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Dietary sugar intake: International time trends in intake levels among children and adolescents and aspects of its relevance for subclinical inflammation and insulin sensitivity among adults

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Karen Aimee Della Corte
from Utah, USA

1. Gutachterin: Prof. Dr. Anette E. Buyken, Paderborn University
2. Gutachterin: Prof. Dr. Sarah Egert, University of Hohenheim

“Work is the miracle by which talents rise to the surface and dreams become a reality.”
(Gordon B. Hinckley)

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SUMMARY

Dietary sugar intake: International time trends in intake levels among children and adolescents and aspects of its relevance for subclinical inflammation and insulin sensitivity among adults

It has been proposed that dietary sugar intake plays a causal role in the development of type 2 diabetes (T2D) and systemic inflammation. It is unclear whether these effects are more specific to dietary fructose or other sugar types. There is rising interest in trends and changes in diet over time, specifically those of sugar-sweetened beverages (SSB) and other dietary sugars, which may influence the development of these diseases and such world intake trend data were lacking. Therefore, the **1st aim** of this thesis was to investigate world trends in the consumption of SSB and dietary sugar among children and adolescents. The **2nd aim** was to pool together evidence from human intervention studies in order to assess the relevance of dietary fructose and dietary glucose as a comparator for biomarkers of subclinical inflammation. The **3rd aim** was to examine the prospective relevance of dietary sugar intake (based on dietary data and urinary biomarkers) in adolescence for insulin sensitivity and systemic inflammation in adulthood.

Two of these aims were based on data obtained through 1) a systematic review, 2) a systematic review/meta-analysis and 3) a prospective analysis from the DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study. In **Study I**, it was revealed that SSB and dietary sugar consumption among US children and adolescents rose substantially in the decades preceding 2000, followed by a faster and continued decline as compared to other international countries where trends changed only slightly across three decades. In **Study II**, limited evidence did not support the hypothesis that dietary fructose, as found alone or in HFCS, contributes more to subclinical inflammation than other dietary sugars. Meta-evidence for this review was graded as low. **Study III** did not conclude that dietary sugar in adolescence is associated with adult risk factors for T2D. The one potential exception to this was dietary fructose (as measured by 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.

In conclusion, the findings presented in this thesis suggest that SSB and dietary sugar intake trends from the US differ starkly form those of other countries around the world. The implications of these dietary shifts on human health are yet unclear, and require further research. In order to be able to draw firm conclusions on the effects of dietary sugar on T2D and systemic inflammation, more large-scale intervention and prospective studies are needed that investigate the relevance of dietary sugar intake distinguished by type, amount, source and form.

ZUSAMMENFASSUNG

Zuckerzufuhr: Internationale Zeittrends in der Zufuhrhöhe bei Kindern und Jugendlichen sowie Aspekte der Relevanz für subklinische Entzündungsneigung und Insulinsensitivität im Erwachsenenalter

Es wird diskutiert, dass die Zuckerzufuhr eine kausale Rolle in der Entwicklung von Typ 2 Diabetes (T2D) und subklinischer Entzündungsneigung spielt. Bislang wurde nicht untersucht, ob diese Effekte spezifisch für Fruktose oder andere Zuckerarten sind. Zudem besteht ein steigendes Interesse an Zeittrendanalysen, v.a. zum Konsum von zuckergesüßten Getränke (ZG) und der Zufuhr an anderen Zuckern, die für die Entstehung chronischer Erkrankungen relevant sein könnten. Jedoch fehlen hierzu Daten zu globalen Verzehrsmustern. Daher war das **1. Ziel** dieser Arbeit die Analyse weltweiter Zeittrends im Konsum von ZG und anderen Zuckern bei Kindern und Jugendlichen. Das **2. Ziel** war die systematische Beleuchtung von Evidenz aus Interventionsstudien hinsichtlich möglicher Effekten von Fruktose bzw. Glukose auf Biomarker der subklinischen Entzündungsneigung. Das **3. Ziel** war die Analyse der prospektiven Relevanz der Zuckerzufuhr in der Adoleszenz (basierend auf Ernährungsdaten und Biomarkern aus 24-h Urinen) für die Insulinsensitivität und subklinische Entzündungsneigung im Erwachsenenalter.

Zwei dieser Ziele basierten auf Daten aus einem systematischen Review, bzw. einem systematischen Review/Meta-Analyse, das dritte Ziel auf Daten der DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Studie. **Studie I** zeigte, dass der Konsum von ZG und Zucker bei US-amerikanischen Kindern und Jugendlichen in den Jahrzehnten vor 2000 substanziell anstieg, gefolgt von einer schnelleren und kontinuierlicheren Abnahme als in anderen Ländern, in denen sich das Zufuhrniveau in drei Jahrzehnten nur leicht änderte. **In Studie II** reichten die verfügbaren begrenzten Hinweise nicht aus, um die Hypothese zu unterstützen, dass Fruktose, isoliert oder in Form von Glucose-Fructose-Sirup, mehr zur subklinischen Entzündungsneigung beiträgt als andere Zucker. Die Meta-Evidenz für dieses Review wurde als niedrig eingestuft. **Studie III** lieferte keinen Beleg für eine prospektive Relevanz der Zuckerzufuhr von Jugendlichen für T2D Risikofaktoren im Erwachsenenalter. Allerdings war eine höhere Fruktosezufuhr geschätzt anhand des Biomarkers der 24-h Ausscheidung im Urin im Kontext eines moderaten Fruktosezufuhrniveaus bei Frauen mit einer günstigeren Insulinsensitivität und niedrigeren Nüchterninsulinspiegeln assoziiert.

Die Ergebnisse dieser Arbeit zeigen, dass sich die Zeittrends in der Zufuhr von ZG- und Zucker in den USA stark von den Trends anderer Länder unterscheiden. Die Konsequenzen dieser veränderten Verzehrsgewohnheiten für die Gesundheit sind noch unklar und erfordern weitere Untersuchungen. Um bessere Schlussfolgerungen hinsichtlich des Effektes der Zuckerzufuhr T2D und subklinische Entzündungsneigung zu ziehen sind weitere groß angelegte und robuste Interventionsstudien sowie prospektive Beobachtungsstudien von Nöten. Diese sollten auch die Art, Menge, Quelle und Form der Zuckerzufuhr untersuchen.

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ABBREVIATIONS

%En	percentage of total energy intake
24-h recall	24-hour dietary recall
AAP	American Academy of Pediatrics
AHA	American Heart Association
ATO	age at take-off
AS	added sugar
BHS	Bogalusa Heart Study
BMI	body mass index
BMR	basal metabolic rate
CHIS	California Health Interview Survey
CI	confidence interval
(hs)CRP	high sensitivity C-reactive protein
CVD	cardiovascular Disease
df	degrees of freedom
DNL	De Novo Lipogenesis
DONALD	Dortmund Nutritional and Anthropometric Longitudinally Designed study
EAT	Eating Among Teens study
FFA	free fatty acids
FFQ	food frequency questionnaire
FITS	Feeding Infants and Toddlers Studies
FKE	Forschungsinstitut für Kinderernährung (Research Institute of Child Nutrition)
FVMM	Fruits and Vegetables Make the Marks study
GI	glycemic index
GL	glycemic load
HbA1c	hemoglobin A1c
HFCS	high fructose corn syrup
HOMA	homeostatic model assessment
HOMA2-%S	HOMA of insulin sensitivity
I^2	inconsistency between study results
ICQC	International Carbohydrate Quality Consortium
IOTF	international Obesity Task Force
IL	interleukin

IL-1RA	interleukin-1 receptor antagonist
IQR	interquartile range
IR	insulin resistance
LEBTAB	in-house nutritional database (Lebensmitteltabelle)
MCP-1	monocyte chemoattractant protein 1
MD	mean difference
MeSH	medical subject headings
Mo/Di	mono- and disaccharides
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcohol steatohepatitis
NFCS	National Food Consumption Surveys
NHANES	National Health and Nutrition Examination Survey
NMES	non-milk extrinsic sugars
NR	not reported
PKC	protein kinase C
SACN	Scientific Advisory Committee on Nutrition
SD	standard deviation
SE	standard error
(s)ICAM-1	(soluble) intercellular cell adhesion molecule 1
RCTs	randomized controlled trials
ROBINS-I	Risk Of Bias In Nonrandomised Studies – of Interventions
SAS	statistical analysis system
sE-selectin	soluble E-selectin
SSB	sugar-sweetened beverages
SEM	standard error of the mean
Suc	sucrose intake
T1-T3	tertiles 1 thru 3
T2D	type 2 diabetes mellitus
TNF-α	tumor necrosis factor – alpha
TS	total sugar intake
VLDL	very low density lipoprotein
WHO	World Health Organization

1 INTRODUCTION

Type 2 diabetes mellitus (T2D) along with the other cardio-metabolic diseases and the accompanying comorbidity of chronic inflammation are leading causes of premature disease and death.[1, 2] As the rates and onset of these diseases occur ever increasingly among younger age groups, [3-13] there is rising interest in primary prevention measures as well as trends and changes in diet over time that may play causal roles in their development. Dietary sugars and sugar-sweetened beverages (SSB) are consumed in considerable amounts in the Westernized diet especially among children and adolescents [14, 15] and are thus discussed as a major culprit. The effect of dietary sugar intake on health has, however, been a topic of continual controversy and numerous questions remain unclear.

In the area of research concerning sugar and adolescent health, an estimation of world dietary sugar and SSB intake trends is needed and lacking in order to provide valuable insights into the evolution of sugar intake trends over time in various geographical regions of the world. This had been undertaken in the past only by evaluating either supply or apparent consumption data [16] or national dietary surveys from different sources to determine within-country trends.[17] The world trends review researched and outlined in this thesis had a unique aim to provide an overview of world sugar intake trends by collating data from longitudinal follow-up studies and repeated cross-sectionals through a formal systematic review in order to comprehensively assess all available trend data on dietary sugar and SSB intake specifically among children and adolescents.

Further questions involving the effect of sugar intake have been posed relating to T2D risk and inflammatory processes in humans. While the existing data is conflicting, [18, 19] a growing body of evidence from well-powered prospective cohort studies has shown a consistent and relatively strong association between SSB consumption and increased risk of T2D among adolescents [20-22] as well as subclinical inflammation measured by the inflammatory marker C-reactive protein (CRP).[23-26] Evidence for the relevance of other dietary sugar types (e.g. free, added, total sugars) is more conlicting. The specific effects of dietary fructose versus dietary glucose have been heatedly discussed due to conflicting evidence that suggests that dietary fructose has unique metabolic effects and therefore contributes more to cardiometablic risk than do other dietary sugars. Important distinctions are made that analyze sugar categories and sources when investigating associated disease outcomes, e.g. intake of dietary fructose in modest amounts (often from whole foods) compared to fructose from SSB consumed in large amounts leading consistently to adverse effects including increased T2D risk and systemic inflammation. Clarifications are needed concerning the role of dietary fructose and other sugar types in developing T2D and inflammation from both human intervention trials and prospective follow-up studies. In addition, a more comprehensive method that investigates various forms of sugar

present in foods (added, free, total) as well as sugars on a chemical level (fructose, glucose, sucrose, etc.) should be employed.

Therefore, the **1st aim** of this thesis was to investigate world trends in the consumption of SSB and dietary sugar among children and adolescents. The **2nd aim** was to pool together evidence from published human interventional studies in order to assess the relevance of dietary fructose (alone or found in sucrose or HFCS) and dietary glucose as a comparator for biomarkers of subclinical inflammation. The **3rd aim** was to examine the prospective association between the intake of dietary sugar (on the basis of chemical sugar types (fructose, glucose, sucrose) and uses of sugar (total, added and free sugar) in adolescence (from both dietary data and urinary excretion data) and the target outcomes of insulin sensitivity and fasting insulin as well as systemic inflammation in adulthood.

2 THEORETICAL BACKGROUND

2.1 Dietary sugar among children and adolescents – Intake trends/levels and implications for health

2.1.1 Definitions of sugar and assessment methods

Sugar intake can be defined and quantified in multiple ways. In a nutrient-based method, the mono- or disaccharides such as glucose, fructose and sucrose themselves are evaluated. A sourced-based method measures sugar intake based on major high-sugar food sources such as SSB or candy and desserts. Sugar intake can also be determined by assessing the amount of "added sugar" or "free sugar" in a diet. The Food and Drug Association has defined added sugar to be "sugars and syrups that are added to foods during processing or preparation" excluding naturally-occurring sugars found in foods such as fruits and dairy products.[27] The World Health Organization (WHO) uses the term "free sugars" and defines them to be monosaccharides and disaccharides added to foods and drinks by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates. The term 'total sugars' includes all mono- and disaccharides; namely glucose, fructose, galactose, lactose, sucrose and maltose. In the UK, the term 'non-milk extrinsic sugars' (NMES) has been used, which is designed to be a measure of sugars that are not contained within the cell wall of fruits, vegetables, etc., excluding those in milk.[28] The NMES term is no longer used and the definition of free sugars has been adopted in the UK.[29] These variations and differences in definitions of the term 'sugar' can lead to consumer and researcher confusion, also causing deviation in whether and how data on free, added or total sugar intakes are being gathered into databases. Additionally, attempting to estimate and assign sugars within foods to being either free or not free is extremely complex. Due to the fact that only total sugars are subject to mandatory labelling in Europe, databases often do not distinguish between free sugars and other sugars in foods. To this end, researchers have to estimate the levels of free sugars in foods and beverages themselves and append them to their databases.[30]

There is an array of categories and uses by which dietary sugar is defined and tested for in nutritional research. Broken down on the 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 assess intake levels in various forms and quantities that may each be of physiological relevance.

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 of sugar but also of the various dietary assessment methods used. Intake estimates can be made using indirect or

direct assessment methods. Indirect methods include trends in sales of beverages or supply and availability data,[16] whereas food diary or record methods, diet histories, FFQ or 24h recall are considered direct methods at the individual level. Indirect methods can provide an indication of per capita availability of sugars but do not account for wastage, which is known to be significant in developing countries.[31, 32] Hence, such data tend to report much higher intake levels than those measured at the individual level. Furthermore, such data cannot inform on the actual consumption amounts of specific subpopulations such as children and adolescents.[33, 34] Indirect methods are unable to estimate free or added sugar levels.[35] Direct methods of assessment can be applied at the individual level and estimate actual food consumption. Such methods can then be aggregated and summarized at subpopulations of interest, namely children and adolescents. When assessing sugar intake, it is best to use assessment methods that can quantify individual outcomes in gram amounts or percent energy intake. As regards to FFQ, this method does not allow for a quantification of consumption levels and is rather used for ranking intakes. An acceptable exception to this would be the use of FFQs that address servings of SSB so that the intakes are comparable between studies.

While direct methods allow more accurate assessments of food consumption, the varying methodologies for using direct methods can cause difficulties in making international comparisons of intake amounts. For example, in the United States, a 24-hour recall is used by the US Department of Agriculture, whereas in the UK, a 7-day weighed food record is the chosen method. These differences could curtail the ability to make meaningful comparisons. This is a reason why food supply data is believed to permit trends at the broadest level geographically. Another limitation for direct methods is that they are not available for all countries. Many countries in the developing world lack the expertise or resources to undertake nutrition surveying at the individual level.

To avoid measurement error in self-reported sugars (a long-standing obstacle for determining the true association between sugars and chronic disease risk) biomarkers for assessment of dietary sugar intake have been introduced [36, 37] and hold a lot of promise as objective markers in diet-related studies. Twenty-four hour (24 h) urinary sucrose and fructose have been suggested as predictive biomarkers for sugars intake. The use of urinary biomarkers to measure dietary sugar intake may produce more reliable results since dietary assessment methods are prone to measurement errors [38] and sugars are among the nutrients that are frequently underreported.[39, 40] Although this biomarker has great potential, it is still debated in the existing literature which sugars (extrinsic, intrinsic, total, added, free, etc.) are really captured by urinary sucrose and fructose excretion.[36, 37, 41, 42]

2.1.2 Recommended levels and trends of sugar intake

As the world has become increasingly aware of the role of dietary sugar and health, government and health organizations have reacted by issuing dietary guidelines. In the past, these guidelines have diverged greatly, yet with time there has risen a general consensus from major authorities. Examples include: a maximum 10% of total energy from free sugars intake from the WHO [43] and Scientific Advisory Committee on Nutrition (SACN) guidelines with a conditional 5% maximum free sugars intake [28]; a similar maximum on added sugar intake from the Dietary Guidelines for Americans of 10% total caloric intake [44]; and a maximum added sugar intake of half of discretionary energy allowance from the American Heart Association (on average 6-9 tsp/day).[45] On the other hand, diverging guidelines still exist including a maximum of 25% of energy of added sugar from the Institute of Medicine [46] as well as the European Food Safety Authority who has not yet been able to set a dietary reference for sugar due to insufficient data but confirm that when consumed in high amounts (>25%E) is detrimental to health.[47] The variations in these recommendations may arise from differences in the underlying definitions of dietary sugar exposure and classifications, though the insufficiency of evidence in the literature also plays a role.[48]

In the United States, it has been estimated that added sugars are found in approximately 75% of foods and beverages.[49] Although added sugar intake rose during the last decades of the 20th century (in all age groups > 2 years), more recent data showed that with increased public awareness, the consumption of added sugars in the United States decreased between 1999 and 2008 from a mean of 100 g/day to 77 g/day (assessed by 24-h recalls).[50] In Europe, intakes of free sugars vary by country and setting. Estimates from the WHO indicated that adult intake of free sugars ranged from about 7-8% of total energy intake in Hungary and Norway to 16-17% in countries like Spain and the United Kingdom.[43] Free sugar intake in children was generally much higher, ranging from 12% in countries like Slovenia and Sweden, to nearly 25% in Portugal.[43] In a review of the sugar consumption of fifteen developed countries it was reported that between 2000 and 2012, total sugar intake among adolescents aged 12-18 years ranged from 15-29% of total energy intake and for children aged 4-10 years from 23.5-28%.[51] World trend data on sugars intake among children, adolescents and adults reported a percent energy increase in various dietary sugars in Sweden and New Zealand, a decrease in Norway, and a rise and fall in intakes in the United States over a 14-22 year time period between 1971 and 2011.[17] This review revealed that while some subpopulations were experiencing an estimated increase in sugars intake, most reported stable or decreasing trends in dietary sugars intakes. In Germany, the DONALD study performed a time trend analysis of total, added, and free sugar intake among 3-18 y old youth from 1985 to 2016 and confirmed that there was an increase in total and free sugar until 2005, followed by decreases most notably since 2010.[52]

The intake of SSB increased dramatically around the world toward the end of the 20th century.[53, 54] In the United States, the largest source of added sugars is SSB [45, 55, 56] and adolescents have been the largest consumers of them.[57] The definition of SSB includes a wide spectrum of soft drinks, fruit drinks and energy or vitamin water drinks containing added sugars. These beverages contain and are sweetened by high-fructose corn syrup (commonly 55% fructose and 45% glucose), sucrose, or fruit juice concentrates. Trends and levels of individual SSB consumption in higher income countries such as the United States, United Kingdom and Australia increased in the 20th century as measured by 7-day and 24-h recalls,[58-60] while in the most recent decade a moderate decrease in SSB in the United States and Australia has been observed as reported by individual studies and apparent consumption data.[50, 61] SSB sales, however, have increased rapidly in other parts of the world. Sales of SSB in China, for example, have increased from 2000 to 2010 by 145% for Coca Cola and 127% for PepsiCo.[62] Sales figures from Coca Cola's annual report show a 14% and 18% unit case volume growth in India and China respectively in 2007.[63] Food disappearance data from China, Vietnam, India, Thailand and other South Asian countries showed swiftly increasing intakes in SSB toward the end of the 20th century.[64] In Mexico, national survey data reported that between 1999 and 2006 SSB intake more than doubled for adolescents (12-18 y; $p < 0.001$) from 151 to 349 consumed calories from beverages per day.[65] It has been estimated that among those aged 2-19 years in the US, percentage of total daily calories from SSB increased from 4.8% in the late 1970's to 10.3% in 2001.[66] At the same time in the US, milk consumption particularly among children decreased while juice consumption remained stable among all age groups.[67] Trends of SSB intake among children and adolescence may continue to increase in low- and middle-income countries as it appears that a major push toward marketing SSB in these countries exists.[62] There has been a steady increase in many developing countries of SSB intake that rises with rates of urbanization.[63]

2.1.3 Children and adolescent populations

While the negative effects of sugar intake on childhood and adolescent health generally relate to increased weight gain, increased added sugar intake has also been paralleled by decreased nutrient intakes and overall dietary quality in multiple studies.[68-74] Considering the adolescent population, dietary quality is often reduced in puberty, particularly with regard to carbohydrates. Adolescents generally consume more added sugars, principally as soft drinks, fast foods, and less fiber-rich products.[14, 15, 75] Since sweet preference is higher in childhood and adolescence than in adulthood,[13, 14] these age groups are particularly vulnerable to excessive sugar intakes. High sugar intakes in childhood and adolescence are perceived as particularly critical because dietary patterns

track into adulthood [15] and adolescence is a “critical period” for the development of various diseases in later life.[16–19] Public health representatives, politicians, and the general public are increasingly concerned that sugar intake is rising in these susceptible age groups.[20, 21] Adolescence is characterized by substantial hormonal, metabolic and lifestyle changes, which is why this developmental stage is considered a critical period for later cardio-metabolic diseases. Carbohydrate nutrition during periods of physiological insulin resistance such as puberty may have impact on future risk of type 2 diabetes.[76] This period also represents a window of opportunity for preventive measures and lifestyle modifications including reduced sugar intake.

2.1.4 Obesity and chronic disease risk in children and adolescents

After leading-edge work done by Mattes and colleagues [77-80] and Rolls and colleagues [81-84] showing that consumption of SSB does not suppress the intake of other food calories to suppress weight gain, public health and biomedical scholars have focused on the relationship between SSB and increasing obesity trends particularly in children and adolescents.[58, 65, 67, 85-87] The intake of dietary sugars has been associated with the development of T2D, a growing public health problem that has increasingly been identified in children and adolescents.[88-92] While the existing data is conflicting regarding dietary sugars and T2D,[18, 19] a growing body of evidence from well-powered prospective cohort studies has shown a consistent and relatively strong association between SSB consumption and increased risk of T2D among adolescents.[20-22] Two randomized controlled trials lasting 1½ to 2 years conducted in children and adolescents have shown that weight gain can be slowed by replacing SSB with non-caloric beverages.[93, 94] Longitudinal studies have observed that a long-term consumption of SSB in childhood and adolescence is a predictor of overweight and body fatness in adulthood.[95, 96] Furthermore, avoiding overweight and obesity in younger years by limiting excessive energy intake of SSB and dietary sugars is relevant for risk of later cardio-metabolic diseases.[97-100]

2.1.5 Longitudinal and repeat cross-sectional study designs

For Study I, two study designs were investigated that assess survey data uniquely: repeated cross-sectional and longitudinal prospective studies. Each of these approaches has its own merits and both quantitatively measure change with an element of time. The cohort method asks the same information to the same group of individuals over time and the cross-sectional method asks the same information to different samples of individuals in a population at successive time points. Cohort surveys follow participants from an identical point in their lives onwards (often from birth) and thereby

provide useful data to study child habitual intake amounts or habits among people living in the same generation over time. Cross-sectional data do not measure individual participant change but rather analyse groups or population changes at different points over time. This method provides data that better reflect a changing community. The inclusion of both of these types of survey data helps to provide a more comprehensive overview of dietary sugar intake habits and changes over time.

2.2 The influence of dietary sugar intake on systemic inflammation

2.2.1 Definition, development and prevalence of systemic inflammation

Chronic, low-grade inflammation, as reflected by elevated circulating levels of inflammatory cytokines, is a key factor in the pathogenesis of cardiovascular disease,[101] dementia,[102] depression,[103] and certain types of cancer.[104] In addition to a higher risk of all-cause mortality in old age,[105] low-grade inflammation has also been implicated in promoting insulin resistance[105, 106] and is associated with the risk of developing diabetes.[107, 108] Both obesity[109] and T2D[107] are now considered inflammatory diseases.

2.2.2 Inflammatory markers

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 and their risk factors.[110-114] However, some of these associations appear to be non-causal according to mendelian randomization studies. Other inflammatory markers were excluded from the analyses because they could not be clearly attributed to pro- or anti-inflammatory reactions or because they were more closely related to CVD. Two such parameters, omentin and IL-1 receptor antagonist (IL-1ra), which were available in the study were excluded from the analyses as their inclusion seemed impossible to due conflicts in measured effects. Omentin was initially understood to be an anti-inflammatory, insulin-sensitizing adipokine [115-118] with cardio-protective effects, but has recently been reported to be directly associated with higher risk of T2D and stroke in prospective studies.[117, 118] IL-1ra displays a counter-regulatory function by inhibiting the pro-inflammatory and insulin-desensitizing action of cytokine IL-1β.[115] Therefore, the net inflammatory effect of IL-1ra may only be estimated from its ratio to IL-1β.

The goal of an inflammatory score was to determine a value that reflects the overall inflammatory status, which is determined by complex counter-regulatory mediators. A pro-inflammatory score was assumed to be more predictive of inflammation than single markers,[119] and was obtained in the following way: 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 markers, and 3) average of all. Such a holistic approach is generally recommended,[119] however some aspects of its implementation prove to be inconsistent because features and functions of several inflammatory markers are incompletely characterized and partly conflicting. For example, chemerin and leptin are generally considered as pro-inflammatory cytokines, yet they appear to exhibit anti-inflammatory and cardio-protective effects in certain circumstances.[120-123] Other examples of inconsistencies are explained below, which together illustrate the complexity of inflammatory processes and convey that despite providing insight into the overall inflammatory status, the pro-inflammatory score could to too simplistic and should be interpreted with caution. Further detailed characterization and research of relevant markers and their pathophysiological role for cardiometabolic diseases will help to establish a more valid, robust overall inflammatory score.[119]

C-Reactive Protein

C-reactive protein (CRP) is an acute-phase protein responsible for activating the complement system in an immune response. It is synthesized in the liver [124] in response to factors released by macrophages and adipocytes.[125] CRP has been associated with various metabolic diseases such as obesity, insulin resistance, and hyperglycaemia that exhibit ongoing acute-phase inflammatory responses.[126-129] CRP has been related to both T2D[130, 131] and cardiovascular disease (CVD) risk [112, 132, 133] in cohort studies. According to observational data reports, it has been reported that dietary sugar intake (more particularly SSB) may be one stimulus of subclinical inflammation as measured by the inflammatory marker CRP.[23-26] Mendelian randomization studies, however, indicated that the association between CRP and risk of CVD is not causal[134, 135] because reverse causation or other confounding may be at play. Proponents say that despite its non-causality CRP may still be informative as it may be a strong correlate of causal factors related to the onset of a disease outcome. The findings from mendelian randomization analyses might also apply to inflammatory markers' role in T2D development.

Interleukin 6

The secretion of the pro-inflammatory cytokine interleukin-6 (IL-6) stimulates an immune response during an infection or injury and acts as a mediator of the acute-phase response. IL-6 has been related to increased T2D [130, 131] and CVD risk[132, 133] in cohort studies. In contrast to CRP, evidence for a causal role in CVD development exists for IL-6.[136, 137] Conflicting features and functions of IL-6 have also been reported. The function of IL-6 depends on the source and target tissue and the duration of the signal. This means that the chronic secretion of IL-6 from liver and adipose tissues leads to the perpetuation of inflammation, the accumulation of hepatic fat, insulin resistance, and endothelial dysfunction while acute secretion of IL-6 from skeletal muscle stimulates muscle metabolism.[138, 139]

Interleukin 18

The pro-inflammatory cytokine interleukin-18 (IL-18) is involved in activating cell-mediated immunity in response to infections. Compared to the previous markers, there are limited studies regarding IL-18. The MONICA/KORA Augsburg Study showed, however, a positive association between IL-18 concentrations and T2D risk,[140] but not coronary events.[132] It is generally considered that IL-18 is an independent predictor of cardiometabolic diseases (T2D, CVD, metabolic syndrome, insulin resistance, dyslipidaemia and obesity).[141-146] Yet these findings do not harmonize with other observations that IL-18 shows anti-obesity and insulin-sensitizing activities.[144, 145]

Adiponectin

Adiponectin is an anti-inflammatory hormone derived from adipose tissue. Adiponectin attenuates inflammatory responses by modulating signalling pathways.[147] Adiponectin has a beneficial effect on atherosclerosis and insulin resistance and is downregulated in obesity.[147] As a prognostic marker, meta-analyses showed a prospective association between increased adiponectin levels and a lowered T2D risk,[148] but no consistent relation with coronary heart disease and CVD risk.[149-151] Other studies reported an inverse association with T2D, IR and certain CVD (hypertension, myocardial infarction, coronary artery disease),[115, 116, 121, 122, 138, 139] yet reports of a direct association between high adiponectin levels and all-cause and cardiovascular mortality complicate its use as a marker of cardiometabolic risk.[116, 138, 139]

Chemerin

Chemerin is a potent macrophage chemoattractant and adipokine that regulates adipocyte development as well as glucose metabolism in liver and muscle tissues.[152] Chemerin promotes

chemotaxis and retention of macrophages at sites of inflammation.[153] Chemerin levels are elevated in patients with obesity. Thus the role of chemerin in inflammation and metabolism might provide a link between chronic inflammation and obesity.[152]

Leptin

In addition to the generally well-known role of leptin as the "satiety hormone" produced by adipose cells to regulate energy balance, it has a well-established involvement in the regulation of the inflammatory response.[154-156] It is theorized that the role of leptin as an inflammatory marker is to respond to adipose-derived inflammatory cytokines.[157] Leptin is also associated with obesity as well as overeating and inflammation-related diseases. Leptin upregulates inflammatory cytokines like TNF-α, IL-6, and IL-12,[158-160] and TNF-α and IL-1β subsequently increase the expression of leptin mRNA in adipose tissue, thus creating a loop of acute-phase reactants that influence each other in the promotion chronic inflammation.[160]

2.2.3 Possible pathophysiological mechanisms

It has been postulated that dietary sugar consumption leads to increased inflammatory processes in the body as explained by the possibility of multiple pathophysiological mechanisms. Central to one potentially relevant mechanism is the fact that dietary sugar promotes de novo synthesis of free fatty acids (FFA) in the liver,[161-163] which according to the lipotoxicity theory produces FFA metabolites that may trigger inflammatory processes and ROS formation.[164, 165] Another identified mechanism is hyperglycaemia which has been found to be induced by the consumption of diets high in dietary sugar leading to an altered oxidative state.[166, 167] According to Brownlee et al. (2001), hyperglycaemia leads to mitochondrial superoxide production in endothelial cells [168] triggering the release of pro-inflammatory markers. Elevated chronic inflammation may also be caused by advanced glycation end-products (AGE) which are increased by sugar consumption.[169] Glucose or fructose can eventually react with free amino groups of proteins to form glycated products and elicit damage to organelles such as mitochondria and endoplasmic reticulum causing widespread histopathological damage. Because of its fructofuranose ring, fructose is much more reactive compared to the more stable 6-membered ring of glucose, generating up to 100 times more ROS than glucose.[170] High ROS and AGE levels, common in the Western diet, exacerbate inflammation through oxidative stress and cytokine synthesis in animal model studies.[171]

2.2.4 Evidence from interventional and observational studies

Intervention studies

There is some evidence from human intervention trials that points towards pro-inflammatory effects of sucrose and fructose versus glucose based on effects on various biomarkers of subclinical inflammation such as CRP and MCP-1.[172, 173] As will be detailed in this thesis, our 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. These results were inconsistent due to the heterogeneous and small nature of the trials available.

Observation studies

Observational studies link the consumption of SSB to increased chronic inflammation. More specifically, the relationship between SSB intake and the inflammatory biomarker CRP has been consistently reported on in observational studies.[174-177] In a prospective cohort study of over 42,000 men, participants in the top-quartile of SSB intake had a 20% higher relative risk of developing coronary heart disease and SSB intake was significantly associated with CRP, IL-6 and TNF-α.[174] Recent studies have also linked SSB intake to the inflammatory disease rheumatoid arthritis.[178, 179] This observational evidence, however, is confined to SSB intake and the relevance of sugars beyond SSB is still unknown.

2.3 The influence of dietary sugar intake on type 2 diabetes

2.3.1 Definition, development and prevalence of type 2 diabetes

Type 2 diabetes mellitus (T2D) represents a growing public health problem. Incidence and prevalence rates along with comorbidities are increasing rapidly. The International Diabetes Federation estimates that there are currently over 300 million people with diabetes[180]. The prevalence of diabetes among adults 18 years of age or older rose from 4.7% in 1980 to 8.5% in 2014 worldwide.[181] T2D was once thought to be a metabolic disorder of adulthood but in the last two decades has become increasingly present among obese adolescents and children.[91] T2D is a major risk factor for the development of CVD) which is the most common cause of death among adults with diabetes mellitus.[182] In addition to increasing CVD risk, T2D is a major cause of stroke, kidney failure, blindness, and lower limb amputation.[181] Therefore, identifying modifiable risk factors that could lower T2D in addition to inflammatory markers is pivotal in order to contribute to the prevention of chronic disease.

2.3.2 Diabetes markers

HOMA – Insulin sensitivity

The homeostatic model assessment (HOMA) is a method for assessing β-cell function and insulin resistance or sensitivity by measuring basal fasting glucose and insulin or C-peptide concentrations. For the assessment of insulin sensitivity within the DONALD study, HOMA of insulin sensitivity (HOMA2-%S) was used which is the reciprocal equivalent of HOMA-IR (insulin resistance).[183] The HOMA2-%S model has proven to be a robust clinical tool and widely used in large-scale epidemiological studies.[184, 185] It is calculated from fasting glucose and fasting insulin levels to represent steady-state insulin sensitivity.[183] HOMA-2 is an improved model recalibrated to modern insulin assays which better reflects human physiology. The original equation model has been updated by a computer-based recalibrated model, however the original method is unfortunately still the one most often applied.[183] Because HOMA is modelled for a steady state, fasting glucose concentrations reflecting hypoglycaemia (<2.5 mmol/L) should not be included in the calculation.[183] The results for HOMA2-%S and fasting insulin presented in study 3 are alike. Indeed, HOMA is criticised for providing only limited added value for the estimation of insulin sensitivity in the normal population beyond what is reflected by fasting insulin concentrations.[183] Yet, the benefit of calculating HOMA lies in the possibility to compare its values with those of subjects with impaired glucose tolerance and to track if subjects are progressing toward or developing this impairment.[183]

The application of the hyperinsulinaemic-euglycaemic clamp, the declared gold standard used as a reference method for the assessment of insulin sensitivity, is limited. Due to how expensive, time-consuming and laborious the clamp is, HOMA2-%S is among available surrogate measures of insulin sensitivity that provides one of the highest correlations with the clamp method.[183, 186] HOMA represents an easily applicable and well-validated measure of insulin sensitivity that allows inter-study comparisons.[183, 187]

Fasting insulin

Fasting insulin can be used as a predictor of insulin resistance and risk for T2D.[188] Elevated fasting insulin is predictive factor for the development of the metabolic syndrome,[189] which has been strongly associated with the development of T2D.[190] Dietary fructose elicits a lowered insulin secretion compared to dietary glucose, [191-193] and there is some evidence indicating that fructose intake/substitution can beneficially affect blood glucose levels.[194, 195] Clarifications from prospective studies concerning the role of dietary fructose and other sugar types for fasting insulin and the development of insulin sensitivity are needed. In Study III, plasma concentrations of insulin were

analysed at the Laboratory for Translational Hormone Analytics of the University of Giessen using an immunoradiometric assay (IRMA, DRG Diagnostics, Marburg, Germany).

2.3.4 Possible pathophysiological mechanisms

Multiple possible pathways have been identified to explain putative negative effects of dietary sugar intake on risk of developing T2D. There is a clear association between dietary sugar intake FFA levels [196] and it has been suggested that increased FFA levels may result in intramyocellular lipid accumulation and subsequent skeletal muscle insulin resistance.[197] Additionally, human trials have shown that fructose increases visceral adipose tissue,[198, 199] which actively secretes adipokines. The dysregulation of adipokines causes inflammatory responses and has been shown as a major mechanism to induce peripheral insulin resistance.[200] There is some evidence from human trials that dietary sugar intake increases systemic inflammation;[172, 173] this may eventually lead to β-cell damage and reduced insulin secretion.[157] Additionally, a human trial observed that a high-fructose diet impaired insulin binding and insulin sensitivity, which may be a result of decreased cellular adenosine triphosphate caused by fructose intake, leading to reduced cellular binding of insulin and possible reduction in number of insulin receptors.[201] Animal models have also indicated that fructose may be directly involved in increasing hepatic insulin resistance by increasing expression of protein tyrosine phosphatase 1B (PTPB1),[202] which negatively regulates the insulin receptor.[203] A high-fructose diet was also observed to increase c-Jun N-terminal kinase (JNK) activity in rodents,[204, 205] which can interfere with steps in the insulin signalling pathway.[206] Increased hepatic lipid supply from large influxes of substrate for FFA as caused by the metabolism of fructose or sucrose [199] may also induce hepatic insulin resistance.[200] This phenomenon is possibly caused by the increased levels of diacylglycerol, which activates novel protein kinase C (PKC)[207] and which is also increased in high-fructose fed rats.[163] Novel PKC decreases tyrosine phosphorylation of the insulin receptor, resulting in increased hepatic glucose production, impaired glucose tolerance, and increased fasting glucose and insulin concentrations.[200] These findings observed in animal models need to be further validated in epidemiological studies.

2.3.5 Evidence from interventional and observational studies

Intervention studies

In small human intervention studies, isocaloric replacement of starch with sucrose or fructose has been found to cause increased fasting insulin levels,[208, 209] and reduced insulin sensitivity.[201] A meta-analysis of diet intervention trials found that short-term consumption of fructose in isocaloric

exchange or hypercaloric supplementation promotes the development of hepatic insulin resistance in non-diabetic adults, while muscle or peripheral sensitivity was not affected.[210] On the other hand, a meta-analysis reported that isocaloric exchange of fructose for other carbohydrate improved long-term glycemic control (hemoglobin A1c- HbA1c) but had no effect on insulin in people with diabetes.[211] A similar meta-analysis indicated that small doses of fructose in isoenergetic exchange improves HbA1c and fasting blood glucose but has no effect on insulin resistance.[212] Due to its unregulated uptake and metabolism, the fructose component of high-sugared foods has been singled out as a key promotor of adverse cardiometabolic health outcomes when consumed in high amounts.[213, 214] High intake levels of fructose administered in such intervention and feeding trials do not represent common intake patterns consumed habitually over time. Amounts as well as types/sources of ingested fructose are of importance when considering its relation to risk factors of T2D.[215] Dietary fructose elicits a lowered insulin secretion compared to dietary glucose, [191-193]and there is some evidence indicating that fructose intake/substitution can beneficially affect blood glucose levels.[194, 195] Clarifications from human intervention studies concerning the role of dietary fructose and other sugar types in the development of insulin sensitivity are needed.

Observation studies

Although experimental evidence from randomized controlled trials is lacking due to feasibility considerations, a growing body of evidence from prospective cohort studies has shown a consistent and relatively strong association in well-powered studies between SSB consumption and increased risk of diabetes.[20] A meta-analysis of 8 prospective cohort studies evaluating SSB intake and risk of diabetes found that individuals in the highest quantile of SSB intake (1-2 servings/day) had a 26% greater risk of developing type 2 diabetes and weight gain than those in the lowest quantile (none or <1 serving/month) (relative risk: 1.26).[21] Global observational data has reported that dietary sugars elevate diabetes risk independent of their effect on body weight.[22] This study found that HFCS intake elevates diabetes risk even when being adjusted for overall sugar availability and total caloric intake. There is however some controversy related to dietary sugar and risk of T2D based on whether it is dietary sugar from SSB itself that has diabetogenic effects or whether it is SSB's association with other negative lifestyle factors, including weight gain, that increases risk for diabetes. Data indicating positive associations between dietary sugar intake and diabetes markers are also limited to SSB as a sugar source; data on the association of total added or free sugar intake on diabetes markers is lacking. In addition, available evidence is largely based on adult studies. Insulin resistance and chronic subclinical inflammation, however, emerge already in adolescence.[216-218] Other reported

observational evidence suggests that fructose consumption improved HOMA2-S% and insulin levels.[219, 220] Further sources reporting on large cohorts found no association between fructose-containing sugars and incident T2D[221-223] 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.[224] These studies report findings that emerged when investigating chemical sugar types, not SSB. The observational studies referenced here adjusted for anthropometric measures and energy intake but did not measure sugar intake by means of urinary biomarkers as was done in our study. When consumed in high amounts, dietary fructose has been associated in cohort studies with increased risk of T2D.[225, 226] 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.[227]

3 AIMS AND RESEARCH QUESTIONS

As summarized in the previous chapters, dietary sugar intake may represent a risk factor for subsequent development of T2D and systemic inflammation. However, the evidence supporting this is inconsistent and requires further research, particularly when considering the metabolic differences between dietary fructose and other dietary sugars. Additionally, it is of public health interest to ascertain how intakes of SSB and dietary sugar among children and adolescents have developed and changed over recent decades and how these trends compare between countries. The general assumption that sugar intakes are increasing has not been sufficiently substantiated in the literature nor been reviewed in children and adolescents. To address these issues, the following three aims were formulated for this thesis:

Aim 1: To investigate world trends in the consumption of SSB and dietary sugar among children and adolescents.

Research questions:

- **1.1** How have intake trends of SSB and dietary sugar changed over the past several decades among children and adolescents?
- **1.2** How do intake trends compare between various countries around the world, in particular between the United States and other international countries?

Aim 2: To assess the effect of dietary sugar intake (in particular fructose versus glucose) on biomarkers of subclinical inflammation by means of a systematic review and meta-analysis.

Research question:

- **2.1** Based on evidence from human interventional controlled studies, does the intake of dietary fructose (alone or in HFCS) contribute more to systemic inflammation than other dietary sugars?

Aim 3: To examine the prospective influence of dietary sugar intake in adolescence as measured by dietary records and urinary sugar biomarkers on the development of type 2 diabetes and systemic inflammation.

Research questions:

- **3.1** Is a higher intake of certain dietary sugars on the chemical level (fructose, glucose, sucrose) or sugar categories (free sugar, added sugar, total sugar) or sugar sources (SSB, juice, sweet products) in adolescence associated with a higher risk of developing T2D (as measured by

insulin sensitivity and increased fasting insulin) and systemic inflammation (as measured by an inflammatory score) in adulthood?

- **3.2** Is a higher excretion of urinary sugars (urinary sucrose, urinary fructose and the sum of both) in adolescence associated with a higher risk of developing T2D (as measured by insulin sensitivity and increased fasting insulin) and systemic inflammation (as measured by an inflammatory score) in adulthood?

Table 3-1. Research questions assessed by Studies I-III

Aim	Study	Chapter
I	I: World trends in sugar and SSB	5.1
II	II: Meta-analysis: sugar and inflammation	5.2
III	III: Dietary sugar and T2D/inflammation	5.3

4 GENERAL METHODOLOGY

4.1 Systematic literature review and meta-analysis

4.1.1 Methods and definition of systematic search and meta-analysis

The purpose of a systematic review is to provide a transparent and objective overview of all evidence available on a particular scientific question and attempts to identify, appraise and synthesize all the empirical data that meet pre-specified eligibility criteria on a certain scientific question.[228] An explicit, systematic and standardized method is selected, aimed at minimizing bias in order to produce more reliable findings.[228] Firstly, a question is defined and an objective method for answering this question is agreed upon. A search for relevant data begins, which can come from various selected study designs, including for example data from prospective cohorts or human intervention trials. If the research source meets eligibility criteria then further details from that study are extracted, e.g. outcomes, exposure, participants, methods, funding sources, etc. Extracted data can then be compiled to give an overall result, usually qualitatively by means of a narrative review. A systematic review presents findings from multiple sources of evidence in a summarized overview, making it more reliable and robust than individual studies. The more articles there are available to include in a review and the more comparable they are to each other, the higher the quality of evidence. This overall quality of evidence is rated by the researchers (as was done in Study II) who can then determine what reliable overall conclusions can be drawn.

A meta-analysis is a quantitative approach for systematically assessing the pooled results of previously published research in order to make conclusions about the body of research on a given subject.[229] Instead of using individuals as the basic units within analyses, studies themselves are used as units. Pre-defined inclusion and exclusion criteria are determined by means of a search to identify included studies in the meta-analysis. The results of the studies dealing with the hypothesis in question and the statistical analyses of these results are thoroughly reviewed. The results are typically displayed graphically as a pooled measure of association, with the individual estimates of the included studies. As seen in Study II, the diamond represents the summary or pooled measure of association.

4.1.2 Search strategy and terms

The following terms were used to identify all potentially relevant publications published in the English language used for the world SSB and sugar trends review (Study 1):

((“children” OR "Adolescent"[Mesh] OR adolescent* OR “adolescence” OR “youth” OR “school-aged” OR “pre-school aged” OR “toddler” OR “girls” or “boys” OR teenager* OR "Child"[Mesh] OR “child”) **AND**

(“Dietary Sucrose”[Mesh] OR "Fructose"[Mesh] OR "Dietary Sugars"[Mesh] OR “dietary sugar” OR “dietary fructose” OR “dietary glucose” OR sugar*[ti] OR “total sugar” OR “free sugars” OR “free sugar” OR “added sugar” OR “added sugars” OR “sugar intake” OR “sugars intake” OR "soft drink" OR “soft drinks” OR “sugar-sweetened beverage” OR “sugar-sweetened beverages” OR “SSB” OR "High Fructose Corn Syrup"[Mesh] OR “sweets” OR “food intake” OR “food intakes” OR “food consumption” OR “beverage consumption” OR “dietary intake” OR “nutrient intake” OR “nutrient intakes” OR “dietary intakes” OR “nutritional intake” OR “nutritional intakes” OR “dietary habits” OR “eating habits” OR “dietary pattern” OR “dietary patterns”) **AND** (“24-hour recall” OR “24-h recall” OR “24 hour recall” OR “twenty-four-hour dietary recalls” OR “twenty-four-hour dietary recall” OR “24-hour recalls” OR “24-h recalls” OR “24 hour recalls” OR “dietary record” OR “dietary records” OR “diet record” OR “diet records” OR “dietary recall” OR “dietary recalls” OR “diet recall” OR “diet recalls” OR “food recall” OR “food recalls” OR “dietary history” OR “diet history” OR “FFQ” OR “food frequency questionnaire” OR “frequency questionnaire” OR “urinary fructose” OR “urinary sucrose” OR “food consumption record” OR “food diary” OR “duplicate portion method” OR “nutrition surveillance survey” OR “nutrition survey” OR “nutrition surveys” OR “nutritional survey” OR “nutritional surveys” OR “diet survey” or “diet surveys” OR “dietary surveys” OR “dietary survey” OR “diet assessment” OR “dietary assessment” OR “diet assessments” OR “dietary assessments” OR “nutrition assessment” OR “nutrition assessments” OR “nutritional assessment” OR “nutritional assessments”) **AND** (“cross-sectional” OR “cross sectional” OR “prospective” OR “cohort” OR “cohorts” OR “longitudinal” OR “follow-up” OR “population survey” OR “trends”) **NOT** (“case-control”[tiab] OR “review”[tiab] OR animal* OR “mouse” OR “mice” OR rodent* OR “rats” OR “breastfeeding” OR “pregnancy” OR “trial”[ti] OR “randomized”[tiab] OR “controlled”[ti] OR “intervention”[tiab]))

The following terms were used to identify all potentially relevant publications published in the English language for Study II:

(“Dietary Sucrose”[Mesh] OR "Fructose"[Mesh] OR "sugar"[tiab] OR “sugars”[tiab] OR "sucrose" OR “fructose” OR "soft drink" OR “soft drinks” OR “sugar-sweetened beverage” OR “sugar-sweetened beverages” OR “SSB”OR "High Fructose Corn Syrup"[Mesh] OR “high fructose corn syrup” OR "HFCS" OR “dietary glucose” OR “glucose intake”) **AND** (("Inflammation"[Mesh] OR “inflammation” OR “inflammation” OR “inflammatory” OR "C-Reactive Protein"[Mesh] OR “CRP” OR “hs-CRP” OR “C-reactive protein” OR “high sensitivity C-reactive protein” OR "Interleukin-6"[Mesh] OR “IL-6” OR “interleukin 6” OR “interleukin-6” OR “IL6” OR "Interleukin-18"[Mesh] OR “IL-18” OR “interleukin 18” OR “interleukin-18” OR "IL18" OR "adiponectin"[Mesh] OR “adiponectin” OR “ADPN” OR "Intercellular Adhesion Molecule-1"[Mesh] OR “ICAM-1” OR "ICAM1" OR “Intercellular adhesion molecule-1" OR “CD54” OR "E-Selectin"[Mesh] OR “E-selectin” OR "ELAM-1" OR "selectin E" OR "CD62E" OR "Interleukin 1 Receptor Antagonist Protein"[Mesh] OR “IL-1RA” OR “IL1RA” OR “Interleukin-1 receptor antagonist” OR "Chemokine CCL2"[Mesh] OR “monocyte chemoattractant protein 1” OR “MCP-1” OR “MCP1” OR "CCL2" OR “monocyte chemoattractant

protein 1" OR "Tumor Necrosis Factor-alpha"[Mesh] OR "TNF-alpha" OR "TNFα" OR "Tumor necrosis factor alpha" OR "cachectin")) **AND** (randomized controlled trial[pt] OR controlled clinical trial[pt] OR randomized[tiab] OR randomised[tiab] OR randomization[tiab] OR randomisation[tiab] OR placebo[tiab] OR randomly[tiab] OR trial[tiab] OR group*[tiab] OR study[title]) **NOT** ("mouse"[tiab] OR "mice"[tiab] OR "rat"[tiab] OR "rats"[tiab] OR "exercise"[title])

4.1.3 Study selection process and inclusion/exclusion criteria

The study selection process for the systematic reviews conducted in Studies I and II are displayed below (see Figure 4-1 and Figure 4-2). Following the initial search of three databases, studies were removed based on listed exclusion criteria and their relativity. Full text reviews were made of the remaining reports, of which further reports were excluded based on additional criteria. Finally, all remaining studies plus independently found ones were included in the review.

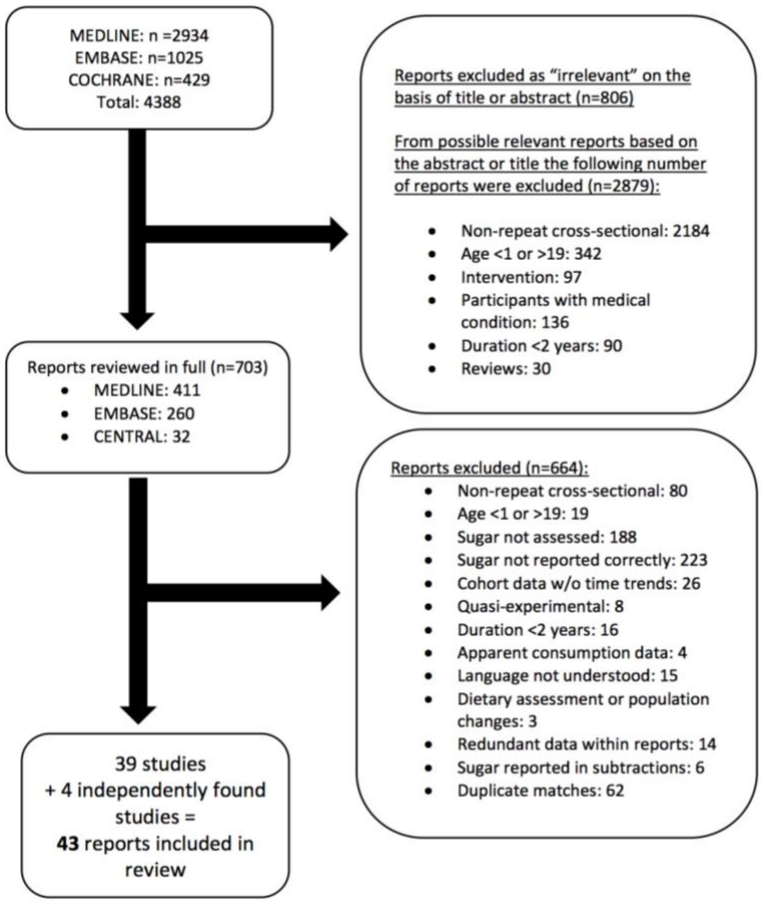

Figure 4-1. The study selection process for the world sugar trends systematic review (Study I)

For Study I, the search was restricted to repeat cross-sectional studies reporting intakes for a sample representative of the background population of the respective country or region or prospective studies, generally not representative, but which assessed time trends in intakes based on repeated data

of the same individual. Only children or adolescents were included within the age range of 1-19 years. Sugar measurements had to have been reported for a period of at least 2 years. Studies that used dietary assessment methods administered at an individual level (excluding FFQs) were included.

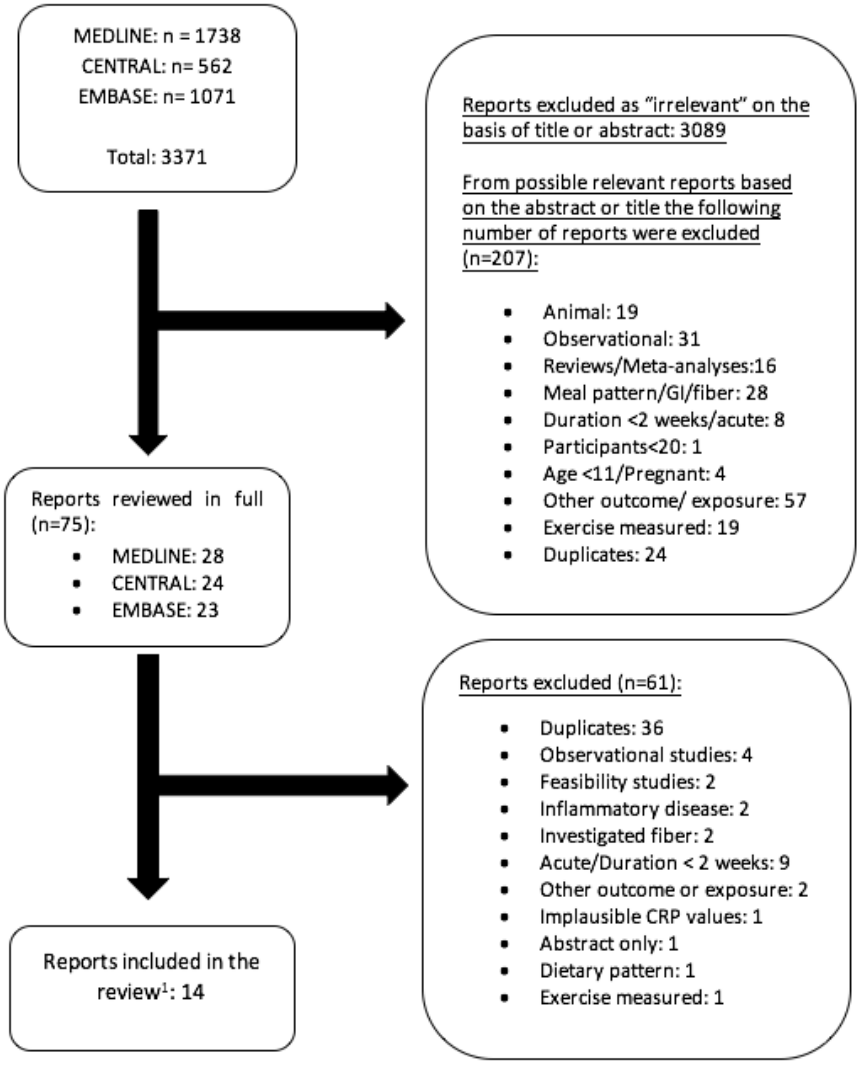

Figure 4-2. The study selection process for the systematic review and meta-analysis (Study II).

For Study II, the search was restricted to human intervention studies (controlled, parallel, or crossover design). Inclusion criteria limited search to either (1) healthy, overweight, or obese adults or adolescents (age 11 and up), (2) with or without diseases for which inflammation is not a major symptomatic factor. Inclusion criteria further limited search to studies in which (3) dietary fructose, glucose, or sucrose was administered as predictors (including information on intake amounts of respective sugars), and with (4) C-reactive protein (CRP); the proinflammatory cytokines IL-6, IL-18, and TNF-α; the anti-inflammatory IL-1RA; the chemokine MCP-1/CCL2; the soluble adhesion molecules ICAM-1 and E-selectin; and adiponectin as outcome measures.

4.1.4 Statistical methods of meta-analysis

A meta-analysis pools together either an absolute or a relative measure (e.g., hazards ratio, odds ratio) of association.[229] Further, a meta-analysis can use a fixed-effects model, or a random-effects model, the differences between the two relating to how well results can be generalized. The fixed-effects model is applied to the specific study populations used in the meta-analysis, and the random-effects model is used for a hypothetical population of studies making it statistically more conservative, as it takes into account not only within-study variance but also the variance between studies.[229, 230] A main assumption when pooling results from different studies is that results are homogeneous or consistent. When there is substantial heterogeneity between studies then neither the fixed- or the random-effects methods are advisable.[231, 232] There are various homogeneity tests available.

As detailed in the original publication, Study II applied a random effects model. Post-intervention means and corresponding standard deviations (if not available, standard errors or 95% confidence intervals) for intervention and control groups were pooled. As recommended by the Cochrane Handbook, post-intervention means were extracted where possible, or change from baseline values if the means were not available.[228] As the main outcome to be analysed in the meta-analysis, pooled effects of the different interventions were investigated as mean difference (MD) by subtracting control group mean values from intervention group mean values. A standard χ^2 test was used to test the heterogeneity between trial results. To measure inconsistency between study results, the I^2 parameter was used: $I^2 = 100\% \times (Q - df)/Q$, where Q is the χ^2 statistic and df is its degrees of freedom.[233] An I^2-value of greater than 50% was considered to indicate substantial heterogeneity.[234]

While quantitative approaches to summarize data are considered to provide stronger evidence, there are numerous problems related to the variability between the quality of included studies in a meta-analysis and the fact that different studies use different data collection methods and participant selection processes leading to possible forms of bias.[229] Therefore, results of meta-analyses should be reviewed judiciously and understood together with qualitative reviews of the literature. In Study II, to evaluate the quality of meta-evidence for the association between dietary sucrose, fructose, glucose, and HFCS on subclinical inflammation we applied the NutriGrade scoring system.[235] NutriGrade comprises the following items for meta-analysis of randomized controlled trials: (0–10 points): (1) risk of bias, (2) precision, (3) heterogeneity, (4) directness, (5) publication bias, (6) funding bias, (7) and

study design. Based on this scoring system, four categories were recommended to judge the meta-evidence: high (≥8 points), moderate (6 to 7.99 points), low (4 to 5.99), and very low (0 to 3.99).[235]

4.2 Population and design of the DONALD Study (Study III)

The DOrtmund Nutritional and Anthropometric Longitudinally Designed (DONALD) Study is an ongoing longitudinal (open cohort) study conducted by the Research Institute of Child Nutrition in Dortmund, Germany. In this study, detailed information concerning the development, diet, and metabolism of participants between infancy and adulthood has been collected since 1985. On the basis of these data, certain research questions can be examined. The main research aims of the study are:

- the collection of longitudinal data describing intra-individual and inter-individual dietary intake of healthy infants, children, adolescents and their families,
- the examination of complex interactions between dietary behaviour, food intake, health during growth development, nutritional status, and metabolism in individuals,
- the development and evaluation of nutritional recommendations and needs for infants, children and adolescents by taking medical and behavioural aspects into account and derivation of metabolic reference data from healthy individuals.[236, 237]

For these purposes, since 1985 approximately 40-50 infants have been newly recruited every year from the city of Dortmund and surrounding communities via personal contacts, maternity wards or paediatric practices. Their parents must be prepared to participate in a longitudinal study, and at least one parent must have sufficient knowledge of the German language. Due to the time and work expenses required for the regular participation in DONALD visits, mainly parents of higher education and socio-economic status enter into the study.

Children are invited to come to the study center (Research Institute of Child Nutrition in Dortmund (FKE)) for the first examination at the age of three months. Normally, each child comes in three more times for return visits in the first year, two visits in the second year and then only one time annually following that until adulthood. Generally, each visit includes anthropometric measurements, interviews on lifestyle, medical examinations, socio-demographic status and health issues. In addition, the participants are asked to fill out a 3-day weighed dietary record at home. These records should give information about the dietary habits, food and nutrient intake of the children. From the age of 3 years onward, the children are additionally asked to collect a 24-hour urine on the 3rd day of the 3-day weighed dietary record. The DONALD Study is entirely observational and no invasive measurements occur until the participants are 18 years of age and older, at which time fasting blood samples are taken at each visit (this has occurred since January 2005). Furthermore, 5 visits are scheduled for parents

wherein anthropometric measurements, medical examinations including blood pressure, as well as interviews about the parent's socio-demographic data, life-style and health status are collected.[236]

The DONALD study was approved by the Ethics Committee of the University of Bonn. All examinations were and continue to be performed with parental written consent and later on with children's written consent. The design of the DONALD Study is summarised in Figure 4-3 (adopted from Kroke et al.[236]).

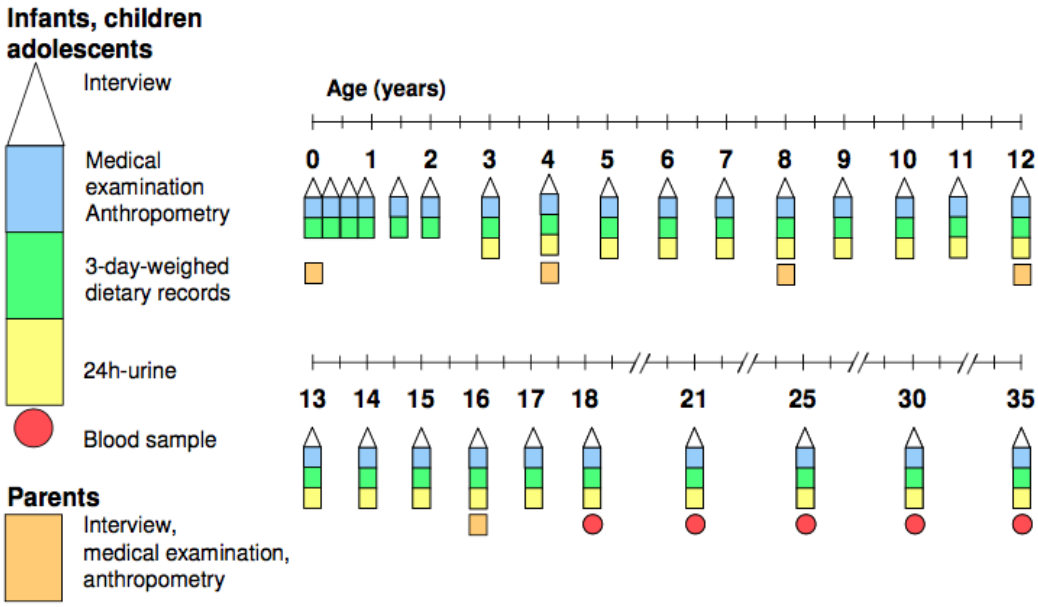

Figure 4-3 Design of the DONALD Study

4.3 Analytical study populations

The sample size for the analysis of the research question in the present thesis was determined by the DONALD Study design. Since 1985, more than 1300 children have been recruited and most children in the cohort actively and continuously participate until early adulthood, with a relatively low drop-out rate,[236] yet a small number of children drop out especially during puberty.[236] During the initial, beginning years of the study, children who were recruited into the DONALD Study differed considerably in age, i.e. information on the first few years of life was not always available. In addition, as the study is ongoing, some children have not yet reached the age at which specific outcomes are assessed. To answer the research questions (outlined in chapter 3), this thesis is based on different sub-samples of the total DONALD cohort. The following basic inclusion criteria applied to Study III:

- Singleton birth,
- Term born, i.e. gestational age 37-42 weeks,

- Birth weight >2,500g.

Multiple births, preterm infants or infants with a low birth weight were not considered, the reason being the concern that those children could differ in health and developmentally, which may lead to deviating growth patterns (e.g. catch-up growth) and special feeding regimes. These differences may have resulted in confounding of the diet-outcome relationships that are of interest. In order to be included in the final sample size, participants had to provide information on the influencing and outcome variables in question, as well as important confounders, which depended on the purpose of the analysis. For information on how the study sample for Study III was derived, please see the Methods section in Chapter 5 of Study III.

4.4 24-hour urine samples

Starting at the age of 3-4 years (when children have learned to use the toilet), 24-h urine samples are collected on the 3rd day of the 3-day weighed dietary record according to standardised procedures. Parents and children are instructed carefully and extensively on how to collect complete 24-h urine samples. The first micturition in the morning is discarded, defining the start of the-collection, and the full 24 hours end with the collection of the first urine the following morning. After the first micturition, all urine passed over the 24-hour period is collected in a container, including the first void on the following morning. If there are any omitted collections, they are specified. All micturitions are stored immediately in preservative-free, Extran-cleaned (Extran, MA 03; Merck Darmstadt, Germany) 1l plastic containers at <–12°C before they are transported to the research institute by a trained dietician. The exact time of each urination, as well as any other additional information on drug or supplement intake is noted on a urine collection form. After arriving at the institute, the containers are stored at ≤–20°C before being analysed. All urine samples undergo routine check using a commercial test strip (combur 9, Roche Diagnostics GmbH, Mannheim, Germany), after thawing and stirring, in order to determine volume, pH, osmolarity and creatinine. Aliquots of the urine samples are afterwards frozen for potential future analysis at –22°C.

For the purpose of Study III, the completeness of the 24-h urines was determined via sex- and age-specific body-weight-related reference values of creatinine [238] i.e. samples with a daily creatinine excretion rate below 0.1mmol/kg body weight were not considered. Furthermore, samples that were reported to contain incomplete micturitions (according to the urine collection diary) were excluded. The samples were thawed immediately before analysis.

Urinary fructose and sucrose were measured in the laboratory of the Department of Food & Nutritional Sciences at the University of Reading using LC-MS and quantified using stable-isotope

labelled 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. 100 µL of urine was combined with 100 µL acetonitrile containing the internal standards, vortex-mixed, centrifuged at 13000g for 10 min and the supernatant transferred into a 96-well plate for LC-MS/MS analysis. Samples were separated by HPLC (Acquity BEH Amide 2.1 x 50 mm, 1.7 µm column (Waters, Milford, MA, USA), kept at 35°C), using 80/20 (v/v) acetonitrile/water with 0.2 % NH_4OH as mobile phase (250 µL/min) using an Acquity UPLC binary solvent manager, sampler manager and column manager (Waters, Milford, MA, USA), and detected by tandem mass spectrometry using a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK). The mass spectrometer was operated with electrospray ionisation (ESI) in positive ion mode in multiple reaction monitoring mode. Nitrogen was used as the desolvation gas and argon was used as the collision gas. The following generic source conditions were used: capillary voltage, 3.6 kV; cone voltage, 35 V; desolvation temperature, 400 °C; source temperature, 120 °C, desolvation gas flow, 500 L/hr; cone gas flow, 100 L/hr. The concentration range was 0.1 to 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.

4.5 Dietary assessment

In the DONALD Study, dietary intake is assessed using weighed 3-day dietary records. The participants chose the first day of dietary recording within 8 weeks after the visit at the study center. Parents of the children are asked to weigh and record all foods and beverages as well as leftovers consumed by their child over three consecutive days to the nearest 1 gram with the help of regularly calibrated electronic food scales (initially Soehnle Digita 8000, Leifheit SG, Nassau, Germany; now WEDO digi 2000, Werner Dorsch GmbH, Muenster/Dieburg, Germany).

Parents are instructed by trained dieticians and a written example of a 1-d dietary record is left with the families as an example. Recipes for meals prepared at home are recorded and semi-quantitative recording (e.g. number of teaspoons, tablespoons, cups, pieces) is allowed when exact weighing is not possible, e.g. foods eaten away from home or snacks. However, previous analyses revealed that more than 90% of all foods consumed were weighed on more than 80% of the recording days.[239] Participants are asked to record the time and location of where and when the food was eaten. The packaging of commercial food products is collected and information on recipes for meals prepared at home are also recorded. At the end of the 3-day record period, a dietician visits the family,

checks the plausibility of the record using a structured questionnaire and enquires about unusual events which might have affected eating behavior during the data collection period.

Energy and nutrient intakes are calculated and analyzed at the FKE with the help of nutrient database LEBTAB ("Lebensmitteltabelle"),[240] which is continuously updated to include all recorded food items. It includes all food items (e.g. commercial food products, basic food items, infant food products or dietary supplements) ever recorded by DONALD participants and is based on information from the German standard nutrient tables, product labels or recipe simulation based on the labelled ingredients and nutrients.[241, 242] About 15% of the food items in LEBTAB are common basic foods, 75% are complex products. The data of the dietary records is coded and linked to LEBTAB to calculate the intake of 30 nutrients and energy for each child [368]. At the time of this analysis, LEBTAB contained more than 13,100 entries including additives, supplements and medicine, i.e. 1,207 basic food items and 10,832 composite foods.[236]

4.5.1 Dietary sugars

According to the purpose of the research questions, the following dietary variables were examined in Study III: fructose, glucose, sucrose, added sugar, free sugar, and total sugar. The weighed records allow the consideration of brand-specific sugar content in commercial products as well as sugars or sweetening agents like honey and syrups, which are used for home-based food preparation.

Table 4.1. Sugar definitions

Term	Definition
Total sugar	Sum of all monosaccharides and disaccharides in foods, therefore including sources such as fruits and vegetables, fruit/vegetable juices, and dairy products. [243]
Added sugar	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)[244]
Free sugar	All monosaccharides and disaccharides added to foods by the manufacturer, cook, or consumer, plus sugars naturally present in honey, syrups, and fruit juices, as well as sugars from vegetable juices, juice spritzers and smoothies [245, 246]

Sugar content and type recorded in the dietary record was calculated using the LEBTAB database.[240] Because free sugar is not included in LEBTAB, we expanded the definition from the WHO of free sugar as suggested by the SACN [76, 247] 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 vegetable juices, juice spritzers and smoothies were also considered to be free sugars in our study. In LEBTAB, the following foods were defined as added sugars: white sugar, brown sugar, raw sugar, corn syrup, corn-syrup solids, high-fructose corn syrup, malt syrup, maple syrup, pancake syrup, fructose sweetener, liquid fructose, honey, molasses, anhydrous dextrose, and crystal dextrose. Fruit syrups commonly used as sweeteners in Germany were also considered. Conversely, naturally occurring sugars such as lactose in milk or fructose in fruits were not included in the free sugars or added sugars definitions.

4.5.2 Energy underreporting

In Study III, only dietary records considered plausible were included. The reported energy intake was used as a surrogate measure for the overall quality of the dietary records by relating it to the basal metabolic rate (BMR). BMR was estimated using the equations of Schofield [248] that include, age, sex, weight and height. Age- and sex-specific cut-offs for the ratio between energy intake and BMR were used to identify implausible records. These cut-offs were calculated for children by Sichert-Hellert et al. [249] for the three recording days. Adolescents are prone to underreporting energy intake, therefore records were checked for energy underreporting. In Study III, the number of records in which energy levels were underreported came to 209 (12.6%), were collected from 109 participants, and were excluded for sensitivity analyses; i.e. sensitivity analyses were based on 1446 records from 277 participants. Underreporters were not excluded from the main 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.

4.6 Anthropometric measurements

In the DONALD Study, anthropometric measurements are performed according to standard procedures [250] by trained nurses, who undergo an annual quality control check in which intra- and inter-observer agreement is carefully monitored. In addition, all instruments are routinely calibrated.

The age of the participant determines the number of anthropometric measurements. Those that were relevant for this thesis, however, are performed at each visit and include weight, height, and skinfold thickness with the children dressed in underwear only and barefoot. Body weight is assessed to the nearest 100 g with a supine infant weighing scale (Mettler PS 15, METTLER TOLEDO, Columbus, OH, USA) or an electronic scale for subjects in standing position (Seca 753 E, Seca GmbH & Co KG, Hamburg, Germany). Standing height is measured to the nearest 0.1 cm with a digital telescopic wall-mounted stadiometre. Skinfold thickness is measured from the age of 6 months

onwards on the right side of the body at the biceps, triceps, subscapular and suprailiac sites to the nearest 0.1 mm using a Holtain calliper (Holtain Ltd., Crymych, UK).

In study III, BMI was calculated using the formula weight / height^2 (kg/m^2). Overweight was defined according to the International Obesity Task Force (IOTF) BMI cut-offs for children, which correspond to an adult BMI of 25 kg/m^2.[251] Percent body fat (%BF) was calculated using the Slaughter equations.[252] Sex- and age-independent standard deviation scores (SDS) were calculated using the German reference curves for BMI.[253]

4.7 Medical examination, interview and parental data

A typical medical examination conducted at each visit included routine questions, some of which related to acute illnesses since the last visit, physical activity, preventive measures and the use of medical services sleeping habits, and food preferences. If children are found to suffer from a disease that could impact growth and development then they are excluded from the study. At certain ages additional medical examinations are performed, including blood pressure measurements, assessment of pubertal stage, developmental, lung and muscle function tests, which are, however, irrelevant for this thesis.

At several points during the course of their participation, parents are asked to provide information about family characteristics (e.g. number of people living in household, smoking in household), their educational status and employment, and children's behaviour and attitudes. High educational status is defined in this thesis as greater than 12 years of schooling. Parental weight and height are measured at regular intervals by the same trained nurses who assess the anthropometrics of the participating children. Information on birth weight and length as well as gestational age is extracted from the "Mutterpass", a standardised document given to all pregnant women in Germany.

4.8 Collection of blood parameters

Venous blood samples were drawn after an overnight fast, centrifuged at 4°C within 15 min 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 was calculated.[238]

4.9 Statistical considerations (Study III)

All statistical analyses were carried out using the Statistical Analyses System SAS (versions 8.2 and 9.1.3, SAS Institute Inc., Cary, NC). Due to the fact that many predictors were used which could lead to multiple testing issues, a p-value of <0.01 was considered statistically significant, and a p-value of <0.05 was considered to be a trend.

4.9.1 Exposure and outcome variables

The main exposure variables in Study III were dietary fructose, glucose and sucrose, free sugar, added sugar, and total sugar intake as well as urinary sugars in adolescence. These sugars were selected on the basis of chemical sugar types (fructose, glucose, sucrose), and sugar use (total sugar, added sugar, free sugar), as well as excretions of urinary sugars (excreted fructose and excreted fructose + sucrose as a sum). The outcome variables included the parameters of insulin sensitivity (fasting insulin and HOMA2-%S) and separately for the pro-inflammatory score.

4.9.2 Statistical models

Linear regression models (PROC GLM in SAS)

The method of linear regression is a statistical technique used to assess the association between two continuous variables, i.e. to determine whether they are linearly associated.[229] Such a technique assesses whether these variables are statistically compatible with a perfect straight line and estimates the best fitting straight line to describe an association.[254] Simple linear regression models consider the effect of one independent exposure variable on a dependent outcome variable and multiple linear regression models consider more than one independent variables.[255] A linear regression gives the equation of the straight line that best describes how the outcome variable increases or decreases with an increase (of 1 unit) in the exposure variable(s).[256] Multiple regression techniques allow the examination of the effects of all exposure variables and simultaneously adjust for all the other variables in the model.[229] This approach has been used in Study III and details on the used linear regression models can be found in the methods section of Chapter 5.3.

4.9.3 Consideration of potential confounding

A confounder distorts the effect that is of direct interest, because the observed association can be attributed to a confounding variable or factor. Confounding variables associate with the exposure and the outcome, yet do not lie on the exposure outcome causal pathway.[254, 257] This is particularly the case in all non-randomised research, such as in the observational data used in Study III. In order for variables to qualify as confounders, a factor must (**Figure 4-4**) [256]:

- be causally associated with the outcome (i.e. even in the absence of the exposure of interest),
- be associated with the exposure of interest (non-causally or causally), and
- not be an intermediate between exposure and the outcome, i.e. it must not lie on the causal pathway.

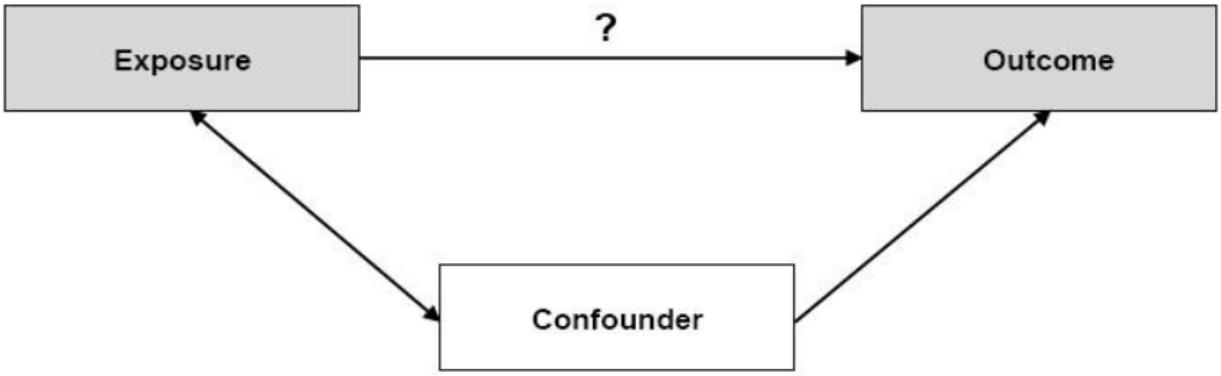

Figure 4-4 Definition of confounding

Confounding can lead to either an overestimation of the true strength of an association (positive confounding) or an underestimation of the association (negative confounding).[229] Confounding has the potential to hide an association that really exists as well as reverse the direction of an effect.[256] In the prospective Study III, a hierarchical approach based on a conceptualized framework similar to what has been proposed by Victora et al.[258] was employed for the selection of relevant confounding variables. This approach is intended to adequately reflect the complex hierarchical interrelationships of multivariable models.[258] The conceptual hierarchical framework was constructed by individual examination of potential influencing covariates and hierarchical inclusion 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, gestational age, maternal age at birth, full breastfeeding ≥ 4 months, and gestational weight gain (kg)], (2) socioeconomic factors and parental health status [smokers in the household, paternal school education ≥12 years, parental overweight and parental history of diabetes, (3) predictor-specific

adolescent data [BMI, percent body fat, age, flavonoid intake, glycemic index (GI) and energy-adjusted fiber intake in models with the dietary predictors sugar intake]. For biomarker analyses, urinary variables (24h-creatinine excretion (mmol/d), 24h-urea excretion, urine volume, excreted hippuric acid were also considered. In conditional models we additionally included adult body fat (%) to investigate whether observed associations were partly attributable to body composition in adulthood. For more details on these statistical methods see Chapter 5.3.

Residual confounding occurs when adjustment does not completely remove the effect of confounding due to a given variable or set of variables, thus causing confounding to persist after adjustment for inadequately measured confounding variables. It can occur when either the categories of the confounder controlled for are too broad, resulting in imperfect adjustment, or when some confounding variables remain unaccounted for. Measurement error in confounding variables that leads to residual confounding as well as the failure to measure confounding variables (leading to unmeasured/unaccounted confounding) are major challenges for valid inferences in epidemiological studies.[257, 259] As such confounding variables can affect and even falsify the exposure-outcome association,[254] careful choice of potential confounding variables, and their accurate measurement and adjustment in multivariate analysis are critical steps to minimize the likelihood of confounding.

4.9.4 Effect modifiers and mediators

Effect modification (or "interaction") is considered present when the effect of the exposure on the outcome differs based on whether a third factor is present or not (dichotomous variables), or when it depends on the level of this factor (continuous variables).[256] Since, as a result, the association of interest is not the same at different levels of an effect modifier, the relationship has to be presented at each level of the effect modifier. In Study III, effect modification by sex was evaluated using appropriate interaction terms and by conducting stratified analyses (for details, see Chapter 5.3). 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.

By contrast, mediators are intermediate variables lying on the pathway between exposure and outcome, and can thus serve to explain the underlying mechanisms of their association. For this reason, adjusting for intermediary variables has to be treated with caution and should only be conducted in a separate step as was done in a conditional model in study III. If an association is eliminated by adjustment for a mediator, this suggests a potential mechanism involving the mediator, but does not question the result obtained before. In this thesis, adult body fat at the time of blood withdrawal

represents a potential mediator between diabetes and inflammatory markers and adolescent dietary sugar intake (Study III).

4.9.5 Energy adjustment methods

Dietary sugar intakes were adjusted for energy intake in study III, since total energy intake is an important potential confounder when diet-disease relationships are investigated in epidemiological studies. Since most nutrients, especially the energy-contributing macronutrients such as carbohydrates, are positively correlated with total energy intake, any association with the outcome could thus simply be due to confounding by energy intake (the effect of a nutrient per se is assumed to best be addressed when isocaloric diets are compared). This is further complicated by the observation that the composition of diets may vary by level of total energy intake, depending on the behaviour of the population. These reasons further highlight the need to consider total energy intake when interpreting associations between specific nutrients and disease outcomes in epidemiologic studies.

Nutritional factors can be examined in terms of absolute intake (crude intake) or in relation to total caloric intake, depending on the biologic relationship and other public health considerations. If a nutrient is metabolized in proportion to total energy intake (such as with dietary sugar), nutrient intake is most likely biologically important in relation to energy intake. If a nutrient affects the organ of a system selectively that is uncorrelated with body size or if physical activity does not affect its metabolism then absolute intake may be most relevant.

Willett [260] introduced four methods to adjust for total energy intake: the residual method, the multivariate nutrient density method, as well as the standard multivariate and the energy partition method. In the residual method, nutrient intakes are computed as the residuals from the regression model, with total energy intake as the independent variable and absolute nutrient intake as the dependent variable (see Figure 4-5). The resulting residuals represent the differences between actual intake and intake expected for each individual with mean caloric intake. The nutrient residuals by definition provide a measure of nutrient intake uncorrelated with energy intake, i.e. in this way any variation in nutrient intake is due only to the nutrient composition of the diet. In study III, the residual method (the studentized (mean=0, standard deviation=1) residuals) was used for the examination of the relation of dietary sugars with risk markers for T2D.

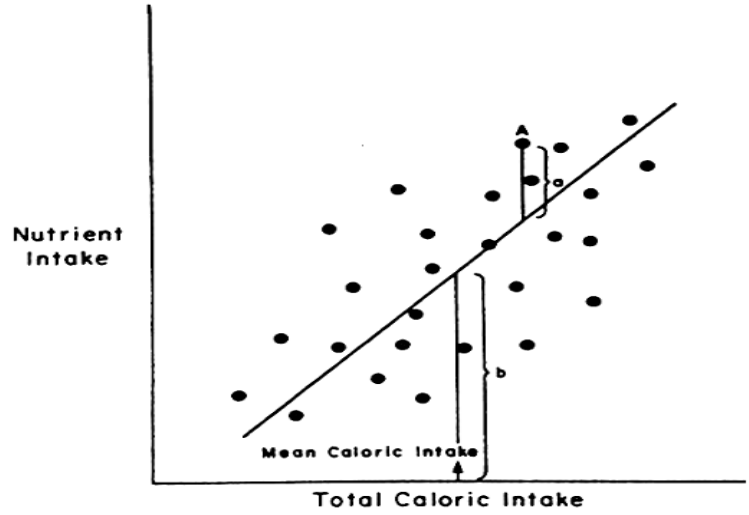

Figure 4-5. Adjusting for total energy intake – the residual method. Calorie-adjusted intake = a + b, where a = residual for subject from regression model with nutrient intake as the dependent variable and total caloric intake as the independent variable and b = the expected nutrient intake for a person with mean caloric intake.[260]

5 STUDIES

5.1 Study I - World Trends in Sugar-Sweetened Beverage and Dietary Sugar Intakes in Children and Adolescents: A Systematic Review

5.1.1 Summary

This review aims to provide a systematic overview of world dietary sugar and SSB intake trends in children and adolescence. Medline, Embase and the Cochrane Central Register of Controlled Trials in the Cochrane Library were searched through January 2019 to identify longitudinal follow-up studies with time trend data and repeat cross-sectional studies. Data from studies reporting ≥ 2 measurements (sugar, SSB or sweets/candy) over a time period ≥ 2 years and included ≥ 20 healthy, normal- or overweight children or adolescents aged 1-19 years. Data from 43 articles (4 prospective cohort studies; 39 repeat cross-sectional studies) from 15 countries (8 European countries, Australia, Canada, China, Korea, Mexico, Russia, and US) are presented narratively. According to the 'risk-of-bias-in-non-randomised-studies-of-interventions' (ROBINS-I)-tool 34 studies were judged to have a moderate risk of bias, and 5 to have a serious risk of bias. Consumption among US children and adolescents rose substantially in the decades preceding 2000, followed by a faster and continued decline. As a whole, other international intake trends did not reveal drastic increases and decreases in SSB and dietary sugars but tended to change only slightly across three decades.

5.1.2 Introduction

The effect of dietary sugar intake on health has been a topic of continual controversy, especially among children and adolescents where intake values are thought to be high. The magnitude of increase in child and adolescent obesity[261] gives rise to these concerns. As rates of chronic disease incidence among children and adolescents continue to increase,[3-11] there is rising interest in trends and changes in diet over time that may play causal roles in the development of these diseases. Dietary sugars and SSB are discussed as a major culprit since they are consumed in considerable amounts in the Westernized diet and adolescents generally consume more added sugars (i.e. discretionary sugars and those added to processed foods) than other age groups.[14, 15] Furthermore, avoiding overweight and obesity in younger years by limiting excessive energy intake of SSB and dietary sugars is relevant for risk of later cardio-metabolic diseases.[97-100]

Some national data suggest decreases in sugar and SSB consumption in the US,[50] yet this has not been systematically established and it is not clear whether dietary sugar and SSB intakes from

other international countries have decreased in tandem with the US or whether their consumption is still on the rise as a characteristic of a continued adoption of a Westernized diet. Furthermore, as revised dietary guidelines become available regarding consumption of dietary sugars, estimations of world dietary intake trends are increasingly required in order to evaluate progress of public health initiatives taken to implement these recommendations. In the past, monitoring often drew on either supply or apparent consumption data,[16] or on national dietary surveys to determine within-country trends.[17] Supply data are, however, not suitable for assessing consumption patterns for children and adolescents as it is not possible to measure different subgroups of the population,[34] apart from the fact that food waste is not accounted for. A study by Wittekind et al.[17] on world trends in dietary sugar intake assessed within country trends using national dietary survey data; however, this was not a systematic review. World intakes of SSB and dietary sugar have yet to be reviewed systematically in children and adolescents.

Therefore, the present review aims to provide a systematic overview of world dietary sugar (i.e. total, added, free, etc.) and SSB intake trends by collating repeated cross-sectional trend data together with some longitudinal follow-up studies with time trend data specifically among children and adolescents. US data are analysed as a starting point due to the broader amount of available trend data that exist, followed by other international data. This timely effort provides estimations on the direction of sugar and SSB intake trends in order to inform national nutrition policy, monitor progress towards meeting dietary guidelines, and provide a benchmark for ongoing surveillance in the critical age periods of childhood and adolescence.

5.1.3 Methods

This systematic review has been registered in PROSPERO (ID: CRD42018111823) and was conducted according to MOOSE guidelines for systematic reviews of observational studies.

Search Strategy

For a comprehensive review of the literature, we conducted a systematic search of the Medline (http://www.ncbi.nlm.nih.gov/pubmed/), the Cochrane Central Register of Controlled Trials (CENTRAL; in the Cochrane Library; http://onlinelibrary.wiley.com/cochranelibrary/search/) and Embase (https://www.elsevier.com/solutions/embase-biomedical-research) library databases on January 11, 2019. The following terms were used to identify all potentially relevant publications: sugar category terms (dietary) sugar, SSB, sucrose, high fructose corn syrup (HFCS), fructose, sweets), together with terms referring to children and adolescents, as well as dietary assessment methods and study design.

The literature search was conducted independently by three investigators (KDC, BM, AG). The detailed study selection process is illustrated as a flow diagram (see Figure 4-1 in methodology section).

Selection of studies

Inclusion criteria were:

- repeat cross-sectional studies reporting intakes for a sample representative of the background population of the respective country or region or prospective studies, generally not representative, but which assessed time trends in intakes based on repeated data of the same individual.
- normal or overweight healthy (i.e. without medical condition). Studies solely focused on obese and unhealthy children were excluded.
- children and adolescents between the ages of 1 and 19; i.e. all data on adults >19 y or infants <1 y were excluded.
- studies with at least two reported sugar measurements over a time period of at least 2 years.
- studies with diet assessed by a method administered at the individual level (such as weighed food record, 24 h recall, dietary history). Reports that assessed sugar intake by food frequency questionnaires (FFQ) were excluded because they lack detailed information on how food was prepared, which can lead to inaccurate assessments of grams of sugar consumption.[262, 263] The exception to this was when servings of SSB were reported, as such values can be converted and quantified.

All included studies were available in the public domain and reported summarized data (no analysis of raw data). We excluded studies that:

- estimated population intakes through apparent consumption data such as supply and demand reports.
- had quasi-experimental designs.
- were performed on participants with a medical condition (e.g. coeliac disease, diabetes, inflammatory bowel disease, etc.).
- had a duration between assessment years or follow-up times <2 years.
- included <20 participants.

- did not assess any of the included sugar categories, or did not report absolute sugar intake amounts in units per time (studies which solely performed statistical calculations on reported intake values).
- reported sugar intakes as portions sizes (with the exception of SSB) or fractions of total daily intake of sugar.
- had redundant or identical data that had already been published in other included reports.
- reported results in languages other than English except for those that had data summarized and reported in published reviews.
- Abstract only papers and unpublished studies.

A detailed listing of all inclusion and exclusion criteria can be found in our current PROSPERO registration.

Data extraction and presentation

Three investigators (KDC, JF, BM) independently reviewed and extracted relevant data from each included report. Extracted data included information on name of study, study type (national, regional or local), sugar category, location, assessment year, duration of covered period including start and end year, sample size, sex, age or age range, dietary assessment method, mean daily sugar intake or servings per day of SSB, and units of intake. Where available, information on total daily energy intake, percentage of total energy of intake from sugar (%E), distribution parameters, performed statistical analyses, statistical significance, funding source and conflicts of interest were also extracted.

Data are presented as provided in the reports and publications. In studies of question, authors were contacted to determine if other cross-sectional data existed, no such studies qualified for inclusion. Divisions by age range and sex are presented as reported in original publications. Sugar categories include total sugars, added sugars, SSB (non-diet), sucrose, mono- and disaccharides, free sugar, non-milk extrinsic sugars (NMES) and sweets. Presentations of data in tables and graphs are made by dividing studies into two geographical categories: “US” and “other international.” Extracted data are presented in three forms: tables, narrative text descriptions, and graphs.

All tables (Table 5-1 and Tables S1-S4 in supplementary material) present sugar intake values per age group and assessment year. Mean results are summarized for each assessment period and distribution parameters such as 95% CI, interquartile range, standard deviations or standard error of the mean are also included in the representation of dietary sugar intake data. Where mean values are not provided, median values are presented. All energy intakes that were not reported in kcal/day were converted to this unit. If %E intakes from sugars were not provided and total energy intake was

reported, a calculation was made to determine %E from sugar. Due to differences in dietary assessment methodologies and categorization of sugars as well as age/sex categorical differences between studies, statistical analyses were not performed. Type of study, sugar category, covered period of time, dietary assessment method, reported units and distribution data are also presented in the tables. In order to estimate time trends, the direction of change in absolute sugar amounts was determined and represented in tables by the following signs: ↑, intakes increased; ↓, intakes decreased; ↔, intakes remained stable. An asterisk (*) positioned next to an arrow indicates that a statistical test was performed in the original study and the change between years was reported as statistically significant.

Graphs (Figures 5-1 through 5-6) were designed to judge trends and provide an optical overview. The Figures 5-1 and 5-3 display trends from all age groups in order to compare intakes as a whole between the US and the rest of the world. Figures 5-2, 5-4, 5-5 and 5-6 display intakes of SSB separated by age so as to compare intake trends between age groups. The figures/graphs were derived from available data, therefore the range and number of countries depicted varied. Age groupings also varied based on available data, but could generally be separated into younger childhood, middle childhood and adolescence for the US, and childhood and adolescence for other international reports. Years are listed on the X-axis, and daily consumption in kcal on the Y-axis. If intake levels were measured over multiple years per cross-sectional, then the year number plotted was the average of the range of years reported. Conversions were made in order to present all studies graphically in the same units of kcal. Dietary sugars were converted from grams to kcal by a conversion factor of '× 4'. For SSB that were not reported in kcal, 1 serving was assumed to be 360 ml (1 can), the density 394 g, and energy 150 kcal per can.[264]

Narrative text descriptions of the findings focus on the depiction of the results in the graphs. Additional narrative summaries of the tables can be found in the supplementary material where details on the observed consumption levels are recorded (see Tables S1-S4 in supplementary material). In Table S5, additional information about the statistical significance is presented, including which analysis test was performed (if reported in the original publication), as well as information on the full name of each study and explanations of sugar categories if reported.

Risk of bias assessment

Risk of bias (RoB) was assessed using the 'Risk Of Bias In Nonrandomised Studies – of Interventions' (ROBINS-I) tool,[265-267] taking into account the following domains: Bias due to confounding, bias in selection of participants into the study, bias in classification of the intervention (exposure), bias due to deviations from the intended intervention (exposure), bias due to missing data,

bias in measurement of outcomes, and bias in selection of the reported results. These domains were judged as being at 'low', 'moderate', 'serious', 'critical' or 'unclear' risk of bias and reasons were documented for each bias classification. RoB was assessed by one author (KDC), and verified by a second authors LS. Information on sources of funding as well as conflicts of interest in each study were also extracted and included in Table 5-1 and in Table S5 in the appendix.

5.1.4 Results

Study selection process and characterization of studies

A total of 4388 articles were identified through search databases and of these, 703 were eligible for full text reviews on the basis of titles and abstracts. A total of 664 of these full texts were excluded. Four additional articles were found through independent/hand search by October 2019 after the formal search in January of 2019 was conducted. Hence, 43 articles were identified as matches and included in this review, 39 of which are repeat cross-sectional studies and 4 prospective studies. Of the 39 cross-sectional reports, 24 addressed SSB (10 US, 14 other international), 21 addressed other dietary sugars (14 other international, 7 US), while 9 studies reported on sweets/candy (5 other international, 4 US) (see Table S4 on sweets & candy in appendix). Of the 4 prospective studies (3 other international, 1 US), 2 addressed dietary sugars and 2 SSB (see Table 5-1).

The 43 included studies reported sugar intakes from 15 countries around the world, comprising 8 European countries together with 3 Asian countries, Australia, the US, Canada and Mexico. Of the 17 US reports, 13 were national, 1 regional and 3 local. Of the 26 other international reports, 16 were national, 5 regional and 5 local. In the US, reported years ranged from 1965 to 2016, in Europe from 1950 to 2012, in Asia from 1991 to 2011, in Australia from 1995-2012, in Canada from 2004-2015 and in Mexico from 1999-2012. Age of participants of both US and other international studies ranged between 1-19 years and reported age groups within this range varied. Eighteen studies reported the age range of 1 to ~6 years, 25 studies reported the age range of ~5 to 11 years, and 25 studies reported the age range of ~11 to 19 years. A broader age group ranging from about 2-19 years included 9 reports. Types of dietary sugars identified by the search include total sugar, added sugar, mono-/disaccharides, free sugar, NMES, sucrose, fructose, candy and sweets. Types of SSB included sugary drinks, soft drinks, soda, and added sugar/NMES from beverages.

Trends in SSB Consumption

United States

The years reported by the 10 US cross-sectional reports assessing SSB intakes ranged from 1965-2014.[60, 268-276] Of these 10 studies, 7 reported on SSB, 2 on soft drinks and one on added sugars from beverages.

Overall, Figure 5-1 illustrates that SSB consumption among US children and adolescents increased from the 1960's until the turn of the century (~year 2000) followed by a substantial and continued decrease since then (Figure 5-1: data on females in green, data on males in blue, and data on both males and females in black). These trends were similar for all three age groups (see Figure 5-2, same color coding as Figure 5-1), though the increase and decrease in SSB occurred on a higher level and were more pronounced for 11-19 y old adolescents. Trends for wide age groups of 2-18 y are analogous to those of the separate childhood and adolescent age groups. For details on the observed consumption levels please refer to Table S1 and accompanying narrative review in the appendix.

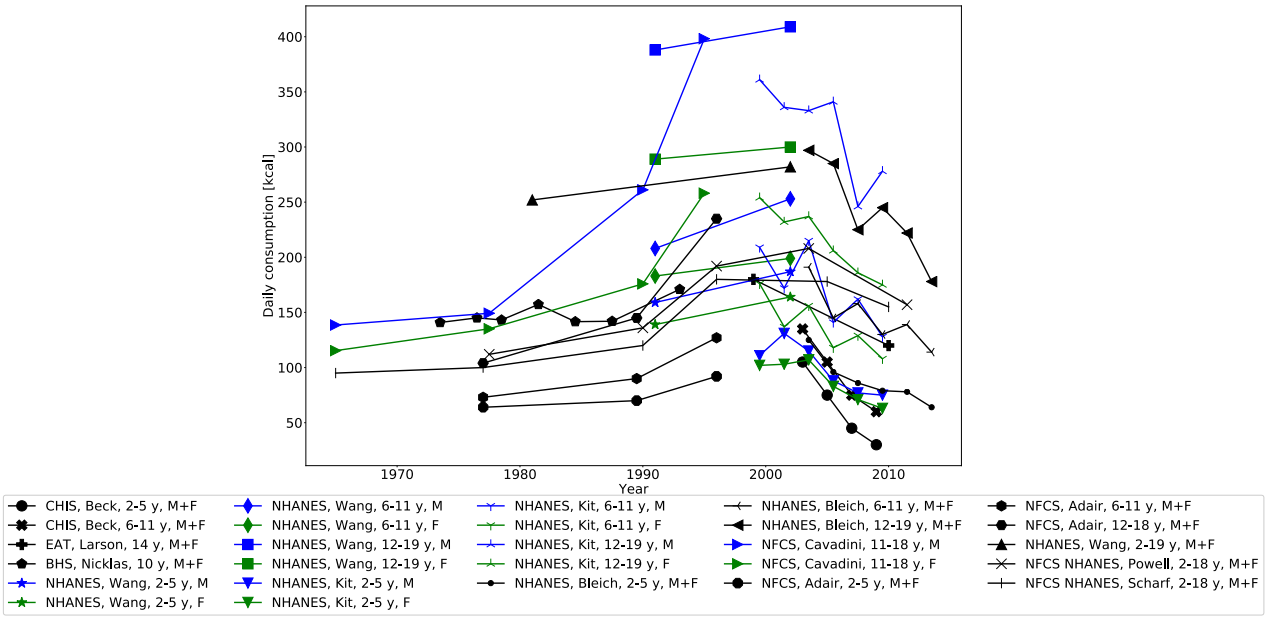

Figure 5-1 US intake trends of sugar-sweetened beverages (in kcal) among all age groups between 2 and 19 years from 1965 to 2014.

Intakes increased until peaking at year 2000, then reversed and declined rapidly. Abbreviations: BHS, Bogalusa Heart Study; CHIS, California Health Interview Survey; EAT, Eating Among Teens; F, female; M, male; NFCS, National Food Consumption Surveys; NHANES, National Health and Nutrition Examination Survey.

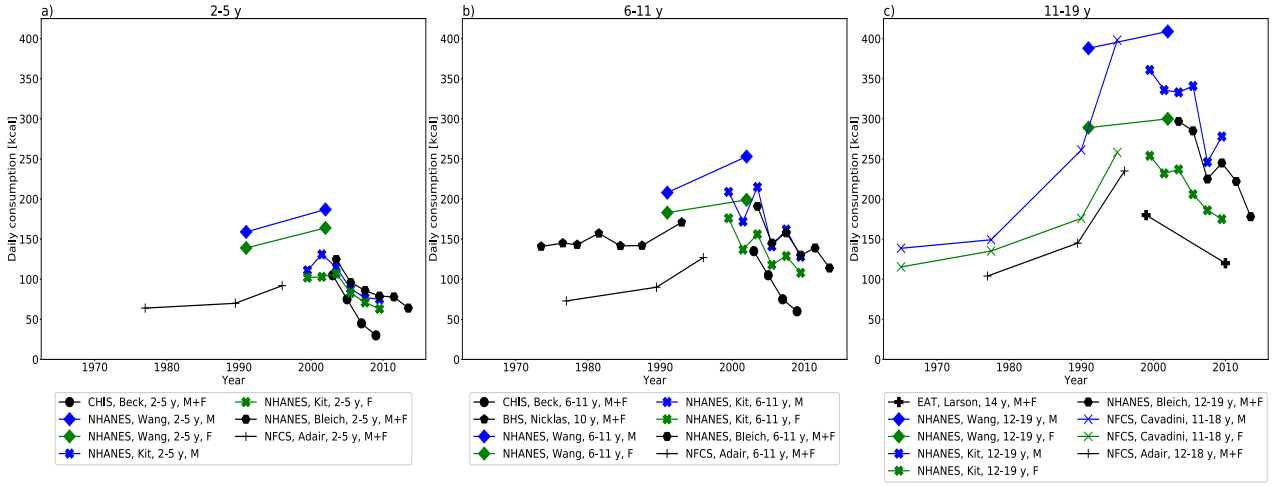

Figure 5-2 US intake trends of sugar-sweetened beverages (in kcal) separated into age group panels.

(A) 2–5 years, (B) 6–11 years, and (C) 11–19 years are shown. Intake amounts increased with age, as indicated on the y-axis. (C) The rise and fall of intake trends was most dramatic among the adolescent age group. Abbreviations: BHS, Bogalusa Heart Study; CHIS, CHIS, California Health Interview Survey; EAT, Eating Among Teens; F, female; M, male; NFCS, National Food Consumption Surveys; NHANES, National Health and Nutrition Examination Survey.

Other International Countries

The years reported by the 14 cross-sectional reports assessing SSB intakes in countries outside the US ranged from 1991-2015 [87, 269, 277-286] (excepting one report from year 1950).[287] Of these, 7 reported on SSB, 5 on soft drinks, 1 on NMES from soft drinks and 1 on soda.

Trends in SSB consumption varied by age and country (see Figures 3 & 4). Looking at childhood intakes, SSB consumption tended to decrease or stay stable from 1990 to 2015 in the 1-5 y and 6-11 y old children of Canada, UK, Russia, Australia, Korea, and China (see Figure 4). Intakes of SSB among children of 4 y in the UK increased steadily from 1950 to 1993.[287] In Mexico, however, intakes decreased in 1-5 y old children only and increased significantly among 6-11 y old children from 1999 to 2012.[279] As shown in Figure 4, SSB consumption for adolescents ages 11-19 years tended to increase or stay stable across 1991 to 2012 in Australia,[278] UK,[281] Spain,[282] China,[269] Russia,[269] Mexico[279] and Korea.[285] In Norway, there was a clear decrease from 2001 to 2008.[283] For Mexico and one Korean report intakes increased significantly from 1999-2012 and from 2001 to 2009, respectively.[279, 285] Wide age range studies from Australia, the UK, and China all reported decreases in SSB covering the years of 1995 to 2012, [284, 286] (Brand-Miller et al.[278] study not included in world SSB figures, because it only reported %E changes). For details on the observed consumption levels of other international SSB studies please refer to Table S2 and accompanying narrative review in the appendix.

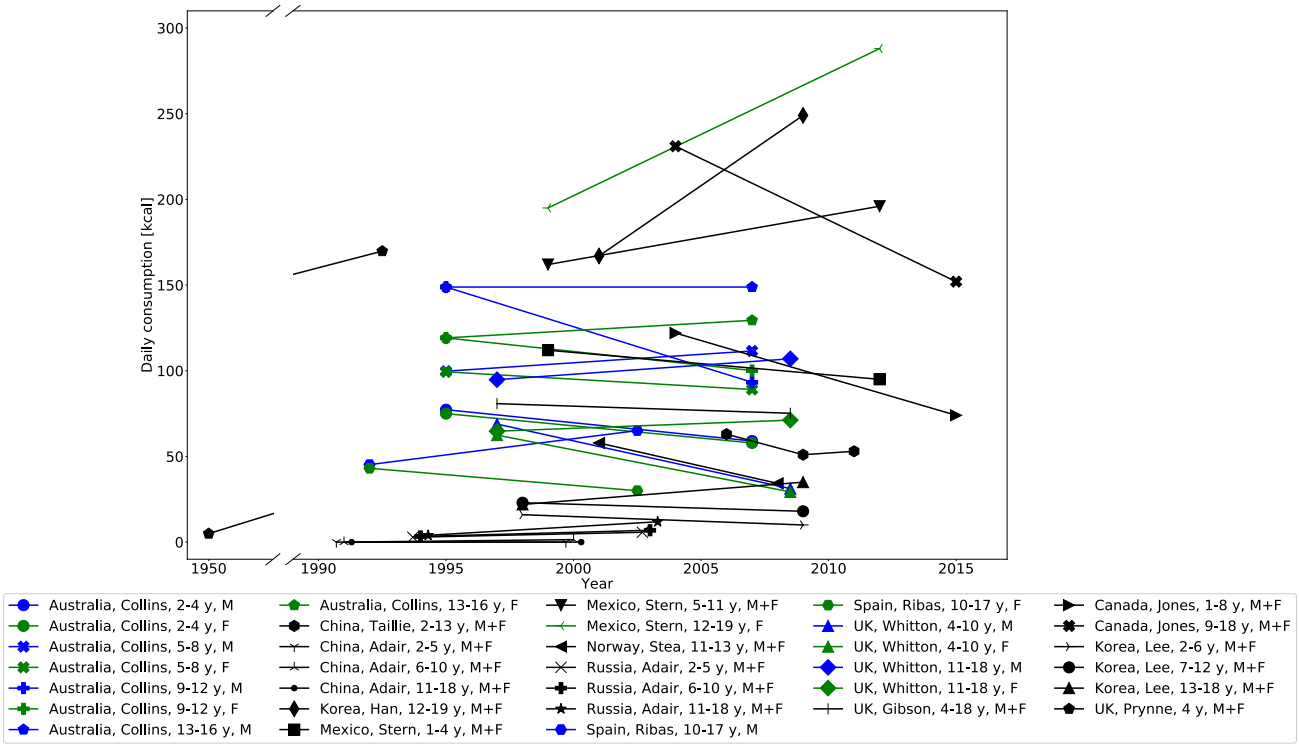

Figure 5-3 Other international intake trends of sugar-sweetened beverages from 1950 to 2015 among all age groups between 1 and 19 years are shown.

Trend data from Australia, China, Mexico, South Korea, Norway, Russia, Spain, the United Kingdom, and Canada are depicted. Intake trends tended to remain stable among most countries. In Mexico and South Korea, intakes increased substantially in the years after 2000, whereas in Canada, intake decreased. Intake levels among 4-year-old old children increased considerably from 1950 to the 1990s. Abbreviations: F, female; M, male.

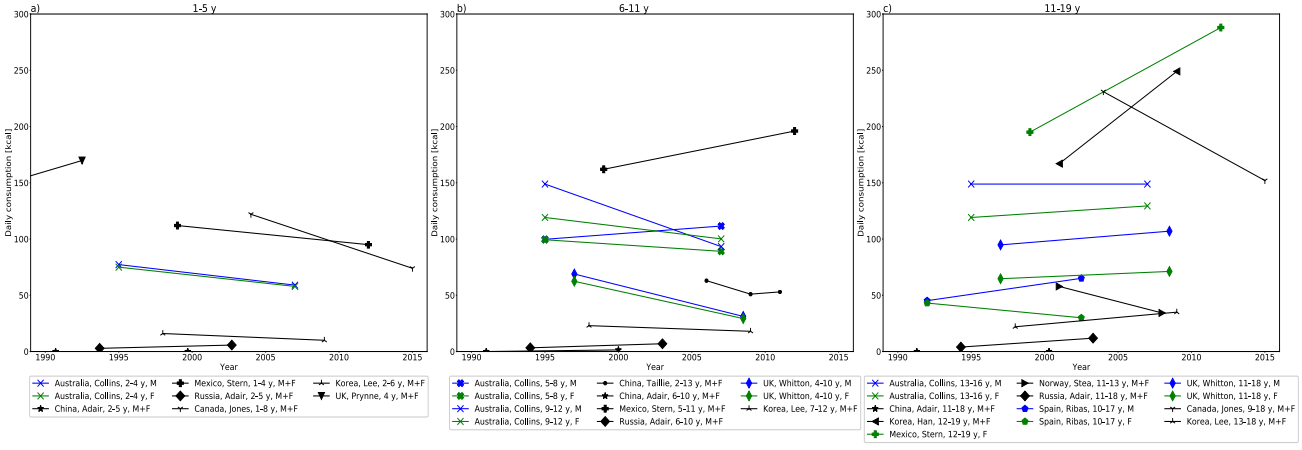

Figure 5-4 Other international intake trends of sugar-sweetened beverages separated into age group panels.

(A) 1–5 years; (B) 6–11 years, (C) 12–19 years. Intake trends decreased or stayed level among the younger age groups, except in Mexico among children ages 5–11 years, where they increased. (C) Caloric levels are highest among adolescents, and intake trends did not decrease but remained generally stable, whereas they increased in Mexico and South Korea. In Canada, intake levels decreased among 9- to 18-year-old youth. Abbreviations: F, female; M, male.

Trends in Dietary Sugars Consumption

United States

The years reported by the 7 US cross-sectional reports[15, 50, 275, 288-291] assessing dietary sugar intakes ranged from 1973 to 2016. Three studies reported on added sugar and 3 on total sugar.

Included US dietary sugar intake data are not as comprehensive compared to that of SSB, yet some trends can be identified in Figure 5. For added sugar (depicted in green), national data for 2-5 y and 2-18 y report that consumption increased significantly from the 1970's until late 1990's/early 2000[275] when intakes peaked then began to decline significantly[50] (Kranz et al.[290], not included in graph, reported similar trends in %E among 2-5 y old children in this time period). The Bogalusa Heart Study on 10 y old children reported overall stable intakes in total sugar (depicted in red) from 1974 to 1994,[15] which when compared to NHANES data in 2003, continued to remain stable.[291] For 2-5 y and 6-11 y old children, national data reported an increase in added sugar from 1994 to 2004 followed by a decrease until 2010.[289] For 12-19 y old adolescents of this study, the decrease in added sugar started as early as 1994 and continued through 2010. Caloric intake levels of added sugar were nearly the same for 6-11 y and 12-19 y old children from 2003 to 2016.[291] Decreases in added sugar and total sugar occurred in all studies starting ~year 2000 onward [50, 275, 288-291] (excepting 2-4 y olds in one study where intakes remained stable).[288] For details on the observed consumption levels please refer to Table S1 and accompanying narrative review in the appendix.

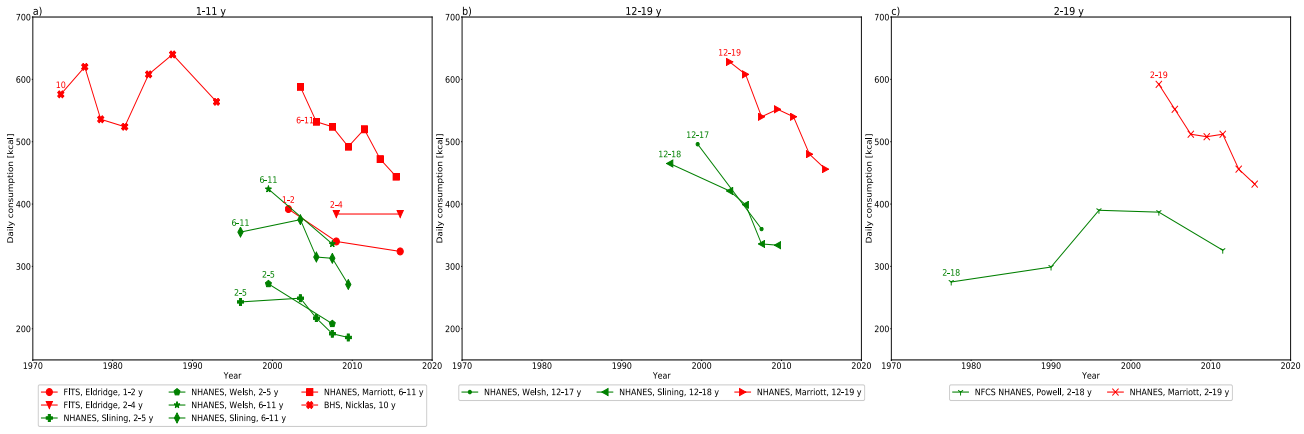

Figure 5-5 US intake trends of total and added sugars (in kcal) among children and adolescents aged 1 and 19 years from 1973 to 2016.

Age groups are separated into panels as follows: (A) 1–11 years; (B) 12–19 years, and (C) 2–19 years to improve comparability among world intake trends displayed in Figure 6. Data on added sugar are depicted in green and data on total sugar in red. Ages are noted next to each line. Consumption trends in added sugar increased until the late 1990s (as seen among the broad age group in panel C) and then

decreased in all studies among all ages groups along with post-2000 total sugar intakes. The Bogalusa Heart Study reported high total sugar intakes among 10-year-old children in the 1970s to 1990s. All studies reported on boys and girls. Abbreviations: BHS, Bogalusa Heart Study; FITS, Feeding Infants and Toddlers Studies; NHANES, National Health and Nutrition Examination Survey.

Other International Countries

Dietary sugar intakes were reported by 14 repeat cross-sectional studies originating from countries other than the US (9 national, 3 regional, 2 local).[278, 281, 286, 287, 292-301] Besides the country of Australia, all of these studies reported from European countries, including Denmark, Finland, Germany, Ireland, the Netherlands, Spain, and the UK. Dietary sugars assessed within these studies varied (6 total sugar, 2 added sugar, 2 sucrose, 2 mono-/disaccharides and 2 NMES). The years reported by the 12 European studies ranged from 1980 to 2012 with the exception of one study reporting as far back as 1950. The two Australian-based studies reported on the years 1995-2012.

Overall, Figure 6 illustrates that intake amounts of dietary sugars tended to remain stable in many of the included European countries over time (color-coded as data on females in green, data on males in blue, and data on both males and females in black). Only in Ireland were drastic increases in total sugar intake reported in the 1990's.[296] Among wide age groups, on the other hand, steady declines in dietary sugars were observed from 1995 to 2012 in Australia, 1995-2006 in Denmark, and from 1997 to 2012 in the UK.[278, 292, 293, 301] For details on the observed consumption levels please refer to Table S3 and accompanying narrative review in the appendix.

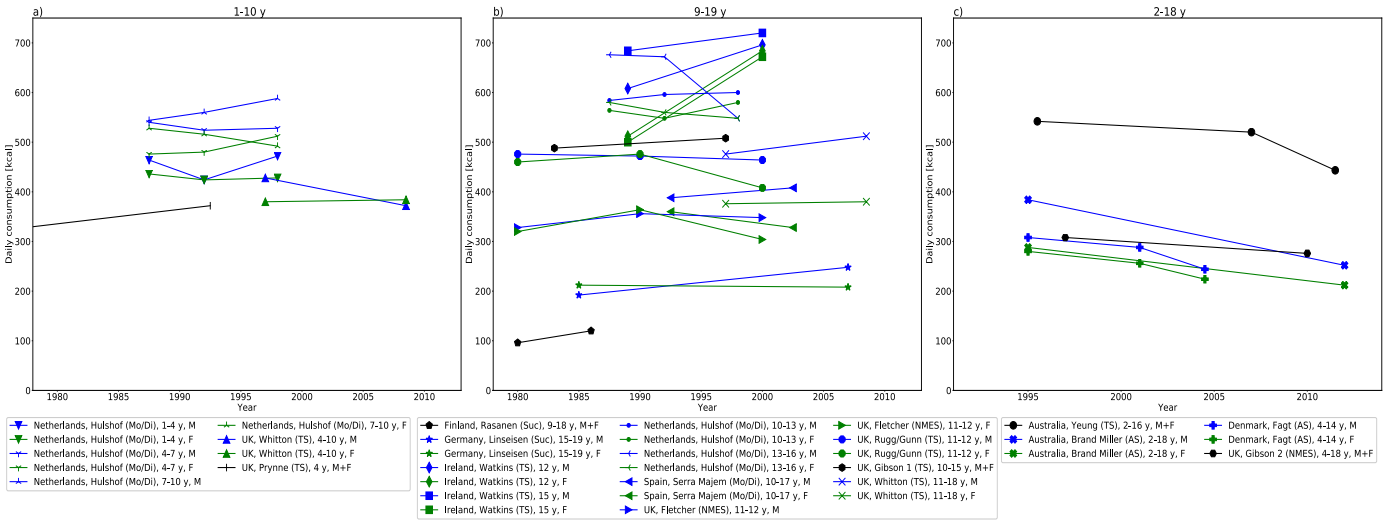

Figure 5-6 Other international intake trends of various dietary sugars from 1950 to 2012 separated into panels according to age groups.

(A) 1–10 years, (B) 9–19 years, and (C) 2–18 years. Trend data from Australia, Denmark, Finland, Germany, Ireland, Netherlands, Spain, and the United Kingdom are depicted. Intake trends tended to remain stable among most countries in both children and adolescents. In Ireland, dietary sugar intakes

increased sharply. (C) Intake levels in Australia, Denmark, and the United Kingdom decreased gradually among broad age groups. Abbreviations: AS, added sugar; F, female; M, male; Mo/Di, mono- and disaccharides; NMES, non-milk extrinsic sugars; Suc, sucrose; TS, total sugar.

Longitudinal Reports

Dietary sugar and SSB trends have been assessed by prospective longitudinal studies in which statistical time trends analyses were performed, controlling for concurrent age trends. Four such studies were identified by this systematic review, one of which reports from the US,[302] one from Norway,[303] and two from a cohort in Germany.[52, 304] In the US, the Eating Among Teens (EAT) study reported an increase in SSB from about 1999 to 2004.[302] In Norway, intakes of SSB decreased from 2001 to 2005 among 12-15 y old participants of the Fruits and Vegetables Make the Marks (FVMM) study.[303] In Germany, the Dortmund Nutritional and Anthropometric Longitudinally Designed (DONALD) study performed a time trend analysis on 8-16 y old children from 1988 to 2007 where intakes of added sugar from sources other than beverages or sweets increased, yet intakes of total added sugar and added sugar from beverages rose significantly only among males. Another more recent DONALD analysis on time trends of total, added, and free sugar intake among 3-18 y old youth from 1985 to 2016 confirmed an increase in total and free sugar until 2005, followed by decreases most notably since 2010.[52]

Table 5-1. Prospective time trend data on consumption of SSB and other dietary sugars from Germany, the US, and Norway

Germany (DONALD) [304]	Local 8-16 y	Total added sugar (Total AS) AS from beverages AS from sweets AS from other sources Single 3d weighed record Mean (SD); %E 1988-97	Baseline: M: 1982 F: 1672	 M: F: M: F: M: F: M: F:	Total added sugar: (n=100): 65 (34) g; 12.9% (n=116): 59 (29) g; 13.9% Added sugar from beverages: (n=100): 18 g (21); 3.6% (n=116): 17 g (19); 3.8% Added sugar from sweets: (n=100): 30 (18) g; 6% (n=116): 27 (17) g; 6.4% Added sugar from other sources: (n=100): 17 g (13); 3.3% (n=116): 15 g (10); 3.6%	Agency	Total AS: M: *↑ F: ↔ AS from beverages: M: *↑ F: ↔ AS from sweets: M: ↔ F: *↓ AS from other sources: M: *↑ F: *↑
	8-16 y	1998/02	M: 2078 F: 1648	 M: F: M: F: M: F: M: F:	Total added sugar: (n=100): 76 (41) g; 14.5% (n=116): 64 (32) g; 15.2% Added sugar from beverages: (n=100): 26 g (29); 4.9% (n=116): 19 g (21); 4.5% Added sugar from sweets: (n=100): 29 (22) g; 5.5% (n=116): 28 (18) g; 6.8% Added sugar from other sources: (n=100): 21 g (14); 4% (n=116): 16 g (12); 3.8%		
	8-16 y	2003/07	M: 2126 F: 1696	M: F: M: F: M: F: M: F:	Total added sugar: (n=100): 84 (37) g; 15.8 % (n=116): 62 (32) g; 14.2 % Added sugar from beverages: (n=100): 24 (25) g; 4.5% (n=116): 17 g (24); 3.7% Added sugar from sweets: (n=100): 32 (23) g; 5.9% (n=116): 22 (15) g; 5.2% Added sugar from other sources: (n=100): 29 g (19); 5.4% (n=116): 22 g (13); 5.1%		

Germany (DONALD) [52]	Local 3-18 y	Total sugars Median (IQR); %E 1985-1994	Baseline: M: 1596 F: 1395	M: (n=280) F: (n=291)	Total sugars: 27.0 (8) Added sugars: 12.7 (7) Free sugars: 16.1 (8) Total sugars: 27.3 (8) Added sugars: 12.4 (8) Free sugars: 16.2 (9)	Agency / Agency-related conflict of interest	Since 2010: TS: *↓ AS: *↓ FS: *↓
	3-18 y	1995-2004	M: 1722 F: 1481	M: (n=334) F: (n=354)	Total sugars: 27.5 (9) Added sugars: 13.2 (8) Free sugars: 17.9 (9) Total sugars: 27.6 (9) Added sugars: 12.9 (7) Free sugars: 17.4 (8)		
	3-18 y	2005-2016	M: 1745 F: 1527	M: (n=406) F: (n=374)	Total sugars: 26.1 (9) Added sugars: 12.1 (6) Free sugars: 16.5 (9) Total sugars: 25.8 (10) Added sugars: 12.0 (12) Free sugars: 16.3 (8)		
US (EAT) [302]	Local 12.8 y	SSB FFQ Mean serv/d 1999	--	M+F:	0.86	Agency	↑
	17.8 y	2004	--		0.97		
Norway (FVMM) [303]	Regional 11.8 y	SSB Single questionnaire Mean times/week 2001	Baseline	M+F:	(M: n=195, F: n=242): 2.8	Agency	↓
	15.5 y	2005	Year 4		(M: n=195, F: n=242): 2.4		

* Significant change in respective sugar/SSB intake between years tested. See Table S5 in the appendix for further details. %E Percentage of total energy intake. -- Not reported. † Funding sources. Agency: funding from government, university, or not-for-profit health agency sources. Perrar et al reports conflict of interest from author AEB as a member of the International Carbohydrate Quality Consortium (ICQC). [x] See Table S5 in the appendix for full study name. [1] Unless otherwise indicated

Risk of Bias

With the use of the ROBINS tool, 34 studies were judged as having a moderate risk of bias, 5 were judged as having a serious risk of bias, and 4 did not provide enough information to make a judgement. We defined all observational studies to be moderately biased as a default in at least two domains which caused there to be no study judged as having a low risk of bias. The bias due to confounding was generally rated as moderate because of the inherent potential confounding bias of observational studies. Bias due to the measurement of the outcome was also generally rated as moderate because results were based on self-reported sugar intake data. The domain dealing with exposure assessment could not be determined since the exposure was time. The bias due to misclassification could also not be determined since the outcome did not involve any classification (e.g. disease onset yes/no). The 5 studies which had a serious risk of bias reported intake trends that follow trends from other studies published in the same period of time and location.

Conflict of Interest

Ten of the 43 included studies declared either industry-based funding or an industry-related conflict of interest that at least one author from each study reported. In analysing the results of those 10 studies that were either industry-funded or simply industry-affiliated separately and comparing them to similar studies published in the same period of time, it was found that they do follow the same expected trends as reported by studies with agency-related CoI.

5.1.5 Discussion

Estimations on the direction of SSB and dietary sugar intakes in the US among children and adolescents can be confirmed systematically. SSB consumption among US children and adolescents rose substantially in the decades preceding the peak year 2000, followed by a faster and continued decline, with changes occurring on a higher level for adolescents compared to children. For added sugar levels in the US there was also a rise and fall observed yet not as marked as SSB. By contrast, other international intake trends as a whole did not reveal drastic increases and decreases in SSB and dietary sugar consumption as observed in the US but tended to remain stable.

Other International vs US

Patterns similar to SSB consumption trends in the US were only observed in the countries of Canada, UK and Australia. Therefore, health campaigns to reduce SSB consumption may have only been effective in these countries. Other influences such as economic changes, availability, and cultural norms may have also had an impact on observed trends. SSB intakes in other countries across the world tended to remain stable from about 1990 to year 2010. These differences may in part be attributable to the fact that baseline levels of SSB intakes in kcal were remarkably higher in the US at least among adolescents when compared to other regions of the world. Of note among adolescents from other international countries, rather than observing declines in SSB intakes in the decade following year 2000 as seen in the US, intakes were generally stable, yet among adolescents in Korea and Mexico they markedly increased (i.e. for these countries the concern that SSB consumption may be on the rise does indeed hold true).

Similarly, other international dietary sugar intakes tended to rise gradually or remain stable from 1980 through the year 2007/09 (except for Ireland, where increases rose quickly in the 1990's) among both children and adolescents. In Australia, the UK, and Denmark, gradual declines in dietary sugar intake were observed from the mid 1990's to 2010/12 among broad age groups spanning childhood and adolescence.[278, 286, 292] This contrasts with added sugar intakes in the US where increases peaked between the years 1995-2000 among all childhood and adolescent age groups[50, 289] then declined substantially until 2010, remaining higher overall than baseline intake levels from 1977.[275] In addition, studies from other regions of the world report on intake levels until 2010 only, however, a recent longitudinal study from Germany [52] (see Table 5-1) indicates that while sugar intake levels increased until 2005 then started to decrease, the most notable decrease was since 2010. It is hence possible that decreases observed in the US will occur within the next decade in many other countries around the world.

Dietary sugar trends vs SSB trends

For SSB, consumption levels rose substantially in the decades preceding the peak year 2000, followed by a dramatic drop until the year 2012. Similar – yet less pronounced – declines were also seen for total and added sugar, presumably driven by the decrease in SSB consumption. After seminal work by Mattes and colleagues [77-80] and Rolls and colleagues [81-83] showing that consumption of SSB does not suppress the intake of other food calories to prevent weight gain, public health and biomedical scholars have focused on the relationship

between SSB and increasing obesity trends particularly in children and adolescents.[58, 65, 67, 85-87] While the cause for the reversal and decline in SSB was surely multifactorial based on economic fluctuations, social norms, and availability of sugar worldwide, it can be conjectured that this public focus on SSB and weight gain and resulting public health initiatives may have effectively influenced changes in consumption levels as reported in this review. The increased public concern and interest surrounding the obesity epidemic in the US, spurred by a Surgeon General's report on the topic of obesity released in 2001,[305] may have influenced trends in SSB and added sugar intakes leading to the reversal in trends around the year 2000. Other efforts from scientific communities were active in this period of time and promoted the awareness of the negative effects of SSB on health.[306] In 2001 California began to impose legislation on schools to restrict access to SSB, and according to a review in 2005, 34 additional states in the US also had restricted access.[307] Although intake levels have decreased in the US and other countries, worldwide reported intakes almost always exceed the recommendations given by either the local authorities or the World Health Organization.

Strengths and Limitations

There were notable strengths and limitations in this review. The authors acknowledge that there is a marked absence of available data from developing countries where it is debated that large changes in sugar consumption are occurring.[308] No studies from Africa were identified by our search where, according to cross-sectional data from South Africa, sugar intake levels are were 11%E among 1-9 y old children in 1999[309] and SSB intake levels among 15-24 y olds were 20 %E in 2012.[310] These adolescent/young adult intake levels are greater than average adolescent SSB consumption levels in the US from 2000 to 2010.[60, 274] It can be argued that the data from this review derive from relatively few countries in the developed world, limiting the degree to which these findings may be considered worldwide. We also acknowledge the post-hoc nature of our decision to compare other international countries only to the US. The high number of included studies from the US (17 out of 43) and the fact that these studies contrasted so greatly from those of other international countries led us in making this decision. Another limitation is the absence of statistical analyses performed on the extracted data. This was due to the variability of the categorization/definitions of sugars as well as the age/sex categorical differences between studies. Only statistical significance of changes in intakes as reported in the original studies are included. Additionally, years reported by the included studies varied and the terms employed to define sugar and SSB were diverse, limiting the ability to compare trends between countries.

Notwithstanding the weaknesses, the present study provides international estimates on intake trends across multiple decades of not only dietary sugar but also SSB consumption from countries around the world including North America, Asia, Europe, and Australia. Our review allows some comparisons between the US and other parts of the world, unlike other reviews where only within-country trends were analysed.[17, 51] Moreover, this initiative was performed by means of a systematic review, whereas other reviews on sugar intake only used national reports from a specific source,[17, 51] and did not include SSB trends in their reviews. A strength of this review is its specific focus on childhood and adolescence, thus targeting vulnerable age groups where research on intake trends over time is sparse. Furthermore, US intake trends are reported, providing a broad overview not only from national data but other studies in order to confirm trends systematically. This review uses dietary intake data assessed at an individual level using dietary assessment methods with the highest reliability in order to increase accuracy. No food supply data or apparent consumption data is included as it does not reflect actual intake amounts and cannot report by age. Methods of dietary analyses employed in the included studies did not change between assessment periods (two exceptions being Linseisen et al.[295] and Prynne et al.,[287] both assessed as having serious risks of bias for this reason), meaning that small changes in dietary intakes across assessment years could not be attributed to changes in the method of dietary assessment.

The ROBINS tool reported that the majority of our included studies had a moderate risk of bias, and several had a more serious risk of bias. Accordingly, the certainty of evidence has to be judged as limited since no study was rated with a low RoB. It should however be noted that observational studies relying on self-reported sugar intake data will by definition be rated as moderate RoB; yet this is presently the only source of data available for trend analyses. Cross-sectionals generally carry a moderate risk of bias due to the variation of the study population across time and unreliability of dietary assessment methods across time. We did however, also consider evidence from prospective cohort studies, which are vulnerable to selection and attrition bias, yet intake levels are assessed in the same persons across time. We regarded it informative to consider both types of studies which are affected by different potential sources of error, so as to have a more solid basis for our conclusions.

There is a possibility that these trend data are biased by underreporting added sugar intake, this was not measured or accounted for in most of the studies. Underreporting may be specifically strong for SSB which became increasingly undesirable socially as described above. However, it appears implausible that this misreporting should be a phenomenon largely restricted to the US, Australia, Canada and the UK. It could be argued that this review may be

affected by a publication bias because unpublished studies were not included and a 'NOT' statement was used excluding animal and intervention studies, thereby decreasing the breadth of reported trend data. In view of the extraordinarily large number of publications on sugar we deemed it necessary to refrain from attempting to include unpublished studies because identifying and accessing unpublished food intake reports from countries all around the world is an overwhelming task and the availability/findability of the studies is unpredictable compared to a systematic search (also considering that reports are often not available in English). Although funding sources as well as conflicts of interest that are industry-related have been reported to bias the results due to industry sponsors' financial interest in research conclusions,[311] we observed among our results that industry-sponsored studies followed the same expected trends as reported by agency-related studies.

Public Health Conclusions

As decreases in dietary sugar and SSB consumption in the US, the UK, Australia and other countries are observed, the implications of these dietary shifts on human health are unclear. Further research into whether rates of chronic disease have declined in conjunction with decreased intakes of dietary sugar and SSB is warranted. In view of dietary guidance concerning the consumption of dietary sugars, it is important to continually assess and review recent sugar and SSB intake trends from different countries to determine whether populations are responding to public health initiatives. In addition, cross-country comparisons would be informative in order to learn why intakes are decreasing in some countries while increasing or staying stable in others. This appraisal and monitoring is especially needed among children and adolescents who are in critical stages of physical development.

For future dietary surveys, it would be beneficial for policy guidance if employed assessment methods used a uniform way of expressing sugars that has public health significance and can compare to other areas in the world. The most useful terminology has been suggested to be added and/or free sugar. Measuring total sugar is of little informative value for public health nutrition because total sugar (and mono-/disaccharides) can come from sources such as milk and fruits, the consumption of which should be encouraged. As health recommendations are mostly given for added and free sugar, these sugars should be appraised separately. Future research providing more insights into the actual food groups providing these sugars is needed, as well as research on whether these food sources have changed, so as to learn which public health measures have been effective. There is also a call for biomarker data from urinary sugars in order to reduce measurement errors from self-reported methods of dietary assessment.

Conclusion

In conclusion, trends in SSB consumption among US children and adolescents rose substantially in the decades preceding the peak year 2000, followed by a faster and continued decline. The rise and fall of US intake trends of added sugar were not as marked as SSB. As a whole, other international intake trends did not reveal drastic increases and decreases in SSB and dietary sugars as observed in the US but tended to remain stable or change slightly across three decades. The ROBINS tool judged 34 of the studies to have a moderate risk of bias, and 5 to have a serious risk of bias. Further research on world SSB and sugar intake trends in children and adolescents is needed in order to determine whether populations are responding to public health initiatives and to assess the implications of these dietary shifts on human health.

Resulting publication:
Della Corte, K., Fife, J., Gardner, A., Murphy, B. L., Kleis, L., Della Corte, D., Schwingshackl, L., LeCheminant, J. D., & Buyken, A. E. (2020). World trends in sugar-sweetened beverage and dietary sugar intakes in children and adolescents: a systematic review. Nutrition reviews, nuaa070. https://doi.org/10.1093/nutrit/nuaa070
KDC Contribution: KDC organized and conducted the systematic search, extracted the data, assimilated the results and wrote the manuscript.

5.2 Study II - Effect of dietary sugar intake on biomarkers of subclinical inflammation: A systematic review and meta-analysis

5.2.1 Summary

It has been postulated that dietary sugar consumption contributes to increased inflammatory processes in humans and that this may be specific to fructose (alone, in sucrose or in HFCS). Therefore, we conducted a meta-analysis and systematic literature review to evaluate the relevance of fructose, sucrose, HFCS and glucose consumption for systemic levels of biomarkers of subclinical inflammation. MEDLINE, EMBASE, and Cochrane libraries were searched for controlled intervention studies that report the effects of dietary sugar intake on (hs)CRP, IL-6, IL-18, IL-1RA, TNF-α, MCP-1, sICAM-1, sE-selectin or adiponectin. Included studies were conducted on adults or adolescents with ≥20 participants and ≥2-wk duration. 13 studies investigating 1141 participants were included in the meta-analysis. Sufficient studies (≥ 3) to pool were only available for (hs)CRP. Using a random effects model, pooled effects of the interventions (investigated as mean difference (MD)) revealed no differences in (hs)CRP between fructose intervention and glucose control groups (MD: -0.03 mg/L (95% CI: -0.52, 0.46), I^2= 44%). Similarly, no differences were observed between HFCS and sucrose interventions (MD: 0.21 mg/L (-0.11, 0.53), I^2= 0%). The quality of evidence was evaluated using Nutrigrade and was rated low for these two comparisons. The limited evidence available to date does not support the hypothesis that dietary fructose, as found alone or in HFCS, contributes more to subclinical inflammation than other dietary sugars.

5.2.2 Introduction

Chronic, low-grade inflammation is a key factor in the pathogenesis of cardiovascular disease [312] and is associated with the risk of developing diabetes [313, 314], dementia **[315]**, and depression **[316]**. Also, low-grade inflammation is related to a higher risk of all-cause mortality in old age **[317]**. Therefore, identifying modifiable risk factors that could effectively lower chronic inflammation would contribute to the prevention of chronic disease.

According to observational data reports, it has been consistently reported that dietary sugar intake (more specifically sugar-sweetened beverages (SSB)) may be one stimulus of subclinical inflammation as measured by the inflammatory marker C-reactive protein (CRP) **[23-26]**. Dietary sugar is consumed in significant amounts in Western diets. In a review of the sugar consumption of 18 developed countries it was found that total sugar intake as a percentage of energy ranged between 13.5-24.6% in adults **[51]**. In the United States, Nationwide Food Consumption Surveys (NHANES) have suggested that the percentage of sweeteners from high-

fructose corn syrup (HFCS) increased from 16% in 1978 to 42% in 1998 and then stabilized [318]. A similar trend pattern was also observed for total fructose intake as a percentage of carbohydrates **[53]**. The most recent data has shown that with increased public awareness, the consumption of added sugar in the United States has actually decreased between 1999 and 2008 from a mean of 18.1% of total energy to 14.6% **[50]**. Overall sugar energy intakes are, however, still much higher than the United Kingdom's Scientific Advisory Committee on Nutrition (SACN) guidelines, which recommend a maximum free sugars intake of 5% daily energy intake [28], and the World Health Organization (WHO) which recommends a maximum of 10% (5% for further health benefits) **[43]**.

It has been postulated that dietary sugar consumption contributes to increased inflammatory processes in humans. Central to the potentially relevant mechanisms is the fact that dietary sugar promotes de novo synthesis of free fatty acids (FFA) in the liver **[319-321]**, which according to the lipotoxicity theory would produce FFA metabolites that may trigger inflammatory processes and ROS formation **[322, 323]**.

The differences in the metabolism of fructose (alone or found in sucrose) versus that of glucose should be considered in order to distinguish what potential role these monosaccharides may play in increasing inflammatory processes. In contrast to glucose, which can be metabolized by any cell in the body, fructose must be metabolized in the liver. Because there are no negative feedback mechanisms that control for and prevent excess substrate supply of fructose to liver mitochondria, fructose is independently partly converted to acetyl-CoA, which is a building block for fatty acid synthesis**[321]**. This metabolic pathway of fructose supports the lipotoxicity theory, however, it remains to be established whether dietary fructose/sucrose is more important than dietary glucose for promoting inflammation in human studies.

Therefore, the aim of the current meta-analysis and systematic review was to evaluate the evidence from published human interventional studies regarding the relevance of dietary fructose (alone or found in sucrose or HFCS) and dietary glucose as a comparator for biomarkers of subclinical inflammation. This evaluation was done quantitatively through a meta-analysis and qualitatively through a brief narrative review. Quality of meta-evidence was also assessed **[235]**. We selected the acute-phase protein high-sensitivity C-reactive protein (hsCRP), pro-inflammatory cytokines (interleukin-6 (IL-6), interleukin-18 (IL-18), interleukin-1 receptor antagonist (IL-1RA), tumor necrosis factor-α (TNF-α)), the chemokine monocyte chemoattractant protein 1 (MCP-1), soluble adhesion molecules (soluble E-selection (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1)) and the anti-inflammatory adipokine adiponectin as biomarkers of subclinical and vascular inflammation, because they

are the most commonly measured inflammation-related biomarkers in clinical and epidemiologic studies with established associations with cardiometabolic diseases [116, 324-328].

5.2.3 Methods

Study selection

In order to review the literature comprehensively, we conducted a systematic literature search of the MEDLINE library database (http://www.ncbi.nlm.nih.gov/pubmed/), and the EMBASE (https://www.elsevier.com/solutions/embase-biomedical-research) library database, and the Cochrane Library [Cochrane Central Register of Controlled Trials (CENTRAL)] (http://onlinelibrary.wiley.com/cochranelibrary/search/) databases from January 1990 through April 18, 2018. The search was limited to this time frame because hsCRP assays and sensitive assays for low-abundance cytokines such as IL-6 were not available before this time period. This review was registered with PROSPERO (registration number: CRD42017081171). The guidelines found in the Cochrane handbook for systematic reviews of interventions were used in writing this review [228]. The following terms were used to identify all potentially relevant publications published in the English language: (dietary) sucrose, glucose, and fructose/ HFCS together with (high sensitivity) C-reactive protein, ((hs-) CRP), interleukin 6 (IL-6), interleukin 18 (IL-18), interleukin-1 receptor antagonist (IL-1RA), tumor necrosis factor-α (TNF-α) monocyte chemoattractant protein 1 (MCP-1)/CCL2, E-selectin, intercellular adhesion molecule 1 (ICAM-1) or adiponectin (for details on the search terms used see the appendix. The search was restricted to human intervention studies (controlled, parallel or crossover design). Inclusion criteria limited search to either 1) healthy, overweight, or obese adults or adolescents (age 11 and up) 2) with or without diseases for which inflammation is not a major symptomatic factor. Inclusion criteria further limited search to studies in which 3) dietary fructose, glucose or sucrose was administered as predictors (including information on intake amounts of respective sugars) and with 4) C-reactive protein (CRP); the pro-inflammatory cytokines IL-6, IL-18 and TNF-α; the anti-inflammatory IL-1RA; the chemokine MCP-1/CCL2, the soluble adhesion molecules ICAM-1 and E-selectin; and adiponectin as outcome measures. A detailed listing of all inclusion and exclusion criteria was included in our PROSPERO registration. Because we were interested in the specific effects of dietary fructose, sucrose and glucose on low-grade inflammation, we excluded studies that analysed dietary patterns, effects of glycemic index (GI), treatment studies, or studies on pregnant women (for n-numbers see appendix A Figure 1). Studies that assessed the effects of fiber intake

simultaneously with sugar intake were also excluded. We additionally excluded intervention studies on participants with major inflammatory diseases such as arthritis, hepatitis, or irritable bowel syndrome, i.e., diseases for which inflammation or oxidative stress with clinical symptoms are major symptomatic factors in their progression or development.

Furthermore, because we were interested in investigating the potential impact of dietary sugars on chronic inflammation rather than short-term responses to diet, we chose to include only studies with a duration of at least 2 weeks. The literature search was conducted independently by two investigators (KDC and IP). The study selection process is illustrated in appendix A Figure 1.

Data extraction

Two investigators (KDC and IP) independently reviewed and extracted relevant data from each report. Extracted data included information on study design, duration, location, sample size, participant characteristics (sex, age, BMI, health status), type of intervention, and dietary sucrose, fructose, HFCS or glucose intake amounts (see Tables 2 and 3). If available, data on CRP, IL-6, IL-18, TNF-α, IL-1RA, MCP-1, sICAM-1, sE-selectin and adiponectin were extracted as the biomarkers of subclinical inflammation of interest. We extracted data from baseline and change and/or endpoint of these outcome measurements in order to calculate percent changes to include into the tables describing the studies. Post-intervention means, standard deviations and number of participants were collected and the statistical software tool Review Manager 5.3 (Nordic Cochrane Center, Copenhagen, Denmark) was used for the statistical analyses. Where post-intervention means were not available, change from baseline data was extracted. A comparator subgroup (e.g. free fructose vs free glucose) was only included in the meta-analysis if there were at least three studies available to report on. Information on whether diets were hypercaloric, eucaloric, hypocaloric or isocaloric was also extracted. Hypercaloric diets were determined to be those in which energy intakes were ad libitum and unregulated in addition to offering sugar-sweetened liquids or foods that caused an increase in energy intake compared to baseline. Implausible data were corrected and confirmed by contacting original authors in one case. Data were also extracted for the purposes of conducting a bias assessment using the Cochrane Collaboration's assessment tool to elucidate the risk of bias and attached either a low, unclear, or high risk of bias in eight areas to each study (see appendix A Figure 2).

Statistical analysis

Investigation of the effects of dietary sucrose, fructose, glucose or HFCS on biomarkers of subclinical inflammation was done using a random effects model. In this model, post-intervention mean values and corresponding standard deviations (if not available, standard errors or 95% confidence intervals were used to calculate the standard deviation) for intervention and control groups were pooled. As recommended by the Cochrane Handbook, we extracted post-intervention means where possible. If they were not available, change from baseline values were extracted [228]. As the main outcome to be analysed in the meta-analysis, pooled effects of the different interventions were investigated as mean difference (MD) by subtracting control group mean values from intervention group mean values. A standard χ^2 test was used to test the heterogeneity between trial results. To measure inconsistency between study results, the I^2 parameter was used: $I^2 = 100\% \times (Q - df)/Q$, where Q is the χ^2 statistic and df is its degrees of freedom [233]. The observed numerical value for I^2 depends on the direction and magnitude of the effect and the strength of evidence for heterogeneity (e.g. confidence interval for I^2 or P-value from the chi-squared test) [228]. An I^2-value of greater than 50% was considered to indicate substantial heterogeneity [234].

Assessment of quality of meta-evidence

To evaluate the quality of meta-evidence for the association between dietary sucrose, fructose, glucose and HFCS on subclinical inflammation we applied the NutriGrade scoring system **[235]**. NutriGrade comprises the following items for meta-analysis of RCTs (0-10 points): (1) risk of bias (2) precision, (3) heterogeneity, (4) directness, (5) publication bias, (6) funding bias, (7) and study design. Based on this scoring system, four categories were recommended to judge the meta-evidence: high ($\geqslant$8 points), moderate (6 to 7.99 points), low (4 to 5.99) and very low (0 to 3.99) [235].

5.2.4 Results

Description of studies

The literature search was conducted on April 18, 2018. The detailed steps of the systematic search and selection process are given as a flow diagram (see appendix A Figure 1). Taken together, 13 studies were identified by the search as matches and were included in the review (one study [329] resulted in two publications: Cox et al. [330] and Rezvani et al. [331] each covering different outcomes). Of these 13 studies, 8 addressed fructose, 3 HFCS, 7 sucrose, and 6 glucose (see Tables 5-3 and 5-4).

The 13 intervention trials that addressed dietary fructose, sucrose, glucose or HFCS intake as a nutritional exposure variable [329-343] lasted between 2 weeks and 3 months and included a total of 1141 participants (range: 20-355), aged 11-72 years, with a BMI ranging from 19 to 40 kg/m². Two studies were performed on men only [334, 336], and one study included women only [337]. The dietary fructose intake ranged from 17 grams daily to 217 grams daily in the eight fructose intervention studies. Six of the 8 studies on fructose compared free fructose to free glucose isocalorically. The other 2 fructose studies investigated the effects of low-fructose, hypocaloric diets. Dietary sucrose intake for the intervention groups in the seven sucrose studies ranged from 50 to 203 grams of daily intake. These sucrose studies had diverse exposures in the control groups which they compared to dietary sucrose intake. Three of them compared sucrose-sweetened beverages to glucose-, fructose-, or HFCS-sweetened beverages in isocalorically matched or differing intake amounts [335, 336, 340]. Two of these three administered sugars in a milk medium: sucrose-sweetened low-fat milk was compared to HFCS-, fructose-, and glucose-sweetened low-fat milks in Angelopoulos et al. [344] and sucrose-sweetened milk was compared to HFCS-sweetened milk in Lowndes et al. [340].

Two studies compared sucrose to either an artificial sweetener control group [343] or to sugar-reformulated products [342], two additional sucrose intervention studies had either a honey-intake control group [341], or both fructose and honey control groups [339].

Meta-analysis results (see Table 5-2)

Owing to the different designs of the intervention trials, they were classified in subgroups for the meta-analysis according to the types of dietary sugar interventions and controls as follows (n = number of studies in each subgroup):

- Fructose vs glucose (n=6 studies; n=8 study arms);
- (b) HFCS vs sucrose (n=3 studies; n=6 study arms).

The analyses of the comparators high-fructose vs low-fructose, fructose vs sucrose and glucose vs sucrose could not be performed because these subgroups had two or less comparisons to report on. For the same reason, the outcomes of IL-6, MCP-1, sICAM1, sE-selectin, adiponectin and TNFα were not included in the meta-analysis. No studies were found that reported effects of dietary sugar on IL-18 or IL-1RA.

(a) Fructose vs glucose

The comparator of fructose interventions vs glucose control groups showed no differences for CRP (MD: -0.03 mg/L 95% CI -0.52 to 0.46, I^2= 44%) (appendix A Figure 3).

(b) HFCS vs sucrose

In the comparator of HFCS interventions vs sucrose control groups, changes in CRP were non-significantly higher in HFCS compared to sucrose groups (MD: 0.21 mg/L 95% CI -0.11 to 0.53, I^2= 0%) (see appendix Figure 4).

Table 5-2 The effects of dietary sugar on hs(CRP)

hs(CRP) (mg/L) Intervention vs control	No. of studies	No. of partici -pants	MD	95% CI	P-value	I^2 (%) (95% CI)[1]	Quality of meta-evidence (NutriGrade)[2]
Fructose vs glucose	6	403	-0.03	-0.52, 0.46	(p=0.90)	44 (0, 75)	Low
HFCS vs sucrose	3	677	0.21	-0.11, 0.53	(p=0.19)	0 (0, 75)	Low

Pooled estimates of effect sizes (95% confidence intervals) expressed as mean differences (MD). MD = mean of intervention group – mean of control group. MD = 0: no difference between groups. MD > 0: greater value of respective outcome measured in intervention (first) groups. MD < 0: greater value of respective outcome measured in control (second) groups. [1]I^2 value represents degree of heterogeneity within comparison groups. I^2-value of greater than 50% was considered to indicate substantial heterogeneity. [2]Nutrigrade is an applied scoring system to judge the quality of meta-evidence [235].

NutriGrade

Overall, the quality of meta-evidence for the association between dietary fructose vs. glucose, and comparing HFCS vs. sucrose on CRP was rated as "low", (i.e. confidence in the effect estimate is low). Further research will provide additional evidence and will likely change the effect estimate. This judgement was mainly based on the low number of identified studies and study participants, the study limitations, and the imprecise effect estimates.

Comprehensive narrative overview

Due to the fact that the quality of meta-evidence as assessed by NutriGrade was low, the studies were heterogeneous in nature, and only a portion of the studies were able to be quantitatively assessed by the meta-analysis, an additional narrative review may provide further insights not captured by the meta-analysis.

The six studies included in the meta-analysis compared free fructose intake intervention groups with free glucose intake control groups isocalorically [329-336], and therefore warrant further scrutiny. Two of these studies reported significant effects on biomarkers of subclinical inflammation: Jin et al. [332] found a difference between the fructose and glucose group in hsCRP (increase by 4% vs decrease by 23%, respectively). Cox/Rezvani et al. (both reporting

from the original study by Stanhope et al. [329]) reported a treatment effect confined to MCP-1, where values in the fructose group increased by 38%, while decreasing in the glucose group by 9%. No treatment effects were seen for hsCRP, IL-6, sE-selection or adiponectin in this study. While Aeberli et al. [336] did not observe a significant overall effect, hsCRP increased in all interventions (by 82-109%) comparing varying amounts of fructose, glucose or sucrose with the greatest increase in the high-fructose and the high-sucrose group (109% and 105%, respectively). In the study by Angelopoulous et al. [335], increases in CRP were confined to the fructose and the glucose interventions, both amounting to approximately 24%, yet overall effects did not differ between the four intervention groups. The study by Johnston et al. [334] did not show any significant differences between the high-fructose and high-glucose groups for CRP and IL-6 outcomes. Silbernagel et al. [333] did not report significant differences between the glucose or fructose intervention groups, yet a substantial increase of 57% was only found for CRP in the glucose group.

The additional two studies on fructose that were not included in the meta-analysis used a low-fructose, [337, 338] low-calorie diet and measured the effects on CRP and sICAM values, which decreased in both intervention and control groups. Sorensen et al. [343] conducted a 10-week long study and reported an insignificant difference in CRP concentrations in the sucrose-added group (increase of 6%) and a decrease in the artificial sweetener group (decrease of 26%). None of the other intervention studies reported an effect of sucrose on the investigated biomarkers of subclinical inflammation. Of note, in the study by Angelopoulos et al., the fructose- and glucose-intake groups reported a 24% increase in CRP concentrations while the sucrose-intake group experienced no increase [335].

Table 5-3: Dietary intervention studies investigating the effect of fructose/HCFS, sucrose or glucose on biomarkers of subclinical inflammation. Extracted data on participants' characteristics, study designs, dietary interventions, form of sugar and feeding control.

First author, year, Country	Study Design	Participants' characteristics	Duration (weeks)	Intervention	Energy Intake	Sugar form §	Feeding control‡
Aeberli et al. (2011) [336] Switzerland	Crossover Double-blind	29 healthy males Age 20-50 y, BMI 19-25 kg/m^2	3	Intervention: ● high-fructose (80 g/d) ● medium-fructose (40 g/d) ● high-sucrose (80 g/d) ● high glucose (80g/d) ● medium glucose (40g/d) Control: ● low-fructose diet (33 g/day)	Hypercaloric*	Liquid	Supp/DA
Angelopoulos et al. (2016) [335] USA	Parallel Double-blind Randomized	267 healthy participants (96 m/171 w) Age 37.7 ± 12.1 y BMI 26.3 ± 3.3 kg/m^2	10	Intervention: ● sucrose-sweetened low-fat milk (18%En = 203.4 ± 53.7 g) ● HFCS-sweetened low-fat milk (18%En = 203.0 ± 56.9 g) ● fructose-sweetened low fat milk (9%En = 171.6 ± 63.6 g) ● glucose-sweetened low-fat milk (9%En = 160.7 ± 51.2 g)	Hypercaloric*	Liquid	Supp/DA
Cox/Rezvani et al.[†] (2009) [330, 331] USA	Parallel Blinded	31 overweight/obese participants (16 m/15 w) Age 40-72 y BMI 25-35 kg/m^2	10	Intervention: ● fructose-sweetened beverage (n=16) (175 g/d) (25%En Control: ● glucose-sweetened beverage (n=15) (175 g/d) (25%En)	Hypercaloric*	Liquid	Met/Supp
Jin et al. (2014) [332] USA	Parallel Double-blind Randomized	24 overweight Hispanic-American adolescents with hepatic fat > 8%, Age 11-18 y BMI ≥85th percentile	4	Intervention: ● fructose-sweetened beverage (n=11) (99 g/d) Control: ● glucose-sweetened beverage (n=13) (99 g/d)	Eucaloric	Liquid	Supp

Johnson et al. (2015) [337] Finland	Parallel Randomized	51 morbidly obese women with polycystic ovarian syndrome Age 18–40 y BMI ≥ 40 or 35-40kg/m^2	8	Intervention: ● moderate-fructose, low-calorie diet (LCD) (85 g fructose/day) Control: ● low-fructose LCD (17 g fructose/day)	Hypocaloric	Liquid	Supp/DA
Johnston et al. (2013) [334] UK	Parallel Double-blind Randomized	32 healthy overweight males Mean age 33-35 y BMI 25-32 kg/m^2	2	Intervention: ● high-fructose diet (n=15) (25%En =217 g/d) Control: ● high-glucose diet (n=17) (25%En = 215 g/d)	Eucaloric and Hypercaloric* (2 weeks each)	Liquid	Supp/Met
Lowndes et al. (2014) [340] USA	Parallel Blinded Randomized	355 overweight or obese participants (165 m/190 w) Age 20-60 y BMI 23-35 kg/m^2	10	Intervention: ● 8%En sucrose (n=58) ● 18%En sucrose (n=64)), ● 30%En sucrose (n=53), ● 8%En HFCS (n=69), ● 18%En HFCS (n=60), ● 30%En HFCS (n=51)	Hypercaloric Sugars administered in milk medium.	Liquid	Supp
Madero et al. (2011) [338] Mexico	Parallel Randomized	131 obese participants (102 w/29 m) Age 38.8± 8.8 y BMI 32.4± 4.5 kg/m^2	6	Intervention: ● Low-fructose diet (<20 g/d) (n=66) Control: ● moderate natural fructose diet (50-70 g/d) (n=65)	Hypocaloric	Solid	DA
Markey et al. (2013) [342] UK	Crossover Double-blind Randomized	50 normal or overweight participants (16 m/ 34 w) Age 20-49 y BMI 18.5-30 kg/m^2	8	Intervention: ● diet with regular sugar products (75.1 g non-milk extrinsic sugars/d) (n=28) ● diet with sugar-reduced (reformulated) products (28.9 g non-milk extrinsic sugars/d) (n=22)	Eucaloric	Mixed	Supp/DA

Raatz et al. (2015) [339] USA	Crossover Randomized	55 participants (39 w/ 16 m): group 1 with normal glucose tolerance (NGT) (n=28), group 2 with impaired glucose tolerance (IGT) (n=27) Mean age of NGT 39 y, Mean age of IGT 52 y BMI of NGT 26 kg/m^2, BMI of IGT 31.5 kg/m^2	2	Intervention: ● 50 g daily intake of HFCS (HFCS55) ● 50 g of honey ● 50 g of sucrose	Eucaloric	Liquid	Supp
Silbernagel et al. (2014) [333] Germany	Parallel Single-blinded Randomized	20 healthy participants (12 m/8 w) Mean age 30 y BMI of 26±0.5 kg/m^2	4	Intervention: ● 150 g fructose intake (n=10) Control: ● 150 g glucose intake (n=10)	Hypercaloric*	Liquid	Supp
Sorensen et al. (2005) [343] Denmark	Parallel	41 overweight participants (6 m/35 w) Age 33-37 y BMI 27-28 kg/m^2	10	Intervention: ● 125-175 g/d sucrose intake (n = 21) ● artificial sweetener intake (n=20)	Hypercaloric	Mixed	Supp
Yaghoobi et al. (2008) [341] Iran	Parallel Randomized	55 overweight or obese participants (24 m/31 w) Age 20-60 y BMI > 25 kg/m^2	≈ 4	Intervention: ● sucrose intake (70 g) (n=17) ● honey intake (70 g) (n=38)	Eucaloric	Liquid	Supp

† Both studies report from one original study by Stanhope et al.[329] and each study (Cox et al., Rezvani et al.) reports on different inflammatory markers measured in the original study. ‡ Feeding control. Met: Metabolic feeding control was the provision of all meals, snacks, and study supplements (test sugars and foods) consumed during the study under controlled conditions. Sup: Supplement feeding control was the provision of study supplements. DA: Dietary advice is the provision of counselling on the appropriate test and control diets. § Sugar form. Dietary sugar was provided in 1 of 3 forms. Liquid: all or most of the dietary sugar was provided as beverages or crystalline sugars to be added to beverages. Solid: dietary sugar was provided as solid foods. Mixed: all or most of the dietary sugar was provided as a mix of beverages, solid foods (not fruit), and crystalline sugars. * Denotes hypercaloric studies in which fructose vs glucose interventions were administered isocalorically.

Table 5-4: Dietary intervention studies investigating the effect of fructose/HCFS, sucrose or glucose on biomarkers of subclinical inflammation. Extracted data on baseline concentrations, results and funding sources.

First author, year, Country	Outcome baseline concentrations[1]	Results								Funding Source††
		Percent changes in inflammatory marker after completion of intervention							Statistical tests Comment	
		hsCRP/CRP	IL-6	TNF-α	MCP-1	sICAM-1	sE-selectin	Adiponectin		
Aeberli et al. (2011) [336] Switzerland	hsCRP (ng/mL) 205.6 ± 430.7 Adiponectin (μg/mL) 6.44±7.69	<u>High fructose</u>: **+ 109.19** % <u>Moderate fructose</u>: + 82.2% <u>High sucrose</u>: **+ 105.2%** <u>High glucose</u>: **+ 89.74** %	N/A	N/A	N/A	N/A	N/A	<u>High fruc:</u> **+ 18.6** % <u>Mod. fruc:</u> + 14.8 % <u>High suc</u>: + 17.8 % <u>High gluc</u>: **+ 20.6** %	No treatment effect for hsCRP and adiponectin reported. hsCRP increased significantly after all of the interventions - highest increase observed in high fructose group.	NR
Angelopoulos et al. (2016) [335] USA	CRP (mg/L) Fructose group: 1.74 ± 1.74 HFCS group: 1.92 ± 2.10 Sucrose group: 1.74 ± 1.78 Glucose group: 1.21 ± 1.43	<u>Fructose:</u> **+ 24.1**% <u>HFCS:</u> - 3.1 % <u>Sucrose:</u> - 1.7 % <u>Glucose:</u> **+ 23.9** %	N/A	N/A	N/A	N/A	N/A	N/A	No significant between-group changes in CRP for fructose, HFCS, sucrose and glucose as compared to each other. P-values not reported.	Industry
Cox/Rezvani et al. (2009) [330, 331] USA	MCP-1 (pg/ml) 144.7±18.8 sE-selectin (ng/dl) 45.0± 5.5	<u>Fructose</u>: - **16.2** % <u>Glucose</u>: - **22.8** %	<u>Fructose</u>: - **11.4** % <u>Glucose</u>: + **18.2** %	<u>Fructose</u>: **-12.8 %** <u>Glucose</u>: **+ 0.3 %**	<u>Fructose</u>: **+37.7 %** <u>Glucose</u>: **- 8.6 %**	<u>Fructose</u>: **+ 2.9 %** <u>Glucose</u>: - **1** %	<u>Fructose</u>: **+ 14.4** % <u>Glucose</u>: **- 1.6 %**	<u>Fructose</u>: **-14.8 %** <u>Glucose</u>: - **9.1 %**	Significant between-group change in MCP-1 (P = 0.03).	Agency

	sICAM-1 (ng/ml) 221.9 ± 6.3 CRP (mg/L) 3.7 ± 0.8 IL-6 (pg/ml) 3.5 ± 0.7 Adiponectin(ug/ml) 7.7 ± 1.1 TNF-α: NR								Significant within-group change in sE-selectin (P = 0.048). But no significant between-group difference (P = 0.17). No significant between-group change in sICAM-1 (P=0.22) CRP (P = 0.33) IL-6 (P=0.31) adiponectin (P=0.10) TNF-α (P=0.42)	
Jin et al. (2014) [332] USA	hsCRP (mg/L) 6.78 ± 3.16	Fructose: **+ 4.13** % Glucose: **- 23.4** %	N/A	N/A	N/A	N/A	N/A	N/A	Significant between-group change in hsCRP (P= 0.019).	Agency
Johnson et al. (2015) [337] Finland	CRP (mg/L) Low-fructose: 6.8 ± 7.4 Moderate-fructose: 10.9 ± 10.2	Low-fructose: - 8.8 % Moderate-fructose: -29.3 %	N/A	N/A	N/A	N/A	N/A	N/A	No significant between-group change in CRP (P=0.278) Confounder (low-calorie diet → weight loss)	Agency
Johnston et al. (2013) [334] UK	CRP (mg/L) 1.01 ± 1.08 IL-6 (pg/mL) 3.56 ± 4.84 TNF-α (pg/ml) 1.92 ± 0.5	Isocaloric period: Fructose: **- 21.8** % Glucose: **- 11.4** % Hyper-caloric period: Fructose: **- 8.9** % Glucose:	Isocaloric period: Fructose: **- 4.2** % Glucose: **- 5.8** % Hyper-caloric period: Fructose: **+ 23.8** %	Isocaloric period: Fructose: - 0.5 % Glucose: - 2.5 % Hyper-caloric period: Fructose:	N/A	N/A	N/A	N/A	No significant between-group change in CRP (P=0.37), IL-6 (P=0.23) or TNF-α (P=.36) in isocaloric or hypercaloric periods	Agency Industry – related conflict of interest

		+ 40 %	Glucose: **- 39.6** %	- 4.7 % Glucose: - 0.5 %						
Lowndes et al. (2014) [335] USA	CRP (mg/L) HFCS 8%En: 1.9 ± 1.9 HFCS 18%En: 1.6 ± 1.6 HFCS 30%En: 2.1 ± 2.1 Sucrose 8%En: 1.5 ±1.6 Sucrose 18%En: 2.0 ± 1.8 Sucrose 30%En: 1.5 ± 1.8	HFCS: 8 %En: + 26.3 % 18 %En: + 25 % 30 %En: 0 % Sucrose: 8 %En: + 40 % 18 %En: + 5 % 30 %En: + 6.7 %	N/A	N/A	N/A	N/A	N/A	N/A	No significant between-group change (HFCS vs sucrose) (P=0.679) No significant between-group changes in CRP between various intake amounts (8% vs 18% vs 30%) (P=0.597)	Industry
Madero et al. (2011) [338] Mexico	sICAM (ng/dL) Low-fructose: 4.44 ± 0.11 Moderate-fructose: 4.37 ± 0.11	N/A	N/A	N/A	N/A	Low-fructose: - 6.3 % Moderate-fructose: - 9.6 %	N/A	N/A	No significant between-group change in sICAM-1 (P= 0.19) Significant within-group decrease for sICAM-1 in low-fructose (P = 0.01) and moderate-fructose (P< 0.0001). (weight loss)	Agency
Markey et al. (2013) [342] UK	CRP (mg/L) Regular sugar intake: 0.93 ± 0.94 Reduced sugar intake: 1.05 ± 1.35	Regular sugar: + 6.5 % Re-formulated sugar: + 15.2 %	N/A	N/A	N/A	N/A	N/A	N/A	No treatment effect for sucrose (P = 0.593)	Agency
Raatz et al. (2015) [339] USA	hsCRP (mg/L) Glucose tolerant: 2.2± 0.5 Glucose impaired: 4.6±0.8 IL-6 (pg/ml)	HFCS: NGT: - 5 % IGT: + 29.6% Sucrose: NGT: - 20 %	HFCS: NGT: + 7.7 % NGT: + 6.7 % Sucrose: IGT: - 22.2 %	N/A	N/A	N/A	N/A	N/A	No treatment effect for hsCRP or IL-6.	Agency

	Glucose tolerant: 1.6± 0.2 Glucose impaired: 2.6 ±0.5	IGT: + 15.8% Honey: NGT: +8.7% IGT: +41.2%	NGT: + 3.5 % Honey: NGT: + 23.1% IGT: + 19.4%							
Silbernagel et al. (2014) [333] Germany	CRP (mg/dl) 0.13±0.06 MCP-1 (pg/ml) 275±34 E-selectin (ng/ml) 31.8 ±5.1	Fructose: **- 7.7** % Glucose: **+ 57** %	N/A	N/A	Fructose: - **16.7** % Glucose: - **9.1** %	N/A	Fructose: - **7.8** % Glucose: + **3.5** %	N/A	No significant between-group change in CRP (P = 0.284), MCP-1 (P = 0.803) or E-selectin (P = 0.311)	Agency
Sorensen et al. (2005) [343] Denmark	CRP (mg/L) 1.8 (0.9-3.0)	Sucrose: + 6 % Artificial sweetener: - 26 %	N/A	N/A	N/A	N/A	N/A	N/A	No significant between-group change (P = 0.1)	Industry
Yaghoobi et al. (2008) [341] Iran	hsCRP (mg/dl) Healthy subjects (normal hsCRP levels): 4.8 ± 3.2 Subjects with elevated hsCRP 9.9 ± 3.6	Sucrose: - 1 % Honey: - 3.3 %	N/A	N/A	N/A	N/A	N/A	N/A	No significant between-group effect observed (P>0.5).	Agency

Bold print represents studies in which fructose or sucrose was isocalorically compared to glucose. [1] Data refer to mean ± SD unless otherwise indicated; N/A: not investigated NR: not reported. [2] Both studies report from one original study by Stanhope et al. [329] and each study (Cox et al., Rezvani et al.) reports on different inflammatory markers measured in the original study. †† Funding sources. Agency: funding from government, university, or not-for-profit health agency sources. Industry: funding from companies that utilize dietary sugar for profit. NR: not reported. Johnston et al. reports conflict of interest of the author, IA Macdonald, who is on the Scientific Advisory Boards for Mars, Inc and Coca Cola Inc.

5.2.5 Discussion

The current systematic review and meta-analysis identified 13 intervention studies that addressed the relevance of dietary fructose, sucrose and glucose for biomarkers of subclinical inflammation as assessed by (hs)CRP, IL-6, TNF-α, MCP-1, sE-selectin, sICAM-1 and adiponectin. Pooled effects of the different interventions (investigated as mean differences) revealed that fructose intervention groups showed no significant differences in (hs)CRP when compared to glucose control groups. Similarly, no differences were observed between HFCS vs sucrose interventions. As summarized narratively, effects were observed in a small number of studies, but the overall picture is inconsistent, the effect sizes are variable, and the overall quality of meta-evidence is low. Additional evidence from future intervention studies on this topic are needed in order to draw confident conclusions as to the effects of dietary sugar on subclinical inflammation.

Implications arising from the study design

Evaluation of the impact of dietary sugars is often hampered by the lack of an isocaloric comparator, which makes it therefore unclear whether the effects result from fructose or sucrose itself or simply excessive energy consumption [345]. A strength of most of the fructose intervention studies included in this review is that they do administer fructose with an isocaloric control group of dietary glucose, although most of these studies were hypercaloric trials. The fact that there was often no difference observed between intervention and control groups in studies that administered supra-physiological doses [329, 333-335, 340] may be due to post-prandial stress that activates low-grade inflammation due to extremely high energy intake. If this is the case, then no conclusions can be drawn about the unique effects of fructose versus glucose metabolism from studies that administer supra-physiological doses across comparative groups. In order to be able to draw firm conclusions for health care policy decisions, more large-scale, effective, longitudinal intervention studies that investigate sucrose or HFCS ingested at average intake levels (75-100 grams/day) compared to control groups, as well as studies which ingest sucrose or HFCS at the lower levels recommended by WHO or the UK's SACN guidelines (5% of energy intake or about 25 grams/day), need to be conducted with low-grade inflammation as an outcome measure. Because sugar is typically not consumed in an isolated monosaccharide form, studies which compare glucose and fructose isocalorically at average consumption levels would serve to further understand the unique metabolic differences between fructose and glucose and how they affect inflammation.

The methodological limitations of the two largest studies both lasting 10 weeks are worth mentioning. These two studies used HFCS- [335] or sucrose-sweetened milk [340] as intervention diets

and addressed the effect on CRP only. Both studies did not specify how randomization was done (risk of selection bias) and had relatively high drop-out rates of 27% and 26% respectively. In view of the risk of bias, the absence of effects reported from these studies should be interpreted with caution also because these studies were either funded by the sugar industry or the authors received consulting fees from organizations that market or utilize fructose, HFCS or sucrose. Results from industry- and non-industry-funded research often differ and a recent analysis suggests that industry-funded research tends to underestimate the adverse effects of dietary sugar [346]. On the other hand, personal views from non-industry researchers could present another source of bias. Such bias, however, is difficult to address because there is no marker for this potential problem.

Finally, some methodological aspects in the measurements of inflammatory markers can be considered. Ideally, one would like to have a clear picture of which biomarkers are responsive to fructose and sucrose across all studies, which would in turn allow conclusions on the pathways relevant for disease prevention. This is, however, hampered by methodological problems ranging from the use of very different/non-standardized assays to the large inter-individual variation in inflammatory biomarkers. Such variation is affected by the presence or absence of accompanying metabolic conditions. Repeated measures both pre- and post-intervention would be required in order to ascertain the size of such variations in a study population.

Potentially relevant pathophysiological mechanisms

Evidence from human intervention studies suggests that doses of fructose providing excess energy (+ 21-35% energy) raise liver fat [319, 320]. This effect, however, appears to be confounded by excessive energy intake [319, 320]. As previously discussed, the metabolism of dietary fructose (alone or found in sucrose) has been reported to promote de novo synthesis of FFA in the liver when consumed in high amounts [319, 321]. While hepatic triglyceride storage and accumulation seem to be a benign symptom of steatosis, there is preliminary evidence that FFA metabolites may contribute to the progression of non-alcoholic fatty liver disease (NAFLD) to non-alcoholic steatohepatitis (NASH) by triggering inflammatory processes, ROS formation and apoptosis [322, 323]. Studies involving tissue biopsies indeed showed a gradual increase in systemic levels of biomarkers of inflammation such as CRP, IL-6 and IL-1RA from healthy adipose tissue to adipose tissue heavily infiltrated by immune cells [347] and from healthy livers to NASH [348]. Therefore, it is plausible to hypothesize that specifically supplementary fructose exerts adverse effects. Hence, future studies should use both outcome measures of hepatic fat/de novo lipogenesis as markers for NAFLD while simultaneously measuring more specific inflammatory markers ideally related to hepatic fat, e.g. fetuin A etc. There is

some evidence from human and animal studies that suggests a relationship between fructose consumption and increased visceral adipose tissue [349], which is metabolically active and produces numerous inflammatory cytokines including TNF-α, and IL-6 [350], which may in turn induce CRP release in the liver. In addition, other mechanistic animal studies suggest that fructose leads to intestinal bacterial overgrowth and increased intestinal permeability, which causes endotoxin levels of lipopolysaccharides to translocate and activate Toll-like receptor 4 in liver Kupffer cells [351, 352], whose activation leads to the release of several cytokines including mainly TNF-α [353]. In another animal study, Gersch et al. reported that a high-fructose diet significantly increased the renal expression of MCP-1 in rats [354]. An in vitro study in human epithelial tubular cells suggested a response of MCP-1 production induced by fructose, but not glucose [355].

Looking at the effects of glucose, there is evidence for a specific role of glucose on oxidative markers, which can in turn lead to increases in biomarkers of chronic inflammation. High dietary GI has been linked to increased inflammatory responses by means of recurrent hyperglycemic responses in the early postprandial phase as well as elevated levels of free fatty acids in the late postprandial phase, both of which are considered to result in an overproduction of free radicals and releases of pro-inflammatory cytokines, which may in turn induce inflammation and vascular damage[356]. Fructose has a low glycemic index (GI) and there is evidence that suggests that consumption of foods with a lower dietary GL/GI is associated with anti-inflammatory effects. Accordingly, ingestion of fructose may contribute to a reduction of chronic inflammation due to the avoidance of glycemic spikes[357]. This may be one potential explanation for the absence of consistent signals or harm in response to fructose intake.

Because obesity and sugar intake are closely linked (at least with respect to SSB) on the one hand, and obesity and inflammation are closely linked on the other, it is well possible that weight gain is a potential mediator in the association between sugar intake and inflammation. In this context, it is of interest to separately investigate the eucaloric and hypercaloric effects of dietary sugars on inflammatory markers as the former can give relevant information on the metabolic pathways linking sugar and inflammation, whereas the latter informs on the public health relevance for inflammatory biomarkers in a hypercaloric setting as is often the case in real-world intakes. The question of whether it is excess energy intake or excess sugar intake that leads to adverse health outcomes requires further research. Similar work was done in a review by Sievenpiper et al. who found that fructose intake only affected weight gain in hypercaloric versus isocaloric trials [358].

Considering the relationship between obesity and low-grade inflammation, a discussion of the change in body weight in the included trials would be relevant. Two studies included in this review observed

that weight loss resulting from energy-restricted diets was associated with greater improvements in inflammatory markers (regardless of fructose-intake amounts) [337, 338]. The remainder of the studies did not take changes in weight into account. Future studies interested in the association between dietary sugar intake and low-grade inflammation should bear in mind that weight loss may mediate the results [359].

Interpretation in the context of evidence from observational studies

One could argue that our data disagree with evidence from observational studies since the relationship between SSB intake and the inflammatory biomarker CRP has been consistently reported on in observational studies [24-26]. SSB intake was also associated with IL-6 and TNF-α[23] and recent studies link SSB intake to the inflammatory disease rheumatoid arthritis [360, 361]. Due to concerns that SSB intake could be a marker for an undesirable diet [362, 363], there is dispute whether results from observational data linking SSB intake to adverse health outcomes are impacted by residual confounding, i.e. that failure to adjust for various (non-measured) lifestyle factors could lead to an overestimation of the strength of positive associations. Since in practice fructose and glucose are consumed together (HFCS, sucrose, sugar from fruits), even separate appraisals of fructose and glucose intake on inflammatory outcomes and/or liver outcomes in observational studies may provide only limited information on how these sugars contribute to health outcomes. Hence, it is not possible to state whether our results from intervention studies agree with observational data.

Strengths and limitations

The strengths of this meta-analysis and systematic review include its standard to only analyse controlled human intervention trials and its inclusion of six interventions that had similar isocaloric control groups. The inclusion of a broad range of biomarkers of subclinical inflammation is also a strength and allows for a more thorough evaluation of the effects of dietary sugar on low-grade inflammation. In analysing the main findings, quantification of the observed associations was possible and is a strength of this review. The objective quantification of the effects in this meta-analysis was reinforced by additionally carrying out a risk of bias assessment and performing an analysis on the quality of meta-evidence. The limitations of this review lie in the fact that the data have substantial heterogeneity and some of the comparisons had only a small number of studies. The studies range in duration from two weeks to three months. Most of the fructose studies compared glucose to fructose isocalorically (n=6), but comparators in the rest (n=7) vary. Intake amounts of the investigated sugars varied widely as well. Most of the studies are generally similar in design, exposures, interventions and

outcome measurements but there are several that do not compare easily. Two of the studies measured the effects of artificial sugars, which may have unique metabolic effects. The study populations differed from each other in age, body weight and health status. The included interventions had generally shorter durations, and therefore do not reflect the overconsumption of fructose over years in the general population, as is better depicted in observation studies.

Conclusions

In conclusion, the overall findings as collectively analysed by a meta-analysis do not support the hypothesis that dietary fructose (alone or in HFCS) is more detrimental with respect to subclinical inflammation than dietary glucose or sucrose. However, the studies included in this review were heterogeneous and several of the comparisons had only small numbers of studies providing limited evidence. Consequently, the grading of meta-evidence was low for all comparisons. In order to draw more confident conclusions about the pro-inflammatory effects of free fructose or fructose found in sucrose versus glucose or other non-fructose containing carbohydrates (fructose vs glucose, sucrose, vs maltose, sucrose vs refined carbohydrates), further human intervention studies with larger sample sizes, longer follow-up periods, better controlled designs, and with subclinical inflammation as a priori planned outcome are required.

Resulting publication:
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. Nutrients. 2018;10(5):606. Published 2018 May 12. doi:10.3390/nu10050606
KDC contribution: KDC helped conceive the project, performed the systematic search, extracted the data, helped interpret the results, conducted the meta-analysis and wrote the manuscript.

5.3 Study III - The prospective association of dietary sugar intake in adolescence with type 2 diabetes and inflammatory markers in young adulthood

5.3.1 Summary

To examine the prospective relevance of dietary sugar intake (based on dietary data as well as urinary excretion data) independent of food source in adolescent years for insulin sensitivity and biomarkers of inflammation in young adulthood. 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 and added sugars 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 y, males: 10-16 y) 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). On the dietary intake level, no prospective associations were observed between adolescent fructose, sucrose, glucose, added, free or total 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 ($p>0.01$). The present study cannot provide support that dietary sugar consumed in adolescence is associated with adult insulin sensitivity. The one potential exception is the moderate dietary consumption of fructose, which showed a beneficial association with adult fasting insulin and insulin sensitivity.

5.3.2 Introduction

It has been proposed that dietary sugar intake plays a causal role in the development of type 2 diabetes (T2D),[20, 364-366] yet data on this topic is conflicting.[367, 368] 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.[369, 370] 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,[371, 372] therefore amounts as well as types/sources of ingested fructose are of importance when considering its relation to risk factors of T2D.[373] Dietary fructose elicits lower insulin secretion as compared to dietary glucose,[192, 374, 375] and there is some

evidence indicating that fructose intake/substitution can beneficially affect blood glucose levels.[376, 377] 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 versus glucose,[378, 379] 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.[380] Observational studies link the consumption of SSB to increased chronic inflammation,[20, 381-383] 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.[384, 385] 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.[386] Dietary assessment methods are prone to measurement errors[387] and sugars are among the nutrients that are frequently underreported[388, 389] especially by adolescents who may be susceptible to socially desired reporting. Therefore, dietary biomarkers of 24-hour urinary sucrose and urinary fructose have been introduced,[390, 391] 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), and sugar use (total sugar, added sugar, free 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.

5.3.3 Methods

Study population

The present analysis is 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 four years. All examinations are performed with parental and later on, children's written consent. Since 2005, participants are invited for follow-up in adulthood including fasting blood withdrawal. The study has been previously described in more detail,[392] and was approved by the Ethics Committee of the University of Bonn 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 y) 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 y, males: 10-16 y), participants additionally had to have provided at least two 3-day weighed dietary records in the period of adolescence (with >50% plausible records)[249] (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.[390, 393] 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).[394] To identify energy underreporting pediatric cutoffs from Sichert-Hellert et al. were used.[249] 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 5-5 and 5-6**) (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 are weighed to the nearest gram or alternatively are recorded semi-quantitatively if weighing is not possible. The calculation of the intake of energy and nutrients based on the dietary records occurs with the help of the food database LEBTAB, an in-house database which is continuously updated.[392] 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 are estimated by recipe simulation using labelled 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 is 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)[386, 395] 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. 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)[253] and overweight during adolescence were defined and calculated according to the International Obesity Task Force.[251] 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 are asked to void their bladders upon getting up in the morning and this micturition is 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).[396]

Urinary fructose and sucrose excretions were measured in the laboratory of the Department of Food & Nutritional Sciences at the University of Reading using LC-MS and quantified using stable-isotope labelled 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 to 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.[397]

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 analysed 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 was calculated.

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 are the most commonly measured inflammation-related biomarkers in clinical and epidemiologic studies with established associations with cardiometabolic diseases.[398-402]

A pro-inflammatory score, assumed to be more predictive of inflammation than single markers,[400] 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.[111, 403]

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 5-5 and 5-6**).

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 and thereby avoid mixing potential errors arising from dietary record assessments with more biomarker measurements, as they are differently biased.

Prospective associations between dietary sugar intake (total sugar, added sugar, free sugar, sucrose, fructose, glucose) 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

analysed 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 blood withdrawal. 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/m2 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 (24h-creatinine excretion (mmol/d), 24h-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 investigate whether observed associations were partly attributable to body composition in adulthood. 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. 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.[55] We only present substitution models for associations identified as significant in fully adjusted analyses.

As mentioned in the methods section, adolescents are susceptible to underreporting energy intake. 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 1446 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). 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-values < 0.01 were considered to indicate statistical significance, p-values <0.05 were considered to indicate a trend.

5.3.4 Results

Characteristics of the participants at baseline and at follow-up are presented in Tables 1 and 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 5-7, 5-8, and 5-9.**

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 5-7). Similarly, there were no independent associations between total, free, or added sugar intakes in adolescence and adult HOMA2-S% or insulin levels (all p>0.01, Table 5-8).

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 5-9, model C; Figure 1). Among males, sugar excretion levels were not associated with adult insulin sensitivity markers

Adolescent sugar intake and adult systemic inflammation

Intakes of glucose, fructose or sucrose as well as total sugar, free sugar or added sugar were not independently associated with the pro-inflammatory score in adulthood (all p>0.01; See supplementary material). Similarly, sugar excretion levels during adolescence were not associated with pro-inflammatory score in adulthood (all p>0.01, SM Table S3).

Table 5-5 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)	220	9.0 (8.3, 10.2)	7.2 (6.6, 8.1)
Fat (%E)	254	35.3 ± 3.8	36.1 ± 3.5	220	34.9 ± 3.4	36.2 ± 3.5
Protein (%E)	254	13.2 ± 1.3	12.9 ± 1.7	220	13.2 ± 1.3	12.9 ± 1.7
Fiber (g/MJ)	254	2.4 (2.1, 2.7)	2.5 (2.2, 2.8)	220	2.4 (2.2, 2.8)	2.5 (2.1, 2.8)
Carbohydrate (%E)	254	51.3 ± 3.8	51.1 ± 4.3	220	51.5 ± 3.9	51.1 ± 4.4
Total sugar (%E)	254	26.8 ± 5.0	27.1 ± 5.0	220	27.0 ± 5.1	27.0 ± 4.9
Added sugar (%E)	254	14.3 ± 4.3	14.1 ± 4.7	220	14.2 ± 4.4	14.1 ± 4.7
Free sugar (%E)	254	18.2 ± 4.6	17.7 ± 5.0	220	18.4 ± 4.6	17.6 ± 5.0
Sucrose (%E)	254	14.4 ± 3.8	14.6 ± 3.9	220	14.4 ± 3.8	14.5 ± 3.8
Fructose (%E)	254	11.3 ± 2.6	11.4 ± 2.5	220	11.4 ± 2.6	11.3 ± 2.4
Glucose (%E)	254	11.5 ± 2.5	11.8 ± 2.7	220	11.4 ± 2.5	11.8 ± 2.7
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 wks (%)	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 (Cole et al. 2000 [1]); 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 5-6 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[1]	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.[1] Based on energy expenditure levels.

Table 5-7 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 blood withdrawal. 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.

Table 5-8 Sex-stratified prospective associations of total dietary sugar, added sugar, and free sugar intake during adolescence with markers of insulin sensitivity 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	75.6	67.9	0.86	66.6	76.6	70.1	0.72	69.0	76.4	68.6	0.78

	(59.4; 81.5)	(65.3; 86.0)	(57.4; 78.4)		(55.6; 77.6)	(66.6; 86.6)	(59.7; 80.5)		(57.5; 80.4)	(66.3; 86.6)	(58.3; 78.9)	
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 blood withdrawal. 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).

Table 5-9 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 blood withdrawal. 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).

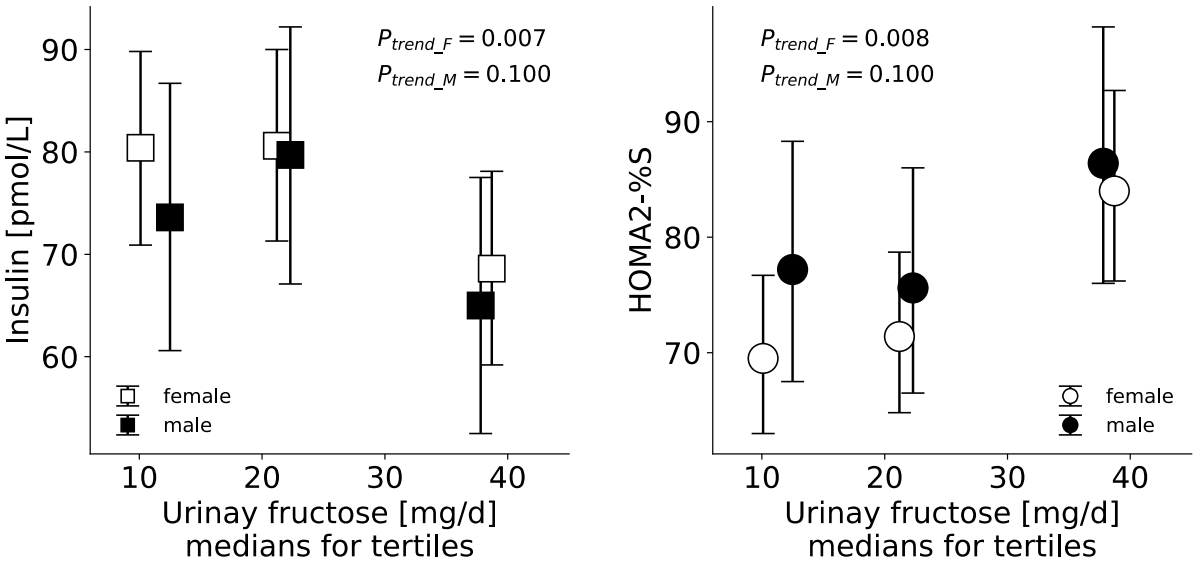

Figure 5-7: Serum levels of fasting insulin in early and insulin sensitivity (HOMA2-%S) by tertiles of excreted urinary fructose among males and females in adolescence.
Data are generic means and 95% CI adjusted for age at blood withdrawal, paternal education, pubertal percent body fat, gestational weight gain and adult percent body fat.

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).

5.3.5 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 sugar is 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 is consistent with our prospective association between fructose intake and improved HOMA2-S% and insulin levels,[219, 220] and further

sources reporting on large cohorts found no association between fructose-containing sugars and incident T2D[221-223] 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.[224] These studies report findings that emerged when investigating chemical sugar types, not SSB. The observational studies referenced here similarly adjusted for anthropometric measures and energy intake but did not measure sugar intake by means of urinary biomarkers as was done in our study. When consumed in high amounts, dietary fructose has been associated in cohort studies with increased risk of T2D.[225, 226] 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.[227]

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[387] and sugars are among the nutrients that are frequently underreported,[388, 389] objective dietary biomarkers of 24-hour urinary sucrose and urinary fructose have been introduced.[390, 391] 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.[371-373] 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.[404, 405] 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. Of note, this DONALD population had a relatively low SSB intake and most of the consumed fructose came from healthy food sources (i.e. fruits and vegetables).

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.[192, 375] 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.[226] On the other hand, our finding that indicates a beneficial association of fructose intake with insulin sensitivity is 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.[406-409] 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.[410] When assessing the effect of dietary fructose, a distinction needs to be made between trials that administer high versus low doses. Of note, our DONALD population consumed low amounts of fructose, primarily from healthy sources. The comparisons made thus far between our findings and those above are, however, not helpful in explaining our results because the amounts consumed in the DONALD study were smaller/from healthy sources, and 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.

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.[411, 412] It has been reported that women show more dramatic changes than men in hormones and body composition due to reproductive factors,[413] which may cause them to react more sensitively to changes in dietary influences. 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.[414] Women generally have lowered insulin sensitivity [414-416](as was also observed in this present study) or increased impaired glucose tolerance than do males,[413] which may increase their susceptibility or sensitivity to dietary influences.

Our results pertaining to biomarkers of inflammation indicated no relationship with sugar intake. Sugar in the form of SSB has been linked with increased chronic inflammation

(more specifically, the biomarker CRP) in observational studies.[20, 381-383] This observational evidence could, however, be subject to residual confounding. SSB was not considered in our analysis, and none of our other six sugar categories were significantly associated with systemic inflammation, as measured by the pro-inflammatory score. While some evidence from human intervention trials points toward pro-inflammatory effects of sucrose and fructose versus glucose,[378, 379] our previously published systematic review based on limited evidence found that dietary fructose does not contribute more to subclinical inflammation than other dietary sugars.[380]

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.[388, 389] 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.[417, 418] 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.[419] 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.[390, 391, 420, 421] In a previous DONALD publication, it was found that dietary total sugar was more strongly associated with excreted fructose than dietary added sugar.[422]

The main strength of the present study is the 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 is 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 allows the consideration of fructose, glucose and sucrose as well as different types of fructose-containing sugars (total sugar, free sugar, added sugar). Our study is 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 24h-urine samples.

Our study is limited by the availability of only one blood sample in young adulthood. A further limitation in the methods used is the handling of our urine samples, which in contrast to previous studies[390, 391] were frozen without preservatives for a long period of time (the earliest 24h-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 less than -12 ºC during the collection period at home as well as at -22 ºC in the study institute, our samples remained frozen until use. The generalizability of our results is limited due to the relatively high SES of the DONALD study population and high SES is known to correlate with lower dietary sugar intake.[423] Nevertheless, our sugar intake data is similar to sugar intake in representative German nutrition surveys[424, 425] as well as our sugar excretion data is similar to sugar excretion in other study populations.[393, 426, 427]

In conclusion, these observational findings do not confirm that dietary sugar consumption in adolescence is related to insulin sensitivity in adulthood. The one potential exception to this is 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.

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KDC Contribution: KDC conducted the statistical analysis, helped interpret the results, and wrote the manuscript.

6. General Discussion

The overall aim of this thesis was to investigate international time trends in intake levels among children and adolescents and aspects of its relevance for subclinical inflammation and insulin sensitivity among adults. According to the results, our findings indicate that SSB and dietary sugar intakes from the United States differ starkly form those of other international countries and intake levels appear to be decreasing in some parts of the world among children and adolescence. Further results suggest that dietary fructose does not illicit more deleterious effects on subclinical inflammation than other dietary sugars as measured by limited evidence from a meta-analysis of human intervention trials. Lastly, this thesis could not provide support that dietary sugar consumed in adolescence is associated with adult insulin sensitivity, one potential exception to this being dietary fructose, which in the context of a moderate consumption pattern had a beneficial association with adult fasting insulin and insulin sensitivity. Below, the central findings of Studies I-III will be expanded by additional considerations on the broadened topics. Following this, the aims will be outlined and results presented as well as directions for future research. Finally, the public health relevance and possible practical implications will be presented, followed by a stated conclusion.

6.1 Additional considerations

6.1.1 Additional considerations (Study I)

Trend reviews that estimate dietary intake across time are integral to informing national nutrition policy and providing benchmarks to monitor progress towards dietary guidelines. Intakes of SSB and dietary sugar is a controversial public health issue, charged from one side by industrial interests and disorientated by the other side through inconsistent guidance by health organizations adding to a state of uncoordinated public health efforts. When approaching this topic, health authorities need to understand from the outset where current intake levels of sugar and SSB stand, how they have evolved over time, and how they compare to other countries around the world. A further investigation into whether and how concerted public health efforts have influenced the decreased trends in intake can then ensue. Published data relating to trends in sugars intake have been relatively sparse, and that which existed was only based off of national data and had never investigated SSB trends. Our world trends review tracked the eating habits and evolution of SSB and dietary sugar intake in the US and other

international countries allowing for between-country comparisons, making it a timely and needed contribution.

As government and health organisations worldwide recognize the impact that an uncontrolled intake of dietary sugar and SSB can have on the health of their populations, especially in children and youth, they have issued dietary guidelines for sugar intake, which vary considerably. These guidelines differ in regard to the type and amount of sugars recommended, what procedures they used to establish their evidence base, and the health outcomes on which the evidence is based.[428] Nonetheless, these public health efforts over the past two decades to reduce sugar consumption may be a critical causal factor for why SSB and dietary sugar intakes in many developed countries have decreased over time.

The timing and implementation of these public health efforts are examined here. In 2005, the recommendations from the Dietary Guidelines for Americans suggested to limit total calorie intake as discretionary calories to a range of 6% to 10% depending on a child's age, sex, and level of physical activity. Discretionary values are those from added sugars and solid fats once the daily nutrient requirements are met. Also in 2005, this recommendation was supported by the American Heart Association (AHA) in collaboration with the American Academy of Pediatrics (AAP). Considering how the decreases observed in other international countries may have been influenced by public guidelines, the World Health Organization has since 2003 recommended that the intake of free sugars be limited to <10% of total daily energy intake. The WHO advised in 2015 that a further reduction to <5% of total daily energy intake would have additional benefits in reducing the risk of excess weight gain and dental caries in children and adults. Additonally, the Scientific Advisory Committee on Nutrition in the UK recommended a 5% limit on sugar intake based on findings that this limit has benefits for dental health and total energy intake.[429]

Other efforts in the US that may have contributed to the drastic decreases in sugar include a focus on nutrition assistance programs which through policy changes began to discourage the consumption of sugary drinks. SSB are no longer included in the Women, Infants and Children program and the Child Adult Care Food program and are prohibited under the Health, Hunger-Free Kids Act of 2010.[430] They further specified that "sweetened beverages and naturally sweet beverages, such as fruit juice, should be limited to 4 to 6 ounces per day for children 1 to 6 years old and to 8 to 12 ounces per day for children 7 to 18 years old."[431]

Another initiative to reduce sugar intake has been school nutrition programs.[432] Considering that childhood is a critical period in establishing life-long eating habits that influence potential risk of overweight and disease,[433-435] and that the majority of children and youth consume a large proportion of their daily food at schools (often between one-third to one-half), school lunch is a crucial setting for potential intervention.[436] In their policy statement on soft drinks in schools in 2004, AAP summarized potential health risks of soft drinks consumption during adolescence and childhood and advocated for strict and clearly defined school district policies that restricted the sale of soft drinks in schools. The US Department of Agriculture school policy prohibits school districts from selling sugary drinks at school. These are some of the changes in public policies that may explain why the US, SSB and dietary sugar intake trends experienced swift declines following the turn of the century.

More recently, in March 2019, the AHA and the AAP released public health policy recommendations designed to reduce the consumption of sugary drinks in children. In contrast to the WHO that has limited warnings attached to sugar consumption mainly to risk of overweight, the AAP warns that soft drink consumption additionally correlates with cardiovascular disease, hypertension, dyslipidaemia, insulin resistance, T2D, and fatty liver disease.[430, 437] The broad-sweeping AAP recommendations targeted federal, state, and local legislators and include sugar taxes, limitations on marketing to children, educational initiatives, and financial incentives for purchasing healthier drink options.[430, 436, 438] The 2015-2020 Dietary Guidelines for Americans also recommended limiting added sugars to 10% total energy.[44] For these reasons and other possible factors, a striking phenomenon has occurred in which consumer attitudes toward sugar have changed.

Another important factor that has contributed to decreased sugar intake amounts is the involvement of the sugar, food, and beverage industries. Reducing sugar content and replacing it with artificial sweeteners or natural alternatives has become a priority for food innovators and manufacturers across the world, as discussed in international food and beverage conferences.[439, 440] Some of the top contributors of added sugars to total energy intake include soda, fruit-flavoured and sports drinks, cakes and cookies.[45] Driven by consumer demand, these products and others ranging from breakfast cereals to infant nutrition have seen changes in sugar content and type. It is a considerable challenge for the food industry to reduce sugars because as an ingredient sugar has exceptional attributes both for texture, product flow, taste, and shelf life. Their efforts to overcome these challenges and reduce sugar levels have had rippling effects throughout the global market and continue to influence sugar intake trends

in children and adolescence. Driven by consumer demand and increasing calls for governments to legislate on this issue, large multinational companies soft drink companies have been working on introducing alternative sweeteners for several decades. Also in the confectionary industry, companies like Nestlé have reformulated their products through a variety of methods and have stripped 10% of sugar content from their confectionary portfolio resulting in what they have estimated to be 2.6 billion teaspoons of sugar being removed from well-known brands.[441] Therefore, observed decreases in SSB and sugar are not just due to the population becoming more health conscious or governmental intervention, they are also thanks to a movement within industry to reduce sugar content in food manufacturing and production.

According to a market research report of the global sugar economy, sugar markets are saturated in developed economies such as North America and Europe but they are experiencing rapid growth in developing countries and emerging regions.[442] Such growth and adoption of Westernized eating habits is driven by urbanization, increased disposable incomes and changing food habits. This demand for sugar-based foods is showing strong growth in developing countries such as India, the Middle East and China. In Southern America, Brazil occupies the leading position in the entire global sugar market.[442] The sugar industry is a vital part of the economy in Brazil and makes up a large share of the national GDP. Brazil is followed by India, United States, European Union, China and others. The global sugar market reached a volume of 187.9 million tons in 2018 and is projected to reach 199.6 million tons by 2024.[442] While sugar intake levels in developed countries have been decreasing as reported by our review, the anticipated global sugar market growth will most likely occur in developing countries where intake levels are increasing.

As the world witnesses an epidemic in obesity rates, little is known about the relative contribution of added sugar versus other carbohydrates to this world health problem. Indeed, high glycemic index foods that contain no fructose contribute substantially more calories to typical Western diets than added sugar.[443] In Australia, intakes of added sugars and SSBs have declined since the 1990s while BMI in children and adults has risen, a phenomenon known as the Australian paradox.[61] In America, SSB represents the largest source of fructose-containing sugars in the diet.[444] In the UK, however, high sugar snacks such as confectionary, cakes, and biscuits contribute more to the intakes of free sugars as well as energy than do SSB.[445] Based on data from the National Diet and Nutrition Survey in the UK, average SSB intakes contribute 2% of total energy and 11% of free sugar intake compared to the combined food group of cakes, biscuits and confectionary, which contributes 12% of total

energy intake and 26% of free sugar intake.[446] Therefore, the focus solely on SSB as an intervention medium to reduce sugar intake is not the best strategy for every country. Further, it should not be assumed in every country that dietary sugar contributes more to overweight and obesity than do refined and high glycemic carbohydrates.

6.1.2 Additional considerations (Study II)

In assessing the findings from Study II there were varying and contradictory results between included studies. This may be due to the inherent challenges of studying the effect of sugar in humans who have complex diets and metabolisms. When studying a single nutrient in the diet, it is impossible to completely isolate its effects, especially when considering the known limitations of self-reported diet data and the short duration of the trials. There is also the additional challenge that adding sugars to a diet often results in the intake of excess calories, confounding the direct relationship between specific sugars and the outcome in question. While it is common practice to adjust for energy intake in these studies, adjustment does not reflect or mirror real-world settings among free-living people who typically do not adjust their diet to keep caloric intake at a set level.[45] In reviewing the body of literature on the topic of sugar and inflammation, it is important to include articles that mirror real-world effects of sugar as well as those that attempt to isolate specific sugars to determine their unique metabolic effects.

Important factors to consider when measuring the effects of sugar in human interventions are the metabolic and satiety responses to liquids versus solid sugars. The form in which sugars are consumed may influence absorption rates and metabolic effects. As was the case in our meta-analysis, clinical trials examining the effects of added sugars most often administer SSB as the exposure (as they are composed mainly of only sugar and water and are therefore less confounded by other nutrients). Differing results and insights, however, may be attained when sugars are consumed as solids in foods versus liquids in beverages. Indeed, it has been consistently reported in observational studies that SSB intake may be one stimulus of subclinical inflammation, as measured by CRP.[20, 381-383] It was reported by several short-term trials that carbohydrates consumed as solids increased satiety more than those consumed as liquids.[78, 372, 447, 448] Subsequent calorie balance is compensated for by additional solid-based sugar intake, which is not the case for liquid sugars.[78, 449] A 10-year follow-up study of adolescent girls investigated the association between liquid versus solid sugars on measures of adiposity.[450] After adjustment for energy intake, the association between each additional teaspoon of added sugars consumed as either a beverage or food remained significant

only for liquid added sugars and waist circumference (P = 0.02; 0.16mm/tsp). This provides support that sugars consumed as a liquid may uniquely affect the distribution of body fat. Our meta-analysis included trials that administered sugars that were liquid, solid, and a mixture of both, which when considering the unique effects that particular sugars exhibit cannot be easily compared to each other. For future research efforts in this area that examine the effects of specific sugars on inflammation, trials administering liquid sugars should be considered separately from those that administer solid sugar forms.

Another challenge in nutritional research is distinguishing the effects measured in hypercaloric trials versus eucaloric trials. Other studies found that fructose-containing sugars consumed at normal levels do not adversely affect health.[344, 358] In this context, there should be separate investigations of eucaloric and hypercaloric effects of dietary sugars on inflammatory markers, as eucaloric trials can give relevant information on the metabolic pathways linking sugar and inflammation, and hypercaloric trials inform on the public health relevance for inflammatory biomarkers in a hypercaloric setting, as is often the case in real-world intakes. Most of the interventions included in our review did administer fructose with an isocaloric control group of dietary glucose, and most of these were hypercaloric trials. There was no difference observed between comparison groups of the hypercaloric trials that administered very high doses. The lack of difference here may be due to post-prandial stress that is activated by high energy intake regardless of the sugar source and leads to triggered low-grade inflammatory processes in both groups. These findings from hypercaloric trials would then not allow conclusions to be drawn about the unique effects of fructose versus glucose metabolism.

One major debate in the topic of sugar and health is whether fructose and glucose, calorically matched monosaccharides, have metabolic effects and fates in the body that strongly differ from one another. After absorption through portal circulation, both fructose and glucose are taken up by the liver, which plays a role in controlling the amount of glucose that reaches peripheral areas in the body. Rising glucose levels stimulate the release of insulin, increase glycogen synthesis and increase fatty acid synthesis. Fructose, on the other hand, does not directly stimulate insulin secretion and is metabolized primarily by the liver where the process of de novo lipogenesis (DNL) is stimulated when consumed in high amounts.[451, 452] Fructose and glucose may contribute to inflammatory processes through their own unique pathways. High levels of fructose intake can promote DNL leading to increased liver fat and thereby triggering ROS formation and inflammatory processes. Large intakes of dietary

glucose, on the other hand, can lead to postprandial hyperglycemic spikes and there is evidence that hyperglycemia induces immune responses and endothelial activation.[453-457] Therefore, it is not necessarily correct to hypothesize that it is either dietary fructose or dietary glucose that contributes more to systemic inflammation because both of these sugars have been implied in inflammatory processes in humans through their own unique metabolic pathways. In this context, it is also of interest to consider that anti-oxidants can hinder some of the inflammatory effects stimulated by hyperglycemia,[458-460] which when consumed in food matrices together with dietary sugar may also impact how sugar affects inflammatory processes.

Different aspects of carbohydrate quality have also been discussed in the development of chronic inflammation. A likely intermediate between carbohydrate quality and chronic disease risk is chronic low-grade inflammation.[461] Whole-grain foods have anti-inflammatory bioactive compounds that scavenge for free radicals and modify redox status of cells and tissues.[462] Viscous fiber slows down the rates of glucose absorption.[463] Such a post-prandial ranking of glycemic potency is best described by the GI. It has been found that a high-GI consumption can contribute to increased chronic low-grade inflammation.[464] The evidence for the anti-inflammatory effects of low-GI/GL diets is more consistent than for higher fiber or whole-grain diets.[461] Based on these findings, the low GI of dietary fructose may be a reason why there was no clear evidence in our meta-analysis for a pro-inflammatory effect of fructose versus glucose. Ingestion of fructose may even contribute to a reduction of chronic inflammation due to the avoidance to glycemic spikes. This may provide some explanation for the absence of consistent harm in response to fructose intake.

Many health professionals believe that avoiding sugar and SSB would have a greater beneficial impact on post-prandial glycemia than avoiding high GI foods like starchy breads or potatoes. Starchy foods have a GI greater than 70 while sugary foods are most often less than 70.[465] This misconception puts a disproportionate focus on dietary sugars as the main culprit, possibly missing more impactful findings regarding the effects of high-GI/low quality carbohydrates and disease development. What is often overlooked is that altogether, we consume twice as much energy in the form of starch than added sugar.[466] Leading experts of carbohydrate nutrition state that carbohydrate quality or type play a more important role in the health of populations than does carbohydrate amount as a percentage of dietary energy.[467] These experts reviewed that the consumption of potato products, high GL grains and added sugars (as liquids in drinks) are causally related to diabetes, obesity, cardiovascular disease and some cancers, but whole fruits, legumes, whole kernel grains and non-starchy vegetables appear

protective.[467-470] This may be explained in part by how these carbohydrates differently affect the degree of postprandial glycemia or insulinemia.[468]

6.1.3 Additional considerations (Study III)

Study design and population

A main strength of the DONALD Study lies in its prospective nature covering a period from birth until young adulthood. This study design gives an indication of the temporal relationship between exposure and outcome (one important causality criterion).[259, 471] The repeated, closely-spaced, and comprehensive measurements are a further strength, making it feasible to determine habitual behaviour by combining data from multiple assessments. Since the longitudinal design of the DONALD Study covers the period of childhood and adolescent development, these potentially critical periods in the programming of later diseases are considered. However, prospective studies may still suffer from other issues that limit the validity of causation inferences: selection bias, measurement error, and confounding.[259] Selection bias, which encompasses biased associations resulting from the selection of study participants,[472] may have been introduced into the DONALD study by recruitment of a non-representative sample of healthy children from the region of Dortmund. Due to the demanding assessment procedures of the DONALD Study (including anthropometric measurements, 24-hours urine sampling and 3-days weighed records each annually over a period of about 20 years) the study sample is characterised by a higher educational and socio-economic status with a generally high interest in health-related topics that render the population non-representative.[236, 249] There is a burden placed on the participants and their parents due to the detailed and frequently repeated assessments that influenced the selection of the population. However, the BMI of DONALD participants differed only slightly from German reference percentile curves and dietary intakes resemble those of nationwide German studies.[236, 473] Finally, daily macronutrient intakes from the nationwide VELS (Consumption Survey of Food Intake Among Infants and Young Children) survey in early childhood were comparable in DONALD i.e., about 50% carbohydrates, 14% protein and 35% fat.[474]

Dietary assessment

The DONALD Study uses a 3-day weighed dietary record,[475] which is considered to be the most accurate dietary assessment method for large study populations. Through this direct

and prospective nutrition assessment, a reliable and detailed description of dietary intake can be determined. In addition, the continuously updated in-house nutrient database LEBTAB allows the consideration of fructose, glucose and sucrose as well as different types of fructose-containing sugars (total sugar, free sugar, added sugar). This study is 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.

Due to the highly detailed and accurate dietary records requiring constant weighing, there is a burden placed on study participants during the time of assessment, which may lead to reactivity and adjustment of dietary behaviour.[476] The changed diet resulting from this reactivity could vary widely from the habitual diet leading to biased results. It is possible, however, that reactivity could merely lead to misreporting with unchanged dietary behaviour.[476] Misreporting is a serious challenge for all dietary assessment methods and expected to be specifically relevant among adolescents.[84]

Underreporting

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.[39, 40] 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.

Underreporting is the most widespread form of misreporting and shows associations with BMI, gender, and age[249, 475, 477-482] as well as socio-economic status, education and lifestyle factors.[478] Systematic differential misreporting concerning select foods may weaken associations or bias results.[478, 479][353, 354]. It is possible that such selective underreporting could more likely occur with sugar-rich foods such as sugar and SSB. There is to date no reliable method to identify selective sugar underreporting.[22] However, our sensitivity analyses that excluded underreporters of energy intake, i.e. dietary records that had implausible energy intake values, yielded similar results.

Unlike many other observational studies of this nature, it is 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.

Urine Collection and Analysis of Sugar Excretion

The 24-h urine samples collected in the DONALD Study allow for the quantification of urinary metabolites and are highly valuable for the measurement of sucrose and fructose excretion. The use of 24-h urine samples in this study is also advantageous because the output of metabolites over a 24-h period provides a more robust monitoring method of daily physiological metabolism than single spot urines or blood samples.[483] Likewise, 24-h urine collections include total daily concentrations of metabolites and are independent of urine volume. However, a single 24-h urine sample does not provide information about habitual metabolite excretion. Of note, the urine samples were quality-checked before analysis to determine that 24-h samples were complete (estimated by anthropometry-specific 24-h urinary creatinine excretion references [238]) without collection errors (e.g. presence of faeces in the samples) and from healthy children (e.g. absence of urinary tract infections). 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.

The use of urinary biomarkers to measure 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 in part 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.[475, 484] 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.[485] Earlier work has shown that small amounts of sucrose pass unchanged across the small intestine and are excreted in the urine.[486] Of the two cleavage products of sucrose, glucose is under tight insulin control and is effectively taken up in the renal tubule, and under normal conditions is not found in urine samples. Fructose, however, has been detected in the urine after oral administrations of sucrose.[487] 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 the 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. A study found that the mean daily excretion of fructose and sucrose was significantly lower after a low-sucrose diet

compared with a base diet, finding that sucrose intake was significantly associated with excreted fructose and sucrose.[391] 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.[36, 37, 41, 42]

Outcome measurements

There is the possibility of measurement error in the assessment of the outcome variables.[259] Certain limitations in the general assessment of the outcomes investigated in Study III should be considered: (1) the assessment of risk factors instead of diabetic or cardiometabolic endpoints, such as T2D incidence, and (2) the single measurement of risk factors in one blood sample taken in early adulthood. The assessment of risk factors instead of discrete endpoints was necessary because the follow-up of the DONALD study was too short for cardiometabolic diseases to manifest. Although risk factors may not guarantee the final development of cardiometabolic diseases, they are well-accepted predictors of chronic diseases such as T2D and CVD based on consistent prospective associations and mechanistic proof of their role in the cardiometabolic pathophysiology. Risk factors could shed light on the potential mechanisms by which dietary sugar associates with the hard endpoint of T2D. Simultaneous investigation of diverse risk factors or markers relevant for early stages of disease development is recommended.[139] The single measurement of risk factors in adulthood may in some extent have led to measurement error because measurements of blood lipids, glucose and insulin are known to vary considerably.[488] T2D diagnosis requires a confirmation by repeated tests [181, 489] and it is recommended that HOMA estimation in individuals is based on three fasting insulin samples.[183] On a population level, however, single measurements are reported and expected to be sufficient. As later follow-up measurements become available, a re-examination of this analysis including more blood samples would be of interest.

Further considerations

With all of the biological mechanisms in the literature used to explain the link between fructose-containing sugars and risk of T2D,[375, 490-493] it may be difficult to reconcile our result of a protective effect of urinary fructose on risk makers for T2D. Looking at unhealthy sources, in our population SSB intake was lower than average adolescent intake values. The limitation of liquid sources of fructose in our population may have helped to prevent any

negative effects arising from the overconsumption of rapidly absorbable sugars. Fructose-containing sugars, as measured by urinary fructose excretion, were consumed moderately over time and the sources of fructose were more healthful. In this context, it appears that fructose intake had a beneficial association with risk markers for T2D. SSB intake is a marker for an overall unhealthy lifestyle, [383, 494-496] the consumption of which is associated with lower physical activity, poorer diet, and higher risk of smoking.[20, 497, 498] It could be on the other hand that the limitation of SSB (as observed in Study III) is a marker for an overall more healthy lifestyle. These factors may be difficult to measure and adjust for in cohort studies. The inability to completely rule out residual confounding in observational studies is an inherent limitation of this study design.

An explanation for why SSB is so strongly associated with diabetes in the literature is excessive and uncompensated energy intake. Multiple meta-analyses and systematic reviews of controlled feeding trials have shown that the adverse effects of dietary sugars on cardiometabolic risk factors are mediated by excessive energy intake.[148, 162, 213, 358, 499-501] A harmful effect is most commonly seen when liquid sugars are supplemented on top of background diets as additional or excessive energy (hypercaloric diets). In addition, sugars in liquid form elicit a lower satiety response and are less likely to be compensated by a decrease in energy intake at subsequent meals compared to solid sugars.[502] This could be a mechanism by which SSB leads to a positive energy balance and weight gain,[78] increasing the risk of developing T2D. Besides SSB, there are other sources of fructose-containing sugars that contribute to overall sugar intake like grain products, fruit products and dairy products.[318, 503] However, it has been reported that these sources sweetened with sucrose such as whole-grain cereals, fruit, and yogurt have a protective association with T2D.[504-506] In understanding the role of dietary sugar in disease development it is crucial to separately appraise sugar predictors based on what form they are in (liquid versus solid), the intake amount (considering the confounding factor of excessive energy), and their source.

6.2 Research Aims

The following paragraphs summarize the results of Studies I-III with respect to the research aims of this thesis. For a more detailed and specific discussion, including comparisons with findings from the literature and potential mechanisms, see the corresponding discussion sections in Chapters 5.1 - 5.3.

6.2.1 Aim 1: World trends in SSB and dietary sugar

Study I aimed to investigate world trends in the consumption of SSB and dietary sugar among children and adolescents. We set out to answer how intake trends of SSB and dietary sugar have changed over the past several decades in these age groups and how these trends compare between various countries around the world, particularly between the United States and other international countries. The following paragraphs will attempt to answer these questions based on findings from Study III.

SSB consumption among US children and adolescents increased from the 1960's until approximately the year 2000. These trends were similar for all three age groups, though the increase in SSB was greatest among adolescents aged 11-19 y. Among international trends, SSB consumption varied by age and country, tending to decrease or remain stable from 1990 to 2015 in 1-5 y and 6-11 y age groups in Canada, the United Kingdom, Russia, Australia, South Korea, and China (with intakes in Mexico being an exception). For adolescents aged 11-19 years, SSB consumption tended to slowly increase or remain stable from 1991 to 2012 in Australia, the United Kingdom, Spain, China, Russia, Mexico and South Korea. In Norway, there was a clear decrease in SSB intake from 2001 to 2008. For Mexico and in one South Korean report, intake increased significantly from 1999 to 2012 and 2001 to 2009, respectively. Studies from Australia, the United Kingdom and China that covered wide age ranges all reported decreases in SSB intake from 1995 to 2012.

Looking now at trends in dietary sugars consumption, national US data for the age groups 2-5 y and 2-18 y indicate consumption increased significantly from the 1970s until late 1990s and early 2000, when intake peaked and began to decline significantly. For adolescents 12-19 y, the decrease in added sugar started as early as 1994 and continued until 2010. Decreases in added sugar and total sugar intakes occurred in all studies starting approximately in 2000 onward. In other international countries mainly from Europe (one being from Australia), types of dietary sugars varied. Dietary sugar tended to remain stable in many of the included European countries over time. Only in Ireland were large increases in total sugar reported in the 1990s. Within wide age groups, on the other hand, declines in intake of dietary sugars were observed from 1995-2012 in Australia, 1995-2006 in Denmark, and 1997-2012 in the United Kingdom.

Patterns similar to SSB consumption trends in the US were only observed in Canada, the United Kingdom and Australia. SSB intakes in other countries across the world tended to remain stable from about 1990 to 2010. Of note, among adolescents from other countries, rather

than observing declines in SSB intakes in the decade after year 2000, as seen in the United States, intakes were generally stable. This was however not the case among adolescents in South Korea and Mexico, where intakes markedly increased (ie, for these countries, the concern that SSB consumption may be on the rise is valid).

Similarly, levels of dietary sugar intakes in non-US countries tended to rise gradually or remain stable from 1980 through 2009 (except for Ireland, where intake increased rapidly in the 1990s) among children and adolescents. In Australia, the United Kingdom, and Denmark, gradual declines in dietary sugar intake were observed from the mid 1990s to 2010–2012 among broad age groups spanning childhood and adolescence. This contrasts with levels of added sugar intakes in the United States, where increases peaked between the years 1995 and 2000 among all childhood and adolescent age groups and then declined substantially until 2010, remaining higher overall than baseline intake levels from 1977. In addition, studies from other regions of the world reported on intake levels until 2010 only; however, a recent longitudinal study from Germany indicated that although sugar intake levels increased until 2005 and then started to decrease, the most notable decrease was since 2010. Therefore, it is possible that decreases as observed in the United States will occur within the next decade in many other countries around the world.

Unique contribution of this review:

Some other national data suggested that there were decreases in sugar and SSB consumption in the United States,[50] yet this had not been systematically established, and it was not clear whether dietary sugar and SSB intakes in other countries had decreased in tandem with those of the US or whether their consumption was still increasing, as is characteristic of continued adoption of a Western diet. In the past, monitoring often drew on either supply or apparent consumption data,[54] or on national dietary surveys to determine within-country trends.[17] Supply data, however, are not suitable for assessing consumption patterns for children and adolescents, because it is not possible to measure different subgroups of the population[34] and food waste is not accounted for. In a study of world trends in dietary sugar intake, Wittekind et al.[17] assessed within-country trends using some national dietary survey data; however, theirs was not a systematic review and did not include SSB trends. Therefore, our contribution was to provide a systematic overview of world dietary sugar (eg, total, added, free) and SSB intake trends by collating repeated cross-sectional trend data together with some longitudinal follow-up studies. Data from US studies were analyzed as a starting point because

of the broader amount of available trend data, followed by data from other countries. This review provides international estimates on intake trends across multiple decades of not only dietary sugar but also SSB consumption from countries around the world, including Australia, North America, Asia, and Europe. Our review allows some comparisons between the United States and other parts of the world, unlike other reviews in which only within-country trends were analyzed or only reported from a specific source[17, 51] and did not at all include SSB trends.

A strength of this review is its specific focus on childhood and adolescence, thus targeting vulnerable age groups for which research on intake trends over time is sparse. Furthermore, US intake trends are reported, providing a broad overview not only from national data but other studies to confirm trends systematically. This review uses dietary intake data assessed at an individual level using dietary assessment methods with the highest reliability to increase accuracy. No food-supply data or apparent consumption data are included, because these do not reflect actual intake amounts and cannot report by age. Methods of dietary analyses used in the included studies did not change between assessment periods (with 2 exceptions), meaning that small changes in dietary intakes across assessment years could not be attributed to changes in the method of dietary assessment. This timely effort will help to provide estimations on the direction of sugar and SSB intake trends to inform national nutrition policy, monitor progress toward meeting dietary guidelines, and provide a benchmark for ongoing surveillance in the critical periods of childhood and adolescence.

Direction for future research

It is estimated and projected by the global sugar market that sugar sales will continue to increase rapidly over the next several years, especially in emerging and developing countries. As reliable dietary data from these countries become available, a more complete review of sugar and SSB trends will need to be undertaken to assess these rapid changes in developing countries. Furthermore, this review may help provide a springboard for future research that attempts to compare intake trends with rates of obesity, diabetes, or cardiovascular disease in children and adolescents. More insights will be able to be gained and continue to unfold in future research to help ascertain how the intake trends reported by this review have been impacted by public health initiatives to reduce sugar and SSB consumption. As we determine which interventions (governmental, industrial, etc.) have produced the most promising results,

we will be able to identify public health programs that are most effective in reducing dietary sugar and SSB consumption in communities and nations.

6.2.2 Aim 2: Dietary sugar and inflammation

Study II aimed to assess the effect of dietary sugar intake (in particular fructose versus glucose) on biomarkers of subclinical inflammation by means of a systematic review and meta-analysis. The research question set out to answer whether based on evidence from human interventional studies the intake of dietary fructose contributes more to systemic inflammation than does dietary glucose. The following section will attempt to answer this question based on findings from Study II.

It has been postulated that dietary sugar consumption contributes to increased inflammatory processes in humans. The differences in the metabolism of fructose (alone or found in sucrose) versus that of glucose should be considered in order to distinguish what potential role these monosaccharides may play in increasing inflammatory processes. Our meta-analysis results were classified into subgroups according to the types of dietary sugar interventions and controls as follows: (a) Fructose vs glucose (n = 6 studies; n = 8 study arms) and (b) HFCS vs sucrose (n = 3 studies; n = 6 study arms). The comparator of fructose interventions vs glucose control groups showed no differences for CRP. In the comparator of HFCS interventions vs sucrose control groups, changes in CRP were non-significantly higher in HFCS compared to sucrose groups. Overall, the quality of meta-evidence for the association between dietary fructose vs glucose, and comparing HFCS vs sucrose on CRP was rated as "low". This judgement was mainly based on the low number of identified studies and study participants, the study limitations, and the heterogeneous nature of the studies. Further research of similar human intervention trials will provide additional evidence, and would likely change the effect estimate. As summarized narratively, effects were observed in a small number of studies, but the overall picture is inconsistent and the effect sizes are variable. Additional evidence from future intervention studies on this topic are needed in order to draw confident conclusions as to the effects of dietary sugar on subclinical inflammation.

<u>Strengths of this meta-analysis</u>

The evaluation of the impact of dietary sugars is often hampered by the lack of an isocaloric comparator, which makes it therefore unclear whether the effects result from fructose or sucrose themselves.[345] A strength of most of the fructose intervention studies included in this review is that they do administer fructose with an isocaloric control group of dietary glucose, although most of these studies were hypercaloric trials. The strengths of this meta-analysis and systematic review include its standard to only analyze controlled human intervention trials, and its inclusion of six interventions that had similar isocaloric control groups. The inclusion of a broad range of biomarkers of subclinical inflammation is also a notable strength, allowing for a more thorough evaluation of the effects of dietary sugar on low-grade inflammation. In analyzing the main findings, quantification of the observed associations was possible, another advantage of this review. The objective quantification of the effects in this meta-analysis was reinforced by additionally carrying out a risk of bias assessment and assessing the quality of meta-evidence.

Directions for future research

The fact that there was often no measured difference between intervention and control groups in studies that administered supra-physiological doses may be due to post-prandial stress that activates low-grade inflammation due to extremely high energy intake. Therefore, in order to be able to draw firm conclusions for health care policy decisions, more large-scale, effective, longitudinal intervention studies that investigate sucrose or HFCS ingested at average intake levels (75–100 grams/day) compared to control groups, as well as studies which ingest sucrose or HFCS at the lower levels recommended by WHO or the UK's SACN guidelines (5% of energy intake or about 25 grams/day), need to be conducted with low-grade inflammation as an outcome. Since sugar is typically not consumed in an isolated monosaccharide form, studies which compare glucose and fructose isocalorically at average consumption levels would serve to further understand the unique metabolic differences between fructose and glucose, and how they affect inflammation.

Results from industry- and non-industry-funded research often differ, and a recent analysis suggests that industry-funded research tends to underestimate the adverse effects of dietary sugar.[346] The personal views from industry researchers could present another source of bias and should be interpreted with caution. Furthermore, some methodological aspects in the measurements of inflammatory markers can be considered. Ideally, one would like to have a clear picture of which biomarkers are responsive to fructose and sucrose across all studies,

which would allow conclusions on the pathways relevant for disease prevention. This is, however, hampered by methodological problems, ranging from the use of very different/non-standardized assays to the large inter-individual variation in inflammatory biomarkers. Such variation is affected by the presence or absence of accompanying metabolic conditions. Repeated measures both pre- and post-intervention would be required in order to ascertain the size of such variations in a study population. Including study participants who are similar in age, body weight and health status is also important in order to further reduce bias from inter-individual variation.

It is certainly possible that weight gain is a potential mediator in the association between sugar intake and inflammation. In this context, it is of interest for future research efforts to separately investigate the eucaloric and hypercaloric effects of dietary sugars on inflammatory markers, as the former can give relevant information on the metabolic pathways linking sugar and inflammation, whereas the latter informs on the public health relevance for inflammatory biomarkers in a hypercaloric setting, as is often the case in real-world intakes. The question of whether it is excess energy intake or excess sugar intake that leads to adverse health outcomes requires further research. Similar work was done in a review by Sievenpiper et al., who found that fructose intake only affected weight gain in hypercaloric versus isocaloric trials. Considering the relationship between obesity and low-grade inflammation, a discussion of the change in body weight in the included trials would be relevant.

Considering how the effects of sucrose or fructose differ based on whether it is consumed as a liquid or a solid, future research in this area should attempt to separate findings based on food form or source of dietary sugar. In order to draw more confident conclusions about the proinflammatory effects of fructose-containing sugars versus glucose, or other non-fructose containing carbohydrates (e.g. fructose vs glucose, sucrose vs maltose, or sucrose vs refined carbohydrates), further human intervention studies with larger sample sizes, longer follow-up periods, better controlled designs, and repeated measures (to reduce bias of inter-individual variation) of subclinical inflammation as a planned outcome are required.

6.2.3 Aim 3: Dietary sugar and T2D/inflammation

Study III aimed to examine the prospective relevance of dietary sugar intake in adolescence, as measured by dietary records and urinary sugar biomarkers, for the development of risk markers for T2D. Research questions set out to answer whether a higher intake of certain dietary

sugars in adolescence associated with a higher risk for developing risk factors for T2D in adulthood and whether a higher excretion of urinary sugars in adolescence also associated with a higher risk of developing risk factors for T2D (insulin sensitivity, fasting insulin and systemic inflammation as measured through a pro-inflammatory score).

High intake levels of fructose administered in intervention and acute studies that report adverse effects of fructose do not represent common intake patterns consumed habitually over time. Observational studies link the consumption of SSB to increased chronic inflammation, yet it is unclear whether a modest and habitual sugar intake in adolescence is associated with later development of systemic inflammation. Therefore, our study examined the prospective association between the intake of dietary sugar in adolescent years and the target outcomes of T2D risk factors by using a comprehensive approach on the basis of chemical sugar types (fructose, glucose, sucrose), sugar use (total sugar, added sugar, free sugar), as well as urinary sugar excretion levels. This unique approach allowed 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.

The results of our analysis reported that the intakes of dietary fructose, glucose or sucrose in adolescence were not independently associated with adult HOMA2-S% or insulin levels (all $p>0.01$). Similarly, there were no independent associations between total, free, or added sugar intakes in adolescence and adult HOMA2-S% or insulin levels (all $p>0.01$). 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). Reporting on adult systemic inflammation as an outcome, intakes of glucose, fructose or sucrose as well as total sugar, free sugar or added sugar were not independently associated with the pro-inflammatory score in adulthood (all $p>0.01$). Similarly, sugar excretion levels during adolescence were not associated with pro-inflammatory score in adulthood (all $p>0.01$). The main finding suggests that dietary sugar is 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. The assumption that dietary sugar intake or excretion in adolescence increases risk for developing T2D in adulthood could not be confirmed. The context in which fructose-containing sugars were consumed (moderately and habitually) should be considered when interpreting these results.

Strengths of this analysis

The main strength of Study III is the 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 is 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 allows the consideration of fructose, glucose and sucrose as well as different types of fructose-containing sugars (total sugar, free sugar, added sugar). Our study is 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 24h-urine samples. This allowed for a unique database compiled from self-reported sugar intake data and urinary sugar excretion as biomarkers to be used to investigate the role of dietary sugars.

Direction for future research

When consumed in high amounts, dietary fructose has been associated in cohort studies with increased risk of T2D.[225, 226] Inconsistent findings related to sugar intake and diabetes risk may be due to the varying levels of sugar intake and the possibility that different sugars elicit different metabolic effects.[227] Our finding indicating a beneficial association of fructose intake with insulin sensitivity is not confirmed by many intervention studies in which high proportions of fructose were consumed. When assessing the effect of dietary fructose, a distinction needs to be made between trials that administer high versus low doses. Of note, the DONALD population from Study III was healthier than average and consumed only moderate amounts of fructose, and we investigated longer-term relevance which is different from short- or medium-term responses to fructose consumption (evidence from available randomized controlled trials).

With regards to future research using similar study designs and follow-up periods, it would be of interest to investigate the associations of sugar intake among children and adolescents with average intake levels of fructose-containing sugars (especially regarding SSB intake) from more representative study populations. It is preferred that sugar intake be assessed by weighed dietary records and where possible urinary biomarkers. Further research is needed for the comparison of various food sources of fructose, especially comparing liquid fructose-

containing SSB versus solid sources of fructose. Trials should attempt to include particpants that are similar to each other in weight, age and overall health status. Weight gain should also be avoided so as to better isolate the effects of sugar independent of changes in weight. Since sugar is typically not consumed in an isolated monosaccharide form, evidence reporting on real world sugar types in which fructose is co-consumed with glucose in same or similar ratios are needed and would be most relevant to public health.

6.3 Public health relevance and practical implications

As advised by the WHO, countries need to promote policies that help curtail the overconsumption of sugars in order to slow the epidemic of diabetes and obesity.[507] For this, they need comprehensive plans of action that combine initiatives in taxation, education, and restriction of the marketing of sugary products to children. The importance of prevention early in life is widely acknowledged,[508, 509] and there is much that public health organizations can do to protect this vulnerable population.

Firstly, awareness can be raised about the health issues associated with excessive sugar intake especially from SSB sources. Social marketing initiatives provide information in various formats to change attitudes and raise awareness.[510] This accompanies the need to improve labelling information. If labels are complicated or inconsistent, they have the potential to do more harm by confusing the consumer than good. Health claims should also be monitored to ensure that high-sugar and low-fat processed foods are not promoted as heart-healthy products. Empty calories in the diets of children and adolescence should be limited to the amount that fits their energy and nutrient needs. The AAP advises the use of a minimum amount of added sugars to promote the enjoyment, palatability and consumption of foods that are nutrient-dense.[511] SSBs, confectionary and sweetened refined grains are more likely to negatively affect health, while sweetened high-fiber and whole grain cereals likely have a beneficial impact. Sugar is best used when it enhances the intake of nutrient-rich foods. Increasing the availability of healthy snacks especially from fruits in schools and homes helps in the avoidance of unhealthy and processed alternatives. Also, providing water and restricting portion sizes of SSB can help to reduce the intake of unfavourable sources of fructose-containing sugars. This was the case for participants of Study III where intakes of SSB were limited and fructose-containing sugars came mainly from more healthful sources.

Other public health initiatives include the reformulation of sugar and changes in portion sizes. When endeavouring to reduce the sugar content of SSB, food manufacturers can replace sugars with artificial sweeteners without substantially changing the texture and taste. This is evidenced in the wide range of diet drinks available, leading to an overall lower energy content of these products. In order to decrease the energy content of solid foods, the focus is more often on fat because it is energy dense and is often replaced with sugar, while replacing sugar with other nutrients would either increase or not change the energy content of the product. Sugar also has chemical properties that are difficult to replace with artificial sweeteners. Therefore, for foods such as cakes and other baked goods the best option is to reduce the portion size. Portions of meals, snacks and drinks (especially SSB in the US) have been increasing over the past decades. A comprehensive Cochrane review assessed the impact of increased portion sizes and concluded that the result has been an increase in energy intake by 12-16%.[512, 513] Instead of restricting the choice of portions available to consumers which would likely be unpopular to the public, work needs to be done to change the food culture and general attitudes of customers to ask for and expect smaller portions. Along with this attitude change, consumers can be encouraged to avoid mindless eating and snacking throughout the day, but rather eat at designated times and places. The AAP 2019 policy statement also called for the recommendation to increase the price of sugary drinks. This could entail the introduction of an excise tax with revenues going to educational campaigns and efforts to reduce socioeconomic and health disparities.[430] Taxes on sugary drinks are associated with significant decreases in the purchases of SSBs and increase the consumption of water.[430, 514] In addition, state governments should implement school policies that restrict and even prohibit school districts from selling sugary drinks at school. These efforts initiated by health advocates in public health nutrition hold promise for population-wide dietary improvements and can be designed on social, environmental and policy levels.[436]

7. Conclusions and perspectives

In summary, the results presented in this thesis suggest that SSB and dietary sugar intakes from the United States differ starkly form those of other countries around the world and intake levels appear to be decreasing in some parts of the world among children and adolescence. Further results suggest that dietary fructose does not illicit more deleterious effects on subclinical inflammation than other dietary sugars as measured by limited evidence from a meta-analysis of human intervention trials. Lastly, this thesis did not provide support that dietary sugar consumed in adolescence is associated with adult insulin sensitivity, one potential exception to this was dietary fructose, which in the context of a moderate consumption pattern had a beneficial association with adult fasting insulin and insulin sensitivity.

In order to be able to draw firm conclusions for health care policy decisions, more large-scale and effective intervention studies that investigate dietary sugar ingested at average intake levels compared to control groups, as well as studies which ingest dietary sugar at the lower levels recommended by WHO or the UK's SACN guidelines need to be conducted. Since sugar is typically not consumed in an isolated monosaccharide form, studies which compare glucose and fructose isocalorically at average consumption levels would serve to further understand the unique metabolic differences between fructose and glucose, and how they affect disease outcomes such as systemic inflammation. In addition, large and more representative longitudinal studies that distinguish types, sources and form of dietary sugar consumed at average levels in adolescence need to be carried out with risk markers of T2D as outcomes.

As decreases in dietary sugar and SSB consumption in the US, the UK, Australia and other countries are observed, the implications of these dietary shifts on human health are unclear. In view of dietary guidance concerning the consumption of dietary sugars, it is important to continually assess and review recent sugar and SSB intake trends from different countries to determine whether populations are responding to public health initiatives. In addition, further cross-country comparisons that inform on why intakes are decreasing in some countries while increasing or staying stable in others would be important to conduct. Future research is needed to investigate how intake trends are changing in the developing world where sources indicate that intakes are on the rise. In developed countries, further research into whether rates of chronic disease have declined in conjunction with decreased intakes of dietary sugar and SSB is warranted.

8. References

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9. Appendix

The original studies included the below appendix items (too large to include in this thesis).

The following appendix items for Study II are available at the following url:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5986486/

Study II:

Figure S1. Risk of bias table (Study II)
Figure S2. Forest plot for HFCS vs sucrose and (hs)CRP as an outcome (Study II)
Figure S3. Forest plot for fructose vs glucose and (hs)CRP as an outcome (Study II)

The following appendix items from Study I and Study III are made available in a separate supplementary material digital document:

Study I:

Table S1. US trend data on SSB and dietary sugars consumption: repeated cross-sections (Study I)
Table S2. International trend data on SSB consumption: repeated cross-sections (Study I)
Table S3. International trend data on dietary sugars consumption: repeated cross-sections (Study I)
Table S4. Trend data on candies, sweets and desserts consumption (Study I)
Table S5. Name of study, statistical analysis and significance, sugar categories, funding and conflict of interest (Study I)
Table S6. Risk of bias overview: ROBINS-I (Study I)
Table S7. Risk of bias assessment: ROBINS-I (Study I)

Study III:

Table S8. Sex-stratified prospective associations of total dietary fructose, sucrose and glucose intake during adolescence with the inflammatory score in early adulthood (dietary fructose, sucrose and glucose and inflammatory score (Study III)
Table S9. Sex-stratified prospective associations of total dietary sugar, added sugar, and free sugar intake during adolescence with markers of insulin sensitivity early adulthood (Study III)
Table S10. Sex-stratified prospective associations of urinary fructose, urinary sucrose, and the sum of urinary fructose and sucrose excretion during adolescence with the inflammatory score in early adulthood (Study III)

10. Versicherung

„Ich erkläre: Ich habe die vorgelegte Dissertation selbständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt, die ich in der Dissertation angegeben habe. Alle Textstellen, die wörtlich oder sinngemäß aus veröffentlichten Schriften entnommen sind, und alle Angaben, die auf mündlichen Auskünften beruhen, sind als solche kenntlich gemacht. Bei den von mir durchgeführten und in der Dissertation erwähnten Untersuchungen habe ich die Grundsätze guter wissenschaftlicher Praxis eingehalten“

Datum: Mar 10, 2021 Unterschrift Karen A. Della Corte