	72.7 (62.5; 82.9)	0.87	73.8 (62.2; 85.5)	73.5 (63.4; 83.5)	67.3 (56.7; 77.9)	0.79
Model C (conditional)	71.6 (60.1; 82.0)	78.7 (68.3; 89.1)	64.7 (54.5; 75.0)	0.96	68.3 (57.2; 79.4)	73.3 (62.8; 83.9)	72.2 (61.9; 82.4)	0.90	73.4 (61.7; 85.1)	74.3 (64.2; 84.4)	66.7 (56.1; 77.3)	0.75
HOMA2-%S												
Model A	77.8 (69.4; 87.1)	76.9 (68.5; 86.3)	86.5 (77.3; 96.8)	0.90	79.9 (71.0; 90.0)	84.9 (75.8; 95.0)	76.6 (68.5; 85.6)	0.98	75.2 (66.8; 84.7)	80.9 (72.4; 90.4)	84.8 (75.6; 95.1)	0.40
Model B	78.1 (69.0; 88.3)	76.7 (68.2; 86.2)	86.0 (76.5; 96.6)	0.95	75.2 (66.8; 84.7)	80.9 (72.4; 90.4)	84.8 (75.6; 95.1)	0.89	75.0 (65.8; 85.4)	80.2 (71.7; 89.8)	84.7 (75.3; 95.4)	0.76
Model C (conditional)	74.4 (67.6; 82.0)	72.0 (65.1; 79.5)	79.1 (71.9; 87.2)	0.89	80.8 (71.4; 91.5)	83.4 (74.2; 93.8)	77.1 (68.8; 86.4)	0.92	75.4 (66.3; 85.9)	79.3 (70.8; 88.7)	85.5 (76.0; 96.2)	0.72

Values are adjusted least-squares means (95% CIs) unless otherwise indicated. Linear trends (P_{trend}) were obtained in sex-stratified linear regression models with the transformed and energy-adjusted predictors dietary fructose, sucrose, and glucose adolescent intakes as continuous variables. Model A adjusted for adult age at time of blood draw. Model B, with outcomes HOMA2-%S and fasting insulin, additionally adjusted for paternal education, birth weight, gestational weight gain, smoking in the household, parental overweight and pubertal percent body fat. Model C, the conditional model, additionally adjusted for adult percent body fat for all predictors and outcomes. Transformations of variables for analysis: log_e for HOMA2-%S, fasting insulin, dietary sucrose and glucose; square root for dietary fructose. HOMA2-%S: updated homeostasis model assessment of insulin sensitivity. [a]Values are unadjusted medians (25th, 75th percentile). Fructose intake is defined to be free fructose plus 50% of sucrose. Glucose intake is defined to be free glucose plus 50% of sucrose. Bold values indicate significant findings ($p < 0.01$) or trends ($p < 0.05$).

TABLE 4 | Sex-stratified prospective associations of total dietary sugar, added sugar, and free sugar intake during adolescence with markers of insulin sensitivity in early adulthood [$n = 254$: (124 males, 130 females)].

	Tertiles of total sugar intake				Tertiles of added sugar intake				Tertiles of free sugar intake			
Females	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Dietary sugar (g/d)[a]	88 (79; 105)	113 (103; 129)	143 (128; 160)		39 (33; 47)	61 (50; 73)	78 (70; 95)		52 (43; 60)	76 (70; 86)	103 (98; 121)	
Insulin (pmol/L)												
Model A	81.3 (71.5; 91.0)	80.2 (70.1; 90.3)	70.7 (60.9; 80.6)	**0.05**	84.0 (74.2; 93.7)	76.9 (66.9; 86.8)	71.0 (61.0; 81.0)	0.24	81.1 (71.3; 90.9)	78.8 (68.6; 88.9)	72.3 (62.4; 82.2)	0.28
Model B	77.7 (67.6; 87.7)	78.8 (68.7; 88.9)	72.2 (62.4; 81.9)	0.23	79.2 (68.8; 89.6)	75.8 (66.0; 85.7)	73.5 (63.3; 83.6)	0.79	76.7 (66.7; 86.6)	78.7 (68.6; 88.7)	73.2 (63.4; 83.0)	0.51
Model C (conditional)	76.6 (66.6; 86.6)	80.5 (70.3; 90.6)	71.7 (62.0; 81.4)	0.28	79.6 (69.3; 89.9)	76.3 (66.6; 86.1)	72.6 (62.5; 82.7)	0.66	76.8 (67.0; 86.7)	79.1 (69.1; 89.0)	72.7 (63.0; 82.4)	0.54
HOMA2-%S												
Model A	71.1 (64.4; 78.6)	71.3 (64.3; 79.1)	80.5 (72.7; 89.0)	0.06	68.5 (62.0; 75.7)	75.7 (63.3; 83.9)	79.0 (71.3; 87.5)	0.28	70.8 (64.1; 78.3)	72.5 (65.4; 80.5)	79.4 (71.7; 87.9)	0.32
Model B	74.2 (67.0; 82.1)	72.3 (65.3; 80.1)	78.9 (71.5; 87.1)	0.27	72.5 (65.2; 80.5)	76.4 (69.2; 84.4)	76.6 (69.1; 84.9)	0.87	74.6 (67.5; 82.5)	72.3 (65.4; 80.0)	78.5 (71.1; 86.6)	0.57
Model C (conditional)	75.0 (67.9; 83.0)	71.0 (64.1; 78.6)	79.4 (72.0; 87.5)	0.33	72.1 (65.0; 80.1)	76.0 (68.9; 83.9)	77.3 (69.9; 85.6)	0.73	74.5 (67.5; 82.2)	72.0 (65.2; 79.6)	78.9 (71.6; 87.0)	0.61
Males	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Dietary sugar (g/d)[a]	110 (95, 128)	148 (135, 172)	173 (150, 200)		51 (42, 68)	73 (67, 89)	102 (86, 125)		66 (55, 77)	92 (81, 111)	129 (110, 141)	
Insulin (pmol/L)												
Model A	70.3 (60.0; 80.6)	74.5 (64.4; 84.5)	67.7 (57.5; 77.9)	0.91	65.9 (55.4; 76.4)	76.9 (67.2; 86.6)	68.9 (58.9; 79.0)	0.92	68.4 (57.8; 78.9)	76.2 (66.4; 86.1)	67.6 (57.6; 77.6)	0.28
Model B	70.5 (59.4; 81.5)	75.6 (65.3; 86.0)	67.9 (57.4; 78.4)	0.86	66.6 (55.6; 77.6)	76.6 (66.6; 86.6)	70.1 (59.7; 80.5)	0.72	69.0 (57.5; 80.4)	76.4 (66.3; 86.6)	68.6 (58.3; 78.9)	0.78
Model C (conditional)	70.5 (59.5; 81.5)	76.0 (65.7; 86.3)	67.5 (57.0; 78.0)	0.87	66.6 (55.6; 77.6)	76.8 (66.8; 86.8)	69.8 (59.4; 80.2)	0.79	68.5 (57.0; 79.9)	77.2 (67.0; 87.4)	68.1 (57.9; 78.4)	0.89
HOMA2-%S												
Model A	78.8 (70.2; 88.5)	79.5 (71.0; 89.1)	82.8 (73.8; 92.9)	0.85	84.0 (74.3; 94.5)	76.5 (68.5; 85.4)	81.4 (72.6; 91.2)	0.97	80.4 (71.4; 90.6)	77.8 (69.6; 87.0)	83.0 (74.1; 93.0)	0.93
Model B	79.2 (70.0; 89.7)	78.6 (70.0; 88.3)	83.0 (73.7; 93.3)	0.93	83.9 (74.1; 95.0)	77.2 (68.8; 86.4)	80.4 (71.5; 90.4)	0.69	80.4 (70.7; 91.5)	78.1 (69.7; 87.6)	82.1 (73.1; 82.2)	0.79
Model C (conditional)	79.2 (70.0; 89.7)	78.2 (69.6; 87.8)	83.5 (74.2; 93.9)	0.95	83.9 (74.2; 95.0)	77.0 (68.8; 86.1)	80.8 (71.9; 90.8)	0.77	81.0 (71.3; 92.1)	77.2 (68.9; 86.6)	82.7 (73.7; 92.8)	0.89

Values are adjusted least-squares means (95% CIs) unless otherwise indicated. Linear trends (P_{trend}) were obtained in sex-stratified linear regression models with the transformed and energy-adjusted predictors dietary fructose, sucrose, and glucose adolescent intakes as continuous variables. Model A adjusted for adult age at time of blood draw. Model B, with both outcomes HOMA2-%S and fasting insulin additionally adjusted for paternal education, birth weight, gestational weight gain, smoking in the household, parental overweight and pubertal percent body fat. Model C, the conditional model, additionally adjusted for adult percent body fat for all predictors and outcomes. Transformations of variables for analysis: log_e for HOMA2-%S, fasting insulin, total sugar intake; square root for added sugar and free sugar intakes. HOMA2-%S: updated homeostasis model assessment of insulin sensitivity. [a]Values are unadjusted medians (25th, 75th percentile). Bold values indicate significant findings ($p < 0.01$) or trends ($p < 0.05$).

TABLE 5 | Sex-stratified prospective associations of urinary fructose, urinary sucrose, and the sum of urinary fructose and sucrose excretion during adolescence with markers of insulin sensitivity in early adulthood [(n = 221: (109 males, 112 females))].

	Tertiles of urinary fructose				Tertiles of urinary fructose + sucrose			
Females	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Urinary sugar (mg/d)[a]	10.1 (7.9, 13.3)	21.2 (19.0, 24.5)	38.7 (32.3, 54.8)		27.0 (21.7, 34.3)	46.1 (41.0, 52.7)	79.4 (67.4, 110.9)	
Insulin (pmol/L)								
Model A	80.6 (70.7; 90.4)	82.2 (72.4; 91.9)	69.4 (59.5; 79.2)	**0.013**	78.2 (68.2; 88.2)	78.0 (67.9; 88.2)	76.1 (66.3; 85.9)	0.29
Model B	79.7 (70.0; 89.4)	81.9 (72.3; 91.4)	67.8 (58.1; 77.5)	**0.011**	76.9 (67.0; 86.9)	77.0 (67.1; 86.9)	75.7 (66.1; 85.3)	0.24
Model C (conditional)	80.4 (70.9; 89.8)	80.6 (71.3; 90.0)	68.6 (59.2; 78.1)	**0.007**	76.2 (66.6; 85.9)	79.0 (69.3; 88.8)	74.8 (65.5; 84.2)	0.18
HOMA2-%S								
Model A	69.3 (62.5; 76.9)	70.5 (63.6; 78.1)	83.0 (74.8; 92.1)	**0.015**	71.7 (64.4; 79.7)	73.9 (66.3; 82.4)	76.3 (68.8; 84.7)	0.31
Model B	70.0 (63.3; 77.4)	70.6 (64.0; 78.0)	84.7 (76.6; 93.6)	**0.013**	72.5 (65.4; 80.5)	74.9 (67.5; 83.1)	76.7 (69.3; 84.8)	0.25
Model C (conditional)	69.5 (63.0; 76.7)	71.4 (64.8; 78.7)	84.0 (76.2; 92.7)	**0.008**	73.0 (66.0; 80.9)	73.5 (66.3; 81.5)	77.3 (70.0; 85.3)	0.19
Males	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}	**Low (T1)**	**Moderate (T2)**	**High (T3)**	P_{trend}
Urinary sugar (mg/d)[a]	12.5 (9.9, 14.2)	22.3 (18.3, 23.2)	37.8 (32.5, 51.7)		31.6 (24.8, 37.1)	52.0 (44.7, 56.0)	89.7 (75.7, 117.8)	
Insulin (pmol/L)								
Model A	72.5 (59.8; 85.3)	79.9 (67.6; 92.1)	65.2 (52.7; 77.8)	0.20	76.2 (63.6; 88.9)	74.6 (62.1; 87.1)	67.2 (54.4; 79.9)	0.53
Model B	72.2 (59.4; 85.0)	80.7 (68.3; 93.1)	65.6 (53.2; 78.1)	0.23	75.2 (62.3; 88.0)	76.0 (63.4; 88.6)	67.3 (54.7; 80.0)	0.74
Model C (conditional)	73.6 (60.6; 86.7)	79.7 (67.1; 92.2)	65.0 (52.5; 77.5)	0.10	76.6 (63.7; 89.5)	77.1 (63.6; 88.7)	65.5 (52.6; 78.3)	0.18
HOMA2-%S								
Model A	79.0 (69.1; 90.2)	74.6 (65.7; 84.8)	85.6 (75.1; 97.6)	0.20	76.4 (66.9; 87.1)	78.7 (69.1; 89.6)	83.7 (73.3; 95.6)	0.50
Model B	79.4 (69.5; 90.8)	74.1 (65.1; 84.4)	85.4 (75.0; 97.2)	0.23	77.4 (67.7; 88.4)	77.6 (68.1; 88.5)	83.7 (67.7; 88.4)	0.71
Model C (conditional)	77.2 (67.5; 88.3)	75.6 (66.5; 86.0)	86.4 (76.0; 98.2)	0.10	75.5 (66.1; 86.1)	77.5 (68.2; 88.1)	86.4 (75.8; 98.5)	0.29

Values are adjusted least-squares means (95% CIs) unless otherwise indicated. Linear trends (P_{trend}) were obtained in sex-stratified linear regression models with the predictors urinary fructose, urinary sucrose, and sum of urinary fructose and sucrose as continuous variables. Model A adjusted for adult age at time of blood draw. Model B, with outcomes HOMA2-%S and fasting insulin, additionally adjusted for paternal education, pubertal percent body fat and gestational weight gain. The conditional Model C additionally adjusted for adult percent body fat for all predictors and outcomes. Transformations of variables for analysis: log_e for HOMA2-%S, fasting insulin; square root for excreted urinary fructose; $log_e(log_e)$ for sum of excreted fructose and sucrose. HOMA2-%S: updated homeostasis model assessment of insulin sensitivity. [a]Values are unadjusted medians (25th, 75th percentile). Bold values indicate significant findings ($p < 0.01$) or trends ($p < 0.05$).

Adolescent Sugar Intake and Adult Systemic Inflammation

Intakes of glucose, fructose, sucrose, total sugar, free sugar or added sugar as well as total sugar intakes from SSB, juice and sweets/sugar were not independently associated with the pro-inflammatory score in adulthood (all $p > 0.01$; see **Supplementary Tables 1, 2, 4**). Similarly, sugar excretion levels during adolescence were not associated with the pro-inflammatory score in adulthood (all $p > 0.01$, **Supplementary Table 3**).

Sensitivity Analyses

All sensitivity analyses yielded similar results as the main investigation, i.e., did not significantly change any observed associations. The results from the substitution analyses indicate that the replacement of each sugar type for non-sugar carbohydrates did not result in any significant associations for the outcomes of pro-inflammatory score (see **Supplementary Table 5**), fasting insulin and insulin sensitivity (see **Supplementary Table 6**).

DISCUSSION

In the present longitudinal study, a unique database compiled from self-reported sugar intake data and urinary fructose and sucrose excretion as dietary sugar intake biomarkers was used to investigate the role of dietary sugars in adolescence for adult risk markers of T2D. The main finding suggests that dietary

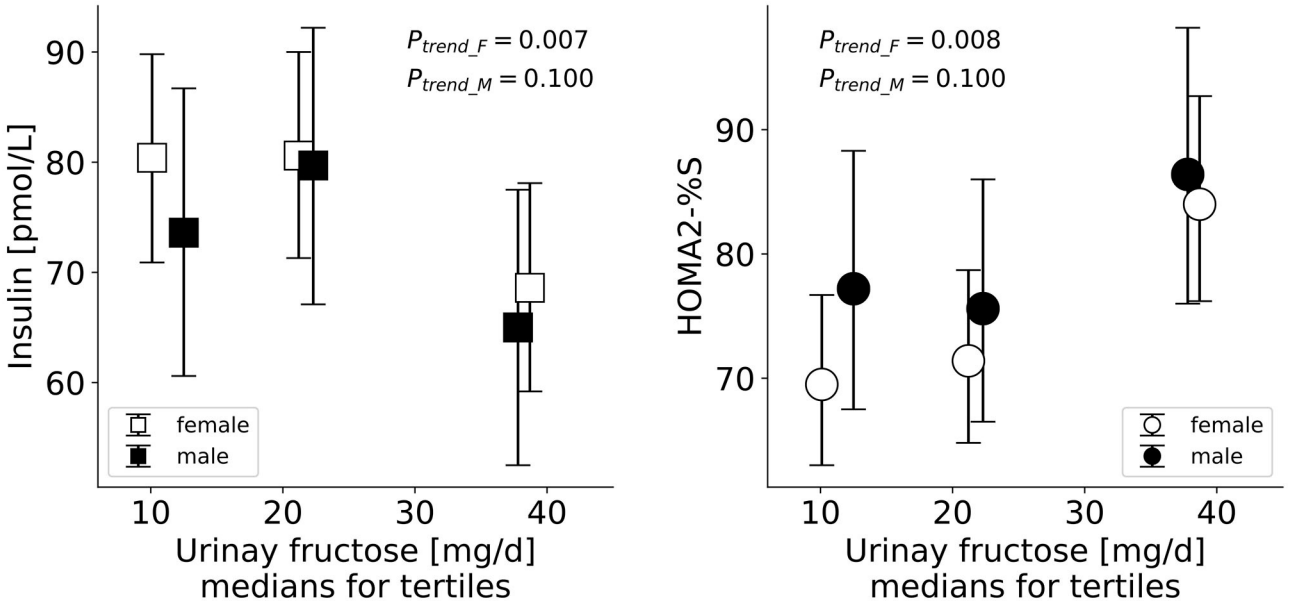

FIGURE 1 | Serum levels of fasting insulin and insulin sensitivity (HOMA2-%S) in early adulthood by tertiles of excreted urinary fructose among females and males in adolescence. Data are generic means and 95% CI adjusted for age at time of blood draw, paternal education, pubertal percent body fat, gestational weight gain and adult percent body fat.

sugar was not consistently related to adult T2D risk factors. The only exception was the urinary fructose biomarker, which was beneficially associated with HOMA2-S% and fasting insulin levels among females only. No other associations were found between the various dietary/urinary sugars and insulin sensitivity or chronic inflammation.

Other reported observational evidence was consistent with our prospective association between fructose intake and improved HOMA2-S% and insulin levels (49, 50), and further sources reporting on large cohorts found no association between fructose-containing sugars and incident T2D (51–53) contrary to the popular opinion that sugar intake increases risk for T2D. A meta-analysis of 15 prospective cohort studies reported no association of total sugar and fructose intake with T2D, and a higher sucrose consumption was associated with a decreased risk in T2D (54). The main predictors in these studies reported findings that emerged when investigating chemical sugar types, as was similarly done in our study. The observational studies referenced here similarly adjusted for anthropometric measures and energy intake as was done in our study but did not measure sugar intake by means of urinary biomarkers. When consumed in high amounts, dietary fructose has been associated in cohort studies with increased risk of T2D (55, 56). Inconsistent findings related to sugar intake and diabetes risk may result from varying levels of sugar intake and the possibility that different sugars elicit different metabolic effects (57). Our results pertaining to biomarkers of inflammation indicated no relationship with sugar intake. Only when analyzing sugar as a source of SSB was it associated with an increased pro-inflammatory score among females ($P_{\text{trend}} < 0.05$). This is consistent with observational evidence that consistently links SSB intake with increased chronic inflammation (more specifically CRP) (1, 20–22).

There is an array of categories and uses by which dietary sugar is defined and tested for in nutritional research. Broken down on a chemical level, the monosaccharides fructose and glucose and the disaccharide sucrose are assumed to have unique metabolic effects on outcomes of health. Other sugar categories of total, added, or free sugars may each be of physiological relevance, i.e., causing varying effects on absorption, satiety, caloric compensation, or insulin response. Since dietary assessment methods are prone to measurement errors (26) and sugars are among the nutrients that are frequently underreported (27, 28), objective dietary biomarkers of 24 h urinary sucrose and urinary fructose have been introduced (29, 30). The inconsistencies often found in epidemiological studies that investigate links between sugars and chronic disease may in part be due to the ambiguity of not only the definition and type of sugar but the sugar source as well (9–11). When the main sources of dietary fructose are fruits and vegetables in their whole form and not as juice, prospective studies have shown inverse associations with the risk of incident diabetes (58, 59). This may be related to factors specifically associated with fruit and vegetable intake, such as particular micronutrients or dietary patterns that are related to a lower risk of diabetes. Although fruit/vegetable juices contain bioactive compounds such as vitamins and phytochemicals, they are stripped of the fiber once had in their whole food form and have sugar and energy contents similar to SSB (60). Additionally, liquid sources of sugar affect satiety differently than solid sources (61). A distinction is made between different types of fruit juices; sugar-sweetened fruit juice has been reported to increase the risk of developing T2D in some prospective studies (55, 62), while in others 100% fruit juice showed no association (63–65) as confirmed by a meta-analysis (66). Sugar-sweetened fruit juice was defined as an SSB in our study, and our juice variable came

from fruit and vegetable sources; no associations were observed for fruit juice intake in our study.

Our finding relating to the inverse association of urinary fructose on insulin levels is in line with evidence from short-term trials that reported decreases in circulating insulin in subjects consuming fructose-sweetened beverages compared to glucose-sweetened beverages (13, 14). Fructose consumption causes smaller excursions in insulin due to its inability to stimulate the secretion of insulin from pancreatic beta cells. This was also confirmed by a meta-analysis of randomized trials wherein iso-energetic replacements of glucose and sucrose with fructose resulted in decreased insulin levels (56). On the other hand, our finding that indicates a beneficial association of fructose intake with insulin sensitivity was not confirmed by many intervention studies in which high proportions of fructose are consumed. These fructose over-consumption trials almost consistently report that higher intakes of fructose lead to decreases in insulin sensitivity (67–70). Many of the studies outlining the biological pathways of fructose administer high levels of pure fructose and the observed outcomes are not applicable to the amount of fructose typically consumed by humans, particularly considering that fructose is most often co-ingested with glucose *via* sucrose or HFCS in ratios similar to sucrose. The human diet rarely encounters fructose as a single nutrient. When looking at the effects of small doses of fructose, a meta-analysis reported that small fructose intakes in iso-energetic exchange improves HbA1c and fasting blood glucose but had no effect on insulin resistance (71). When assessing the effect of dietary fructose, a distinction needs to be made between trials that administer high vs. low doses. Of note, our DONALD population consumed relatively low amounts of fructose. Thus, the comparisons made between our findings and those above are not helpful in explaining our results, also because we investigated longer-term relevance which is different from a short- or medium-term response to fructose consumption (evidence from available randomized controlled trials) unless a metabolic adaptation occurs during adolescence. Considering adult dietary sugar intake in our population, it was unrelated to both the outcomes and the predictors and thus did not change the findings.

In considering why it was only among females that the beneficial association of fructose was observed, other DONALD studies also reported that females were more influenced by dietary changes than men (72, 73). It has been reported that women show more dramatic changes than men in hormones and body composition due to reproductive factors, which may cause them to react more sensitively to changes in dietary influences. Differences between men and women are biology-linked and caused by differences in sex chromosomes, hormones, and gene expression of sex-specific autosomes, which can each have effects on organ systems (74). Especially during adolescence when the fuel economy shifts away from fatty acid composition and ketogenesis toward carbohydrate oxidation, there is reduced metabolic flexibility making puberty a vulnerable period for changes in body composition (75). Women generally have lowered insulin sensitivity (75–77) (as was also observed in this present study) or increased impaired glucose tolerance than do males (74), which may increase their susceptibility or sensitivity to dietary influences.

Sugars are often among the nutrients that are frequently misreported and perceived negatively because they are a source of empty calories and are a common ingredient in unhealthy foods (27, 28). A possible explanation in the present analysis for the contrasting regression results between dietary fructose and urinary fructose is selective underreporting of sugar-rich foods, e.g., sugar sweetened beverages or sweets. There is to date no reliable method to identify selective sugar underreporting. Our sensitivity analyses excluding underreporters of energy intake, i.e., dietary records that had implausible energy intake values, yielded similar results. The use of urinary biomarkers to estimate dietary sugar intake may produce more reliable results as they are less subject to measurement and misreporting errors. The inconsistency in the reported findings of observational studies that investigate relations between sugar and disease outcomes may be due to the ambiguity of the employed dietary assessment methods. This being said, weighed dietary records as used by the DONALD study have been considered to be the most accurate dietary assessment tool for larger study populations, and measurement errors using these records are smaller than for other methods of assessment (78, 79). Evidence based on self-reported intake, however, may be considered lower-grade when compared to objective dietary biomarkers, especially due to selective underreporting of unhealthy foods (80). Neither fructose nor sucrose is endogenously synthesized, therefore urinary excretion has to be of dietary origin. A small amount of sucrose escapes from enzymatic hydrolysis in the small intestine and enters into blood stream before becoming excreted. For ingested fructose, a small proportion derived from free fructose and from hydrolysis of sucrose escapes hepatic fructose metabolism and is likewise excreted through the urine. In the existing literature it is still debated which sugars (extrinsic, intrinsic, total, added, free, etc.) are really captured by urinary sucrose and fructose excretion (29, 30, 81, 82). In a previous DONALD publication, it was found that dietary total sugar was more strongly associated with excreted fructose than dietary added sugar (83). While the relationship between intake and excretion is more complex for 24 h urinary sugars than for recovery biomarkers, they have been shown to reflect intake as so-called predictive biomarkers. Following extensive validation data, Tasevska et al. (84) have shown that it is possible to estimate actual intake from these markers when one considers age and sex. Both dietary and biomarker methods of assessment are analyzed and compared in this study; they have different sources of error and do not necessarily cover the exact same days of assessment (rather the same overall time period).

The main strength of the present study was its longitudinal design, including the long follow-up, which allowed the investigation of the long-term associations between dietary sugar intake in adolescence outcomes in young adulthood. Unlike many other observational studies of this nature, it was a strength that our study allowed comparisons of associations on the dietary as well as the urinary level. The urinary biomarkers are less subject to confounding by other nutrients or underreporting. In addition, our continuously updated in-house nutrient database

LEBTAB allowed the consideration of fructose, glucose and sucrose as well as different types of fructose-containing sugars (total sugar, free sugar, added sugar). Our study was able to consider brand-specific sugar content in commercial products as well as sugars or sweetening agents such as syrups and honey which are used for food preparation at home. Furthermore, the urine analyses were carried out in established laboratories by scientists with years of experience in the measurement of sugar excretion in 24 h-urine samples.

Our study was limited by the availability of only one blood sample in young adulthood. It could be argued that the follow-up time was rather short considering the younger age of the cohort, and therefore endpoints of incidence could not be assessed. Since T2D rates occur ever increasingly in younger populations we justified the decision to measure risk factors for T2D already in early adulthood. A further limitation in the methods used was the handling of our urine samples, which in contrast to previous studies (29, 30) were frozen without preservatives for a long period of time (the earliest 24 h urine was collected in 1985), which may have caused sucrose hydrolysis. Such a possible hydrolysis of sucrose would, however, query the successful application of urinary sucrose as a biomarker in large epidemiological studies in which urine samples are mostly stored without preservatives. Luceri et al. (58) were the first to examine urinary biomarkers for sugar intake referring only to the instability of sucrose in urine samples stored at room temperature. Since our samples were stored at $< -12^{\circ}C$ during the collection period at home as well as at $-22^{\circ}C$ in the study institute, our samples remained frozen until use. The generalizability of our results was limited due to the relatively high SES of the DONALD study population and high SES is known to correlate with lower dietary sugar intake (85). Nevertheless, our sugar intake data were similar to sugar intake in representative German nutrition survey (86, 87) as well as our sugar excretion data, which were similar to sugar excretion in other study populations (33, 88, 89).

In conclusion, these observational findings did not confirm that dietary sugar consumption in adolescence is related to insulin sensitivity in adulthood. The one potential exception to this was dietary fructose (as measured by a urinary fructose biomarker), which had a beneficial association with HOMA2-S% and fasting insulin levels among females in the context of a moderate fructose consumption pattern. No other associations were found between the various dietary/urinary sugars and insulin sensitivity or systemic inflammation.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the University of Bonn, Germany. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

INFORMED CONSENT

All assessments in the DONALD study were performed with parental and later with participants written informed consent.

AUTHOR CONTRIBUTIONS

AB, UA, and TR conceived the research project. CH and MR supervised laboratory measurements of blood analytes. GK carried out the sugar analyses of the urine samples. SW measured the insulin and glucose levels. KP prepared parts of the data set. KDC conducted the statistical analysis and wrote the manuscript. AB supervised the project and had primary responsibility for the final content. All authors made substantial contributions, critically read and revised the manuscript as well as approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2020.615684/full#supplementary-material

REFERENCES

1. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. *Circulation.* (2012) 125:1735–41. doi: 10.1161/CIRCULATIONAHA.111.067017
2. Malik VS, Popkin BM, Bray GA, Després J-P, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2

diabetes: a meta-analysis. *Diabetes Care*. (2010) 33:2477–83. doi: 10.2337/dc10-1079
3. Goran MI, Ulijaszek SJ, Ventura EE. High fructose corn syrup and diabetes prevalence: a global perspective. *Global Public Health*. (2013) 8:55–64. doi: 10.1080/17441692.2012.736257
4. O'Connor L, Imamura F, Lentjes MA, Khaw K-T, Wareham NJ, Forouhi NG. Prospective associations and population impact of sweet beverage intake and type 2 diabetes, and effects of substitutions with alternative beverages. *Diabetologia*. (2015) 58:1474–83. doi: 10.1007/s00125-015-3572-1
5. White JS. *Challenging the Fructose Hypothesis: New Perspectives on Fructose Consumption and Metabolism*. Oxford, UK: Oxford University Press (2013). doi: 10.3945/an.112.003137
6. Rippe JM, Angelopoulos TJ. *Sucrose, High-Fructose Corn Syrup, and Fructose, Their Metabolism and Potential Health Effects: What Do We Really Know?* Oxford, UK: Oxford University Press (2013). doi: 10.1007/978-1-4899-8077-9
7. Wang DD, Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, et al. Effect of fructose on postprandial triglycerides: a systematic review and meta-analysis of controlled feeding trials. *Atherosclerosis*. (2014) 232:125–33. doi: 10.1016/j.atherosclerosis.2013.10.019
8. Stanhope KL, Bremer AA, Medici V, Nakajima K, Ito Y, Nakano T, et al. Consumption of fructose and high fructose corn syrup increase postprandial triglycerides, LDL-cholesterol, and apolipoprotein-B in young men and women. *J Clin Endocrinol Metabolism*. (2011) 96:E1596–605. doi: 10.1210/jc.2011-1251
9. Flood-Obbagy JE, Rolls BJ. The effect of fruit in different forms on energy intake and satiety at a meal. *Appetite*. (2009) 52:416–22. doi: 10.1016/j.appet.2008.12.001
10. Houchins JA, Tan S-Y, Campbell WW, Mattes RD. Effects of fruit and vegetable, consumed in solid vs beverage forms, on acute and chronic appetitive responses in lean and obese adults. *Int J Obesity. (*2013) 37:1109–15. doi: 10.1038/ijo.2012.183
11. O'Connor L, Imamura F, Brage S, Griffin SJ, Wareham NJ, Forouhi NG. Intakes and sources of dietary sugars and their association with metabolic and inflammatory markers. *Clin Nutr*. (2018) 37:1313–22. doi: 10.1016/j.clnu.2017.05.030
12. Bohannon NV, Karam JH, Forsham PH. Endocrine responses to sugar ingestion in man. Advantages of fructose over sucrose and glucose. *J Am Diet Assoc*. (1980) 76:555–60.
13. Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metabolism*. (2004) 89:2963–72. doi: 10.1210/jc.2003-031855
14. Teff KL, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, et al. Endocrine and metabolic effects of consuming fructose-and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. *J Clin Endocrinol Metabolism*. (2009) 94:1562–9. doi: 10.1210/jc.2008-2192
15. Livesey G, Taylor R. Fructose consumption and consequences for glycation, plasma triacylglycerol, and body weight: meta-analyses and meta-regression models of intervention studies. *Am J Clin Nutr*. (2008) 88:1419–37. doi: 10.3945/ajcn.2007.25700
16. Evans RA, Frese M, Romero J, Cunningham JH, Mills KE. Fructose replacement of glucose or sucrose in food or beverages lowers postprandial glucose and insulin without raising triglycerides: a systematic review and meta-analysis. *Am J Clin Nutr*. (2017) 106:506–18. doi: 10.3945/ajcn.116.145151
17. Jin R, Welsh JA, Le N-A, Holzberg J, Sharma P, Martin DR, et al. Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. *Nutrients*. (2014) 6:3187–201. doi: 10.3390/nu6083187
18. Cox CL, Stanhope KL, Schwarz JM, Graham JL, Hatcher B, Griffen SC, et al. Circulating concentrations of monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and soluble leukocyte adhesion molecule-1 in overweight/obese men and women consuming fructose-or glucose-sweetened beverages for 10 weeks. *J Clin Endocrinol Metabolism*. (2011) 96:E2034–8. doi: 10.1210/jc.2011-1050
19. Della Corte KW, Perrar I, Penczynski KJ, Schwingshackl L, Herder C, Buyken AE. Effect of dietary sugar intake on biomarkers of subclinical inflammation: a systematic review and meta-analysis of intervention studies. *Nutrients*. (2018) 10:606. doi: 10.3390/nu10050606
20. Kosova EC, Auinger P, Bremer AA. The relationships between sugar-sweetened beverage intake and cardiometabolic markers in young children. *J Acad Nutr Diet*. (2013) 113:219–27. doi: 10.1016/j.jand.2012.10.020
21. Hert KA, Fisk PS II, Rhee YS, Brunt AR. Decreased consumption of sugar-sweetened beverages improved selected biomarkers of chronic disease risk among US adults: 1999 to 2010. *Nutr Res*. (2014) 34:58–65. doi: 10.1016/j.nutres.2013.10.005
22. Schulze MB, Hoffmann K, Manson JE, Willett WC, Meigs JB, Weikert C, et al. Dietary pattern, inflammation, and incidence of type 2 diabetes in women–. *Am J Clin Nutr*. (2005) 82:675–84. doi: 10.1093/ajcn/82.3.675
23. St-Onge M-P, Keller KL, Heymsfield SB. Changes in childhood food consumption patterns: a cause for concern in light of increasing body weights. *Am J Clin Nutri*. (2003) 78:1068–73. doi: 10.1093/ajcn/78.6.1068
24. Nicklas TA, Elkasabany A, Srinivasan SR, Berenson G. Trends in nutrient intake of 10-year-old children over two decades (1973–1994) The Bogalusa Heart Study. *Am J Epidemiol*. (2001) 153:969–77. doi: 10.1093/aje/153.10.969
25. Goletzke J, Herder C, Joslowski G, Bolzenius K, Remer T, Wudy SA, et al. Habitually higher dietary glycemic index during puberty is prospectively related to increased risk markers of type 2 diabetes in younger adulthood. *Diabetes Care*. (2013) 36:1870–6. doi: 10.2337/dc12-2063
26. Biro G, Hulshof K, Ovesen L, Cruz JA. Selection of methodology to assess food intake. *Eur J Clin Nutr*. (2002) 56:S25–32. doi: 10.1038/sj.ejcn.1601426
27. Krebs-Smith S, Graubard B, Kahle L, Subar A, Cleveland L, Ballard-Barbash R. Low energy reporters vs. others: a comparison of reported food intakes. *Eur J Clin Nutr*. (2000) 54:281–7. doi: 10.1038/sj.ejcn.1600936
28. Price G, Paul A, Cole T, Wadsworth MJ. Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Br J Nutr*. (1997) 77:833–51. doi: 10.1079/BJN19970083
29. Tasevska N, Runswick SA, McTaggart A, Bingham SA. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev*. (2005) 14:1287–94. doi: 10.1158/1055-9965.EPI-04-0827
30. Luceri C, Caderni G, Lodovici M, Spagnesi MT, Monserrat C, Lancioni L, et al. Urinary excretion of sucrose and fructose as a predictor of sucrose intake in dietary intervention studies. *Cancer Epidemiol Biomarkers Prev*. (1996) 5:167–71.
31. Kroke A, Manz F, Kersting M, Remer T, Sichert-Hellert W, Alexy U, et al. The DONALD study. *Eur J Nutr*. (2004) 43:45–54. doi: 10.1007/s00394-004-0445-7
32. Sichert-Hellert W, Kersting M, Schöch G. Underreporting of energy intake in 1 to 18 year old German children and adolescents. *Z Ernährungswiss*. (1998) 37:242–51. doi: 10.1007/s003940050023
33. Kuhnle GG, Joosen AM, Wood TR, Runswick SA, Griffin JL, Bingham SA. Detection and quantification of sucrose as dietary biomarker using gas chromatography and liquid chromatography with mass spectrometry. *Rapid Commun Mass Spectrom*. (2008) 22:279–82. doi: 10.1002/rcm.3355
34. Schofield W. Predicting basal metabolic rate, new standards and review of previous work. *Hum Nutr Clin Nutr*. (1985) 39:5–41.
35. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ*. (2000) 320:1240. doi: 10.1136/bmj.320.7244.1240
36. Swan GE, Powell NA, Knowles BL, Bush MT, Levy LB. A definition of free sugars for the UK. *Publ Health Nutr*. (2018) 21:1636–8. doi: 10.1017/S136898001800085X
37. Kromeyer-Hauschild K, Wabitsch M, Kunze D, Geller F, Geiß HC, Hesse V, et al. Perzentile für den Body-mass-Index für das Kindes-und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschrift Kinderheilkunde*. (2001) 149:807–18. doi: 10.1007/s001120170107
38. Remer T, Neubert A, Maser-Gluth C. Anthropometry-based reference values for 24 h urinary creatinine excretion during growth and their use in endocrine and nutritional research. *Am J Clin Nutr*. (2002) 75:561–9. doi: 10.1093/ajcn/75.3.561
39. Perrar I, Gray N, Kuhnle GG, Remer T, Buyken AE, Alexy U. Sugar intake among German adolescents: trends from 1990 to 2016

based on biomarker excretion in 24 h urine samples. *Br J Nutr*. (2020). doi: 10.1017/S0007114520000665. [Epub ahead of print].
40. OCDEM. *HOMA2 Calculator*. (2020). Available online at: https://www.dtu.ox.ac.uk/homacalculator/download.php
41. Wang X, Bao W, Liu J, OuYang Y-Y, Wang D, Rong S, et al. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care*. (2013) 36:166–75. doi: 10.2337/dc12-0702
42. Herder C, Carstensen M, Ouwens D. Anti-inflammatory cytokines and risk of type 2 diabetes. *Diabetes Obes Metab*. (2013) 15:39–50. doi: 10.1111/dom.12155
43. Herder C, Baumert J, Thorand B, Koenig W, De Jager W, Meisinger C, et al. Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984–2002. *Diabetologia*. (2006) 49:921. doi: 10.1007/s00125-006-0190-y
44. Muris DM, Houben AJ, Schram MT, Stehouwer CD. Microvascular dysfunction is associated with a higher incidence of type 2 diabetes mellitus: a systematic review and meta-analysis. *Arterioscler Thromb Vasc Biol*. (2012) 32:3082–94. doi: 10.1161/ATVBAHA.112.300291
45. Kaptoge S, Seshasai SRK, Gao P, Freitag DF, Butterworth AS, Borglykke A., et al. Inflammatory cytokines and risk of coronary heart disease: new prospective study and updated meta-analysis. *Eur Heart J*. (2014) 35:578–89. doi: 10.1093/eurheartj/eht367
46. Penczynski KJ, Herder C, Krupp D, Rienks J, Egert S, Wudy SA, et al. Flavonoid intake from fruit and vegetables during adolescence is prospectively associated with a favourable risk factor profile for type 2 diabetes in early adulthood. *Eur J Nutr*. (2019) 58:1159–72. doi: 10.1007/s00394-018-1631-3
47. Diederichs T, Herder C, Roßbach S, Roden M, Wudy SA, Nöthlings U, et al. Carbohydrates from sources with a higher glycemic index during adolescence: is evening rather than morning intake relevant for risk markers of type 2 diabetes in young adulthood? *Nutrients*. (2017) 9:591. doi: 10.3390/nu9060591
48. Hu FB, Malik VS. Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. *Physiol Behav*. (2010) 100:47–54. doi: 10.1016/j.physbeh.2010.01.036
49. Ahmadi-Abhari S, Luben RN, Powell N, Bhaniani A, Chowdhury R, Wareham NJ, et al. Dietary intake of carbohydrates and risk of type 2 diabetes: the European Prospective Investigation into Cancer-Norfolk study. *Br J Nutr*. (2014) 111:342–52. doi: 10.1017/S0007114513002298
50. Hodge AM, English DR, O'Dea K, Giles GG. Glycemic index and dietary fiber and the risk of type 2 diabetes. *Diabetes Care*. (2004) 27:2701–6. doi: 10.2337/diacare.27.11.2701
51. Schulze MB, Schulz M, Heidemann C, Schienkiewitz A, Hoffmann K, Boeing H. Carbohydrate intake and incidence of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Br J Nutr*. (2008) 99:1107–16. doi: 10.1017/S0007114507853360
52. Villegas R, Liu S, Gao Y-T, Yang G, Li H, Zheng W, et al. Prospective study of dietary carbohydrates, glycemic index, glycemic load, and incidence of type 2 diabetes mellitus in middle-aged Chinese women. *Arch Intern Med*. (2007) 167:2310–6. doi: 10.1001/archinte.167.21.2310
53. Janket S-J, Manson JE, Sesso H, Buring JE, Liu S. A prospective study of sugar intake and risk of type 2 diabetes in women. *Diabetes Care*. (2003) 26:1008–15. doi: 10.2337/diacare.26.4.1008
54. Tsilas CS, de Souza RJ, Mejia SB, Mirrahimi A, Cozma AI, Jayalath VH, et al. Relation of total sugars, fructose and sucrose with incident type 2 diabetes: a systematic review and meta-analysis of prospective cohort studies. *CMAJ*. (2017) 189:E711–20. doi: 10.1503/cmaj.160706
55. Montonen J, Jarvinen R, Knekt P, Heliovaara M, Reunanen A. Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. *J Nutr*. (2007) 137:1447–54. doi: 10.1093/jn/137.6.1447
56. Wu T, Giovannucci E, Pischon T, Hankinson SE, Ma J, Rifai N, et al. Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentrations in US women. *Am J Clin Nutr*. (2004) 80:1043–9. doi: 10.1093/ajcn/80.4.1043
57. Kim HS, Paik HY, Lee KU, Lee HK, Min HK. Effects of several simple sugars on serum glucose and serum fructose levels in normal and diabetic subjects. *Diabetes Res Clin Pract*. (1988) 4:281–7. doi: 10.1016/S0168-8227(88)80030-5
58. Harding A-H, Wareham NJ, Bingham SA, Khaw K, Luben R, Welch A, et al. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: the European prospective investigation of cancer–Norfolk prospective study. *Arch Intern Med*. (2008) 168:1493–9. doi: 10.1001/archinte.168.14.1493
59. Bazzano LA, Li TY, Joshipura KJ, Hu FB. Intake of fruit, vegetables, and fruit juices and risk of diabetes in women. *Diabetes Care*. (2008) 31:1311–7. doi: 10.2337/dc08-0080
60. Pepin A, Stanhope KL, Imbeault P. Are fruit juices healthier than sugar-sweetened beverages? A review. *Nutrients*. (2019) 11:1006. doi: 10.3390/nu11051006
61. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. *Int J Obes*. (2000) 24:794–800. doi: 10.1038/sj.ijo.0801229
62. Muraki I, Imamura F, Manson JE, Hu FB, Willett WC, van Dam RM, et al. Fruit consumption and risk of type 2 diabetes: results from three prospective longitudinal cohort studies. *BMJ*. (2013) 347:f5001. doi: 10.1136/bmj.f5001
63. Fagherazzi G, Vilier A, Saes Sartorelli D, Lajous M, Balkau B, Clavel-Chapelon F. Consumption of artificially and sugar-sweetened beverages and incident type 2 diabetes in the Etude Epidémiologique auprès des femmes de la Mutuelle Générale de l'Education Nationale–European Prospective Investigation into Cancer and Nutrition cohort. *Am J Clin Nutr*. (2013) 97:517–23. doi: 10.3945/ajcn.112.050997
64. Palmer JR, Boggs DA, Krishnan S, Hu FB, Singer M, Rosenberg L. Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. *Arch Intern Med*. (2008) 168:1487–92. doi: 10.1001/archinte.168.14.1487
65. Eshak ES, Iso H, Mizoue T, Inoue M, Noda M, Tsugane S. Soft drink, 100% fruit juice, and vegetable juice intakes and risk of diabetes mellitus. *Clin Nutr*. (2013) 32:300–8. doi: 10.1016/j.clnu.2012.08.003
66. Xi B, Li S, Liu Z, Tian H, Yin X, Huai P, et al. Intake of fruit juice and incidence of type 2 diabetes: a systematic review and meta-analysis. *PLoS ONE*. (2014) 9:e93471. doi: 10.1371/journal.pone.0093471
67. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest*. (2009) 119:1322–34. doi: 10.1172/JCI37385
68. Hallfrisch J, Ellwood KC, Michaelis OE IV, Reiser S, O'Dorisio TM, Prather ES. Effects of dietary fructose on plasma glucose and hormone responses in normal and hyperinsulinemic men. *J Nutr*. (1983) 113:1819–26. doi: 10.1093/jn/113.9.1819
69. Beck-Nielsen H, Pedersen O, Lindskov H. Impaired cellular insulin binding and insulin sensitivity induced by high-fructose feeding in normal subjects. *Am J Clin Nutr*. (1980) 33:273–8. doi: 10.1093/ajcn/33.2.273
70. Celermajer DS, Ayer JG. Childhood risk factors for adult cardiovascular disease and primary prevention in childhood. *Heart*. (2006) 92:1701–6. doi: 10.1136/hrt.2005.081760
71. Noronha JC, Braunstein CR, Blanco Mejia S, Khan TA, Kendall CW, Wolever T, et al. The effect of small doses of fructose and its epimers on glycemic control: a systematic review and meta-analysis of controlled feeding trials. *Nutrients*. (2018) 10:1805. doi: 10.3390/nu10111805
72. Joslowski G, Remer T, Assmann KE, Krupp D, Cheng G, Garnett SP, et al. Animal protein intakes during early life and adolescence differ in their relation to the growth hormone-insulin-like-growth-factor axis in young adulthood. *J Nutr*. (2013) 143:1147–54. doi: 10.3945/jn.113.175877
73. Krupp D, Johner SA, Kalhoff H, Buyken AE, Remer T. Long-term dietary potential renal acid load during adolescence is prospectively associated with indices of nonalcoholic fatty liver disease in young women. *J Nutr*. (2012) 142:313–9. doi: 10.3945/jn.111.150540
74. Kautzky-Willer A, Harreiter J, Pacini G. Sex and gender differences in risk, pathophysiology and complications of type 2 diabetes mellitus. *Endocr Rev*. (2016) 37:278–316. doi: 10.1210/er.2015-1137
75. Cominetti O, Hosking J, Jeffery A, Pinkney J, Martin F-P. Contributions of fat and carbohydrate metabolism to glucose homeostasis in childhood change with age and puberty: a 12-years Cohort study (EARLYBIRD 77). *Front Nutri*. (2020) 37:278–31. doi: 10.3389/fnut.2020.00139
76. Jeffery SC, Hosking J, Jeffery AN, Murphy MJ, Voss LD, Wilkin TJ, et al. Insulin resistance is higher in prepubertal girls but switches to become higher in boys at age 16: A Cohort Study (EarlyBird 57). *Pediatr Diabetes*. (2018) 19:223–30. doi: 10.1111/pedi.12571

77. Jeffery AN, Metcalf BS, Hosking J, Streeter AJ, Voss LD, Wilkin TJ. Age before stage: insulin resistance rises before the onset of puberty: a 9-year longitudinal study (EarlyBird 26). *Diabetes Care*. (2012) 35:536–41. doi: 10.2337/dc11-1281
78. Bingham S, Cassidy A, Cole T, Welch A, Runswick S, Black A, et al. Validation of weighed records and other methods of dietary assessment using the 24 h urine nitrogen technique and other biological markers. *Br J Nutr*. (1995) 73:531–50. doi: 10.1079/BJN19950057
79. Gibson RS. *Principles of Nutritional Assessment*. Oxford, UK: Oxford University Press (2005).
80. Poppitt S, Swann D, Black A, Prentice A. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes*. (1998) 22:303–11. doi: 10.1038/sj.ijo.0800584
81. Tasevska N, Runswick S, Welch A, McTaggart A, Bingham S. Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr*. (2009) 63:653–9. doi: 10.1038/ejcn.2008.21
82. Beasley J, Jung M, Tasevska N, Wong W, Siega-Riz A, Sotres-Alvarez D, et al. Biomarker-predicted sugars intake compared with self-reported measures in US Hispanics/Latinos: results from the HCHS/SOL SOLNAS study. *Publ Health Nutr*. (2016) 19:3256–64. doi: 10.1017/S1368980016001580
83. Johner SA, Libuda L, Shi L, Retzlaff A, Joslowski G, Remer T. Urinary fructose: a potential biomarker for dietary fructose intake in children. *Eur J Clin Nutr*. (2010) 64:1365–70. doi: 10.1038/ejcn.2010.160
84. Tasevska N, Midthune D, Potischman N, Subar AF, Cross AJ, Bingham SA, et al. Use of the predictive sugars biomarker to evaluate self-reported total sugars intake in the Observing Protein and Energy Nutrition (OPEN) study. *Cancer Epidemiol Biomarkers Prev*. (2011) 20:490–500. doi: 10.1158/1055-9965.EPI-10-0820
85. Thompson FE, McNeel TS, Dowling EC, Midthune D, Morrissette M, Zeruto CA. Interrelationships of added sugars intake, socioeconomic status, and race/ethnicity in adults in the United States: National Health Interview Survey, 2005. *J Am Diet Assoc*. (2009) 109:1376–83. doi: 10.1016/j.jada.2009.05.002
86. T H. Zuckerkonsum in Deutschland. *Aktuel Ernahrungsmed*. (2018) 43:S8–11. doi: 10.1055/a-0659-8828
87. Mensink GBM., Heseker H, Stahl A, Richter A, Vohmann C. Die aktuelle Nährstoffversorgung von Kindern und Jugendlichen in Deutschland. *Ernährungsumschau*. (2007) 54:636–46.
88. Intemann T, Pigeot I, De Henauw S, Eiben G, Lissner L, Krogh V, et al. Urinary sucrose and fructose to validate self-reported sugar intake in children and adolescents: results from the I. Family study. *Eur J Nutr*. (2019) 58:1247–58. doi: 10.1007/s00394-018-1649-6
89. Joosen A, Kuhnle G, Runswick S, Bingham S. Urinary sucrose and fructose as biomarkers of sugar consumption: comparison of normal weight and obese volunteers. *Int J Obes*. (2008) 32:1736–40. doi: 10.1038/ijo.2008.145

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ORIGINAL RESEARCH
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Changes in Added Sugar Intake and Body Weight in a Cohort of Older Australians: A Secondary Analysis of the Blue Mountains Eye Study

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Background: The evidence regarding the association between added sugar (AS) intake and obesity remains inconsistent. The aim of this study was to investigate the association between changes in the percentage of energy intake from AS (EAS%) and changes in body weight in a cohort study of older Australians during 15 years of follow-up. In addition, associations were assessed according to whether EAS% intake was provided from beverage or non-beverage sources.

Methods: Data were analyzed from the participants of the Blue Mountains Eye Study Cohort. Dietary data were collected at baseline (1992–94) and three five-yearly intervals using a 145-item food frequency questionnaire. Participants' body weight was measured at each time point. Five-yearly changes in EAS% intake and body weight were calculated (n = 1,713 at baseline). A generalized estimating equation (GEE) model was used to examine the relationship between the overall five-yearly changes in EAS% intake and body weight, adjusted for dietary and lifestyle variables.

Results: In each time interval, the EAS% intake decreased by ∼5% in the lowest quartile (Q1) and increased by ∼5% in the highest quartile (Q4). The mean (SD) body weight change in Q1 and Q4 were 1.24 (8.10) kg and 1.57 (7.50) kg (first time interval), 0.08 (6.86) kg and −0.19 (5.63) kg (second time interval), and −1.22 (5.16) kg and −0.37 (5.47) kg (third time interval), respectively. In GEE analyses, the overall five-yearly change in EAS% intake was not significantly associated with body weight change (P_{trend} = 0.837). Furthermore, no significant associations were observed between changes in EAS% intake from either beverage or non-beverage sources and changes in body weight ($P_{trend\ for\ beverage\ sources}$ = 0.621 and $P_{trend\ for\ non\text{-}beverage\ sources}$ = 0.626).

Conclusion: The findings of this older Australian cohort do not support the association between changes in EAS% intake and body weight, regardless of AS food sources (beverage or non-beverage).

Keywords: added sugar intake, added sugar food sources, body weight, Blue Mountains Eye Study, older adults, cohort study

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INTRODUCTION

Obesity is a major public health issue in all age groups, including the elderly. Several physical and mental health complications (e.g., cardiovascular disease and depression) are associated with obesity in this population (1). Obesity has a negative impact on the overall quality of life and chronic disease-free life expectancy of older adults (2). Therefore, there is a global focus on the prevention of obesity to reduce the financial and psychosocial burden of the obesity-associated chronic diseases, particularly among the older sectors of the population (3–5). Of the multifactorial causes of obesity, diet is a modifiable risk factor and a target for many weight loss programs (6).

Diets high in energy-dense foods have been linked to obesity (7), and added sugar (AS) has been identified as a major component in many energy-dense foods (8). AS is defined as sugars and syrups added to foods during processing and preparation (9). Honey and concentrated fruit juices used as an ingredient in the multi-ingredient foods are also considered to be AS (10). This is similar to the World Health Organization free sugar definition where all monosaccharaides and disaccharides added to foods (by the manufacturer, cook, or consumer) and sugars naturally present in honey, syrups, fruit juices, and fruit juice concentrates are considered in the free sugar definition (11).

Several intervention studies have investigated the effect of AS intake on the weight gain or development of obesity. These studies accounted for individual types of sugars (e.g., fructose or glucose) or specific food sources of AS (i.e., sugar-sweetened beverages) (12–15), but did not consider the contribution of total AS intake. In addition, some studies were short-term (<1 month) (12, 13, 15), therefore, the generalization of the outcomes of these studies to the general population and everyday diets is limited. Conversely, cohort studies with longer follow-up periods have tended to focus on sugar-sweetened beverages as major sources of AS. In most cases, these studies have reported a positive association between the intake of these beverages and indicators of obesity (16, 17). Nevertheless, the association has not been confirmed in all studies nor across all age groups, including older adults (18, 19).

Few cohort studies have been conducted to investigate the association between baseline AS intake and change in body weight or body mass index (BMI) (19, 20). A longitudinal study that investigated the change in AS intake in children and adolescents reported no significant associations between AS intake, or AS intake from liquid or solid food sources, and BMI when the analysis was adjusted for total energy intake (21). This was contrary to findings from other cohort studies, in which increases in the intake of sugar sweetened beverages were associated with an increased BMI (22, 23). In addition to inconsistent findings for the liquid food sources of AS, results of studies on the association between other AS food sources (e.g., sweets, desserts, and other solid sources) and obesity indicators (24, 25) were also contradictory.

To our knowledge, the longitudinal association between change in AS intake and body weight change over time has not been investigated in adults, including older populations. The aim of the current study is, therefore, to investigate the associations between change in the percentage of energy intake from AS (EAS%) and body weight change in a longitudinal cohort of older Australians during 15 years of follow-up. In addition, associations were assessed according to whether EAS% intake was provided from beverage or non-beverage sources.

METHODS

Study Population

This study is a secondary analysis of data from the Blue Mountains Eye Study (BMES) cohort. Details of the BMES have been described in detail elsewhere (26). Briefly, participants of the BMES were aged ≥49 years old at baseline and lived in two postcode areas within the Blue Mountains region, New South Wales, Australia. Baseline data collection (BMES 1) was conducted between 1992 and 1994 and subsequent data were collected every 5 years (BMES 2:1997–99, BMES 3:2002–04, and BMES 4:2007–09). The BMES has ethics approval from the Sydney West Area Health Services and the University of Sydney Human Research Ethics Committees. Written informed consent was obtained from all participants.

Dietary Data Collection

Dietary data were collected using a validated 145-item semi-quantitative food frequency questionnaire (FFQ) (27) and analyzed using corresponding food composition data from the Australian Nutrient Tables (NUTTAB) to assess nutrient intakes. NUTTAB 1990 was used for the BMES 1, NUTTAB 1995 for BMES 2 and 3 and NUTTAB 2010 for BMES 4, to align with the food supply at each follow up time-points. The NUTTAB databases contain the nutrient content of various foods, including total sugars, but do not contain AS values. Therefore, the AS content of FFQ food items was estimated for each NUTTAB using a stepwise method (28). In this 10-step systematic method, foods with zero total sugar and natural/unprocessed foods were considered to have zero AS. Foods containing 100% AS (i.e., no naturally occurring sugar), such as regular soft drinks, were considered to have an AS content equivalent to the total sugar content (28). The AS content of other foods was estimated from recipes, comparisons of the total sugar content of sweetened products with unsweetened varieties, analytical data for individual sugar types (i.e., lactose and maltose), adoption of values from other countries' AS databases or using food labels (28).

FFQ dietary intake data were cleaned for implausible energy and nutrient intakes (26). For example, FFQs with 12 blank questions or blank page(s) were excluded. Participants with implausible energy intakes of <2,500 kJ and >18,000 kJ or extreme AS intakes of more than the mean ± 4 Standard Deviation (SD) were also excluded. For this study, only participants who provided both dietary and body weight data at two consecutive time points (paired observations) were included in the analyses. These paired observations were provided by 1,713 participants in the first time interval (BMES 1–2), 1,209 participants in the second time interval (BMES 2–3) and 747 participants in the third time interval (BMES 3–4). Thus, the total number of paired observations were 3,669. Throughout this

study, the beginning of each time interval is referred to as the "initial" time point.

Classification of Beverage vs. Non-beverage Added Sugar Sources

Food groups were developed (29) based on the 1995 Australian National Nutrition Survey (NNS1995) (30). For AS analyses, food groups were modified to represent AS food groups (31) and were then classified to beverage (liquid) or non-beverage (non-liquid: semi-solid/solid) categories. The beverage category included the sugar-sweetened beverages group and was formed based on the definition of sugar-sweetened beverages. According to this definition, water-based non-alcoholic beverages containing AS, including regular soft drinks, cordial, electrolyte and energy drinks, fruit, and vegetable drinks (excluding milk, 100% fruit juice, and artificially sweetened drinks) are considered sugar-sweetened beverages (32, 33). Thus, in this study, the beverage category included sugar-sweetened beverages (sweetened juices, cordial and soft drinks) and the non-beverage category included cereal products (breakfast cereals), cereal-based products and dishes (biscuits, cakes, buns and scones, pastries, and mixed dishes), dairy products and dishes (yogurt, custard, ice cream, and dairy-based desserts), sugar products and dishes (discretionary sugar, honey, jam, and syrup), confectionary (sweets and chocolate), savory sauces, meat (processed), and vegetables (processed/canned varieties).

Assessment of Body Weight and Covariates

For the BMES anthropometry assessments, participants' body weight (kg) was measured with electronic scales (without shoes or heavy clothes) (34, 35) at each time point. Since physical stature declines in aging populations (36), change in weight over time was used as an outcome rather than change in BMI. For assessing the covariates, information was collected about the participants' medical history and socio-demographic and lifestyle factors by trained interviewers at all BMES time points using extensive questionnaires (37, 38). Some of these questions were about participants' smoking status (i.e., never smoked, past smoker, or current smoker), and physical activity (39). Physical activity questions collected information regarding walking exercises and moderate-to-vigorous activities over the previous 2 weeks (39). Metabolic equivalents (METs) over 1 week were calculated based on participants' responses to the questions (39), using the International Physical Activity Questionnaire scoring protocol (40). This information was used in the current study to adjust data for smoking and physical activity.

Statistical Analyses

The changes in EAS% intake were reported as quartiles at each 5-year time interval (BMES 1–2, BMES 2–3, and BMES 3–4). The longitudinal associations between the overall 5-yearly changes in EAS% and changes in body weight during the 15 years of follow-up were investigated using a generalized estimating equation (GEE) model. The unstructured correlation matrix was selected for the GEE model to consider the within-individual correlations between the repeated observations for the same participant. In this longitudinal analysis, the first and last quartiles represented the largest decrease and the largest increase in EAS% intakes for each time interval, respectively. The change in EAS% intake was used as a continuous variable to assess *P* for trends.

The GEE analysis was adjusted for gender and the initial age of participants in each paired observation (Model 1). Further adjustments were made for the initial data for weight, diabetes status and EAS% intake, and both the initial and the changes in each time interval for the following variables: fiber intake, total energy intake, glycaemic index, physical activity, and smoking status (Model 2). Similar adjustment models were used for AS food source analyses by replacing EAS% intake variable with EAS% intakes from beverage and non-beverage sources. All analyses were performed in SPSS software (Version 21, SPSS Inc., Chicago, IL, USA) and statistical significance was set at $P < 0.05$.

RESULTS

Participants Characteristics

The baseline characteristics of the BMES 1 participants are shown in **Table 1**. The mean age of BMES 1 participants was 63.8 years. The baseline mean EAS% intake was 9.40% (1.02% from beverage and 8.38% from non-beverage sources) and their mean BMI was 26.22 kg/m^2. A higher proportion of the participants in the lowest quartile were smokers compared to those in other quartiles. Participants in quartile 1 had higher EAS% intakes and BMI compared to those in quartile 4. In addition, their EAS% intakes from beverage and non-beverage sources were higher than other participants. Baseline characteristics of BMES 1 participants who provided paired observations in BMES 2 vs. those without paired observations are presented in **Supplementary Table 1**. Participants with paired observations had a slightly lower EAS% intake compared to those without paired observations (9.40 vs. 10.14%), but their BMI was similar.

Changes in Added Sugar and Weight in Each Time Interval

Change in dietary intake and weight across quartiles of changes in EAS% intake at the first, second and third time intervals are presented in **Table 2**. The EAS% intake at 5-year intervals decreased by ∼5% in the lowest quartile and increased by ∼5% in the highest quartile. The mean of change in energy intake was <200 kJ in the lowest quartiles and <75 kJ in the highest quartiles. The median change in AS intake for the largest decrease (−4.85%) was −26.39 g in the third time interval and for the largest increase (5.19%) was 26.59 g in the first time interval.

In each time interval, participants in the lowest quartile who decreased their EAS% intake, increased their protein, other carbohydrates, fat or alcohol intakes; whereas participants in the highest quartile who increased their EAS% intakes decreased their protein, other carbohydrates, alcohol, or fat intakes. The weight gain occurred across all quartiles in the first and the second time intervals (except quartile 4 in the second time interval), and the mean body weight change in the lowest quartile was <1.3 kg and in the highest quartile was <1.6 kg. In the third time interval, those who increased their EAS% intake experienced less weight loss compared to those who decreased

TABLE 1 | Baseline characteristics of BMES participants according to quartiles of change in percentage of energy from added sugar (EAS%) intake during the first 5-year time interval (BMES 1–2).

	Change in EAS% intake				
	All	**Q1**	**Q2**	**Q3**	**Q4**
Median	0.22	−4.50	−0.83	1.51	5.19
n	1,713	428	428	429	428
Women (*n*, %)	978 (57.09)	227 (53.04)	226 (52.80)	256 (59.67)	269 (62.85)
Age, years	63.8 (8.2)	63.5 (8.6)	64.3 (7.9)	63.0 (8.1)	64.4 (8.2)
Current smoker (*n*, %)	199 (11.62)	66 (15.42)	43 (10.05)	40 (9.32)	50 (11.68)
Diabetes (*n*, %)	104 (6.07)	25 (5.84)	28 (6.54)	29 (6.76)	22 (5.14)
Married (*n*, %)	1,186 (69.24)	278 (64.95)	308 (71.96)	311 (72.49)	289 (67.52)
Qualification after leaving school (*n*, %)	1,040 (60.71)	248 (57.94)	269 (62.85)	265 (61.77)	258 (60.28)
Home ownership (*n*, %)	1,567 (91.48)	389 (90.89)	394 (92.06)	401 (93.47)	383 (89.49)
Living alone (*n*, %)	381 (22.24)	105 (24.53)	94 (21.96)	80 (18.65)	102 (23.83)
Energy, kJ	8,599 (2,481)	8,614 (2,675)	8,727 (2,494)	8,739 (2,345)	8,318 (2,385)
Fat, E%	32.82 (6.21)	32.44 (6.04)	32.97 (6.07)	32.90 (6.33)	32.96 (6.39)
Protein, E%	17.76 (3.08)	16.70 (2.86)	17.80 (2.77)	18.45 (2.99)	18.09 (3.39)
Alcohol, E%	3.81 (5.61)	3.22 (4.76)	4.11 (5.71)	4.26 (5.95)	3.65 (5.88)
Carbohydrate, E%	46.91 (7.64)	49.08 (7.13)	46.44 (7.27)	45.60 (7.93)	46.51 (7.80)
Added sugar, E%	9.40 (5.16)	13.75 (5.31)	8.93 (4.14)	7.16 (3.77)	7.76 (4.51)
Beverage AS, EAS%	1.02 (1.98)	1.83 (3.01)	0.75 (1.30)	0.61 (1.02)	0.88 (1.74)
Non-beverage AS, EAS%	8.38 (4.67)	11.93 (4.89)	8.18 (3.88)	6.54 (3.55)	6.87 (4.17)
Fiber, g	29.07 (12.00)	27.03 (12.06)	29.29 (11.11)	31.36 (12.75)	28.59 (11.66)
Glycaemic index	56.56 (4.34)	57.30 (3.93)	56.75 (4.30)	55.72 (4.23)	56.47 (4.72)
BMI, kg/m^2	26.22 (4.28)	26.39 (4.56)	26.28 (4.37)	26.01 (4.18)	26.20 (4.00)
Body weight, kg	72.14 (13.51)	72.67 (14.09)	73.33 (13.93)	71.48 (13.10)	71.10 (12.80)
Physical activity, MET	1,446 (2,404)	1,367 (2,269)	1,428 (2,410)	1,544 (2,384)	1,442 (2,546)

EAS%, percentage of energy from added sugar; AS, added sugar; BMI, body mass index; MET, metabolic equivalent.
All data were presented as mean (SD) except data for women, current smoker, diabetes, married, qualification after leaving school, home ownership and living alone, where data were presented as n (%).

their intake, but weight loss occurred across all quartiles of change in EAS% intakes.

Changes in body weight across the quartiles of change in EAS% intake from beverage and non-beverage sources in each 5-year time interval are presented in **Table 3**. The largest increase in EAS% from beverage and non-beverage sources were 2.09 and 4.18% in the first time interval, respectively. The mean (SD) change in AS and energy intake for a 2.09% increase in EAS% from beverage sources were 15.92 (15.72) g and 224 (2,369) kJ, and for a 4.18% increase in EAS% from non-beverage sources were 24.10 (20.34) g and −35 (2,436) kJ, respectively (data not shown). For both AS sources, the mean of change in body weight in the first and fourth quartile was <1.8 kg. For EAS% intakes from non-beverage sources in the third time interval, those who had the highest increase in EAS% intake from non-beverage sources experienced less weight loss compared to those who decreased their EAS% intakes from these sources.

Overall Changes in Added Sugar and Weight

The longitudinal analyses for the associations between the overall 5-yearly changes in EAS% intake and body weight during 15 years of follow-up are presented in **Table 4**. In both adjusted models, the association between change in EAS% intake and body weight change was not statistically significant (Model 1 $P_{\text{trend}} = 0.079$; Model 2 $P_{\text{trend}} = 0.837$). Similar results were observed for the association between changes in EAS% intake from either beverage or non-beverage sources and changes in body weight ($P_{\text{trend}} = 0.621$ and $P_{\text{trend}} = 0.626$, respectively). It is also worth noting that analyses in Model 2 were repeated by the exclusion of the initial and the changes in total energy intakes from the adjustment model and the results of these reanalyses provided similar findings.

DISCUSSION

The availability of cohort data provided an opportunity to explore the longitudinal associations between changes in EAS% intake, EAS% intake from both beverage and non-beverage sources and changes in weight over a 15-year period. In this prospective cohort, changes in EAS% intakes were not significantly associated with changes in body weight, regardless of whether the source of AS came from beverages or non-beverages.

Changes in the total energy intakes will result in body weight change (if energy expenditure remains unchanged). During all BMES time intervals, changes in the EAS% intakes were

TABLE 2 | Mean (SD) changes (Δ) in macronutrient intake and body weight according to quartiles of change in percentage of energy from added sugar (EAS%) during three 5-year time intervals in the BMES cohort.

	Change in EAS% intake			
	Q1	**Q2**	**Q3**	**Q4**
First time interval (BMES 1–2)				
Median	−4.50	−0.83	1.51	5.19
n	428	428	429	428
ΔEnergy, kJ	−198 (2,622)	−223 (2,185)	40 (2,311)	53 (2,431)
ΔCarbohydrate (excluding AS), g	14.61 (63.02)	1.98 (53.46)	4.79 (61.70)	−8.21 (60.25)
ΔFat, g	−2.65 (29.42)	−4.66 (25.57)	−4.01 (26.76)	−5.08 (28.31)
ΔProtein, g	5.75 (29.39)	−0.14 (26.21)	−1.48 (26.72)	−4.41 (28.31)
ΔAlcohol, g	−0.05 (9.67)	−0.66 (11.28)	−0.42 (10.99)	−2.40 (11.76)
ΔBeverage AS, EAS%	−0.72 (2.63)	0.10 (1.23)	0.55 (1.37)	1.83 (3.39)
ΔNon-beverage AS, EAS%	−4.80 (3.50)	−1.01 (1.37)	0.98 (1.48)	4.55 (3.86)
ΔBody weight, kg	1.24 (8.10)	0.86 (8.03)	2.29 (6.12)	1.57 (7.50)
Second time interval (BMES 2–3)				
Median	−4.56	−1.13	1.08	4.71
n	302	302	303	302
ΔEnergy, kJ	93 (2,424)	431 (2,417)	127 (2,085)	−36 (2,265)
ΔCarbohydrate (excluding AS), g	11.21 (61.33)	9.13 (60.80)	−6.40 (53.37)	−15.57 (55.91)
ΔFat, g	4.79 (26.39)	5.16 (27.64)	4.59 (24.38)	−1.26 (27.03)
ΔProtein, g	8.45 (26.80)	7.95 (28.41)	−0.60 (26.84)	−5.03 (26.52)
ΔAlcohol, g	0.78 (9.37)	0.47 (10.54)	−1.19 (9.27)	−2.71 (10.71)
ΔBeverage AS, EAS%	−1.69 (3.26)	−0.26 (1.47)	0.07 (1.28)	1.31 (2.82)
ΔNon-beverage AS, EAS%	−4.06 (3.48)	−0.93 (1.60)	1.08 (1.37)	4.28 (3.50)
ΔBody weight, kg	0.08 (6.86)	0.84 (6.58)	0.60 (5.15)	−0.19 (5.63)
Third time interval (BMES 3–4)				
Median	−4.85	−1.19	0.96	4.57
n	186	187	187	187
ΔEnergy (kJ)	−94 (2,554)	−260 (2,206)	−4 (2,166)	74 (1,988)
ΔCarbohydrate (excluding AS), g	4.87 (61.14)	−11.88 (53.56)	−9.29 (57.69)	−18.62 (56.38)
ΔFat, g	2.99 (31.60)	−0.30 (26.55)	1.68 (24.28)	0.79 (24.67)
ΔProtein, g	9.14 (30.14)	1.43 (26.07)	0.67 (25.39)	−5.01 (25.35)
ΔAlcohol, g	−0.75 (7.68)	−0.08 (8.84)	−1.69 (10.86)	−1.71 (7.91)
ΔBeverage AS, EAS%	−1.22 (2.38)	−0.29 (1.14)	−0.05 (1.36)	1.12 (2.59)
ΔNon-beverage AS, EAS%	−4.50 (3.36)	−0.95 (1.29)	1.09 (1.41)	4.39 (3.72)
ΔBody weight, kg	−1.22 (5.16)	−1.76 (6.16)	−0.57 (4.99)	−0.37 (5.47)

EAS%, percentage of energy from added sugar; AS, added sugar.

not accompanied by substantial changes in total energy intake (mostly <260 kJ across quartiles). By definition, changes in EAS% intakes were accompanied by compensatory changes in intakes of other macronutrient energy sources, such as fat, protein, alcohol, and other carbohydrates. The AS intake can result in weight gain if it contributes to excess energy intake; however, its isocaloric replacement by other macronutrient sources of energy may not result in weight change (6, 17).

Our results support the concept that when dietary changes are required, the focus should be on a range of macronutrients and overall energy intake, rather than one single nutrient. Recommendations for AS consumption suggest reduction from 10% energy to 5% energy (11, 41), however, this recommendation should consider the possibility that people may consume other macronutrient sources to compensate for the energy reduction. Our findings suggest that those who decreased their EAS% intake by 5% replaced this with increases in energy intake from other macronutrients, such as protein, fat, alcohol, and other carbohydrates. Of course, AS replacement by some nutrients, particularly protein, would provide other health benefits, such as the maintenance of muscle mass and strength (42) but replacement by alcohol may not be preferable.

A cohort study that used baseline AS intake and its food sources to investigate the association with weight gain over time reported different findings (20) compared to our study. High free sugar intake at baseline was associated with significant weight gain in Japanese men during 10 years of follow-up (20). Japanese participants in the highest quartile of free sugar intake had a

TABLE 3 | Mean (SD) changes (Δ) in body weight according to quartiles of change in percentage of energy from added sugar (EAS%) from beverage and non-beverage sources during three 5-year time intervals in the BMES cohort.

	Change in EAS% intake			
	Q1	**Q2**	**Q3**	**Q4**
Δ*Beverage AS*				
First time interval (BMES1-2)				
Median	−0.71	0.00	0.32	2.09
n	428	428	429	428
ΔBody weight, kg	1.22 (7.94)	1.11 (6.99)	2.11 (7.87)	1.52 (7.10)
Second time interval (BMES 2-3)				
Median	−1.89	−0.18	0.06	1.37
n	302	302	303	302
ΔBody weight, kg	0.77 (7.43)	0.41 (5.96)	0.14 (5.25)	0.03 (5.53)
Third time interval (BMES 3-4)				
Median	−1.47	−0.21	0.02	1.09
n	186	187	187	187
ΔBody weight, kg	−1.13 (5.48)	−0.45 (5.36)	−1.11 (4.99)	−1.23 (6.05)
Δ*Non-beverage AS*				
First time interval (BMES1-2)				
Median	−4.40	−1.00	0.86	4.18
n	428	428	429	428
ΔBody weight, kg	1.01 (9.02)	1.25 (7.40)	1.96 (4.93)	1.74 (7.99)
Second time interval (BMES 2–3)				
Median	−3.92	−0.89	1.04	4.15
n	302	302	303	302
ΔBody weight, kg	−0.03 (6.69)	0.57 (4.66)	0.99 (7.26)	−0.19 (5.39)
Third time interval (BMES3-4)				
Median	−4.26	−0.83	1.09	3.78
n	186	187	187	187
ΔBody weight, kg	−1.69 (5.93)	−1.37 (5.25)	−0.70 (5.15)	−0.16 (5.48)

EAS%, percentage of energy from added sugar; AS, added sugar.

weight gain of 0.20 kg over 10 years (20). Although the weight gain for the highest quartile of change in EAS% in our study was 0.15 kg, it was not statistically significant. It is worth mentioning that in both studies, this small weight gain over a long period of time may not be clinically significant.

The non-significant results for the association between AS intake and body weight in the BMES cohort is consistent with findings from studies on AS intake and BMI (19, 21). Lee et al. (21) found no significant association between changes in AS and BMI, but reported significant findings for increases in waist circumference (WC), and reported a significant positive association between AS from beverage sources and WC. The major sources of AS intake in the BMES population were non-beverage food sources (e.g., sugar, sweet spreads, and confectionary) (31). However, our non-significant findings for the association between changes in AS intake from non-beverage sources and body weight change in the BMES population is consistent with other studies on solid AS food sources and indicators of obesity (19, 23). It is worth mentioning that in the first 5-year time interval, BMES participants experienced weight gain across the quartiles of change in EAS% sources, however, in the third time interval there was an overall weight loss which could be due to the nature of the aging cohort and the expected body composition changes in the older stages of life (43).

Regarding the beverage AS food sources, most studies have focused on sugar-sweetened beverages. Several cohort studies reported that increased intake of sugar-sweetened beverages was associated with obesity indicators (e.g., weight gain and increases in BMI or WC) (22, 23, 44). AS provided by liquid food sources may play a more powerful role in the development of obesity than AS from solid food sources. This is because the consumption of liquid AS food sources appears to have a weaker satiety effect and may not result in compensation for energy intake to the same extent as solid AS food sources (45), leading to excess food consumption and resultant weight gain. There is also some evidence that carbon dioxide in carbonated beverages (i.e., soft drinks) increases the secretion of ghrelin, the hunger hormone, leading to hunger stimulation and, consequently, increased food intake and weight gain (46).

In contrast, our findings from older Australians did not support the association between AS intake from beverage sources

TABLE 4 | The overall 5-yearly change (Δ) in body weight according to quartiles of change in energy from added sugar intake (EAS%) in the BMES cohort during 15 years of follow-up.

	Change in EAS% intake[a]				Estimate (95% CI)[b]	P_{trend}[c]
	Q1	Q2	Q3	Q4		
	Δ Body weight (kg: Mean [95% CI]) for change in EAS%					
Model 1[d]	0.35 (−0.08, 0.78)	0.38 (−0.07, 0.82)	1.08 (0.74, 1.42)	0.70 (0.29, 1.12)	0.050 (−0.006, 0.105)	0.079
Model 2[e]	0.22 (−0.65, 1.08)	−0.05 (−0.85, 0.75)	0.51 (−0.31, 1.34)	0.15 (−0.81, 1.12)	0.006 (−0.048, 0.060)	0.837
	Δ Body weight (kg: Mean [95% CI]) for change in EAS% from beverage sources					
Model 1[d]	0.50 (0.05, 0.95)	0.66 (0.27, 1.06)	0.82 (0.41, 1.22)	0.53 (0.13, 0.92)	0.023 (−0.067, 0.112)	0.618
Model 2[f]	0.06 (−0.85, 0.97)	0.27 (−0.54, 1.08)	0.43 (−0.51, 1.37)	0.10 (−0.71, 0.91)	−0.028 (−0.141, 0.084)	0.621
	Δ Body weight (kg: Mean [95% CI]) for change in EAS% from non-beverage sources					
Model 1[d]	0.18 (−0.28, 0.64)	0.46 (0.09, 0.84)	1.07 (0.71, 1.43)	0.80 (0.37, 1.22)	0.057 (−0.007, 0.121)	0.081
Model 2[g]	−0.08 (−0.96, 0.80)	0.11 (−0.69, 0.90)	0.55 (−0.26, 1.36)	0.28 (−0.70, 1.25)	0.018 (−0.054, 0.090)	0.626

[a] *Q1 represents the largest decrease and Q4 represents the largest increase in percentage of energy from added sugar (EAS%) intake in each 5-year time interval. Q1 includes participants who had the largest decrease in intake (Q1) in the first, second and third 5-year time interval, Q2 includes those who were in Q2 in the first, second and third 5-year time interval, Q3 includes participants who were in Q3 in the first, second and third 5-year time interval and Q4 includes those who had the largest increase (Q4) in the first, second, and third 5-year time interval.*

[b] *Estimated change in body weight per 1% increase in EAS% intake.*

[c] *P for trend was assessed by using change in EAS% intake as a continuous variable.*

[d] *Model 1: GEE analysis adjusted for gender and initial age.*

[e] *Model 2: GEE analysis adjusted for gender, initial age, weight, diabetes status, EAS%, and both initial and change in each time interval for fiber intake, total energy intake, glycaemic index, physical activity, and smoking status.*

[f] *Model 2: GEE analysis adjusted for gender, initial age, weight, diabetes status, EAS% from beverage food sources and both initial and change in each time interval for fiber intake, total energy intake, glycaemic index, physical activity and smoking status.*

[g] *Model 2: GEE analysis adjusted for gender, initial age, weight, diabetes status, EAS% from non-beverage food sources and both initial and change in each time interval for fiber intake, total energy intake, glycaemic index, physical activity, and smoking status.*

and weight gain. This may be explained by the limited sugar-sweetened beverage intake and small changes (15.9 g) in the overall intake of these beverages among the BMES cohort of older people. Studies show that an increase in sugar-sweetened beverage intake by more than one drink per day (>40g AS or 600 kJ) is positively associated with indicators of obesity (22, 47). In a study conducted by Schulze et al. (22), frequent consumers of these beverages increased their total energy intake by 358 kcal (1,496 kJ) per day and had a weight gain of 4.7 kg over 4 years. However, in BMES participants, the increase in AS in the highest quartile of EAS% change from sugar-sweetened beverages (2%) was 15.9 g and the increase in total energy intake was 224 kJ. Similarly, a cohort of older Spanish adults also observed no significant association, likely due to the occasional intakes of sugar-sweetened beverages in this age group (18).

In BMES participants, the average BMI was <30 kg/m^2 and the average EAS% was <10%. Therefore, our findings may not be generalizable to populations with high rates of obesity or those with high EAS% intake. Nonetheless, since both the BMI and EAS% intake of BMES participants are similar to national Australian older population (48), our findings may be generalizable to the older Australians. Our study has several strengths, which includes a long follow-up period and a relatively large sample size. However, despite the relatively large sample, we cannot rule out the possibility of lack of sufficient statistical power to detect the significant body weight change as this study was based on the secondary analysis of an existing cohort. Other advantages of this study include dietary assessment at each 5-year follow-up period, accompanied by measurement of body weight (i.e., not self-reported). Moreover, a consistent systematic method was used to estimate the AS content of foods in each time point.

We acknowledge that the possibility of recall bias and the over- or under-reporting of food intake (a limitation of most dietary assessment methods) cannot be ruled out. Nonetheless, implausible intakes were excluded from the dataset and changes in intake were investigated in the same participants during the 15 years, hence any over- or under-reporting may have had a minimal effect on the change in intake, and presumably any bias that may exist would have been consistent over time. Additionally, it is possible that the change in the EAS% categories reflects a statistical phenomenon of regression to the mean (49), noting that those people who reduced their EAS% intake the most (Q1) had the highest baseline EAS% intake (13.7%). This also could reflect they have scope to reduce their intake further than people with a more moderate level of baseline EAS% intake. Furthermore, although the analyses were adjusted for several covariates, the possibility of residual confounders cannot be excluded. However, as the analyses were adjusted for both initial and changes in energy intake over time, medical conditions which influence energy intake have generally been accounted for.

In conclusion, our results do not support the association between changes in EAS% intakes and body weight change in the cohort of older Australians during 15 years of follow-up. This result was similar for beverage and non-beverage AS food sources. Our findings suggest that population-level messages specifically targeted to address weight loss by reductions in AS may be less applicable to older Australians who already consume

AS at moderate levels. The findings also provide insights into the importance of including multi-faceted population health messages for weight reduction, such as limiting fat (particularly saturated fat) and alcohol intake, along with any recommendations to reduce AS intake.

DATA AVAILABILITY STATEMENT

The data analyzed in this study are not readily available because of ethical and privacy considerations. Requests to access the datasets should be directed to PM, paul.mitchell@sydney.edu.au.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Sydney West Area Health Services Ethics Committee and the University of Sydney Human Research Ethics Committee. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HM conducted the research, estimated added sugar values, analyzed the data, and drafted the manuscript. VF and PM were involved in the collection of original BMES data. All authors critically reviewed the manuscript and approved the final version submitted for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.629815/full#supplementary-material

REFERENCES

1. Han TS, Tajar A, Lean ME. Obesity and weight management in the elderly. *Br Med Bull.* (2011) 97:169–96. doi: 10.1093/bmb/ldr002
2. Stenholm S, Head J, Aalto V, Kivimaki M, Kawachi I, Zins M, et al. Body mass index as a predictor of healthy and disease-free life expectancy between ages 50 and 75: a multicohort study. *Int J Obes.* (2017) 41:769–75. doi: 10.1038/ijo.2017.29
3. Thompson D, Oster G, Brown JB, Nichols GA, Elmer PJ. Body mass index and future healthcare costs: A retrospective cohort study. *Obes Res.* (2001) 9:210–8. doi: 10.1038/oby.2001.23
4. Zamboni M, Mazzali G, Zoico E, Harris TB, Meigs JB, Di Francesco V, et al. Health consequences of obesity in the elderly: a review of four unresolved questions. *Int J Obes Relat Metab Disord.* (2005) 29:1011–29. doi: 10.1038/sj.ijo.0803005
5. Arterburn DE, Crane PK, Sullivan SD. The coming epidemic of obesity in elderly Americans. *J Am Geriatr Soc.* (2004) 52:1907–12. doi: 10.1111/j.1532-5415.2004.52517.x
6. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev.* (2006) 27:750–61. doi: 10.1210/er.2006-0032
7. Prentice AM, Jebb SA. Fast foods, energy density and obesity: a possible mechanistic link. *Obes Rev.* (2003) 4:187–94. doi: 10.1046/j.1467-789X.2003.00117.x
8. Rangan AM, Schindeler S, Hector DJ, Gill TP, Webb KL. Consumption of /'extra/' foods by Australian adults: types, quantities and contribution to energy and nutrient intakes. *Eur J Clin Nutr.* (2008) 63:865–71. doi: 10.1038/ejcn.2008.51
9. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids*. Washington, DC: National Academies Press (2005).
10. US Department of Health Human Services Food and Drug Administration. *Food Labeling: Revision of the Nutrition and Supplement Facts Labels; Proposed Rule*. (2014). Available online at: https://www.federalregister.gov/articles/2014/03/03/2014-04387/food-labeling-revision-of-the-nutrition-and-supplement-facts-labels
11. World Health Organization. *Guideline: Sugars Intake for Adults and Children*. Geneva (2015).
12. Reid M, Hammersley R, Duffy M. Effects of sucrose drinks on macronutrient intake, body weight, and mood state in overweight women over 4 weeks. *Appetite.* (2010) 55:130–6. doi: 10.1016/j.appet.2010.05.001
13. Reid M, Hammersley R, Hill AJ, Skidmore P. Long-term dietary compensation for added sugar: effects of supplementary sucrose drinks over a 4-week period. *Br J Nutr.* (2007) 97:193–203. doi: 10.1017/S0007114507052705
14. Stanhope KL, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, et al. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest.* (2009) 119:1322–34. doi: 10.1172/JCI37385
15. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. *Am J Clin Nutr.* (2011) 94:479–85. doi: 10.3945/ajcn.111.013540
16. Hu FB, Malik VS. Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. *Physiol Behav.* (2010) 100:47–54. doi: 10.1016/j.physbeh.2010.01.036
17. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ.* (2013) 346:e7492. doi: 10.1136/bmj.e7492
18. Ferreira-Pêgo C, Babio N, Bes-Rastrollo M, Corella D, Estruch R, Ros E, et al. Frequent consumption of sugar- and artificially sweetened beverages and natural and bottled fruit juices is associated with an increased risk of metabolic syndrome in a mediterranean population at high cardiovascular disease risk. *J Nutr.* (2016) 146:1528–36. doi: 10.3945/jn.116.230367
19. Wang J, Light K, Henderson M, O'Loughlin J, Mathieu M-E, Paradis G, et al. Consumption of added sugars from liquid but not solid sources predicts impaired glucose homeostasis and insulin resistance among youth at risk of obesity. *J Nutr.* (2014) 144:81–6. doi: 10.3945/jn.113.182519

20. Yamakawa M, Wada K, Koda S, Mizuta F, Uji T, Oba S, et al. High intake of free sugars, fructose, and sucrose is associated with weight gain in japanese men. *J Nutr.* (2019) 150:322–30. doi: 10.1093/jn/nxz227
21. Lee AK, Chowdhury R, Welsh JA. Sugars and adiposity: the long-term effects of consuming added and naturally occurring sugars in foods and in beverages. *Obes Sci Pract.* (2015) 1:41–9. doi: 10.1002/osp4.7
22. Schulze MB, Manson JE, Ludwig DS, Colditz GA, Stampfer MJ, Willett WC, et al. Sugar-sweetened beverages, weight gain, and incidence of type 2 diabetes in young and middle-aged women. *JAMA.* (2004) 292:927–34. doi: 10.1001/jama.292.8.927
23. Nissinen K, Mikkilä V, Männistö S, Lahti-Koski M, Räsänen L, Viikari J, et al. Sweets and sugar-sweetened soft drink intake in childhood in relation to adult BMI and overweight. The Cardiovascular Risk in Young Finns Study. *Public Health Nutr.* (2009) 12:2018–26. doi: 10.1017/S1368980009005849
24. Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. Changes in diet and lifestyle and long-term weight gain in women and men. *N Engl J Med.* (2011) 364:2392–404. doi: 10.1056/NEJMoa1014296
25. Hendriksen MA, Boer JM, Du H, Feskens EJ, van der A DL. No consistent association between consumption of energy-dense snack foods and annual weight and waist circumference changes in Dutch adults. *Am J Clin Nutr.* (2011) 94:19–25. doi: 10.3945/ajcn.111.014795
26. Flood VM, Smith W, Rochtchina E, Wang JJ, Mitchell P. Assembling a nutrient database for a large cohort study: the Blue Mountains Eye Study. *Food Aust.* (2008) 60:37–40. Available online at: https://ro.uow.edu.au/cgi/viewcontent.cgi?article=1355&context=hbspapers
27. Smith W, Mitchell P, Reay EM, Webb K, Harvey PWJ. Validity and reproducibility of a self-administered food frequency questionnaire in older people. *Aust N Z J Public Health.* (1998) 22:456–63. doi: 10.1111/j.1467-842X.1998.tb01414.x
28. Louie JCY, Moshtaghian H, Boylan S, Flood VM, Rangan AM, Barclay AW, et al. a systematic methodology to estimate added sugar content of foods. *Eur J Clin Nutr.* (2015) 69:154–61. doi: 10.1038/ejcn.2014.256
29. Flood VM, Burlutsky G, Webb KL, Wang JJ, Smith WT, Mitchell P. Food and nutrient consumption trends in older Australians: a 10-year cohort study. *Eur J Clin Nutr.* (2010) 64:603–13. doi: 10.1038/ejcn.2010.34
30. McLennan W, Podger A. *National Nutrition Survey Nutrient Intakes and Physical Measurements*. Canberra: Australian Bureau of Statistics (1998).
31. Moshtaghian H, Louie JCY, Charlton KE, Probst YC, Gopinath B, Mitchell P, et al. Trends in added sugar intake and food sources in a cohort of older Australians: 15 years of follow-up from the Blue Mountains Eye Study. *J Hum Nutr Dietetics.* (2017) 30:339–48. doi: 10.1111/jhn.12425
32. Clifton P, Chan L, Moss C, Miller M, Cobiac L. Beverage intake and obesity in Australian children. *Nutr Metab.* (2011) 8:87. doi: 10.1186/1743-7075-8-87
33. Brand-Miller JC, Barclay AW. Declining consumption of added sugars and sugar-sweetened beverages in Australia: a challenge for obesity prevention. *Am J Clin Nutr.* (2017) 105:854–63. doi: 10.3945/ajcn.116.145318
34. Younan C, Mitchell P, Cumming R, Rochtchina E, Panchapakesan J, Tumuluri K. Cardiovascular disease, vascular risk factors and the incidence of cataract and cataract surgery: the Blue Mountains Eye Study. *Ophthalmic Epidemiol.* (2003) 10:227. doi: 10.1076/opep.10.4.227.15905
35. Flood VM. *Folate, Vitamine B12 and Homocysteine Status Among Older Australians*, Dissertation, University of Sydney (2004).
36. Fernihough A, McGovern ME. Physical stature decline and the health status of the elderly population in England. *Econ Hum Biol.* (2015) 16:30–44. doi: 10.1016/j.ehb.2013.12.010
37. Chua B, Flood V, Rochtchina E, Wang JJ, Smith W, Mitchell P. Dietary fatty acids and the 5-year incidence of age-related maculopathy. *Arch Ophthalmol.* (2006) 124:981–6. doi: 10.1001/archopht.124.7.981
38. Townend BS, Townend ME, Flood V, Burlutsky G, Rochtchina E, Wang JJ, et al. Dietary macronutrient intake and five-year incident cataract: the Blue Mountains Eye Study. *Am J Ophthalmol.* (2007) 143:932–9.e1. doi: 10.1016/j.ajo.2007.03.006
39. Gopinath B, Liew G, Burlutsky G, Mitchell P. Physical activity and the 15-year incidence of age-related macular degeneration. *Invest Ophthalmol Vis Sci.* (2014) 55:7799–803. doi: 10.1167/iovs.14-15575
40. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc.* (2003) 35:1381–95. doi: 10.1249/01.MSS.0000078924.61453.FB
41. Johnson RK, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, et al. Dietary sugars intake and cardiovascular health: a scientific statement From the American Heart Association. *Circulation.* (2009) 120:1011–20. doi: 10.1161/CIRCULATIONAHA.109.192627
42. Sahni S, Mangano KM, Hannan MT, Kiel DP, McLean RR. Higher protein intake is associated with higher lean mass and quadriceps muscle strength in adult men and women. *J Nutr.* (2015) 145:1569–75. doi: 10.3945/jn.114.204925
43. Santanasto AJ, Goodpaster BH, Kritchevsky SB, Miljkovic I, Satterfield S, Schwartz AV, et al. Body composition remodeling and mortality: the health aging and body composition study. *J Gerontol Ser A Biol Sci Med Sci.* (2017) 72:513–9. doi: 10.1093/gerona/glw163
44. Barrio-Lopez MT, Martinez-Gonzalez MA, Fernandez-Montero A, Beunza JJ, Zazpe I, Bes-Rastrollo M. Prospective study of changes in sugar-sweetened beverage consumption and the incidence of the metabolic syndrome and its components: the SUN cohort. *Br J Nutr.* (2013) 110:1722–31. doi: 10.1017/S0007114513000822
45. DiMeglio DP, Mattes RD. Liquid versus solid carbohydrate: effects on food intake and body weight. *Int J Obes Relat Metab Disord.* (2000) 24:794–800. doi: 10.1038/sj.ijo.0801229
46. Eweis DS, Abed F, Stiban J. Carbon dioxide in carbonated beverages induces ghrelin release and increased food consumption in male rats: implications on the onset of obesity. *Obes Res Clin Pract.* (2017) doi: 10.1016/j.orcp.2017.02.001
47. Palmer JR, Boggs DA, Krishnan S, Hu FB, Singer M, Rosenberg L. Sugar-sweetened beverages and incidence of type 2 diabetes mellitus in African American women. *Arch Intern Med.* (2008) 168:1487–92. doi: 10.1001/archinte.168.14.1487
48. Lei L, Rangan A, Flood VM, Louie JCY. Dietary intake and food sources of added sugar in the Australian population. *Br J Nutr.* (2016) 115:868–77. doi: 10.1017/S0007114515005255
49. Barnett AG, van der Pols JC, Dobson AJ. Regression to the mean: what it is and how to deal with it. *Int J Epidemiol.* (2004) 34:215–20. doi: 10.1093/ije/dyh299

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SODA MAPS: A Framework for Understanding Caffeinated Sugary Drink Consumption Among Children

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Excess sugary drink (SD) consumption is associated with childhood obesity and development of cardiometabolic disease. In addition to having high added sugar content, many SDs also contain caffeine, which may further encourage excess SD consumption among children. The objective of this study was to develop a conceptual framework of children's caffeinated SD consumption using group concept mapping, an applied social research multimethodology that collectively harnesses qualitative and quantitative data from participants to generate a visual representation of their ideas and input. Children, 8–14 years old, who reported consuming ≥12 ounces of caffeinated SDs (e.g., sodas, sweet teas) per day were recruited throughout Washington, D.C. and invited to participate. Concept mapping included three participant-driven activities: (1) brainstorming ($n = 51$), during which children reported reasons for their SD consumption, from which 58 unique reasons were identified; (2) sorting ($n = 70$), during which children sorted each of the reported reasons into categories and named each category; and (3) rating ($n = 74$), during which children rated the influence of each reason on their own caffeinated SD consumption. Similarity matrices, multidimensional scaling, and hierarchical cluster analysis were used to generate concept maps (hereafter "SODA MAPS"), which display the 58 reasons organized within eight overarching clusters. Among these eight clusters, *Taste and Feel*, *Something to Do*, and *Energy* were rated as particularly influential. Children's caffeinated SD consumption is encouraged not only by the palatable taste and reported preferences for these beverages (e.g., *Taste and Feel*), but also by psychological (e.g., *Mood and Focus*), biological (e.g., *Energy*), social (e.g., *Something to Do*) and environmental reasons (e.g., *Nothing Better Available*). Thus, the SODA MAPS can inform the development of tailored, multi-level SD reduction interventions that incorporate strategies to address important and currently overlooked reasons for caffeinated SD consumption among children.

Keywords: youth, sugar-sweetened beverages, diet, caffeine, obesity, concept mapping

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INTRODUCTION

Excess sugary drink (SD) consumption is a key contributor to excess weight gain and obesity in children (1–3). Weight gain and obesity during childhood increase the risk of multiple health issues, including type 2 diabetes (4, 5), cardiovascular diseases (6), fatty liver, and dyslipidemia (7, 8), as well as bone and joint issues (9), dental decay (10), and psychological problems (11–14). Therefore, limiting SD intake is an urgent public health priority (15).

Contrary to recommendations to limit SD intake to <8 ounces per week or to avoid SDs altogether (16), 63% of children in the U.S. drink one or more SDs per day (17). SD consumption increases with age in both girls and boys and differs by race/ethnicity and socioeconomic status (18–21), with minority and low-income populations reporting the highest SD intakes. While the palatability and accessibility of SDs are well-described reasons for SD consumption (22, 23), children's SD intake is influenced by a variety of factors, including parenting practices (22, 24, 25), nutritional knowledge (20, 26), availability of SDs at home (23), screen time (27), and fast food consumption (28).

The large quantities of added sugar in SDs are not the only cause for concern. Certain sugary drinks, such as colas and sweet teas, are also predominant contributors to caffeine intake among U.S. children (28–30). Caffeine consumption is known to elicit behavioral and psychological effects that can lead to dependence (31), and the combination of added sugar and caffeine in SDs may uniquely reinforce SD consumption behaviors among children. However, determinants specifically pertaining to caffeinated SD intake among children have not yet been studied, except with regard to energy drinks and sugary coffee beverages, which constitute only a small fraction of children's total caffeinated SD intake (32).

We recently reported physical, cognitive, emotional, and interpersonal reasons for children's caffeinated SD consumption based on qualitative data from focus group discussions with children from predominantly minority and/or low-income backgrounds (33). While these findings call attention to the complex interconnection of biological, psychological, and socio-environmental factors associated with children's SD consumption, the relative significance and interrelatedness of these reasons were not evaluated. This study, therefore, aimed to comprehensively examine multifactorial reasons for children's caffeinated SD intake using group concept mapping, an applied social research mixed methodology, which resulted in a novel, participant-driven conceptual framework, hereafter referred to as SODA MAPS. We specifically focused on children from minority and/or low-income backgrounds, who report the highest intakes of SDs and are disproportionately burdened by obesity and cardiometabolic disease (19, 34).

MATERIALS AND METHODS

Study Design

Children, 8–14 years old participated in concept mapping, a mixed-method approach, which involves a series of participant-driven activities, including brainstorming, sorting, and rating. For brainstorming, children were recruited from pediatric primary care clinics and District of Columbia public schools. For sorting and rating, children were recruited from District of Columbia public schools, as well as afterschool programs and local community events. Depending on the location (primary care clinics and community events vs. schools and after school programs), consent forms were either given directly to parents, or students were asked to bring them home to be signed by their parent or guardian (hereafter parent). Children with signed consent forms provided assent and then were assessed for study eligibility using a brief eligibility screener questionnaire. Inclusion criteria included that the child (a) was between 8 and 14 years old; (b) consumed ≥12 ounces of caffeinated, sugary, non-diet drinks (e.g., Coca-Cola™, Pepsi™, Mountain Dew™, Arizona Iced Tea™) per day; and (c) spoke English fluently. Exclusion criteria included child-reported consumption of regular, caffeine-containing coffee, hot tea, or energy drinks (e.g., Red Bull™, Monster™) ≥1 time per week. We selected the 8-to-14-year-old age range in order to focus on children in elementary and middle school, who are less likely to consume coffee and/or energy drinks, compared with older adolescents (28).

After providing assent, participants self-reported their age, sex, and race/ethnicity and then completed the concept mapping activities. All study procedures were conducted in a pre-determined designated private space (e.g., school classroom or vacant conference room). While some participants contributed to brainstorming and also to sorting and/or rating, concept mapping methodology does not require participants to take part in all three activities (35).

All study materials were approved by the Institutional Review Boards at the George Washington University [Protocol 18091] and Children's National Hospital [Protocol 00011014]. Given the minimal time commitment required of participants for brainstorming, financial compensation was not provided; however, participants who completed the sorting and/or rating activities received a $10 gift card as compensation.

TABLE 1 | Participant characteristics for brainstorming and sorting/rating.

	Brainstorming	Sorting/Rating
N	51	77[a,b]
Age (mean ± SD)	10.7 ± 2.0	10.6 ± 1.8
Sex (*n*, %)		
Male	31, 61%	41, 53%
Female	20, 39%	36, 47%
Race/Ethnicity (*n*, %)[c]		
Non-Hispanic Black	31, 61%	56, 73%
Hispanic	10, 20%	12, 16%
Non-Hispanic White	6, 12%	
Other	4, 8%	9, 12%

[a] *n = 7 completed rating and not sorting.*
[b] *n = 3 completed sorting and not rating.*
[c] *Percentages do not add up to 100% due to rounding.*

TABLE 2 | Rank ordering of reasons for caffeinated sugary drink intake based on rating values.[a]

Ranking	Reason (reason number)	Mean Rating Values
1	They taste good (19)	4.76 ± 0.59
2	They have good flavor (16)	4.54 ± 0.81
3	They are sweet (58)	4.51 ± 0.86
4	They are good (56)	4.41 ± 1.03
5	I love drinking them (11)	4.30 ± 1.11
6	They are my favorite drinks (26)	4.07 ± 1.31
7	They are good for parties (10)	4.00 ± 1.37
8	They have a nice aftertaste (13)	3.93 ± 1.20
9	I am thirsty (3)	3.88 ± 1.26
10	There are different types of flavors (33)	3.84 ± 1.43
11	I need something sweet (17)	3.78 ± 1.08
12	They are refreshing (1)	3.74 ± 1.18
13	They give me energy (41)	3.68 ± 1.52
14	They make me hype (44)	3.55 ± 1.57
15	They have lots of sugar (28)	3.51 ± 1.42
16	It is hot out (53)	3.49 ± 1.63
17	I like them on road trips (25)	3.38 ± 1.59
18	The sweetness is addictive (39)	3.34 ± 1.57
19	I need a boost (35)	3.32 ± 1.61
20	They are better than other drinks (37)	3.23 ± 1.53
21	Kids like them (54)	3.18 ± 1.72
22	My energy is low (38)	3.05 ± 1.63
23	I like them better than water (22)	3.00 ± 1.64
24	They are filling (9)	2.99 ± 1.40
25	They keep me awake (12)	2.99 ± 1.58
26	Water does not have a taste (57)	2.99 ± 1.70
27	My family drinks them (14)	2.97 ± 1.58
28	I cannot stop drinking them (29)	2.95 ± 1.57
29	They help me play (24)	2.92 ± 1.62
30	They are fizzy (27)	2.92 ± 1.62
31	They make me ready for a hard day (51)	2.86 ± 1.62
32	They are fruity (7)	2.78 ± 1.61
33	There is not water available (55)	2.77 ± 1.56
34	They help me concentrate (52)	2.72 ± 1.66
35	They give me a sugar rush (21)	2.72 ± 1.53
36	They are caffeinated (40)	2.69 ± 1.59
37	They are like coffee for kids (48)	2.68 ± 1.71
38	There may not be juice available (4)	2.66 ± 1.32
39	I need to calm down when I am angry (46)	2.65 ± 1.66
40	They wake me up (18)	2.64 ± 1.52
41	I like the bubbles (49)	2.51 ± 1.56
42	There is nothing else I like to drink (42)	2.47 ± 1.57
43	They make me run faster (36)	2.41 ± 1.50
44	They make me burp so my stomachache goes away (43)	2.38 ± 1.65
45	They get rid of burning from eating spicy food (15)	2.34 ± 1.58
46	I like the acid (47)	2.30 ± 1.56
47	I am bored (23)	2.27 ± 1.49
48	I want to burp (45)	2.24 ± 1.54
49	They make me ready to go to school (32)	2.19 ± 1.51
50	I do not like water (34)	2.19 ± 1.53
51	They keep me the perfect size (2)	2.18 ± 1.36
52	They are sour (5)	1.90 ± 1.26
53	I am sleepy (6)	1.89 ± 1.32
54	They make my headache go away (8)	1.85 ± 1.31
55	They keep me in shape (50)	1.82 ± 1.30
56	They are healthy for you (30)	1.61 ± 0.98
57	They help with my cramps (20)	1.55 ± 1.11
58	They make me smart (31)	1.55 ± 1.09

(Continued)

TABLE 2 | Continued

[a]*Values are means ± SDs. Each of the numbers in parentheses after each reason is the identifying number on the point map and point-cluster map; these numbers do not signify any value. Mean rating values ranged from a low of 1 (not at all important) to 5 (extremely important).*

Brainstorming

For brainstorming, each child ($n = 51$) completed the focus prompt "I drink sugar-sweetened sodas and sweet teas such as Coke™, Pepsi™, Mountain Dew™, Dr. Pepper™, and Nestea™ because…" and were encouraged to list all of the reasons they could think of for consumption. Each child completed brainstorming separately with supervision from a trained research assistant, who collected the responses on a laptop computer using the Concept System® Global MAX™ web-based platform. Brainstorming took approximately 3–5 minutes per participant. Saturation was reached after 51 participants completed the activity, at which point 121 reasons for caffeinated SD consumption had been reported and no new reasons were generated. The original list of 121 reasons was condensed using idea synthesis, a form of qualitative content analysis that combines redundant ideas to create a condensed list of independent reasons using the participants' original wording (36, 37). Idea synthesis resulted in a final list of 58 reasons, which were edited for syntactic consistency and represented the original set of reasons for caffeinated SD consumption reported by the participants.

Sorting

For sorting, the 58 reasons were printed and laminated onto cards so that each child ($n = 70$) could manually sort each of the reasons (generated during brainstorming) into piles based on their perceived meaning. Prior to beginning the sorting activity, a trained research assistant (RA) presented each child with the stack of 58 cards, each containing a single reason, and instructed them to individually sort each reason into mutually exclusive piles in a way that made sense to them. Children were instructed not to (1) create piles such as "Miscellaneous" or "Other;" (2) sort reasons by personal relevance; or (3) leave any reasons unsorted. Children were also asked to name each of the piles to reflect their collective meaning, even if a pile contained only one card. The sorting activity typically lasted between 25 and 35 minutes per child.

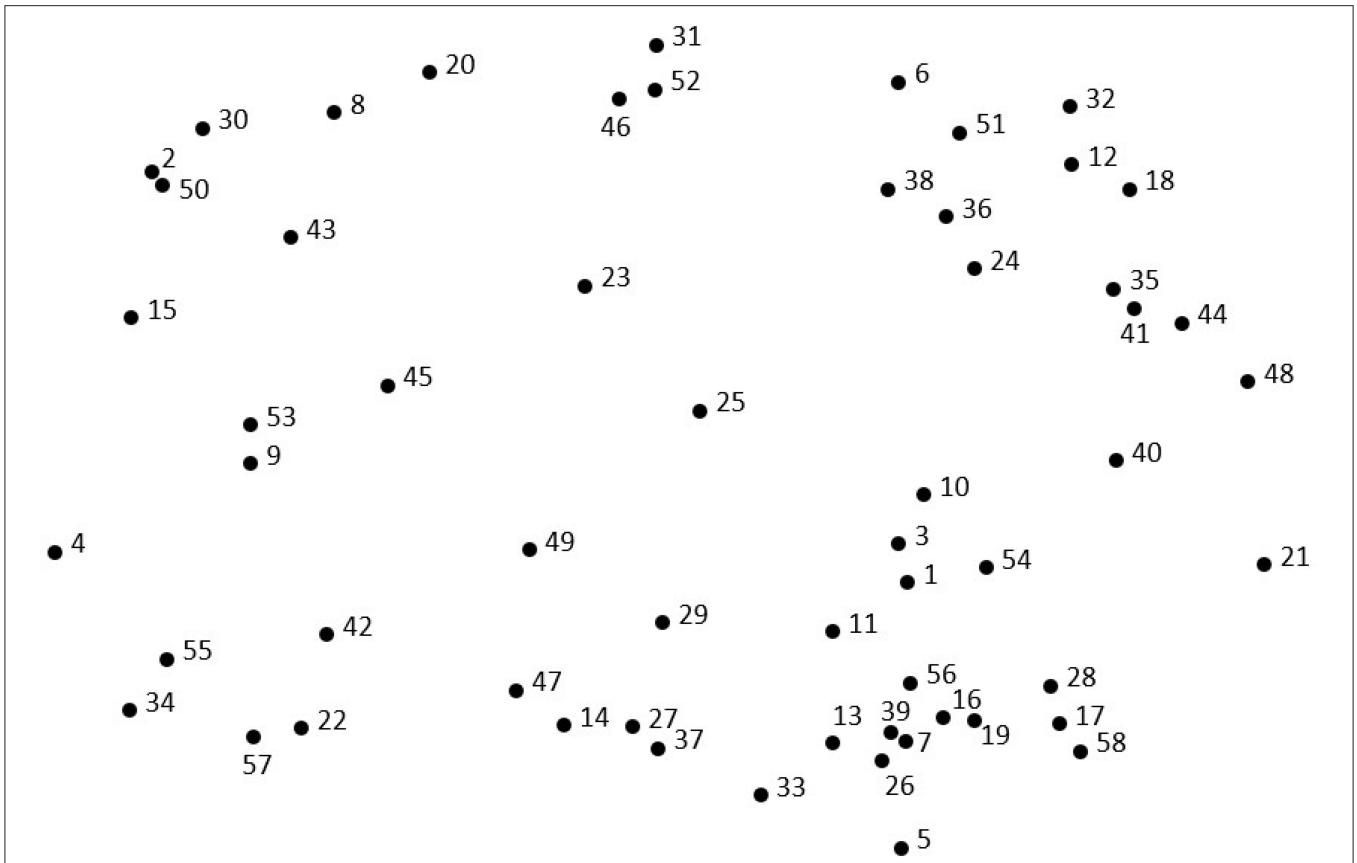

FIGURE 1 | Point map of the 58 reasons for caffeinated SD consumption. Each point represents 1 of the 58 reasons that were brainstormed and sorted by the participants. Point location is an indicator of that point's relation to all other points; points located closer together were conceptually grouped together more frequently than points located distally. The numbers that appear next to each point on the map are not an indication of quantitative value, but instead serve to identify each specific reason (randomly assigned).

Rating

For rating, each child ($n = 74$) completed a paper survey, administered by a trained RA, on which they were instructed to rate on a five-point Likert-style scale (0 = not at all important to 5 = extremely important) the relative importance of each of the 58 reasons for their consumption of caffeinated SDs. Rating took approximately 10 minutes per participant.

Statistical Analysis

The sorting and rating data were entered into the Concept System® Global MAX™ web-based platform, after which the data were analyzed in an iterative process (38, 39). First, multidimensional scaling (MDS) was used to a generate a point map, which was the basis for the subsequent concept maps, described below. The point map's goodness-of-fit was assessed using stress values. Stress values below 0.39 for MDS two-dimensional maps ensure a <1% probability of the matrix having a random structure or no structure (40). Based on a prior pooled analysis, the mean stress value for concept mapping studies is 0.28 (41). The SODA MAPS yielded a stress value of 0.25, indicative of a structured, non-random point map that represented the multivariate data collected, and thus was suitable for continued analyses and generation of subsequent concept maps (42).

Second, a hierarchical cluster analysis using Ward's algorithm was conducted to derive point-cluster maps. Cluster replay maps, which successively display cluster maps of fewer and fewer cluster solutions, were reviewed to determine which cluster maps offered the best conceptual fit of the data. Based on observation, cluster maps with a seven-, eight-, nine-, and ten-cluster solution appeared to be a better fit conceptually. These maps were then examined in greater detail, and points within each of the clusters on each map were carefully examined to ensure appropriate fit. Based on the conceptual meaning of each cluster, and the research team's expertise and prior qualitative findings related to children's SD intake (33, 43), it was determined that the eight-cluster map provided the best fit. Specifically, the eight-cluster map removed the need for themes to be unnecessarily divided (e.g., two energy clusters) and most clearly represented the participants' conceptualization of their caffeinated SD consumption.

Spanning analysis was then conducted, and bridging indices (BI) were calculated to examine the degree to which each point was an anchor on the eight-cluster map or a bridge to other thematic content (36, 38). The BI values reflect whether a reason

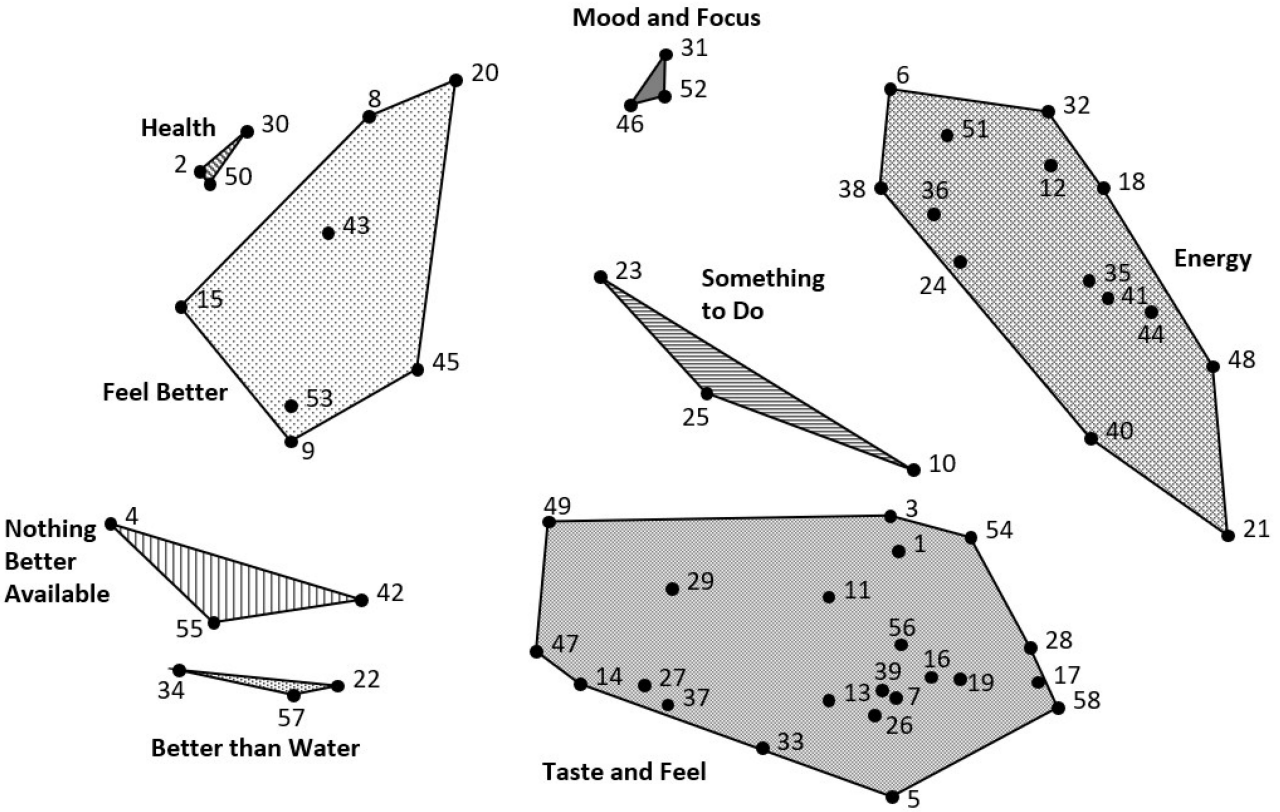

FIGURE 2 | Point-cluster map of caffeinated SD consumption. Each of the eight clusters indicates a dimension of thematically similar content, conceptually grouped together from the 58 reasons for consumption. The clusters include *Health, Mood and Focus, Something to Do, Energy, Taste and Feel, Nothing Better Available, Better than Water*, and *Feel Better*. [1]The cluster names reflect the names provided by the participants when sorting the reasons into piles.

was generally sorted with other nearby reasons (values closer to 0) or with items located further away on the concept map (values closer to 1). Based on these quantitative analyses (SEH, AJV, ACS) and expert judgement (AJV, ACS), cluster lines were redrawn to reflect optimal conceptual fit, resulting in the redistribution of 21 reasons to the closest adjacent cluster without altering each reason's original location on the map (38). Once the eight-cluster map was finalized, cluster names were generated using the original pile names provided by participants during the sorting activity.

Third, mean cluster rating values, computed from the mean rating values of each reason within a cluster, were added to create three-dimensional cluster rating maps.

RESULTS

Characteristics of participants in brainstorming ($n = 51$) and sorting/rating ($n = 77$) are shown in **Table 1**. The mean rating values for the 58 reasons for caffeinated SD consumption are shown in **Table 2**. The highest rated reasons included: "They taste good," "They have good flavor," "They are sweet," "They are good," "I love drinking them," "They are my favorite drinks," and "They are good for parties."

The point map (**Figure 1**) represents the inter-relatedness of the 58 reasons for caffeinated SD consumption. The relative proximity of the reasons reflected their perceived similarity during the sorting activity. Reasons frequently sorted together were located closer together on the point map, while reasons sorted together infrequently were located further apart. Among the eight clusters (**Figure 2**), the clusters with the lowest BI values, indicating more narrowly focused thematic content, were *Taste and Feel* (0.19), *Something to Do* (0.36), and *Energy* (0.5), as illustrated by the relatively compressed shapes on the cluster map. The mean BI for each cluster is shown in **Table 3**.

The three-dimensional cluster rating map, based on the mean of the mean of the participants' ratings of each reason within a cluster, is shown in **Figure 3**. Mean cluster ratings are represented by a layering system; the greater the number of layers, the higher the mean cluster rating. The three highest rated clusters were *Taste and Feel* (3.52), *Something to Do* (3.22), and *Energy* (2.83).

DISCUSSION

In this study, children informed the development of a participant-driven conceptual framework (SODA MAPS) that

TABLE 3 | Rating and bridging indices for the 58 reasons for caffeinated sugary drink consumption by cluster.

Cluster	No.[a]	Reasons	Rating value	Bridging value
Taste and Feel			3.52	0.19
	19	They taste good	4.76	0.05
	16	They have good flavor	4.54	0.02
	58	They are sweet	4.51	0.21
	56	They are good	4.41	0.04
	11	I love drinking them	4.30	0.02
	26	They are my favorite drinks	4.07	0.06
	13	They have a nice aftertaste	3.93	0.00
	3	I am thirsty	3.88	0.20
	33	There are different types of flavors	3.84	0.22
	17	I need something sweet	3.78	0.28
	1	They are refreshing	3.74	0.09
	28	They have lots of sugar	3.51	0.22
	39	The sweetness is addictive	3.34	0.10
	37	They are better than other drinks	3.23	0.26
	54	Kids like them	3.18	0.27
	14	My family drinks them	2.97	0.37
	29	I cannot stop drinking them	2.95	0.22
	27	They are fizzy	2.92	0.27
	7	They are fruity	2.78	0.03
	49	I like the bubbles	2.51	0.33
	47	I like the acid	2.30	0.41
	5	They are sour	1.90	0.46
Something to Do			3.22	0.36
	10	They are good for parties	4.00	0.19
	25	I like them on road trips	3.38	0.34
	23	I am bored	2.27	0.53
Energy			2.83	0.50
	41	They give me energy	3.68	0.40
	44	They make me hype	3.55	0.47
	35	I need a boost	3.32	0.40
	38	My energy is low	3.05	0.46
	12	They keep me awake	2.99	0.43
	24	They help me play	2.92	0.38
	51	They make me ready for a hard day	2.86	0.47
	21	They give me a sugar rush	2.72	0.67
	40	They are caffeinated	2.69	0.47
	48	They are like coffee for kids	2.68	0.71
	18	They wake me up	2.64	0.46
	36	They make me run faster	2.41	0.43
	32	They make me ready to go to school	2.19	0.59
	6	I am sleepy	1.89	0.62
Better than Water			2.73	0.80
	22	I like them better than water	3.00	0.69
	57	Water does not have a taste	2.99	0.77
	34	I do not like water	2.19	0.94
Nothing Better Available			2.64	0.76
	55	There is not water available	2.77	0.75
	4	There may not be juice available	2.66	0.98
	42	There is nothing else I like to drink	2.47	0.54
Feel Better			2.41	0.73
	53	It is hot out	3.49	0.80
	9	They are filling	2.99	0.82
	43	They make me burp so my stomachache goes away	2.38	0.65
	15	They get rid of burning from eating spicy food	2.34	1.00
	45	I want to burp	2.24	0.53
	8	They make my headache go away	1.85	0.66
	20	They help with my cramps	1.55	0.66
Mood and Focus			2.31	0.66
	52	They help me concentrate	2.72	0.58
	46	I need to calm down when I am angry	2.65	0.69
	31	They make me smart	1.55	0.70
Health			1.87	0.77
	2	They keep me the perfect size	2.18	0.77
	50	They keep me in shape	1.82	0.78
	30	They are healthy for you	1.61	0.76

[a]The numbers in the column left of the reasons serve to identify each reason as identified on the point map and are randomly assigned. These numbers do not indicate quantitative value.

provides a comprehensive understanding of the reasons for their caffeinated SD consumption. This framework, developed through participants brainstorming, sorting, and rating 58 distinct reasons for caffeinated SD intake, offers a unique and more nuanced conceptualization of children's caffeinated SD intake behaviors, as compared with prior studies (33, 44).

The findings demonstrate that children consume caffeinated SDs for a variety of reasons, the most influential being related to the drinks' palatability. This is demonstrated by the *Taste and Feel* cluster (which contained reasons such as "They taste good" and "They are sweet") having the highest rating. This finding is unsurprising, as caffeinated SDs contain large quantities of added sugars (e.g., a 12-oz Coca-Cola™ contains 39 g of sugar), and children report a heightened preference for sweetness compared with adults (45–48). In addition to high added sugar content, other reported reasons for caffeinated SD consumption within the *Taste and Feel* cluster pertained to common drink properties, including carbonation (e.g., "I like the bubbles") and acidity (e.g., "I like the acid"). Reasons reported within the cluster *Better than Water* (e.g., "Water does not have a taste") also relate to palatability, and as such, were located in close proximity to the *Taste and Feel*

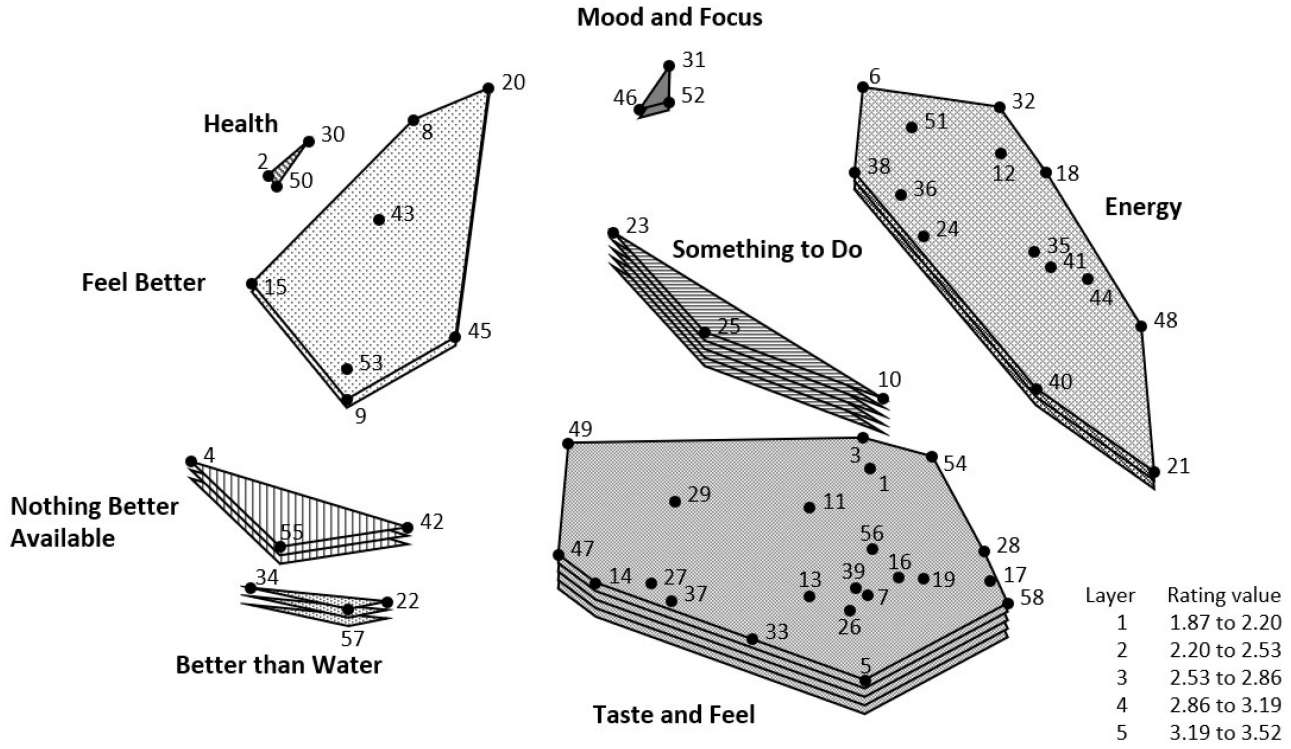

FIGURE 3 | Cluster-rating map of reasons for caffeinated SD consumption. The cluster-rating map illustrates the mean importance rating influencing consumption for each cluster; clusters with a greater number of layers were rated as more important to participants' consumption. The top three rated factors (in order from highest to lowest) include *Taste and Feel, Something to Do*, and *Energy*.

cluster on the SODA MAPS. While most children reported liking water in a prior study with a demographically similar sample of children 8–14 years old (33), the higher perceived palatability of SDs relative to water emphasizes the need to take further actions to limit children's access to SDs. This finding also supports ongoing public health campaigns to offer children water in place of SDs whenever possible (49), consistent with the concept of changing environmental conditions to promote the selection of "optimal defaults" (50).

Another key finding was that, consistent with our recent qualitative findings (33), children described perceived increases in energy as a key reason for their caffeinated SD consumption. While there are previous reports of child hyperactivity resulting from caffeinated SD intake (33), the deliberate use of SDs to achieve a desired outcome, as demonstrated by reasons within the *Energy* cluster such as "They help me stay awake" and "They make me ready for a hard day," suggests that children's caffeinated SD consumption behaviors may parallel well-described behavioral patterns surrounding coffee consumption in adults (51). The purposeful consumption of SDs also reflects established patterns of caffeine use in adolescents (52). Use of caffeinated SDs to boost energy may also suggest that children and adolescents get inadequate sleep, perhaps as a result of excess screen time (53). While our study design did not allow us to distinguish whether reported reasons for caffeinated SD intake were due to their sugar content, caffeine content, or both, our findings highlight the need to investigate the likelihood that sugar and caffeine in SDs may independently and synergistically promote their continued consumption. This is consistent with recent evidence demonstrating that some children may become physically and/or psychologically dependent on caffeinated SDs (54, 55).

While reasons within the *Mood and Focus* and *Feel Better* clusters were not rated as highly compared to those within the *Taste and Feel* or *Energy* clusters, children also reported reasons for caffeinated SD intake related to affective regulation (e.g., "I need to calm down when I am angry"). Withdrawal-like symptoms, both affective (e.g., irritability, sadness) and physical (e.g., headache, stomachache), have been previously reported among children in response to highly processed foods (56–58). Additionally, abstinence from habitual caffeine doses as low as 100 mg per day (comparable to the amount found in two cans of caffeinated soda) has been shown to induce withdrawal symptoms (e.g., headaches) in adults (59). Thus, reasons for children's caffeinated SD intake within *Mood and Focus* and *Feel Better* may reflect important and currently overlooked barriers to sustained reduction in children's caffeinated SD intakes.

While the majority of the reasons for SD consumption reported in the present study were at the individual level, children's dietary behaviors are also strongly influenced by environmental and situational factors (60), such as the availability and accessibility of SDs relative to alternative beverages (61). The cluster *Nothing Better Available* calls attention to environmental

and community influences (62, 63), which may be particularly critical in urban, low-income communities, where access to fast food and junk food is often high relative to healthier options (64–67). Furthermore, reasons within the *Something to Do* cluster call attention to the importance of normative behaviors (e.g., "Good for parties," "I like them on road trips") in influencing children's caffeinated SD intake. Consumption of SDs as a means of alleviating boredom, for example, also suggests that encouraging participation in activities, such as afterschool programs or youth sports, may help to reduce children's caffeinated SD intake. Furthermore, provision of unsweetened, carbonated beverages, such as flavored seltzer water, instead of plain water, may offer a healthy and "less boring" substitute for caffeinated SDs. The influence of cultural and social norms is well described for other dietary behaviors among children (33, 68, 69), and altering norms surrounding risk behaviors has shown promise in initiating lifestyle behavior change among children (70–72).

As the first study to use concept mapping to elucidate reasons for children's caffeinated SD intake, SODA MAPS provide a novel framework for conceptualizing the multifactorial reasons for children's caffeinated SD consumption. The use of concept mapping methodology allowed for the quantitative and qualitative evaluation of the reasons for children's caffeinated SD consumption. However, while the results of this study provide novel insights into caffeinated SD consumption among children, the analysis was subject to several limitations. The sample population was geographically limited (all recruited from Washington, D.C.), as well as racially/ethnically limited (primarily non-Hispanic Black and Hispanic participants). While these could be viewed as strengths, especially given the well-documented disparities in SD consumption and related cardiometabolic health outcomes in minority populations (13), our sample is not representative of all children who consume caffeinated SDs. In addition, selection bias may have affected the makeup of the study population, as it was a convenience sample. Intakes of other, non-beverage, sources of caffeine (e.g., chocolate, dietary supplements), which may influence reasons for children's caffeinated SD consumption, were also not evaluated.

The findings of this study provide a comprehensive conceptual framework for understanding children's caffeinated SD consumption, which is encouraged by a variety of biological (e.g., *Energy*), psychological (e.g., *Mood and Focus*), normative (e.g., *Something to Do*), and environmental factors (e.g., *Nothing Better Available*), as well as the palatability of caffeinated SDs (e.g., *Taste and Feel*). Collectively, these findings support the need for multi-level interventions aimed at addressing individual, sociocultural, and environmental influences on children's SD intake and contribute to informing the development of tailored interventions to reduce SD consumption among children.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Review Board at The George Washington University [Protocol 18091], and the Institutional Review Board at Children's National Hospital [Protocol 00011014]. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

ACS, AJV, and JS designed the research. SEH performed the analyses. SEH, ACS, and AJV interpreted the data. SEH wrote the first draft of the manuscript. All authors were involved in editing the manuscript and approved the final version.

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REFERENCES

1. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. *Am J Clin Nutr*. (2013) 98:1084–102. doi: 10.3945/ajcn.113.058362
2. Hu FB. Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases. *Obes Rev*. (2013) 14:606–19. doi: 10.1111/obr.12040
3. Scharf RJ, DeBoer MD. Sugar-Sweetened Beverages and Children's Health. *Annu Rev Public Health*. (2016) 37:273–93. doi: 10.1146/annurev-publhealth-032315-021528
4. Cruz ML, Shaibi GQ, Weigensberg MJ, Spruijt-Metz D, Ball GD, Goran MI. Pediatric obesity and insulin resistance: chronic disease risk and implications for treatment and prevention beyond body weight modification. *Annu Rev Nutr*. (2005) 25:435–68. doi: 10.1146/annurev.nutr.25.050304.092625
5. Sinha R, Fisch G, Teague B, Tamborlane WV, Banyas B, Allen K, et al. Prevalence of impaired glucose tolerance among children

and adolescents with marked obesity. *N Engl J Med*. (2002) 346:802–10. doi: 10.1056/NEJMoa012578
6. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. (2006) 444:875–80. doi: 10.1038/nature05487
7. Davis JN, Le KA, Walker RW, Vikman S, Spruijt-Metz D, Weigensberg MJ, et al. Increased hepatic fat in overweight Hispanic youth influenced by interaction between genetic variation in PNPLA3 and high dietary carbohydrate and sugar consumption. *Am J Clin Nutr*. (2010) 92:1522–7. doi: 10.3945/ajcn.2010.30185
8. Vos MB, Goran MI. Sugar, Sugar . . . Not So Sweet for the Liver. *Gastroenterology*. (2017) 153:642–5. doi: 10.1053/j.gastro.2017.07.029
9. Kiess W, Galler A, Reich A, Müller G, Kapellen T, Deutscher J, et al. Clinical aspects of obesity in childhood and adolescence. *Obes Rev*. (2001) 2:29–36. doi: 10.1046/j.1467-789x.2001.00017.x
10. Chi DL, Scott JM. Added sugar and dental caries in children: a scientific update and future steps. *Dent Clin North Am*. (2019) 63:17–33. doi: 10.1016/j.cden.2018.08.003
11. Pulgarón ER. Childhood obesity: a review of increased risk for physical and psychological comorbidities. *Clin Ther*. (2013) 35:A18–32. doi: 10.1016/j.clinthera.2012.12.014
12. Hebebrand J, Herpertz-Dahlmann B. Psychological and psychiatric aspects of pediatric obesity. *Child Adolesc Psychiatr Clin N Am*. (2009) 18:49–65. doi: 10.1016/j.chc.2008.08.002
13. Puder JJ, Munsch S. Psychological correlates of childhood obesity. *Int J Obes*. (2010) 34(Suppl. 2):S37–43. doi: 10.1038/ijo.2010.238
14. Puhl RM, Latner JD. Stigma, obesity, and the health of the nation's children. *Psychol Bull*. (2007) 133:557–80. doi: 10.1037/0033-2909.133.4.557
15. World Health Organization. *Sugar Intake for Adults and Children*. Geneva: World Health Organization (2015).
16. U.S. Department of Health and Human Services and U.S. Department of Agriculture. *2015–2020 Dietary Guidelines for Americans*. 8th ed. Washington, DC: United States Department of Agriculture (2015).
17. Vos MB, Kaar JL, Welsh JA, Van Horn LV, Feig DI, Anderson CAM, et al. Added sugars and cardiovascular disease risk in children: a scientific statement from the American heart association. *Circulation*. (2017) 135:e1017–34. doi: 10.1161/CIR.0000000000000439
18. Rosinger A, Herrick K, Gahche J, Park S. Sugar-sweetened beverage consumption among U.S. youth, 2011-2014. *NCHS Data Brief*. (2017) 271:1–8. Available online at: https://www.cdc.gov/nchs/products/databriefs/db271.html
19. Mendez MA, Miles DR, Poti JM, Sotres-Alvarez D, Popkin BM. Persistent disparities over time in the distribution of sugar-sweetened beverage intake among children in the United States. *Am J Clin Nutr*. (2019) 109:79–89. doi: 10.1093/ajcn/nqy123
20. Tipton JA. Caregivers' psychosocial factors underlying sugar-sweetened beverage intake among non-Hispanic black preschoolers: an elicitation study. *J Pediatr Nurs*. (2014) 29:47–57. doi: 10.1016/j.pedn.2013.06.006
21. Bleich SN, Wolfson JA. Trends in SSBs and snack consumption among children by age, body weight, and race/ethnicity. *Obesity*. (2015) 23:1039–46. doi: 10.1002/oby.21050
22. Bogart LM, Cowgill BO, Sharma AJ, Uyeda K, Sticklor LA, Alijewicz KE, et al. Parental and home environmental facilitators of sugar-sweetened beverage consumption among overweight and obese Latino youth. *Acad Pediatr*. (2013) 13:348–55. doi: 10.1016/j.acap.2013.02.009
23. Couch SC, Glanz K, Zhou C, Sallis JF, Saelens BE. Home food environment in relation to children's diet quality and weight status. *J Acad Nutr Diet*. (2014) 114:1569–79 e1. doi: 10.1016/j.jand.2014.05.015
24. Zahid A, Davey C, Reicks M. Beverage Intake among Children: associations with parent and home-related factors. *Int J Environ Res Public Health*. (2017) 14:929. doi: 10.3390/ijerph14080929
25. Lopez NV, Ayala GX, Corder K, Eisenberg CM, Zive MM, Wood C, et al. Parent support and parent-mediated behaviors are associated with children's sugary beverage consumption. *J Acad Nutr Diet*. (2012) 112:541–7. doi: 10.1016/j.jand.2011.11.013
26. Zytnick D, Park S, Onufrak SJ. Child and caregiver attitudes about sports drinks and weekly sports drink intake among U.S. youth. *Am J Health Promot*. (2016) 30:e110–9. doi: 10.4278/ajhp.140103-QUAN-8
27. Bradbury KM, Turel O, Morrison KM. Electronic device use and beverage related sugar and caffeine intake in US adolescents. *PLoS ONE*. (2019) 14:e0223912. doi: 10.1371/journal.pone.0223912
28. Ahluwalia N, Herrick K. Caffeine intake from food and beverage sources and trends among children and adolescents in the United States: review of national quantitative studies from 1999 to 2011. *Adv Nutr*. (2015) 6:102–11. doi: 10.3945/an.114.007401
29. Drewnowski A, Rehm CD. Sources of caffeine in diets of us children and adults: trends by beverage type and purchase location. *Nutrients*. (2016) 8:154. doi: 10.3390/nu8030154
30. Mitchell DC, Knight CA, Hockenberry J, Teplansky R, Hartman TJ. Beverage caffeine intakes in the U.S. *Food Chem Toxicol*. (2014) 63:136–42. doi: 10.1016/j.fct.2013.10.042
31. Meredith SE, Juliano LM, Hughes JR, Griffiths RR. Caffeine use disorder: a comprehensive review and research Agenda. *J Caffeine Res*. (2013) 3:114–30. doi: 10.1089/jcr.2013.0016
32. Owens JA, Mindell J, Baylor A. Effect of energy drink and caffeinated beverage consumption on sleep, mood, and performance in children and adolescents. *Nutr Rev*. (2014) 72(Suppl. 1):65–71. doi: 10.1111/nure.12150
33. Sylvetsky AC, Visek AJ, Halberg S, Rhee K, Ongaro Z, Essel KE, et al. Beyond taste and easy access: physical, cognitive, interpersonal, and emotional reasons for sugary drink consumption among children and adolescents. *Appetite*. (2020) 155:104826. doi: 10.1016/j.appet.2020.104826
34. Guerrero AD, Mao C, Fuller B, Bridges M, Franke T, Kuo AA. Racial and ethnic disparities in early childhood obesity: growth trajectories in body mass index. *J Racial Ethn Health Disparities*. (2016) 3:129–37. doi: 10.1007/s40615-015-0122-y
35. Trochim WM, McLinden D. Introduction to a special issue on concept mapping. *Eval Program Plann*. (2017) 60:166–75. doi: 10.1016/j.evalprogplan.2016.10.006
36. Kane M, Trochim W. *Concept Mapping for Planning and Evaluation*. Thousand Oaks, CA: Sage Publications (2007). doi: 10.4135/9781412983730
37. Trochim W, Kane M. Concept mapping: an introduction to structured conceptualization in health care. *Int J Qual Health Care*. (2005) 17:187–91. doi: 10.1093/intqhc/mzi038
38. Visek AJ, Achrati SM, Mannix H, McDonnell K, Harris BS, DiPietro L. The fun integration theory: toward sustaining children and adolescents sport participation. *J Phys Act Health*. (2015) 12:424–33. doi: 10.1123/jpah.2013-0180
39. Visek AJ, Blake EF, Otterbein M, Chandran A, Sylvetsky AC. SWEET MAPS: a conceptualization of low-calorie sweetener consumption among young adults. *Curr Develop Nutr*. (2018) 3:nzy103. doi: 10.1093/cdn/nzy103
40. Sturrock K, Rocha J. A multidimensional scaling stress evaluation table. *Field Methods*. (2000) 12:49–60. doi: 10.1177/1525822X0001200104
41. Rosas SR, Kane M. Quality and rigor of the concept mapping methodology: a pooled study analysis. *Eval Program Plann*. (2012) 35:236–45. doi: 10.1016/j.evalprogplan.2011.10.003
42. Burke MV, Small DM. Physiological mechanisms by which non-nutritive sweeteners may impact body weight and metabolism. *Physiol Behav*. (2015) 152:381–8. doi: 10.1016/j.physbeh.2015.05.036
43. Sylvetsky AC, Visek AJ, Turvey C, Halberg S, Weisenberg JR, Lora K, et al. Parental concerns about child and adolescent caffeinated sugar-sweetened beverage intake and perceived barriers to reducing consumption. *Nutrients*. (2020) 12:885. doi: 10.3390/nu12040885
44. Eck KM, Dinesen A, Garcia E, Delaney CL, Famodu OA, Olfert MD, et al. "Your body feels better when you drink water": parent and school-age children's sugar-sweetened beverage cognitions. *Nutrients*. (2018) 10:1232. doi: 10.3390/nu10091232
45. Desor JA, Beauchamp GK. Longitudinal changes in sweet preferences in humans. *Physiol Behav*. (1987) 39:639–41. doi: 10.1016/0031-9384(87)90166-1
46. Maller O, Desor JA. Effect of taste on ingestion by human newborns. *Symp Oral Sens Percept*. (1973) 4:279–91.
47. Bobowski N, Mennella JA. Personal variation in preference for sweetness: effects of age and obesity. *Child Obes*. (2017) 13:369–76. doi: 10.1089/chi.2017.0023
48. Desor JA, Greene LS, Maller O. Preferences for sweet and salty in 9- to 15-year-old and adult humans. *Science*. (1975) 190:686–7. doi: 10.1126/science.1188365

49. Patel AI, Hampton KE. Encouraging consumption of water in school and child care settings: access, challenges, and strategies for improvement. *Am J Public Health*. (2011) 101:1370–9. doi: 10.2105/AJPH.2011.300142
50. Brownell KD, Schwartz MB, Puhl RM, Henderson KE, Harris JL. The need for bold action to prevent adolescent obesity. *J Adolesc Health*. (2009) 45:S8–17. doi: 10.1016/j.jadohealth.2009.03.004
51. Juliano LM, Griffiths RR. A critical review of caffeine withdrawal: empirical validation of symptoms and signs, incidence, severity, and associated features. *Psychopharmacology (Berl)*. (2004) 176:1–29. doi: 10.1007/s00213-004-2000-x
52. Bryant Ludden A, Wolfson AR. Understanding adolescent caffeine use: connecting use patterns with expectancies, reasons, and sleep. *Health Edu Behav*. (2010) 37:330–42. doi: 10.1177/10901981093 41783
53. Martin KB, Bednarz JM, Aromataris EC. Interventions to control children's screen use and their effect on sleep: A systematic review and meta-analysis. *J Sleep Res*. (2020):e13130. doi: 10.1111/jsr.13130
54. Sylvetsky AC, Parnarouskis L, Merkel PE, Gearhardt AN. Children's sugar-sweetened beverage consumption: striking parallels with substance use disorder symptoms. *Front Pediatr*. (2020) 8:594513. doi: 10.3389/fped.2020. 594513
55. Falbe J, Thompson HR, Patel A, Madsen KA. Potentially addictive properties of sugar-sweetened beverages among adolescents. *Appetite*. (2019) 133:130–7. doi: 10.1016/j.appet.2018.10.032
56. Gearhardt AN, White MA, Masheb RM, Morgan PT, Crosby RD, Grilo CM. An examination of the food addiction construct in obese patients with binge eating disorder. *Int J Eat Disord*. (2012) 45:657–63. doi: 10.1002/eat.20957
57. Schulte EM, Avena NM, Gearhardt AN. Which foods may be addictive? The roles of processing, fat content, and glycemic load. *PLoS ONE*. (2015) 10:e0117959. doi: 10.1371/journal.pone.0117959
58. Schulte EM, Yokum S, Potenza MN, Gearhardt AN. Neural systems implicated in obesity as an addictive disorder: from biological to behavioral mechanisms. *Prog Brain Res*. (2016) 223:329–46. doi: 10.1016/bs.pbr.2015.07.011
59. Striley CL, Griffiths RR, Cottler LB. Evaluating dependence criteria for caffeine. *J Caffeine Res*. (2011) 1:219–25. doi: 10.1089/jcr.2011. 0029
60. Scaglioni S, De Cosmi V, Ciappolino V, Parazzini F, Brambilla P, Agostoni C. Factors influencing children's eating behaviours. *Nutrients*. (2018) 10:706. doi: 10.3390/nu10060706
61. Watts AW, Miller J, Larson NI, Eisenberg ME, Story MT, Neumark-Sztainer D. Multicontextual correlates of adolescent sugar-sweetened beverage intake. *Eat Behav*. (2018) 30:42–8. doi: 10.1016/j.eatbeh.2018. 04.003
62. Brown CL, Halvorson EE, Cohen GM, Lazorick S, Skelton JA. Addressing childhood obesity: opportunities for prevention. *Pediatr Clin North Am*. (2015) 62:1241–61. doi: 10.1016/j.pcl.2015.05.013
63. Fulkerson JA, Friend S, Horning M, Flattum C, Draxten M, Neumark-Sztainer D, et al. Family home food environment and nutrition-related parent and child personal and behavioral outcomes of the healthy Home Offerings via the Mealtime Environment (HOME) Plus program: a randomized controlled trial. *J Acad Nutr Diet*. (2018) 118:240–51. doi: 10.1016/j.jand.2017. 04.006
64. Zenk SN, Schulz AJ, Israel BA, James SA, Bao S, Wilson ML. Fruit and vegetable access differs by community racial composition and socioeconomic position in Detroit, Michigan. *Ethn Dis*. (2006) 16:275–80.
65. Block JP, Scribner RA, DeSalvo KB. Fast food, race/ethnicity, and income: a geographic analysis. *Am J Preventive Med*. (2004) 27:211–7. doi: 10.1016/S0749-3797(04)00139-4
66. Algert SJ, Agrawal A, Lewis DS. Disparities in access to fresh produce in low-income neighborhoods in Los Angeles. *Am J Prev Med*. (2006) 30:365–70. doi: 10.1016/j.amepre.2006.01.009
67. Kwate NO, Loh JM. Separate and unequal: the influence of neighborhood and school characteristics on spatial proximity between fast food and schools. *Prev Med*. (2010) 51:153–6. doi: 10.1016/j.ypmed.2010.04.020
68. Bruening M, MacLehose R, Eisenberg ME, Nanney MS, Story M, Neumark-Sztainer D. Associations between sugar-sweetened beverage consumption and fast-food restaurant frequency among adolescents and their friends. *J Nutr Educ Behav*. (2014) 46:277–85. doi: 10.1016/j.jneb.2014.02.009
69. Stead M, McDermott L, Mackintosh AM, Adamson A. Why healthy eating is bad for young people's health: identity, belonging and food. *Soc Sci Med*. (2011) 72:1131–9. doi: 10.1016/j.socscimed.2010.12.029
70. Dohnke B, Weiss-Gerlach E, Spies CD. Social influences on the motivation to quit smoking: main and moderating effects of social norms. *Addict Behav*. (2011) 36:286–93. doi: 10.1016/j.addbeh.2010.11.001
71. Chung SJ, Ersig AL, McCarthy AM. The influence of peers on diet and exercise among adolescents: a systematic review. *J Pediatr Nurs*. (2017) 36:44–56. doi: 10.1016/j.pedn.2017.04.010
72. Klassen KM, Borleis ES, Brennan L, Reid M, McCaffrey TA, Lim MS. What people "like": analysis of social media strategies used by food industry brands, lifestyle brands, and health promotion organizations on Facebook and Instagram. *J Med Internet Res*. (2018) 20:e10227. doi: 10.2196/10227

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Chronic Fructose Substitution for Glucose or Sucrose in Food or Beverages and Metabolic Outcomes: An Updated Systematic Review and Meta-Analysis

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Despite the publication of several of meta-analyses in recent years, the effects of fructose on human health remains a topic of debate. We previously undertook two meta-analyses on post-prandial and chronic responses to isoenergetic replacement of fructose for sucrose or glucose in food or beverages (Evans et al. 2017, AJCN 106:506–518 & 519–529). Here we report on the results of an updated search with a complete re-extraction of previously identified studies and a new and more detailed subgroup-analysis and meta-regression. We identified two studies that were published after our previous analyses, which slightly altered effect sizes and conclusions. Overall, the isoenergetic substitution of fructose for glucose resulted in a statistically significant but clinically irrelevant reduction in fasting blood glucose, insulin, and triglyceride concentrations. A subgroup analysis by diabetes status revealed much larger reductions in fasting blood glucose in people with impaired glucose tolerance and type 2 diabetes. However, each of these subgroups contained only a single study. In people with a healthy body mass index, fructose consumption was associated with statistically significant, but clinically irrelevant reductions in fasting blood glucose and fasting blood insulin. Meta-regression of the outcomes by a number of pre-identified and *post-hoc* covariates revealed some sources of heterogeneity, such as year of publication, age of the participants at baseline, and participants' sex. However, the small number of studies and the large number of potential covariates precluded detailed investigations of effect sizes in different subpopulations. For example, well-controlled, high quality studies in people with impaired glucose tolerance and type 2 diabetes are still lacking. Taken together, the available data suggest that chronic consumption of fructose is neither more beneficial, nor more harmful than equivalent doses of sucrose or glucose for glycemic and other metabolic outcomes.

Keywords: fructose, glucose, sucrose, meta-analysis, glycemia

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INTRODUCTION

Historically, as reviewed by Sievenpiper (1), fructose was considered a healthy choice for people with diabetes (1). For the last several decades, however, fructose has instead been seen as a primary driver of adverse health outcomes (2–12). In 2004, a retrospective observational correlation of increasing dietary intake of high fructose corn syrup (HFCS) with increasing obesity was published (13). Despite the findings being observational, and the authors' own analysis that HFCS "may play a role" in the increase in obesity due to a "temporal relation," this analysis led to a large number of studies being done to investigate not if, but how fructose causes harm, even though HFCS contains, at most, only 55% fructose. The recent publication of several meta-analyses demonstrating neutral or even positive effects of isoenergetic fructose consumption, both for short-term and chronic exposures (14–23), have not, despite significant media coverage (24–30), changed the views of many in the scientific community and the general public. This is perhaps because the epidemiological trials, real-world interventional trials, and isoenergetic randomized controlled trials are addressing different questions.

Our previous meta-analyses were the first to concentrate on potential "real world" changes in the use of fructose, i.e., the isoenergetic substitution of fructose for current uses of sucrose or glucose in food or beverages (14, 15). In addition, these analyses were restricted to studies that used double-blind methodology, provided participants with all foods, and/or kept a detailed analysis of the participants' food intakes. This allowed us to be more confident in the interpretation of the results. For example, we had fewer concerns that participants altered their behavior based on the knowledge of their allocation. Our previous analysis of post-prandial studies included 47 studies reporting on 62 individual study arms (14). The data overwhelmingly showed that fructose reduces post-prandial peak blood glucose, especially in people with overweight or obesity, and in people with impaired glucose tolerance, type 1 diabetes, and type 2 diabetes. Other changes were significant reductions in the post-prandial blood glucose area under the curve (AUC) and peak post-prandial insulin concentrations. No changes were observed in peak post-prandial triglyceride concentrations (14). Our acute and chronic findings gained some traction, at least in the public domain (24–30). However, several narrative reviews that were published well after our meta-analyses, failed to mention that, at least for the outcomes mentioned here, fructose does not seem to be a specific cause of disease above and beyond that of other sugars (31–36).

Given that over 4 years had elapsed since our previous search, and that the debate over the role of fructose in health is still ongoing, we undertook an updated search for chronic studies matching our previous search criteria.

METHODS

Study Design

This update followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (37). The PICOTS question was: in people with normal glucose tolerance, impaired glucose tolerance, or diabetes, with healthy body weight, overweight, or obesity, does fructose, isoenergetically substituted for sucrose or glucose in food or beverages, alter measures of longer-term glycemic control [glycated hemoglobin (HbA1c), homeostatic model assessment (HOMA), fasting blood glucose, fasting blood insulin], blood lipids [fasting total cholesterol, low density lipoproteins (LDL), high density lipoproteins (HDL), and triglycerides], and obesity (measured as body weight), over a period of two or more weeks in a double-blind, food-controlled, or strict dietary analysis setting?

Participants

Participants in the studies could be children, teenagers, or adults, with normal glucose tolerance, impaired glucose tolerance, or diabetes. People with healthy body weight, or with overweight, or obesity were included. No restrictions were placed on ethnicity of the participants, or the country in which the study took place.

Interventions

Included interventions were purified fructose (i.e., not fructose-containing foods such as fruits), provided to participants in either foods (e.g., baked into cakes, dissolved in jams or yogurts) or beverages.

Comparators

Acceptable comparators were purified glucose or sucrose provided to participants in the same vehicle and at the same caloric value as fructose.

Systematic Review Protocol

We followed the same search strategy, inclusion and exclusion criteria, and largely the same subgroup analyses outlined in the original protocol registered previously (38). The PROSPERO registration number of the present study is CRD42015029385.

Data Sources

The search terms from the original analysis were reused for the updated search (15). The Cochrane Library, MEDLINE, EMBASE, the World Health Organization (WHO) International Clinical Trials Registry and clinicaltrials.gov databases were searched. The search was restricted to the time frame from the day before the date of the previous search (April 26, 2016) until September 23, 2020; no other restrictions were applied. All citations were uploaded into Covidence (39). Duplicates were removed and the remaining studies were subjected to double-blind coding at the title and abstract level; conflicts were resolved by consensus. The remaining studies were obtained as full texts and subjected to double-blind coding for inclusion in the review; again, any conflicts were resolved by consensus.

Study Selection and Data Extraction

The following selection criteria were applied to each citation and full text: randomized controlled trials in humans of at least 2 weeks' duration that compared fructose with either sucrose or glucose; the study was double-blind or blinded to participants; the diet was monitored or provided, or both; and data on any blood glucose outcome were provided. The studies could include people with or without diabetes, but not people who were acutely

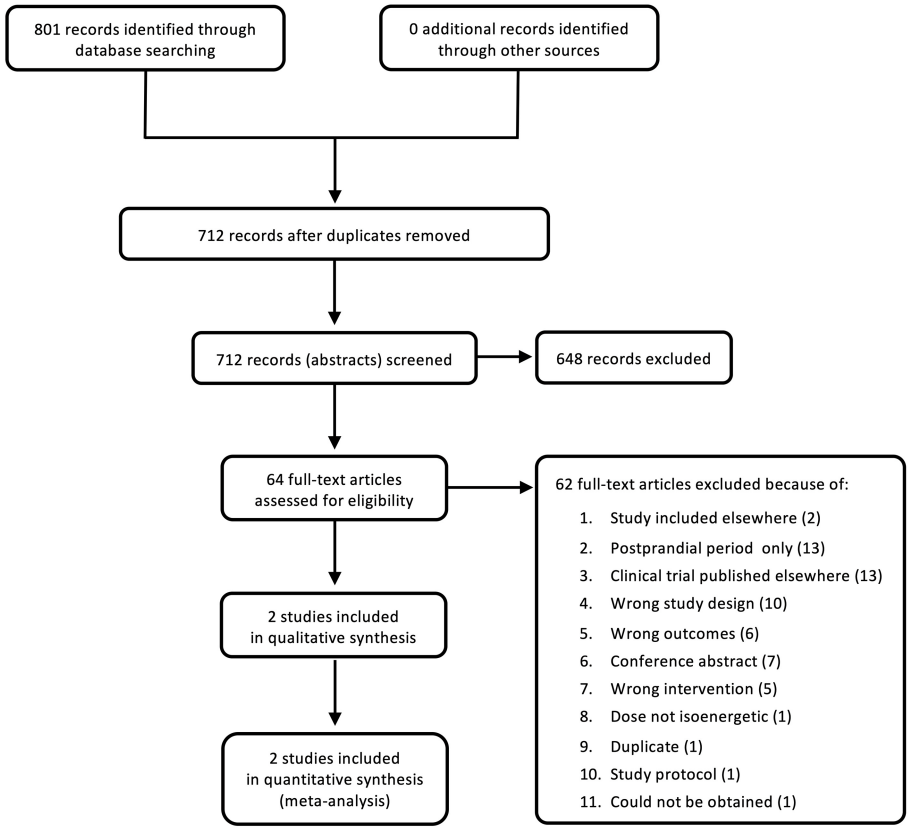

FIGURE 1 | PRISMA flow diagram of searches and study selection.

ill. Studies were excluded if they were of <2 weeks in duration, were unblinded or the diet was not isoenergetic (demonstrated through monitoring of the diet or providing all participants with their food), or if blood glucose data were not reported.

Data from all included studies (previously included studies and new studies) were extracted into an Excel spreadsheet by one author and checked by another. We extracted data on study characteristics (study type, substituted sugar, age, weight, diabetes status of participants etc.) along with changes in HbA1c, HOMA, fasting blood glucose, fasting blood insulin, fasting total, HDL and LDL cholesterol, fasting triglycerides, and body weight.

The definitions of normoglycemia, impaired glucose tolerance, and type 2 diabetes were taken from General Practice Management of Diabetes (40). If stated, we used the study authors' baseline values and classification of their study population. If this information was not provided, fasting blood glucose values were defined as the mean blood glucose value at time 0 of the intervention.

Data Analysis

Data presented in different units (e.g., μIU/mL, pmol/mL, or g/L for insulin concentrations) were standardized using EndMemo.com (41). When required, data were converted using the statistical algorithms reported by the Cochrane Collaboration (42). Where data were given as means and standard deviations (SD), these were converted to standard errors (SE), using the following formula:

$$SD = SE \times \sqrt{N},$$

where N = the number of participants in the study arm. Where neither SD or SE was given, and could not be calculated by other means, the SE was imputed by taking the mean of the SEs from all other studies of the same kind reporting the same outcome.

Mean differences (MD) and standard errors (SE) of the mean differences were calculated for crossover studies as follows:

$$MD = Outcome_{(end\ of\ intervention\ period)} - Outcome_{(end\ of\ control\ period)}$$

and the SE as:

$$SE = \sqrt{\left[\left(SEendi^2 + SEendc^2\right) - 2r\left(SEendi \times SEendc\right)\right]},$$

where r is the intrapersonal correlation coefficient of the individual outcome, SE endi = the standard error of the end value of the intervention period, and SE endc = the standard error of the end value of the control period (14).

For parallel studies, the mean differences were calculated as follows:

$$MD = Outcome_{(end\ of\ intervention\ period)} - Outcome_{(end\ of\ control\ period)}$$

TABLE 1 | Characteristics of included studies.

Study ID (reference)	Study kind	Study length	*N* Int.	*N* Cont.	Dose	Type of substitution	Glucose status	Presentation	Country	Blinding	Sex	Mean age	BMI category	Mean BMI	Funding
Aeberli 2011 G40 (56)	X-over	6 × 3 w	29	29	40	Glucose	Norm	Beverage	Switzerland	Yes	M	22.8	Healthy	22.5	Government
Aeberli 2011 G80 (56)	X-over	6 × 3 w	29	29	80	Glucose	Norm	Beverage	Switzerland	Yes	M	22.8	Healthy	22.5	Government
Aeberli 2011 S80 (56)	X-over	6 × 3 w	29	29	80	Sucrose	Norm	Beverage	Switzerland	Yes	M	22.8	Healthy	22.5	Government
Aeberli 2013 G (57)	X-over	4 × 3 w	9	9	80	Glucose	Norm	Beverage	Switzerland	Yes	M	26.3	Healthy	22.4	Government
Aeberli 2013 S (57)	X-over	4 × 3 w	9	9	80	Sucrose	Norm	Beverage	Switzerland	Yes	M	26.3	Healthy	22.4	Government
Angelopolous 2016 (58)	Parallel	10 w	92	94	9% of EEI	Glucose	Norm	Beverage	USA	Yes	Both	37.7	Overweight	26.3	Private
Bantle 2000 (59)	X-over	2 × 6 w	24	24	70	Glucose	Norm	Beverage	USA	Unclear	Both	41.3	Overweight	25.1	Government
Bossetti 1984 (60)	X-over	2 × 2 w	8	8	78.5	Sucrose	Norm	Food	USA	Unclear	Both	26.7	Healthy	22.7	Government
Heden 2014/1 (61)	X-over	2 × 2 w	9	9	35	Glucose	Norm	Beverage	USA	Yes	M	18.3	Healthy	23.5	Government
Heden 2014/2 (61)	X-over	2 × 2 w	11	11	35	Glucose	Norm	Beverage	USA	Yes	M	17.1	Obese	30.6	Government
Heden 2014/3 (61)	X-over	2 × 2 w	11	11	35	Glucose	Norm	Beverage	USA	Yes	F	18.3	Healthy	24.2	Government
Heden 2014/4 (61)	X-over	2 × 2 w	9	9	35	Glucose	Norm	Beverage	USA	Yes	F	17.8	Obese	31.0	Government
Heden 2015 6666 (62)	X-over	2 × 2 w	7	7	35	Glucose	Norm	Beverage	USA	Unclear	Both	18.0	Obese	34.6	Government
Jin 2014 (63)	Parallel	4 w	9	12	99	Glucose	Norm	Beverage	USA	Yes	Both	13.5	Obese	32.6	Government
Koh 1988 I (64)	X-over	2 × 4 w	9	9	15% of EEI	Glucose	Impaired	Food	USA	Unclear	Both	54.0	Overweight	27.3	Government

(Continued)

TABLE 1 | Continued

Study ID	Study kind	Study length	*N* Int.	*N* Cont.	Dose	Type of substitution	Glucose status	Presentation	Country	Blinding	Sex	Mean age	BMI category	Mean BMI	Funding
Koh 1988 N (64)	X-over	2 × 4 w	9	9	15% of EEI	Glucose	Norm	Food	USA	Unclear	Both	50.0	Healthy	23.3	Government
Kuzma 2019 N (65)	X-over	3 × 8 d	12	12	25% of EEI	Glucose	Norm	Beverage	USA	Yes	Both	33.0	Healthy	23.7	Government
Kuzma 2019 O (65)	X-over	3 × 8 d	12	12	25% of EEI	Glucose	Norm	Beverage	USA	Yes	Both	39.0	Obese	31.0	Government
Lowndes 2015 (66)	Parallel	10 w	41	44	9% of EEI	Glucose	Norm	Beverage	USA	Yes	Both	35.7	Overweight	26.0	Private
Malerbi 1996 (67)	X-over	3 × 4 w	16	16	19-20% EEI	Sucrose	Type 2 diabetes	Food	Brazil	Unclear	Both	54.2	Overweight	25.6	Both
Silbernagel 2011 (68)	Parallel	4 w	10	10	150	Glucose	Norm	Beverage	Germany	Yes	Both	30.5	Overweight	25.9	Government

G40, fructose/glucose 40 g/day; G80, fructose/glucose 80 g/day; G, glucose; S, sucrose; I, impaired glucose tolerance; N, normal glucose tolerance/body weight; O, overweight; X-over, crossover; EEI, estimated energy intake; M, males; F, females; Int., intervention; Cont., control; d, days; w, weeks; m, months.

and the SE as:

$$SE = \sqrt{(SE - endi^2 + SE - endc^2)},$$

where SEendi = the standard error of the end value of the intervention arm, and SEendc = the standard error of the end value of the control arm.

Outcome data were copied into Review Manager 5.4 and analyzed using a generic inverse variance, random effects model with 95% confidence intervals (CI) (43). The use of this model was chosen in order to combine crossover and parallel trials. A random effects model was chosen over a fixed effects model, as a random effects model is the appropriate statistical model for combining studies that differ in the participant characteristics (e.g., age, body weight, dose of sugar, etc.). Most outcomes were reported as mean differences; standardized mean differences were used where studies reported outcomes in different ways that could not be converted to single scale.

Where a study had more than two arms, both arms were included in separate subgroups with full participant numbers for all study arms. However, in these cases, the totals were removed from the meta-analyses, and only subtotals were included. If a study was included in a single subgroup, the number of participants in the repeated study arm was halved to avoid double-counting (44). If studies gave data as medians and ranges, or medians and inter-quartile ranges (IQR), these were converted to means and standard deviations following the work of Luo et al. and Wan et al. (45, 46).

Some studies gave participants fructose, sucrose, or glucose as a percent of daily energy requirements rather than a specific dose. In these cases, the doses were calculated from the baseline data (weight, height, BMI), using national averages where required.

Subgroups analyses were undertaken to determine the effect of study design (crossover compared with parallel design), publication date, blinding, dose of sugar used, funding source, diabetes status, body weight, sex, age, and sugar presentation (meal compared with beverage).

In some analyses, substantial heterogeneity was present. Subgroup analysis explained some, but not all of the heterogeneity. We therefore undertook meta-regression to identify the extent to which both factorial and continuous covariates altered the results.

Meta-regression was carried out in cases in which 10 or more studies were available for each covariate in an analysis. Outcomes with <10 studies were considered to be insufficient to enable a meaningful interpretation of outcomes (44). Where practical, meta-regression was undertaken using OpenMetaAnalyst with a random-effects model (47). Given the small number of studies under investigation, we could not undertake multivariate meta-regression, so each covariate was examined individually.

Heterogeneity of 0–40% as measured by I^2 was defined as potentially unimportant, 30–60% was considered to be potentially moderate heterogeneity, 50–90% was defined as potentially substantial heterogeneity, and 85–100% was defined as potentially considerable heterogeneity (48).

Study Quality

As all included studies were randomized controlled trials (RCTs), study quality was assessed using the Risk of Bias tool in Review Manager 5.4, based on the Cochrane Handbook for Systematic Reviews (49). The risk of bias was assessed in seven areas: (i) random sequence generation, (ii) allocation concealment, (iii) blinding of participants and personnel, (iv) blinding of outcome assessment, (v) incomplete outcome data (attrition bias), (vi) selective reporting (reporting bias), and (vii) other bias.

Clinical Relevance

The minimum clinically important differences (MCID) for changes in metabolic measures was taken as follows: HbA1c: 1% (50), fasting blood glucose: 23% (51), fasting blood triglycerides: 30% (52), fasting LDL: 10% (53), fasting HDL: 10% (53), body weight: 5% (54). For standardized mean differences, a change of 0.5 units was taken to be a meaningful change (55). No MCIDs were found for the following outcomes: fasting insulin, HOMA-IR, HOMA2, fasting total cholesterol.

RESULTS

Study Search

The search was carried out on September 23, 2020 and yielded 801 references, of which 89 were duplicates. The remaining 712 studies were screened at title and abstract level. From these, 648 studies were deemed to be irrelevant. The remaining 64 full texts were analyzed at full text level. Of these, only two new studies were identified and included into the updated analysis (**Figure 1**). The majority of full texts were excluded as they dealt with acute, post-prandial effects of fructose, were clinical trials that had not been published, had an inappropriate study design, did not include a measure of glycemic control, were conference abstracts, or had an inappropriate intervention.

Study Characteristics

The study characteristics of the included studies are shown in **Table 1**. In addition to the previously identified studies (56, 57, 59–64, 66–68), two new studies were included (58, 65), both of which were carried out in adults without diabetes. Angelopoulos et al. (58) included adults with an average BMI just into the overweight range (26.3 kg/m^2), whereas Kuzma et al. (65) had two groups of participants, one in the healthy BMI range (average = 23.7) and one in the obese range (average = 31.0). Angelopoulos et al. (58) substituted 9% of each participant's weight-maintaining energy intake with fructose or glucose, whereas Kuzma et al. (65) substituted 25% of each participant's energy needs with fructose or glucose. Both studies were undertaken in adults.

Quality Assessment and Risk of Bias

Data on study quality as determined by the Cochrane 7-item risk of bias analysis is shown in **Supplementary Figure 1**. The inclusion criteria for study design were restrictive; hence the risk of bias was low for most outcomes. However, as reported in the original analysis (15), not all measures of bias were reliably reported.

Fasting Blood Glucose

Twenty-one studies/study arms reported on the change in fasting blood glucose following fructose substitution for glucose (17 study arms) or sucrose (4 studies). The addition of the new studies changed the effect size slightly, but not the direction or significance. The substitution of fructose for glucose reduced fasting blood glucose by 0.11 mmol/L (95% CI: −0.18, −0.05; $p = 0.0005$) (**Figure 2**), but this result was not clinically relevant. There were no significant differences between fructose and sucrose. When grouped by diabetes status, all three groups (normal glucose tolerance, impaired glucose tolerance, type 2 diabetes) showed statistically significant reductions in fasting blood glucose (**Supplementary Figure 2**). The single studies in people with impaired glucose tolerance (64) and type 2 diabetes (67) showed much larger reductions in fasting blood glucose (−0.61 mmol/L; −0.80 mmol/L, respectively), which were reduced to a statistically significant but not clinically relevant degree. No differences were observed between subgroups when divided by dose or baseline BMI (**Supplementary Figures 3**, **4**).

HbA1c

Because most studies were done in people without impaired glucose tolerance or diabetes, change in HbA1c was reported by only two studies (**Supplementary Figure 5**). As each of these studies reported change in HbA1c in a different way, we calculated the standardized mean differences. We found that Koh et al. (64) reported a statistically significant and meaningful difference in HbA1c [SMD = −2.51 (95% CI: −3.44, −1.57), $p < 0.00001$], whereas the change HbA1c reported by Malerbi et al. (67) was not significant (58).

HOMA

The 13 studies/study arms that reported on HOMA used both HOMA-IR and HOMA2 as outcomes. In order to combine the HOMA results of all studies, we used a standardized mean difference analysis (**Supplementary Figures 6–9**). There were no significant differences between fructose and glucose [SMD = 0.11 (95% CI: −0.34, 0.56); $p = 0.64$]. A single study (56) that compared fructose with sucrose found a statistically significant increase in HOMA2 after fructose consumption.

Fasting Blood Insulin

Sixteen studies/study arms reported on changes in fasting blood insulin following fructose substitution for glucose (13 study arms) or sucrose (three studies). Fasting blood insulin reduced significantly following fructose consumption compared with glucose consumption [MD = −1.29 μIU/mL (95% CI: −2.22, −0.36), $p = 0.007$] (**Figure 3**). The comparison with sucrose revealed similar results but was not statistically significant. Fasting insulin was also statistically significantly lowered in studies using lower doses (30–40 g/day) [MD = −1.00 μIU/mL (95% CI: −1.84, −0.16), $p = 0.02$] and in studies using doses >80 g/day [MD = −1.49 μIU/mL (95% CI: −2.55, −0.44), $p = 0.005$]. Studies in people without diabetes at baseline also showed statistically significant reductions in fasting blood insulin [MD = −0.82 μIU/mL (95% CI: −1.52, −0.12), $p = 0.02$]

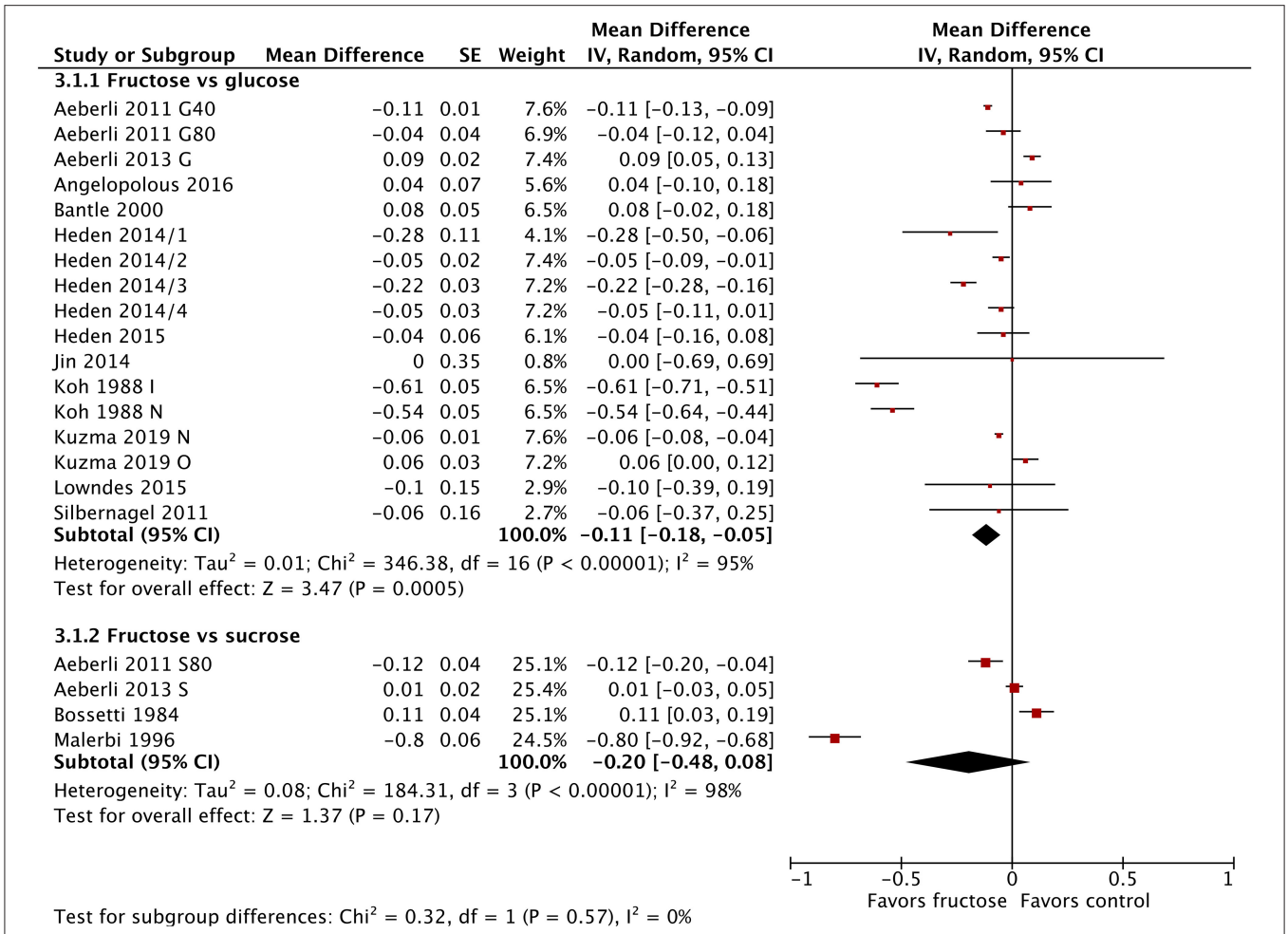

FIGURE 2 | Subgroup meta-analysis of fasting blood glucose following isoenergetic substitution of glucose or sucrose by fructose in food or beverages by substituted sugar. Values are mean differences (95% CIs) (expressed as mmol/L) between fasting blood glucose after fructose consumption and fasting blood glucose following glucose or sucrose consumption. IV, inverse variance; SE, standard error; G40, fructose/glucose 40 g/day; G80, fructose/glucose 80 g/day; G, glucose; S, sucrose; I, impaired glucose tolerance, N, normal glucose tolerance/body weight; O, overweight.

(**Supplementary Figures 10–12**). Baseline BMI did not influence blood insulin concentrations.

Fasting Blood Lipids

Total Cholesterol

The substitution of fructose for glucose or sucrose did not result in any significant changes in total cholesterol (**Figure 4A**). This did not differ when subgrouped by baseline BMI, dose, or diabetes status (**Supplementary Figures 13–15**).

Low Density Lipoproteins

The substitution of fructose for glucose or sucrose did not result in any significant changes in LDL cholesterol (**Figure 4B**), except when subgrouped by diabetes status (**Supplementary Figure 16**). The single study in people with type 2 diabetes showed a statistically but not clinically significant reduction in LDL following fructose consumption. This subgroup was also statistically different from the subgroup of studies in people without diabetes. No differences were apparent when subgrouping by BMI or dose (**Supplementary Figures 17, 18**).

High Density Lipoproteins

No changes in HDL were apparent (**Figure 4C**). No statistically significant differences emerged between any subgroups, by BMI, dose, or diabetes status (**Supplementary Figures 19–21**).

Triglycerides

The substitution of fructose for glucose or sucrose showed no significant changes in fasting triglyceride concentrations, except in the three studies comparing fructose with sucrose consumption (**Figure 4D**); this change was not clinically relevant. Subgrouping by baseline BMI or dose did not reveal any significant differences (**Supplementary Figures 22, 23**). When subgrouped by diabetes status, people with impaired glucose tolerance and those with type 2 diabetes showed statistically but not clinically relevant reductions in fasting triglycerides; however,

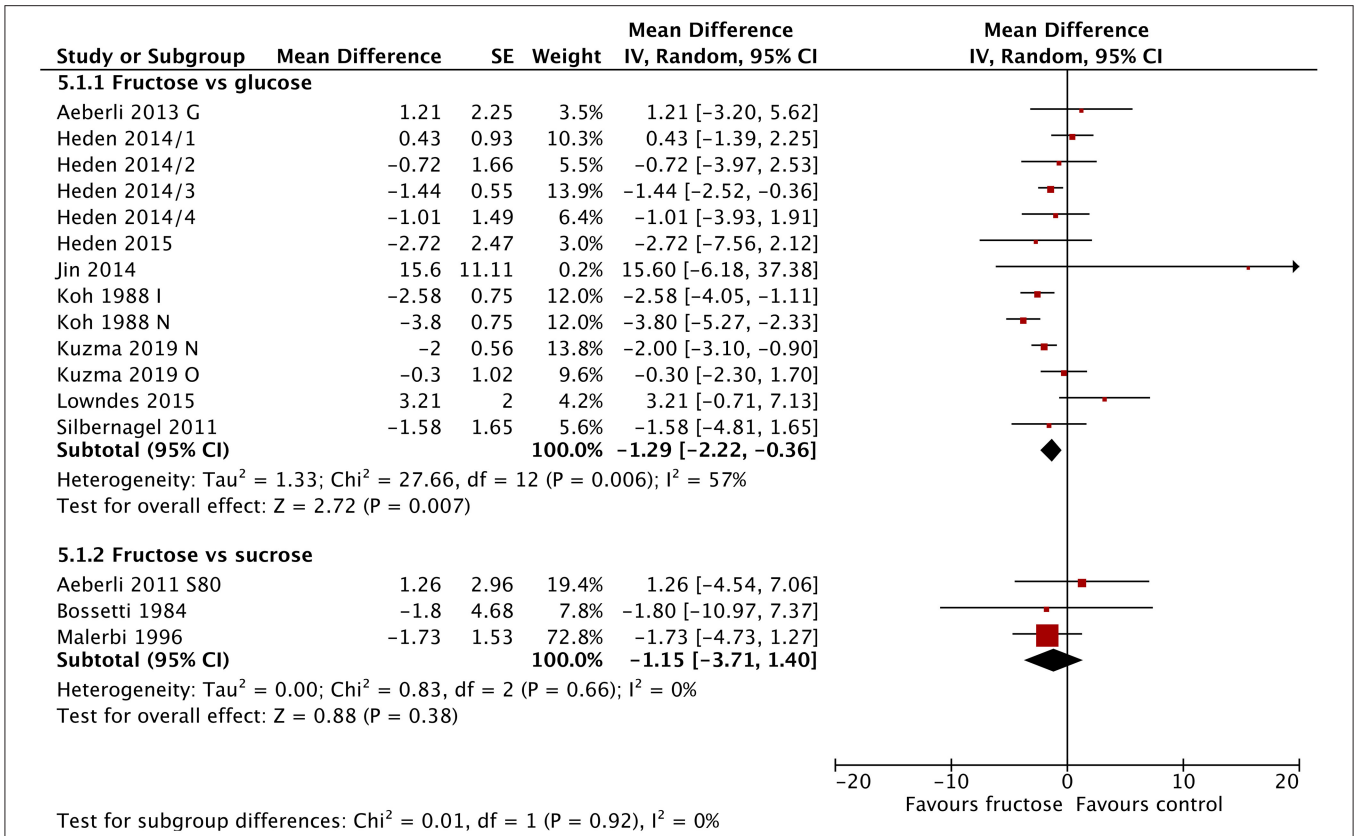

FIGURE 3 | Subgroup meta-analysis of fasting blood insulin following isoenergetic substitution of glucose or sucrose by fructose in food or beverages by substituted sugar. Values are mean differences (95% CIs) (expressed as μIU/mL) between fasting blood glucose after fructose consumption and fasting blood glucose following glucose or sucrose consumption. IV, inverse variance; SE, standard error; G, glucose; S, sucrose; I, impaired glucose tolerance, N, normal glucose tolerance/body weight; O, overweight.

each group was represented by only a single study in each group (**Supplementary Figure 24**).

Body Weight

Body weight was not significantly influenced by the substitution of fructose for glucose or sucrose (**Figure 5**). Similarly, subgroup analysis found no differences in body weight by baseline BMI or diabetes status (**Supplementary Figures 25, 26**), with the exception of dose. Studies using very high doses of fructose (>80 g/day) resulted in a statistically significant reduction in body weight [MD = −1.20 kg (95% CI: −2.11, −0.29), $p = 0.01$] (**Supplementary Figure 27**). This difference was not clinically significant.

Meta-Regression

The results of our meta-regression analyses are presented in **Table 2** and **Supplementary Tables 1–3**. For fasting blood glucose, a number of significant results came from single studies (e.g., impaired glucose tolerance, type 2 diabetes, funding from both industry, and government); these were ignored. However, a statistically significant difference was observed between the studies that provided food as the source of sugar rather than beverages, and for studies that blinded the participants to their allocation compared with those that provided food or kept account of the participants' diets. Similarly, both age of participants and year of publication were significantly associated with changes in fasting blood glucose. Unfortunately, the four food-based study arms were also among the studies causing a great deal of heterogeneity in the meta-regression by age of study (60, 64, 67), thus it is not clear if the difference came from the use of food, or simply from the age of the study.

Meta-regression of the studies for fasting insulin similarly revealed differences arising from covariates (**Supplementary Table 1**). Unclear blinding, age of participants and year of publication all influenced the outcomes. Interestingly, studies in males were more likely to be associated with an increase in fasting blood insulin, compared with those in females.

Meta-regression of body weight by the same covariates revealed only dose as a significant influence on the outcome. The coefficient was very small, however, and this result is likely to be of limited relevance.

Meta-regression of fasting triglyceride concentrations showed several significant covariates. As seen in our previous analyses, the use of food instead of beverages as the vehicle for

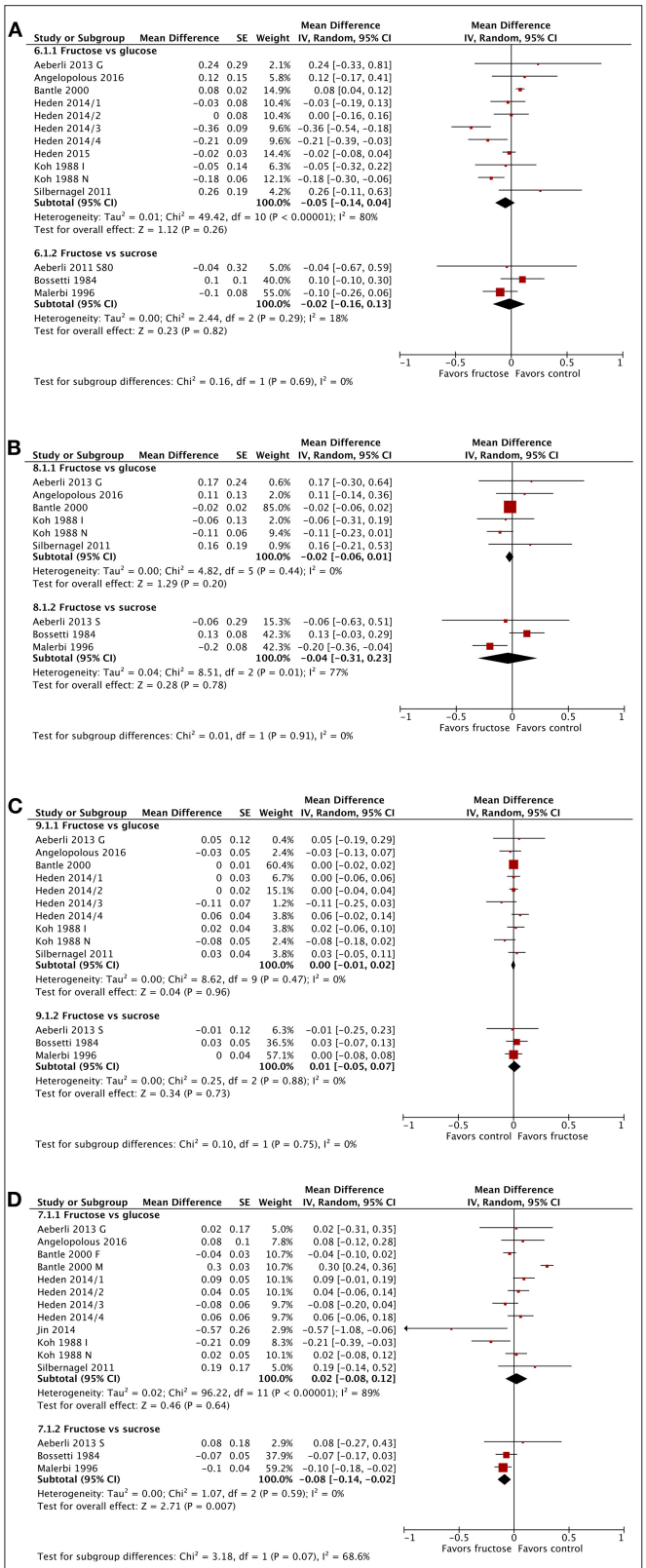

FIGURE 4 | Subgroup meta-analysis of fasting total cholesterol **(A)**, low density lipoprotein **(B)**, high density lipoprotein **(C)**, and triglycerides **(D)**

(Continued)

FIGURE 4 | following isoenergetic substitution of glucose or sucrose by fructose in food or beverages by substituted sugar. Values are mean differences (95% CIs) (expressed as mmol/L) between fasting blood glucose after fructose consumption and fasting blood glucose following glucose or sucrose consumption. IV, inverse variance; SE, standard error; G, glucose; S, sucrose; I, impaired glucose tolerance, N, normal glucose tolerance/body weight; O, overweight.

fructose delivery significantly reduced fasting triglycerides following fructose consumption compared with sucrose or glucose consumption. Interestingly, males were again more likely to have increased fasting triglyceride concentrations following fructose consumption compared with mixed or female-only studies, in whom significant reductions in fasting triglycerides were observed.

DISCUSSION

The results of our updated meta-analysis repeat and strengthen our previous findings on the lack of harmful effects specific to fructose consumption (15) in line with similar analyses (18–20, 22, 23), at least in the outcomes reported by these reviews. A reiteration and new discussion of these findings is warranted because many narrative reviews still propagate the view that fructose is more harmful than other sugars (31–36, 69, 70).

Our findings did show statistically significant reductions in fasting blood glucose (FBG) concentrations, fasting blood insulin (FBI) concentrations, and body weight. However, none of these differences was clinically relevant. Meta-regression did reveal some interesting findings, which require further investigation. For example, we found that presenting the sugar in foods rather than in beverages considerably altered the effect size for changes in FBG and FBI. However, we also found that the age of the study had a similar effect. The answer as to which of these covariates is causing this change is obscured by the fact that the food studies were also older.

Another interesting correlation to emerge from our meta-regression was the finding that the sex of the participants was associated with quite different outcomes. For example, overall fructose consumption lowered FBI and had no significant effect on triglycerides. Meta-regression by sex, however, found that female sex was associated with a reduction in FBI, whereas male sex was associated with no reduction in FBI. Similarly, female sex was associated with a statistically significant reduction in fasting triglycerides, whereas male sex was associated with a statistically significant increase in fasting triglycerides. Differences by sex in glucose homeostasis and lipoproteins have been described previously (71, 72). It is therefore possible that changes in these outcomes are also influenced by sex.

Even after subgroup meta-analysis and meta-regression, much heterogeneity remained. The lack of studies that covered multiple potential covariates, along with a small number of studies in total, did not allow us to investigate the sources of heterogeneity. We are also aware that undertaking multiple analyses will increase the

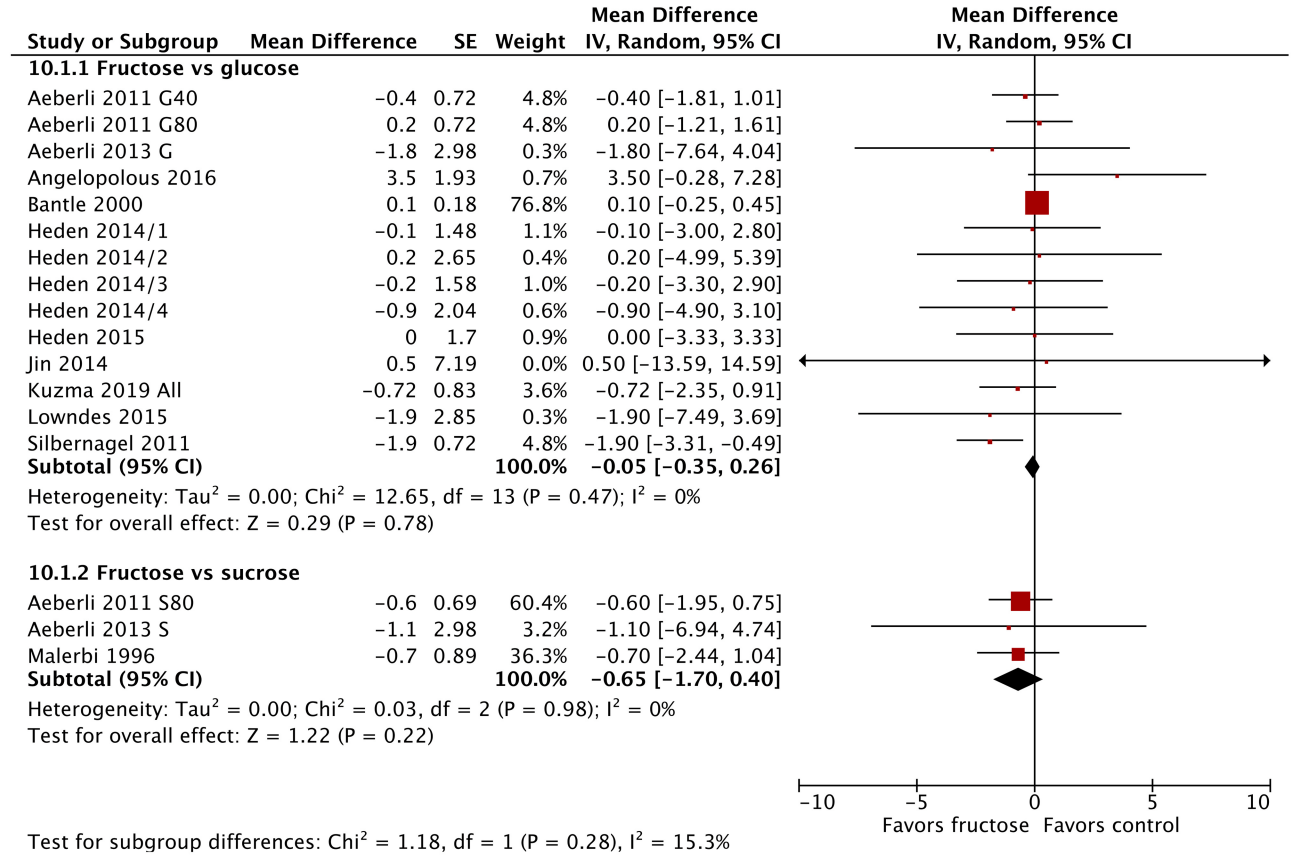

FIGURE 5 | Subgroup meta-analysis of body weight following isoenergetic substitution of glucose or sucrose by fructose in food or beverages by substituted sugar. Values are mean differences (95% CIs) (expressed as kg) between fasting blood glucose after fructose consumption and fasting blood glucose following glucose or sucrose consumption. IV, inverse variance; SE, standard error; G40, fructose/glucose 40 g/day; G80, fructose/glucose 80 g/day; G, glucose; S, sucrose; I, impaired glucose tolerance, N, normal glucose tolerance/body weight; O, overweight.

possibility of statistical significance arising by chance. One should therefore be careful not to overinterpret some of our findings.

Of note, we are not stating that consumption of sugar, especially as refined carbohydrates, is advisable or beneficial. Consumption of highly energy-dense foods without significant fiber and/or micronutrient content is certainly inadvisable (73). For this reason, the WHO recommends that <10% of daily energy should come from free sugars (74). We argue purely that ascribing harmful effects to fructose in particular is counter to the evidence. Where the use of sugar will continue (e.g., as a preservative or in home-made cakes and other treats), information on the post-prandial benefits of fructose (e.g., a reduction in peak post-prandial blood glucose and insulin concentrations), particularly in those with impaired glucose tolerance, type 1 and type 2 diabetes, should be provided.

Our study design deliberately selected for studies that kept the diets between the groups isocaloric. However, there are other factors at play that influence real world weight gain. For example, it has been shown that fructose increases the subjective sensation of hunger and food-seeking behavior in functional MRI studies (45, 75) although recent work comparing the actual food intakes following glucose-, fructose-, high fructose corn syrup-, and aspartame-sweetened beverages found no difference between any sugar in the total number of calories consumed over 8 days (76). Furthermore, Silbernagel et al. found a statistically significant reduction in body weight following 4 weeks of fructose consumption (68). These partially contradictory findings suggest that further research should be conducted before any conclusions are made.

Interestingly, it appears that a significant proportion of ingested fructose is converted to glucose in the small intestine (77, 78), with only very large doses spilling over to the liver (79). This was first shown over 50 years ago in an elegant study by Öckerman and Lundborg (80). In this study, the authors administered fructose or galactose to humans directly into the jejunum at doses ranging from 37.5 to 150 g. Up to 70% of the fructose could be recovered as glucose in the mesenteric veins, while an administration of galactose did not result in the recovery of glucose. More recently, it has been established that the small intestine expresses fructokinase along with other fructolytic and gluconeogenic enzymes (77) and that their expression is regulated by GLUT5 (a glucose transporter protein) and KHK (ketohexokinase) (81). Thus, it is unlikely that

TABLE 2 | Meta-regression of mean differences in fasting blood glucose concentrations between consumption of fructose compared with sucrose or glucose by factor and continuous covariates.

Potential covariant	*N* study arms	Model coefficient	Lower bound	Upper bound	*p*-value	
Factor covariates						
Control sugar	Glucose	17				
	Sucrose	4	−0.079	−0.338	0.180	0.552
Diabetes status	Normoglycaemia	19				
	Impaired glucose tolerance	1	−0.545	−0.833	−0.256	**<0.001**
	Type 2 diabetes	1	−0.735	−1.031	−0.439	**<0.001**
Study type	Cross-over	17				
	Parallel	4	0.119	−0.184	0.421	0.442
Food vs. beverage	Beverage	17				
	Food	4	−0.405	−0.600	−0.220	**<0.001**
Gender	Males	7				
	Both	12	−0.107	−0.328	0.114	0.343
	Females	2	−0.069	−0.431	0.293	0.703
Funding	Government	18				
	Both	1	−0.697	−1.076	−0.319	**<0.001**
	Industry	2	0.086	−0.216	0.389	0.575
Blinding	Yes	15				
	Unclear	6	−0.239	−0.444	−0.034	**0.022**
Continuous covariates						
BMI	Range: 22.4–34.6	21	0.004	−0.025	0.034	0.766
Age	Range: 13.5–54.2	21	−0.012	−0.019	−0.005	**<0.001**
Dose	Range: 35–150	21	0.001	−0.003	0.004	0.633
Year of publication	Range: 1984–2019	21	0.011	0.003	0.020	**0.009**

All data, including model coefficients, confidence intervals, and p-values were obtained using random-effect models and 95% confidence intervals in OpenMetaAnalyst. Values in bold represent statistically significant results.

small or moderate amounts of fructose are necessarily be more harmful than equivalent amounts of glucose, because at usual levels of consumption, most of the fructose simply never reaches the liver.

In the 4 years since our earlier search for articles on the effects of chronic fructose consumption, only two studies were published on isoenergetic fructose replacement for sucrose or glucose for periods longer than the immediate post-prandial period. However, 17 new fructose studies were published that were concerned with the post-prandial time period, despite little chance of these studies significantly changing the effect sizes already generated by previous meta-analyses.

The two new chronic studies (58, 65) were carried out in people with normal fasting blood glucose concentrations. In one study (65) only 24 participants were enrolled, half of whom had a healthy weight at baseline. The other study (58) enrolled more (i.e., 186) participants, but unfortunately, the average BMI at baseline was only just into the overweight range, and people with diabetes were actively excluded. Given the paucity of evidence in people with impaired glucose tolerance or diabetes, we find this disappointing. We therefore renew our call for high quality, isoenergetic studies to be carried out in people with a lack of glycemic control; else evidence-based dietary advice for these populations will continue to be lacking.

This updated systematic review and meta-analysis has confirmed our previous findings, i.e., that even high doses of fructose consumed daily do not adversely affect health when compared with isoenergetic amounts of sucrose or glucose. The absence of high-quality studies in people with, or at risk of diabetes hampers our ability to make specific recommendations based on diabetes status. Similarly, the large number of covariates and small number of studies did not allow us to investigate residual confounding through multivariate meta-regression. The little evidence we have in populations with diabetes does not support the claim that fructose is harmful for people with this condition; indeed, the opposite seems true. Whether these beneficial effects are real, however, can only be established with more evidence.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

KM designed the study, carried out the search, and drafted the manuscript. KM and MZ did title/abstract and full text inclusion, data extraction/checking, and data analysis. KM and

MF designed the analyses. MF and MZ critically analyzed the manuscript and suggested edits. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2021.647600/full#supplementary-material

REFERENCES

1. Sievenpiper JL. Fructose: back to the future? *Am J Clin Nutr*. (2017) 106:439–42. doi: 10.3945/ajcn.117.161539
2. DiNicolantonio JJ, O'Keefe JH, Lucan SC. Added fructose: a principal driver of type 2 diabetes mellitus and its consequences. *Mayo Clin Proc*. (2015) 90:372–81. doi: 10.1016/j.mayocp.2014.12.019
3. Lustig RH. Sickeningly sweet: does sugar cause type 2 diabetes? *Yes Can J Diabetes*. (2016) 40:282–6. doi: 10.1016/j.jcjd.2016.01.004
4. *Fat Chance: Beating the Odds Against Sugar, Processed Food, Obesity, and Disease*. Lustig, Robert H: Amazon.com.au: Books. Available online at: https://www.amazon.com.au/Fat-Chance-Beating-Against-Processed/dp/0142180432 (accessed December 26, 2020).
5. *The Case Against Sugar by Gary Taubes: 9780307946645*. PenguinRandomHouse.com: Books. PenguinRandomhouse.com. Available online at: https://www.penguinrandomhouse.com/books/213737/the-case-against-sugar-by-gary-taubes/ (accessed December 26, 2020).
6. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am J Clin Nutr*. (2014) 100:65–79. doi: 10.3945/ajcn.113.081521
7. Teff KL, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, et al. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab*. (2004) 89:2963–72. doi: 10.1210/jc.2003-031855
8. Saito H, Kato M, Yoshida A, Naito M. The ingestion of a fructose-containing beverage combined with fat cream exacerbates postprandial lipidemia in young healthy women. *J Atheroscler Thromb*. (2015) 22:85–94. doi: 10.5551/jat.22681
9. Hallfrisch J, Ellwood KC, Michaelis OE, Reiser S, O'Dorisio TM, Prather ES. Effects of dietary fructose on plasma glucose and hormone responses in normal and hyperinsulinemic men. *J Nutr*. (1983) 113:1819–26. doi: 10.1093/jn/113.9.1819
10. Montonen J, Järvinen R, Knekt P, Heliövaara M, Reunanen A. Consumption of sweetened beverages and intakes of fructose and glucose predict type 2 diabetes occurrence. *J Nutr*. (2007) 137:1447–54. doi: 10.1093/jn/137.6.1447
11. Goran MI, Ulijaszek SJ, Ventura EE. High fructose corn syrup and diabetes prevalence: a global perspective. *Glob Public Health*. (2013) 8:55–64. doi: 10.1080/17441692.2012.736257
12. de Koning L, Malik VS, Kellogg MD, Rimm EB, Willett WC, Hu FB. Sweetened beverage consumption, incident coronary heart disease, and biomarkers of risk in men. *Circulation*. (2012) 125:1735–41.S1. doi: 10.1161/CIRCULATIONAHA.111.067017
13. Bray GA, Nielsen SJ, Popkin BM. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Clin Nutr*. (2004) 79:537–43. doi: 10.1093/ajcn/79.4.537
14. Evans RA, Frese M, Romero J, Cunningham JH, Mills KE. Fructose replacement of glucose or sucrose in food or beverages lowers postprandial glucose and insulin without raising triglycerides: a systematic review and meta-analysis. *Am J Clin Nutr*. (2017) 106:506–18. doi: 10.3945/ajcn.116.145151
15. Evans RA, Frese M, Romero J, Cunningham JH, Mills KE. Chronic fructose substitution for glucose or sucrose in food or beverages has little effect on fasting blood glucose, insulin, or triglycerides: a systematic review and meta-analysis. *Am J Clin Nutr*. (2017) 106:519–29. doi: 10.3945/ajcn.116.145169
16. Braunstein CR, Noronha JC, Khan TA, Mejia SB, Wolever TM, Josse RG, et al. Effect of fructose and its epimers on postprandial carbohydrate metabolism: a systematic review and meta-analysis. *Clin Nutr Edinb Scotl*. (2020) 39:3308–18. doi: 10.1016/j.clnu.2020.03.002
17. Fattore E, Botta F, Bosetti C. Effect of fructose instead of glucose or sucrose on cardiometabolic markers: a systematic review and meta-analysis of isoenergetic intervention trials. *Nutr Rev*. (2020) 79:209–26. doi: 10.1093/nutrit/nuaa077
18. Ha V, Sievenpiper JL, de Souza RJ, Chiavaroli L, Wang DD, Cozma AI, et al. Effect of fructose on blood pressure: a systematic review and meta-analysis of controlled feeding trials. *Hypertens Dallas Tex 1979*. (2012) 59:787–95. doi: 10.1161/HYPERTENSIONAHA.111.182311
19. David Wang D, Sievenpiper JL, de Souza RJ, Cozma AI, Chiavaroli L, Ha V, et al. Effect of fructose on postprandial triglycerides: a systematic review and meta-analysis of controlled feeding trials. *Atherosclerosis*. (2014) 232:125–33. doi: 10.1016/j.atherosclerosis.2013.10.019
20. Sievenpiper JL, Chiavaroli L, de Souza RJ, Mirrahimi A, Cozma AI, Ha V, et al. "Catalytic" doses of fructose may benefit glycaemic control without harming cardiometabolic risk factors: a small meta-analysis of randomised controlled feeding trials. *Br J Nutr*. (2012) 108:418–23. doi: 10.1017/S0007114512 00013X
21. Sievenpiper JL, de Souza RJ, Mirrahimi A, Yu ME, Carleton AJ, Beyene J, et al. Effect of fructose on body weight in controlled feeding trials: a systematic review and meta-analysis. *Ann Intern Med*. (2012) 156:291–304. doi: 10.7326/0003-4819-156-4-201202210-00007
22. Chiavaroli Laura, de Souza Russell J, Vanessa H, Cozma Adrian I, Arash M, Wang David D, et al. Effect of fructose on established lipid targets: a systematic review and meta-analysis of controlled feeding trials. *J Am Heart Assoc*. 4:e001700. doi: 10.1161/JAHA.114.001700
23. Choo VL, Viguiliouk E, Blanco Mejia S, Cozma AI, Khan TA, Ha V, et al. Food sources of fructose-containing sugars and glycaemic control: systematic review and meta-analysis of controlled intervention studies. *BMJ*. (2018) 363:k4644. doi: 10.1136/bmj.k4644
24. *Fruit-Based Sugar Found to be Healthier Option than "Table Sugar"_Health_Asia Pacific Daily*. Available online at: https://www.apdnews.com/e-lifestyle/health/669461.html (accessed December 28, 2020).
25. *Fruit-Based Sugar Found to be Healthier Option than "Table Sugar": Aussie Research*. Xinhua, English.news.cn. Available online at: http://www.xinhuanet.com//english/2017-06/08/c_136349346.htm (accessed December 28, 2020).
26. *Apples and Diabetes: Benefits, Nutrition, and Other Fruits*. (2019). Available online at: https://www.medicalnewstoday.com/articles/321882 (accessed December 28, 2020).
27. Connery G. *University of Canberra Study Proves Fructose no Worse than Any Sugar*. Brisb Times (2017). Available online at: https://www.brisbanetimes.com.au/national/act/university-of-canberra-study-proves-fructose-no-worse-than-any-sugar-20170608-gwn1ng.html (accessed December 28, 2020).
28. Nation TLT. *Tip: Stop Blaming Fructose*. T Nation. Available online at: https://www.t-nation.com/diet-fat-loss/tip-stop-blaming-fructose (accessed December 28, 2020).
29. *Fructose Hits Sweet Spot*. Australasian Science Magazine. Available online at: http://www.australasianscience.com.au/article/issue-julyaugust-2017/fructose-hits-sweet-spot.html (accessed December 28, 2020).

30. *Fructose: Harmful or Healthy? Break Muscle*. Available online at: https://breakingmuscle.com/fitness/fructose-harmful-or-healthy (accessed December 28, 2020).
31. Hannou SA, Haslam DE, McKeown NM, Herman MA. Fructose metabolism and metabolic disease. *J Clin Invest*. (2018) 128:545–55. doi: 10.1172/JCI96702
32. Taskinen M-R, Packard CJ, Borén J. Dietary fructose and the metabolic syndrome. *Nutrients*. (2019) 11:1987. doi: 10.3390/nu11091987
33. Hernández-Díazcouder A, Romero-Nava R, Carbó R, Sánchez-Lozada LG, Sánchez-Muñoz F. High fructose intake and adipogenesis. *Int J Mol Sci*. (2019) 20:2787. doi: 10.3390/ijms20112787
34. Mirtschink P, Jang C, Arany Z, Krek W. Fructose metabolism, cardiometabolic risk, and the epidemic of coronary artery disease. *Eur Heart J*. (2018) 39:2497–505. doi: 10.1093/eurheartj/ehx518
35. Jensen T, Abdelmalek MF, Sullivan S, Nadeau KJ, Green M, Roncal C, et al. Fructose and sugar: a major mediator of non-alcoholic fatty liver disease. *J Hepatol*. (2018) 68:1063–75. doi: 10.1016/j.jhep.2018.01.019
36. Tappy L. Fructose-containing caloric sweeteners as a cause of obesity and metabolic disorders. *J Exp Biol*. (2018) 221:164202. doi: 10.1242/jeb.164202
37. Moher D, Liberati A, Tetzlaff J, Altman DG, Group TP. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. (2009) 6:e1000097. doi: 10.1371/journal.pmed.1000097
38. Evans RA, Lithander FE, Frese M, Cunningham JH, Mills KE. *Fructose Substitution of Glucose or Sucrose in Food for Normoglycaemic Persons or People with Impaired Glucose Tolerance or Diabetes [Cochrane Protocol]*. York: University of York (2015). Available online at: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42015029385 (accessed December 29, 2020).
39. *Covidence Systematic Review Software*. Melbourne, VIC: Veritas Health Innovation (2020). Available online at: www.covidence.org (accessed December 29, 2020).
40. Royal Australian College of General Practitioners, Diabetes Australia. *General Practice Management of Type 2 Diabetes: 2016-18*. Royal Australian College of General Practitioners, Diabetes Australia (2016).
41. *EndMemo—Online Converters, Calculators and Tutorials*. Available online at: http://endmemo.com/ (accessed December 26, 2020).
42. Deeks JJ, Higgins JP, Statistical Methods Group. *Statistical Algorithms in Review Manager 5*. The Cochrane Collaboration (2010). Available online at: https://training.cochrane.org/handbook/current/chapter-10 (accessed December 29, 2020).
43. The Nordic Cochrane Centre. *Review Manager (RevMan)*. Copenhagen: The Cochrane Collaboration (2014).
44. Higgins JP, Greeen S. *Cochrane Handbook for Systematic Reviews of Interventions*. Copenhagen: The Cochrane Collaboration (2011).
45. Luo D, Wan X, Liu J, Tong T. Optimally estimating the sample mean from the sample size, median, mid-range, and/or mid-quartile range. *Stat Methods Med Res*. (2018) 27:1785–805. doi: 10.1177/0962280216669183
46. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol*. (2014) 14:135. doi: 10.1186/1471-2288-14-135
47. Wallace, Byron C, Dahabreh, Issa J, Trikalinos, Thomas A, et al. Closing the gap between methodologists and end-users: R as a computational back-end. *J Stat Softw*. (2012) 49:1–15. doi: 10.18637/jss.v049.i05
48. Deeks JJ, Higgins JPT, Altman DG. *Chapter 10: Analysing Data and Undertaking Meta-analyses*. Available online at: /handbook/current/chapter-10 (accessed December 26, 2020).
49. Higgins JPT, Thomas J. *Cochrane Handbook for Systematic Reviews of Interventions*. (2020) Available online at: https://training.cochrane.org/handbook/current (accessed May 24, 2019).
50. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. (2000) 321:405–12. doi: 10.1136/bmj.321.7258.405
51. ClinLab Navigator. *Lab Test Significant Change*. Available online at: http://www.clinlabnavigator.com/test-significant-change.html (accessed May 1, 2020).
52. Bradley R, Kozura E, Buckle H, Kaltunas J, Tais S, Standish LJ. Description of clinical risk factor changes during naturopathic care for type 2 diabetes. *J Altern Complement Med*. (2009) 15:633–8. doi: 10.1089/acm.2008.0249
53. Magno S, Ceccarini G, Pelosini C, Jaccheri R, Vitti J, Fierabracci P, et al. LDL-cholesterol lowering effect of a new dietary supplement: an open label, controlled, randomized, cross-over clinical trial in patients with mild-to-moderate hypercholesterolemia. *Lipids Health Dis*. (2018) 17:124. doi: 10.1186/s12944-018-0775-8
54. Magkos F, Fraterrigo G, Yoshino J, Luecking C, Kirbach K, Kelly SC, et al. Effects of moderate and subsequent progressive weight loss on metabolic function and adipose tissue biology in humans with obesity. *Cell Metab*. (2016) 23:591–601. doi: 10.1016/j.cmet.2016.02.005
55. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Routledge (2013). doi: 10.4324/9780203771587
56. Aeberli I, Gerber PA, Hochuli M, Kohler S, Haile SR, Gouni-Berthold I, et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. *Am J Clin Nutr*. (2011) 94:479–85. doi: 10.3945/ajcn.111.013540
57. Aeberli I, Hochuli M, Gerber PA, Sze L, Murer SB, Tappy L, et al. Moderate amounts of fructose consumption impair insulin sensitivity in healthy young men: a randomized controlled trial. *Diabetes Care*. (2012) 36:150–6. doi: 10.2337/dc12-0540
58. Angelopoulos TJ, Lowndes J, Sinnett S, Rippe JM. Fructose containing sugars at normal levels of consumption do not effect adversely components of the metabolic syndrome and risk factors for cardiovascular disease. *Nutrients*. (2016) 8:179. doi: 10.3390/nu8040179
59. Bantle JP, Raatz SK, Thomas W, Georgopoulos A. Effects of dietary fructose on plasma lipids in healthy subjects. *Am J Clin Nutr*. (2000) 72:1128–34. doi: 10.1093/ajcn/72.5.1128
60. Bossetti BM, Kocher LM, Moranz JF, Falko JM. The effects of physiologic amounts of simple sugars on lipoprotein, glucose, and insulin levels in normal subjects. *Diabetes Care*. (1984) 7:309–12. doi: 10.2337/diacare.7.4.309
61. Heden TD, Liu Y, Park Y-M, Nyhoff LM, Winn NC, Kanaley JA. Moderate amounts of fructose- or glucose-sweetened beverages do not differentially alter metabolic health in male and female adolescents. *Am J Clin Nutr*. (2014) 100:796–805. doi: 10.3945/ajcn.113.081232
62. Heden TD, Liu Y, Park Y-M, Winn NC, Kanaley JA. Walking reduces postprandial insulin secretion in obese adolescents consuming a high-fructose or high-glucose diet. *J Phys Act Health*. (2015) 12:1153–61. doi: 10.1123/jpah.2014-0105
63. Jin R, Welsh JA, Le N-A, Holzberg J, Sharma P, Martin DR, et al. Dietary fructose reduction improves markers of cardiovascular disease risk in Hispanic-American adolescents with NAFLD. *Nutrients*. (2014) 6:3187–201. doi: 10.3390/nu6083187
64. Koh ET, Ard NF, Mendoza F. Effects of fructose feeding on blood parameters and blood pressure in impaired glucose-tolerant subjects. *J Am Diet Assoc*. (1988) 88:932–8.
65. Kuzma JN, Cromer G, Hagman DK, Breymeyer KL, Roth CL, Foster-Schubert KE, et al. Consuming glucose-sweetened, not fructose-sweetened, beverages increases fasting insulin in healthy humans. *Eur J Clin Nutr*. (2019) 73:487–90. doi: 10.1038/s41430-018-0297-5
66. Lowndes J, Sinnett SS, Rippe JM. No effect of added sugar consumed at median american intake level on glucose tolerance or insulin resistance. *Nutrients*. (2015) 7:8830–45. doi: 10.3390/nu7105430
67. Malerbi DA, Paiva ES, Duarte AL, Wajchenberg BL. Metabolic effects of dietary sucrose and fructose in type II diabetic subjects. *Diabetes Care*. (1996) 19:1249–56. doi: 10.2337/diacare.19.11.1249
68. Silbernagel G, Machann J, Unmuth S, Schick F, Stefan N, Häring HU, et al. Effects of 4-week very-high-fructose/glucose diets on insulin sensitivity, visceral fat and intrahepatic lipids: an exploratory trial. *Br J Nutr*. (2011) 106:79–86. doi: 10.1017/S000711451000574X
69. Havel PJ. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. *Nutr Rev*. (2005) 63:133–57. doi: 10.1111/j.1753-4887.2005.tb00132.x
70. Bray GA. How bad is fructose? *Am J Clin Nutr*. (2007) 86:895–6. doi: 10.1093/ajcn/86.4.895
71. Mauvais-Jarvis F. Gender differences in glucose homeostasis and diabetes. *Physiol Behav*. (2018) 187:20–3. doi: 10.1016/j.physbeh.2017.08.016
72. Godsland IF, Wynn V, Crook D, Miller NE. Sex, plasma lipoproteins, and atherosclerosis: prevailing assumptions and outstanding questions.

Am Heart J. (1987) 114:1467–503. doi: 10.1016/0002-8703(87)90552-7

73. Sievenpiper JL. Low-carbohydrate diets and cardiometabolic health: the importance of carbohydrate quality over quantity. *Nutr Rev*. (2020) 78:69–77. doi: 10.1093/nutrit/nuz082
74. WHO. *Guideline: Sugars Intake for Adults and Children*. World Health Organization (2015). Available online at: https://www.who.int/publications/i/item/9789241549028
75. Page KA, Chan O, Arora J, Belfort-Deaguiar R, Dzuira J, Roehmholdt B, et al. Effects of fructose vs. glucose on regional cerebral blood flow in brain regions involved with appetite and reward pathways. *JAMA*. (2013) 309:63–70. doi: 10.1001/jama.2012.116975
76. Kuzma JN, Cromer G, Hagman DK, Breymeyer KL, Roth CL, Foster-Schubert KE, et al. No difference in ad libitum energy intake in healthy men and women consuming beverages sweetened with fructose, glucose, or high-fructose corn syrup: a randomized trial1. *Am J Clin Nutr*. (2015) 102:1373–80. doi: 10.3945/ajcn.115.116368
77. Lee H-J, Cha J-Y. Recent insights into the role of ChREBP in intestinal fructose absorption and metabolism. *BMB Rep*. (2018) 51:429–36. doi: 10.5483/BMBRep.2018.51.9.197
78. Merino B, Fernández-Díaz CM, Cózar-Castellano I, Perdomo G. Intestinal fructose and glucose metabolism in health and disease. *Nutrients*. (2020) 12:94. doi: 10.3390/nu12010094
79. Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab*. (2018) 27:351–61.e3. doi: 10.1016/j.cmet.2017.12.016
80. Öckerman PA, Lundborg H. Conversion of fructose to glucose by human jejunum absence of galactose-to-glucose conversion. *Biochim Biophys Acta BBA Enzymol Biol Oxid*. (1965) 105:34–42. doi: 10.1016/S0926-6593(65)80173-4
81. Patel C, Douard V, Yu S, Tharabenjasin P, Gao N, Ferraris RP. Fructose-induced increases in expression of intestinal fructolytic and gluconeogenic genes are regulated by GLUT5 and KHK. *Am J Physiol-Regul Integr Comp Physiol*. (2015) 309:R499–509. doi: 10.1152/ajpregu.00128.2015

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Increased Added Sugar Consumption Is Common in Parkinson's Disease

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Objectives: There is limited information about the dietary habits of patients with Parkinson's Disease (PD), or associations of diet with clinical PD features. We report on nutritional intake in an Australian PD cohort.

Methods: 103 PD patients and 81 healthy controls (HCs) completed a validated, semi-quantitative food frequency questionnaire. Food and nutrient intake was quantified, with consideration of micronutrients and macronutrients (energy, protein, carbohydrate, fat, fibre, and added sugar). Participants also completed PD-validated non-motor symptom questionnaires to determine any relationships between dietary intake and clinical disease features.

Results: Mean daily energy intake did not differ considerably between PD patients and HCs (11,131 kJ/day vs. 10,188 kJ/day, $p = 0.241$). However, PD patients reported greater total carbohydrate intake (279 g/day vs. 232 g/day, $p = 0.034$). This was largely attributable to increased daily sugar intake (153 g/day vs. 119 g/day, $p = 0.003$) and in particular free sugars (61 g/day vs. 41 g/day, $p = 0.001$). PD patients who (1) experienced chronic pain, (2) were depressed, or (3) reported an impulse control disorder, consumed more total sugars than HCs (all $p < 0.05$). Increased sugar consumption was associated with an increase in non-motor symptoms, including poorer quality of life, increased constipation severity and greater daily levodopa dose requirement.

Conclusions: We provide clinically important insights into the dietary habits of PD patients that may inform simple dietary modifications that could alleviate disease symptoms and severity. The results of this study support clinician led promotion of healthy eating and careful management of patient nutrition as part of routine care.

Keywords: Parkinson's disease, diet, nutrition, carbohydrates, sugars

INTRODUCTION

Parkinson's Disease (PD) is the second most common neurodegenerative disease and is associated with significant morbidity (1). It is characterised by the loss of dopaminergic neurons in the substantia nigra pars compacta, and a deficiency of dopamine in the striatum and other basal ganglia structures. A growing body of evidence suggests that nutrition may play an important role in PD (2).

PD patients are more frequently underweight (3, 4), have a higher risk of malnutrition (5) and tend to have a lower body mass index (BMI) (6) that inversely associates with disease duration, disease severity and levodopa-related motor complications (7). Furthermore, it has been suggested that lower dietary intake of poly-unsaturated fatty acids, vitamin A, vitamin E, vitamin B12, vitamin D and folic acid are associated with an increased risk of developing PD (8, 9), although this remains controversial. Nevertheless, throughout the disease course, weight gain and loss may fluctuate, being influenced by both changes in food intake and energy expenditure (10). Interestingly, PD patients are also purported to display a preference for sweet foods, such as cakes (11), chocolate (12), ice cream (13), milk puddings and custards (14), consistent with an increased consumption of carbohydrates (7, 15, 16).

Emerging research suggests that the complex bidirectional communication between the gut and brain is influenced by dietary patterns and may contribute to the development and progression of PD (17, 18), as well as levodopa metabolism (19). Therefore, the predominance of gastrointestinal dysfunction in PD may further influence the diet of PD patients and vice versa. For example, PD patients are three-times more likely to experience constipation than control subjects and they reported increased occurrence and severity of indigestion, nausea, excessive fullness and bloating (20), which negatively impact on PD health-related quality of life (QoL) (21). Additionally, constipation associates with higher levodopa requirements (7), likely due to gastroparesis and impaired intestinal motility, which hinders drug absorption.

Despite ethnic variability in dietary habits (22), general improvement in nutritional condition has been shown to improve PD patient QoL (23). Furthermore, adherence to a healthy diet may reduce the occurrence of non-motor symptoms that often precede PD diagnosis (24) and may lead to optimisation of levodopa therapy to minimise disease-associated complications (7). Due to limited information about the dietary habits of PD patients in Australia, we aimed to characterise the nutritional intake of an Australian PD cohort, and investigate potential associations between diet and clinical disease features.

MATERIALS AND METHODS

Study Settings and Subjects

Subjects were recruited from the movement disorder and neurology clinics at Royal North Shore Hospital, Sydney, Australia, between 2018 and 2019, as described previously (20). Inclusion criteria were; >18 years of age, a clinical diagnosis of idiopathic PD according to the UK Parkinson's Disease Society Brain Bank Diagnostic Criteria (25), regardless of disease duration, and being managed by a specialist neurologist. The healthy control (HC) inclusion criteria were; >18 years of age, exhibiting no clinical indication of PD, and were a spouse, sibling or child of a respective PD patient with similar dietary habits. Exclusion criteria included secondary Parkinsonism, tube feeding, medical or surgical disorders preventing completion of questionnaires and significant cognitive impairment demonstrated by incapacity to provide consent. Ethical approval was granted by the Northern Sydney Local Health District Human Research Ethics Committee and the North Shore Private Hospital Ethics Committee, HREC/18/HAWKE/109, NSPHEC 2018-LNR-009, respectively.

Data Collection

Dietary and lifestyle data were collected for all participants through a 145-item, semi-quantitative food frequency questionnaire (FFQ), modified for Australian diet and vernacular from an early Willett FFQ (26, 27) and originally developed and validated as part of the Blue Mountains Eye Study (28, 29). The FFQ was later updated to reflect new foods commonly available in the Australian food supply (30). A nine-category frequency scale was used to indicate the usual frequency of consumption of food items during the past year, and included portion size estimates. Nutrient content of food items was calculated using the Australian Food Composition Database (31) multiplied by the frequency and portion size, using a purpose-built Microsoft Access program. Nutrient analysis included calculations for energy, protein, carbohydrate, sugars, fats and fibre. "Added sugars" include sucrose, fructose, dextrose, lactose and sugar syrups (such as glucose syrup), which are added during the manufacture of foods or by the consumer in the preparation of food and beverages (32). "Free sugars" extends the definition of added sugars to include sugars naturally present in honey, fruit juice and fruit juice concentrates (33). Total average daily consumption of macronutrients and micronutrients were calculated. Macronutrients were also calculated as a percentage of total energy intake and micronutrients per 1,000 kJ (34).

Demographic data was collected on patient age, gender, ethnicity, marital status, socioeconomic status and medical history, including co-morbidities, medication use, alcohol consumption and smoking history. Physical activity was assessed using the International Physical Activity Questionnaire (35). The Leeds Dyspepsia Questionnaire (36) assessed upper gastrointestinal symptoms and the Rome-IV criteria (37) and the Cleveland Constipation Score (38) were used to determine constipation severity and gut motility. QoL was evaluated using the PDQ-39 (39), mood was assessed by the Beck Depression Inventory (40), cognitive function was gauged by the Montreal Cognitive Assessment (MoCA) (41) and non-motor symptoms were assessed by the Non-Motor Symptoms Scale (NMSS) (42). Quantitative and qualitative motor severity assessment was evaluated with the Movement Disorder Society—Unified Parkinson's Disease Rating Scale—Part III (MDS-UPDRS III) and the Modified Hoehn & Yahr scale (43). Medications were compared following standard methods for calculating daily levodopa equivalent dose (LED) (44), whilst chronic pain severity was assessed by the Visual Analogue Scale (45).

Statistical Analysis

Normal distribution of all data was confirmed using the Shapiro-Wilk test. Independent *t*-tests were used to analyse differences between groups for continuous variables. Chi-squared (χ^2) tests were used to compare differences between categorical variables. Logistic and linear regression models were constructed to evaluate differences in dietary intake between the PD and

TABLE 1 | Cohort demographic and clinical characteristics.

	Parkinson's disease	Healthy control	Test statistic	*p*-value
Number of Patients, $n =$	103	81		
Mean age (years) [SD, range]*	67.1 [12.2, 33–88]	62.4 [15.6, 18–90]	$t = 2.3$ (182)^	**0.023**
Gender - n (%)*			$\chi^2 = 10.7$ (1)∞	**0.001**
Male	58 (56.3)	26 (32.1)		
Female	45 (43.7)	55 (67.9)		
Marital status - n (%)*			$\chi^2 = 4.2$ (3)∞	0.244
Married/de facto	79 (76.7)	69 (85.1)		
Single	10 (9.7)	8 (9.9)		
Widowed	6 (5.8)	1 (1.2)		
Other	8 (7.7)	3 (3.7)		
Ethnicity - n (%)*			$\chi^2 = 2.3$ (3)∞	0.506
Caucasian	81 (78.6)	64 (79.0)		
Asian	4 (3.9)	5 (6.2)		
Middle Eastern	7 (6.8)	2 (2.5)		
Other	11 (10.7)	10 (12.3)		
Body mass index [SD]	25.7 [5.2]	26.2 [4.6]	$t = -0.7$ (182)^	0.485
Smoking History - n (%)				
Current smoker	2 (1.9)	3 (3.7)	$\chi^2 = 0.6$ (1)∞	0.457
Prior smoker	38 (36.9)	27 (33.7)	$\chi^2 = 0.2$ (1)∞	0.659
Never smoked	65 (63.1)	53 (66.3)	$\chi^2 = 0.2$ (1)∞	0.659
Type of tobacco (%)			$\chi^2 = 2.6$ (2)∞	0.268
Cigarettes	84.2	96.3		
Cigars	10.5	3.7		
Pipe	5.3	0		
Pack year history, [SD]	13.3 [13.8]	14.4 [14.6]	$t = -0.3$ (63)^	0.758
Caffeine consumption (coffee/tea) (%)	88 (85.4)	74 (91.4)	$\chi^2 = 1.5$ (1)∞	0.219
Number of daily cups, [SD]	2.3 [1.7]	3.1 [1.8]	$t = 3.0$ (182)^	**0.003**
History of diabetes (%)	5 (4.9)	5 (6.2)	$\chi^2 = 0.2$ (1)∞	0.695
Self-Reported HbA1c%, [SD]	6.1 [0.2]	7.3 [1.0]	$t = -1.9$ (6)^	0.095
Biochemical characteristics [SD]*				
Erythrocyte sedimentation rate (mm/h)	9.5 [13.4]	9.5 [10.4]	$t = -0.1$ (181)^	0.991
C-Reactive protein (mg/L)	3.9 [10.8]	2.2 [2.5]	$t = 1.4$ (182)^	0.177
Total cholesterol (mmol/L)	4.8 [0.9]	5.2 [1.1]	$t = -2.5$ (182)^	**0.014**
Low density lipoprotein (mmol/L)	2.7 [0.7]	2.9 [0.9]	$t = -1.5$ (178)^	0.132
High density lipoprotein (mmol/L)	1.4 [0.4]	1.6 [0.4]	$t = -2.2$ (181)^	**0.033**
Triglycerides (mmol/L)	1.3 [1.0]	1.5 [0.9]	$t = -1.2$ (182)^	0.239
Random glucose (mmol/L)	5.8 [0.6]	5.9 [0.9]	$t = -0.8$ (182)^	0.438
HbA1c%	5.3 [0.4]	6.0 [5.2]	$t = -1.2$ (182)^	0.217
Albumin (g/L)	38.7 [3.5]	39.8 [3.1]	$t = -2.3$ (182)^	**0.023**
Dietary variables				
Vegetarian diet, n (%)	3 (2.9)	2 (2.5)	$\chi^2 = 0.1$ (1)∞	0.865
Energy with dietary fibre (k/J), [SD]	11,131 [5782.6]	10,188 [4799.9]	$t = 1.2$ (181)^	0.241
Energy without dietary fibre (k/J), [SD]	10,778 [5546.6]	9,861 [4624.4]	$t = 1.2$ (181)^	0.235
Protein (g/day) [SD]	118 [79.3]	117 [74.5]	$t = 0.1$ (181)^	0.883
Total fat (g/day) [SD]	102 [49.7]	96 [43.6]	$t = 0.9$ (181)^	0.392
Carbohydrate (g/day) [SD]	279 [161.8]	232 [124.8]	$t = 2.1$ (181)^	**0.034**
Total sugars (g/day) [SD]	153 [86.3]	119 [60.6]	$t = 3.0$ (181)^	**0.003**
Free sugars g/day [SD]	61 [48.0]	41 [23.2]	$t = 3.5$ (181)^	**0.001**
Added sugars g/day [SD]	53 [43.3]	35 [20.4]	$t = 3.5$ (181)^	**0.001**
Fibre (g/day) [SD]	41 [31.2]	38 [22.7]	$t = 0.7$ (181)^	0.475

(Continued)

TABLE 1 | Continued

	Parkinson's disease	Healthy control	Test statistic	*p*-value
Moisture (mL/day) [SD]	2,878 [1236.2]	3,044 [1050.6]	$t = -0.1$ (181)^	0.337
Alcohol (g/day) [SD]	9 [12.6]	13 [15.8]	$t = -2.1$ (181)^	**0.038**
Calcium (mg/day) [SD]	1,158 [590.4]	1,129 [593.6]	$t = 0.3$ (181)^	0.739
Iron (mg/day) [SD]	15 [11.0]	14 [8.3]	$t = 0.4$ (181)^	0.677
Magnesium (mg/day) [SD]	478 [270.0]	479 [223.4]	$t = -0.1$ (181)^	0.973
Potassium (mg/day) [SD]	4,965 [3359.4]	4,761 [2551.1]	$t = 0.5$ (181)^	0.652
Sodium (mg/day) [SD]	2,145 [1421.4]	2,075 [1344.2]	$t = 0.3$ (181)^	0.733
Zinc (mg/day) [SD]	14 [8.5]	14 [7.8]	$t = 0.2$ (181)^	0.880
Retinol (ug/day) [SD]	634 [675.0]	533 [545.7]	$t = 1.1$ (181)^	0.281
Beta carotene (ug/day) [SD]	6,703 [7046.0]	6,703 [5805.7]	$t = 0.1$ (181)^	1
Vitamin A (ug/day) [SD]	1,957 [1671.6]	1,867 [1412.3]	$t = 0.4$ (181)^	0.702
Thiamine (mg/day) [SD]	2 [1.0]	2 [0.9]	$t = 0.1$ (181)^	0.955
Riboflavin (mg/day) [SD]	2 [1.2]	2 [1.1]	$t = 0.1$ (181)^	0.663
Vitamin B12 (ug/day) [SD]	7 [4.7]	7 [4.5]	$t = 0.1$ (181)^	0.925
Vitamin C (mg/day) [SD]	158 [147.1]	145 [110.7]	$t = 0.6$ (181)^	0.525
Dietary folate (DFE) (ug/day) [SD]	788 [490.2]	755 [422.2]	$t = 0.5$ (181)^	0.626

*^Independent sample t test; ∝Pearson's chi-squared test; df, degrees of freedom; [SD], Standard Deviation. *This data is partially reproduced from Lubomski et al. (20). The bold values indicate clinical significance.*

HC groups, as well as within the PD cohort, after controlling for demographic and clinical variables. Correlation of clinically relevant variables was evaluated using Pearson's correlation test. $p < 0.05$ was considered statistically significant. Data analysis was performed using SPSS, version 26 (SPSS Inc, Chicago, Illinois, USA), as described earlier (20).

RESULTS

Demographic and Clinical Characteristics

Demographic information pertaining to the cohort studied here has been reported previously (20). In summary, a total of 103 PD patients (56.3% male, mean age 67) and 81 healthy controls (32% male, mean age 62; comprised of 73 spouses, 7 children and 1 sibling) completed the FFQ. Demographic, anthropometric, clinical and nutritional features of the study population are reported in **Table 1**.

The mean BMI of the combined cohort was 26.0 (SD 4.90). PD patients were not underweight and their BMI (25.7 [SD 5.2]) did not differ significantly from HC (26.2 [SD 4.6], $p = 0.485$). 5.5% of subjects reported a history of diabetes, with no statistically significant difference observed between the groups for this measure. More PD patients reported chronic pain over the preceding year than HC (72.8 vs. 39.5%, $p < 0.001$). PD patients were also more depressed, as measured by the Beck's Depression Inventory (total score 11.9 [SD 8.8] vs. 5.2 [SD 5.5], $p < 0.001$). PD patients also reported more constipation, as measured by the Cleveland Constipation Score (7.2 [SD 4.7] vs. 3.1 [SD 2.9], $p < 0.001$) and Rome IV Criteria (4.4 [SD 3.5] vs. 1.1 [SD 1.4], $p < 0.001$). Furthermore, PD patients reported more dyspepsia as measured by the Leeds Dyspepsia Questionnaire (score 8.3 [SD 7.7] vs. 4.6 [6.1], $p = 0.001$). Physical activity, assessed by the IPAQ, identified that PD patients undertook considerably less physical activity (1823.6 metabolic-equivalent [MET]-minutes/week [SD 1693.6]) compared to the HC group (2942.4 MET-minutes/week [SD 2620.9], $p = 0.001$). Further clinical characteristics of the PD cohort including the utilisation of standard and device assisted therapies, physical activity and frequency and severity of other non-motor symptoms (NMS) are outlined in **Table 2**.

Dietary Characteristics

Mean daily energy intake did not differ significantly between PD patients (1130.9 kJ/day [SD 5782.6]) and HC (10188.2 kJ/day [SD 4800.0], $p = 0.241$). When total energy intake was evaluated in terms of gender, the difference between males (11052.4 kJ/day [SD 5486.4]) and females (10435.7 kJ/day [SD 5302.4]) was not statistically different across the whole cohort ($p = 0.7$) or PD cohort alone (males 11350.6 kJ/day [SD 5998.3], females 10847.7 kJ/day [SD 5546.4], $p = 0.8$).

PD patients reported greater total carbohydrate intake compared to HCs (278.8 g/day [SD 161.8] vs. 232.2 g/day [SD 124.8], $p = 0.034$), which was largely attributable to increased daily total sugar intake (153.3 g/day [SD 86.3] vs. 118.7 g/day [SD 60.6], $p = 0.003$; **Table 1**). Consistently, PD patients consumed more total free sugar (61.2 g/day [SD 48.0] vs. 40.6 g/day [SD 23.2], $p = 0.001$) and total added sugar (52.9 g/day [SD 43.3] vs. 34.7 g/day [SD 20.4], $p = 0.001$) compared to HC. Among people with PD, beverages provide 19.6% of free sugars, compared to about half this among HCs (10.4%). The main contributors to free sugars among both groups were: chocolate, jam/marmalade/honey, cordial, sugar, soft drinks, cake, cold breakfast cereal, and yoghurt.

TABLE 2 | Parkinson's disease clinical characteristics.

Mean age at diagnosis (years) [SD, range]*	58.8 [13.6, 24–88]
Mean Parkinson's disease duration (years) [SD, range]*	9.2 [6.5, 1–30]
Parkinson's disease phenotype - n (%)*	
Tremor dominant	31 (30.1)
Postural instability and gait impairment	21 (20.4)
Akinetic rigid	40 (38.9)
Young onset (<40 years)	11 (10.7)
Late onset (>60 years)	51 (49.5)
Disease complications - n (%)*	
Motor fluctuations	60 (58.3)
Dyskinesia	60 (58.3)
Wearing off	84 (81.6)
Impulse control disorder	20 (19.4)
REM sleep behaviour disorder	50 (48.5)
Parkinson's disease therapy - n (%)*	
Treatment naïve	5 (4.9)
Oral levodopa	92 (89.3)
Dopamine agonist	36 (35.0)
Monoamine oxidase B inhibitor	19 (18.4)
Anticholinergic	13 (12.6)
Catechol-O-methyl transferase inhibitor	24 (23.3)
Amantadine	13 (12.6)
Levodopa/carbidopa intestinal gel	9 (8.7)
Deep brain stimulation	11 (10.7)
Apomorphine (subcutaneous infusion)	7 (6.8)
Levodopa equivalent daily dose (mg) [SD, range]*	834.8 [527.3, 0–2,186]
Mean MDS unified Parkinson's disease rating scale-III ("on" state) [SD, range]*	32.9 [17.7, 5–91]
Gastrointestinal symptoms*	
Mean cleveland constipation score [SD]	7.2 [4.7]
Mean Rome-IV criteria constipation score [SD]	4.4 [3.5]
Functional constipation as per Rome-IV criteria (%)	78.6
Mean leeds dyspepsia questionnaire (LDQ) score [SD]*	8.3 [7.7]
Chronic pain over last 3 months (%)*	75 (72.8)
Mean pain score (visual analogue scale) [SD]	4.9 [2.5]
Mean international physical activity questionnaire (IPAQ) score (MET-minutes/week) [SD]*	1823.6 [1693.6]
PDQ-39 summary index [SD]	29.2 [17.3]
Depression characteristics	
Mean Beck's depression inventory total score [SD]	11.9 [8.8]
Clinically depressed (>13 for Parkinson's disease) - n (%)	40 (38.9)
Mean MDS total non-motor symptoms score (NMSS), [SD]	62.7 [42.9]
Montreal cognitive assessment (MoCA), [SD]	24.4 [4.8]
Mild cognitive impairment (<26/30) - n (%)	50 (48.6)
Parkinson's disease dementia (<21/30) - n (%)	17 (16.5)

*[SD], Standard Deviation. *This data is partially reproduced from Lubomski et al. (20).*

The total intake of vitamins and other macronutrients did not differ between the groups, and there were no macronutrient or micronutrient differences noted between the genders in both the PD and HC groups. When subjects with diabetes within the PD and combined cohorts were excluded from analysis, PD patients still consumed significantly more carbohydrates, total sugar, added sugar and free sugar than healthy controls (all $p < 0.05$). Excluding PD dementia patients also demonstrated a persistent increased sugar intake compared to HCs.

Logistic regression modelling evaluated the significance of dietary differences between the PD and HC groups. Statistical significance between the two groups persisted after controlling for age, sex, physical activity and constipation (Rome-IV criteria), for the following dietary variables: carbohydrates (Wald $\chi^2 = 3.6$, df = 3, $p = 0.044$); total sugars (Wald $\chi^2 = 3.9$, df = 3, $p = 0.036$), free sugars (Wald $\chi^2 = 3.5$, df = 3, $p = 0.049$), added sugars (Wald $\chi^2 = 3.6$, df = 3, $p = 0.046$) and alcohol (Wald $\chi^2 = 4.8$, df = 3, $p = 0.029$).

PD patients reported less alcohol consumption compared to the HCs (8.9 g/day [SD 12.6] vs. 13.3 g/day [SD 15.8], $p = 0.038$), with male PD patients consuming more alcohol than female PD patients (12 g/day [SD 14.4] vs. 5 g/day [SD 8.4], $p = 0.005$). Over 90% of participants reported daily caffeine consumption, although PD patients reported lower daily intake (2.3 cups/day [SD 1.7] vs. 3.1 cups/day [SD 1.8], $p = 0.003$). No associations between PD phenotype, standard or advanced therapy use, motor severity (assessed by the MDS-UPDRS-III score), or any of the measured dietary parameters were identified.

Evaluating macronutrient intake calculated as a percentage of total energy intake, PD patients consumed less protein than HCs (18.0% [SD 3.5] vs. 19.2% [SD 3.1], $p = 0.011$), as well as more carbohydrates (40% [SD 6.1] vs. 36.2% [SD 6.6], $p < 0.001$) and more added sugar (7.6% [SD 4.7] vs. 5.6% [SD 2.9], $p = 0.001$). PD patients also consumed more total sugars (21.9% [SD 5.9] vs. 18.7% [SD 5.0], $p < 0.001$) and more free sugars (8.8% [SD 5.1] vs. 6.5% [SD 3.3], $p = 0.001$), when expressed as a percentage of total energy intake (**Figure 1** and **Table 3**). Moreover, the percentage total energy consumption of free sugars showed that 28.2% of PD patients compared with 7.5% of HC had >10% of energy intake attributed to free sugars (**Figure 2**). The assessment of micronutrients, expressed per 1,000 kJ energy intake, identified that PD patients consumed less magnesium, potassium and zinc than HC (**Table 3**). Ten participants (6 PD and 4 HC) reported a very high energy consumption. Reanalysis of the combined cohort when excluding these individuals did not alter the significance of the macro- and micronutrient findings between the PD and HC groups, both in terms of total intake and percentage of total energy intake. When controlling for participant age and sex, using linear regression analysis with Bonferroni correction for multiple testing (new α threshold; $p < 0.016$), all statistically significant comparisons remained, aside from potassium (**Table 3**).

Biochemical Characteristics

Biochemical analysis showed that PD patients had lower total cholesterol levels (4.8 mmol/L [SD 0.9] vs. 5.2 mmol/L [SD 1.1], $p = 0.014$), lower high density lipoprotein (HDL) levels (1.4 mmol/L [SD 0.4] vs. 1.6 mmol/L [SD 0.4], $p = 0.033$), and lower albumin levels (38.7 mmol/L [SD 3.5] vs. 39.8 mmol/L [SD 3.1], $p = 0.023$), although all measures were still within normal physiological ranges. A full biochemical description is provided in **Table 1**.

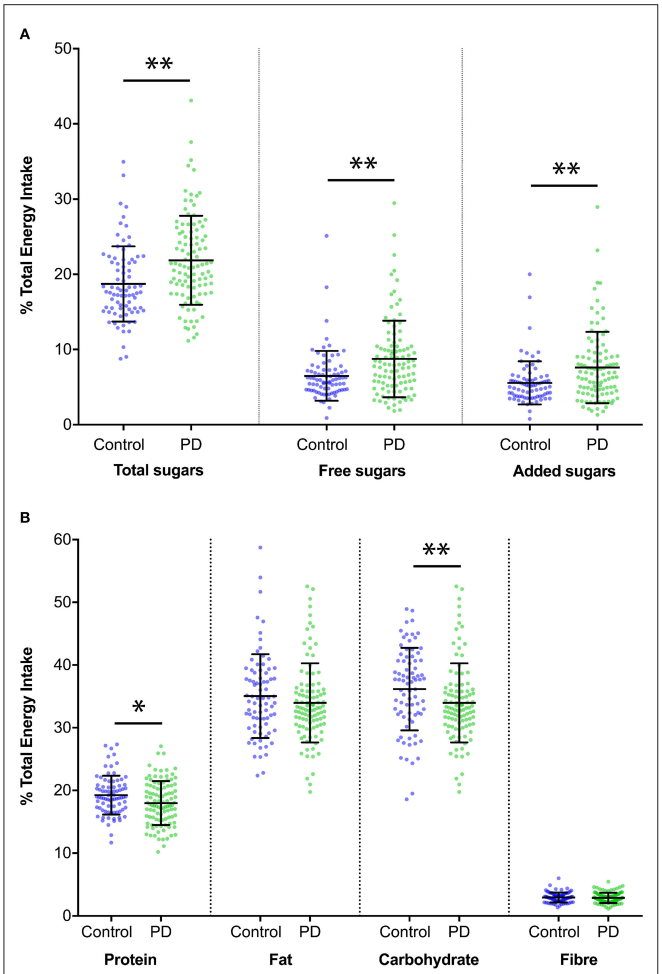

FIGURE 1 | Comparison of macronutrient intake as a percentage of total energy intake. **(A)** Comparison of sugar intake expressed as a percentage of total energy intake between Parkinson's disease and healthy control groups (Mean ± [SD]). Patients with Parkinson's disease consumed a greater amount of total sugars (percentage of total energy intake; 21.9% [5.9] in Parkinson's disease patients vs. 18.7% [5.0] in healthy controls, $p < 0.001$). Similar findings were also noted for free sugars (8.8% [5.1] vs. 6.5% [3.3], $p = 0.001$) and added sugars (7.6% [4.7] vs. 5.6% [2.9], $p = 0.001$). Statistically significant comparisons of $p \leq 0.001$ are indicated with**. **(B)** Comparison of protein, fat, carbohydrate and fibre intake for Parkinson's disease and Healthy control groups, expressed as a percentage of total energy intake (Mean ± [SD]). Statistically significant comparisons of $p \leq 0.05$ are indicated with*.

Dietary Intake of the Parkinson's Disease Cohort

Impulse Control Disorders

Mean energy intake was significantly greater for PD patients who reported an impulse control disorder compared to those without (13544.3 kJ/day [SD 8357.8] vs. 10549.3 kJ/day [SD 4862.8], $p = 0.037$), after adjusting for age, sex and PD duration ($\beta = -0.203$, $r^2 = 0.073$, $p = 0.041$). This was mainly attributable to increased consumption of carbohydrates (353.9 g/day [SD 246.3] vs. 260.8 [SD 129.7] g/day, $p = 0.020$), increased total sugar intake (199.5 g/day [SD 120.2] vs. 142.2 g/day [SD 72.7], $p = 0.007$) and increased consumption of total fibre (57.3 g/day [SD 43.0] vs. 37.2 g/day [SD 26.7], $p = 0.009$) by PD patients with an impulse control disorder. Linear regression modelling validated that the increased carbohydrate and total sugar consumption in impulse control disorder patients persisted after controlling for patient age, sex and PD duration ($\beta = -0.229$, $r^2 = 0.084$, $p = 0.021$, and $\beta = -0.263$, $r^2 = 0.101$, $p = 0.008$, respectively). PD patients with an impulse control disorder also consumed greater amounts of a variety of vitamins and minerals, as outlined in **Supplementary Table 1**. When micronutrient intake was assessed per 1,000 kJ energy intake, PD patients with an impulse control disorder compared to PD patients without an impulse control disorder consumed more potassium (483.7 mg/day [SD 76.7] vs. 429.6 mg/day [SD 82.8], $p = 0.009$), more beta carotene (723.4 ug/day [SD 348.1] vs. 522.7 ug/day [SD 314.5], $p = 0.035$) and more vitamin C (17.3 mg/day [SD 11.2] vs. 12.8 mg/day [SD 6.7], $p = 0.022$).

Depression

PD patients who were depressed (BDI >13) (46), consumed more added sugars compared to those who were not depressed (63.7 g/day [SD 43.6] vs. 46.1 g/day [SD 42.0], $p = 0.043$), after controlling for patient age, sex and PD duration ($\beta = -0.192$, $r^2 = 0.062$, $p = 0.040$). Interestingly, depressed PD patients consumed less alcohol than those who did not report depression (5.6 g/day [SD 9.1] vs. 11.0 g/day [SD 14.1], $p = 0.034$), after controlling for patient age, sex and PD duration ($\beta = 0.195$, $r^2 = 0.061$, $p = 0.044$).

Cognition

Those PD patients meeting the criteria for PD dementia (MoCA <21/30) and loss of one or more instrumental activities of daily living (47), consumed significantly more total sugar per day (195.1 g/day [SD 67.8] vs. 145.1 g/day [SD 87.5], $p = 0.028$), total free sugar (87.5 g/day [SD 53.5] vs. 56 g/day [SD 45.4], $p = 0.013$), and total added sugars (77.3 g/day [SD 51.1] vs. 48.1 g/day [SD 40.1], $p = 0.010$) compared to PD patients without dementia, after controlling for patient age, sex and PD duration ($\beta = -0.207$, $r^2 = 0.111$, $p = 0.033$; $\beta = -0.213$, $r^2 = 0.111$, $p = 0.031$; $\beta = -0.225$, $r^2 = 0.117$, $p = 0.023$, respectively).

Chronic Pain and Other Clinical Features

PD patients with chronic pain consumed more total sugar than PD patients without chronic pain (164.0 g/day [SD 92.2] vs. 124.6 g/day [SD 60.8], $p = 0.039$; controlling for age, sex and PD duration, $\beta = -0.202$, $r^2 = 0.087$, $p = 0.040$). Patients with REM sleep behaviour disorder (RBD) reported significantly more total sugar consumption per day compared with PD patients without RBD (174.2 g/day [SD 96.6] vs. 133.6 g/day [SD 70.8], $p = 0.016$; $\beta = -0.208$, $r^2 = 0.087$, $p = 0.020$) after controlling for patient age, sex and PD duration. PD patients with RBD consumed more total free sugars (77.6 g/day [SD 59.5] vs. 45.8 g/day [SD 26.0], $p = 0.001$) and total added sugars (67.0 g/day [SD 54.3] vs. 39.6 g/day [SD 22.9], $p = 0.001$) compared to those without RBD, after controlling for patient age, sex and PD duration ($\beta = -0.320$, $r^2 = 0.132$, $p = 0.001$ and $\beta = -0.306$, $r^2 = 0.125$, $p = 0.002$ respectively). PD patients with motor

TABLE 3 | Intake of macronutrients expressed as percentage of energy intake and intake of micronutrients expressed per 1,000 kJ intake.

	Parkinson's disease	Healthy control	Test statistic	*p*-value
Number of patients (n =)	103	81		
Dietary variables				
Protein % [SD]	18.0 [3.5]	19.2 [3.1]	$t = -2.6$ (181)^	**0.011***
Total fat % [SD]	34.0 [6.3]	35.0 [6.7]	$t = -1.1$ (181)^	0.262
Carbohydrate % [SD]	40.0 [6.1]	36.2 [6.6]	$t = 4.0$ (181)^	**<0.001***
Total Sugars % [SD]	21.9 [5.9]	18.7 [5.0]	$t = 3.8$ (181)^	**<0.001***
Free sugars % [SD]	8.8 [5.1]	6.5 [3.3]	$t = 3.4$ (181)^	**0.001***
Added sugars % [SD]	7.6 [4.7]	5.6 [2.9]	$t = 3.4$ (181)^	**0.001***
Fibre % [SD]	2.9 [0.8]	2.9 [0.8]	$t = -0.5$ (181)^	0.606
Alcohol % [SD]	2.5 [3.6]	4.1 [4.7]	$t = -2.6$ (181)^	**0.010***
Calcium (mg/day per 1,000 kJ) [SD]	106.8 [33.5]	111.3 [30.1]	$t = 1.0$ (181)^	0.343
Iron (mg/day per 1,000 kJ) [SD]	1.3 [0.3]	1.3 [0.2]	$t = -1.5$ (181)^	0.141
Magnesium (mg/day per 1,000 kJ) [SD]	43.0 [8.0]	47.0 [6.5]	$t = -3.6$ (181)^	**<0.001***
Potassium (mg/day per 1,000 kJ) [SD]	440.1 [84.1]	465.4 [67.1]	$t = -2.2$ (181)^	**0.029**
Sodium (mg/day per 1,000 kJ) [SD]	192.3 [51.3]	201.7 [49.3]	$t = -1.3$ (181)^	0.213
Zinc (mg/day per 1,000 kJ) [SD]	1.2 [0.2]	1.3 [0.2]	$t = -3.0$ (181)^	**0.003***
Retinol (ug/day per 1,000 kJ) [SD]	61.4 [83.1]	53.4 [57.1]	$t = 0.7$ (181)^	0.490
Beta carotene (ug/day per 1,000 kJ) [SD]	585.9 [326.6]	648.3 [434.7]	$t = -1.1$ (181)^	0.269
Vitamin A (ug/day per 1,000 kJ) [SD]	177.0 [100.7]	183.0 [100.9]	$t = -0.4$ (181)^	0.690
Thiamine (mg/day per 1,000 kJ) [SD]	0.2 [0.0]	0.2 [0.0]	$t = -1.9$ (181)^	0.060
Riboflavin (mg/day per 1,000 kJ) [SD]	0.2 [0.1]	0.2 [0.1]	$t = -0.9$ (181)^	0.372
Vitamin B12 (ug/day per 1,000 kJ) [SD]	0.6 [0.3]	0.7 [0.2]	$t = -0.9$ (181)^	0.365
Vitamin C (mg/day per 1,000 kJ) [SD]	13.7 [8.0]	14.2 [7.8]	$t = -0.5$ (181)^	0.643
Dietary folate (DFE) (ug/day per 1,000 kJ) [SD]	70.5 [17.0]	73.6 [16.0]	$t = -1.3$ (181)^	0.203

*^Independent sample t test; df, degrees of freedom; [SD], Standard Deviation. *Indicates dietary variables that remain statistically significant after Bonferroni correction ($p < 0.016$) of multiple testing with linear regression modelling when controlling for participant age and sex, as potential confounders. The bold values indicate clinical significance.*

fluctuations consumed less alcohol than those without motor fluctuations (6.3 g/day [SD 9.7] vs 12.6 g/day [SD 15.2], $p = 0.013$), after controlling for patient age, sex and PD duration ($\beta = 0.161$, $r^2 = 0.379$, $p = 0.049$), possibly due to alcohol further exacerbating their brittle PD motor features. When adjusted for energy intake per 1,000 kJ, PD patients with dyskinesia consumed more beta carotene (8060.4 ug/day [SD 8759.2]) compared to those without dyskinesia (4809.3 ug/day [SD 2547.1], $p = 0.024$).

Dietary and Clinical Correlations

PD patient age was negatively correlated with the amount of daily protein intake ($r = -0.277$, $p = 0.005$). Furthermore, increasing PD duration was associated with a lower albumin level ($r = -0.208$, $p = 0.004$). Increased alcohol consumption was associated with increased age at diagnosis ($r = 0.201$, $p = 0.042$) and older age at commencing treatment ($r = 0.200$, $p = 0.026$). Higher PDQ-39 SI scores (suggesting poorer QoL) were associated with lower total alcohol consumption ($r = -0.31$, $p = 0.001$) and higher total free sugar consumption ($r = 0.248$, $p = 0.012$). That is, PD individuals with a worse QoL consumed less alcohol, but ingested more sugar. Increased constipation severity was associated with increased free and added sugar consumption ($r = 0.211$, $p = 0.032$; $r = 0.201$, $p = 0.042$), respectively. Increased total sugar consumption was associated with greater daily levodopa dose (LED) requirement ($r = 0.272$, $p = 0.005$) and greater burden of non-motor symptoms as measured by the NMSS ($r = 0.213$, $p = 0.031$). The above associations of excess

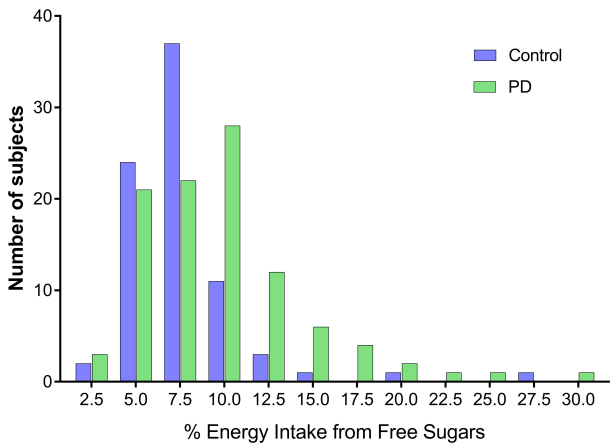

FIGURE 2 | Comparison of relative energy intake from free sugars between Parkinson's disease patients and healthy controls. Parkinson's disease patients consumed more free sugars as a proportion of energy intake compared to healthy controls.

sugar consumption in patients with more severe constipation, higher LED and worse NMSS scores can be partially explained by features suggestive of advancing PD severity. Higher NMSS total scores were also associated with higher total fat intake ($r = 0.292$, $p = 0.003$), increased protein consumption ($r = 0.232$, $p = 0.018$) and overall higher mean energy intake ($r = 0.257$, $p = 0.009$), suggesting that individuals who were more burdened by NMS required an increased food intake that was higher in protein and fat. Lastly, the effects of free sugar intake were also associated with gastrointestinal dysfunction in PD, with individuals who consumed more free sugars also reporting worse constipation, noted by the Rome-IV criteria ($r = 0.195$, $p = 0.049$), and Cleveland Constipation Score ($r = 0.211$, $p = 0.032$), as well as worse upper gastrointestinal dysfunction, indicated by a higher Leeds Dyspepsia Questionnaire score ($r = 0.202$, $p = 0.040$).

DISCUSSION

The results of this study provide novel and clinically important insights into the dietary habits of Australian PD patients. Consistent with other reports (7, 15, 16), PD patients consumed greater amounts of carbohydrates, which was largely attributable to increased daily sugar intake. However, in contrast, Barichella et al. (7) found increased consumption of many other macro and micronutrients by PD patients, rather than carbohydrates and sugars alone, as in our cohort. There are several reasons why PD patients may consume more sugar. It has been suggested that carbohydrates and sweets, through insulin, may increase brain dopamine as somewhat of a compensatory mechanism for disease-related dopamine loss (14, 15). A variety of mechanisms contribute to altered eating behaviour in PD, such as alterations in hypothalamic regulation, energy expenditure and dopaminergic signalling (48). Food reward alterations seem to be present in PD, and these may influence eating behaviours (49). In addition, non-motor complications affecting taste and olfaction, cognition, mood and reward may impair food perception, eating behaviours and motivation toward food consumption (49). Perhaps seeking more sugary foods is related to a decrease in taste function in PD patients. As demonstrated by Cecchini et al. (50), PD patients have reduced olfactory function and taste performance compared with controls. A chemosensory interaction has been proposed, where olfactory loss leads to a decrease in taste function (51).

Concerningly, we have shown a generally unhealthy diet in many PD patients in our study. In 2015 the World Health Organisation (WHO) issued a recommendation that both adults and children reduce their intake of free sugars to $<$ 10% of total dietary energy to help reduce the non-communicable disease burden from unhealthy weight gain and dental caries (33). Notably, in this study, 28.2% of PD patients compared to 7.5% of HCs had $>$10% energy intake attributed to free sugars (**Figure 2**), which is outside of the recommended WHO guidelines for healthy eating (52). Reassuringly, the PD cohort sampled did not have greater prevalence of diabetes, or a higher HbA1c than HCs. However, these measures should be monitored carefully throughout. Further evidence of an unhealthy diet is demonstrated by our PD patients consuming lower levels of certain micronutrients (when expressed per 1,000 kJ energy intake), despite high levels of sugar consumption. This is consistent with previous research, which has shown that intake of added sugar greater than the recommended level of 10% is associated with lower micronutrient intakes, indicating micronutrient dilution (53). The macronutrient distribution in this study cohort is similar to those reported for an Australian population, as per The Australian Health Survey 2011–13 (54). Poor diet in PD has been shown in a number of previous studies, for example in a study by van Steijn et al. (55) of Dutch elderly PD patients, 22.5% had unfavourable nutritional status. Patients in this study consumed less protein than HCs (when expressed as a percentage of energy intake), which is consistent with the finding here of slightly lower serum albumin levels, suggesting poorer nutritional status. Lower protein consumption by PD patients has not consistently been found (7, 16), despite a low protein or protein redistribution diet being recommended for PD patients with motor fluctuations (56, 57). It is known that the absorption of one of the most commonly used oral medications for PD, Levodopa, is impaired by simultaneous protein ingestion, and thus may be a potential reason why more PD patients eat less protein routinely (57).

In this study, patients who reported impulse control disorders consumed more sugar. Eating disorders are common in PD, and 21.6% of PD patients experience episodes of out-of-control eating with a large quantity of food in short time (58). The existence of a food addiction profile has been described in PD patients, and more specifically compulsive eating symptomatology (58). It is possible that the PD patients in our cohort binge eat, although this was not evident from the measures used and is not a feature considered in the impulse control disorder questionnaire. Dietary intake and compulsive eating in PD patients with impulse control disorders warrants further investigation.

Furthermore, increased sugar consumption was associated with chronic pain and depression. Depression is prevalent in PD, with 38.9% of our PD cohort reporting depression, which is almost double the proportion of depressed HCs (59). More PD patients in our study reported depression and chronic pain than healthy controls, which may contribute to the increased sugar consumption of these patients compared to controls. Depression may alter appetite, food intake and weight regulation. Serotonin plays a role in eating behaviour, and as discussed in a review by Kistner et al. (48), neurodegeneration of the serotonergic system, with low levels of serotonin in PD, may explain the pronounced preference for sweet foods. The association between sugar consumption and indicators of disease severity (e.g., greater LED, more non-motor symptoms) may suggest the possibility of comfort eating behaviour. Furthermore, cognition plays an important role in eating behaviour (49) meaning our results may have been influenced by the inclusion of patients with cognitive impairment and dementia, whereas many other studies exclude patients with MMSE $<$24. However, when subjects with dementia are excluded from analysis, the finding of increased sugar consumption by PD patients compared with HCs persisted.

PD patients in our cohort consumed less alcohol than healthy controls, which is consistent with previous findings (7, 13, 16). A possible explanation for this is that PD patients may be replacing alcoholic drinks with sugar sweetened beverages, as in previous research where higher added sugar intake has been associated with lower alcohol intake (53). Another proposed explanation for lower alcohol consumption by PD patients is that they may fear potential interactions between alcohol and medications (14). PD patients who reported motor fluctuations were found to consume less alcohol, perhaps suggesting a reluctance to consume alcohol for fear of worsening tremor or other motor features. Likewise, PD patients who were depressed potentially consumed less alcohol due to suspected medication interactions or perceptions of alcohol worsening their mood or PD management.

The mean BMI in our cohort was 26 and suggests that participants were overweight, consistent with epidemiological data in Australia (50). However, BMI did not differ between PD patients and HC. This is contrary to prior findings of lower weight and BMI in PD patients (3, 4, 6, 7), but consistent with other studies showing no difference in BMI (16). A possible explanation for this may be the relatively affluent socioeconomic standing of our cohort and the fact that our HC group were spouses of the PD patients.

The findings of this study are limited by its relatively small cohort size and cross-sectional design. Dietary habits may change over time, are typically influenced by seasonal availability of certain foods and are influenced by multiple disease factors. No significant relationship was seen between PD duration and sugar consumption. Over the course of the disease, nutritional requirements may change, body weight may fluctuate, with changes in both energy expenditure and food intake (10). Longitudinal studies, with larger sample size, are needed to further evaluate these dietary trends. Another limitation of this study is the potential for selection bias, with the population drawn from a single specialist PD centre, and in an area of relatively high socioeconomic status in metropolitan Sydney, Australia. Whereas, previous Australian studies have shown PD patients from regional areas to be comparably older with an older age of diagnosis and comparatively lower socioeconomic status (60, 61). Furthermore, the FFQ is subject to recall bias, particularly the reliance on long-term memory and errors in estimating frequencies and serving sizes. Memory recall may be unreliable in patients with cognitive impairment. Furthermore, the FFQ has also been shown to have a tendency to overestimate total carbohydrate and sugar (26), which may be relevant given our findings, although partially controlled for as both PD and HC cohorts completed the same FFQ. Mean fibre intake in our cohort is generally high and may be overestimated by the FFQ assessment. However, even when subjects with very high energy consumption are excluded from analysis, the significant findings observed in this study persist. Additionally, the comparability of these results to other studies is limited by the different dietary assessment tools utilised. Dietary habits vary significantly depending on ethnicity (22), limiting the generalizability of our findings. However, this also highlights the importance of this research, being the only dietary data to our knowledge for an Australian population of PD patients.

Important clinical correlations were identified in this study, such as increased sugar consumption being associated with an increase in non-motor symptoms, poorer QoL, increased constipation severity and greater levodopa requirements. Adherence to a healthy diet has recently been shown to reduce the occurrence of non-motor symptoms that predate PD diagnosis (24). It therefore remains to be determined if a reduction in dietary intake of added sugar can consequently reduce disease complications and non-motor features. Further research on dietary variations and their associations with clinical PD features and complications is warranted. Additionally, the high consumption of added sugar in our cohort highlights the need to carefully monitor PD patients for the development of diabetes.

CONCLUSION

Evaluating the dietary habits of an Australian PD cohort has provided valuable insights into important clinical associations between diet and disease characteristics. Thorough management of patient nutrition should be considered integral to patient care, as nutrition associates with many disease complications. We encourage clinicians to promote healthy eating as part of routine clinical care. The WHO strongly recommend reducing free sugar intake to $< 10\%$ to provide health benefits (52), and PD patients are at particular risk of the consequences of excess sugar consumption shown here. PD patients with impulse control disorders, RBD, depression, cognitive impairment, chronic pain and motor fluctuations are at risk of specific variations in nutritional intake, in particular excess consumption of added sugars. PD patients would benefit from dietitian input as part of routine clinical management.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Northern Sydney Local Health District Human Research Ethics Committee and the North Shore Private Hospital Ethics Committee, HREC/18/HAWKE/109, NSPHEC 2018-LNR-009, respectively. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

NP and VF: study design, analysed data, drafted, and reviewed the manuscript. ML: study design, reviewed patients, collected and analysed data, drafted, and reviewed the manuscript. RD and CS: study design, supervision, drafted, and reviewed the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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REFERENCES

1. Lubomski M, Rushworth RL, Tisch S. Hospitalisation and comorbidities in Parkinson's disease: a large Australian retrospective study. *J Neurol Neurosurg Psychiatry.* (2015) 86:324–30. doi: 10.1136/jnnp-2014-307822
2. Seidl SE, Santiago JA, Bilyk H, Potashkin JA. The emerging role of nutrition in Parkinson's disease. *Front Aging Neurosci.* (2014) 6:36. doi: 10.3389/fnagi.2014.00036
3. Bachmann CG, Trenkwalder C. Body weight in patients with Parkinson's disease. *Mov Disord.* (2006) 21:1824–30. doi: 10.1002/mds.21068
4. Kashihara K. Weight loss in Parkinson's disease. *J Neurol.* (2006) 253(Suppl. 7):VII38–41. doi: 10.1007/s00415-006-7009-0
5. Fereshtehnejad SM, Ghazi L, Sadeghi M, Khaefpanah D, Shahidi GA, Delbari A, et al. Prevalence of malnutrition in patients with Parkinson's disease: a comparative study with healthy controls using Mini Nutritional Assessment (MNA) questionnaire. *J Parkinsons Dis.* (2014) 4:473–81. doi: 10.3233/JPD-130323
6. van der Marck MA, Dicke HC, Uc EY, Kentin ZH, Borm GF, Bloem BR, et al. Body mass index in Parkinson's disease: a meta-analysis. *Parkinson Relat Disord.* (2012) 18:263–7. doi: 10.1016/j.parkreldis.2011.10.016
7. Barichella M, Cereda E, Cassani E, Pinelli G, Iorio L, Ferri V, et al. Dietary habits and neurological features of Parkinson's disease patients: implications for practice. *Clin Nutr.* (2017) 36:1054–61. doi: 10.1016/j.clnu.2016.06.020
8. Agim ZS, Cannon JR. Dietary factors in the etiology of Parkinson's disease. *Biomed Res Int.* (2015) 2015:672838. doi: 10.1155/2015/672838
9. Gao X, Chen H, Fung TT, Logroscino G, Schwarzschild MA, Hu FB, et al. Prospective study of dietary pattern and risk of Parkinson disease. *Am J Clin Nutr.* (2007) 86:1486–94. doi: 10.1093/ajcn/86.5.1486
10. Barichella M, Cereda E, Pezzoli G. Major nutritional issues in the management of Parkinson's disease. *Mov Disord.* (2009) 24:1881–92. doi: 10.1002/mds.22705
11. Lorefalt B, Granerus AK, Unosson M. Avoidance of solid food in weight losing older patients with Parkinson's disease. *J Clin Nurs.* (2006) 15:1404–12. doi: 10.1111/j.1365-2702.2005.01454.x
12. Wolz M, Kaminski A, Lohle M, Koch R, Storch A, Reichmann H. Chocolate consumption is increased in Parkinson's disease. Results from a self-questionnaire study. *J Neurol.* (2009) 256:91–2. doi: 10.1007/s00415-009-0118-9
13. Meyers C, Amick MA, Friedman JH. Ice cream preference in Parkinson's disease. *Med Health R I.* (2010) 93:91–2.
14. Cassani E, Barichella M, Ferri V, Pinelli G, Iorio L, Bolliri C, et al. Dietary habits in Parkinson's disease: adherence to mediterranean diet. *Parkinson Relat Disord.* (2017) 42:40–6. doi: 10.1016/j.parkreldis.2017.06.007
15. Aden E, Carlsson M, Poortvliet E, Stenlund H, Linder J, Edstrom M, et al. Dietary intake and olfactory function in patients with newly diagnosed Parkinson's disease: a case-control study. *Nutr Neurosci.* (2011) 14:25–31. doi: 10.1179/174313211X12966635733312
16. Marczewska A, De Notaris R, Sieri S, Barichella M, Fusconi E, Pezzoli G. Protein intake in Parkinsonian patients using the EPIC food frequency questionnaire. *Mov Disord.* (2006) 21:1229–31. doi: 10.1002/mds.20888
17. Jackson A, Forsyth CB, Shaikh M, Voigt RM, Engen PA, Ramirez V, et al. Diet in Parkinson's disease: critical role for the microbiome. *Front Neurol.* (2019) 10:1245. doi: 10.3389/fneur.2019.01245
18. Lubomski M, Tan AH, Lim SY, Holmes AJ, Davis RL, Sue CM. Parkinson's disease and the gastrointestinal microbiome. *J Neurol.* (2020) 267:2507–23. doi: 10.1007/s00415-019-09320-1
19. Lubomski M, Davis RL, Sue CM. The gut microbiota: a novel therapeutic target in Parkinson's disease? *Parkinson Relat Disord.* (2019) 66:265–6. doi: 10.1016/j.parkreldis.2019.08.010
20. Lubomski M, Davis RL, Sue CM. Gastrointestinal dysfunction in Parkinson's disease. *J Neurol.* (2020) 267:1377–88. doi: 10.1007/s00415-020-09723-5
21. Lubomski M, Davis R, Sue C. Health-related quality of life for Parkinson's disease patients and their caregivers. *J Mov Disord.* (2020) 14:42–52. doi: 10.14802/jmd.20079
22. Sauerbier A, Schrag A, Martinez-Martin P, Hall LJ, Parry M, Mischley LK, et al. Dietary variations in a multiethnic Parkinson's disease cohort and possible influences on nonmotor aspects: a cross-sectional multicentre study. *Parkinsons Dis.* (2018) 2018:7274085. doi: 10.1155/2018/7274085
23. Sheard JM, Ash S, Mellick GD, Silburn PA, Kerr GK. Improved nutritional status is related to improved quality of life in Parkinson's disease. *BMC Neurol.* (2014) 14:212. doi: 10.1186/s12883-014-0212-1
24. Molsberry S, Bjornevik K, Highes K, Healy B, Schwarzchild M, Ascherio A. Diet pattern and prodromal features of Parkinson's disease. *Neurology.* (2020) 95:e2095–108. doi: 10.1212/WNL.0000000000010523
25. Hughes AJ, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry.* (1992) 55:181–4. doi: 10.1136/jnnp.55.3.181
26. Barclay AW, Flood VM, Brand-Miller JC, Mitchell P. Validity of carbohydrate, glycaemic index and glycaemic load data obtained using a semi-quantitative food-frequency questionnaire. *Public Health Nutr.* (2008) 11:573–80. doi: 10.1017/S1368980007001103
27. Willett WC, Sampson L, Browne ML, Stampfer MJ, Rosner B, Hennekens CH, et al. The use of a self-administered questionnaire to assess diet four years in the past. *Am J Epidemiol.* (1988) 127:188–99. doi: 10.1093/oxfordjournals.aje.a114780
28. Flood VM, Smith W, Rochtchina E, Wang JJ, Mitchell P. Assembling a nutrient database for a large cohort study: Blue Mountains Eye Study. *Food Aust.* (2008) 60:37–40. Available online at: https://ro.uow.edu.au/hbspapers/344/
29. Smith W, Mitchell P, Reay EM, Webb K, Harvey PW. Validity and reproducibility of a self-administered food frequency questionnaire in older people. *Austr N Zeal J Public Health.* (1998) 22:456–63. doi: 10.1111/j.1467-842X.1998.tb01414.x
30. Gopinath B, Flood VM, Kifley A, Louie JC, Mitchell P. Association between carbohydrate nutrition and successful aging over 10 years. *J Gerontol A Biol Sci Med Sci.* (2016) 71:1335–40. doi: 10.1093/gerona/glw091
31. Food Standards *Australia New Zealand (2019). Australian Food Composition Database - Release 1.* Canberra, ACT: FSANZ. Available online at: www.foodstandards.gov.au

32. Food Standards Australia New Zealand. *Determining the Amount of Added Sugars and Free Sugars in Foods Listed in the AUSNUT 2011-13 Dataset*. (2016) Canberra: Australian Government.
33. World Health Organization. *Guideline: Sugars Intake for Adults and Children*. (2015) Geneva: WHO.
34. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr.* (1997) 65(Suppl. 4):1220S−8. doi: 10.1093/ajcn/65.4.1220S
35. Hagstromer M, Oja P, Sjostrom M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr.* (2006) 9:755–62. doi: 10.1079/PHN2005898
36. Moayyedi P, Duffett S, Braunholtz D, Mason S, Richards ID, Dowell AC, et al. The leeds dyspepsia questionnaire: a valid tool for measuring the presence and severity of dyspepsia. *Aliment Pharmacol Ther.* (1998) 12:1257–62. doi: 10.1046/j.1365-2036.1998.00404.x
37. Sood R, Ford AC. Diagnosis: Rome IV criteria for FGIDs - an improvement or more of the same? *Nat Rev Gastroenterol Hepatol.* (2016) 13:501–2. doi: 10.1038/nrgastro.2016.110
38. Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD. A constipation scoring system to simplify evaluation and management of constipated patients. *Dis Colon Rectum.* (1996) 39:681–5. doi: 10.1007/BF02056950
39. Jenkinson C, Fitzpatrick R, Peto V, Greenhall R, Hyman N. The Parkinson's Disease Questionnaire (PDQ-39): development and validation of a Parkinson's disease summary index score. *Age Ageing.* (1997) 26:353–7. doi: 10.1093/ageing/26.5.353
40. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry.* (1961) 4:561–71. doi: 10.1001/archpsyc.1961.01710120031004
41. Nasreddine ZS, Phillips NA, Bedirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc.* (2005) 53:695–9. doi: 10.1111/j.1532-5415.2005.53221.x
42. Chaudhuri KR, Martinez-Martin P, Brown RG, Sethi K, Stocchi F, Odin P, et al. The metric properties of a novel non-motor symptoms scale for Parkinson's disease: results from an international pilot study. *Mov Disord.* (2007) 22:1901–11. doi: 10.1002/mds.21596
43. Goetz CG, Tilley BC, Shaftman SR, Stebbins GT, Fahn S, Martinez-Martin P, et al. Movement disorder society-sponsored revision of the unified Parkinson's disease rating scale (MDS-UPDRS): scale presentation and clinimetric testing results. *Mov Disord.* (2008) 23:2129–70. doi: 10.1002/mds.22340
44. Tomlinson CL, Stowe R, Patel S, Rick C, Gray R, Clarke CE. Systematic review of levodopa dose equivalency reporting in Parkinson's disease. *Mov Disord.* (2010) 25:2649–53. doi: 10.1002/mds.23429
45. McCormack HM, Horne DJ, Sheather S. Clinical applications of visual analogue scales: a critical review. *Psychol Med.* (1988) 18:1007–19. doi: 10.1017/S0033291700009934
46. Schrag A, Barone P, Brown RG, Leentjens AF, McDonald WM, Starkstein S, et al. Depression rating scales in Parkinson's disease: critique and recommendations. *Mov Disord.* (2007) 22:1077–92. doi: 10.1002/mds.21333
47. Dalrymple-Alford JC, MacAskill MR, Nakas CT, Livingston L, Graham C, Crucian GP, et al. The MoCA: well-suited screen for cognitive impairment in Parkinson disease. *Neurology.* (2010) 75:1717–25. doi: 10.1212/WNL.0b013e3181fc29c9
48. Kistner A, Lhommee E, Krack P. Mechanisms of body weight fluctuations in Parkinson's disease. *Front Neurol.* (2014) 5:84. doi: 10.3389/fneur.2014.00084
49. Aiello M, Eleopra R, Rumiati RI. Body weight and food intake in Parkinson's disease. A review of the association to non-motor symptoms. *Appetite.* (2015) 84:204–11. doi: 10.1016/j.appet.2014.10.011
50. Cecchini MP, Osculati F, Ottaviani S, Boschi F, Fasano A, Tinazzi M. Taste performance in Parkinson's disease. *J Neural Transm.* (2014) 121:119–22. doi: 10.1007/s00702-013-1089-7
51. Landis BN, Scheibe M, Weber C, Berger R, Bramerson A, Bende M, et al. Chemosensory interaction: acquired olfactory impairment is associated with decreased taste function. *J Neurol.* (2010) 257:1303–8. doi: 10.1007/s00415-010-5513-8
52. Organization Geneva: Wold Health. *Guideline: Sugars Intake for Adults and Children* (2015).
53. Moshtaghian H, Louie JC, Charlton KE, Probst YC, Gopinath B, Mitchell P, et al. Added sugar intake that exceeds current recommendations is associated with nutrient dilution in older Australians. *Nutrition.* (2016) 32:937–42. doi: 10.1016/j.nut.2016.02.004
54. The Australian Health Survey 2011-13. Available online at: https://www.abs.gov.au/statistics/health/health-conditions-and-risks/australian-health-survey-nutrition-first-results-foods-and-nutrients/latest-release#energy-and-nutrients
55. van Steijn J, van Harten B, Flapper E, Droogsma E, van Walderveen P, Blaauw M, et al. The nutritional status of Dutch elderly patients with Parkinson's disease. *J Nutr Health Aging.* (2014) 18:601–7. doi: 10.1007/s12603-014-0444-1
56. Cereda E, Barichella M, Pedrolli C, Pezzoli G. Low-protein and protein-redistribution diets for Parkinson's disease patients with motor fluctuations: a systematic review. *Mov Disord.* (2010) 25:2021–34. doi: 10.1002/mds.23226
57. Wang L, Xiong N, Huang J, Guo S, Liu L, Han C, et al. Protein-restricted diets for ameliorating motor fluctuations in Parkinson's disease. *Front Aging Neurosci.* (2017) 9:206. doi: 10.3389/fnagi.2017.00206
58. de Chazeron I, Durif F, Chereau-Boudet I, Fantini ML, Marques A, Derost P, et al. Compulsive eating behaviors in Parkinson's disease. *Eat Weight Disord.* (2019) 24:421–9. doi: 10.1007/s40519-019-00648-1
59. Lubomski M, Davis RL, Sue CM. Depression in Parkinson's Disease: perspectives from an Australian cohort. *J Affect Disord.* (2020) 277:1038–44. doi: 10.1016/j.jad.2020.09.032
60. Lubomski M, Louise Rushworth R, Lee W, Bertram KL, Williams DR. Sex differences in Parkinson's disease. *J Clin Neurosci.* (2014) 21:1503–6. doi: 10.1016/j.jocn.2013.12.016
61. Lubomski M, Rushworth RL, Lee W, Bertram K, Williams DR. A cross-sectional study of clinical management, and provision of health services and their utilisation, by patients with Parkinson's disease in urban and regional Victoria. *J Clin Neurosci.* (2013) 20:102–6. doi: 10.1016/j.jocn.2012.05.015

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No Effect of Added Sugars in Soft Drink Compared With Sugars in Fruit on Cardiometabolic Risk Factors: Results From a 4-Week, Randomized Controlled Trial

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High intakes of added sugar from soft drinks are associated with negative health outcomes such as the increased risk of gout and type 2 diabetes, weight gain and cardiovascular disease. Fruits are naturally high in sugars but their effect on cardiometabolic risk remains unknown. We examined the effect on cardiometabolic risk factors of consuming natural sugars from fruit or added sugars from sugar-sweetened soft drinks in overweight adults. Forty-eight healthy, overweight (BMI $\geq$ 28 kg/m^2) men (n = 21) and women (n = 20) were randomized to either a fruit (n = 19) or sugar-sweetened soft drink (n = 22) intervention for 4 weeks. The fruit group received 6 items of fresh and dried fruit per day and the sugar-sweetened soft drink group received 955 ml of sugar-sweetened soft drink per day. The interventions were matched for both energy (fruit: 1,800 kJ/d; soft drink: 1,767 kJ/d) and fructose content (fruit: 51.8 g/d; soft drink: 51.7 g/d). The soft drink intervention provided 101 g total sugars, which was all added sugar and the fruit intervention provided 97 g total sugars, which were all natural sugars. Dietary intakes were otherwise *ad libitum*. Despite being asked to consume additional sugar (up to 1,800 additional kJ/d), there were no changes in weight, blood pressure or other cardiometabolic risk factors, except by uric acid, in any of the intervention groups. In conclusion, our findings do not provide any evidence that short-term regular intake of added sugars is linked to higher cardiometabolic risks, with exception of uric acid in overweight men. Public health interventions to prevent obesity and related diseases should focus on the quality of the whole diet rather than only focusing on reducing sugary drinks or sugar intakes.

Keywords: fructose, fruit, sugar, cardiometabolic risk, sugar-sweetened soft drink, beverage, dietary intervention

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INTRODUCTION

Increased consumption of added or free sugars is associated with increased risk of obesity and related diseases worldwide (1–3). The evidence linking sugar intakes with obesity-related diseases relates largely to the provision of extra calories with causes weight. The World health organization (WHO) recommends that free sugars

(all monosaccharides and disaccharides added to foods by the manufacturer, cook or consumer, plus the sugars that are naturally present in honey, syrups and fruit juices) are limited to <10% of daily energy intake (4). This recommendation is mostly based on findings related to dental caries given relatively limited evidence of increased risk of cardiovascular disease or other negative health outcomes. There has been considerable interest in whether the associations between fructose and negative health outcomes are specifically linked with high intakes of fructose (1, 5–7). High fructose intake has indeed been linked with hyperuricemia (8, 9), risk of gout (5), but has not been found to be associated with risk for type 2 diabetes (10). However, fructose is infrequently consumed in the absence of equal amounts of glucose and therefore reduction of free or added sugars tends to be the target of public health interventions (11).

Sugars in the human diet are mostly presented as glucose, fructose, galactose, lactose, and sucrose (12). Sucrose, commonly consumed as table sugar, is a disaccharide constituted by equal parts of fructose and glucose (12). Fructose-containing sweeteners, such as high fructose corn syrup are presented in different forms but contain fructose and glucose in relatively similar proportions to sucrose (13). Added sugars are most commonly consumed globally in the form of sucrose or high fructose syrups which are commonly used to sweeten soft drinks, desserts and bakery items, confectionary, and other processed foods (11, 14).

Fruit is also an important source of dietary sugars glucose and sucrose and particularly, fructose. Chemically the sugars in fruit and added sugars are indistinguishable and the ratio of glucose to fructose equivalents is similar (1:1) (12). This has led to concerns in some quarters that fruit should also be limited. The strongest evidence for limiting fruit comes from studies which have examined the associations between fruit intake, and gout and hyperuricaemia. Generally, observational studies of varied design have indicated a reduced risk of incident gout (5) or experiencing gout attacks (15) with higher fruit consumption. Contrary to these findings, in an analysis of the Health Professionals Follow-up Study (n = 46,393 men), higher fruit juice and fruit intakes were associated with an *increased* risk of incident gout after a 12 years follow-up (5). Acute feeding studies in humans involving fruit or fruit juice have also produced mixed results, with an immediate rise in serum uric acid seen after consuming apples (16) or apple juice (17); a lowered plasma urate level after cherry consumption, and no effect of grapes, strawberries or kiwifruit on plasma urate (18). Finally, in a 6 week weight reduction trial, energy-restricted diets providing either a relatively high intake of fructose from fruit (50–70 g/d) or a low amount of fructose (<10–20 g/d) led to significantly lowered serum uric acid concentrations, although no difference was seen between the diets and the reductions in serum uric acid could have been explained by the weight achieved in both interventions (19). However, fruit is high in micronutrients, antioxidants and dietary fiber, and has a relatively low energy density and fruit intake has been associated with a reduced risk of several chronic diseases (9) as well as better glycemic control in people with type 2 diabetes (20). Clearly there is a need for research to clarify whether the sugars naturally found in fruits have similar effects on disease risks as added sugars.

In the present study, we sought to compare the effect of consuming sugars from either whole fruit or sugar sweetened soft drink (soft drink) on serum cardiometabolic risk factors, over 4 weeks, matched for both energy, fructose and total sugars in addition to an *ad libitum* diet. We hypothesized that due to its more favorable nutritional properties, sugars in fruit would have a more favorable effect on cardiometabolic risk factors than sugars from sugar-sweetened soft drinks.

MATERIALS AND METHODS

Subjects

In September 2012, overweight men and women were recruited using a local newspaper advertisement and University of Otago, Dunedin, New Zealand email lists. Two-hundred and sixty-seven (267) respondents were assessed via an online survey or telephone interview, 48 of whom appeared to meet inclusion criteria: body mass index (BMI) $\geq$ 28 kg/m^2; aged between 20 and 75 years; no established diabetes, liver or kidney disease, gout or a history of other major chronic illnesses; no diagnosed mental disorders; not currently taking medications affecting blood pressure, blood lipids, blood glucose or mood/mental state; not currently pregnant or breastfeeding; no intolerance to study fruit or fructose; able to remain in Dunedin for the duration of the intervention period; and willing to consume either fruit or soft drink for 4 weeks. Of the 48 respondents invited to attend a screening visit to confirm their eligibility and obtain written informed consent, seven did not meet inclusion criteria. A total of 41 participants were randomized to consume either fruit (n = 19) or soft drink (n = 22). Following randomization, but prior to receiving their allocated beverage, three participants withdrew from the study. One participant in the fruit group moved away from Dunedin midway through the intervention and was lost to follow-up, resulting in a final total of 37 participants (n = 18 fruit; n = 19 soft drink) completing the study by December 2012. This study was approved by University of Otago Human Ethics Committee (Ref: 12/197). The trial was registered with the Australian New Zealand Clinical Trials Registry: ACTRN12612000874819; http://www.anzctr.org.au.

Experimental Protocol

Computer-generated block randomization was stratified by sex (to account for potential differences uric acid in response to treatment), performed before recruitment, and concealed from researchers in sealed, numbered envelopes. After establishing a participant's eligibility at the screening visit, and obtaining written informed consent, the next envelope was opened and the participant's group allocation revealed. Due to the nature of the study the researcher responsible for delivering the interventions, and participants, could not be blinded to group allocation.

Approximately 1–2 weeks after their initial screening visit, participants attended a baseline visit at the Department of Human Nutrition Clinic, University of Otago, Dunedin, New Zealand. Between screening and baseline, and during the final

week of the intervention, participants completed a 3-day weighed diet record, recorded on non-consecutive days and including a weekend day. Electronic scales were provided along with written instructions, and a trained researcher verbally explained how to complete the diet records. The researcher was available by email or telephone to answer questions that arose during completion of the diet record. Dietary intakes of macronutrients, fructose; vitamin C; potassium and dietary fiber were determined using Kaiculator dietary assessment software (University of Otago, Dunedin, NZ), which uses the New Zealand food composition database (21). Participants were informed of their group allocation at baseline. Those randomized to fruit were provided with one Cavendish banana (128 g), three Braeburn or Jazz apples (149 g each) and two 14 g boxes of Sunmaid seedless raisins per day. Those assigned to soft drinks were provided with one 355 mL can and one 600 mL bottle of sugar (sucrose) sweetened soft drink (either Coca-Cola or Sprite) per day. The interventions were matched as closely as possible for energy (fruit: 1,800 kJ/d; soft drink: 1,767 kJ/d), fructose (fruit: 51.8 g/d; soft drink: 51.7 g/d), and totals sugars (fruit: 97 g/d soft drink: 101 g/d) content and all participants were instructed to consume their usual diet as normal. Participants collected fruit and soft drinks weekly, and were asked to record daily consumption in a log booklet, and to return empty beverage containers and any unconsumed fruit/beverages on a weekly basis.

Participants attended a clinic at baseline and at the end of study after an overnight (10–12 h) fast. Anthropometric measurements were made by one trained researcher, during which participants wore light clothing and no shoes. Weight to the nearest 0.1 kg, body fat percentage to the nearest 0.1% and BMI to the nearest 0.1 kg/m^2 were measured in duplicate using a calibrated Tanita Wedderburn bioimpedance analyzer. Height was measured using a Seca fixed stadiometer, and waist circumference was measured underneath clothing with a non-stretching anthropometric tape according to the International Society for the Advancement of Kinanthropometry protocols (22). Height and waist circumference were measured to the nearest 0.1 cm in duplicate, or in triplicate if measurements differed by >0.5 cm or >1%, respectively. Seated blood pressure was measured after a 5 min rest in triplicate using an Omron digital blood pressure monitor in mm Hg. Fasting blood samples (8 mL) were drawn by a research nurse using ethylenediaminetetraacetic acid (EDTA)-treated vacutainers. Blood samples were kept at <4°C for ∼1 h, then centrifuged at 1,650 g for 15 min. Plasma samples were then frozen in polyethylene cryovials at −80°C until analysis.

TABLE 1 | Baseline demographic and clinical measures of participants randomized to fruit or soft drink[a].

	Fruit (n = 19)	Soft drink (n = 22)
Female, *n* (%)	9 (47.4)	11 (50.0)
Age, years	34.7 (12.5)	33.2 (12.8)
Weight, kg	91.0 (21.4)	94.5 (15.2)
Height, cm	170.8 (9.4)	170.8 (9.9)
BMI, kg/m^2	31.0 (5.3)	32.2 (3.4)
Waist circumference, cm	97.3 (17.2)	99.4 (11.4)
Body fat, %	34.4 (8.4)	35.7 (7.6)
Self-reported physical activity, *n* (%)		
Inactive	3 (15.8)	4 (20.0)
Moderately active	6 (31.6)	6 (30.0)
Active	10 (52.6)	10 (50.0)
Systolic blood pressure, mm Hg	122 (15)	120 (13)
Diastolic blood pressure, mm Hg	70 (11)	69 (9)
Triglycerides, mmol/L	1.29 (0.65)	1.19 (0.45)
LDL-cholesterol, mmol/L	2.93 (0.97)	3.19 (0.89)
HDL-cholesterol, mmol/L	1.26 (0.22)	1.32 (0.37)
Total cholesterol, mmol/L	4.78 (1.10)	5.05 (0.97)
Plasma insulin, mIU/ml	12.67 (10.32)	11.61 (4.70)
Plasma glucose, mmol/L	5.44 (0.60)	5.29 (0.37)
Plasma uric acid, μmol/L	334.2 (66.5)	385.5 (79.7)
Insulin sensitivity index[b]	7.23 (2.03)	7.02 (1.31)

[a]*Data are means (SD) unless otherwise indicated; soft drink, sugar-sweetened soft drink.*
[b]*McAuley Insulin sensitivity index: Mffm/I = exp[2.63 – 0.28 ln(insulin) – 0.31 ln(TAG)].*

Laboratory Analysis

Plasma insulin was measured using a specific electrochemiluminescence immunoassay for the Elecsys analyzer (Roche Diagnostics, Mannheim, Germany), with a coefficient of variation (CV) of 1.1%. Total cholesterol (CV: 1.0%), triglyceride (CV: 0.9%), glucose (CV: 0.8%), and plasma uric acid (CV: 0.9%) concentrations were measured enzymatically with kits and calibrators supplied by Roche Diagnostics on a Cobas Mira analyzer (Roche Diagnostics). High-density lipoprotein (HDL; CV: 1.5%) was measured in the supernatant after precipitation of apolipoprotein B-containing lipoproteins with phosphotungstate/magnesium chloride solution (23). Low-density lipoprotein (LDL) was calculated using the Friedewald equation (24). High sensitivity C-reactive protein (CRP) was measured using a latex-enhanced immunoturbidimetric method (Roche Diagnostics) with a CV of 6.4%.

Statistical Analysis

The primary outcome measure was serum uric acid and insulin sensitivity measured by the McAuley Index (25). Using an estimated change in mean plasma uric acid of 50 μmol/L (SD of 75 μmol/L) and a correlation between repeated measures of 0.75 (26), it was estimated that 15 participants per group would be required for analysis of covariance (ANCOVA) with one baseline and one follow-up measurement, at 80% power and alpha = 0.05. To allow for attrition, n = 40 was the overall recruitment goal.

Change in body composition and clinical measures from baseline to week 4 were compared within treatment groups using Student's t-tests. Data were checked for normality and equal variance, with non-normal data compared using Mann–Whitney tests, and data with unequal variance compared using Welch's t-tests. The effect of treatment on body composition and clinical measures was analyzed by ANCOVA with baseline values as a covariate. Other participant characteristics likely to affect outcomes (baseline BMI and age) and a potential interaction effect (sex by group) were included as covariates

TABLE 2 | Change in dietary intake from baseline to week 4 and difference between treatments.

	Fruit		Soft drink		Overall difference between the treatments[a]	
	Baseline (mean, SD)	Change (mean, SD)	Baseline (mean, SD)	Change (mean, SD)	Difference (Mean, 95%CI)	*P*-value for difference
Energy (kJ)						
Males	12,409	−879 (6,639)	12,316	218 (3,592)	−1,031 (−3,899, 1,837)	0.467
Females	8,178	930 (1,461)	8,364	891 (2,723)	−93 (−2,881, 2,694)	0.946
Carbohydrate (g)						
Males	307	41 (148)	357	14 (162)	−11 (−89, 65)	0.754
Females	227	48 (42)	245	36 (88)	−2 (−76, 72)	0.959
Total sugars (g)						
Males	120	52 (80)	153	64 (63)	−40 (−83, 3)	0.068
Females	101	52 (34)	119	51 (63)	−13 (−54, 27)	0.506
Sucrose (g)						
Males	53	−4 (36)	78	32 (43)	−54 (−79, −29)	<0.001
Females	49	0 (23)	50	32 (31)	−34 (−57, −10)	0.006
Fructose (g)						
Males	22	29 (16)	23	21 (19)	8 (−3, 20)	0.139
Females	17	31 (7)	27	11 (15)	12 (0, 24)	0.044
Glucose (g)						
Males	22	22 (5)	23	20 (6)	3 (−4, 11)	0.538
Females	16	23 (3)	26	9 (5)	5 (−5, 15)	0.33
Vitamin C (mg)						
Males	106	−6 (64)	106	63 (269)	−68 (−212, 76)	0.339
Females	92	28 (61)	90	−25 (91)	55 (−85, 195)	0.426
Dietary fiber (g)						
Males	34	−2 (18)	28	−7 (11)	9 (1, 16)	0.004
Females	25	6 (5)	20	−3 (8)	12 (5, 19)	0.002
Alcohol (g)						
Males	13	−6 (10)	1	9 (11)	−11 (−23, 0)	0.054
Females	4	3 (12)	6	6 (13)	−4 (−14, 7)	0.459

[a]*ANCOVA was used to obtain estimate with adjustment for baseline values.*

individually, and those with a $P < 0.25$ were retained in the final model. All analyses were conducted using Stata 11.1 (Stata Corporation 2010, College Station, Texas, United States), and a two-sided 0.05 level of significance was used in all cases.

RESULTS

Table 1 summarizes baseline characteristics of participants randomized to treatment. Thirty-seven participants completed the study, 19 in the fruit group, and 18 in the soft drink group. Nine women completed each intervention. Participants had a mean age of 33.2 years and 54% were obese with mean BMI of 31.4 kg/m^2.

There were no significant differences in total energy, carbohydrate, total sugars and glucose intakes between treatments (**Table 2**). However, sucrose intake was higher in the soft drink group, and fructose intake was higher in the fruit group amongst women only. The fruit group also consumed significantly more dietary fiber during treatment. On average participants in the fruit group consumed 92% of the fruit provided (5.5 items per day) and participants in the soft drink group consumed 94% of the beverages provided (900 ml per day).

There were no overall significant differences in the effects of treatment on cardiometabolic variables or body composition (**Table 3**). However, there was a significant sex by treatment interaction ($P = 0.032$) for serum uric acid. Amongst men uric acid was 57 μmol/L higher in those in the soft drink group ($P = 0.008$) but there was no effect in women. There was also a significant sex interaction ($P = 0.034$) for insulin sensitivity index. Insulin sensitivity declined amongst men and increased amongst women in the soft drink group compared with the fruit groups but the differences between treatments were not significant for men or for women. In a multivariate adjusted model examining the effect of treatment on serum uric acid soft drink treatment ($P = 0.001$), baseline BMI ($P < 0.001$) and increased alcohol intake ($P = 0.032$) were associated with higher uric acid while female sex ($P = 0.002$) was associated with lower uric acid (**Table 4**).

TABLE 3 | Change in body composition and clinical measures from baseline to week 4 and difference between treatments.

	Fruit Change from baseline[a]	Soft drink Change from baseline[a]	Treatment effect[b]	*P*-value for difference
Plasma uric acid (μmol/L)				
All participants	−6.00 (37.75)	4.37 (49.15)	14.09 (−17.77, 45.96)	0.375
Males	−14.67 (31.89)	22.80 (39.42)	57.17 (16.35, 98.00)[c]	0.008
Females	2.67 (42.92)	−16.11 (52.82)	−1.33 (−6.88, 9.53)[c]	0.295
			P for sex interaction	*0.032*
Insulin sensitivity index[d]				
All participants	−0.21 (0.99)	−0.27 (1.36)	−0.115 (−0.89, 0.66)	0.765
Males	−0.14 (1.24)	−1.01 (1.27)	−0.84 (−1.80, 0.13)[c]	0.088
Females	−0.28 (0.73)	0.55 (0.94)	0.68 (−0.32, 1.69)[c]	0.173
			P for sex interaction	*0.034*
Weight, kg	0.03 (1.02)	0.24 (1.67)	0.26 (−0.67, 1.18)	0.579
Waist circumference, cm				
All participants	0.72 (1.83)	1.48 (1.62)	0.79 (−0.33, 1.91)	0.161
Males	1.07 (1.43)	0.87 (1.58)	−0.12 (–1.68, 1.72)	0.863
Females	0.36 (2.18)	2.17 (1.46)	1.76 (0.17, 3.35)	0.031
			P for sex interaction	*0.093*
Body fat, %	−0.18 (1.06)	0.27 (1.28)	0.46 (−0.32, 1.24)	0.235
Systolic BP, mm Hg	2.17 (9.54)	0.63 (6.62)	−2.18 (−7.16, 2.80)	0.381
Diastolic BP, mm Hg	4.11 (11.50)	0.32 (8.33)	−4.96 (−10.84, 0.92)	0.272
Triglycerides, mmol/L	0.04 (0.36)	0.12 (0.10)	0.07 (−0.18, 0.32)	0.571
LDL-cholesterol, mmol/L	0.03 (0.36)	0.08 (0.42)	0.08 (−0.18, 0.34)	0.526
HDL-cholesterol, mmol/L	−0.04 (0.17)	−0.04 (0.15)	0.004 (−0.08, 0.09)	0.932
Total cholesterol, mmol/L	0.01 (0.41)	0.09 (0.53)	0.12 (−0.19, 0.43)	0.438
Plasma insulin, mlU/ml	0.02 (0.71)	1.94 (5.97)	1.54 (−2.48, 5.56)	0.443
Fasting glucose, mmol/L	0.02 (0.39)	0.02 (0.32)	−0.05 (−0.27, 0.17)	0.632
CRP, mg/L	0.74 (0.39)	0.21 (0.69)	0.10 (−1.54, 1.74)	0.902

[a]Values are mean change (SD); [b]ANCOVA was used to obtain estimate with adjustment for baseline values with fruit as the reference group; [c]ANCOVA was used to obtain estimate with adjustment for baseline uric acid and an interaction effect between treatment and sex; [d]McAuley Insulin sensitivity index: Mffm/I = exp[2.63 – 0.28 ln(insulin) – 0.31 ln(TAG)].

DISCUSSION

We found that amongst overweight people increasing sugars intake either in the form of added sugars in soft drinks, or natural sugars from fruit did not lead to any deleterious changes in body weight or cardiometabolic risk factors and with no difference in effects between the two interventions. The absence of any change in weight despite being provided with additional energy from sugars (∼1,800 kJ/d) in the form of fruit or soft drinks suggest that participants sub-consciously moderated their overall food intake to compensate. In a *post-hoc* analysis, we did find that consumption of soft drink resulted in a non-significant rise in plasma uric acid levels among men, while intake of an equivalent amount of fruit did not, with the difference between interventions being statistically significant. This research suggests that health promotion strategies to reduce the prevalence of non-communicable diseases must consider more than simply recommending the replacement of added sugars with fruit.

The finding that participants did not gain weight as a result being asked to consume additional sugar on a daily basis for 4 weeks was surprising. Many previous studies that have reported that participants gain weight when provided with sugar sweetened foods and drinks in addition to their usual diets, in an *ad libitum* context (2). We recruited participants that were willing to increase their sugars intake and were therefore likely to be relatively unconcerned about gaining weight. Evidence suggests that these type of participants may be more responsive to appetite and satiety cues than participants who are worried about gaining weight (27). However, if our study had been conducted over a longer period of time it is possible that we may have observed weight gain as our participants became habituated to a higher sugar intakes.

Our study has suggests that fructose intake from whole fruit may be handled differently by the body than added sugars from soft drinks leading to the observed rise in uric acid amongst men (7). However, it is also conceivable that differences in alcohol intake, which increased more in the soft drink group, might also explain the effect. Nevertheless fruit is important as fruit provides many beneficial dietary components, and those that would try to restrict fruit on the basis of its high fructose or sugars content would therefore reduce intake of these beneficial nutrients unnecessarily.

In this study the difference in plasma uric acid of 57 μmol/L between treatments amongst males is not only statistically

TABLE 4 | Multivariate analysis of covariance of the effect of treatment and confounding variables on uric acid at week 4.

	β (SE)	*P*-value	R^2
Model 1		<0.0001	0.756
Soft drink	14.10 (15.70)	0.375	
Model 2		<0.0001	0.817
Soft drink	57.17 (20.04)	0.008	
Female	−20.61 (24.64)	0.409	
Model 3		<0.0001	0.871
Soft drink	65.50 (17.28)	0.001	
Female	−38.21 (21.63)	0.087	
Baseline BMI	5.20 (1.45)	0.001	
Model 4		<0.0001	0.893
Soft drink	58.3 (15.5)	0.001	
Female	−65.0 (19.5)	0.002	
Baseline BMI	6.7 (1.3)	<0.001	
Alcohol intake (g)	1.0 (0.5)	0.032	

Model 1 covariate, baseline uric acid; Model 2 covariates, baseline uric acid, sex by group interaction term; Model 3 covariates, baseline uric acid, sex by group interaction term, baseline BMI; Model 4 covariates, baseline uric acid, sex by group interaction term, baseline BMI, change in alcohol intake during intervention period.

significant, but is also large enough to be of clinical importance in the etiology of gout. While this was not a mechanistic study, potential reasons for the difference in uric acid between male groups should be explored. Firstly, it is not surprising that no difference in plasma uric acid was seen between female intervention groups, as high plasma uric acid levels and gout are characteristically more prevalent among men (28). During the intervention period, fiber intake was significantly higher among men and women consuming fruit (by 9–12 g/d), while total energy intake appeared higher (∼1,000 kJ/d) among males consuming soft drinks. Thus, it is possible that fruit, due to its high fiber content, was more satiating and therefore conferred a reduced overall energy intake among men, leading to a lower plasma uric acid level. If continued longer, this difference in energy intake between male groups may have resulted in a significant difference in weight gain. The intrinsic fiber content of fruit may also have slowed the digestion rate of fructose, producing portal fructose concentrations that did not exceed the capacity of the small intestine and liver to metabolize fructose via routes other than those resulting in uric acid production (29–31). In addition, the fruit was consumed on average over 4 occasions per day (1.5 items/occasion) compared with 1.5 occasions for soft drink, further reducing the bolus dose of fructose consumed. When fructose is consumed in conjunction with glucose, as is the case in sugar-sweetened soft drinks, its absorption is enhanced (31) and it is unable to be metabolized down the glycogenic pathway, which is occupied by glucose (32).

A further possibility is that the higher vitamin C content of fruit reduced the effect of fructose on plasma uric acid levels. In a meta-analysis of 13 vitamin C supplementation studies reporting serum uric acid levels, a statistically significant mean reduction in serum uric acid of 21 μmol/L was observed with a median supplementary intake of 500 mg/d vitamin C (33). In this study, however, fruits with low vitamin C contents were chosen, and the difference in vitamin C intakes, although not significantly different, appeared higher in men receiving the soft drink treatment.

A strength of this study is that the amount of additional total sugars (∼100 g/d) and fructose (∼50 g/d) participants were required to consume was within the range consumed by populations, a factor often neglected in studies of this kind (34, 35). The average American consumes ∼75 g/d of fructose (36) while the median usual daily intake of total sugars in NewZealand is ∼120 g for males and 96 g for females (37). The fact that we did not observe the weight gain or other cardiometabolic effects observed in other studies (2, 3) suggests that public health approaches to reducing population obesity focusing only on reducing sugary drink intake may not be particularly effective and should focus on improving the quality of population diets as a whole.

Several factors limit the interpretation of the current study. While it is important to note that a meaningful difference in plasma uric acid in men was observed *without* evidence of a difference in weight gain, this study was underpowered to detect such a difference. In addition, it is possible that the intervention was not of sufficient duration to see an effect of soft drink consumption on other cardiometabolic risk factors also thought to be elevated by high fructose intakes and associated with hyperuricaemia (7, 31). A further, appropriately powered, longer-term study in overweight men is therefore warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

This study was approved by University of Otago Human Ethics Committee (Ref: 12/197). The trial was registered with the Australian New Zealand Clinical Trials Registry: ACTRN12612000874819; http://www.anzctr.org.au. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LT designed the research and had primary responsibility for the final content. SM conducted the research. LT, SM, and FO performed the statistical analysis and wrote the manuscript. All authors have read and approved the final manuscript.

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REFERENCES

1. Choo VL, Viguiliouk E, Mejia SB, Cozma AI, Khan TA, Ha V, et al. Food sources of fructose-containing sugars and glycaemic control: systematic review and meta-analysis of controlled intervention studies. *BMJ.* (2018) 363:k4644. doi: 10.1136/bmj.k4644
2. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ.* (2013) 346:e7492. doi: 10.1136/bmj.e7492
3. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am J Clin Nutr.* (2014) 100:65–79. doi: 10.3945/ajcn.113.081521
4. World Health Organization. *Guideline: Sugars Intake for Adults and Children* (2015).
5. Choi HK, Curhan G. Soft drinks, fructose consumption, and the risk of gout in men: prospective cohort study. *BMJ.* (2008) 336:309–12. doi: 10.1136/bmj.39449.819271.BE
6. Choi JWJ, Ford ES, Gao X, Choi HK. Sugar-sweetened soft drinks, diet soft drinks, and serum uric acid level: the Third National Health and Nutrition Examination Survey. *Arthritis Rheum.* (2008) 59:109–16. doi: 10.1002/art.23245
7. Johnson RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, et al. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. *Am J Clin Nutr.* (2007) 86:899–906. doi: 10.1093/ajcn/86.4.899
8. Gao X, Qi L, Qiao N, Choi HK, Curhan G, Tucker KL, et al. Intake of added sugar and sugar-sweetened drink and serum uric acid concentration in US men and women. *Hypertension.* (2007) 50:306–12. doi: 10.1161/HYPERTENSIONAHA.107.091041
9. Lin WT, Huang HL, Huang MC, Chan TF, Ciou SY, Lee CY, et al. Effects on uric acid, body mass index and blood pressure in adolescents of consuming beverages sweetened with high-fructose corn syrup. *Int J Obes.* (2013) 37:532–9. doi: 10.1038/ijo.2012.121
10. Tsilas CS, de Souza RJ, Mejia SB, Mirrahimi A, Cozma AI, Jayalath VH, et al. Relation of total sugars, fructose and sucrose with incident type 2 diabetes: a systematic review and meta-analysis of prospective cohort studies. *CMAJ.* (2017) 189:E711–E20. doi: 10.1503/cmaj.160706
11. Popkin BM, Hawkes C. Sweetening of the global diet, particularly beverages: patterns, trends, and policy responses. *Lancet Diabetes Endocrinol.* (2016) 4:174–86. doi: 10.1016/S2213-8587(15)00419-2
12. Mann J, Cummings J, Englyst H, Key T, Liu S, Riccardi G, et al. FAO/WHO scientific update on carbohydrates in human nutrition: conclusions. *Eur J Clin Nutr.* (2007) 61:S132–S7. doi: 10.1038/sj.ejcn.1602943
13. White JS. Straight talk about high-fructose corn syrup: what it is and what it ain't. *Am J Clin Nutr.* (2008) 88:1716S–21S. doi: 10.3945/ajcn.2008.25825B
14. Khan TA, Sievenpiper JL. Controversies about sugars: results from systematic reviews and meta-analyses on obesity, cardiometabolic disease and diabetes. *Eur J Nutr.* (2016) 55:25–43. doi: 10.1007/s00394-016-1345-3
15. Nakagawa T, Lanaspa MA, Johnson RJ. The effects of fruit consumption in patients with hyperuricaemia or gout. *Rheumatology (Oxford).* (2019) 58:1133–41. doi: 10.1093/rheumatology/kez128
16. Lotito SB, Frei B. The increase in human plasma antioxidant capacity after apple consumption is due to the metabolic effect of fructose on urate, not apple-derived antioxidant flavonoids. *Free Radic Biol Med.* (2004) 37:251–58. doi: 10.1016/j.freeradbiomed.2004.04.019
17. Godycki-Cwirko M, Krol M, Krol B, Zwolinska A, Kolodziejczyk K, Kasielski M, et al. Uric acid but not apple polyphenols is responsible for the rise of plasma antioxidant activity after apple juice consumption in healthy subjects. *J Am Coll Nutr.* (2010) 29:397–406. doi: 10.1080/07315724.2010.10719857
18. Jacob RA, Spinozzi GM, Simon VA, Kelley DS, Prior RL, Hess-Pierce B, et al. Consumption of cherries lowers plasma urate in healthy women. *T J Nutr.* (2003) 133:1826–9. doi: 10.1093/jn/133.6.1826
19. Madero M, Arriaga JC, Jalal D, Rivard C, McFann K, Perez-Mendez O, et al. The effect of two energy-restricted diets, a low-fructose diet versus a moderate natural fructose diet, on weight loss and metabolic syndrome parameters: a randomized controlled trial. *Metabolism.* (2011) 60:1551–9. doi: 10.1016/j.metabol.2011.04.001
20. Christensen AS, Viggers L, Hasselström K, Gregersen SJ. Effect of fruit restriction on glycemic control in patients with type 2 diabetes-a randomized trial. *Nutr J.* (2013) 12:29. doi: 10.1186/1475-289 1-12-29
21. Plant and Food Research and Ministry of Health (NZ). *New Zealand Food Composition Database from Ministry of Health (NZ) and the New Zealand Institute for Plant and Food Research.* Available online at: http://www.foodcomposition.co.nz/ (accessed June 15, 2021).
22. Stewart A, Marfell-Jones M, Olds T, Ridder H. *International Standards for Anthropometric Assessment*. Lower Hutt: ISAK.
23. Assmann G, Schriewer H, Schmitz G, Hagele EO. Quantification of high-density-lipoprotein cholesterol by precipitation with phosphotungstic acid/$MgCl_2$. *Clin Chem.* (1983) 29:2026–30. doi: 10.1093/clinchem/29.12.2026
24. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* (1972) 18:499–502. doi: 10.1093/clinchem/18.6.499
25. McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, et al. Diagnosing insulin resistance in the general population. *Diabetes Care.* (2001) 24:460–4. doi: 10.2337/diacare.24.3.460
26. McAuley KA, Smith KJ, Taylor RW, McLay RT, Williams SM, Mann JI. Long-term effects of popular dietary approaches on weight loss and features of insulin resistance. *Int J Obes.* (2006) 30:342–9. doi: 10.1038/sj.ijo.0803075
27. Johnson F, Pratt M, Wardle J, Johnson F, Pratt M, Wardle J. Dietary restraint and self-regulation in eating behavior. *Int J Obes.* 36, 665–674. doi: 10.1038/ijo.2011.156
28. Smith EUR, Diaz-Torne C, Perez-Ruiz F, March LM. Epidemiology of gout: an update. *Best Pract Res Clin Rheumatol.* (2010) 24:811–27. doi: 10.1016/j.berh.2010.10.004
29. Jang C, Hui S, Lu W, Cowan AJ, Morscher RJ, Lee G, et al. The small intestine converts dietary fructose into glucose and organic acids. *Cell Metab.* (2018) 27:351–61. doi: 10.1016/j.cmet.2017.12.016
30. Ludwig DS. Examining the health effects of fructose. *JAMA.* (2013) 310:33–4. doi: 10.1001/jama.2013.6562
31. Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. *Physiol Rev.* (2010) 90:23–46. doi: 10.1152/physrev.00019.2009
32. Hudgins LC, Parker TS, Levine DM, Hellerstein MK. A dual sugar challenge test for lipogenic sensitivity to dietary fructose. *J Clin Endocrinol Metab.* (2011) 96:861–8. doi: 10.1210/jc.2010-2007
33. Juraschek SP, Miller ER 3rd, Gelber AC. Effect of oral vitamin C supplementation on serum uric acid: a meta-analysis of randomized controlled trials. *Arthritis Care Res (Hoboken).* (2011) 63:1295–306. doi: 10.1002/acr.20519
34. Wang DD, Sievenpiper JL, de Souza RJ, Chiavaroli L, Ha V, Cozma AI, et al. The effects of fructose intake on serum uric acid vary among controlled dietary trials. *J Nutr.* (2012) 142:916–23. doi: 10.3945/jn.111.151951
35. White JS. Challenging the fructose hypothesis: new perspectives on fructose consumption and metabolism. *Adv Nutr.* (2013) 4:246–56. doi: 10.3945/an.112.003137
36. Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM. Dietary fructose consumption among US children and adults: the Third National Health and Nutrition Examination Survey. *Medscape J Med.* (2008) 10:160.
37. University of Otago and Ministry of Health. *A Focus on Nutrition: Key findings of the 2008/09 New Zealand Adult Nutrition Survey*. Wellington (2011).

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Associations Between Sugars Intakes and Urinary Sugars Excretion and Carbon Stable Isotope Ratios in Red Blood Cells as Biomarkers of Sugars Intake in a Predominantly Māori Population

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Determining the extent to which added sugars intake contribute to non-communicable disease in various populations is challenging because it is difficult to accurately measure intakes. Biomarkers may provide a reliable and easily measured method of assessing intakes. In a predominantly Māori population we compared various sugars intake estimates derived from a 36 item sugar-specific food frequency questionnaire (FFQ) with biomarkers of sugars intake; urinary sugars excretion in random spot collections (n = 153) and carbon stable isotope ratios (n = 36) in red blood cells (RBCs, $\delta^{13}C_{RBC}$) and in the alanine fraction of the RBCs ($\delta^{13}C_{alanine}$). Estimated 24 h urinary sucrose+fructose excretion was statistically significantly correlated with intakes of total sugars (r = 0.23), sucrose (r = 0.26) and added sugars from sugar-sweetened beverages (SSBs; r = 0.26). $\delta^{13}C_{alanine}$ was correlated with added sugars (r = 0.40). In log linear multiple regression models adjusted with HbA1C and eGFR $\delta^{13}C_{alanine}$ predicted added sugars intakes (r^2 = 0.29) and estimated 24 h urinary sucrose+fructose excretion predicted intakes of total sugars (r^2 = 0.14), sucrose (r^2 = 0.17), added sugars (r^2 = 0.17) and sugars from SSBs (r^2 = 0.14). These biomarkers have potential for improving assessment of sugars intake in New Zealand populations enabling monitoring of the effectiveness of sugar reduction strategies designed to reduce risk of NCDs. However, further validation is required to confirm these preliminary findings.

Keywords: added sugars, free sugars, carbon stable isotope ratio, urinary sugars, urinary excretion, Māori, New Zealand, dietary biomarker

INTRODUCTION

Sugars added to the diet are often referred to as added sugars or free sugars. Added sugars are defined as "all monosaccharides and disaccharides added by manufacturer, cook or consumer to sweeten foods or drinks including, sucrose, glucose, honey, syrups, but excludes fruit juices and fruit concentrates. Free sugars include added sugars plus fruit juices and fruit juice concentrates." (1). There is widespread consensus that intakes of added or free sugars should be limited to <10% of total energy intake (1–3) based on evidence that high intakes contribute to excess weight gain (4) and dental caries (5), and are associated with increased risk of non-communicable diseases including type 2 diabetes (6) and cardiovascular disease (CVD) (7, 8). Māori are disproportionally affected by obesity, diabetes and CVD. While socioeconomic factors are major determinants (9), it is likely that excess consumption of added sugars in sugar-sweetened drinks and processed foods contribute to this disease burden. However, determining the extent to which added sugars contribute to disease in various populations is challenging because it is difficult to accurately measure intakes. Dietary assessment at a population level is still largely dependent on self-report methods such as 24 h diet recalls or food frequency questionnaires which are subject to reporting biases (10). Previous research has shown that self-reported intakes of sugars are particularly prone to misreporting (11). Biomarkers of dietary sugars intake may provide an alternative and more reliable method of assessing intakes. This will improve our understanding of the contribution of added sugars intakes to non-communicable diseases in different population groups and our ability to monitor the effectiveness of strategies to reduce added sugars intakes.

Two promising biomarkers of sugars intake have been identified, and validation studies of these biomarkers have developed regression equations to enable these measures to be converted into estimates of sugars intakes (12, 13). The first predictive biomarker assessed sugars excreted in 24-h urine samples. Results from controlled-feeding studies showed that 24-h urinary excretion of fructose and sucrose provided a valid method of measuring intake of total sugars in controlled-feeding studies (13–15), findings supported in cross-sectional studies (16). Applied in the Norfolk cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC), sugars concentrations in spot urine collections were associated with the development of obesity (17, 18). Twenty-four hour urinary fructose excretion has also been used to a limited extent in studies of children, showing moderate correlations between the biomarker and dietary sugars intake assessed by 3 day weighed food records (19).

The second biomarker of sugars intake are carbon stable isotope ratios ($^{13}C/^{12}C$ ratio expressed as $\delta^{13}C$) measured in various tissues. The $\delta^{13}C$ composition of foods reflects the isotope composition of the plant and animals it originates from (20) and the $\delta^{13}C$ composition of corn- and cane-derived sugars is distinctive from other foods and sweeteners. Since humans are unable to change the $\delta^{13}C$ composition of their tissues, the $\delta^{13}C$ in various tissues may reflect the level of consumption of sweetened foods and drinks (21) In studies conducted in the USA $\delta^{13}C$ values were shown to be moderately correlated with intakes of sweeteners derived from corn and cane in cross-sectional analyses in tissues including whole blood (22–24), serum (25–27), plasma (25, 28), red blood cells (RBCs) (12, 29–31) and hair (24, 29, 32). Precision may be further improved by two different approaches. The first involves measuring the $\delta^{13}C$ in the alanine component of the target tissue (29). Such measurements are more complex and time consuming but have increased specificity for sugars intake because alanine is directly involved in sugars metabolism via the Cahill cycle, a shuttle of carbon between plasma glucose and alanine (33). Up to ∼60% of alanine in blood tissues is estimated to originate from intake of dietary sugars. The second approach takes into account that dietary proteins from meat and fish are also an important source of ^{13}C, and could confound the association between $\delta^{13}C$ and sugars intake (31, 34). Nitrogen stable isotope ratios ($^{15}N/^{14}N$; expressed as $\delta^{15}N$) are increased with intakes of fish and meat but not with consumption of cane and corn-derived sugars. Therefore, a dual isotope model, using $\delta^{13}C$ with inclusion of $\delta^{15}N$, has been proposed to control for these confounding dietary effects (12, 30, 31).

In the USA, sugars added to foods are commonly derived from sugar cane, corn and sugar beet (35) with only cane and corn sugars being isotopically distinct from all other plant-derived sweeteners. In contrast, the main sweetener used in foods and drinks commonly available in New Zealand is derived from sugar cane. Thus, $\delta^{13}C$ is a promising biomarker for assessing sugars in New Zealand populations. To date no research has been published on the comparative performance of the urinary sugars excretion and $\delta^{13}C$ as biomarkers for sugars intake in populations like New Zealand where cane sugars predominate.

The ongoing *Gout and Related Conditions in Tairāwhiti: Genes and Environment Study* is examining the genetic relationship between gout and other metabolic diseases such as type 2 diabetes and heart disease and the role environmental factors play in combination with the predisposing genetic factors. As part of this broader study we compared three biomarkers of sugars intake, urinary sugars excretion in random spot collections, carbon stable isotope ratios in red blood cells, and carbon stable isotope ratios in red blood cell alanine against sugars estimates derived from a culturally-appropriate validated semi-quantitative food frequency questionnaire (FFQ) in a predominantly Māori population.

MATERIALS AND METHODS

Subjects

This cross-sectional study recruited a total of 175 participants aged 16 years and over, with and without gout, who were able to give written consent. Participants were recruited via the patients register of Ngāti Porou Hauora Charitable Trust (NPH), the Māori primary health organization (PHO) and health care provider for all in the Ngāti Porou rohe (tribal territory) on the largely rural East Coast area of the Tairāwhiti/Gisborne region in the North Island of New Zealand. Potential participants were either contacted by the research nurse via telephone or mail or approached in person at NPH health centres at Tawhiti, Ruatoria,

Tokomaru Bay, Matakaoa, Uawa, and Puhi Kaiti (Gisborne). Further participants were recruited at community centres and through community groups, events and by word of mouth. The study protocol, risks and benefits were explained to each subject and written consent was given. The study was approved by the University of Otago Human Ethics Committee (13/117). The study was overseen by the NPH Research Coordinator Dr. Harré Hindmarsh, and the research team was advised by the NPH Gout and Related Conditions Research Advisory Group, chaired by Research Coordinator and consisting of a NPH general practitioner, nurse, manager, two community representatives, Professor Merriman, and the research nurse (study recruiter).

Data collection took place between November 2013 and March 2015. To reduce attrition rates, data were collected at a time and location convenient to the participant, usually during daytime working hours and either at local health clinics or in the participants' homes.

Experimental Protocol

Participants completed a sugars-specific FFQ in the presence of the research nurse who was able to provide clarification of questions if necessary. Two 10 ml urine specimen collection containers were provided to the participants for a spot urine collection at the clinic appointment. One container was for analyses of urinary creatinine and urate. The other for urinary sugars analyses and contained 30 mg boric acid as a preservative. Four blood samples were collected in two serum separator vacutainers, for serum analysis, and two vacutainers containing EDTA (an anticoagulant for blood samples) for analysis of plasma and RBCs. A general questionnaire was administered by the research nurse, registered and trained in rural health care, to elicit information on variables including age; sex; educational attainment; employment status; smoking habits; previous diagnosis of metabolic disorders; medical therapies including uric acid-lowering medication, cholesterol lowering medications, diuretics and other antihypertensive medication; family history of gout and diabetes; alcohol and seafood consumption; and physical activity level. Height (m), weight (kg), and waist circumference (cm) were also measured by the research nurse. Missing data were obtained from patient medical records with participant permission.

Assessment of Dietary Intakes

Participants completed a 34-item, semi-quantitative FFQ to assess sugars intake over the past month, which was developed and validated previously in this Māori community (36). Usual daily intakes of total available sugars; added sugars; added sugars in sugar-sweetened beverages; and sugars from fruits were calculated via a pre-developed spreadsheet estimated using Kaiculator© 2013 analysis software and New Zealand food composition data [2010 NZ FOODfiles; (37)].

The FFQ was validated is a previous study conducted by Masters of Dietetics students. Cross-classification agreement of sugars intake quartiles from FFQ and repeated 24-h recalls in 72 participants showed that 95–97% of participants were classified into the same or adjacent quartiles, with weighted kappa values (K_w) ranging from 0.43 to 0.51, which suggests a moderate agreement between the two dietary assessment techniques (36). Correlation coefficients between the FFQ and repeated 24-h recalls ranged from 0.59 for total fructose intake, to 0.76 for total sugars from SSBs intakes.

Sugars intakes were defined six ways using data collected from the FFQ:

1. Total sugars: the sum of all sugars from all foods and beverages
2. Sucrose: the sum of sucrose from all foods and beverages.
3. Added sugars: the sum of all sugars minus lactose derived from beverages except 100% fruit juice, the sum of glucose, fructose and sucrose from dairy foods and total sugars in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.
4. Added sugars from sugar-sweetened beverages (SSBs): the sum of total of fructose, glucose and sucrose from all beverages including fruit juices and alcoholic beverages.
5. Total sugars from sweetened foods (all food items in which sugars are added as a sweetener)
6. Totals sugars from all fresh raw fruit items.

Laboratory Analyses

Blood collection

For serum analyses (lipids, urate, creatinine) blood samples were centrifuged in the field for 15 min at 3,000 rpm at 4°C. The samples were couriered to Dunedin and analysed by Southern Community Laboratories (SLC), an accredited diagnostic laboratory. Haemoglobin A_{1C} (HbA_{1C}) was obtained from whole blood using ion-exchange HPLC (BioRad D-10™, Haemoglobin A_{1C} Program) at TLab, Gisborne. After analysis, the remaining blood sample was centrifuged and RBCs were washed twice with a saline solution (0.9 g sodium chloride (NaCl) with 100 mL deionised water) before transport to the Department of Human Nutrition at the University of Otago in Dunedin at 0°C and after arrival transferred to −20°C until analysis. Serum creatinine was measured by SCL using the 'Roche Cobas 8000 system. Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula (38).

Urinary Measurements

Urine samples were preserved by adding boric acid (30 mg). Urine samples were returned to the Department of Biochemistry at the University of Otago in Dunedin at room temperature. They were then temporarily stored (<2 days) at 4°C and transferred to long-term storage at −20°C in the Department of Human Nutrition laboratories until analysis. Spot urine collections samples were defrosted at room temperature for analysis. Urinary sucrose, fructose and glucose concentrations were estimated using spectrophotometry with an enzymatic kit (K-SUFRG, Megazyme International Ireland). The UV-method is based on the determination of D-glucose before and after hydrolysis of sucrose by β-fructosidase. D-fructose was determined after isomerization by phosphoglucose isomerase. The smallest differentiating absorbance for the assay is 0.010 absorbance units, corresponding to a concentration of 0.69 mg/L of glucose, fructose or sucrose. The initial protocol was modified to run on a microplate reader in 96-well plates. Each run included

fructose, glucose and sucrose standards (50, 100, 200, 300, and 400 mg/L) and samples were measured in duplicates (R^2 for the standard curve was 0.999 for fructose and 0.999 for sucrose for each of the plates measured). When the coefficient of variance (CV%) was more than 10%, samples were re-analysed. Urinary creatinine concentrations were measured using a Roche Modular P (Hitachi) analyser by SLC.

We estimated 24 h urinary excretion of the sum of sucrose and fructose from spot urine samples by creatinine adjustment with spot urinary creatinine concentration using the following formula:

$$24\,h\ urinary\ sucrose\ +\ fructose\ (mg) = spot\ urinary\ sucrose + fructose\ (mg/L)/spot\ creatinine\ (g/L)\ X\ 24\,h\ Creatinine\ (g)$$

where 24 h creatinine was assumed to be 1.7 and 1.0 g for males and females, respectively.

Stable Isotope Measurements of Bulk RBCs

Stable isotope ratios were determined in a convenience sub-sample (the final 36 participants recruited for the study, and from whom we had RBCs) to pilot test the suitability of the method for assessing sugars intakes in New Zealand population. Bulk carbon ($\delta^{13}C_{RBC}$) and nitrogen ($\delta^{15}N_{RBC}$ – measured to adjust for potential confounding by meat and fish intakes) isotopic compositions were determined on ∼0.8 mg of freeze-dried RBCs, weighed into tin capsules. $\delta^{13}C_{RBC}$ and $\delta^{15}N_{RBC}$ were determined by combustion in a NA 1500 Elemental Analyser (CE Instruments, Milan), and measurement of the resulting CO_2 or N_2 gases (respectively) by a Thermo Finnigan Delta Advantage Isotopic Ratio Mass Spectrometer (EA-IRMS) at the Isotrace lab facility (Dunedin, New Zealand). The conventional method of expressing $\delta^{13}C$ or $\delta^{15}N$ at natural abundance is in per mil (‰) abundance of ^{13}C or ^{15}N relative to an international standard (Vienna PeeDee Belemnite, VPDB or atmospheric N_2, respectively), as follows:

$$\delta^{13}C = ((^{13}C/^{12}C_{sample} - ^{13}C/^{12}C_{standard})/^{13}C/^{12}C_{standard}) \times 1000‰$$
$$\delta^{15}N = ((^{15}N/^{15}N_{sample} - ^{15}N/^{15}N_{standard})/^{13}N/^{12}N_{standard}) \times 1000‰ \quad (1)$$

The instrument precision was 0.2‰ for C and 0.2‰ for N, based on multiple measurements of laboratory control material (EDTA). Data were calibrated to the international scales using triplicate measurements of two reference materials (USGS41 and 41) run with each batch of samples.

Stable Isotope Measurements of Alanine in RBCs

Stable carbon isotope ratios of alanine in RBCs ($\delta^{13}C_{alanine}$) were determined after extraction and derivatization of alanine using adapted protocols (39, 40). In brief, aliquots of 50 μL RBCs and 50 μL of internal standard were pipetted into a Kimax© tube. Samples were hydrolysed with 1 mL 6 M HCl. The tube was then filled with N_2, sealed (to prevent drying while heated), shaken, and heated at 150°C for 70 min. Samples were cooled down to room temperature, and centrifuged at 3,000 rpm for 7 min. The supernatant was transferred into a clean Kimax© tube and evaporated to dryness at 60°C in a heating block under a gentle stream of N_2. Alanine was then derivatised following the protocol by Styring et al. (41). $\delta^{13}C_{alanine}$ was measured by gas-chromatography combustion isotope-ratio mass-spectrometer (GC-IRMS), using a Thermo Trace gas chromatograph, the GC-IsoLink combustion interface, and a Delta-XP isotope ratio mass spectrometer (Thermo Fisher Scientific). Two hundred nanoliters aliquots of derivitised alanine were injected at 270°C in splitless mode, carried by helium at 1.4 mL min^{-1} and separated on a VF-35 ms column (0.32 mm ID and a 1.0 μm film thickness). The oxidation reactor was set at 950°C and the reduction reactor was left at room temperature. Samples were analysed in duplicate along with amino acid laboratory standards of known isotopic composition (measured on EA-IRMS). Raw deltas or chromatographic peaks are measured against a CO_2 monitoring gas and corrected to PDB with an internal standard of caffeine ($\delta^{13}C = -26.98‰$) (42). Derivatised $\delta^{13}C_{alanine}$ was corrected relative to the $\delta^{13}C_{alanine}$ of the laboratory standard to account for the exogenous C and kinetic fractionation introduced during derivatisation (42).

Data Analyses and Statistics

All statistical analyses were performed using Stata/IC 14.2 for Mac (StataCorp, College Station, TX, USA). Descriptive data are presented as the mean and standard deviation (SD) unless specified otherwise. Sugars variables were log transformed to account for skewness in correlation and regression analyses. $\delta^{13}C_{RBC}$ and $\delta^{13}C_{alanine}$ variables were inversed (since the values are expressed as negatives) and then log transformed.

Partial correlation coefficients were calculated for the associations between the six definitions of sugars intakes (described above) as a continuous variable and the additive inverse of $\delta^{13}C_{RBC}$ and additive inverse of $\delta^{13}C_{alanine}$. Because the FFQ was designed to rank sugars intakes by quartiles rather than to provide validated estimates of actual intake 2 participants reported very high sugars intake values (e.g., >9 kg/d) therefore intakes were censored at 500 g/d. $\delta^{15}N_{RBC}$ was included as the control variable to account for the effect of meat and fish intake on $\delta^{13}C$ values.Partial correlation coefficients were also calculated for the associations between 24 h urinary sucrose + fructose and the additive inverses of $\delta^{13}C_{RBC}$ and $\delta^{13}C_{alanine}$ with $\delta^{15}N_{RBC}$ included as the control variable. Finally correlation coefficients were calculated for the associations between 24 h urinary sucrose+fructose and sugars intakes.

Log linear multiple regression models were used to determine whether the three sugars intake biomarkers could predict the various measures of dietary sugars intakes. Single variable regression analyses were conducted to test for an association between sugars intake measures and each biomarker. Stepwise regression was used to identify whether covariates for $\delta^{15}N_{RBC,}$ age, sex, BMI, HbA1c and EGFR should be included in multivariate log regression models, up to a maximum of two covariates for the carbon stable isotope models and four covariates for the 24 h urinary sucrose + fructose model with $p < 0.1$. EGFR and HbA1C were selected to account for potentially abnormal urinary sugars excretion in people with impaired

TABLE 1 | Characteristics of study participants of the complete sample and the subset of participants with red blood cell samples.

	Complete sample, $n = 175$	RBC subset, $n = 36$
	Mean (SD) or percent	Mean (SD) or percent
Sex, *n*; % male	175 (67)	36 (72)
Age, y	60.4 (14.9)	60.7 (16.7)
Ethnicity (% Māori)	87%	89%
BMI, kg/m^2	35.2 (9.2)	35.1 (8.5)
Waist circumference (cm)	112 (20)	112 (14)
Systolic blood pressure, mm Hg	135 (20)	138 (21)
Diastolic blood pressure, mm Hg	80 (14)	83 (12)
HbA_{1C}, mmol/mol	45.9 (15.7)	45.7 (16.4)
Serum uric acid, mmol/L	0.43 (0.11)	0.43 (0.13)
Total cholesterol, mmol/L	5.00 (1.11)	4.80 (1.12)
Urinary creatinine, mmol/L	10.8 (5.9)	11.4 (8.0)
eGFR, mL/min/1.73 m^2	69.5 (19.2)	65.6 (22.2)
Estimated 24 h urinary sucrose+fructose excretion (mg)[1]	4.7 (3.6, 4.1)[2]	6.5 (3.3, 12.7)[3]
$\delta^{13}C_{alanine}$, ‰[4]	–	−19.68 (2.08)
$\delta^{13}C_{RBC}$, ‰[5]	–	−22.64 (0.71)
$\delta^{15}N_{RBC}$, ‰[6]	–	7.92 (0.47)

[1] *Urinary excretion of sucrose and fructose measured in spot urine samples and adjusted to estimate 24 h excretion with spot urinary creatinine, reported as geometric mean (95% CI).*
[2] *n = 144.*
[3] *Only n = 28 participants provided both urine and blood samples.*
[4] *The $^{13}C/^{12}C$ ratio in red blood cell alanine.*
[5] *The $^{13}C/^{12}C$ ratio in red blood cells.*
[6] *The $^{15N}/^{14N}$ ratio in red blood cells.*

TABLE 2 | Sugars intakes reported by six definitions estimated from a 36 item food frequency questionnaire (g/day).

	Median	Min	Max	Interquartile range
Total sugars[1]	99.6	9.5	500	(68.6, 184.4)
Sucrose[2]	55.9	1.0	500	(25.3, 101.8)
Added sugars[3]	54.2	4.3	500	(30.7, 85.9)
Added sugars from SSB[4]	20.3	0.0	500	(6.1, 63.3)
Total sugars from sweetened foods[5]	29.1	0.0	272.3	(12.3, 50.6)
Total sugars in raw fruit[6]	18.0	0.0	252.4	(5.2, 54.1)

N = 175.
[1] *Total sugars content of all food items.*
[2] *Total sucrose content of all food items.*
[3] *Total sugars derived from beverages except 100% fruit juice minus lactose, plus the sum of glucose, fructose and sucrose content of dairy foods, and total sugars content in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.*
[4] *SSBs, sugar sweetened beverages; includes the total fructose, glucose and sucrose content in all beverages including fruit juices and alcoholic beverages.*
[5] *Total sugars content in all sweetened food items.*
[6] *Total sugars content in all fresh raw fruit items.*

glycaemic control. We also tested the associations between the three biomarkers with regression analyses.

RESULTS

The study included 175 participants of whom 122 had a previous diagnosis of gout. **Table 1** describes the participant characteristics. The mean (SD) age of the group was 60 (15) years and mean BMI was 35.2 (9.7) kg/m^2 (**Table 1**). Seventy-three percent were obese (BMI >30 kg/m^2). Eighteen percent of participants had hypertension stage 2 (systolic blood pressure ≥145 or diastolic blood pressure ≥90 mm Hg), 79% had high total cholesterol (>4 mmol/L), 32% had impaired glucose tolerance [HbA_{1C} between 40 and 50 mmol/mol; (43)] and 18% had HbA_{1C} above 50 mmol/mol, consistent with a diagnosis of diabetes (43), indicating a high level of comorbidities in the population. There were no statistically significant differences in variables between the complete sample population and the RBC subset ($n = 36$).

Sugars intakes estimated by the food frequency questionnaire for six definitions for sugars are provided in **Table 2**. Extreme values reported by 2 individuals were truncated to a maximum value of 500g/day. Spot urine samples were available for 150 of the 175 participants recruited for the study and urinary creatinine was available for 144 of these. Excretion of estimated 24 h urinary sucrose + fructose was statistically significantly correlated with self-reported intakes of sucrose, added sugars, total sugars from SSBs and total sugars ($p = 0.051$) (**Figure 1**).

We conducted isotope analyses on RBCs from a subsample of 36 participants. $\delta^{13}C_{RBC}$ correlated with $\delta^{15}N_{RBC}$ ($r = 0.348$, $p = 0.038$) but not with $\delta^{13}C_{alanine}$ ($r = 0.012$, $p = 0.948$). $\delta^{13}C_{alanine}$ correlated with intakes of total added sugars after controlling for $\delta^{15}N_{RBC}$ but not for any other definitions of sugar intakes (**Figure 2**). $\delta^{13}C_{RBC}$ was not correlated with any sugars intake variables. Estimated 24 h urinary sugars excretion correlated with $\delta^{13}C_{RBC}$ ($r = 0.41$; $p = 0.0385$) but did not correlate with $\delta^{13}C_{alanine}$ ($r = 0.07$; $p = 0.7275$) after controlling for $\delta^{15}N_{RBC}$. Correlation coefficients between sugars intake variables and biomarkers are presented in **Table 3**.

Unadjusted, single variable, log regression analyses showed a significant association between estimated 24 h urinary sucrose + fructose excretion and total sugars ($p = 0.002$), sucrose ($p = 0.001$), added sugars ($p = 0.007$) and sugars in SSBs ($p = 0.003$). There was also a statistically significant association between $\delta^{13}C_{alanine}$ and added sugars ($P = 0.025$) and sucrose at the 90% confidence level ($P = 0.08$). There were no statistically significant associations between $\delta^{13}C_{RBC}$ or $\delta^{15}N_{RBC}$ and any sugars intake variables. There was a significant association between $\delta^{13}C_{RBC}$ and 24 h urinary sucrose+fructose excretion ($p = 0.049$).

In multivariate log linear regression analyses including HbA1C and eGFR as covariates, estimated 24 h urinary sucrose + fructose excretion was a significant predictor of intakes of total sugars, sucrose, added sugars and sugars in SSBs (**Table 4**). The best predictive model was between 24 h urinary sucrose+fructose excretion and sucrose ($p < 0.001$) with the model explaining 17.4% of the variation in sucrose intake. For each 1% increase

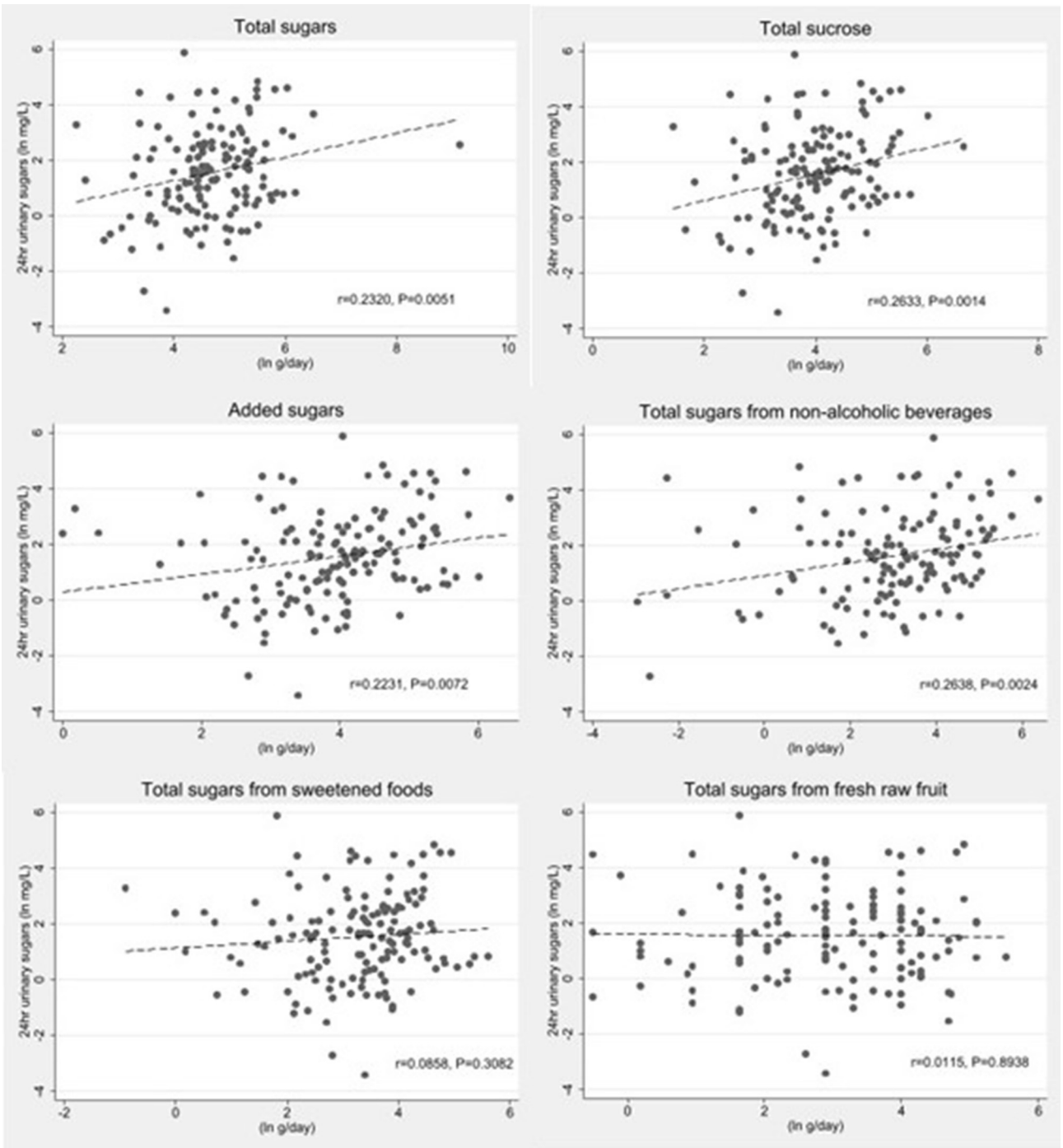

FIGURE 1 | Scatter plots of log transformed estimated 24 h urinary sucrose+fructose excretion by log transformed sugars intakes estimated by a sugar specific FFQ ($n = 140$). The dashes lines represent the linear fit models.

in 24 h urinary sucrose+fructose there was a 0.17% increase in sucrose intake ($P < 0.0001$).

In multivariate log linear regression analyses including HbA1C and eGFR as covariates, $\delta^{13}C_{alanine}$ was a significant predictor of added sugars intakes (**Table 5**). For each 1% increase in $\delta^{13}C_{alanine}$ an increase in added sugars intake of 4.9% is predicted. The adjusted model explained 28.5% of the variation in added sugars intakes estimated by the FFQ also predicted total sugars intake from sweetened foods at the 90% confidence level ($p = 0.072$) in the HBA1c and eGFR adjusted model which was statistically significant ($p = 0.0065$) explaining 30% of the variation in sugars intake. $\delta^{13}C_{RBC}$ did

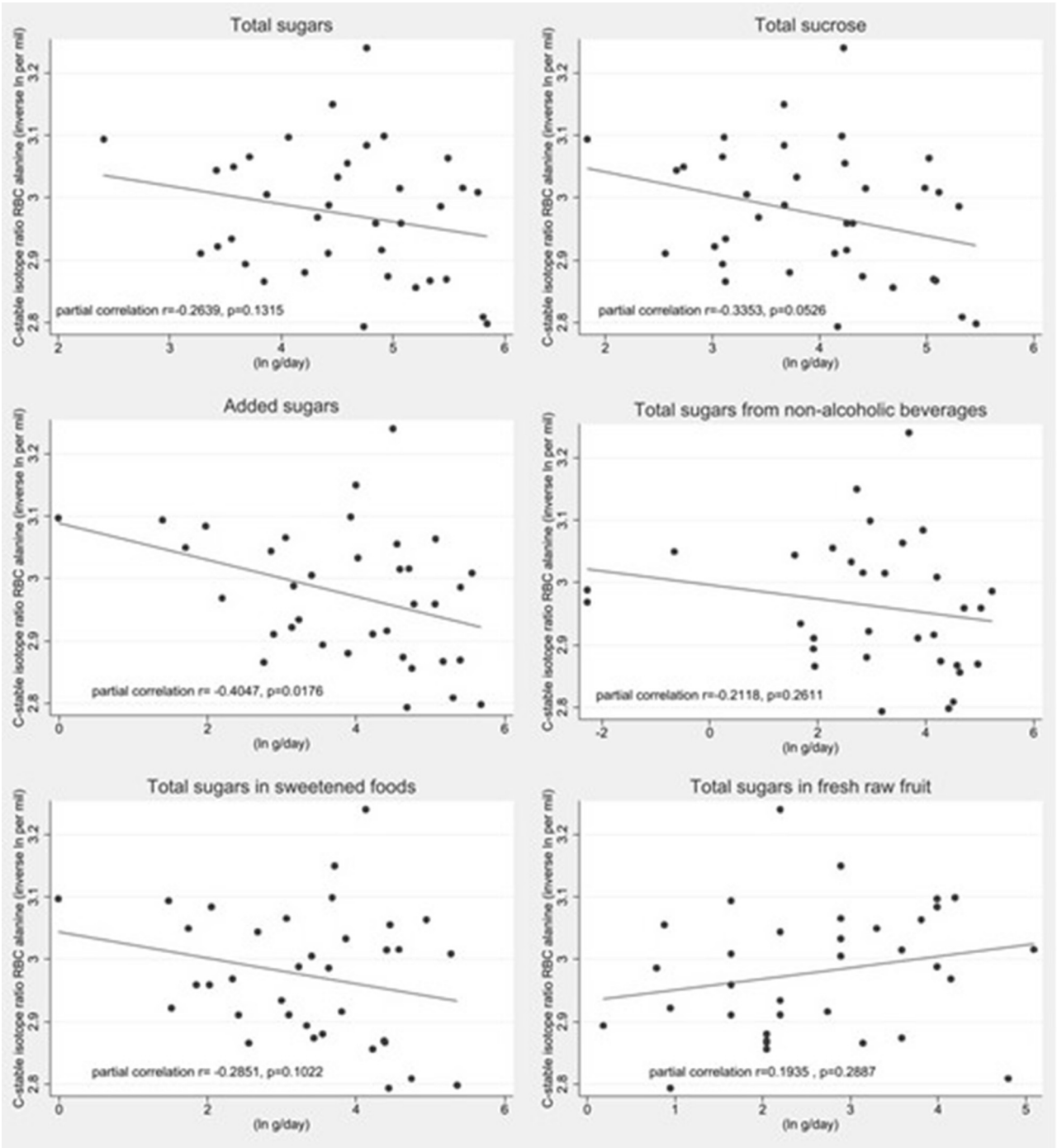

FIGURE 2 | Scatter plots of the log transformed additive inverse $\delta^{13}C_{alanine}$ values by log transformed sugars intake estimates estimated by a sugar specific FFQ (n = 36). The dashed represents the linear fit model. Partial correlation values after controlling for $\delta^{15}N_{RBC}$ are presented within each plot.

not predict sugars intakes in multivariate log regressions models (**Table 6**).

The predictive equation for added sugars intake based on $\delta^{13}C_{alanine}$ is defined as follows:

$$\text{Added sugars (g/d)} = \exp(-4.8042 \times \ln[\delta^{13}C_{alanine} \times -1] + [0.0270777 \times \text{eGFR}] - [0.0349989^{*}\text{HbA1C}]\ 17.99757)$$

Where $\delta^{13}C_{alanineis}$ measured in mg, HbA1C in mmol/mol and eGFR in mL/min/1.73 m^2

DISCUSSION

This is the first study to assess the association between self-reported sugars intake and two different biomarkers

TABLE 3 | Correlation coefficients between dietary sugars intakes estimated by food frequency questionnaire (g/d) and biomarkers for sugars intake from spot urinesamples and red blood cells.

	24 h urinary sucrose + fructose, mg[1]		$\delta^{13}C_{alanine}$, ‰[2]		$\delta^{13}C_{RBC}$, ‰[3]		$\delta^{15}N_{RBC}$, ‰[4]	
	r	*p*	*Partial r*	*p*	*Partial r*	*p*	*r*	*p*
Total sugars[5]	0.232	0.005	0.264	0.132	−0.087	0.620	−0.058	0.739
Sucrose[6]	0.263	0.001	0.335	0.053	−0.090	0.606	0.028	0.876
Added sugars[7]	0.223	0.007	0.405	0.018	−0.108	0.538	0.082	0.635
Added sugars from SSB[8]	0.264	0.002	0.212	0.261	−0.010	0.959	0.152	0.408
Total sugars from sweetened foods[9]	0.086	0.308	0.285	0.102	−0.303	0.077	−0.076	0.661
Total sugars in raw fruit[10]	−0.012	0.894	−0.194	0.289	0.009	0.963	−0.119	0.504

[1]*Urinary excretion of the total of sucrose and fructose measured in spot urine samples and adjusted to estimate 24 h excretion with spot urinary creatinine; n = 144.*
[2]*Partial correlation coefficient for the $^{13}C/^{12}C$ ratio in red blood cell alanine controlling for $\delta^{15}N$, n = 36.*
[3]*Partial correlation coefficient for the $^{13}C/^{12}C$ ratio in red blood cells controlling for $\delta^{15}N$, n = 36.*
[4]*Correlation coefficient for the $^{15}N/^{14}N$ ratio in red blood cells, n = 36.*
[5]*Total sugars content of all food items.*
[6]*Total sucrose content of all food items.*
[7]*Total sugars derived from beverages except 100% fruit juice minus lactose, plus the sum of glucose, fructose and sucrose content of dairy foods, and total sugars content in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.*
[8]*SSBs, sugar sweetened beverages; includes the total fructose, glucose and sucrose content in all beverages including fruit juices and alcoholic beverages.*
[9]*Total sugars content in all sweetened food items.*
[10]*Total sugars content in all fresh raw fruit items.*

TABLE 4 | Associations between dietary sugars intakes (g/d) and estimated 24 h urinary sucrose+fructose excretion from a multivariate log linear regression model[1].

	24 h urinary sugar predictor value[2]	*P* for 24 h urinary sugar	Obs	F(3, 26)	*P* for model	R^2	Adjusted R^2
Total sugars[3]	0.14 (0.06, 0.22)	0.001	133	8.15	0.0001	0.1593	0.1398
Sucrose[4]	0.17 (0.08, 0.26)	<0.001	133	10.23	<0.0001	0.1922	0.1735
Added sugars[5]	0.18 (0.07, 0.29)	0.002	133	10.22	0.0001	0.1920	0.1732
Added sugars from SSB[6]	0.31 (0.11, 0.51)	0.003	133	7.31	0.0002	0.1589	0.1372
Total sugars from sweetened foods[7]	Not estimable						
Total sugars in raw fruit[8]	Not estimable						

[1]*Models include covariates for HbA1C and estimated glomerular filtration rate (eGFR).*
[2]*Percentage change (95% CI) in independent sugars variable due to a 1% increase in $\delta\ ^{13}C_{alanine}$ ($^{13}C/^{12}C$ ratio in red blood cell alanine).*
[3]*Total sugars content of all food items.*
[4]*Total sucrose content of all food items.*
[5]*Total sugars derived from beverages except 100% fruit juice minus lactose, plus the sum of glucose, fructose and sucrose content of dairy foods, and total sugars content in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.*
[6]*SSBs, sugar sweetened beverages; includes the total fructose, glucose and sucrose content in all beverages including fruit juices and alcoholic beverages.*
[7]*Total sugars content in all sweetened food items.*
[8]*Total sugars content in all fresh raw fruit items.*

of sugars intake in a population of New Zealand Māori adults; urinary sucrose and fructose excretion, $\delta^{13}C_{alanine}$ and $\delta^{13}C_{RBC}$. We found that urinary sugars in spot urine samples were very weakly correlated with self-reported intakes of total sugars, sucrose, total added sugars and added sugars in SSBs. In the subset of the population in which we were able to conduct carbon stable isotope analyses we found that $\delta^{13}C_{alanine}$ in RBCs was weakly correlated with self-reported intake of added sugars after partial adjustment for $\delta^{15}N$ to account for potential confounding by meat and fish intake. In log linear multiple regression models adjusted with HbA1C and eGFR $\delta^{13}C_{alanine}$ some predicted added sugars intakes and estimated 24 h urinary sucrose+fructose excretion predicted sucrose and added sugars intakes.

Several studies have shown 24 h urinary sucrose and fructose to be valid predictive biomarkers of sugars intake. In a 30 day controlled-feeding crossover study involving with 12 participants living in a metabolic unit and consuming three different diets varying in sugars content for 10 days each Tasevska et al. (13) showed a strong correlation ($r = 0.89$) between total sugars intake and urinary sucrose and fructose excretion measured from twelve 24 h urine collections per participant. In a study involving twelve subjects living in a metabolic unit and consuming their habitual diets for 30 days Tasevska et al. (15) found that mean daily urinary sucrose and fructose excretion was most strongly associated with intakes of extrinsic sugars ($r = 0.84$) compared to intrinsic sugars ($r = 0.43$). In contrast a recent cohort study of 477 participants in the U.S. self-reported intakes of total sugars were not associated with biomarker-predicted intakes based on single 24-h urine

TABLE 5 | Associations between dietary sugars intakes (g/d) and $\delta\ ^{13}C_{alanine}$ from a multivariate log linear regression model[1].

	$\delta^{13}C_{alanine}$ predictor value[2]	*P* for $\delta^{13}C_{alanine}$	Obs	F(3, 26)	*P* for model	R^2	Adjusted R^2
Total sugars[3]	2.0 (−1.0, 5.0)	0.183	30	2.6	0.0733	0.231	0.1423
Sucrose[4]	2.7 (−0.4, 5.9)	0.09	30	3.19	0.0402	0.2691	0.1847
Added sugars[5]	4.8 (0.7, 8.9)	0.024	30	4.85	0.0082	0.3589	0.285
Added sugars from SSB[6]	4.1 (−3.7, 12.6)	0.296	30	0.99	0.4132	0.1148	−0.0007
Total sugars from sweetened foods[7]	3.6 (−0.3, 7.6)	0.072	30	5.12	0.0065	0.3713	0.2987
Total sugars in raw fruit[8]	−2.3 (−7.3, 2.8)	0.352	30	0.32	0.809	0.0373	−0.0783

[1]*Models include covariates for HbA1C and estimated glomerular filtration rate (eGFR).*
[2]*Percentage change (95% CI) in independent sugars variable due to a 1% increase in* $\delta^{13}C_{alanine}$ *(*$^{13}C/^{12}C$ *ratio in red blood cell alanine).*
[3]*Total sugars content of all food items.*
[4]*Total sucrose content of all food items.*
[5]*Total sugars derived from beverages except 100% fruit juice minus lactose, plus the sum of glucose, fructose and sucrose content of dairy foods, and total sugars content in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.*
[6]*SSBs, sugar sweetened beverages; includes the total fructose, glucose and sucrose content in all beverages including fruit juices and alcoholic beverages.*
[7]*Total sugars content in all sweetened food items.*
[8]*Total sugars content in all fresh raw fruit items.*

TABLE 6 | Associations between dietary sugars intakes (g/d) and $\delta\ ^{13}C_{RBC}$ from a multivariate log linear regression model[1].

	$\delta\ ^{13}C_{RBC}$ predictor value[2]	*P* for $\delta^{13}C_{RBC}$	Obs	F(3, 25)	*P* for model	R^2	adjusted R^2
Total sugars[3]	−6.5 (−16.9, 5.1)	0.323	29	1.91	0.1541	0.231	0.1423
Sucrose[4]	−5.8 (17.2, 7.2)	0.438	29	1.81	0.1708	0.1786	0.1786
Added sugars[5]	−7.2 (−22.5, 11.1)	0.467	29	2.29	0.1027	0.2157	0.1216
Added sugars from SSB[6]	Not estimable						
Total sugars from sweetened foods[7]	−8.9 (−22.3, 6.7)	0.288	29	3.47	0.0311	0.2939	0.2092
Total sugars in raw fruit[8]	0.5 (−17.4, 22.3)	0.874	29	0.02	0.9966	0.0023	−0.1225

[1]*Models include covariates for HbA1C and estimated glomerular filtration rate (eGFR).*
[2]*Percentage change (95% CI) in independent sugars variable due to a 1% increase in* $\delta\ ^{13}C_{RBC}$ *(*$^{13}C/^{12}C$ *ratio in red blood cells).*
[3]*Total sugars content of all food items.*
[4]*Total sucrose content of all food items.*
[5]*Total sugars derived from beverages except 100% fruit juice minus lactose, plus the sum of glucose, fructose and sucrose content of dairy foods, and total sugars content in breakfast cereals, iceblocks, cakes, biscuits, confectionary and chocolate.*
[6]*SSBs, sugar sweetened beverages; includes the total fructose, glucose and sucrose content in all beverages including fruit juices and alcoholic beverages.*
[7]*Total sugars content in all sweetened food items.*
[8]*Total sugars content in all fresh raw fruit items.*

collections ($r = -0.06$) (44). The biomarker predicted intakes were calculated using the formulas developed by Tasevska and colleagues based on feeding studies in a U.K population (13), and were not adapted to a U.S. population. Different sources of sugar, i.e., beet sugars, corn-derived sugars or cane-derived sugars, in the two countries may explain the inconsistent findings.

In our study urinary sugars excretion was assessed using random spot urine collections rather than 24 h urine to reduce respondent burden and we used a sugar specific limited item FFQ that was designed to rank sugar intakes rather to assess actual intakes. More intensive and accurate dietary assessment and 24 h urine collections were not possible in this population study of older Maori adults due to limited funding and our desire to minimize participant burden. It is therefore not surprising that we found weaker correlations between measures than those demonstrated in studies by Tasevska et al. (14, 16) study where multiple days of complete urine samples were collected and analysed. Nevertheless that fact that our analyses showed that are urinary sucrose + fructose biomarker was most strongly associated with sucrose and added sugars, in which glucose and the disaccharides sucrose and fructose would predominate, strengthens our confidence that the biomarker reflects the level of sugars intakes in our population group. The first study reporting on the spot urinary excretion of sucrose and fructose, showed in nine participants in Italy, that the average urinary sucrose excretion of four timed spot urine collections (collected at 8 a.m., 10 a.m., 3 p.m., and 8 p.m.) was correlated with dietary sucrose intake ($r = 0.70$) (45). In a cross-sectional analysis of data from free living participants in the EPIC-Norfolk study ($n = 475$), sucrose intake assessed by FFQ was positively associated with urinary sucrose and fructose excretion in single spot urine samples of individuals with normal body weight (BMI < 25 kg/m^2) ($p < 0.001$). There were, however, no associations shown between urinary sucrose and fructose excretion and self-reported sucrose intake in obese participants (17). Further prospective analysis of prospective data from EPIC Norfolk participants ($n = 1{,}734$) where sucrose intake was assessed using 7-day diet diaries showed a negative association between sucrose and BMI, whereas sugar intake estimated from baseline spot urine samples was positively associated with BMI. Given that our

systematic review of dietary intervention studies showed that sugars intakes are associated with weight gain (8) these findings suggest suggests that urinary excretion of sugars may be a more reliable objective measure of sugars intake than self-report methods in both obese and lean participants. Further previously reported discrepant findings [such as those reported by Bingham et al. (17)] may be due to misreporting of intakes, particularly by obese participants (18). Supporting this theory previous research by Joosen and colleagues, assessing the effect of BMI on urinary sugars excretion, showed that urinary sugars excretion is not affected by BMI (14).

Participants in our study had a high prevalence of overweight and obesity, gout, and dysglycaemia (HbA1C > 40 mmol/mol). Metabolic comorbidities may alter urinary excretion of sugars, affecting the suitability of this biomarker in this group of people. Both diabetes and hyperuricaemia are associated with chronic kidney disease (46, 47) and it is possible that urinary sugars excretion patterns are different for people with these conditions. Furthermore, the mechanism by which sucrose and fructose occurs in the urine is not well-understood (13) except in the case of glucosuria, which is a direct result of elevated blood glucose. Therefore, further research is needed to assess the validity of urinary sugars excretions as a biomarker of sugars intake in participants with metabolic comorbidities such as obesity, diabetes and gout.

Stable isotope ratios in blood have been proposed previously as a biomarker of sugars intake in the U.S. Most of this research evaluated the use of δ^{13}C in whole blood or serum (12, 20, 22–28, 30). However, Choy et al. (29) evaluated carbon stable isotope ratios in RBC's alanine as a more precise marker of sugars intake compared to $\delta^{13}C_{RBC}$ (29). As high intakes of animal proteins also tend to elevate δ^{13}C, this approach attempts to account for confounding of the association between sugar and δ^{13}C by dietary protein (31, 34, 48). Choy et al. (29) found in a Native Alaskan population ($n = 68$) that RBC's $\delta^{13}C_{alanine}$ was strongly correlated with self-reported SSBs intake ($r = 0.70$), added sugars intake ($r = 0.59$) and total sugars intake ($r = 0.57$) independent of animal protein intake. Our study showed weaker but statistically significant associations between RBC $\delta^{13}C_{alanine}$ and added sugars, sucrose and added sugars in sweetened food in multivariate log linear regression models, but not with total sugars, and sugars from SSBs and raw fruit. While the findings are weak this likely reflects the small sample size and the limitations of our dietary assessment method. Further the lack of association with sugars definitions that included sugars from fruit and dairy sources supports the theory that $\delta^{13}C_{alanine}$ has promise as a marker of sugar-sweetened foods in New Zealand where cane sugar is the predominant sweetener. The lack of association, however, between RBC $\delta^{13}C_{alanine}$ and the urinary sugars biomarker is not unexpected at the two biomarkers measure sugars intakes over different timeframes; urinary sugars measure recent intake whereas RBC $\delta^{13}C_{alanine}$ is hypothesized to represent intakes over the previous months.

There are a number of limitations to our study. The primary limitation of this study was its small sample size. Urinary measures were obtained for 153 participants recruited, however we only obtained RBC samples from 36 participants for measurement of $\delta^{13}C_{alanine}$ and $\delta^{13}C_{RBC}$ as this was an initial exploratory analysis. The collection of a single spot urine sample rather than a 24 h urine sample is a major limitation as it assumes that sugars excretion is consistent throughout the day. Spot urine samples must thus be corrected for urine concentration. This is typically achieved by correction with urinary creatinine or specific gravity assuming consistent daily excretion values across a population. We corrected for urine dilution with urinary creatinine concentrations however the high level of comorbidity and obesity in our older Maori population means this assumption may not be valid. Even with correction for urine concentration spot samples are not particularly reliable and bias will attenuate the true association between urinary sugars and dietary intakes. On the other hand 24 h urine collections place a high burden on study participants, are frequently incomplete, and are challenging to collect in large population studies. Additionally dietary assessment by self-report is also affected by a high degree of reporting bias further attenuating potential associations between self-reported intakes and the biomarkers of interest. We assessed dietary intakes using a sugar-specific FFQ that had been previously validated for use among Māori in NZ as our reference method (36). FFQs are limited to a finite list of foods and are constrained by the ability of participants to accurately report their food intake retrospectively over a long period of time, and are therefore subject to reporting errors (49). Further research including controlled feeding studies, or free-living participants using dietary measures such as 7-day weighed food records is needed to further study these associations. Because our FFQ instrument was designed specifically to estimate sugars intakes we were not able to assess intakes of other sources of ^{13}C-enriched foods such as meat and fish intake that may have confounded the associations between $\delta^{13}C_{alanine}$ and $\delta^{13}C_{RBC}$ with sugars intake. Additionally, the FFQ was not designed to distinguish between cane and corn-derived sugars and other sweeteners such as honey, maple syrup and beet sugars that are not enriched in ^{13}C. However, consumption of these other sugar sources in New Zealand is relatively low (50) and thus is unlikely to substantially affect the association between stable isotope ratios and dietary sugars intake.

CONCLUSIONS

In conclusion, these results show that both urinary sugars excretion and $\delta^{13}C_{alanine}$ in red blood cells, but not $\delta^{13}C_{RBC}$, have potential as objective biomarkers of sugars intake in the New Zealand Māori population, where added sugars in foods and beverages are derived predominantly from sugar cane. However, the weak to moderate associations shown indicate that further research is needed in other populations including groups with and without diabetes and pre-diabetes as these conditions may alter the excretion of sugars in urine and sugars metabolism. Further adequately powered research involving more precise methods of dietary assessment such as a 7-day weighed food record is needed in order to confirm these findings.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by University of Otago Human Ethics Committee (13/177). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LT, TM, and DK conceived and designed the study. DK, AS, and XT performed the laboratory analyses. RH and AS contributed expertise and support for the isotope analyses. LT analyzed the data. JH supported the study with access to the community, and guardianship and monitoring of the data collection. LT, JM, and TM provided academic supervision. LT, TM, and JH obtained funding for the study and LT had overall responsibility for the research. LT, DK, and RM wrote the paper. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. World Health Organization. *Guideline: Sugars Intake for Adults and Children*. Geneva: World Health Organization (2015).
2. Casavale KO, Stoody EE, Rihane C, Olson R. Recommendations of the 2015–2020 dietary guidelines for Americans. *FASEB J.* (2016) 30:lb423. doi: 10.1096/fasebj.30.1_supplement.lb423
3. Evans CEL. Sugars and health: a review of current evidence and future policy. *Proc Nutr Soc.* (2017) 76:400–7. doi: 10.1017/S0029665116002846
4. Te Morenga L, Mallard S, Mann J. Dietary sugars and body weight: systematic review and meta-analyses of randomised controlled trials and cohort studies. *BMJ.* (2013) 345:e7492. doi: 10.1136/bmj.e7492
5. Moynihan P, Kelly S. Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines. *J Dent Res.* (2014) 93:8–18. doi: 10.1177/0022034513508954
6. Malik VS, Popkin BM, Bray GA, Després J-P, Willett WC, Hu FB. Sugar-sweetened beverages and risk of metabolic syndrome and type 2 diabetes. *Diabetes Care.* (2010) 33:2477–83. doi: 10.2337/dc10-1079
7. Yang Q, Zhang Z, Gregg EW, Flanders WD, Merritt R, Hu FB. Added sugar intake and cardiovascular diseases mortality among US adults. *JAMA.* (2014) 174:516–24. doi: 10.1001/jamainternmed.2013.13563
8. Te Morenga LA, Howatson AJ, Jones RM, Mann J. Dietary sugars and cardiometabolic risk: systematic review and meta-analyses of randomized controlled trials of the effects on blood pressure and lipids. *Am J Clin Nutr.* (2014) 100:65–79. doi: 10.3945/ajcn.113.081521
9. Theodore R, McLean R, TeMorenga L. Challenges to addressing obesity for Māori in Aotearoa/New Zealand. *Aust N Z J Public Health.* (2015) 39:509–12. doi: 10.1111/1753-6405.12418
10. Gibson RS. *Principles of Nutritional Assessment*. New York, NY: Oxford University Press (2005).
11. Poppitt S, Swann D, Black A, Prentice A. Assessment of selective under-reporting of food intake by both obese and non-obese women in a metabolic facility. *Int J Obes Relat Metab Disord.* (1998) 22:303–11. doi: 10.1038/sj.ijo.0800584
12. Nash SH, Kristal AR, Bersamin A, Hopkins SE, Boyer BB, O'Brien DM. Carbon and nitrogen stable isotope ratios predict intake of sweeteners in a Yup'ik Study Population. *J Nutr.* (2013) 143:161–5. doi: 10.3945/jn.112.169425
13. Tasevska N, Runswick SA, McTaggart A, Bingham SA. Urinary sucrose and fructose as biomarkers for sugar consumption. *Cancer Epidemiol Biomarkers Prev.* (2005) 14:1287–94. doi: 10.1158/1055-9965.EPI-04-0827
14. Joosen A, Kuhnle G, Runswick S, Bingham S. Urinary sucrose and fructose as biomarkers of sugar consumption: comparison of normal weight and obese volunteers. *Int J Obes.* (2008) 32:1736–40. doi: 10.1038/ijo.2008.145
15. Tasevska N, Runswick S, Welch A, McTaggart A, Bingham S. Urinary sugars biomarker relates better to extrinsic than to intrinsic sugars intake in a metabolic study with volunteers consuming their normal diet. *Eur J Clin Nutr.* (2008) 63:653–9. doi: 10.1038/ejcn.2008.21
16. Song X, Navarro SL, Diep P, Thomas WK, Razmpoosh EC, Schwarz Y, et al. Comparison and validation of 2 analytical methods for measurement of urinary sucrose and fructose excretion. *Nutr Res.* (2013) 33:696–703. doi: 10.1016/j.nutres.2013.05.017
17. Bingham S, Luben R, Welch A, Tasevska N, Wareham N, Khaw KT. Epidemiologic assessment of sugars consumption using biomarkers: comparisons of obese and nonobese individuals in the European prospective investigation of cancer Norfolk. *Cancer Epidemiol Biomarkers Prev.* (2007) 16:1651–4. doi: 10.1158/1055-9965.EPI-06-1050
18. Kuhnle GG, Tasevska N, Lentjes MA, Griffin JL, Sims MA, Richardson L, et al. Association between sucrose intake and risk of overweight and obesity in a prospective sub-cohort of the European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk). *Public Health Nutr.* (2015) 18:2815–24. doi: 10.1017/S1368980015000300
19. Johner SA, Libuda L, Shi L, Retzlaff A, Joslowski G, Remer T. Urinary fructose: a potential biomarker for dietary fructose intake in children. *Eur J Clin Nutr.* (2010) 64:1365–70. doi: 10.1038/ejcn.2010.160
20. Jahren AH, Saudek C, Yeung EH, Kao WL, Kraft RA, Caballero B. An isotopic method for quantifying sweeteners derived from corn and sugar cane. *Am J Clin Nutr.* (2006) 84:1380–4. doi: 10.1093/ajcn/84.6.1380
21. O'Brien DM. Stable isotope ratios as biomarkers of diet for health research. *Annu Rev Nutr.* (2015) 35:565–94. doi: 10.1146/annurev-nutr-071714-034511
22. Davy BM, Jahren AH, Hedrick VE, Comber DL. Association of δ13C in fingerstick blood with added-sugar and sugar-sweetened beverage intake. *J Am Diet Assoc.* (2011) 111:874–8. doi: 10.1016/j.jada.2011.03.019
23. Hedrick VE, Davy BM, Wilburn GA, Jahren AH, Zoellner JM. Evaluation of a novel biomarker of added sugar intake (δ 13 C) compared with self-reported added sugar intake and the Healthy Eating Index-2010 in a community-based, rural US sample. *Public Health Nutr.* (2016) 19:429–36. doi: 10.1017/S136898001500107X
24. Hedrick VE, Zoellner JM, Jahren AH, Woodford NA, Bostic JN, Davy BM. A dual-carbon-and-nitrogen stable isotope ratio model is not superior to a single-carbon stable isotope ratio model for predicting added sugar intake in southwest Virginian Adults. *J Nutr.* (2015) 145:1362–9. doi: 10.3945/jn.115.211011

25. Cook CM, Alvig AL, Liu YQD, Schoeller DA. The natural 13C abundance of plasma glucose is a useful biomarker of recent dietary caloric sweetener intake. *J Nutr.* (2010) 140:333–7. doi: 10.3945/jn.109.114777
26. Fakhouri TH, Jahren AH, Appel LJ, Chen L, Alavi R, Anderson CA. Serum carbon isotope values change in adults in response to changes in sugar-sweetened beverage intake. *J Nutr.* (2014) 144:902–5. doi: 10.3945/jn.113.186213
27. Yeung EH, Saudek CD, Jahren AH, Kao WHL, Islas M, Kraft R, et al. Evaluation of a novel isotope biomarker for dietary consumption of sweets. *Am J Epidemiol.* (2010) 172:1045–52. doi: 10.1093/aje/kwq247
28. Nash SH, Kristal AR, Hopkins SE, Boyer BB, O'Brien DM. Stable isotope models of sugar intake using hair, red blood cells, and plasma, but not fasting plasma glucose, predict sugar intake in a Yup'ik Study Population. *J. Nutr.* (2014) 144:75–80. doi: 10.3945/jn.113.182113
29. Choy K, Nash SH, Kristal AR, Hopkins S, Boyer BB, O'Brien DM. The carbon isotope ratio of alanine in red blood cells is a new candidate biomarker of sugar-sweetened beverage intake. *J Nutr.* (2013) 143:878–84. doi: 10.3945/jn.112.172999
30. Nash S, Kristal A, Bersamin A, Choy K, Hopkins S, Stanhope K, et al. Isotopic estimates of sugar intake are related to chronic disease risk factors but not obesity in an Alaska native (YupÄôik) study population. *Eur J Clin. Nutr.* (2014) 68:91–6. doi: 10.1038/ejcn.2013.230
31. Nash SH, Bersamin A, Kristal AR, Hopkins SE, Church RS, Pasker RL, et al. Stable nitrogen and carbon isotope ratios indicate traditional and market food intake in an indigenous circumpolar population. *J Nutr.* (2012) 142:84–90. doi: 10.3945/jn.111.147595
32. Chi DL, Hopkins S, O'Brien D, Mancl L, Orr E, Lenaker D. Association between added sugar intake and dental caries in Yup'ik children using a novel hair biomarker. *BMC Oral Health.* (2015) 15:121. doi: 10.1186/s12903-015-0101-z
33. Bender DA. Energy nutrition – the metabolism of carbohydrates and fat. In: Bender DA, editors. *Introduction to Nutrition and Metabolism.* Boca Raton, FL: CRC Press (2014).
34. Petzke KJ, Boeing H, Klaus S, Metges CC. Carbon and nitrogen stable isotopic composition of hair protein and amino acids can be used as biomarkers for animal-derived dietary protein intake in humans. *J Nutr.* (2005) 135:1515–20. doi: 10.1093/jn/135.6.1515
35. Pomeranz JL. The bittersweet truth about sugar labeling regulations: they are achievable and overdue. *Am J Public Health.* (2012) 102:e14–20. doi: 10.2105/AJPH.2012.300732
36. Furter E. *The Māori Kai Semi-Quantitative Food Frequency Questionnaire: Relative Validity and Reliability for Assessing Usual Sugar Intakes in New Zealand East Coast Māori.* University of Otago (2014). Available online at: http://hdl.handle.net/10523/4662 (accessed June 14, 2021).
37. The New Zealand Institute for Plant & Food Research Limited. *New Zealand Food Composition Database: New Zealand FOODfiles 2010.* The New Zealand Institute for Plant & Food Research Limited and Ministry of Health (2010). Available online at: https://www.foodcomposition.co.nz/foodfiles/ (accessed June 14, 2021).
38. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. *Ann Intern Med.* (1999) 130:461–70. doi: 10.7326/0003-4819-130-6-199903160-00002
39. Popp BN, Graham BS, Olson RJ, Hannides CC, Lott MJ, López-Ibarra GA, et al. Insight into the trophic ecology of yellowfin tuna, *Thunnus albacares*, from compound-specific nitrogen isotope analysis of proteinaceous amino acids. *Terrestrial Ecol.* (2007) 1:173–90. doi: 10.1016/S1936-7961(07)01012-3
40. Walsh RG, He S, Yarnes CT. Compound-specific δ13C and δ15N analysis of amino acids: a rapid, chloroformate-based method for ecological studies. *Rapid Commun Mass Spectrom.* (2014) 28:96–108. doi: 10.1002/rcm.6761
41. Styring AK, Kuhl A, Knowles TD, Fraser RA, Bogaard A, Evershed RP. Practical considerations in the determination of compound-specific amino acid δ15N values in animal and plant tissues by gas chromatography-combustion-isotope ratio mass spectrometry, following derivatisation to their N-acetylisopropyl esters. *Rapid Commun Mass Spectrom.* (2012) 26:2328–34. doi: 10.1002/rcm.6322
42. Sabadel A, Woodward E, Van Hale R, Frew R. Compound-specific isotope analysis of amino acids: a tool to unravel complex symbiotic trophic relationships. *Food Webs.* (2016) 6:9–18. doi: 10.1016/j.fooweb.2015.12.003
43. Braatvedt G, Cundy T, Crooke M, Florkowski C, Mann JI, Lunt H, et al. Understanding the new HbA1c units for the diagnosis of Type 2 diabetes. *N Z Med J.* (2012) 125:70–80.
44. Beasley J, Jung M, Tasevska N, Wong W, Siega-Riz A, Sotres-Alvarez D, et al. Biomarker-predicted sugars intake compared with self-reported measures in US Hispanics/Latinos: results from the HCHS/SOL SOLNAS study. *Public Health Nutr.* (2016) 19:3256–64. doi: 10.1017/S1368980016001580
45. Luceri C, Caderni G, Lodovici M, Spagnesi MT, Monserrat C, Lancioni L, et al. Urinary excretion of sucrose and fructose as a predictor of sucrose intake in dietary intervention studies. *Cancer Epidemiol Biomarkers Prev.* (1996) 5:167–71.
46. Min T, Stephens M, Kumar P, Chudleigh R. Renal complications of diabetes. *Br Med Bull.* (2012) 104:113–27. doi: 10.1093/bmb/lds030
47. Bellomo G, Venanzi S, Verdura C, Saronio P, Esposito A, Timio M. Association of uric acid with change in kidney function in healthy normotensive individuals. *Am J Kidney Dis.* (2010) 56:264–72. doi: 10.1053/j.ajkd.2010.01.019
48. Patel PS, Cooper AJ, O'Connell TC, Kuhnle GG, Kneale CK, Mulligan AM, et al. Serum carbon and nitrogen stable isotopes as potential biomarkers of dietary intake and their relation with incident type 2 diabetes: the EPIC-Norfolk study. *Am J Clin Nutr.* (2014) 100:708–18. doi: 10.3945/ajcn.113.068577
49. Dodd KW, Guenther PM, Freedman LS, Subar AF, Kipnis V, Midthune D, et al. Statistical methods for estimating usual intake of nutrients and foods: a review of the theory. *J Am Diet Assoc.* (2006) 106:1640–50. doi: 10.1016/j.jada.2006.07.011
50. Ministry of Health, New Zealand; University of Otago. *A Focus on Nutrition: Key Findings of the 2008/09 New Zealand Adult Nutrition Survey.* Wellington, NZ: Ministry of Health (2011).

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