# Sugar consumption and cardiometabolic risk. With a focus on the urinary sucrose and fructose biomarkers.

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## With a focus on the urinary sucrose and fructose biomarkers

STINA RAMNE | FACULTY OF MEDICINE | LUND UNIVERSITY

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Berit Lilienthal Heitmann
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**Abstract**

Introduction: In contrast to the intake of sugar-sweetened beverages (SSBs), the evidence linking added sugar intake to the risk of cardiometabolic disease (primarily referring to cardiovascular disease and type 2 diabetes (T2D)) is contradictory.

Aim: The aim of this thesis is to elucidate the role of added sugar intake in the risk for cardiometabolic diseases. To obtain further understanding of such a potential association, the aims include exploring differences between the intake of added sugar and different added sugar sources, studying an objective biomarker of sugar intake and investigating various pathways through which added sugar intake could possibly affect cardiometabolic risk.

Method: In the Malmö Diet and Cancer study and the Malmö Offspring Study, both cross-sectional and prospective associations of intake of added sugar and sugar-rich foods and beverages were investigated along with various cardiometabolic risk markers, cardiometabolic incidence outcomes, the gut microbiota composition and the plasma proteome. Furthermore, the urinary sucrose and fructose biomarkers were investigated from overnight urine samples in the Malmö Offspring Study and from 24-h urine samples in individuals with prediabetes in the PREVIEW study.

Results: U-shaped associations between added sugar intake and all-cause and cardiovascular mortality, T2D incidence and C-reactive protein have been observed, whereas SSB intake was associated with increased all-cause mortality, a higher Firmicutes:Bacteroidetes ratio and a lower abundance of the genus *Lachnobacterium* in the gut, as well as a T2D-related plasma proteomic profile. Furthermore, the urinary sucrose and fructose biomarkers in overnight urine samples was found to be a useful complement to self-reported sugar intake, but the 24-h urinary sucrose and fructose biomarkers did not perform optimally in a population with prediabetes.

Conclusion: The intake of SSBs was consistently associated with higher cardiometabolic risk via various measures, whereas the total intake of added sugars showed a U-shaped association with cardiometabolic risk. Future evaluation of these associations can be aided by the use of the urinary sucrose and fructose biomarkers, except in already metabolically impaired individuals, in whom this biomarker may not provide an accurate enough measure of sugar intake.

**Key words** added sugar intake, sugar-sweetened beverages, cardiometabolic risk, type 2 diabetes, cardiovascular disease, urinary sugar biomarker, sucrose and fructose excretion, gut microbiota, plasma proteome

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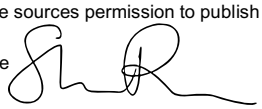
Signature  Date 2021-08-05

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*“I am not young enough to know everything”*

*By rumor, Oscar Wilde,*

*but the true origin of the quote is apparently not known either.*

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# Abbreviations

| | |
|---|---|
| ASB | Artificially sweetened beverage |
| BMI | Body mass index |
| CRP | C-reactive protein |
| CHD | Coronary heart disease |
| CVD | Cardiovascular disease |
| DGA | Dietary Guidelines for Americans |
| EFSA | European Food Safety Authority |
| FFQ | Food frequency questionnaire |
| HDL | High-density lipoprotein |
| HHS | US Department of Health and Human Services |
| HOMA-IR | Homeostatic model assessment of insulin resistance |
| FPG | Fasting plasma glucose |
| LASSO | Least absolute shrinkage and selector operator |
| LDL | Low-density lipoprotein |
| MDC | Malmö Diet and Cancer |
| MDC-CC | Malmö Diet and Cancer-Cardiovascular Cohort |
| MOS | Malmö Offspring Study |
| NNR | Nordic Nutrition Recommendations |
| NSHDS | Northern Swedish Health and Disease Study |
| OGTT | Oral glucose tolerance test |
| PC | Principal component |
| PREVIEW | Prevention of diabetes through lifestyle Intervention and population studies in Europe and around the World |
| RCT | Randomized controlled trial |
| SNP | Single nucleotide polymorphism |
| SSB | Sugar-sweetened beverage |
| T2D | Type 2 diabetes |
| USDA | US Department of Agriculture |
| VLDL | Very low-density lipoprotein |
| WHO | World Health Organization |

# List of papers

**Paper I**

**Association between added sugar intake and mortality is nonlinear and dependent on sugar source in 2 Swedish population-based prospective cohorts.** Stina Ramne, Joana Alves Dias, Esther Gonzalez-Padilla, Kjell Olsson, Bengt Lindahl, Gunnar Engström, Ulrika Ericson, Ingegerd Johansson and Emily Sonestedt. *The American Journal of Clinical Nutrition* 2019;109(2):411-23. doi: 10.1093/ajcn/nqy268

**Paper II**

**Comparing self-reported sugar intake with the sucrose and fructose biomarker from overnight urine samples in relation to cardiometabolic risk factors.** Stina Ramne, Nicola Gray, Sophie Hellstrand, Louise Brunkwall, Sofia Enhörning, Peter M Nilsson, Gunnar Engström, Marju Orho-Melander, Ulrika Ericson, Gunter GC Kuhnle and Emily Sonestedt. *Frontiers in Nutrition* 2020;7. doi: 10.3389/fnut.2020.00062

**Paper III**

**24-hour urinary sucrose and fructose excretion as biomarkers of sugar intake in individuals with prediabetes – a PREVIEW substudy.** Stina Ramne, Emily Sonestedt, Jennie Brand-Miller, Mikael Fogelholm, Thomas Meinert Larsen, Anne Raben and Lars Ove Dragsted. *Manuscript* 2021

**Paper IV**

**Gut microbiota composition in relation to intake of added sugar, sugar-sweetened beverages and artificially sweetened beverages in the Malmö Offspring Study.** Stina Ramne, Louise Brunkwall, Ulrika Ericson, Nicola Gray, Gunter GC Kuhnle, Peter M Nilsson, Marju Orho-Melander and Emily Sonestedt. *European Journal of Nutrition* 2020. doi: 10.1007/s00394-020-02392-0

**Paper V**

**Identification of inflammatory and disease-associated plasma proteins that associate with intake of added sugar and sugar-sweetened beverages and their role in type 2 diabetes risk.** Stina Ramne, Isabel Drake, Ulrika Ericson, Jan Nilsson, Marju Orho-Melander, Gunnar Engström and Emily Sonestedt. *Nutrients* 2020;12(10). doi: 10.3390/nu12103129

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**High versus low added sugar consumption for the primary prevention of cardiovascular disease.** Bergwall S*, Ramne S*, Sonestedt E, Acosta S. *Cochrane Database of Systematic Reviews* 2019(4). doi: 10.1002/14651858.CD013320. *Shared first author

**Association between soft drink consumption and mortality in 10 European countries.** Mullee A, Romaguera D, Pearson-Stuttard J, Viallon V, Stepien M, Freisling H, Fagherazzi G, Mancini FR, Boutron-Ruault MC, Kuhn T, Kaaks R, Boeing H, Aleksandrova K, Tjonneland A, Halkjaer J, Overvad K, Weiderpass E, Skeie G, Parr CL, Quiros JR, Agudo A, Sanchez MJ, Amiano P, Cirera L, Ardanaz E, Khaw KT, Tong TYN, Schmidt JA, Trichopoulou A, Martimianaki G, Karakatsani A, Palli D, Agnoli C, Tumino R, Sacerdote C, Panico S, Bueno-de-Mesquita B, Verschuren WMM, Boer JMA, Vermeulen R, Ramne S, Sonestedt E, van Guelpen B, Holgersson PL, Tsilidis KK, Heath AK, Muller D, Riboli E, Gunter MJ, Murphy N. *JAMA Internal Medicine* 2019. doi: 10.1001/jamainternmed. 2019.2478

**Dietary intake of advanced glycation end products (AGEs) and changes in body weight in European adults.** Cordova R, Knaze V, Viallon V, Rust P, Schalkwijk CG, Weiderpass E, Wagner KH, Mayen-Chacon AL, Aglago EK, Dahm CC, Overvad K, Tjonneland A, Halkjaer J, Mancini FR, Boutron-Ruault MC, Fagherazzi G, Katzke V, Kuhn T, Schulze MB, Boeing H, Trichopoulou A, Karakatsani A, Thriskos P, Masala G, Krogh G, Panico S, Tumino R, Ricceri F, Spijkerman A, Boer J, Skeie G, Rylander C, Borch KB, Quiros JR, Agudo A, Redondo-Sanchez D, Amiano P, Gomez-Gomez JH, Barricarte A, Ramne S, Sonestedt E, Johansson I, Esberg A, Tong T, Aune D, Tsilidis KK, Gunter MJ, Jenab M, Freisling H. *European Journal of Nutrition* 2019. doi: 10.1007/s00394-019-02129-8

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**Replacement of red and processed meat with other food sources of protein and the risk of type 2 diabetes in European populations: the EPIC-InterAct study.** Ibsen DB, Steur M, Imamura F, Overvad K, Schulze MB, Guevara M, Antonio Agudo A, Bendinelli B, Aune D, Amiano P, Barricarte A, Ericson U, Franks PW, Fagherazzi G, Freisling H, Quiros JR, Heath AK, Grioni S, Huybrechts I, Katze V, Laouali N,

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**Associations between added sugar intake and four different cardiovascular diseases in a Swedish population-based cohort.** Janzi S, Ramne S, González-Padilla E, Johnson L, Sonestedt E. *Frontiers in Nutrition* 2020;7. doi: 10.3389/fnut.2020.603653

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**Soft drink and juice consumption and renal cell carcinoma incidence and mortality in the European Prospective Investigation into Cancer and Nutrition**. Heath AK, Clasen JL, Jayanth NP, Jenab M, Tjønneland A, Petersen KE, Overvad K, Srour B, Katzke V, Bergmann MM, Schulze MB, Masala G, Krogh V, Tumino R, Catalano A, Pasanisi F, Brustad M, Standahl Olsen K, Skeie G, Lujan-Barroso L, Rodríguez-Barranco M, Amiano P, Santiuste C, Barricarte Gurrea A, Axelson H, Ramne S, Ljungberg B, Watts EL, Huybrechts I, Weiderpass E, Riboli E, Muller DC. *Cancer Epidemiology, Biomarkers & Prevention* 2021:cebp.1726.2020. doi: 10.1158/1055-9965.Epi-20-1726

**Dietary intake of advanced glycation endproducts and risk of hepatobiliary cancers: a multinational cohort study.** Mayén AL, Aglago EK, Knaze V, Cordova R, Schalkwijk CG, Wagner KH, Aleksandrova K, Fedirko V, Keski-Rahkonen P, Leitzmann MF, Katzke V, Srour B, Schulze MB, Masala G, Krogh V, Panico S, Tumino R, Bueno-de-Mesquita B, Brustad M, Agudo A, López MDC, Amiano P, Ohlsson B, Ramne S, Aune D, Weiderpass E, Jenab M, Freisling H. *International Journal of Cancer* 2021. doi: 10.1002/ijc.33612

**Association between sugar intake and intima media thickness as a marker for atherosclerosis: a cross-sectional study in the Malmö Diet and Cancer Study (Sweden)**. González-Padilla E, Janzi S, Ramne S, Thuneland C, Borné Y, Sonestedt E. *Nutrients* 2021;13(5). doi: 10.3390/nu13051555

**Dietary data in the Malmö Offspring Study – reproducibility, method comparison and validation against objective biomarkers.** Hellstrand S, Ottosson F, Smith E, Brunkwall L, Ramne S, Sonestedt E, Nilsson PM, Melander O, Orho-Melander M, Ericson U. *Nutrients* 2021;13(5). doi: 10.3390/nu13051579

# Abstract

Introduction: In contrast to the intake of sugar-sweetened beverages (SSBs), the evidence linking added sugar intake to the risk of cardiometabolic disease (primarily referring to cardiovascular disease and type 2 diabetes (T2D)) is contradictory.

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The main findings of this thesis are depicted in a graphical abstract (Figure 1).

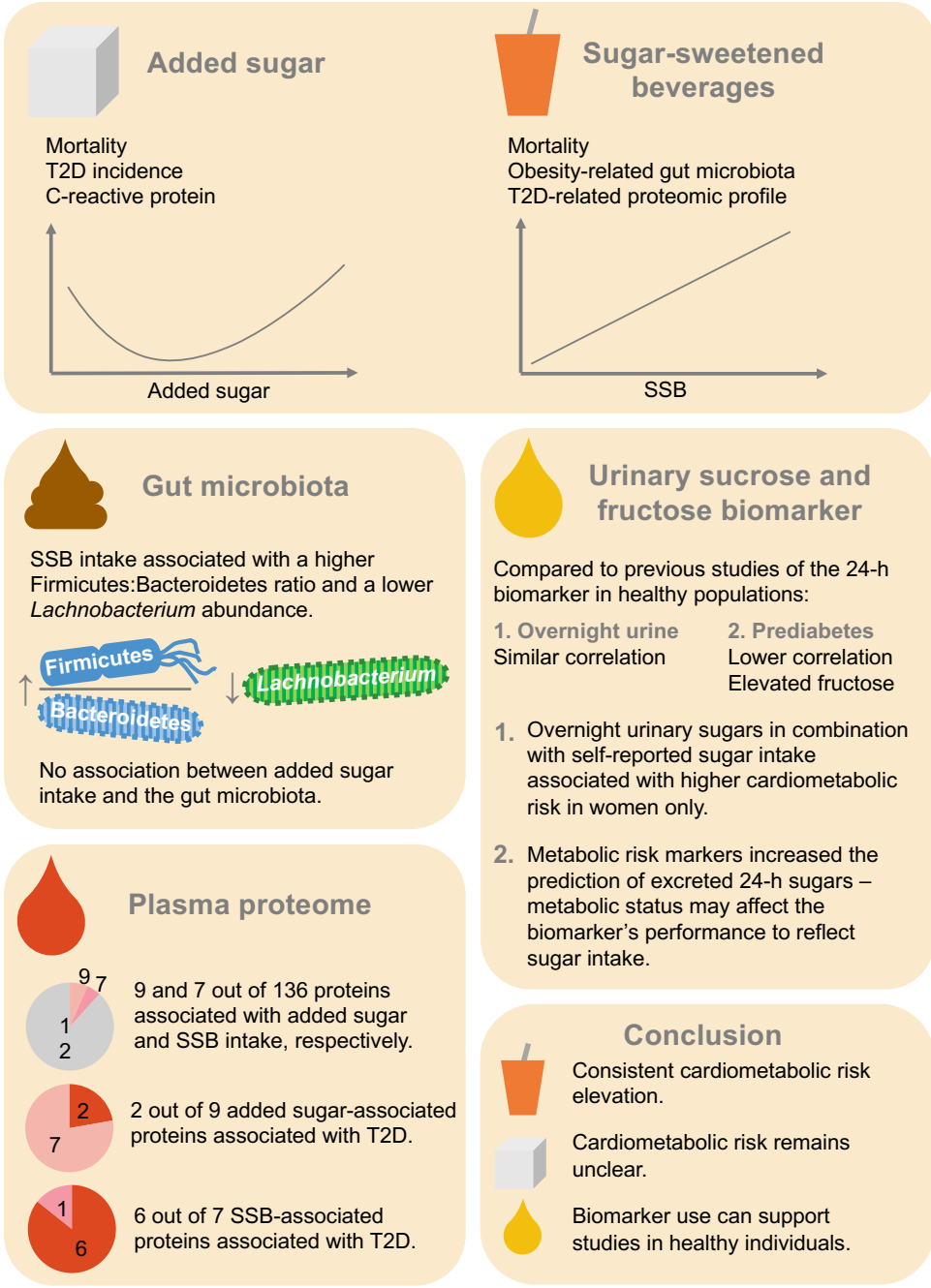


**Figure 1.** Graphical abstract of the main findings of this thesis.

# Populärvetenskaplig sammanfattning

Frågan huruvida mängden socker i vår kost har betydelse för risken att utveckla fetma, typ 2 diabetes och hjärtkärlsjukdom har diskuterats och undersökts flitigt de senaste årtionden. I dagens media målas det faktum att socker ökar risken för dessa kardiometabola sjukdomar ofta upp som en sanning och något som hela forskningsvärlden är överens om. Fast om man tittar närmre på de vetenskapliga studier som faktiskt har publicerats på ämnet, så inser man att så enkelt är det inte.

Trots att vi från djurstudier har stor förståelse om hur socker skulle kunna öka risken för kardiometabola sjukdomar, så har de flesta studier hos människor inte kunnat se ett samband mellan sockerintag och risk för typ 2 diabetes eller hjärtkärlsjukdom. Bara några få studier har sett ökad risk med ökat sockerintag, men lika många studier har sett faktiskt det motsatta, minskad risk. Om man däremot samlar ihop alla studier som specifikt har undersökt intag av läsk och andra sockersötade drycker, så är det tydligt att ett högt intag ökar risken för kardiometabola sjukdomar. Hur både det totala sockerintaget och intaget av sockersötade drycker förhåller sig till hälsa behöver alltså studeras mer, och vi behöver använda förbättrade metoder och undersöka nya potentiella mekanismer som kan vara involverade i sjukdomsutvecklingen.

I denna avhandling har sambanden visats sig vara något U-formade mellan intag av tillsatt socker och total dödlighet, dödlighet från hjärtkärlsjukdom, risk för typ 2 diabetes samt med en markör för inflammation. Detta innebär att risken är som lägst vid medelhögt intag, men att risken är högre vid både högsta och lägsta intaget av tillsatt socker. Däremot så ser vi mycket tydligare samband för intag av sockersötade drycker, precis som andra studier har visat; ju högre intag desto högre risk.

Anledningen till att risken ser ut att vara ökad även vid lågt intag av tillsatt socker är svårförklarat och behöver inte reflektera ett sant samband, det kan också bero på brister i forskningsmetoden. Till exempel är det vanligt att studiedeltagare underskattar sitt matintag, och framförallt av mat som anses mindre hälsosam, såsom sockerrik mat. Därför behövs objektiva mått på hur mycket socker man äter för att förbättra forskningen. Genom att mäta mängden av sockerarterna sackaros och fruktos som utsöndras i urinen under 24 timmar, kan man få en god uppfattning om en individs sockerintag utan att fråga studiedeltagarna om deras kost. Vi undersökte om vi kunde använda denna markör för sockerintag genom att istället mäta nivåerna från ett enda urinprov taget på morgonen och fann att så troligen är fallet. Vi kunde även visa att om

man kombinerar denna markör med det självrapporterade sockerintaget, kan man få ett ännu bättre mått på sockerintaget. Detta kombinerade mått var bland kvinnor förenat med högre grad av övervikt, högre midjemått och blodtryck, samt lägre nivå i blodet av det goda HDL-kolesterolet. Vi har också kunnat konstatera att denna markör troligtvis bör användas med försiktighet bland individer med förstadium till typ 2 diabetes. Detta eftersom utsöndringen av fruktos i urinen visade sig vara förhöjd hos dessa individer och korrelationen mellan urinsackaros- och fruktos med självrapporterat intag av socker var lägre än vad som setts i tidigare studier med friska individer. Dessutom kunde vi visa att olika riskmarkörer för typ 2 diabetes var relaterade till sackaros- och fruktosutsöndringen i urin. Med andra ord, nedsatt metabol hälsa verkar kunna påverka hur bra denna markör fungerar som mått på sockerintag.

För att ytterligare förstå sockrets roll för kardiometabola sjukdomar så undersökte vi sockrets samband med bakteriefloran i tarmen och med ett stort antal sjukdoms-relaterade proteiner i blodet. Vi fann att intag av sockersötad dryck, men inte det totala intaget av tillsatt socker, var kopplat till högre kvot mellan bakteriestammarna Firmicutes och Bacteroidetes, vilket tidigare studier har länkat till högre kardiometabol risk. Av alla enskilda bakterier som undersöktes var det bara *Lachnobacterium* som visade ett samband med intag av sockersötad dryck, medan inget samband mellan intag av tillsatt socker någon bakterie kunde ses. Vad gäller de cirkulerande proteinerna i blodet, så var de flesta proteiner som visade ett samband med intag av sockersötad dryck också kopplade till ökad risk för typ 2 diabetes, medan proteinerna som visade ett samband till intag av tillsatt socker till väldigt liten grad var kopplade till typ 2 diabetes. Båda dessa studier stödjer alltså att intag av sockersötad dryck är förenat med högre kardiometabol risk, vilket inte kunde ses när det totala intaget av tillsatt socker studerades.

Sammanfattningsvis, så är resultaten i denna avhandling i linje med tidigare studier. Vi ser relativt tydliga samband mellan intag av sockersötad dryck och ökad risk för kardiometabola sjukdomar via flera olika mekanismer, medan sambanden inte är lika tydliga för det totala intaget av tillsatt socker. Detta betyder dock inte att vi inte borde sträva efter att få befolkningen att äta mindre tillsatt socker, det borde vi. Vi har även visat att forskningen på intag av socker skulle kunna stärkas genom att även mäta sackaros och fruktos i urinen för att objektivt spegla sockerintaget, även om bara morgonurinprover använts (till skillnad från 24-timmars urinprov), men att denna markör eventuellt inte är helt pålitlig hos personer med förstadium till diabetes.

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# Background

## Cardiometabolic disease

Cardiometabolic disease is the umbrella term used to cover all the different conditions and traits involved in both cardiovascular diseases (CVDs) and metabolic diseases, in general referring to type 2 diabetes (T2D) (1).

### Cardiovascular disease

CVDs are diseases of the heart and vascular system and include conditions such as coronary heart disease (CHD), myocardial infarction, stroke, atrial fibrillation and peripheral artery disease, which in majority are typically caused by atherosclerotic processes in some way. Atherosclerosis is a process initiated by the accumulation of lipids (particularly low-density lipoprotein (LDL) cholesterol) and other particles in the wall of the arteries. These lipid deposits eventually become inflamed and harden into so-called plaques, which stiffen and narrow the arteries, limiting blood flow. When blood flow is limited, so is oxygen delivery, causing cell death. If the plaques rupture, they may flush through the circulation and create a total blockage of oxygen delivery, creating a myocardial infarction (if in the heart) or stroke (if in the brain) (2). CVD is the leading cause of death globally and has been so for many decades. In 2019, 18.6 million people died from CVD (3). However, the trends in CVD are currently improving slightly in developed countries such as Sweden (Figure 2), and especially for CVD mortality. This trend can be attributable to improvements in health care (in both medications and surgical procedures) and of a few risk factors, such as decreased smoking and decreased consumption of trans fats (3).

### Type 2 diabetes

T2D can be defined as elevated blood glucose levels, initially caused by insulin resistance, in contrast to type 1 diabetes, which is characterized by elevated blood glucose caused by a lack of insulin production. The difference in T2D and type 1 diabetes is that the effects of the blood-glucose-lowering hormone insulin are insufficient either because of a lack of the hormone (type 1 diabetes) or that the body

does not respond to the hormone, i.e., resistance (T2D). Eventually, the insulin production is impaired in T2D, but that is not the root cause of the condition (4). Together, these two conditions fall under the name diabetes mellitus, but their pathology is very different. Over 90% of all diabetic patients suffer from T2D, and the risk factors for T2D are modifiable, whereas type 1 diabetes is not considered preventable, as genetics is the most important risk factor (4). Furthermore, new research findings indicate that this division into type 1 and 2 diabetes may be too broad, and further subdiagnoses with differing etiologies could be of additional benefit for the future research and care of diabetes patients (5). In 2019, 463 million people globally suffered from diabetes (6% of the world's population) (6), and the trends in T2D incidence certainly do not suggest improvements as compared to the trends in CVD (Figure 2). In 2045, it is expected that 700 million people will be suffering from diabetes globally, an increase of 50% from today (6).

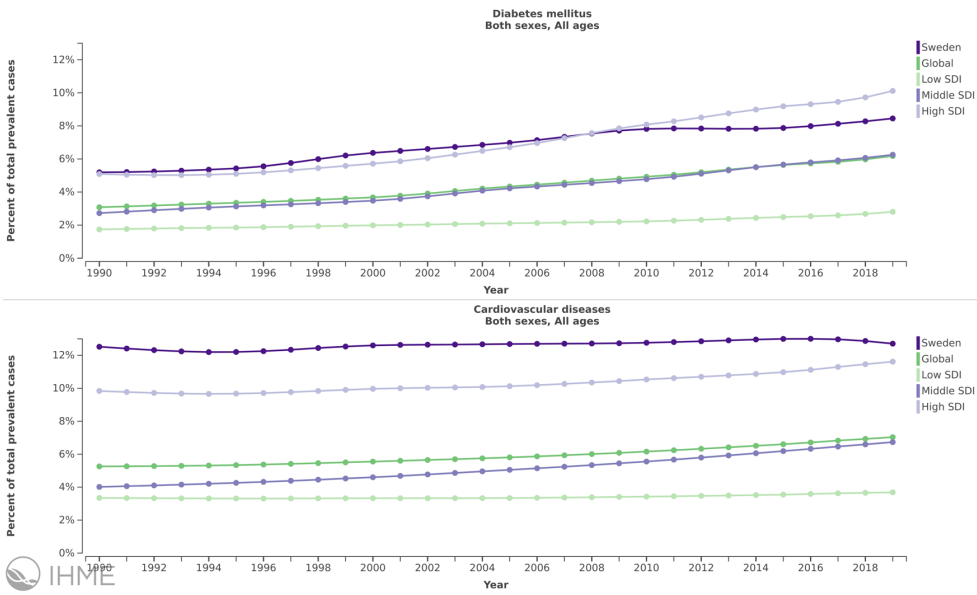


**Figure 2.** Time trends of the prevalence of diabetes mellitus and CVDs from 1990 to 2019 in Sweden, globally and in countries with low, middle and high sociodemographic indices (SDIs). Obtained from the Global Burden of Disease (GBD) Compare Viz Hub, Institute of Health Metrics and Evaluation (7).

## The common ground between CVD & T2D

A main risk factor for both CVD and T2D is weight gain and consequent overweight and obesity, which leads to several preconditions of cardiometabolic disease, such as insulin resistance, dyslipidemia (unfavorable blood lipid profile) and hypertension (elevated blood pressure). However, these preconditions can occur without the presence

of overweight or obesity, but that is much less likely. In contrast, obese individuals may be free of such conditions, but that is also much less likely.

CVD and T2D go hand in hand. Risk factors are highly shared between CVD and T2D, and it is well known that patients with T2D have a severely elevated risk of CVD (8, 9). A term called cardiometabolic multimorbidity has been introduced in the literature and has been defined as the coexistence of two or more out of a combination of three cardiometabolic disorders: either diabetes mellitus, stroke and myocardial infarction (10), or hypertension, diabetes mellitus and CVD (11). Either way cardiometabolic multimorbidity is defined, the more conditions that are present, the higher is the mortality (10, 11).

In cardiometabolic research, when disease incidence or mortality cannot be assessed, markers of cardiometabolic risk must be studied instead. The main and most general cardiometabolic risk markers studied in the literature include various measures of body composition (weight, body mass index (BMI), waist circumference, and waist-to-hip ratio), blood lipids (total cholesterol, triglycerides, high-density lipoprotein (HDL), LDL and various apolipoproteins), glucose homeostasis (fasting glucose and insulin, glycated hemoglobin (HbA1c), and homeostatic model assessment of insulin resistance (HOMA-IR)) and blood pressure (systolic and diastolic).

In the latest Global Burden of Disease report, 11 million deaths globally could be attributed to dietary risk, which corresponds to 22% of all deaths in adults. Out of these 11 million diet-related deaths, approximately 10 million were caused by CVD, and 34,000 deaths were directly caused by T2D. This report covered 15 different dietary aspects, one of which was a high intake of sugar-sweetened beverages (SSBs), but the total intake of sugars was not addressed (12).

## Dietary sugars

Sugar is a highly used ingredient in food production, mainly to provide sweetness in primarily baked goods, deserts, snacks and drinks, but also for preservation and fermentation. From a biological and technical perspective, sugars are the smallest varieties of carbohydrates, consisting of only one or two carbon-hydrogen-oxygen hexose molecules, forming so-called monosaccharides (one molecule) or disaccharides (two paired molecules). Three main monosaccharides exist, glucose, fructose and galactose, and they make up the three main disaccharides. The disaccharide called sucrose is what we normally refer to as table sugar, and it serves as the most common added sugar in foods. Sucrose consists of one glucose molecule paired with one fructose molecule. The disaccharide lactose is composed of one glucose and one galactose molecule, and the disaccharide maltose is made up of two paired glucose molecules.

The monosaccharides glucose, fructose and galactose all have the exact same molecular formula ($C_6H_{12}O_6$); they differ only in their structures (13).

Various definitions of "sugar" are found in the literature, and there is still no consensus on what definitions and types of sugars should be focused on in research and dietary guidelines. Some early research used only the term "sugar intake", and did not always explain exactly what was meant by and included in this term. Therefore, it is of great importance to define exactly what sugars are studied, and a global agreement on what terms should be official sugar terms is warranted (14). Below are the sugar definitions most commonly used in the literature today.

*Total sugar*: The term total sugar include all mono- and disaccharides, meaning the sum of glucose, fructose, galactose (monosaccharides), sucrose, lactose and maltose (disaccharides) (14).

*Added sugar*: The term added sugar refer to only those sugars that are added, and hence, not naturally occurring. To quote the Nordic Nutrition Recommendations (NNR), "added sugar refers to sucrose, fructose, glucose, starch hydrolysates (glucose syrup, high-fructose syrup) and other isolated sugar preparations used as such or added during food preparation and manufacturing" (15, 16). Various interpretations of this term exist, and sometimes honey and syrups are included here (as they are isolated sugar preparations) and sometimes not, but according to the definition used by the Dietary Guidelines for Americans (DGA), honey and syrups are included in the definition of added sugar (17).

*Free sugar*: Free sugar is a term suggested by the World Health Organization (WHO), and they define it as follows: "Free sugars include monosaccharides and disaccharides added to foods and beverages by the manufacturer, cook or consumer, and sugars naturally present in honey, syrups, fruit juices and fruit juice concentrates" (18).

## Sugar intake recommendations

In 2005, the US Institute of Medicine recommended that a maximum intake of 25 percent of total energy intake (E%) be from added sugars. This recommendation was motivated by the desire to avoid a reduction in the intake of micronutrients, which has been observed among high consumers of added sugar (19). Since then, most dietary authorities have considered sugar intake in their dietary guidelines. Both the NNRs 2012 (5$^{th}$ edition) (16) and the DGA 2015 and 2020 (17, 20) recommend that a maximum intake of 10E% be from added sugar. The NNRs and DGA base these recommendations on ensuring adequate intake of other important nutrients while not exceeding energy needs. Furthermore, the NNRs also consider the risk of dental caries associated with a high added sugar intake (16). On the other hand, both the European Food Safety Authority (EFSA) (15) and the German Nutrition Society (21), in 2010

and 2012, respectively, concluded in their systematic review work that not enough evidence existed to set an upper limit of recommended added sugar intake. In contrast, the WHO recommended in 2015 that a maximum intake of 10E% be from free sugar, with a suggested recommendation to encourage further reductions in free sugar intake to below 5E% (18). This was motivated by the risk of both insufficient nutrient intake, dental caries and increased body weight associated with high free sugar intake. The recommendation of <5E% being from free sugar was also adopted by Public Health England in 2015 (22), with the motivation that a high intake of free sugar increases energy intake and the risk of tooth decay (23). The sugar intake recommendations from selected organizations are displayed in Table 1.

**Table 1. Most recent sugar intake recommendations from various selected organizations.**

| Organization (reference) | Year[1] | Sugar definition | Recommendation | Motivation |
|---|---|---|---|---|
| US Institute of Medicine (19) | 2005 | Added sugar | 25E% | Prevent the displacement of foods that are major sources of essential micronutrients. |
| NNR (16) | 2014 | Added sugar | 10E% | Ensure adequate intakes of micronutrients and dietary fiber (nutrient density).<br>Support a healthy dietary pattern.<br>*SSBs have been associated with increased T2D and weight gain and should be limited.**<br>*Sugar-containing foods should be avoided to reduce the risk of dental caries.** |
| DGA (20) | 2020 | Added sugar | 10E% | Help achieve healthy dietary patterns within calorie limits. |
| EFSA (15) | 2010 | Added sugar | - | ”Available data do not allow the setting of a Tolerable Upper Intake Level for total or added sugars, neither an Adequate Intake nor a Reference Intake range.” |
| WHO (18) | 2015 | Free sugar | 10E% (5E%) | Free sugars threaten the nutrient quality of diets by providing significant energy without specific nutrients.<br>Body weight (low and moderate quality evidence).<br>Dental caries (very low and moderate quality evidence). |
| Public Health England (23) | 2015 | Free sugar | 5E% | Improve the management of energy intake.<br>This is expected to beneficially influence the risk of obesity and to improve dental health. |

[1]Refers to latest updated version. *Address the intake of SSBs and sugar-containing foods rather than the added sugar intake.

For reference, on a 2000 kcal diet, 10E% from added sugar would correspond to 50 g, as obtained from approximately 500 ml soda or approximately 80-100 g chocolate or jelly candy per day. 5E% from added sugar would translate to 25 g of added sugar,

which is obtained from approximately 150 g of fruit yoghurt and two slices of Swedish bread (*kavring* or *sirapslimpa*) if less obvious sources of added sugar are considered.

What is interesting is that none of the guidelines above finds evidence to support their recommendations in regard to either T2D or CVD incidence. The only cardiometabolic risk factor mentioned in any of these guidelines in regard to added and free sugar is body weight. There is no mentioning of the possible impairment of either blood lipids, blood pressure or glucose homeostasis because the scientific evidence is not sound enough.

Another point that must be noted is that most of these dietary guidelines, independent of what level of added or free sugar intake they have agreed to recommend, point out a specific risk with a high intake of SSBs and encourage limited intake of such beverages. Here, the risk of T2D is particularly pointed out.

Various disease organizations also set their own dietary recommendations for prevention purposes, e.g. the American Heart Association recommends a maximum intake of 100 and 150 kcal per day from added sugar for women and men, respectively (24), and a maximum intake of 25 g added sugar per day (100 kcal) in children for a reduction of CVD risk factors (25).

## Sugar consumption trends

The consumption of added sugar steadily increased from the 1700s when sugar increased in availability. In Europe, the sugar consumption particularly increased during the late 1800's following the industrialization of food production and the invention of the process to extract sugar from sugar beets instead of sugar cane. Prior to this, sugar had been a tropical treat and, unfortunately, a main driver of the transatlantic slave trade (26). The largest elevations in sugar consumption were seen in Europe and North America between the 1970s and 2000. In the USA, this elevation was primarily characterized by increased use of high-fructose corn syrup, and the matched timing between increased consumption and escalating rates of obesity constitutes the initial hypothesis and basis for the current focus on sugar in relation to obesity and cardiometabolic risk (27).

Only recently can one can see a slight decline in sugar consumption in some countries (28). Continuous mapping of dietary habits in the USA showed a small but clear negative trend in self-reported consumption of sugar and SSBs between 2003 and 2016 (29). In Sweden, the total consumption of sugar (measured as total sales for human consumption) decreased by 73% from 1980 until 2019, although the consumption of chocolate, confectionaries and SSBs continued to increase, with a reduced rate from the year 2000 (30). In the latest Swedish national food survey of adults in 2010-11, 15% of the energy was reported to come from treats, SSBs and snacks. Of the total intake of

sucrose, 15% came from fruit and berries, 15% from pastries, 13% from SSBs, 8% from candy and chocolate and 8% from desserts, marmalade and jam. In comparison to the national food survey from 1997-98, reduced intake was seen for SSBs, pastries and ice cream, but not for candy and chocolate (31). These trends of reductions in added sugar consumption are likely also parallel to increases in the consumption of low-calorie sweeteners (32). The awareness of obesity and unhealthy food habits has started to create a change among the wealthiest countries, but among still developing countries, sugar consumption is expected to increase much before reaching a tipping point.

## Metabolism of sugars

Upon the ingestion of degradable carbohydrates, the chains of carbohydrates of various lengths are enzymatically hydrolyzed throughout the upper gastrointestinal tract. Well in the small intestine, the carbohydrates have reached their smallest state as monosaccharides and are ready for absorption. Sucrose, the main added sugar, is a disaccharide and, hence, only passes one stage of hydrolysis, by the enzyme sucrase, in the small intestine to become absorbable as fructose and glucose (13). However, as will become evident later in this thesis, a tiny share of intact sucrose can actually be absorbed in the small intestine, but the mechanism through which this occurs is not clear.

### *Glucose metabolism*

Glucose is absorbed in the small intestine and reaches the circulation after bypassing the liver. If the liver is in a state of glucose shortage, its glucose levels will be restored in the form of glycogen (the molecule used to store glucose in liver and muscle) along the way, and the remaining glucose will continue throughout the circulation to supply the entire body. The circulating levels of glucose are constantly tightly controlled within the narrow range of 4-8 mmol/L in healthy individuals, primarily by the hormones insulin and glucagon. Insulin is produced by the β-cells in the pancreas and released into the circulation when glucose levels in the circulation increase, i.e. after a meal containing carbohydrates. Insulin signals to cells to take up glucose from the circulation, wherein the energy provided by the glucose is utilized by processes initiated by glycolysis (see Figure 3 for details). Insulin also signals to inhibit glucose production from stored glycogen. This results in a reduction in circulating glucose levels. When the blood glucose levels become lower than optimal, the hormone glucagon is released and signals for increased glucose production from glycogen in the liver to restore normal blood glucose levels. Insulin and glucagon are constantly balanced to keep the blood glucose levels within the optimum range, and this balance is the key to remaining metabolically healthy. If excess glucose remains after the glycogen stores have been filled and it has not been used for other activities, it is converted into lipids to be stored as adipose tissue (13). The glycemic index (GI) of a food is a measure of how much it raises the blood glucose levels following the consumption of 50 g.

*Fructose metabolism*

Fructose is absorbed in the small intestine and then transported to the liver via the portal vein. Unlike glucose uptake, fructose uptake in the liver is not regulated by the liver's energy needs (glycolysis is limited by citrate and ATP), resulting in most fructose being rapidly metabolized in the liver to partly glucose and partly lipids. With larger fructose liver overloads, the proportion of lipids produced is larger, which contributes directly to the hepatic lipid stores and increases the circulating levels of triglycerides and very low-density lipoprotein (VLDL) (33, 34). It should, however, be noted that to determine these pathways, generally very large doses of fructose have been studied (35). The basics of hepatic glucose and fructose metabolism are depicted in Figure 3. Recent research has also elucidated that some fructose metabolism actually takes places already in the small intestinal lumen. The small intestine can metabolize a limited amount of fructose into glucose, but if the ingested fructose dose is large enough, the fructose continues its path to metabolism by the liver (36).

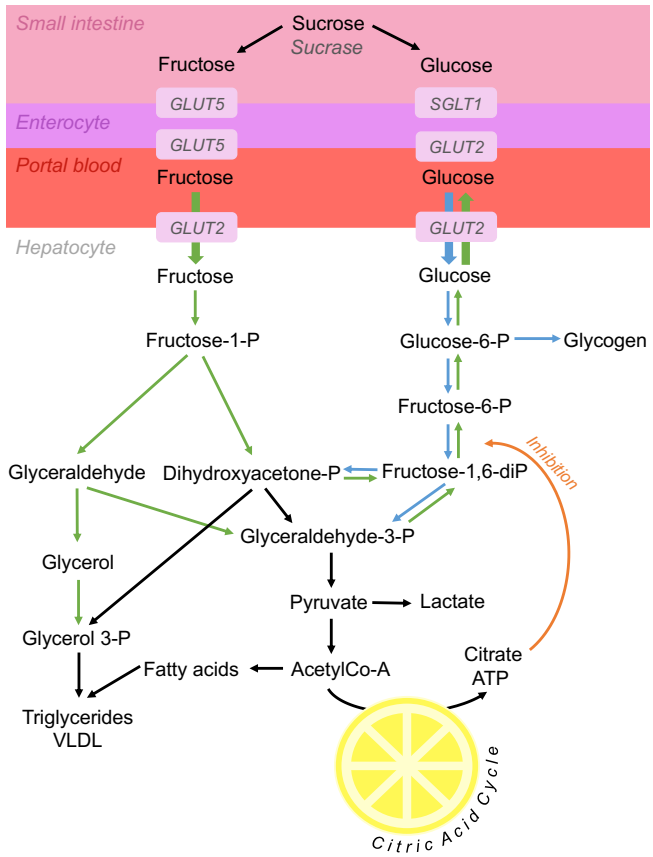


**Figure 3.** Main pathways of hepatic metabolism of fructose and glucose. Green arrows show fructose metabolism, blue arrows show glucose metabolism and the black arrows show their joint pathways.

Worth mentioning is also that this rapid hepatic fructose metabolism results in only very small amounts of fructose reaching the circulation (37), and the small amounts that do are not regulated by insulin and glucagon, in contrast to glucose (38). The small glucose and insulin response following a fructose load is mainly a consequence of fructose being metabolized in part to glucose in the small intestine and liver. Furthermore, the absorption of fructose in the small intestine is not always perfectly efficient. After large and quick ingestion of fructose, the absorption can be saturated, and the fructose passes on to the large intestine. However, fructose absorption is aided by the co-ingestion of glucose, which normally is the case when added sugars such as sucrose or high-fructose corn syrup are consumed (39).

*Glucose & fructose metabolism in cardiometabolic disease*

In brief, during the early phases of T2D development, reduced insulin sensitivity starts to appear, meaning that the cells that normally take in glucose in the presence of insulin, become resistant to insulin signals and do not respond. Consequently, glucose does not enter the cells and remains elevated in the circulation. When cells are not responsive to insulin (insulin resistance), the β-cells start producing more insulin to enhance the signal. As the threshold of how much insulin is needed for cells to be responsive increases, the insulin production continues to increase. This overproduction of insulin, i.e., hyperinsulinemia, wears out the β-cells, which eventually lose their function (40). Logically, the entire system may be burdened, and the process expedited if the amount of glucose that needs to be cleared from the circulation is elevated, such as following high consumption of starch or sugar.

The reasons why cells become insulin resistant in the first place are multifactorial but primarily involve elevated intracellular lipid accumulation in muscle and liver cells (41), which may be a direct effect of excess weight gain but also exacerbated by elevated fatty acid production and accumulation in the liver through excessive metabolism of fructose. Furthermore, the production of lipids from glucose and fructose is stimulated by higher circulation levels of insulin, generating a viscous spiral toward metabolic disturbances (42, 43).

To summarize, excess glucose is believed to be detrimental to cardiometabolic health primarily because the steep and rapid strain it causes to the glucose-insulin homeostasis. Excess fructose, however, is not responsible for a large amount of direct glycemic stress, but is instead believed to be detrimental to cardiometabolic health because of its effects on lipid metabolism. Is this combination of glucose and fructose that comprise added sugars (sucrose or high-fructose corn syrup) the ultimate recipe for strained metabolic systems?

# Added sugar intake & cardiometabolic disease

## Potential mechanisms

*Adiposity*

The main mechanism underlying how a high added sugar intake would contribute to cardiometabolic disease risk is primarily through weight gain and the accumulation of adiposity. A high intake of sugar-rich foods and beverages may promote weight gain because of factors such as their general high energy density and/or high hedonic value, easily resulting in overconsumption and an excess energy intake (44, 45). However, sucrose in itself does not have an extremely high energy density (4 kcal/g) and poses a low risk for overconsumption.

Whether high added sugar intake would cause weight gain independent of elevated energy intake has been frequently discussed, but actual evidence supporting this is insufficient (46, 47). However, plausible mechanisms could revolve around the relatively high postprandial glycemic stress related to sugar intake, which may increase appetite (48), and that fructose does not stimulate leptin production (as compared to glucose) and its hunger-inhibiting effects (33). Nevertheless, these mechanisms would still result in an elevated energy intake but not necessarily from the sugar itself.

Weight gain causes further impairments in generally all additional cardiometabolic risk factors. Additional mechanisms by which high added sugar intake might increase cardiometabolic risk, aside from the obvious effects caused by weight gain on these risk factors, are presented below.

*Glucose & insulin homeostasis*

Due to the postprandial effects of a high-sugar meal, it is reasonable to suggest that a high-sugar diet may cause cardiometabolic disease. Frequent exposure to rapid and large postprandial glucose stressors, as occur after consuming large amounts of sugar, results in frequent and large elevations of circulating insulin, which may affect the cells' sensitivity to insulin and strain the β-cells (48). This effect is considered to be due to glucose, while fructose could contribute to insulin resistance by accumulation of fatty acids in the liver and muscle (33). These are the first steps toward the development of T2D, which in the long run also contributes to the development of CVD (49).

*Blood lipids*

Dyslipidemia directly increases the risk of CVD (50), and a causal role, particularly of LDL cholesterol has been proven in CVD development (51). Higher levels of circulating lipids also contribute to insulin resistance development. As portrayed by the

pathways in which particularly fructose is metabolized, it is feasible to suggest that a high intake of added sugar could induce elevated levels of circulating lipids, especially triglycerides and LDL and VLDL cholesterol (33).

*Liver fat*

Liver fat accumulation has been strongly hypothesized to increase with high sugar intake, and specifically as a result of high fructose intake considering the pathways of fructose metabolism (52). Liver fat accumulation not only is the precursor of nonalcoholic fatty liver disease (NAFLD), but also contributes to reduced insulin resistance in the liver. However, the results of a meta-analyses showed that without increases in energy intake, the intake of sugars did not increase liver fat (53).

*Blood pressure*

The potential effects of high added sugar intake on blood pressure other than those mediated by weight gain are less straightforward to explain mechanistically, but plausible mechanisms involve elevations of uric acid concentrations due to high sugar, particularly fructose, intake (54), which may increase blood pressure mainly by increasing arterial stiffness via several suggested mechanisms (55).

*Inflammation*

The role of chronic inflammation in T2D development and atherosclerotic processes is well established, and it has been suggested that a high added sugar intake would contribute to such chronic inflammation. The main potential proinflammatory effects of a high sugar intake are suggestively induced by weight gain (56), while in addition, the stress of high postprandial plasma glucose, epigenetic changes and excess formulation of reactive oxygen species are other plausible mechanisms through which high sugar intake might cause low-grade inflammation (56, 57). One of the most commonly assessed circulating inflammatory proteins is C-reactive protein (CRP).

*Gut microbiota*

So-called gut microbial dysbiosis has been linked to both obesity and T2D (58). However, how high sugar intake might affect one's gut microbiota composition is far from obvious, since sugars are absorbed already in the small intestine and hence do not reach the colon. This is in contrast to fiber, which we know has important effects on the colonic microbiota (59). However, fructose absorption can vary in efficiency, and it has been postulated that the malabsorption of fructose, which consequently ends up in the colon, may induce alterations in the gut microbiota composition (60). Nevertheless, this is a very new and scarcely studied research area, requiring human investigations.

## Existing evidence

Already in 2013, a prominent nutritional epidemiologist wrote a review article titled the following: "Resolved: there is sufficient scientific evidence that decreasing sugar-sweetened beverage consumption will reduce the prevalence of obesity and obesity-related diseases" (61). That title alone summarizes the SSB research field quite sufficiently; it is clear that high intake of SSBs increases cardiometabolic risk. This statement is supported by several meta-analyses of both interventional and epidemiological evidence that have associated SSB intake with higher body weight (62) and obesity (63), higher fasting glucose and insulin (64), and a higher incidence of T2D (65-69) and CVD, including hypertension, CHD and stroke (70-72). For the total intake of added sugar, on the other hand, the picture looks much different, and we must review the literature in more depth to fully grasp the existing evidence.

*Animal studies*

From rodent studies we have obtained great understanding of the mechanisms by which the intake of added sugars, and specifically high fructose intake, can lead to obesity, impaired glucose control, insulin resistance, dyslipidemia, fatty liver disease and much more. By looking only at the animal studies, there is not much doubt on the link between added sugar intake and cardiometabolic disease risk (34, 73). However, rodents are not humans, and the results from human studies are not nearly as clear.

*Human intervention studies*

Among the human intervention studies on the effect of sugar intake on cardiometabolic health, the focus has mainly been on comparing potential differences between glucose, fructose and sucrose. One can question the rationale for comparing glucose or fructose, since both of them almost never are consumed on their own, but rather consumed jointly as sucrose or as high-fructose corn syrup. Surely, it is important to understand how the different sugars affect us, but such studies lack resemblance to how sugars are consumed in real-life situations. The results of such studies have been summarized in a network meta-analysis by Schwingshackl et al., who concluded that with a very low certainty of evidence, that the exchange of sucrose and fructose for starch may improve LDL cholesterol, the exchange of sucrose with starch may improve fasting glucose levels, the exchange of fructose with glucose may improve HOMA-IR and the exchange of fructose or sucrose with glucose may improve uric acid concentrations (74). Another meta-analysis of randomized controlled trials (RCTs) comparing various sugars concluded that isoenergetic substitution of fructose for glucose or sucrose did not adversely affect health (75).

Intervention studies with a more general approach to "sugars" or sugar-rich diets (here, we start to see the struggle with the various definitions of sugar) that, in one way or another, compare low vs high intake generally suffer from small sample sizes and short

durations. Te Morenga et al. summarized all these studies in meta-analyses which provided evidence suggesting that the intake of sugar increases body weight as a result of a positive energy balance (46) and that the intake of sugar increases blood pressure and blood lipid levels independent of the effects of sugar on body weight (76). However, another systematic review conducted at the same time showed contrastingly that dietary sucrose intake up to 25E% appears to have no adverse effects on cardiometabolic risk factors in healthy adults when substituted for starch (77). The latter study was, however, supported by the World Sugar Research Organization. A more recent meta-analysis concluded that substitution of free sugar for complex carbohydrates increases both LDL and HDL cholesterol but has no effect on blood pressure and body weight in isoenergetic studies (78).

On the basis of the findings from these systematic reviews of human intervention studies, the following summary could be stated: There is evidence to suggest that a high intake of sugars could increase cardiometabolic risk factors, but the evidence is not sufficiently convincing that it can end the lobbying of those with a conflict of interest. Furthermore, it should be noted that the vast majority of these human intervention studies have been performed with interventions of sugar in liquid form, very often as SSBs. What we can actually conclude from these studies regarding a total intake of added sugar, from any type of source, is insufficient. Additionally, what we unfortunately totally lack, is any human intervention study of sugar intake investigating hard outcomes, such as the incidence of T2D or CVD. This is clearly an important limitation. For such outcomes, we can rely only on epidemiological studies.

*Epidemiological studies*

Published prospective cohort studies have mainly focused on SSB intake and found that it is consistently associated with T2D and CVD incidence (65, 67, 69). However, looking only at SSBs as a proxy for sugar intake is not correct. As seen in meta-analyses, the epidemiological studies that have investigated sugar intake, rather than SSB intake, and its association with the incidence in CVD and T2D have a hard time finding positive associations (22, 79, 80). As presented in Table 2, out of 11 prospective studies examining the association between sugar intake and the incidence of T2D, only three have found a positive association (and only so when specifically studying glucose, fructose and total disaccharides, never jointly as sucrose, added sugar, free sugar or total sugar). Three studies also found inverse associations between sugar intake and T2D incidence. Out of the nine epidemiological studies that investigated the association between sugar intake and CVD, only one study showed a positive association between sucrose intake and CHD. Additionally, please note the studies in Table 2 that have studied the exposure to "sugar", and have not given further explanations of how this is defined. Is this total sugar, sucrose or something else?

**Table 2. Prospective epidemiological studies that have investigated the association between sugar intake and T2D and CVD incidence.**

| T2D | | | | |
|---|---|---|---|---|
| Study | n | Exposure | Outcome | Results |
| Feskens 1995 (81) | 338 | Mono + disaccharides | T2D | |
| Meyer 2000 (82) | 35,988 | Sucrose | T2D | |
| | | Glucose | T2D | |
| | | Fructose | T2D | |
| Janket 2003 (83) | 38,480 | Total sugar | T2D | |
| | | Sucrose | T2D | |
| Hodge 2004 (84) | 31,641 | Sugar? | T2D | |
| Barclay 2007 (85) | 1,833 | Sugar? | T2D | |
| Montonen 2007 (86) | 4,304 | Total sugar | T2D | |
| | | Sucrose | T2D | |
| | | Glucose | T2D | |
| | | Fructose | T2D | |
| Schulze 2008 (87) | 25,067 | Sucrose | T2D | |
| | | Glucose | T2D | |
| | | Fructose | T2D | |
| Sluijs 2010 (88) | 37,846 | Sugar? | T2D | |
| Ahmadi-Abhari 2014 (89) | 3,496 | Total sugar | T2D | |
| | | Sucrose | T2D | |
| | | Glucose | T2D | |
| | | Fructose | T2D | |
| Tasevska 2018 (90) | 82,254 | Total sugar | T2D | |
| | | Biomarker-calibrated total sugar | T2D | |
| Olsson 2020 (91) | 26,622 | Added sugar | T2D | |
| | | Sucrose | T2D | |
| | | Monosaccharides | T2D | |
| | | Disaccharides | T2D | |
| CVD | | | | |
| Study | n | Exposure | Outcome | Results |
| Liu 2000 (92) | 75,521 | Sucrose | CHD | |
| | | Fructose | CHD | |
| Beulens 2007 (93) | 15,714 | Mono- + disaccharides | CVD | |
| Sieri 2010 (94) | 44,132 | Sugar? | CHD | |
| Burger 2011 (95) | 19,608 | Sugar? | CHD | |
| | | Sugar? | Stroke | |
| Sieri 2013 (96) | 44,099 | Sugar? | Stroke | |
| Sonestedt 2015 (97) | 26,445 | Sucrose | CVD | |
| Warfa 2016 (98) | 26,190 | Sucrose | CHD | |
| Tasevska 2018 (90) | 82,254 | Total sugar | CVD | |
| | | Total sugar | CHD | |
| | | Total sugar | Stroke | |
| | | Biomarker-calibrated total sugar | CVD | |
| | | Biomarker-calibrated total sugar | CHD | |
| | | Biomarker-calibrated total sugar | Stroke | |
| Janzi 2020 (99) | 25,877 | Added sugar | CHD | |
| | | Added sugar | Stroke | |

Gray denotes no significant linear association. Green denotes an inverse association or protective association. Red denotes a positive association or harmful association.

## Why is the evidence lacking?

Despite a good understanding of the potential underlying mechanisms, the main reasons for why we lack evidence linking added sugar intake to cardiometabolic risk in humans, I believe can be summarized in the following four categories:

1. Unmet assumptions on the effect of sugar intake on metabolic health
2. How do we treat mediators in the causal pathway?
3. Added and free sugar intake cannot be measured
4. Methodological challenges in nutrition research

It is, however, very important to remember that "an absence of evidence is not evidence of absence", meaning that, although there is no compelling evidence to say that added sugar intake causes cardiometabolic disease, this does not mean there is evidence to say that added sugar intake does not cause cardiometabolic disease.

### Unmet assumptions on the effect of sugar intake on metabolic health

One important thing that I have learned during my statistical training is that if your assumptions are not met, you cannot just ignore it; you have to start thinking. Otherwise, detrimentally misleading conclusions may be drawn.

There are many deeply rooted assumptions that a high sugar intake causes T2D, the origins of which can be traced back to the fact that glucose is excreted in urine to a high extent and gives urine a sweet taste in diabetes mellitus, as recognized by Egyptians as early as approximately 1500 BC (100). To resolve the condition of high urinary excretion of glucose, one may either interpret it as the lost sugar must be replenished through the implementation of a high sugar diet (as unfortunately originally was believed (101)) or by avoiding excess sugar intake (as we know is appropriate today). Actually, the etymology of the word diabetes comes from Greek and means to pass through (as a large amount of urine does in untreated diabetic patients), and the word mellitus comes from Latin and means for honey or sweet. The first hypotheses linking a high intake of sugar with T2D were published in the very first decade of the 1900s (102). In Swedish, diabetes mellitus was historically called *sockersjuka* (sugar disease). However, it is not only because of such historic knowledge that we assume that high sugar intake causes T2D. This assumption is still buried deep because we are aware of the effects of a high sugar intake…

… on those who already have T2D. High sugar intake likely increases hyperglycemia and could lead to more severe diabetes complications (although, even this can be discussed to be a preterm assumption (103)). However, there is a very large difference

in this sense between healthy and diabetic individuals. Healthy individuals do not develop hyperglycemia; otherwise they are not healthy.

… on postprandial glucose and insulin response, which is a relatively large postprandial response. However, glucose is tightly regulated, and postprandial peaks are well handled if the individual is healthy. In healthy individuals, we actually do not have strong support for that frequent high postprandial glucose leads to insulin resistance, unless other risk factors for insulin resistance, such as weight gain and, particularly, intracellular fatty acid accumulation, are present (41, 48). However, once insulin resistance is present, the postprandial response is much elevated.

Furthermore, both of these assumptions are based on the assumption that the glycemic strain of sugar consumption is exceptionally high. Surely, the glycemic strain is not low, but it is not as high as for most refined starch. A high GI has been defined as a GI>70 (104); sucrose has a GI of 60, and fructose has a GI of only 23 (glucose, on which the GI is based, has a GI of 100). White bread, rice and potato have a GI of approximately 80 (105). This misunderstanding is enhanced because glucose and sucrose are frequently confused and treated as being the same, which may be because we casually say blood sugar when we refer to blood glucose.

## How do we treat mediators in the causal pathway?

Whether one can conclude whether added sugar intake increases cardiometabolic risk, depends on whether only the direct effects or also the indirect effects are being considered (33). The conclusions of most current literature are that the current support for a direct effect is lacking, while there is more support for added sugar intake causing cardiometabolic disease indirectly via the processes of weight gain and adiposity. Weight gain is, hence, a mediator in this causal pathway between sugar intake and cardiometabolic disease, and the question is whether a high added sugar intake could contribute to increased cardiometabolic risk if weight remains stable. The mediating effects of body weight are well established, and even in crude global ecological studies, researchers have estimated that the association between sugar intake and T2D is to 66% mediated by BMI (106).

Furthermore, whether we can say that high added sugar consumption causes weight gain also depends on whether we are referring to directly or indirectly. Indirectly, via a surplus of energy intake, is the main path, while whether high added sugar intake would directly cause weight gain without an excess energy intake is not well supported. Technically, the calories from added sugar would not cause more weight gain than the calories from any other sources of energy; i.e., an energy intake exceeding energy needs can be achieved with all foods, not just foods containing sugar. In that sense, added sugar does not directly cause weight gain. Nevertheless, if we consider the behavioral

parts of our consumption patterns, sugar-rich foods are often highly palatable and energy dense, and therefore at risk of being overconsumed more than many other foods. In that sense, a high consumption of added sugar may certainly cause weight gain.

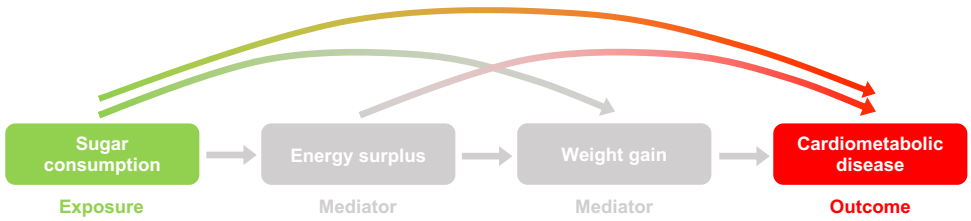


**Figure 4.** Direct and indirect effects of sugar consumption on cardiometabolic disease risk. Gray arrows indicate the indirect, mediated causal pathways. Gradient arrows indicate potential direct effects.

In isocaloric intervention trials, the first gray arrow of Figure 4 is blocked, which blocks the energy-mediated causal pathway. However, in real-life situations, isocaloric exchange between nutrients is rarely the case, and increased intake of sugar-rich foods and beverages serves as an additive energy source. When individuals are randomized to a high sugar diet (ad libitum), they generally increase their energy intake and gain weight as a consequence (46). Furthermore, in epidemiological studies, the associations between added sugar intake and the risk of cardiometabolic disease can vary widely depending on whether we adjust the regression models for either body weight, BMI or weight circumference (blocking the third gray arrow). Furthermore, it is a standard procedure in nutritional epidemiology to always adjust diet-disease regression models for total energy intake due to the many various ways in which energy intake may be a confounder of such an association (107), although we risk blocking parts of an important mediating pathway.

Therefore, on the question whether added sugar causes weight gain and cardiometabolic disease, the answer could be both yes, and no, depending on the perspective. Added sugar may not directly cause weight gain and cardiometabolic risk, but it can still be an indirect cause. An association between two variables can still be causal and just as important, despite passing through mediators.

## Added & free sugar intake cannot be measured

Another reason why we lack evidence supporting our dietary guidelines on sugar is because they all recommend certain maximum levels of either added or free sugar. While, in fact, there is no way to measure the content of added or free sugars in foods because these sugars are not molecularly different from naturally occurring sugars. What we have to do instead is to estimate the amount of sugars that naturally occur and the amount that is classified as either added or free sugar in foods, thus providing

us with very few studies that actually have studied the intake of added or free sugars. More studies have investigated the intake of total sugar, but that is not what the dietary guidelines are addressing.

## Methodological challenges in nutrition research

*Study design limitations*

RCTs are generally considered necessary to provide causal evidence. The randomization in an RCT evenly distributes participants (and their characteristics) between the intervention and control groups, hence, naturally blocking all confounding and making it possible to actually demonstrate causality. In epidemiological studies, however, confounding haunts every result. Unfortunately, it is barely plausible or ethical to conduct the perfect RCT that investigates added sugar intake and cardiometabolic risk. Such an RCT would need to be several years long (preferably up to 10 years) to be able to study incidence in T2D and CVD. Furthermore, it would be a major challenge to get the study participants to adhere to this diet for a very long time, which is much more difficult than getting participants to adhere to drug interventions. Therefore, many dietary intervention studies are limited by a lack of adherence to the dietary intervention, and the longer the intervention is, the likelier it is that the study participants will deviate from their assigned intervention. Additionally, because of the known health effect of a high sugar intake on, for example, the risk of dental caries, a study is unlikely to be granted ethical permission to give a randomized group of people a high daily intake of added sugar over several years. Instead, as in the existing literature, we have to rely on short RCTs where markers of disease are evaluated instead of actual disease incidence, or we have to rely on epidemiological studies that are ultimately limited by confounding. Therefore, the existing evidence on added sugar intake and cardiometabolic risk is not stronger.

*Complexity of diet as an exposure*

On might suggest that dietary intake is the most complex exposure to study, because it unavoidably incorporates all multilevel coexisting dietary exposures. In just one meal, one is exposed to several food items, which all contain several ingredients, multiple nutrients and uncountable numbers of additional compounds that are either known or completely unknown. Furthermore, this is just one meal, for one day, during a week, in a year, in an entire lifetime. In addition, effects may exist from how food or nutrients interact in combination, the timing of the meal or the social aspects of how the meal was consumed. As an example, sucrose, fructose and glucose in fruits and vegetables are very differently associated with health and disease than sucrose, fructose and glucose as added sugars, although they are exactly the same. This difference is likely because when we eat sugar from fruits and vegetables, we also eat fibers, micronutrients, polyphenols

and other dietary components that are healthy. When eating sugars as added sugars, however, the foods are very often accompanied by a high fat content (especially saturated or trans fat), a low content of fibers and micronutrients and a high energy density. Furthermore, the sugars in fruits and vegetables may be more tightly bound into the structure of the plant than if the sugars are added as an ingredient, which is suggested to slow down the digestion of naturally occurring sugars compared to added sugars (108). The totality of a food's or diet's all constituting components, how they interact with one another and the structure of the foods is what we call the food matrix effect (109) or food synergy (110). "The whole is greater than the sum of its parts" is a well-fitting old saying to describe this phenomenon.

Another aspect of the complexity of our diet involves the substitution phenomenon; i.e., if we reduce the intake of a certain nutrient/food, what is consumed in its place? To understand the link between sugar intake and cardiometabolic risk, one must think about what low consumers of added sugar consume instead. Salty snacks? Low-calorie sweeteners? Fruit and vegetables? Furthermore, what are the high consumers of added sugar not consuming? Actually, we have evidence suggesting that high consumers of added sugar generally eat fewer micronutrients, fiber and fruits and vegetables, indicating that sugary foods take their place in the diet (111). Therefore, are the potential harmful effects of high added sugar intake due to the actual sugar or the lack of, say, fruits and vegetables or both?

*Dietary misreporting*

Since no method to perfectly measure our dietary habits currently exists any, nutrition research must often rely on self-reported dietary intake data. There are many different methods to assess dietary intake in this way, the most common ones being food frequency questionnaires (FFQs), food records or 24-h recalls. These methods can be more or less advanced and more or less accurate depending on many different factors, but what they all have in common is that they never can completely escape the issue of dietary measurement error or dietary misreporting. Dietary misreporting means that study participants wrongly estimate or report their dietary intake, which can occur for many various reasons. Most often dietary data are misreported in the direction that less is reported, compared to what is actually consumed – what we call underreporting (112). Underreporting has been shown to be more pronounced in individuals with a higher BMI (113-115) and for snack foods and foods considered less healthy (116), such as foods high in added sugars. This unlucky combination causes the detrimental issue in epidemiological research called differential misclassification (117), meaning that the misclassification of intake is not evenly distributed between cases and noncases of the condition; i.e., the underreporting of added sugar intake is more frequent in individuals with higher cardiometabolic risk, which may create attenuated associations and wrongly drawn conclusions (117).

# The urinary sucrose & fructose biomarkers

As a possible solution to the obstacle of dietary misreporting that is inherent in most nutritional research, the nutrition research field has worked on developing nutritional biomarkers as objective markers of intake in the past few decades. When we learned how to objectively measure energy expenditure using the doubly labeled water technique (118), the nutrition research field took many leaps forward. This technique enabled gold standard validation of dietary assessment methods and the possibility to map out how, where and in who the misreporting of energy intake primarily occurs (113).

The research community early classified nutritional biomarkers into either recovery biomarkers or concentration biomarkers (119). Recovery biomarkers are those in which a fixed proportion of intake is recovered and excreted within a specific time period (119, 120). Recovery biomarkers include the use of the doubly label water method to measure energy expenditure, urinary nitrogen excretion to measure protein intake and urinary potassium and sodium to assess potassium and sodium intake (121). Concentration biomarkers are, on the contrary, markers that simply correlate with intake, but are not a direct recovery of what has been consumed. No exact physiological quantitative relationship between intake and concentration levels is necessary, and the biomarker is not bound to a specific timeframe (119, 120). Such biomarkers are plentiful but include, for example, plasma levels of beta-carotenes and vitamin C as biomarkers of fruit and vegetable intake, urinary excreted polyphenols or various circulating fatty acids, to name a few. Nonetheless, more flexible biomarker classifications have recently been proposed that may be more suitable and informative (122).

The phenomenon of measuring sucrose and fructose in urine to assess sugar intake was first studied by Luceri et al. in 1996 (123). In this study, it was confirmed that the urine excretion of sucrose and fructose was significantly decreased after the consumption of a low-sucrose diet for three days and that urinary excretion of both sucrose and fructose significantly correlated with sucrose intake (123). The mechanisms behind the urinary sucrose and fructose biomarkers are based on that sucrose, which in general is hydrolyzed into glucose and fructose before absorption, in small amounts is absorbed intact in the small intestine. Once whole sucrose is in the circulation, it is believed to be excreted in urine basically unmetabolized, since insulin regulates only circulating levels of glucose, not sucrose or fructose. For fructose, the urinary excreted amounts come from the fractions of fructose that escape metabolism by the liver. It has been estimated that approximately 0.05% of ingested sugar is recovered in 24-h urine samples, summing urinary sucrose and fructose together (124).

Th urinary sucrose and fructose biomarker cannot be classified as a recovery biomarker due to the low recovered amounts of sucrose and fructose in urine. However, the high correlation of this biomarker with intake, its dose-response qualities and its ability to predict sugar intake makes it much more accurate than a concentration biomarker. Therefore, a new biomarker category was introduced – predictive biomarkers. Urinary sucrose and fructose in 24-h urine samples currently serves as the only biomarker that can be classified as a predictive biomarker (125).

## Validation studies

Inspired by the study by Luceri et al (123), Natasha Tasevska et al. conducted the first validation study of the 24-h urinary sucrose and fructose biomarker and published the results in 2005 (125). The validation was performed in two separate studies, both with highly controlled conditions where healthy adults lived in a volunteer suite for 30 days. First, a dose-response relationship was ascertained following a crossover trial of three diets containing three different levels of total sugars (63 g, 143 g and 264 g) for 10 days each. At days 4 and 7 of each diet, 24-h urine was collected. In this study, the correlation between total sugar intake and the sum of urinarily excreted sucrose and fructose was 0.89. Second, a study of habitual intake was performed. Prior to the study, participants had completed a detailed 7-day food record to assess their habitual dietary intake. For 30 days, study participants were fed meals based on what they had reported in their food record. Duplicate meals were saved to assess the amounts eaten, and 24-h urine was collected daily. In this study, the correlation between total sugar intake and the sum of urinary excreted sucrose and fructose was 0.84. Total sugar intake explained 74% of the variation in excreted urinary sucrose and fructose in the dose-response study and 72% in the habitual intake study (125).

The early validation of the biomarker was then advanced to compare normal-weight (BMI<25) and obese (BMI>30) individuals (126). After a crossover design of three different diets containing 13E%, 30E% and 50E% from total sugars for 4 days each in controlled conditions, no effect of BMI on the 24-h urinary sucrose and fructose biomarker performance was observed (126). The 24-h biomarker has also been successfully validated in adolescents, unless the sugar intake was too low (5E% from added sugar, compared to 25E%) (127). In another larger study where the biomarker was validated against duplicate portions and 24-h recalls over long-term consumption (collections of the biomarker, duplicate portions and 24-h recalls were not made simultaneously, but spread out over a long time period), the validation coefficients for the 24-h urinary sucrose and fructose biomarker were shown to be as good as those for the urinary nitrogen biomarker for protein intake if repeated measurements were used (>2 measurements) (128).

Very recently, a new validation study in 98 adults (BMI <35) by Tasevska et al. was published (129). Here, 15-day sugar intake, where all food was prepared and provided to the participants after design on the basis of their 14-day food record, was compared with sucrose and fructose excretion in 8 24-h urine collections, and a correlation coefficient of 0.68 was found. If only one 24-h urine collection was used for comparison with the 15-day mean sugar intake, the correlation was 0.56 (129).

### Correlations with self-reported intakes

After the 24-h urinary sucrose and fructose biomarker were first validated, Natasha Tasevska and colleagues studied it in comparison to self-reported sugar intake in several cohort studies. They predicted "true" sugar intake from the sum of 24-h urinary sucrose and fructose using a prediction equation generated based on the data from the 30-day validation study (125). In the Observing Protein and Energy Nutrition (OPEN) study (n=484), the correlation coefficients between the biomarker-predicted total sugar intake and self-reported total sugar intake were 0.43 for men and 0.16 for women when using an FFQ, and 0.58 for men and 0.25 for women when using an average of two 24-h recalls(130). In the Nutrition and Physical Activity Assessment Study (n=450), the correlation between biomarker-predicted total sugar intake and self-reported total sugar intake was 0.22 using an FFQ, 0.26 using a 4-day food record and 0.26 using an average of three 24-h recalls (131). In contrast, in the Study of Latinos: Nutrition & Physical Activity Assessment Study (n=450), no significant correlation between the biomarker-predicted total sugar intake and self-reported total sugar intake was observed (average of up to five 24-h recalls) (132). However, when only the single 24-h recall closest to the 24-h urinary collection was studied in a subsample, the correlation coefficient between self-reported total sugar intake and the biomarker-predicted total sugar intake was 0.36. In the 2005 Health Survey England, the correlation coefficient between the 24-h urinary sucrose and fructose biomarker and self-reported sugar intake was never reported, but the biomarker was associated with higher BMI, waist circumference and waist-to-hip ratio (133).

### Non-24-hr urine samples

Additionally, a few studies have studied this biomarker in non-24-h urine samples, but the biomarker has never undergone validation in any form other than 24-h urine samples. In the EPIC Norfolk cohort, the urinary sucrose and fructose concentrations from spot urine samples were compared in normal-weight and obese individuals. Cross-sectionally, urinary sucrose excretion was associated with increased odds of obesity, while urinary fructose excretion and self-reported sugar intake were not (134). Later on, in a larger sample of the EPIC Norfolk cohort and with data on BMI after 3 years of follow-up and sugar intake assessed with a 7-day food record, it was concluded that

sucrose measured in urine was associated with higher BMI and increased odds of overweight and obesity, while self-reported sugar intake was associated with lower odds of overweight and obesity (135). Unfortunately, these studies never reported a correlation coefficient between the biomarker and self-reported sugar intake, which would have been useful for understanding if the accuracy of the spot urine sugar biomarker is comparable to that of the 24-h urinary sugar biomarker. In the I.Family study, morning urine samples from children aged 5-18 across several European countries were analyzed for sucrose and fructose concentrations, and correlation coefficients with self-reported sugar intake were found to be 0.27 from a 24-h recall from the day before the urine collection, and 0.23 from the means of repeated 24-h recalls (136).

## Undiscovered territories

On the basis of both the existing and nonexisting literature regarding the sucrose and fructose biomarkers, a few questions arise.

Are there any determinants of the biomarker other than sugar intake? Could physical activity level, gastrointestinal health, kidney function, metabolic health or blood pressure (to name a few examples) potentially alter the relationship between ingested and excreted sucrose and fructose?

Must e 24-h urine samples be used? Since 24-h urine samples are very cumbersome to collect in large cohort studies, this is a vital question. The use of spot or overnight urine samples would greatly enhance data collection. A few studies have examined the biomarker in non-24-h urine samples, but more knowledge is needed.

In which different population groups is this biomarker applicable? In addition to in healthy adults, the biomarker has been validated in both obese individuals (126) and in children (127, 136, 137). However, is this biomarker applicable in individuals with other conditions, such as those who are metabolically impaired?

Will the biomarker help us find an association between sugar intake and cardiometabolic disease? Thus far, the only study that has used this biomarker when actually examining longitudinal T2D and CVD incidence data found no association, while the association between self-reported total sugar intake and T2D was inverse (90). This result indicates that dietary misreporting may not be the only reason why an association between sugar intake and cardiometabolic disease rarely has been seen in epidemiological settings.

# Explorative omics research

Advances in technology have made possible a whole new field of research, which is often referred to as *omics* research. The suffix *ome* is Greek for total or totality, and the suffix *omic* consequently means the study of such totality. For example, the genome is the totality of genes, and genomics is the study of the totality of genes. Other than genome, one could, for example, study the totality of microbes in the gut (gut microbiome) or the totality of circulating proteins (proteome), metabolites (metabolome) or lipids (lipidome), and much more (138). These methodologies create opportunities to obtain knowledge that we have never been able to obtain before. Omics methodologies have the potential to clarify the complex relationships between added sugar intake and cardiometabolic risk in many various ways, for example by highlighting new physiological pathways and mechanisms of action or identifying traits to stratify populations based on their response to high sugar intake for improved prediction of their cardiometabolic risk (139).

The first step in omics research is generally to study the data without any specific hypothesis. This may sound controversial, as textbooks in the philosophy of science clearly emphasize the need for a hypothesis to be tested for rejection; which is how we traditionally define the scientific method and how science always has been done (140). However, we are currently experiencing a paradigm shift, in which technological advances can supply us with so much detailed data that we no longer must limit the research to a specific hypothesis. These explorative omics studies are what we may call hypothesis-generating studies rather than hypothesis-testing studies (140, 141). In practice, this means that instead of framing a hypothesis regarding only one particular (or group of) gene, gut bacterium or plasma protein, for example, we study the associations with all available genes, gut bacteria or plasma proteins and let the results steer our future hypotheses from there.

# Rationale

The existing scientific literature strongly supports that the intake of SSBs increases the risk of cardiometabolic diseases, but the same clear picture is not presented when the literature of total intake of added sugar is summarized, despite that we have a mechanistic understanding of how added sugars could contribute to cardiometabolic disease development. In terms of actually establishing causality, we need RCTs studying sugar intake and cardiometabolic outcomes, which are extremely complicated, impractical and sometimes even unethical. An alternative for establishing causality is to use Mendelian randomization, which takes advantage of the nonmodifiable nature of our genome (142). However, to be able to use Mendelian randomization to study the actual causal relationship between the intake of added sugar and cardiometabolic disease, we need identify genetic variants that are strongly associated with sugar intake. Surely, some genetic variants have been identified (143), but generally not as strongly as is warranted to perform Mendelian randomization studies (yet, at least).

Another alternative to address causality is to study the nine Bradford Hill criteria (144). Using these criteria, the association between SSB intake and T2D has already been considered to indicate causality (145) (Table 3). In Table 3, I have outlined how the intake of added sugar in association with T2D risk fulfills, and does not fulfill, the Bradford Hill criteria from previous literature, and it becomes quite clear that we are far from establishing a causal relationship here.

Considering the Bradford Hill criterion of *consistency*, it is important to study the associations between added sugar intake and cardiometabolic disease in many different populations, although such single association studies cannot establish causality on their own. In other words, to one day be able to answer this question of causality, more epidemiological investigations of the associations between added sugar intake and cardiometabolic disease are needed. We must also study various physiological pathways which could shed new knowledge on potential mechanisms linking added sugar intake to cardiometabolic disease development to address the Bradford Hill criteria of *analogy*, *biological plausibility* and *biological coherence*. Furthermore, we need to develop and utilize new and improved methods to study these associations, considering the many limitations inherent in studying self-reported dietary intake in relation to disease outcomes that otherwise reduce the *strength of associations*.

**Table 3. Bradford Hill criteria for causality (144) for SSB intake and sugar intake increasing the risk of T2D.**

| Bradford Hill criteria | SSB intake | Sugar intake |
|---|---|---|
| 1. Strength of association | "Significant positive association. RR: 1.26 (CI, 1.12, 1.41) for 1-2 servings/day." | Weak associations in epidemiological studies, if any. |
| 2. Consistency | "Consistent data from large prospective cohort studies." | Very inconsistent data from prospective cohort studies. |
| 3. Specificity | "SSB has been shown to increase risk of related metabolic conditions and unrelated conditions such as dental caries and reductions in bone mineral density." | Not applicable as the previous research found contradictory findings. |
| 4. Temporality | "Prospective studies have established temporality." | Prospective studies suggest temporality, but rarely find associations. |
| 5. Biological gradient | "Increase of 1 SSB/day associated with about 15% increased risk of T2D RR: 1.15 (CI, 1.11, 1.20)." | Tendencies for U-shaped associations are not infrequently observed. |
| 6. Plausibility | "Evidence regarding incomplete compensation for liquid calories, glycemic effects of consuming large amounts of rapidly absorbable sugars, and metabolic effects of fructose provide biological plausibility." | Mechanistic understanding of effects on weight gain, glycemic effects and metabolic effects of fructose provide biological plausibility. |
| 7. Coherence | There is coherence between experimental and epidemiological findings. | Low coherence between experimental and epidemiological findings. |
| 8. Experimental evidence | "RCTs with clinical T2D as an end point are logistically difficult; however, experimental evidence from studies of biomarkers of T2D and cardiovascular risk provide support." | RCTs with clinical T2D as an end point are logistically difficult; however, experimental evidence from studies of biomarkers of T2D and cardiovascular risk provide some support. |
| 9. Analogy | Many risk factors for T2D have been identified. | Associations analogous to those observed between SSB intake and T2D are not observed for sugar intake. |

Inspired by, and citations obtained from, Malik et al. 2012 (145), which bases its statements about SSBs on a meta-analysis by Malik et al. 2010 (65).

# Aims

The overarching aim of this thesis is to elucidate the role of added sugar intake in the risk for cardiometabolic diseases. To obtain further understanding of such an association, the aims include exploring differences between the intake of added sugar and different added sugar sources, studying an objective biomarker of sugar intake and evaluating various new physiological pathways in which sugar intake could possibly affect cardiometabolic risk.

Paper I

- To examine the prospective associations between the consumption of added sugar and all-cause and cardiovascular mortality.
- To examine the prospective associations between the consumption of different added sugar sources and all-cause mortality.

Paper II

- To compare the measurement of sucrose and fructose in overnight urine samples with self-reported sugar intake.
- To assess and compare the cross-sectional associations between overnight urine samples, self-reported added sugar intake and their composite measure with cardiometabolic risk markers.

Paper III

- To evaluate 24-h urinary sucrose and fructose excretion as biomarkers of sugar intake in individuals with prediabetes, and the role of metabolic status in the performance of the biomarkers.

Paper IV

- To cross-sectionally examine the associations between the intake of added sugar and SSBs with individual bacterial genera and measures of microbial composition.

Paper V

- To identify plasma proteins that cross-sectionally associate with the intake of added sugar and SSBs and study how those proteins prospectively associate with T2D incidence.
- To study the cross-sectional association between added sugar and SSB intake with CRP and the prospective association with T2D incidence.

# Methods

## Study populations

The majority of this thesis work have been conducted in the following Malmö-based cohorts: the Malmö Diet and Cancer study (MDC), the Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC) and the Malmö Offspring Study (MOS). Basic descriptions of these cohorts and how they are connected are depicted in Figure 5. In addition, the Northern Swedish Health and Disease Study (NSHDS) and the Prevention of diabetes through lifestyle Interventions and population studies in Europe & around the World (PREVIEW) were studied.

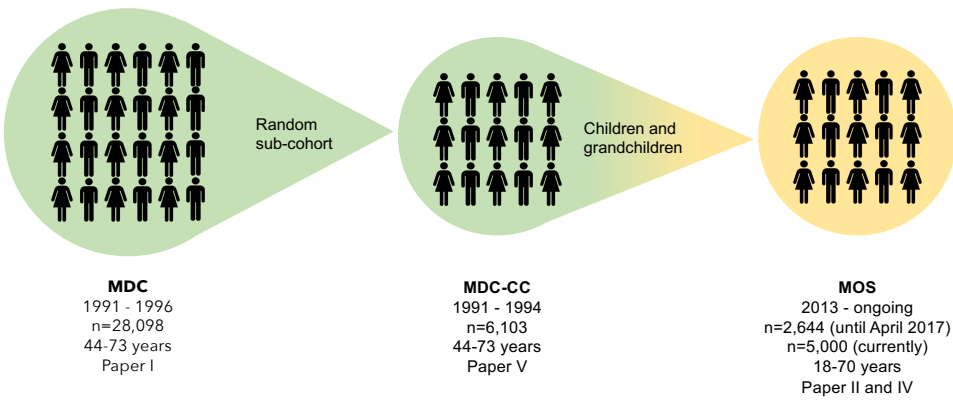


**Figure 5.** Desription of the Malmö-based cohort studies. The color shift represent a generational shift.

### Malmö Diet & Cancer study (MDC)

The MDC is a population based prospective cohort study with baseline examination conducted in the city of Malmö in southern Sweden between 1991 and 1996. All men born between 1923 and 1945 and all women born between 1923 and 1950 residenting in the Malmö area were eligible to participate. Study participants were recruited via personal invitation letters and advertisements in media and public areas. A total of 74,138 individuals constituted the source population and a total of 30,447 individuals participated, giving a total participation rate of 41%. The only exclusion criteria for participation was inability to perform the baseline examination, such as inadequacy in

the Swedish language or inadequate mental capacity. Anthropometrics, blood samples, a lifestyle questionnaire and diet assessment were collected (146). A total of 28,098 had complete data on dietary intake, and in Paper I, we further excluded individuals with diabetes, CVD or cancer prior to baseline and ended up with a total study sample of 24,272 individuals. All participants signed written informed consent before study entry, and MDC was granted ethical approval by the Ethical Committee at Lund University (LU 51/90).

### Malmö Diet & Cancer-Cardiovascular Cohort (MDC-CC)

The MDC-CC is a more deeply phenotyped subcohort within the MDC. During the years of 1991 to 1994, 6,103 randomly selected MDC participants were invited to a second examination. Participants attended this second examination after an overnight fast and fasting plasma samples could be obtained in 5,540 individuals. The fasting blood samples were analyzed for glucose, insulin, total cholesterol, triglycerides and HDL cholesterol, as well as biobanked in -80°C (147). In Paper V, 4,742 individuals had successful plasma protein measurements, from which we excluded individuals with diabetes and CVD prior to baseline, resulting in a study sample of 4,382 individuals.

### Malmö Offspring Study (MOS)

The MOS is the offspring cohort of the MDC-CC participants, in which their children and grandchildren living in the Malmö area and were above 18 years of age were invited to participate. All eligible individuals were sent personal invitation letters which were followed up with phone calls. The baseline data collection started in 2013 and is still ongoing (although, temporarily paused due to the current pandemic). Currently, about 5,000 participants have been recruited and the data collection is expected to last until the end of 2021. Until April 2017, a half-time cohort was compiled and involved the first 2,644 participants. MOS participants made two fasting visits to the research clinic where participants' anthropometrics and fasting blood samples were collected. Blood samples were initially analyzed for glucose and blood lipids, and the remaining were biobanked at -80°C. On the first research visit, participants received detailed instructions on how to, at home, complete a web-based lifestyle questionnaire, a web-based short FFQ and a web-based 4-day food record, as well as how to collect faecal samples and urine samples to be brought on the morning of the second research visit (148). In Paper II, after exclusion of individuals with diabetes and self-reported energy intakes outside the range of 500-6,000 kcal, we studied a total of 991 individuals that had their urine samples sent for analysis, from which 763 individuals had successful measurement of both sucrose and fructose. In Paper IV, the study sample comprised 1,371 individuals that were free from diabetes, had an energy intake within 500-6,000

kcal and had complete data from the 4-day food record, gut microbiome sequencing and model covariates. Ethical approval for the MOS was provided from the Regional Ethics Committee in Lund (dnr.2012/594) and all participants signed written informed consent prior to participation.

### Northern Swedish Health & Disease Study (NSHDS)

The NSHDS, or more specifically the Västerbotten Intervention Programme, is an ongoing cohort study in the county of Västerbotten in the north of Sweden that were initiated in 1985. Everyone in Västerbotten are called to a health check-up when they turn 40, 50 and 60 years of age where anthropometrics, blood samples, lifestyle questionnaires and FFQs were obtained (149). In Paper I, we only used data collected between 1991 and 1996, because a change in the diet assessment method was made thereafter which prevented estimation of added sugar intake. This gave 36,826 eligible participants, but after exclusion of individuals with diabetes or CVD prior to baseline, 24,475 comprised our study sample in Paper I. The NSHDS was ethically approved by the Regional Ethical Review Board of Northern Sweden and all participants gave written informed consent before participating.

### PREVIEW

The PREVIEW RCT is a large multicenter study conducted at 8 different sites across the globe; Denmark, Finland, UK, The Netherlands, Spain, Bulgaria, Australia and New Zealand. The study conducted in Copenhagen, Denmark, comprised 353 individuals and was used in Paper III of this thesis (150). In PREVIEW, individuals with prediabetes were recruited to participate in a randomized diet and lifestyle intervention aimed for weight loss and reduced T2D incidence. The intervention started with 2 months on a low energy diet (Cambridge Weight Plan, Northants, UK), i.e. the weight loss phase. Following the two month of rapid weight loss, the participants were randomized to four different intervention groups for the 34 months long weight maintenance phase: high-protein and low-GI diet with high-intensity exercise; high-protein and low-GI diet with moderate-intensity exercise; moderate-protein and moderate-GI diet with high-intensity exercise; moderate-protein and moderate-GI diet with moderate-intensity exercise. The low-GI diet contained 25E% protein, 45E% carbohydrate and the GI should be maximum 50. The moderate-GI diet contained 15E% protein intake, 55E% carbohydrate and the GI should be at least 56. Both diets should contain 30E% total fat and both diets were healthy (150, 151). Simultaneous 4-day food records and 24-h urine samples were collected at baseline, 6 months and 12 months of the intervention. In Paper III, 268 individuals had complete data on urinary sucrose and fructose measurements and 4-day food records at baseline.

PREVIEW was given ethical approval by the Ethics Committee of the Capital Region in Denmark and all participants provided written informed consent.

## Assessment of lifestyle & socioeconomic factors

In the cohorts MDC, MOS and NSHDS, participants filled out questionnaires at baseline covering a wide range of questions on lifestyle, socioeconomics, family history and disease history. From these questionnaires, many variables for covariate adjustment were obtained, including smoking habits, alcohol habits, leisure-time physical activity and educational level (see *Statistical analyses*, page 57).

In the MDC, leisure-time physical activity was assessed from self-reported time spent in 17 different activities. The metabolic equivalent intensity for each activity was used to translate this to metabolic equivalent hours per week. In the MOS, leisure-time physical activity was studied in Paper II, and was assessed from a question from the lifestyle questionnaire with a four-level answering scale. In Paper IV (MOS), however, physical activity levels were used. These were calculated within the web-based 4-day food record system based on the answers of two questions addressing both leisure-time physical activity and occupational physical activity. In MOS, alcohol habits were assessed on a five-level scale from never to ≥4 times/week, while in the MDC, self-reported alcohol consumption from the dietary assessment was categorized into one category of nonconsumers and quintiles of consumers. Smoking habits was categorized as never smoker, ex-smoker and current smoker in MDC (plus irregular smoker in MOS). Education was categorized in terms of years in school in MDC and highest achieved educational level in MOS.

## Dietary intake assessment

The MDC, including the MDC-CC, used a so-called modified diet history method to obtain dietary intake information. This involved 7-day food records, a 168-item semi-quantitative FFQ and a 45-60 min diet interview. The 7-day food record focused on the cooked meals such as lunch and dinner, the cold beverages consumed, as well as use of dietary supplements. The FFQ focused on the noncooked meals such as breakfast and snacks and covered the past 12 months. The dietary interview elucidated food choices, cooking methods and portion sizes of the 7-day food record. Both in the interview and the FFQ, portion size estimation was aided with a booklet of photograph of different foods at different portion sizes. In 1994, the interview was shortened from 60 to 45 minutes and, consequently, the coding of dietary intakes was somewhat

altered. These three different methods all covered different aspects of the total dietary intake and were used in combination to capture the total intake: the intakes from the FFQ and the food records were summed together, while the interview complemented the food record with additional details. This total reported intake of foods was transformed into daily nutrient intakes using the MDC nutrient database (152). The MDC database was specifically designed for the MDC study, but originated from the food database of the Swedish National Food Agency. The Malmö Food Study serves as the validation study of this dietary assessment method, in which two different dietary assessment methods were validated against a 6×3-day weighed food records. The two studied methods were an extensive FFQ of 350 foods with photography-aided portion size estimation and a combined food record-food frequency method which combined a 130-item FFQ and a 2-week food record. It was concluded that the combined method was superior and was hence incorporated in a modified version in the MDC. The combined method shows good validity to the reference methods in terms of intake of sugar (Pearson's correlation coefficients of 0.74 for women and 0.60 for men) (153).

In the NSHDS between the years of 1991 and 1996, dietary data was collected using an 84-item self-administered FFQ. It addressed the intake of the past 12 months and intake frequencies of food items were reported on a 9-level scale; never, a few times per year, 1-3 times per month, 1 time per week, 2-3 times per week, 4-6 times per week, 1 time per day, 2-3 times per day and more than 4 times per day. Portion size estimation of staple foods such as potatoes, rice, pasta, meat, fish and vegetables were supported with photographs showing various amounts of food on a plate. Fixed age- and sex-specific portion sizes were used for other foods. The food database from the Swedish National Food Agency was used to obtain daily energy and nutrient intake from the portion size-weighted intake frequencies.

The more recent studies, both the MOS and PREVIEW, obtained dietary information using 4-day food records. In the MOS, the 4-day food record was reported in a web-based system called Riksmaten2010 developed by the Swedish National Food Agency. The MOS participants recorded everything they consumed during 4 consecutive days into the web-based system. Estimation of portion sizes was done with the help of photographs of different foods of various portion sizes or using standard household measures. The web-based food record was linked to the Swedish National Food Agency's food database from which daily average energy and nutrient intakes were obtained. Energy intake assessed using the Riksmaten2010 4-day food record has been shown to correlate with doubly-labeled water measurements of energy expenditure of 0.40 (154), and the correlation between two repeated 4-day food record measurements for sucrose intake was 0.41 (155). In the MOS, participants also filled out a short FFQ covering the past 6 months. Intake frequencies of various food items were assessed on an 8-level scale ranging from never/seldom to several times per day. The FFQ data on intake of SSBs and ASBs was combined with the 4-day food record data of intake of

SSBs and ASBs in Paper IV. The correlation between the SSB intake reported with the 4-day food record and the short FFQ has been shown to be 0.42 (155).

In PREVIEW, weighed 4-day food records were collected at baseline, 6 months and 12 months of the RCT. Intake of total sugar (sum of all monosaccharides and disaccharides) were obtained from the Danish diet database Dankost 3000. The GI values for the different foods were obtained from the Diogenes GI tables for Danish foods (156). The formula of van Woudenberg as used to calculate total GI and glycemic load (GL) (157).

## Added sugar intake estimation

In MOS and NSHDS we estimated added sugar intake using the following calculation (all intake variables are expressed in g/day): added sugar = monosaccharides + sucrose - (fruit and berry intake × 0.1 + vegetable intake × 0.03 + juice intake × 0.08). From the sum of all monosaccharides and sucrose, the naturally occurring sugars in fruit and berries, vegetables and fruit juices were subtracted. From the sugar content of the most commonly consumed fruits, vegetables and juices in Sweden, estimated average sugar contents were estimated in fruit and berries to 10 g per 100 g, vegetables to 3 g per 100 g and juices to 8 g per 100 g using the food database from the Swedish National Food Agency (Table 4). This calculation does not include other disaccharides such as lactose and maltose, as they normally never are added to foods as added sugars. It also assumes that all monosaccharides and sucrose in other products than fruit, vegetables and juices are added to the product, hence, assumes that any naturally occurring monosaccharides and sucrose in e.g. cereal products are neglectable.

**Table 4. Sugar content of most common fruits and berries, vegetables and juices in the Swedish diet as basis for the added sugar estimation equation.**

| | Fruit and berries | Sugar g/100g | Vegetables | Sugar g/100g | Juices | Sugar g/100g |
|---|---|---|---|---|---|---|
| | Banana | 13.5 | Lettuce | 2.2 | Orange | 8.0 |
| | Apple | 9.9 | Tomato | 2.8 | Pineapple | 11.8 |
| | Pear | 8.5 | Cucumber | 1.7 | Apple | 10.4 |
| | Orange | 8.9 | Carrot | 6.2 | Carrot | 4.7 |
| | Clementine | 8.2 | Corn | 3.6 | Tomato | 3.2 |
| | Peach | 7.8 | Cabbage | 5.7 | Grape fruit | 7.2 |
| | Watermelon | 8.7 | Onion | 4.5 | | |
| | Grapes | 15.1 | Peas | 5.0 | | |
| | Strawberry | 7.9 | Broccoli | 1.2 | | |
| | Raspberry | 4.1 | Spinach | 0.3 | | |
| Mean | | 9.26 | | 3.32 | | 7.55 |
| Used value* | | 10 | | 3 | | 8 |

These values were used in MOS and NSHDS, additional food groups were used in the equation in MDC. *The used value was determined from the mean and by subjectively weighting for the more common fruits and berries, vegetables and juices.

In MDC (and MDC-CC), we had data available on more detailed food groups, both the food groups fruits and berries and juices were split into non-citrus and citrus, making the added sugar estimation calculation as follows: added sugar = monosaccharides + sucrose - (non-citrus fruit and berry intake × 0.1 + citrus fruit × 0.085 + vegetable intake × 0.03 + non-citrus fruit juice intake × 0.1 + citrus juice intake × 0.08 + carrot juice intake × 0.05 + other vegetable juice intake × 0.03). For Paper I, we also estimated free sugar intake in the MDC using the same calculation, but without subtracting for the sugar present in fruit juices. Table 5 shows the distribution of MDC and MOS participants, respectively, over the six intake categories of added sugar.

**Table 5. Added sugar intake distribution across the six studied categories in the MDC and MOS.**

| | <5E% | 5-7.5E% | 7.5-10E% | 10-15E% | 15-20E% | >20E% |
|---|---|---|---|---|---|---|
| MDC (n=28,098) | 9% | 19% | 26% | 34% | 9% | 3% |
| MOS (n=2,644) | 5% | 10% | 18% | 38% | 20% | 10% |

Added sugar intake appears elevated in MOS compared to MDC.

## Sugar-rich foods & beverages

In MDC, sugar-rich foods and beverages were categorized into the following categories in Paper I: SSBs, treats and toppings, and were studied in servings/week. SSBs included carbonated and noncarbonated sugar-sweetened soft drinks, cordials and fruit drinks (that were not 100% fruit) and was assessed using only the 7-day food record. One serving of SSBs was considered 280 g. Treats included all types of pastries, candies, chocolate and ice cream. One serving was considered 60 g for pastries, candy and chocolate, and 75 g for ice cream. Toppings included table sugar and sugar cubes (added to coffee and oatmeal etc), syrup, honey, jam and marmalade. One serving was considered 10 g for sugar and syrup and 20 g for honey, jam and marmalade. These categories were chosen to 1) differentiate between liquid and solid sources of added sugar and 2) differentiate between solid added sugar sources that generally also are high in fat, and at high risk of being overconsumed (treats), with sources of added sugar that are primarily contributing with carbohydrates and are not generally prone to be binge eaten (toppings).

In Paper V conducted in the MDC-CC, SSB intake was investigated expressed in E% to make it more comparable to the intake of added sugar that also was studied as E%. To calculate the energy contribution from the SSBs, we estimated the standard sugar content per 100 g of SSBs to 10 g based on that soft drinks generally have a sugar content varying between 10-13 g/100 g and cordials have a sugar content around 8 g/100 g.

In Paper IV conducted in the MOS, intake of SSBs was calculated as a combination of the reported intake from the 4-day food record and the short FFQ. In addition, the intake of artificially sweetened beverages (ASBs) was studied in the same manner, as a

few previous studies have indicated a potential link between low-calorie sweeteners intake, gut microbiome dysbiosis and reduced metabolic health (158). Reported intake of SSBs and ASBs from the short FFQ was categorized into three groups based on intake frequency; never/seldom, ≤2 times/week and ≥3 times/week. The reported intake of SSBs and ASBs from the 4-day food record was categorized to match the categories from the short FFQ into 0 ml/d, 0.1-100 ml/day and >100 ml/day (assuming a serving size of 250 ml, the corresponding FFQ cut-offs were at <71 ml/day and >107 ml/day. The two variables of three categories each were then cross tabulated and those reporting zero-consumption using both methods were groups as certain zero-consumers. High consumers were everyone reporting >100 ml/day from the 4-day food record, as well as those reporting 0.1-100 ml/day from the food record, but ≥3times/week from the FFQ. The remaining were considered as medium consumers. The 4-day food record was hence the dominant diet assessment method, but you could be upgraded based on your FFQ report. We designed it in this way because for the purpose of Paper IV, that was to study associations with the gut microbiota, it was thought more relevant to capture the more recent dietary intake rather than long-term, as the gut microbiota can vary quite rapidly to dietary alterations.

## Urinary sucrose & fructose assessment

### Overnight urine samples in MOS

In the MOS, participants were instructed to empty the bladder before going to bed and thereafter collected any urine excreted during the night (if any) and all of the first morning urine in a provided plastic bottle. Participants brought their urine samples to the research clinic where they were stores in a refrigerator for maximum 4 h before aliquoted and frozen at -80°C.

The first 1,500 urine samples collected in the MOS were sent to Gunter Kuhnle's laboratory at Reading University, UK, for analysis of sucrose and fructose concentrations. The urine samples were thawed at 4°C and diluted with an internal standard solution labeled with stable isotopes $^{13}$C12-sucrose at 4 µg/mL and $^{13}$C6-fructose at 10 µg/mL prepared in acetonitrile. Liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis was performed using an Acquity UPLC system (Waters, Milford, MA, USA), coupled to a Quattro Ultima tandem quadrupole mass spectrometer (Micromass, Manchester, UK). The mass spectrometer was operated through electrospray ionization in positive ion mode using multiple reaction monitoring mode. 226 urine samples were not successfully analysed and 61 measurements were outside the calibration range and were thus excluded from statistical analysis.

Since these urine samples were not 24-h urine samples, adjustment for urine dilution must be performed. We did so by expressing the urinary sugars concentrations as ratios to the urine osmolality (mOsm/kg $H_2O$) in units of $(\mu mol \cdot L^{-1})/(mOsm \cdot kg^{-1})$. Urine osmolality was measured with an i-Osmometer basic (Löser, Germany).

The overnight urinary sugars were also combined with self-reported added sugar intake to a composite measure by summing the two variables, each divided by its standard deviation. This is proportional to the first principal component (PC) of the two variables (159). This composite measure is from here on denoted as the PC added×U-sugar in this thesis and was studied in Papers II and IV.

### 24-h urine samples in PREVIEW

In PREVIEW, the participants were given instructions on how to collect 24-h urine samples and were provided with urine collection containers and insulated thermos bag with cooling element and, for women, a funnel. At the end of the 24-h collection period, participants returned their urine collections to the research clinic and weight and density of the total collection was recorded, and volume was calculated. The samples stood refrigerated for maximally two h at 5°C at the research clinic before the aliquots were frozen. In Paper III, sucrose and fructose excretions from the 24-h urine samples collected at baseline and at 6 and 12 months of the intervention period were studied.

The laboratory of Lars Ove Dragsted, University of Copenhagen, Denmark, analyzed the sucrose and fructose concentrations of the 24-h urine samples. Sucrose concentrations were measured with an ultra-high performance liquid chromatography system (UHPLC; Acquity H-class; Waters, Taastrup, Denmark) coupled to a quadruple time-of-flight (QTOF) mass spectrometer (Premier, Waters Corporation, Manchester, UK). A pool of all samples (global pool) and a pool of all samples within each batch (batch pools) were analysed during each batch along with a water sample as blank. Twelve serial dilutions by a factor of two of a sucrose standard (2.92 uM in 50% acetonitrile with 0.9% NaCl) were analysed with every batch. A pooled urine sample (40% urine in acetonitrile) with negligible sucrose content was used for standard dilutions to have a comparable matrix. All samples were prepared by adding 200 uL urine to 50 uL cold acetonitrile. All standards and samples, as well as the blank, were added with 8.23 nM D-Sucrose-13C12 (Cambridge Isotopes Laboratories, Tewksbury, MA) as internal standard. Sucrose concentrations in ng/ml were calculated using the regression equation obtained from the internal standards on each plate, predicting the concentrations from the measured response after correction for the internal standard. Batch correction was performed by multiplying the concentrations with the ratio between the grand mean to the batch mean. Corrected concentrations in ng/ml were

multiplied with the urine volume to obtain the total 24-h urine excretion and was transformed into mg/d.

Fructose concentrations were measured using the EnzyChrom® EFRU-100 fructose assay kit (Bioassay Systems, Hayward, CA) according to the instructions of the provider. Samples with concentrations above the highest standard were diluted five times and re-analysed. Samples below the limit of quantification (3 × the standard deviation above the average blank) were re-analysed using 60 uL sample. Concentrations in uM were transformed to mg/L and multiplied with urine volume to obtain daily fructose excretion in mg/d.

In the tables and figures of this thesis, the urinary excretion of sucrose, fructose and their sum are denoted U-sucrose, U-fructose and U-sugars, respectively.

## Gut microbiota assessment

In the MOS, participants were instructed in person and via an instruction video on how to collect a fecal sample in four provided plastic tubes at home. The instructions were to keep the sample in the freezer until bringing it to the research clinic where samples were frozen at -80°C. Bacterial DNA was extracted with the QIAmp column stool kit and was sequenced using HiSeq Illumina at GATC Biotech (Germany). Sequencing data was binned to operational taxonomic units using QIIME (1.9.1) and linked to the Greengenes database (v.13.8). Data was extracted from Greengenes on genus level. From the total of 542 identified genera, we excluded genera identified in <3 individuals and/or with relative abundance of <0.01%, giving 64 included bacterial genera. MetagenomiqSeq in R was used to normalize the absolute abundances of the included genera using cumulative sum scaling. The Firmicutes:Bacteroidetes ratio was calculated from the relative abundances of the tow phyla in question and the Shannon index of alpha-diversity was calculated using the *vegan* package in R.

## Plasma proteins assessment

In the MDC-CC, fasting blood samples were available in 5,540 participants. These were stored in -80°C until sent to the SciLifeLab (Uppsala, Sweden) for analysis using the Olink Proseek Multiplex proximity extension assays (Olink Proteomics, Uppsala, Sweden). This method utilizes DNA-tagged antibodies that bind in pairs to every target protein. When bound and matched, the DNA-tags hybridize and are extended to an amplicon, which is sequenced with quantitative PCR. Values are presented as normalized protein expressions on the $\log^2$ scale adjusted for any batch effects

(http://www.olink.com). A total of 149 proteins were measured, but we excluded those protein that were available in less than 75% of study population, resulting in 136 studied proteins.

The Tina-quant® CRP latex high sensitivity assay (Roche Diagnostics, Switzerland) on an ADVIA 1650 Chemistry System (Bayer Healthcare, USA) was used to measure CRP concentrations. Values are presented as means of reads in 6 second intervals over 1 minute after 5 minutes of incubation.

## Outcome assessment

### Cardiometabolic risk factors

In all studied populations, cardiometabolic risk markers have been measured in the form of anthropometric measurements, blood pressure and from blood samples, including various blood lipids and measures of glucose homeostasis.

Weight has been measured with light clothing and BMI has been calculated as weight (kg)/height$^2$ (m), and has been categorized as normal weight <25, overweight 25-30 and obese >30. Waist circumference was studied in the MOS and PREVIEW and was assessed between the lowest point of the rib and the highest point of the hip. Systolic and diastolic blood pressure have been measured following a short rest in all studied populations. Triglycerides, total cholesterol and HDL cholesterol have been measured in fasting blood samples using standardized methods, and LDL cholesterol was calculated using the Friedewald equation. Fasting plasma glucose (FPG) have been measured in all studied populations, except the whole MDC, and fasting insulin was measured in MDC-CC and PREVIEW. Using these insulin measurements HOMA-IR was calculated with the following formula: FPG (mmol/L) × fasting insulin (mU/L)/22.5. In PREVIEW, HbA1c was measured using standardized procedures and fat mass was measured using dual-energy X-ray absorptiometry. Participants also conducted an oral glucose tolerance test (OGTT) at each clinical investigation, and 2-h plasma glucose was measured. The dichotomous outcome normoglycemia was defined as FPG <5.6 mmol/L and with a 2-h plasma glucose of <7.8 mmol/L from OGTT, in accordance with previous investigations in PREVIEW.

### Incidence outcomes

For ascertainment of incidence outcomes, various Swedish health registries were mostly used. We obtained date of death from the Swedish National Tax Agency, Statistics in Sweden, and the National Board of Health and Welfare and the cause of death was of

from the Swedish Cause of Death Register. End of follow-up was at 31 December 2014 for mortality in Paper I.

For incidence of T2D, the following registries were used: Swedish National Diabetes Register, the regional Diabetes 2000 register of the Scania region, the Malmö HbA1c register, the Swedish inpatient register, the Swedish outpatient register, and the nationwide Swedish drug prescription register. Diabetes incidence was also assessed via the re-examinations of the MDC-CC in 2007-2012 and the Malmö Preventive Project in 2002-2006. End of follow-up was until 31 December 2016 for T2D in Paper V.

## Statistical analyses

For the most statistical analyses in this thesis, StataSE (version 15) was used. A few analyses have also been performed in SPSS and in R. Generally, two-sided P-values <0.05 were deemed significant, but if multiple variables were studied (64 gut bacterial genera or 136 plasma proteins) a lower P-value was used to control for multiple testing, using either the false discovery rate (FDR) approach or the Bonferroni method. Distribution of studied variables were studied using histograms and if the distributions were severally skewed, the variables were either logarithmically transformed to improve distribution or studied using non-parametric tests, dependent on the situation.

### Correlation analyses

The comparison between the self-reported sugar intake and urinary excretion of sucrose and fructose have primarily been studied using various correlation analyses. In Paper II, we log-transformed the skewed urinary sucrose and fructose concentrations and correlated with self-reported sugar intake using partial correlations adjusted for age, sex energy intake and BMI. In Paper III, however, we instead used unadjusted Spearman correlation to account for the skewed 24-h urinary sucrose and fructose excretion variables.

### Multivariable regression analyses

Various types of multivariable regression analyses have been performed in the work of this thesis to explore the associations between, primarily, various sugar intake variables and various indicators of cardiometabolic health. Normally, a set of different models of covariate adjustment have been studied, most often including a basic adjustment model including age, sex and energy intake (and any cohort-specific methodological variables) and an extended model including lifestyle factors such as smoking habits, alcohol

consumption habits, physical activity habits and sometimes some sort of indicator of socioeconomic status (education level). In addition, covariate adjustment of BMI has been performed, to study the association without the indirect mediating effects of body composition. Furthermore, adjustment of other dietary variables (in addition to energy intake) have been conducted on occasion, as intake of many different dietary constituents often are correlated to each other, as well as to cardiometabolic risk. These included covariates were in general chosen based on a possible association with both the exposure variable and the outcomes variable (i.e. the definition of a confounder), most often explored and displayed using directed acyclic graphs. Table 6 explains the different regression models used to study various measures of cardioembolic risk.

**Table 6. Desription of the investigated regression models in Papers I-V used to study associations with various measures of cardiotmabolic risk.**

| Paper | Design (cohort) | Exposure, categories[1] | Outcome | Model 1 | Model 2 |
|---|---|---|---|---|---|
| I | Prospective (MDC, NSHDS) | Added sugar (E%), 6<br>SSBs (serv/wk), 5<br>Treats (serv/wk), 5<br>Toppings (serv/wk), 5 | Mortality | Age<br>Sex<br>Energy<br>Season[2]<br>Screening date[2] | Education<br>Physical activity<br>Smoking<br>Alcohol<br>Fruit/vegetables<br>Processed meat<br>Coffee<br>Saturated fat<br>Fiber<br>BMI |
| II | Cross-sectional (MOS) | Added sugar (E%)<br>Urinary sugars<br>PC added×U-sugar | Cardiometabolic risk markers | Age<br>Sex<br>Energy | Education<br>Physical activity<br>Smoking<br>Alcohol<br>Fiber |
| III | Longitudinal within an intervention trial (PREVIEW) | Total sugar (g/d)<br>Total sugar (E%)<br>Urinary sucrose<br>Urinary fructose<br>Urinary sugars | Changes in cardiometabolic risk markers during lifestyle intervention | Age<br>Sex<br>Intervention group | - |
| IV | Cross-sectional (MOS) | Added sugar (E%), 3<br>PC added×U-sugar<br>SSBs (g/d), 3<br>ASBs (g/d), 3 | Gut microbiota | Age<br>Sex<br>Energy<br>Physical activity<br>Smoking | Fiber<br>BMI |
| V | Cross-sectional (MDC-CC) | Added sugar (E%)<br>SSB intake (E%) | Plasma proteins | - | Age<br>Sex<br>Energy<br>Season<br>Screening date<br>Education<br>Physical activity<br>Smoking<br>Alcohol |
| V | Prospective (MDC-CC) | Plasma proteins | T2D incidence | Age<br>Sex<br>Education<br>Physical activity<br>Smoking<br>Alcohol | BMI<br>Fasting glucose[3] |

[1]Number of categories (if studied categorically). [2]In MDC only. [3]In an additional 3rd model.

*Survival analyses*

Survival analyses in the form of Cox proportional hazards regressions have been used in Papers I and V for longitudinal analyses of mortality and T2D incidence, respectively. Follow-up time (date from baseline examination to either date of event, emigration or end of follow-up) was used as the time variable.

*Restricted cubic splines*

In Paper I, the fully-adjusted cox proportional hazards regression models were further studied using continuous exposure variables using restricted cubic splines to visualize dose-response curves. Cubic polynomials make out the base of cubic splines, which does not assume linearity. The independent (exposure) variable is split into segments (at the knots), and a single polynomial is fit within each segment. The spline is then fitting a smooth curve to all these segments. If the cubic splines are restricted (as in this case) the first and last segments are linear functions rather than polynomials. In Paper I, splines were created using 4 knots (3 knots for SSBs in MDC due to the large number of zero-consumers) at Harrell's default percentiles. For added sugar intake, the reference was placed at 10E% (since the lowest mortality was observed in the intake category between 7.5-10E% of added sugar) and at 0 servings/week for SSBs, treats and toppings.

*Linear regression*

In Paper I, the categories of added sugar intake were studied cross-sectionally in association with cardiometabolic risk markers in MDC and NSHDS using the general linear model.

In Paper II, multivariable adjusted linear regression was used to study the cross-sectional associations between self-reported added sugar intake, the overnight urinary sugars biomarker and the composite measure PC added×U-sugar and several cardiometabolic risk markers.

In Paper III, the changes in cardiometabolic risk markers between 6 and 12 months during the diet and lifestyle intervention in PREVIEW were studied in relation to self-reported total sugar intake and 24-h urinary excretion of sucrose, fructose and their sum using multivariable adjusted linear regression. Both the absolute intake and excretion at 6 months and the changes in intake and excretion between 6 and 12 months were modelled (Δ-values). The same analyses for the dichotomous outcome normoglycemia at 6 and 12 months was analysed using logistic regression.

*Stepwise backwards linear regression*

In Paper II, stepwise backward linear regression was used to identify significant determinants of the urinary sucrose and fructose excretion from overnight urine samples. In a stepwise fashion, the covariate with the highest *P*-value was excluded from

the linear regressions model from an initial model including all covariates of interest, until all remaining covariates were significant ($P$<0.05). The remaining covariates constituted the model studied using partial correlation.

*Least absolute shrinkage & selector operator (LASSO) regression*

In Paper III, LASSO regression was used to identify the most important predictors of the 24-h urinary excreted amounts of sucrose and fructose. In contrast to the stepwise backward linear regressions used in Paper II, LASSO regression is not limited by issues related to multicollinearity. LASSO regression utilizes a penalty term, called lambda, for shrinking the covariate coefficients. The covariates with the smallest coefficients are shrunk to zero, resulting in a model selection of the most important predictors. 10-fold cross-validation was used to determine the lambda that provided the smallest mean squared prediction error, and the LASSO regression was conducted with this lambda (160). The presented data is from linear (ordinary least squares) regression using the model selected by the LASSO regression. In these analyses, both absolute values of the cardiometabolic risk markers at the different timepoints, as well as changes in cardiometabolic risk markers from baseline to the particular timepoints was modelled (Δ-values).

*Negative binomial regression*

Due to the skewed distribution of many of the bacterial genera studied in Paper IV, negative binomial regressions were used to account for the lack of normal distribution and high variance of the outcome variables when studying the associations between intake of added sugar, SSB and ASB, as well as the PC added×U-sugar, with individual gut bacterial genera.

*Two-step iterative resampling*

In Paper V, a two-step iterative resampling approach was used to internally replicate the findings of proteins associated with added sugar and SSB intake, respectively, among 136 studied proteins. The dataset was randomly split into 2/3 (discovery cohorts) and 1/3 (replication cohorts) 100 times. A protein was considered internally replicated if it associated significantly with added sugar or SSB intake in both the discovery and replication cohorts at least 20 out of the 100 iterations using multivariable adjusted linear regression. Three different significance levels ($\alpha_1$) were evaluated in the discovery cohorts (0.05, 0.01 and the $P$-values corresponding the FDR of 0.05 using the Benjamini-Hochberg method) and the significance level of 0.05 was always used in the replication cohorts. This methodology was inspired by Kang et al (161).

### Sensitivity analyses

In the MDC and MDC-CC, i.e. in Papers I and V, we have conducted sensitivity analyses excluding individuals who are classified as low- or high energy reporters. Low- or high reporting of energy can be used as a proxy for misreporting of energy intake, although it cannot be properly distinguished from an actual low or high energy intake (for example with the aim to lose weight). Low- and high energy reporting was determined with the Black and Goldberg method (162) using individual physical activity levels that were calculated from self-reported activity levels during work, leisure-time and household work as previously described by Mattisson et al. (163). In the NSHDS in Paper I, we instead excluded the lowest 15% and top 2.5% values of energy intake/basal metabolic rate to match the percentages excluded in the MDC.

Low- and high energy reporters were also identified with the same method in Paper II in MOS, with values of physical activity level determined from two questionnaire questions about work-related activity and leisure-time physical activity. It was studied as a potential effect modifier on the associations between self-reported added sugar intake and cardiometabolic risk markers.

Sensitivity analyses excluding those who have reported a past food habit change was also conducted in Papers I and V, based on the answer to the following baseline questionnaire question "Have you substantially changed your eating habits because of illness or some other reasons?" (164).

# Results

## Paper I

This study aimed at studying the association between added sugar intake and mortality were studied in 24,272 participants in the MDC and 24,475 participants in the NSHDS. The average intake of added sugar was 10.1E% in MDC and 8.2E% in NSHDS. In both the studied cohorts, as shown in Table 7 and Figure 6, the lowest all-cause mortality risk where observed at added sugar intake between 7.5-10E% after a median of 20 years of follow-up. In reference to this intake category with the lowest mortality, significantly increased all-cause mortality risks of 30% and 31% in MDC and NSHDS, respectively, were observed at the highest added sugar intakes ≥20E% after full covariate adjustment. Furthermore, the all-cause mortality risk was also increased at the lowest added sugar intakes <5E%, with 23% in MDC and 9% in NSHDS (not significant). Cardiovascular mortality in MDC was increased with 40% and 22% at added sugar intake ≥20E% and <5E%, respectively (Table 7). Very similar U-shaped associations were seen when free sugar intakes were studied instead of added sugar intakes (Figure 6). The associations between added sugar intake and all-cause mortality were somewhat attenuated in both ends after exclusion of individuals that had reported previous diet changes. However, exclusion of low and high energy reporters was mainly attenuating the risk at the higher end in MDC, while in the lower end in NSHDS.

When studying different sugar-rich foods and beverages, once again were the results very similar in MDC and NSHDS. In both cohorts, intake of SSBs associated linearly with increased mortality risk in the restricted cubic splines (Figure 7), however, this association was not significant in the fully adjusted categorical analysis in NSHDS (Table 8). Intake of treats associated inversely with mortality risk in both cohorts and intake of toppings was negatively associated with mortality in MDC, while no association was observed in NSHDS.

We also studied the associations between added sugar intake and cardiometabolic risk markers to obtain further understanding (Table 9). Only HDL cholesterol (inversely) and the apoB:apoA1 ratio associated significantly and linearly with the added sugar intake categories in MDC. In NSHDS, borderline significant inverse association were observed between added sugar intake categories and total cholesterol and triglycerides.

**Table 7. Associations between the intake of added sugar and mortality in the MDC and NSHDS.**

| | MDC | | | | | | | NSHDS | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Added sugar intake categories (E%) | | | | | | | Added sugar intake categories (E%) | | | | | | |
| All-cause mortality | <5 | 5-7.5 | 7.5-10 | 10-15 | 15-20 | ≥20 | *P*-value | <5 | 5-7.5 | 7.5-10 | 10-15 | 15-20 | ≥20 | *P*-value |
| Basic adjusted | 1.29 (1.17, 1.43) | 1.08 (1.00, 1.17) | 1 | 1.05 (0.99, 1.12) | 1.14 (1.04, 1.24) | 1.62 (1.41, 1.87) | 0.033[1] <0.001[2] | 1.13 (1.00, 1.27) | 1.03 (0.92, 1.14) | 1 | 1.08 (0.97, 1.21) | 1.05 (0.89, 1.25) | 1.49 (1.16, 1.91) | 0.284[1] 0.023[2] |
| Fully adjusted | 1.23 (1.11, 1.35) | 1.06 (0.98, 1.14) | 1 | 1.03 (0.96, 1.10) | 1.04 (0.95, 1.15) | 1.30 (1.12, 1.51) | 0.920[1] <0.001[2] | 1.09 (0.97, 1.22) | 1.05) (0.95, 1.17) | 1 | 1.04 (0.93, 1.16) | 0.92 (0.78, 1.10) | 1.31 (1.01, 1.70) | 0.664[1] 0.005[2] |
| Cardiovascular mortality | | | | | | | | | | | | | | |
| Basic adjusted | 1.30 (1.08, 1.55) | 1.12 (0.97, 1.28) | 1 | 1.03 (0.92, 1.16) | 1.07 (0.90, 1.26) | 1.82 (1.43, 2.32) | 0.326[1] <0.001[2] | | | | | | | |
| Fully adjusted | 1.22 (1.02, 1.47) | 1.09 (0.95, 1.25) | 1 | 1.01 (0.89, 1.13) | 0.97 (0.82, 1.16) | 1.40 (1.09, 1.82) | 0.744[1] 0.020[2] | | | | | | | |

Multivariable Cox proportional hazards regression was used to examine the associations. The data are expressed as HRs (95% CI) unless otherwise indicated.
Basic adjusted: adjusted for age, sex and energy intake (and season and screening date in the MDC).
Fully adjusted: adjusted for energy intake, age, sex, educational level, leisure-time physical activity, smoking status, alcohol habits, dietary habits (fruit and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI (and season and screening date in the MDC).
[1] *P*-trend for intake categories using the median in each category.
[2] *P*-overall for intake categories.

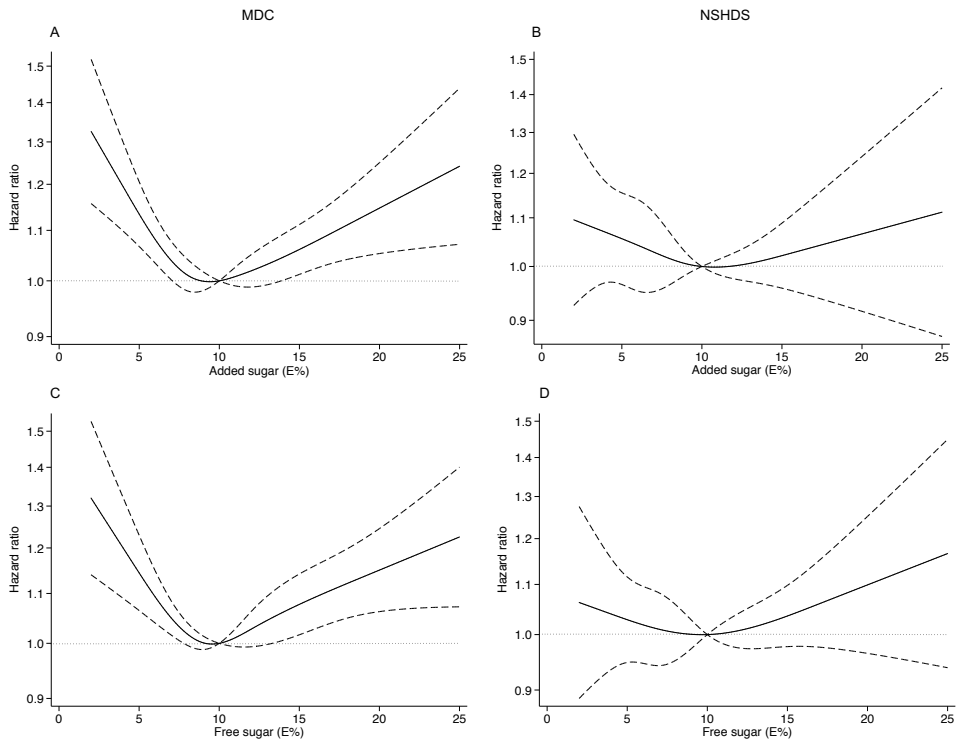


**Figure 6.** Restricted cubic splines of all-cause mortality risk (HR) from added and free sugar intake in the MDC and NSHDS examined using a Cox proportional hazards regression with continuous sugar exposure variables, with 10E% as the reference value, adjusted for energy intake, age, sex, educational level, leisure-time physical activity, smoking status, alcohol habits, dietary habits (fruit and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI (and season and screening date in the MDC). The filled line represents the HR and the dotted lines represent the 95% CI.

**Table 8. Associations between the intake of different sugar-rich foods and beverages and all-cause mortality in the MDC and NSHDS.**

| | MDC | | | | | NSHDS | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treats | Intake categories* | | | | *P*-value | Intake categories* | | | | *P*-value |
| Basic adjusted | 1 | 0.78 (0.72, 0.86) | 0.72 (0.66, 0.79) | 0.69 (0.63, 0.75) | 0.76 (0.67, 0.84) | <0.001[1] <0.001[2] | 1 | 0.77 (0.69, 0.86) | 0.64 (0.57, 0.73) | 0.67 (0.59, 0.76) | 0.62 (0.53, 0.73) | <0.001[1] <0.001[2] |
| Fully adjusted | 1 | 0.86 (0.79, 0.93) | 0.83 (0.76, 0.90) | 0.77 (0.70, 0.85) | 0.83 (0.74, 0.93) | 0.001[1] <0.001[2] | 1 | 0.85 (0.76, 0.95) | 0.71 (0.63, 0.81) | 0.75 (0.66, 0.85) | 0.66 (0.56, 0.78) | <0.001[1] <0.001[2] |
| Toppings | | | | | | | | | | | | |
| Basic adjusted | 1 | 0.90 (0.83, 0.99) | 0.87 (0.80, 0.95) | 0.86 (0.79, 0.95) | 1.07 (0.97, 1.20) | 0.022[1] <0.001[2] | 1 | 0.97 (0.87, 1.08) | 0.91 (0.82, 1.01) | 1.08 (0.97, 1.20) | 1.17 (0.99, 1.14) | 0.019[1] 0.012[2] |
| Fully adjusted | 1 | 0.92 (0.84, 1.01) | 0.90 (0.82, 0.99) | 0.86 (0.78, 0.94) | 0.93 (0.78, 1.03) | 0.316[1] 0.033[2] | 1 | 1.00 (0.90, 1.12) | 0.95 (0.85, 1.05) | 0.99 (0.88, 1.10) | 0.97 (0.82, 1.15) | 0.697[1] 0.864[2] |
| SSBs | | | | | | | | | | | | |
| Basic adjusted | 1 | 0.97 (0.90, 1.03) | 1.01 (0.92, 1.10) | 1.11 (1.00, 1.23) | 1.24 (1.13, 1.37) | <0.001[1] <0.001[2] | 1 | 0.95 (0.87, 1.04) | 0.99 (0.88, 1.12) | 1.10 (0.97, 1.24) | 1.26 (1.04, 1.54) | 0.002[1] 0.024[2] |
| Fully adjusted | 1 | 0.97 (0.90, 1.03) | 1.00 (0.91, 1.10) | 1.09 (0.98, 1.21) | 1.14 (1.03, 1.26) | 0.009[1] 0.035[2] | 1 | 0.96 (0.88, 1.05) | 0.97 (0.85, 1.10) | 1.03 (0.91, 1.10) | 1.10 (0.90, 1.35) | 0.177[1] 0.549[2] |

Multivariable Cox proportional hazards regression was used to examine the associations. The data are expressed as HRs (95% CI) unless otherwise indicated.
Basic adjusted: adjusted for age, sex and energy intake (and season and screening date in the MDC).
Fully adjusted: adjusted for energy intake, age, sex, educational level, leisure-time physical activity, smoking status, alcohol habits, dietary habits (fruit and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI (and season and screening date in the MDC).
*Categorizations were performed as follows; treats: ≤2, 2-5, 5-8, 8-14 and >14 servings/week; toppings: ≤2, 2-7, 7-14, 14-28 and >28 servings/week; and SSBs: ≤1, 1-3, 3-5, 5-8 and >8 servings/week.
[1] *P*-trend for intake categories using the median in each category.
[2] *P*-overall for intake categories.

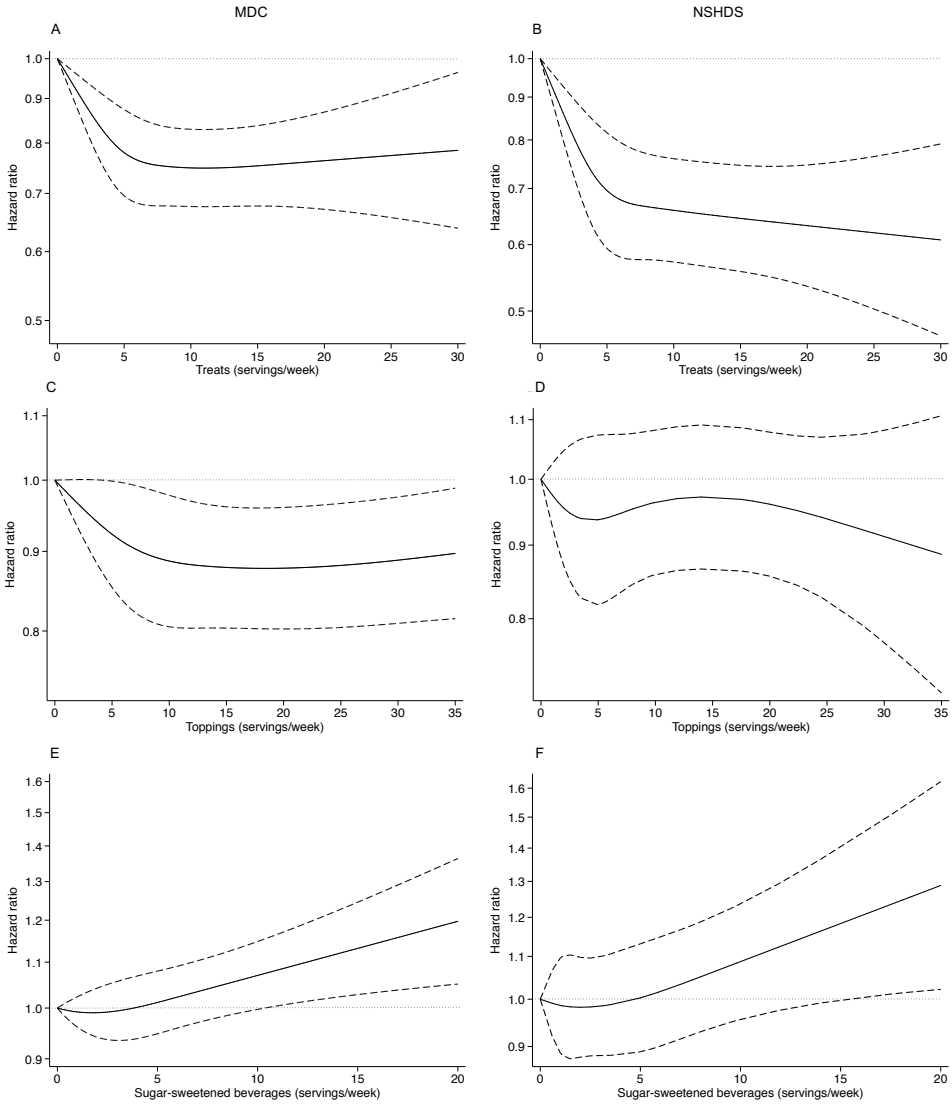


**Figure 7.** Restricted cubic splines of all-cause mortality risk (HR) from intake of treats, toppings and SSBs in the MDC and NSHDS examined using a Cox proportional hazards regression with continuous sugar exposure variables with 0 as the reference value, adjusted for energy intake, age, sex, educational level, leisure-time physical activity, smoking status, alcohol habits, dietary habits (fruit and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI (and season and screening date in the MDC). The filled line represents the HR and the dotted lines represent the 95% CI.

**Table 9. Cross-sectionally analyzed fully adjusted means (SE) of cardiometabolic risk markers according to added sugar intake in 6 intake energy percentage categories in the MDC and NSHDS.**

| | | Added sugar intake categories, E% (median) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MDC | n | <5 (4.0) | 5-7.5 (6.4) | 7.5-10 (8.8) | 10-15 (11.9) | 15-20 (16.6) | ≥20 (22.3) | β | *P*-trend |
| FPG[1], mmol/L | 4651 | 5.67 (0.04) | 5.68 (0.03) | 5.62 (0.03) | 5.65 (0.02) | 5.61 (0.04) | 5.70 (0.08) | -0.002 | 0.519 |
| Fasting plasma insulin[1], pmol/L | 4500 | 47.5 (2.40) | 52.2 (1.71) | 50.3 (1.52) | 51.4 (1.38) | 55.1 (2.45) | 54.6 (4.75) | 0.001* | 0.277* |
| HOMA-IR[1] | 4300 | 1.66 (0.06) | 1.70 (0.04) | 1.66 (0.04) | 1.71 (0.04) | 1.77 (0.06) | 1.84 (0.12) | 0.000* | 0.634* |
| ApoB/ApoA-1[2] | 23709 | 0.724 (0.006) | 0.730 (0.005) | 0.739 (0.005) | 0.742 (0.005) | 0.758 (0.006) | 0.756 (0.009) | 0.002 | <0.001 |
| Total cholesterol[2], mmol/L | 4656 | 6.31 (0.08) | 6.34 (0.07) | 6.40 (0.07) | 6.29 (0.06) | 6.32 (0.08) | 6.22 (0.13) | -0.006 | 0.218 |
| Triglycerides[2], mmol/L | 4656 | 1.48 (0.05) | 1.49 (0.05) | 1.48 (0.04) | 1.49 (0.04) | 1.59 (0.05) | 1.50 (0.09) | 0.005 | 0.113 |
| HDL cholesterol[2], mmol/L | 4615 | 1.40 (0.02) | 1.33 (0.02) | 1.35 (0.02) | 1.33 (0.02) | 1.27 (0.02) | 1.28 (0.04) | -0.007 | <0.001 |
| LDL cholesterol[2], mmol/L | 4558 | 4.25 (0.07) | 4.33 (0.06) | 4.37 (0.06) | 4.29 (0.06) | 4.36 (0.07) | 4.28 (0.12) | 0.000 | 0.944 |
| Systolic blood pressure[3], mmHg | 24249 | 143.2 (0.41) | 143.2 (0.30) | 142.5 (0.27) | 142.9 (0.26) | 143.2 (0.41) | 141.7 (0.73) | 0.027 | 0.408 |
| Diastolic blood pressure[3], mmHg | 24247 | 87.2 (0.22) | 87.4 (0.16) | 86.9 (0.15) | 87.2 (0.14) | 87.4 (0.22) | 87.2 (0.38) | 0.006 | 0.728 |
| NSHDS | n | <5 (4.0) | 5-7.5 (6.3) | 7.5-10 (8.6) | 10-15 (11.7) | 15-20 (16.5) | ≥20 (22.6) | β | *P*-trend |
| FPG[4], mmol/L | 24382 | 5.33 (0.01) | 5.31 (0.01) | 5.31 (0.01) | 5.32 (0.01) | 5.31 (0.03) | 5.29 (0.04) | -0.001 | 0.515 |
| Total cholesterol[5], mmol/L | 24377 | 6.36 (0.14) | 6.09 (0.12) | 6.11 (0.13) | 6.06 (0.13) | 5.80 (0.23) | 6.12 (0.52) | -0.028 | 0.055 |
| Triglycerides[5], mmol/L | 19824 | 1.83 (0.12) | 1.58 (0.10) | 1.63 (0.12) | 1.56 (0.11) | 1.46 (0.19) | 1.45 (0.41) | -0.022 | 0.075 |
| Systolic blood pressure[6], mmHg | 24095 | 138.8 (0.95) | 137.6 (0.83) | 136.7 (0.92) | 137.4 (0.93) | 138.0 (1.6) | 142.6 (3.0) | -0.008 | 0.939 |
| Diastolic blood pressure[6], mmHg | 24092 | 85.7 (0.55) | 84.5 (0.48) | 84.6 (0.53) | 84.8 (0.53) | 85.4 (0.94) | 89.6 (1.7) | 0.040 | 0.485 |

Trends were determined using a general linear model, using the median E% in each category of added sugar intake.
[1] MDC fully adjusted model: adjusted for age, sex, season, screening date, energy intake, educational level, lesiure time physical activity, smoking status, alcohol habits, dietary habits (fruits and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI.
[2] Additional adjustment for the usage of lipid-lowering drugs.
[3] Additional adjustment for the usage of antihypertensive medication.
[4] NSHDS fully adjusted model: adjusted for age, sex, energy intake, educational level, leisure-time physical activity, smoking status, alcohol habits, dietary habits (fruits and vegetables, processed meat, coffee, saturated fat and fiber density) and BMI.
[5] Additional adjustment for the usage of lipid-lowering drugs.
[6] Additional adjustment for the usage of antihypertensive medication.
* Determined using a log10-transformed variable.

# Paper II

The sucrose and fructose excretions from overnight urine samples in 991 diabetes-free participants in the MOS were compared to self-reported sugar intake measures. Significant Pearson's correlations between various measures of dietary sugar from 4-day food records and the sum of urinary sucrose and fructose from overnight urine samples were observed in MOS with correlation coefficients ranging between r=0.2 and r=0.3 (Table 10). This moderate agreement is also visualized in the alluvial plot (Figure 8). In general, these correlations were somewhat higher in men than women, as well as higher for sucrose and added sugar intake, rather than total sugar intake and total sugar density. The overnight urinary sucrose correlated much higher with the dietary sugar measures and most sugar-rich foods and beverages as compared to urinary fructose (Table 10).

Both self-reported added sugar intake and the sum of urinary sucrose and fructose associated positively with BMI and waist circumference in women. In men on the other hand, the sum or urinary fructose and sucrose associated negatively with BMI and waist circumference, while no association was seen with added sugar intake. In women, the sum or urinary sucrose and fructose, but not added sugar intake, associated positively with systolic and diastolic blood pressure and FPG (Table 11). The composite measure of added sugar intake and the sum or urinary sucrose and fructose in the form of their first principal component, associated significantly with increased BMI, waist circumference, systolic blood pressure and reduced HDL cholesterol in women. No significant association between the composite measure and cardiometabolic risk markers were seen in men (Table 11).

Intake of desserts and sweets, FPG and systolic blood pressure were identified as significant determinants of higher urinary sugars in women, while higher intake of added sugar, but lower educational level and waist circumference were identified as determinants of urinary sugars in men (Table 12).

**Table 10. Partial correlations between U-sucrose, U-fructose and U-sugars and different measures and sources of dietary sugars in all, women and men in the MOS.**

| | U-sucrose | | U-fructose | | U-sugars | |
|---|---|---|---|---|---|---|
| | r | *P*-value | r | *P*-value | r | *P*-value |
| All | n=889 | | n=775 | | n=763 | |
| Sucrose (g/d) | 0.27 | <0.01 | 0.13 | <0.01 | 0.13 | <0.01 |
| Total sugar (g/d) | 0.22 | <0.01 | 0.12 | <0.01 | 0.12 | <0.01 |
| Total sugar density (g/1000 kcal) | 0.22 | <0.01 | 0.13 | <0.01 | 0.13 | <0.01 |
| Added sugar (E%) | 0.27 | <0.01 | 0.14 | <0.01 | 0.14 | <0.01 |
| Desserts (g/d) | 0.09 | <0.01 | 0.05 | 0.14 | 0.05 | 0.14 |
| Sweets (g/d) | 0.20 | <0.01 | 0.07 | 0.04 | 0.07 | 0.04 |
| Toppings (servings/d) | 0.03 | 0.31 | -0.01 | 0.78 | -0.01 | 0.78 |
| SSBs (g/d) | 0.18 | <0.01 | 0.09 | 0.01 | 0.09 | 0.01 |
| Juice (g/d) | 0.04 | 0.25 | 0.11 | <0.01 | 0.11 | <0.01 |
| Fruits (g/d) | -0.04 | 0.21 | -0.04 | 0.25 | -0.04 | 0.25 |
| Women | n=467 | | n=421 | | n=412 | |
| Sucrose (g/d) | 0.23 | <0.01 | 0.11 | 0.03 | 0.11 | 0.03 |
| Total sugar (g/d) | 0.19 | <0.01 | 0.13 | <0.01 | 0.13 | <0.01 |
| Total sugar density (g/1000 kcal) | 0.15 | <0.01 | 0.12 | 0.02 | 0.12 | 0.02 |
| Added sugar (E%) | 0.21 | <0.01 | 0.13 | <0.01 | 0.13 | <0.01 |
| Desserts (g/d) | 0.05 | 0.25 | 0.005 | 0.92 | 0.005 | 0.92 |
| Sweets (g/d) | 0.21 | <0.01 | 0.12 | 0.02 | 0.12 | 0.02 |
| Toppings (servings/d) | -0.02 | 0.65 | -0.05 | 0.33 | -0.05 | 0.33 |
| SSBs (g/d) | 0.08 | 0.08 | 0.01 | 0.79 | 0.01 | 0.79 |
| Juice (g/d) | 0.02 | 0.71 | 0.05 | 0.27 | 0.05 | 0.27 |
| Fruits (g/d) | -0.05 | 0.33 | 0.0007 | 0.99 | 0.0007 | 0.99 |
| Men | n=422 | | n=354 | | n=351 | |
| Sucrose (g/d) | 0.30 | <0.01 | 0.14 | <0.01 | 0.14 | <0.01 |
| Total sugar (g/d) | 0.25 | <0.01 | 0.11 | 0.03 | 0.11 | 0.03 |
| Total sugar density (g/1000 kcal) | 0.27 | <0.01 | 0.12 | 0.02 | 0.12 | 0.02 |
| Added sugar (E%) | 0.31 | <0.01 | 0.13 | 0.01 | 0.13 | 0.01 |
| Desserts (g/d) | 0.13 | <0.01 | 0.11 | 0.05 | 0.11 | 0.05 |
| Sweets (g/d) | 0.18 | <0.01 | 0.01 | 0.83 | 0.01 | 0.83 |
| Toppings (servings/d) | 0.08 | 0.12 | 0.02 | 0.68 | 0.02 | 0.68 |
| SSBs (g/d) | 0.26 | <0.01 | 0.16 | <0.01 | 0.16 | <0.01 |
| Juice (g/d) | 0.05 | 0.33 | 0.15 | <0.01 | 0.15 | <0.01 |
| Fruits (g/d) | -0.04 | 0.37 | -0.12 | 0.03 | -0.12 | 0.03 |

The partial correlations are adjusted for age, sex, energy intake and BMI (not adjusted for sex in sex-specific analyses). The urinary sugar variables are log10-transformed.

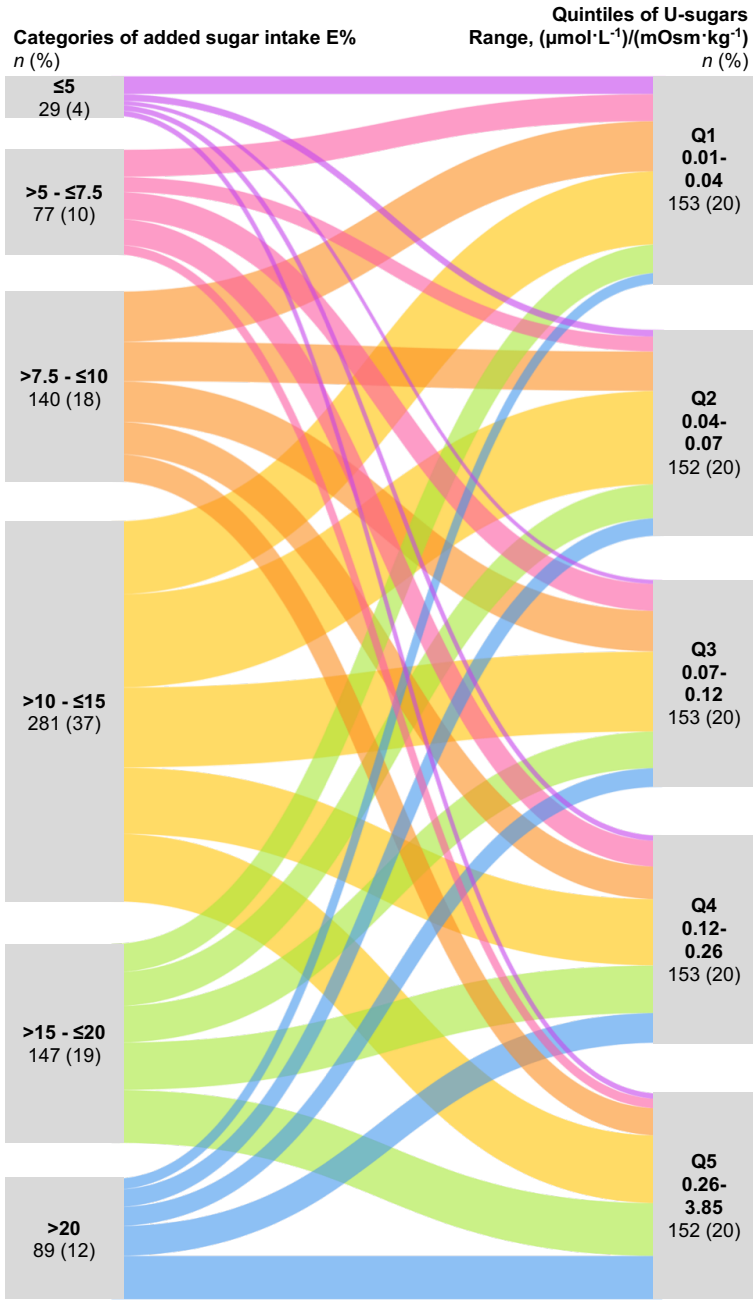


**Figure 8**. Alluvial plot demonstrating the agreement based on crosstabulation of the 6 categories of reported added sugar intake (≤5, 5-7.5, 7.5-10, 10-15, 15-20, and >20E%) and quintiles of U-sugars (Q1–Q5).

**Table 11. Linear regression of added sugar intake, U-sugars and their composite measure (PC added×U-sugar) on cardiometabolic risk markers in the MOS.**

| | All (n=889[1], 677[2]) | | Women (n=493[1], 831[2]) | Men (n=369[1], 296[2]) |
|---|---|---|---|---|
| | ß (95% CI) | $P_{interaction}$ sex | ß (95% CI) | ß (95% CI) |
| BMI (kg/m$^2$) | | | | |
| U-sugars | 0.08 (-0.58, 0.74) | <0.01 | 1.05 (0.12, 1.97) | -1.45 (-2.40, -0.51) |
| Added sugar | 0.03 (-0.03, 0.09) | 0.03 | 0.10 (0.01, 0.19) | -0.03 (-0.10, 0.05) |
| PC added×U-sugar | 0.26 (0.04, 0.48) | <0.01 | 0.50 (0.22, 0.79) | -0.24 (-0.59, 0.11) |
| Waist circumference (cm) | | | | |
| Added sugar | 0.15 (0.01, 0.29) | 0.05 | 0.25 (0.03, 0.46) | 0.05 (-0.14, 0.24) |
| U-sugars | -0.20 (-1.84, 1.45) | <0.01 | 2.02 (-0.23, 4.28) | -3.79 (-6.19, -1.39) |
| PC added×U-sugar | 0.76 (0.22, 1.30) | <0.01 | 1.19 (0.51, 1.88) | 0.19 (-1.09, 0.70) |
| Total cholesterol (mmol/L) | | | | |
| Added sugar | -0.009 (-0.02, 0.004) | 0.41 | -0.02 (-0.03, 0.0003) | -0.001 (-0.02, 0.02) |
| U-sugars | -0.14 (-0.28, 0.01) | 0.91 | -0.12 (-0.30, 0.06) | -0.15 (-0.40, 0.10) |
| PC added×U-sugar | -0.03 (-0.07, 0.02) | 0.69 | -0.03 (-0.08, 0.03) | -0.02 (-0.11, 0.08) |
| Triglycerides (mmol/L) | | | | |
| Added sugar | 0.003 (-0.01, 0.01) | 0.49 | -0.004 (-0.01, 0.01) | 0.006 (-0.01, 0.02) |
| U-sugars | 0.02 (-0.08, 0.11) | 0.96 | 0.007 (-0.09, 0.11) | -0.01 (-0.20, 0.18) |
| PC added×U-sugar | 0.02 (-0.01, 0.05) | 0.49 | 0.003 (-0.03, 0.03) | 0.03 (-0.03, 0.10) |
| HDL cholesterol (mmol/L) | | | | |
| Added sugar | -0.01 (-0.02, -0.01) | 0.04 | -0.02 (-0.02, -0.01) | -0.009 (-0.02, -0.002) |
| U-sugars | -0.04 (-0.11, 0.02) | 0.16 | -0.07 (-0.17, 0.02) | 0.01 (-0.07, 0.09) |
| PC added×U-sugar | -0.03 (-0.05, -0.01) | 0.32 | -0.03 (-0.06, -0.001) | -0.03 (-0.06, 0.002) |
| LDL cholesterol (mmol/L) | | | | |
| Added sugar | 0.002 (-0.01, 0.01) | 0.68 | -0.003 (-0.02, 0.01) | 0.008 (-0.01, 0.03) |
| U-sugars | -0.13 (-0.26, 0.01) | 0.66 | -0.09 (-0.26, 0.07) | -0.16 (-0.39, 0.07) |
| PC added×U-sugar | -0.006 (-0.05, 0.04) | 0.90 | -0.006 (-0.06, 0.04) | 0.008 (-0.07, 0.09) |
| Systolic blood pressure (mmHg) | | | | |
| Added sugar | -0.09 (-0.26, 0.08) | 0.87 | -0.02 (-0.27, 0.22) | -0.09 (-0.31, 0.13) |
| U-sugars | 2.95 (0.99, 4.92) | 0.22 | 4.63 (1.96, 7.30) | 1.30 (-1.55, 4.16) |
| PC added×U-sugar | 0.46 (-0.19, 1.12) | 0.55 | 1.01 (0.17, 1.85) | -0.09 (-1.13, 0.96) |
| Diastolic blood pressure (mmHg) | | | | |
| Added sugar | -0.01 (-0.12, 0.10) | 0.98 | -0.02 (-0.18, 0.14) | 0.003 (-0.15, 0.16) |
| U-sugars | 1.30 (-0.01, 2.62) | 0.43 | 1.81 (0.07, 3.55) | 0.58 (-1.48, 2.64) |
| PC added×U-sugar | 0.45 (0.02, 0.88) | 0.86 | 0.48 (-0.06, 1.02) | 0.41 (-0.34, 1.16) |
| FPG (mmol/L) | | | | |
| Added sugar | -0.008 (-0.02, 0.001) | 0.04 | -0.002 (-0.01, 0.01) | -0.01 (-0.03, 0.001) |
| U-sugars | 0.10 (0.0003, 0.20) | 0.22 | 0.16 (0.05, 0.26) | 0.03 (-0.17, 0.22) |
| PC added×U-sugar | -0.0004 (-0.03, 0.03) | 0.11 | 0.01 (-0.02, 0.05) | -0.03 (-0.10, 0.04) |

[1]Study sample for added sugar intake. [2]Study sample for U-sugars and PC added×U-sugar. U-sugars are log10-transformed. The PC added×U-sugar is the first PC of the two variables U-sugars and added sugars. Linear regressions are adjusted according to model 2: age, sex educational level, leisure-time physical activity, smoking status, alcohol habits, fiber density (and energy intake for added sugar and the PC added×U-sugar). Regressions with total cholesterol, triglycerides, HDL, and LDL cholesterol are additionally adjusted for usage of lipid lowering drugs and regressions with systolic and diastolic blood pressure are additionally adjusted for usage of antihypertensive drugs.

**Table 12. Partial correlation coefficients between U-sugars (not adjusted for urine osmolality) and its potential predictors in women and men of the MOS.**

| | Women (n=373) | | | | Men (n=295) | | | |
|---|---|---|---|---|---|---|---|---|
| | Separate models | | Multivariate model | | Separate models | | Multivariate model | |
| | r | *P* | r | *P* | r | *P* | r | *P* |
| Added sugar (E%) | 0.23 | <0.01 | | | 0.27 | <0.01 | 0.31 | <0.01 |
| Desserts (g/d) | 0.09 | 0.08 | 0.10 | 0.04 | 0.14 | <0.01 | | |
| Sweets (g/d) | 0.22 | <0.01 | 0.21 | <0.01 | 0.16 | <0.01 | | |
| Toppings (servings/d) | -0.02 | 0.72 | | | 0.05 | 0.35 | | |
| SSBs (g/d) | 0.07 | 0.15 | | | 0.25 | <0.01 | | |
| Fruits (g/d) | -0.10 | 0.05 | | | -0.14 | 0.01 | | |
| Juice (g/d) | 0.02 | 0.68 | | | 0.10 | 0.07 | | |
| Education level | -0.12 | 0.03 | | | -0.12 | 0.05 | -0.13 | 0.02 |
| Smoking status | 0.05 | 0.35 | | | 0.06 | 0.28 | | |
| Alcohol habits | -0.09 | 0.09 | | | -0.005 | 0.93 | | |
| Leisure-time physical activity | -0.05 | 0.34 | | | -0.03 | 0.56 | | |
| BMI ($kg/m^2$) | 0.11 | 0.02 | | | -0.08 | 0.12 | | |
| Waist circumference (cm) | 0.09 | 0.08 | | | -0.08 | 0.12 | -0.18 | <0.01 |
| Systolic blood pressure (mmHg) | 0.18 | <0.01 | 0.17 | <0.01 | 0.08 | 0.13 | | |
| FPG (mmol/L) | 0.13 | <0.01 | 0.12 | 0.01 | 0.01 | 0.78 | | |
| Urine osmolality (mOsm/kg) | 0.41 | <0.01 | 0.41 | <0.01 | 0.39 | <0.01 | 0.40 | <0.01 |
| e-GFR ($ml/min/1.73\ m^2$) | -0.05 | 0.33 | | | -0.08 | 0.16 | | |

All partial correlations are adjusted for age and energy intake. The multivariate partial correlation model was determined through stepwise backward linear regression. All covariates were added simultaneously to a linear regression model and the covariate with the highest *P*-value was excluded in a stepwise manner from the model until all covariates were deemed significant. U-sugars are log10-transformed.

# Paper III

In 268 participants with prediabetes of the PREVIEW RCT, sucrose and fructose 24-h excretion was evaluated as biomarkers of sugar intake. The self-reported intakes of total sugar were at baseline on average 71.5 g, and was reduced during the intervention to 52.0 g at 6 months and 53.5 g 12 months. The median 24-h urinary excretion of sucrose and fructose were at baseline 4.1 mg/d and 50.7 mg/d respectively. The sucrose excretions were reduced in line with total sugar intake to 2.1 mg/d at 6 months and 2.7 mg/d at 12 months, but fructose excretion remained high at 54.5 mg/d at 6 months and 54.9 mg/d at 12 months (Table 13). Furthermore, neither the sucrose and fructose excretion or the total sugar intake was significantly different between the two diet groups during the intervention (Table 14). The fructose excretion levels observed in this population of individuals with prediabetes are much elevated in comparison to previous observations in validation studies of healthy individuals. The relationships between total sugar intake and 24-h sucrose and fructose excretion in this study and previous validation studies are visualized in Figure 9.

At baseline, self-reported total sugar intake correlated significantly with urinary sucrose excretion (rho=0.18, $P$=0.003) and fructose excretion (rho=0.16, $P$=0.008). During the intervention, no correlation with sucrose excretion were seen at 6 months (rho=0.01, $P$=0.876), when the correlation with fructose excretion was enhanced (rho=0.25, $P$<0.001), but correlations were weak but significant for both sucrose and fructose excretion at 12 months (rho=0.18, $P$=0.015 and rho=0.17, $P$=0.022, respectively) (Table 15). The variance explained by total sugar intake in sucrose and fructose excretion was overall very low.

From LASSO regression analysis, total sugar intake, weight and serum insulin levels were identified as positive predictors of urinary sucrose excretion at baseline ($R^2$=9.1%), while total sugar intake, weight and male sex predicted higher fructose excretion ($R^2$=4.1%). During the intervention, several metabolic risk markers (both absolute values and changes from baseline) improved the predictions of the sucrose and fructose excretion, mostly in the positive direction, but with exceptions. We could not identify any pattern of which metabolic markers that were most influential (Table 16).

During the weight maintenance phase of the intervention, self-reported intake of total sugar did not associate with any changes in metabolic outcomes, while higher urinary sucrose excretion at 6 months associated with increases in weight and HbA1c from 6 to 12 months, and increases in urinary sucrose excretion from 6 to 12 months associated with increases in fat mass, but reductions of HbA1c. On the other hand, increases in urinary fructose excretion from 6 to 12 months associated with reductions in fasting serum insulin and HOMA- IR. No associations with odds of achieving normoglycemia were observed for total sugar intake or sucrose or fructose excretion (Table 17).

**Table 13. Descriptives of all PREVIEW Copenhagen participants at baseline, 6 months and 12 months.**

| | Baseline | 6 months | 12 months |
|---|---|---|---|
| n | 268 | 243 | 188 |
| Age, y | 54.9 (10.0) | - | - |
| Sex, % women | 58.2% | - | - |
| Smokers, % | 11.2% | - | - |
| Normoglycemia, % | 15.7% | 27.2% | 30.3% |
| BMI, kg/m$^2$ | 34.5 (5.1) | 29.9 (4.7) | 30.6 (5.1) |
| Weight, kg | 102.4 (18.6) | 88.3 (17.0) | 90.5 (17.4) |
| Waist circumference, cm | 113.6 (12.8) | 100.3 (12.3) | 102.6 (12.4) |
| Fat mass, kg | 44.8 (12.1) | 33.4 (11.4) | 35.4 (12.1) |
| Fat mass, % | 44.8 (6.8) | 38.5 (8.1) | 39.8 (8.2) |
| FPG, mmol/L | 6.1 (0.6) | 5.8 (0.5) | 5.8 (0.6) |
| Insulin, mU/L | 11.6 (6.0) | 7.2 (3.4) | 7.9 (3.9) |
| HbA1c, mmol/mol | 35.7 (3.7) | 34.1 (2.9) | 34.9 (3.2) |
| HOMA-IR | 3.2 (1.8) | 1.9 (1.0) | 2.1 (1.1) |
| Systolic blood pressure, mmHg | 133.4 (15.5) | 129.1 (15.6) | 129.8 (15.5) |
| Diastolic blood pressure, mmHg | 85.5 (8.1) | 80.4 (8.8) | 81.5 (8.3) |
| Energy, kcal | 2167 (654) | 1666 (537) | 1625 (570) |
| Total sugar, g/d | 71.5 (35.5) | 52.0 (27.8) | 53.5 (27.1) |
| Total sugar, E% | 13.3 (5.2) | 12.5 (5.0) | 13.3 (5.5) |
| U-sucrose, mg/d* | 4.1 (1.8, 8.5) | 2.1 (1.2, 4.2) | 2.7 (1.3, 5.4) |
| U-fructose, mg/d* | 50.7 (29.8, 88.0) | 54.5 (28.6, 114.2) | 54.9 (31.9, 124.6) |
| U-sugars, mg/d* | 59.1 (36.1, 95.2) | 58.2 (32.2, 120.5) | 61.6 (35.6, 129.0) |

Data is presented as mean (SD), unless stated otherwise. *Data is presented as median (IQR).

**Table 14. Self-reported intake and 24-h urinary sucrose and fructose excretion in the low- and moderate-GI diet groups.**

| | Low-GI diet | Moderate-GI diet | *P*-value |
|---|---|---|---|
| Baseline | n=137 | n=131 | |
| GI | 61.9 (5.55) | 62.1 (5.24) | 0.755 |
| GL | 133.1 (43.7) | 128.1 (44.5) | 0.353 |
| Total sugar, g/d | 74.0 (36.1) | 68.9 (34.7) | 0.233 |
| Total sugar, E% | 13.5 (5.02) | 13.0 (5.43) | 0.516 |
| U-sucrose, mg/d* | 3.78 (1.95, 8.55) | 4.27 (1.81, 8.50) | 0.819 |
| U-fructose, mg/d* | 55.1 (31.7, 89.3) | 47.8 (27.5, 87.3) | 0.549 |
| U-sugars, mg/d* | 61.1 (31.7, 89.3) | 55.0 (34.6, 97.1) | 0.620 |
| 6 months | n=123 | n=120 | |
| GI | 57.3 (5.42) | 63.2 (4.70) | <0.001 |
| GL | 87.8 (34.9) | 107.1 (38.6) | <0.001 |
| Total sugar, g/d | 54.7 (30.4) | 49.3 (24.8) | 0.137 |
| Total sugar, E% | 12.4 (4.93) | 12.5 (5.08) | 0.953 |
| U-sucrose, mg/d* | 2.05 (1.13, 3.50) | 2.19 (1.17, 4.81) | 0.687 |
| U-fructose, mg/d* | 55.8 (32.0, 110.7) | 51.9 (27.1, 125.9) | 0.585 |
| U-sugars, mg/d* | 57.8 (33.5, 112.7) | 58.6 (29.6, 127.4) | 0.635 |
| 12 months | n=96 | n=92 | |
| GI | 57.6 (6.90) | 61.8 (7.59) | <0.001 |
| GL | 91.9 (37.6) | 105.6 (43.5) | 0.022 |
| Total sugar, g/d | 53.8 (26.9) | 53.3 (27.5) | 0.893 |
| Total sugar, E% | 12.5 (5.54) | 14.2 (6.20) | 0.037 |
| U-sucrose, mg/d* | 3.14 (1.49, 6.11) | 2.42 (1.22, 4.91) | 0.175 |
| U-fructose, mg/d* | 70.9 (33.1, 143.6) | 50.6 (31.7, 114.1) | 0.293 |
| U-sugars, mg/d* | 76.8 (35.5, 146.9) | 52.7 (35.9, 114.9) | 0.258 |

Data is presented as mean (SD) and *P*-values are determined using t-tests, unless stated otherwise. *Data is presented as median (IQR) and *P*-values are determined using Mann-Whitney U tests.

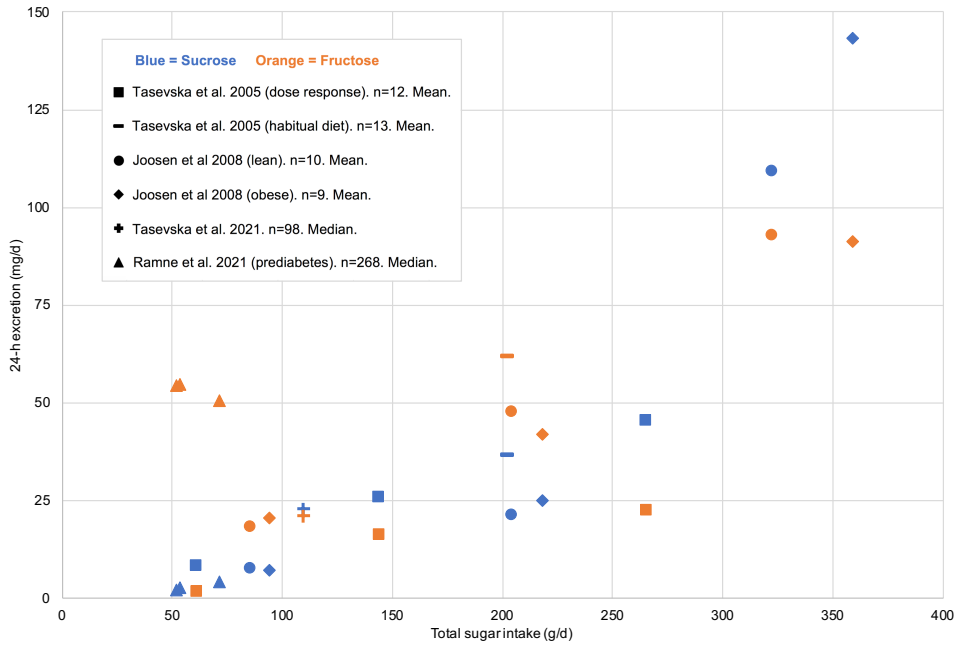


**Figure 9.** Comparison of the relationships between total sugar intake and 24-h urinary sucrose (blue) and fructose (orange) excretion in previous validation studies of healthy individuals with those of this study in individuals with prediabetes.

**Table 15. Spearman correlations between self-reported total sugar intake and 24-h urinary sucrose and fructose excretion at baseline, 6 months and 12 months.**

| | Total sugar, g/d | | | Total sugar, E% | | |
|---|---|---|---|---|---|---|
| | rho | *P* | $R^{2*}$ | rho | *P* | $R^{2*}$ |
| Baseline (n=268) | | | | | | |
| U-sucrose, mg/d | 0.18 | 0.003 | 3.1% | 0.16 | 0.009 | 2.0% |
| U-fructose, mg/d | 0.16 | 0.008 | 1.4% | 0.09 | 0.143 | 0.1% |
| U-sugars, mg/d | 0.19 | 0.002 | 1.9% | 0.11 | 0.069 | 0.5% |
| 6 months (n=243) | | | | | | |
| U-sucrose, mg/d | 0.01 | 0.876 | -0.3% | -0.01 | 0.845 | -0.3% |
| U-fructose, mg/d | 0.25 | <0.001 | 4.2% | 0.16 | 0.015 | 1.4% |
| U-sugars, mg/d | 0.24 | <0.001 | 3.8% | 0.15 | 0.021 | 1.1% |
| 12 months (n=188) | | | | | | |
| U-sucrose, mg/d | 0.18 | 0.015 | 0.4% | 0.05 | 0.479 | -0.5% |
| U-fructose, mg/d | 0.17 | 0.022 | 2.9% | 0.05 | 0.467 | 0.01% |
| U-sugars, mg/d | 0.18 | 0.012 | 2.7% | 0.06 | 0.423 | -0.1% |

*The adjusted $R^2$-values are obtained from linear regressions of log-transformed urinary excretion variables.

**Table 16. LASSO regression for determining predictors of urinary sucrose and fructose excretion at baseline, 6 months and 12 months.**

| | U-sucrose*, mg/d | | U-fructose*, mg/d | |
|---|---|---|---|---|
| | ß | *P* | ß | *P* |
| Baseline | | | | |
| Sex (men) | | | 0.236 | 0.075 |
| Age, years | | | | |
| Total sugar, g | 0.006 | 0.002 | 0.003 | 0.055 |
| Weight, kg | 0.006 | 0.147 | 0.006 | 0.099 |
| FPG, mmol/L | | | | |
| Insulin*, mU/L | 0.468 | 0.002 | | |
| HbA1c, mmol/mol | | | | |
| Systolic blood pressure, mmHg | | | | |
| $R^2$ | 9.1% | | 4.1% | |
| 6 months | | | | |
| Sex (men) | | | | |
| Age, years | | | | |
| Total sugar, g | | | 0.009 | <0.001 |
| Weight, kg | 0.011 | 0.025 | | |
| Δ Weight, kg | 0.045 | <0.001 | | |
| FPG, mmol/L | | | | |
| Δ FPG, mmol/L | | | 0.560 | <0.001 |
| Insulin*, mU/L | 0.159 | 0.373 | | |
| Δ Insulin, mU/L | | | -0.073 | <0.001 |
| HbA1c, mmol/mol | | | | |
| Δ HbA1c, mmol/mol | | | -0.030 | 0.376 |
| Systolic blood pressure, mmHg | | | | |
| Δ Systolic blood pressure, mmHg | | | 0.005 | 0.335 |
| Diet group (moderate-GI) | | | | |
| $R^2$ | 8.2% | | 12.9% | |
| 12 months | | | | |
| Sex (men) | | | | |
| Age, years | | | | |
| Total sugar, g | 0.003 | 0.350 | 0.008 | 0.006 |
| Weight, kg | 0.008 | 0.087 | | |
| Δ Weight, kg | 0.015 | 0.296 | | |
| FPG, mmol/L | -0.138 | 0.354 | 0.353 | 0.014 |
| Δ FPG, mmol/L | | | | |
| Insulin*, mU/L | | | | |
| Δ Insulin, mU/L | 0.038 | 0.086 | | |
| HbA1c, mmol/mol | | | | |
| Δ HbA1c, mmol/mol | 0.042 | 0.357 | | |
| Systolic blood pressure, mmHg, | | | | |
| Δ Systolic blood pressure, mmHg | 0.014 | 0.220 | | |
| Diet group (moderate-GI) | -0.191 | 0.164 | | |
| $R^2$ | 8.8% | | 5.5% | |

The lambda providing the minimum mean squared prediction error was determined using 10-fold cross validation. The presented data is from the ordinary least squares regression with predictor variables selected from the LASSO regression. *Log-transformed variable due to skewed distribution.

**Table 17. Associations between sugar intake and 24-h urinary sucrose and fructose excretion at both 6 months and change from 6-12 months, and change in cardiometabolic outcomes between 6-12 months.**

| | Absolute intake/excretion at 6 months | Δ Intake/excretion from 6-12 months |
|---|---|---|
| | β (95% CI) | β (95% CI) |
| Δ Weight, kg | n=201 | n=176 |
| Total sugar, g | 0.01 (-0.01, 0.03) | 0.004 (-0.01, 0.02) |
| Total sugar, E% | -0.04 (-0.13, 0.06) | 0.002 (-0.08, 0.09) |
| U-sucrose, mg/d* | 0.55 (0.12, 0.98) | 0.062 (-0.02, 0.15) |
| U-fructose, mg/d* | 0.23 (-0.20, 0.67) | -0.001 (-0.004, 0.003) |
| U-sugars, mg/d* | 0.29 (-0.17, 0.76) | -0.0005 (-0.004, 0.003) |
| Δ Fat mass, kg | | |
| Total sugar, g | 0.003 (-0.01, 0.02) | 0.004 (-0.01, 0.02) |
| Total sugar, E% | -0.04 (-0.14, 0.05) | 0.03 (-0.05, 0.11) |
| U-sucrose, mg/d* | 0.24 (-0.17, 0.66) | 0.10 (0.02, 018) |
| U-fructose, mg/d* | 0.30 (-0.12, 0.71) | -0.0005 (-0.004, 0.003) |
| U-sugars, mg/d* | 0.33 (-0.12, 0.78) | -0.0003 (-0.004, 0.003) |
| Δ FPG, mmol/L | | |
| Total sugar, g | -0.00004 (-0.002, 0.002) | 0.001 (-0.001, 0.004) |
| Total sugar, E% | 0.0007 (-0.01, 0.01) | -0.001 (-0.01, 0.01) |
| U-sucrose, mg/d* | -0.006 (-0.07, 0.05) | 0.005 (-0.01, 0.02) |
| U-fructose, mg/d* | -0.05 (-0.11, 0.01) | -0.0001 (-0.001, 0.0004) |
| U-sugars, mg/d* | -0.06 (-0.13, 0.004) | -0.0001 (-0.001, 0.0004) |
| Δ Insulin, mU/L | | |
| Total sugar, g | 0.003 (-0.01, 0.02) | -0.01 (0.02, 0.01) |
| Total sugar, E% | -0.007 (-0.08. 0.07) | -0.02 (-0.08, 0.05) |
| U-sucrose, mg/d* | 0.02 (-0.32, 0.36) | 0.01 (-0.05, 0.08) |
| U-fructose, mg/d* | 0.31 (-0.03, 0.65) | -0.003 (-0.01, -0.0004) |
| U-sugars, mg/d* | 0.36 (-0.005, 0.73) | -0.003 (-0.01, -0.0004) |
| Δ HbA1c, mmol/mol | | |
| Total sugar, g | -0.001 (-0.01, 0.01) | 0.005 (-0.004, 0.01) |
| Total sugar, E% | -0.04 (-0.09, 0.01) | 0.04 (-0.01, 0.08) |
| U-sucrose, mg/d* | 0.43 (0.22, 0.64) | -0.05 (-0.09, -0.01) |
| U-fructose, mg/d* | 0.18 (-0.04, 0.40) | 0.0005 (-0.001, 0.002) |
| U-sugars, mg/d* | 0.22 (-0.01, 0.46) | 0.0004 (-0.001, 0.002) |
| Δ HOMA-IR | | |
| Total sugar, g | 0.002 (-0.003, 0.01) | -0.002 (-0.01, 0.002) |
| Total sugar, E% | 0.003 (-0.02, 0.03) | -0.01 (-0.03, 0.01) |
| U-sucrose, mg/d* | -0.01 (-0.11, 0.10) | 0.01 (-0.01, 0.03) |
| U-fructose, mg/d* | 0.07 (-0.04, 0.18) | -0.001 (-0.002, -0.0001) |
| U-sugars, mg/d* | 0.08 (-0.03, 0.20) | -0.001 (-0.002. -0.0001) |
| | | |
| | OR (95% CI)[1] | OR (95% CI)[2] |
| Normoglycemia | 6 months (n=243) | 12 months (n=188) |
| Total sugar, g | 1.00 (0.99, 1.01) | 0.99 (0.98, 1.004) |
| Total sugar, E% | 1.02 (0.96, 1.08) | 0.99 (0.94, 1.05) |
| U-sucrose, mg/d* | 0.87 (0.67, 1.12) | 1.01 (0.96, 1.07) |
| U-fructose, mg/d* | 0.97 (0.74, 1.28) | 1.00 (0.997, 1.002) |
| U-sugars, mg/d* | 0.94 (0.70, 1.26) | 1.00 (0.997, 1.002) |

All regression models are adjusted for sex, age and intervention group. *The absolute urinary sugar excretion variables at 6 months are log-transformed to achieved a normal distribution.
[1]Association between absolute intake/excretion at 6 months and normoglycemia at 6 months.
[2]Association between Δ intake/excretion from 6-12 months and normoglycemia at 12 months.

# Paper IV

In 1,371 participants in the MOS, associations between measures of sugar intake and 64 gut bacteria were studied in individuals free from diabetes. Out of the 64 studied bacterial genera, various genera were nominally associated with added sugar intake, PC added×U-sugars, SSB intake and ASB intake (Table 18, Figure 10). After full adjustment for fiber intake and BMI, added sugar intake was nominally associated with *Streptococcus*, *Succiniclasticum*, *Paraprevotella*, *Oxalobacter* and *Odoribacter*. Out of these, *Succiniclasticum* and *Odoribacter* were also nominally associated with the PC added×U-sugars, in addition to *Lactobacillus*. SSB intake was nominally associated with *Lachnobacterium*, *Dialister*, *Lactobacillus* and *Cetobacterium*, while ASB intake was nominally associated with *Prevotella*, *Suterella*, *Lachnospira* and an unknown genus in the RF16 family. However, only the inverse association between SSB intake and *Lachnobacterium* remained statistically significant after correction for multiple testing using an FDR of 0.05.

A significant positive association between SSB intake (but not any other studied exposure) and the Firmicutes:Bacteroidetes ratio could also be observed, even after adjustment for fiber intake and BMI ($P$=0.048) (Table 19). No associations with the Shannon index were observed after full covariate adjustments.

**Table 18. Adjusted means (SD) of normalized abundances over three groups of the exposures for those bacteria with *P*-trend <0.05 over those three exposure groups.**

| | | | Model 1 | | | | | | Model 2 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Added sugar | <10E% | 10-20E% | >20E% | ß-trend | *P*-trend | *P'*-trend | Added sugar | <10E% | 10-20E% | >20E% | ß-trend | *P*-trend | *P'*-trend |
| n=1,371 | 455 | 780 | 136 | | | | n=1371 | 455 | 780 | 136 | | | |
| *Streptococcus* | 7.72 (0.13) | 8.08 (0.10) | 8.66 (0.25) | 0.05 | 0.001 | 0.060 | *Streptococcus* | 7.75 (0.14) | 8.07 (0.10) | 8.56 (0.26) | 0.05 | 0.005 | 0.314 |
| *Oxalobacter* | 2.81 (0.19) | 2.32 (0.12) | 2.10 (0.28) | -0.16 | 0.015 | 0.355 | *Succiniclasticum* | 0.19 (0.09) | 0.06 (0.02) | 0.06 (0.05) | -0.81 | 0.029 | 0.518 |
| *Paraprevotella* | 4.03 (0.29) | 3.19 (0.17) | 3.21 (0.41) | -0.16 | 0.019 | 0.355 | *Paraprevotella* | 4.00 (0.29) | 3.19 (0.17) | 3.29 0.43) | -0.14 | 0.036 | 0.518 |
| *Lachnobacterium* | 4.13 (010) | 4.03 (0.07) | 3.60 (0.17) | -0.05 | 0.025 | 0.355 | *Oxalobacter* | 2.77 (0.19) | 2.33 (0.12) | 2.19 (0.30) | -0.14 | 0.038 | 0.518 |
| *Odoribacter* | 2.01 (0.16) | 1.88 (0.11) | 1.26 (0.19) | -0.17 | 0.033 | 0.355 | *Odoribacter* | 2.02 (0.16) | 1.88 (0.11) | 1.27 (0.20) | -0.17 | 0.040 | 0.518 |
| *Succiniclastisum* | 0.17 (0.07) | 0.06 (0.02) | 0.06 (0.05) | -0.74 | 0.033 | 0.355 | | | | | | | |
| PC added×U-sugars | T1 | T2 | T3 | ß-trend | *P*-trend | *P'*-trend | PC added×U-sugars | T1 | T2 | T3 | ß-trend | *P*-trend | *P'*-trend |
| n=577 | 193 | 192 | 192 | | | | n=577 | 193 | 192 | 192 | | | |
| *Odoribacter* | 2.15 (0.24) | 2.37 (0.26) | 1.36 (0.16) | -0.22 | 0.012 | 0.507 | *Odoribacter* | 2.13 (0.24) | 2.34 (0.26) | 1.39 (0.17) | -0.21 | 0.020 | 0.822 |
| *Streptococcus* | 7.89 (0.21) | 8.56 (0.21) | 8.64 (0.22) | 0.05 | 0.016 | 0.507 | *Succiniclasticum* | 0.26 (0.16) | 0.11 (0.05) | 0.05 (0.03) | -0.79 | 0.032 | 0.822 |
| *Succiniclasticum* | 0.25 (0.14) | 0.11 (0.06) | 0.05 (0.03) | -0.78 | 0.024 | 0.507 | *Lactobacillus* | 2.43 (0.19) | 2.84 (0.21) | 3.08 (0.24) | 0.12 | 0.039 | 0.822 |
| *Lactobacillus* | 2.44 (0.19) | 2.85 (0.21) | 3.06 (0.23) | 0.11 | 0.044 | 0.711 | | | | | | | |
| SSBs | Non | Medium | High | ß-trend | *P*-trend | *P'*-trend | SSBs | Non | Medium | High | ß-trend | *P*-trend | *P'*-trend |
| n=1,086 | 314 | 425 | 347 | | | | n=1086 | 314 | 425 | 347 | | | |
| *Lachnobacterium* | 4.23 (0.13) | 4.12 (0.10) | 3.61 (0.11) | -0.08 | 0.0003* | 0.020 | *Lachnobacterium* | 4.19 (0.13) | 4.11 (0.10) | 3.65 (0.11) | -0.07 | 0.002 | 0.088 |
| *Dialister* | 8.04 (0.28) | 9.05 (0.25) | 9.35 (0.30) | 0.07 | 0.003 | 0.090 | *Dialister* | 8.04 (0.30) | 9.05 (0.25) | 9.35 (0.30) | 0.07 | 0.003 | 0.088 |
| *Lactobacillus* | 2.36 (0.15) | 2.74 (0.14) | 3.04 (0.19) | 0.13 | 0.007 | 0.111 | *Lactobacillus* | 2.33 (0.15) | 2.73 (0.14) | 3.09 (0.19) | 0.14 | 0.004 | 0.088 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *[Eubacterium]* | 5.12 (0.21) | 5.53 (0.18) | 6.00 (0.23) | 0.08 | 0.007 | 0.111 | *Cetobacterium* | 0.16 (0.27) | 0.02 (0.02) | 0.004 (0.004) | -1.84 | 0.044 | 0.607 |
| *Roseburia* | 9.20 (0.18) | 9.16 (0.15) | 8.58 (0.17) | -0.04 | 0.016 | 0.208 | | | | | | | |
| *Anaerotruncus* | 2.16 (0.13) | 2.48 (0.12) | 2.66 (0.15) | 0.10 | 0.021 | 0.222 | | | | | | | |
| Unknown genus in the Peptostreptococceae family | 6.20 (0.15) | 6.56 (0.13) | 6.68 (0.15) | 0.04 | 0.039 | 0.353 | | | | | | | |

| ASBs | Non | Medium | High | ß-trend | *P*-trend | *P*'-trend | ASBs | Non | Medium | High | ß-trend | *P*-trend | *P*'-trend |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| n=1,085 | 669 | 269 | 147 | | | | n=1085 | 669 | 269 | 147 | | | |
| *Prevotella* | 6.01 (0.17) | 6.82 (0.29) | 7.20 (0.42) | 0.10 | 0.001 | 0.075 | *Prevotella* | 6.04 (0.17) | 6.78 (0.29) | 7.12 (0.42) | 0.09 | 0.004 | 0.257 |
| Unknown genus in the RF16 family | 1.81 (0.12) | 2.32 (0.24) | 2.56 (0.35) | 0.19 | 0.007 | 0.234 | Unknown genus in the RF16 family | 1.82 (0.12) | 2.30 (0.23) | 2.50 (0.34) | 0.18 | 0.017 | 0.525 |
| *Sutterella* | 3.49 (0.15) | 4.44 (0.31) | 4.16 (0.39) | 0.12 | 0.013 | 0.265 | *Sutterella* | 3.51 (0.16) | 4.40 (0.30) | 4.12 (0.39) | 0.11 | 0.025 | 0.525 |
| Unknown genus in the SHA98 order | 2.78 (0.15) | 2.40 (0.22) | 2.06 (0.25) | -0.15 | 0.017 | 0.265 | *Lachnospira* | 5.86 (0.10) | 5.67 (0.15) | 5.40 (0.20) | -0.04 | 0.037 | 0.588 |
| *[Eubacterium]* | 5.32 (0.14) | 5.99 (0.24) | 5.90 (0.33) | 0.07 | 0.021 | 0.268 | | | | | | | |
| Unknown genus in the Christensenellaceae family | 4.31 (0.10) | 4.21 (0.15) | 3.79 (0.19) | -0.05 | 0.032 | 0.296 | | | | | | | |
| *Lachnospira* | 5.86 (0.09 | 5.68 (0.15) | 5.40 (0.19) | -0.04 | 0.032 | 0.296 | | | | | | | |

PC added×U-sugars is divided into equal tertiles. SSBs and ASBs are combinations of 4-day food record and FFQ and are divided into three groups. Negative binomial regressions were used. Model 1 is adjusted for age, sex, energy intake, smoking and physical activity level. Model 2 is additionally adjusted for fiber intake and BMI. *Holds after FDR-correction. *P*' is adjusted for FDR using the Benjamini-Hochberg method.

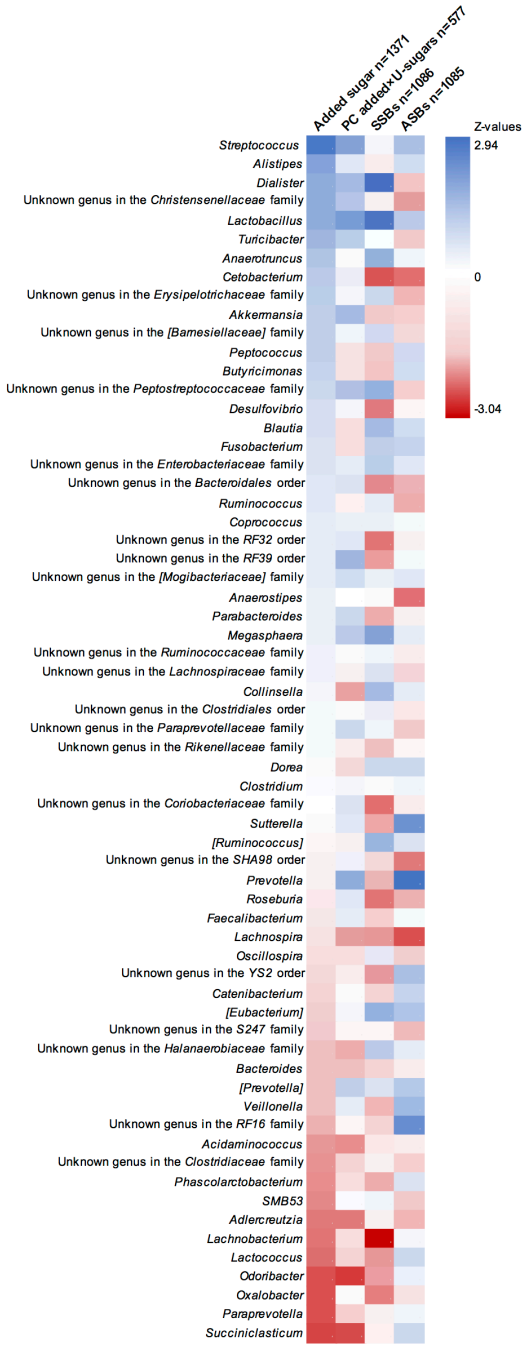


**Figure 10.** Heatmap of z-values from trend over three groups of exposure using negative binomial regressions adjusted according to model 2 (age, sex, energy intake, smoking, physical activity level, fiber intake and BMI). Genera are sorted according to the z-value from regressions with added sugar intake.

**Table 19. Associations between three categories of added sugar intake, PC added×U-sugars, SSB intake and ASB intake and the Firmicutes:Bacteroidetes ratio and the Shannon index.**

| | Added sugar | | PC added×U-sugars | | SSBs | | ASBs | |
|---|---|---|---|---|---|---|---|---|
| | n=1,371 | | n=577 | | n=1,086 | | n=1,085 | |
| | ß | P | ß | P | ß | P | ß | P |
| Firmicutes:Bacteroidetes ratio* | | | | | | | | |
| Model 1 | 0.119 | 0.021 | 0.117 | 0.041 | 0.108 | 0.021 | 0.049 | 0.291 |
| Model 2 | 0.098 | 0.059 | 0.089 | 0.120 | 0.094 | 0.048 | -0.008 | 0.864 |
| Shannon index | | | | | | | | |
| Model 1 | 0.026 | 0.087 | 0.037 | 0.031 | 0.019 | 0.187 | 0.005 | 0.713 |
| Model 2 | 0.019 | 0.213 | 0.031 | 0.080 | 0.014 | 0.352 | -0.005 | 0.743 |

Determined using linear regression. Model 1 is adjusted for age, sex, energy intake, smoking and physical activity level. Model 2 is additionally adjusted for fiber intake and BMI. *Firmicutes:Bacteroidetes ratio is log-transformed.

# Paper V

The associations between measures of sugar intake and 136 plasma proteins was examined in 4,382 individuals from the MDC-CC study, free from diabetes and CVD. As shown in Table 20 and Figure 11, out of the 136 studied plasma proteins, nine proteins were internally replicated to associate with added sugar intake; human epididymis protein 4 (HE4), folate receptor alpha (FRalpha), tumor necrosis factor receptor superfamily member 4 (TNFRSF4), inducible T cell costimulator ligand (ICOGSL), CD40 ligand (CD40L), cadherin 3 (CDH3), chemokine (C-X-C motif) ligand 13 (CXCL13), melanoma-derived growth regulatory protein (MIA) and resistin (RETN). Of these, HE4, FRalpha and TNFRSF4, remained internally replicated at $\alpha_1$<0.01 and HE4 was the only protein internally replicated at $\alpha_1$<FDR of 0.05. Seven proteins were internally replicated to associate with SSB intake; interleukin-1 receptor antagonist (IL1ra), hepatocyte growth factor (HGF), interleukin 12 (IL12), tissue plasminogen activator (tPA), prostasin (PRSS8), furin (FUR) and chitinase-3-like protein 1 (CHI3L1). IL1ra, HGF and IL12, remained internally replicated at $\alpha_1$<0.01 and none of the proteins were internally replicated at $\alpha_1$<FDR of 0.05. No protein was internally replicated to associate with both added sugar and SSB intake.

All proteins that were internally replicated to associate with SSB intake, except for IL12, were strongly associated with increased T2D incidence (*P*-values 6.9E-8 to 2.8E-46). Among the proteins associated with added sugar intake, only two associated with increased T2D incidence (*P*-values 0.00046 to 0.00023) (Table 21).

As visualized in Figure 12, no significant linear associations were seen between either added sugar intake or SSB intake and T2D risk (*P*-trend 0.51 and 0.28, respectively) or CRP concentrations (*P*-trend 0.41 and 0.09, respectively). Although, the associations with SSB intake appeared linear, while the associations with added sugar intake appeared more U-shaped (this U-shape remained after exclusion of low and high energy reporters and past diet changers). However, a significant positive interaction between added sugar and CRP were seen on the association with T2D incidence (*P*=0.014), where added sugar intake was positively associated with T2D at high CRP levels, but not associated at low CRP levels. A similar tendency was seen for SSB intake, but this interaction was not significant (*P*=0.110).

**Table 20. The number of times proteins associated with added sugar intake and SSB intake, respectively, out of 100 iterations of random discovery and replication cohorts at various $\alpha_1$ levels using a two-step iterative resamling approach. A protein must be replicated at least 20 times to pass internal replication.**

| Added sugar, E% | Replicated at $\alpha_1$ <0.05 | Replicated at $\alpha_1$ <0.01 | Replicated at $\alpha_1$ <FDR |
|---|---|---|---|
| Epididymial secretory protein E4 (HE4) | 69 | 56 | 20 |
| Folate receptor alpha (FRalpha) | 60 | 37 | 5 |
| Tumor necrosis factor receptor superfamily member 4 (TNFRSF4) | 51 | 27 | 3 |
| Cadherin 3 (CDH3) | 46 | 17 | 1 |
| Inducible T Cell Costimulator Ligand (ICOSLG) | 46 | 14 | 1 |
| C-X-C motif chemokine 13 (CXCL13) | 41 | 14 | 0 |
| Melanoma-derived growth regulatory protein (MIA) | 40 | 11 | 0 |
| CD40 ligand (CD40L) | 37 | 4 | 0 |
| Resistin (RETN) | 23 | 0 | 0 |
| Immunoglobulin-like transcript 3 (ILT3) | 19 | 0 | 0 |
| Interleukin 12 (IL12) | 17 | 0 | 0 |
| Prostasin (PRSS8) | 16 | 0 | 0 |
| Matrix metalloproteinase-10 (MMP10) | 12 | 1 | 0 |
| C-X-C motif chemokine 1 (CXCL1) | 3 | 0 | 0 |
| Transforming growth factor alpha (TGFalpha) | 2 | 0 | 0 |
| Interleukin-1 receptor antagonist (IL1ra) | 2 | 0 | 0 |
| Adrenomedullin (AM) | 1 | 0 | 0 |
| Renin (REN) | 1 | 0 | 0 |
| Agouti-related protein (AGRP) | 1 | 0 | 0 |
| Cathepsin L1 (CTSL1) | 1 | 0 | 0 |
| Furin (FUR) | 1 | 0 | 0 |
| | | | |
| SSB, E% | | | |
| Interleukin-1 receptor antagonist (IL1ra) | 60 | 46 | 5 |
| Hepatocyte growth factor (HGF) | 44 | 25 | 0 |
| Interleukin 12 (IL12) | 46 | 12 | 0 |
| Prostasin (PRSS8) | 31 | 1 | 0 |
| Tissue-type plasminogen activator (tPA) | 21 | 5 | 0 |
| Furin (FUR) | 24 | 0 | 0 |
| Chitinase-3-like protein 1 (CHI3L1) | 22 | 0 | 0 |
| Cathepsin D (CTSD) | 8 | 0 | 0 |
| Tartrate-resistant acid phosphatase type 5 (TRAP) | 1 | 0 | 0 |
| Parkinson disease protein 7 (PARK7) | 1 | 0 | 0 |
| Proteinase-activated receptor 1 (PAR1) | 1 | 0 | 0 |
| Prolactin (PRL) | 1 | 0 | 0 |
| Lectin-like oxidized LDL receptor 1 (LOX1) | 1 | 0 | 0 |
| Myoglobin (MB) | 1 | 0 | 0 |
| C-X-C motif chemokine 1 (CXCL1) | 1 | 0 | 0 |

Linear regressions were adjusted for age, sex, season, screening date, total energy intake, education, smoking, alcohol and leisure-time physical activity. With the two-step iterative resamling approach, the cutoff was always set to $\alpha_2 < 0.05$ in the replication cohorts. SSB intake is log-transformed.

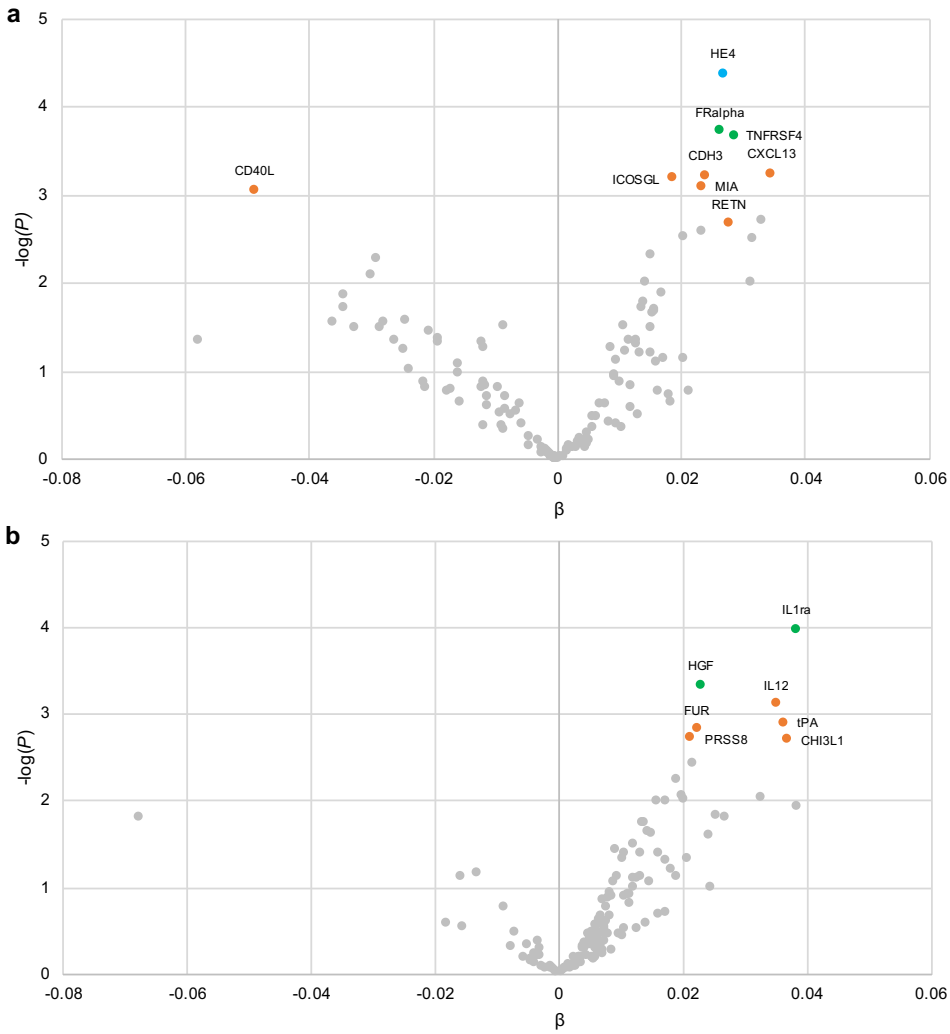


**Figure 11.** Volcano plot of associations between (a) added sugar intake (standardized) and (b) SSB intake (log-transformed and standardized) and 136 plasma proteins in full sample analysis (n=3,351-4,382). Linear regressions were adjusted for age, sex, season, screening date, total energy intake, education, smoking, alcohol and leisure-time physical activity. Blue, internally replicated at $\alpha_1$<FDR of 0.05; green, internally replicated at $\alpha_1$<0.01; orange, internally replicated at $\alpha_1$<0.05; grey, not internally replicated.

**Table 21. Associations with T2D incidence for proteins internally replicated to associate with added sugar intake and SSB intake.**

| | | Lifestyle adjustments | | Lifestyle adjustments + BMI | | Lifestyle adjustments + BMI + FPG | |
|---|---|---|---|---|---|---|---|
| Added sugar | n | HR (95% CI) | *P* | HR (95% CI) | *P* | HR (95% CI) | *P* |
| HE4 | 4,253 | 1.01 (0.93, 1.10) | 0.77 | 1.06 (0.97, 1.16) | 0.16 | 1.02 (0.93, 1.11) | 0.69 |
| FRalpha | 4,253 | 0.94 (0.88, 1.02) | 0.15 | 1.00 (0.93, 1.08) | 0.98 | 0.92 (0.85, 0.99) | 0.029 |
| TNFRSF4 | 4,175 | 1.01 (0.93, 1.09) | 0.84 | 0.97 (0.90, 1.05) | 0.52 | 0.87 (0.81, 0.95) | 0.0012* |
| CDH3 | 4,241 | 0.96 (0.89, 1.03) | 0.23 | 0.98 (0.91, 1.05) | 0.52 | 0.94 (0.87, 1.01) | 0.11 |
| ICOSLG | 4,253 | 1.04 (0.96, 1.12) | 0.35 | 1.06 (0.98, 1.14) | 0.15 | 0.95 (0.88, 1.03) | 0.25 |
| CXCL13 | 4,175 | 1.14 (1.06, 1.22) | 0.00045* | 1.12 (1.04, 1.21) | 0.0033 | 1.07 (0.98, 1.16) | 0.12 |
| MIA | 4,252 | 0.96 (0.89, 1.04) | 0.34 | 1.01 (0.94, 1.09) | 0.79 | 0.99 (0.91, 1.07) | 0.74 |
| CD40L | 4,382 | 1.15 (1.07, 1.24) | 0.00023* | 1.13 (1.05, 1.22) | 0.0012* | 1.08 (1.00, 1.16) | 0.047 |
| RETN | 4,382 | 1.11 (1.03, 1.19) | 0.0057 | 1.08 (1.01, 1.17) | 0.031 | 1.12 (1.04, 1.21) | 0.0021* |
| SSBs | | | | | | | |
| IL1ra | 3,761 | 1.51 (1.42, 1.61) | 4.6E-37* | 1.35 (1.26, 1.45) | 2.1E-16* | 1.27 (1.18, 1.37) | 6.6E-10* |
| HGF | 4,382 | 1.65 (1.53 ,1.77) | 2.6E-38* | 1.48 (1.37, 1.60) | 1.0E-22* | 1.37 (1.27, 1.48) | 5.2E-15* |
| IL12 | 4,252 | 1.05 (0.97, 1.13) | 0.24 | 0.98 (0.90, 1.06) | 0.55 | 0.87 (0.81, 0.95) | 0.0014* |
| PRSS8 | 4,252 | 1.43 (1.31, 1.55) | 7.7E-17* | 1.34 (1.23, 1.46) | 8.2E-12* | 1.14 (1.05, 1.24) | 0.0030* |
| tPA | 4,382 | 1.44 (1.34, 1.55) | 8.1E-22* | 1.33 (1.23, 1.43) | 5.6E-13* | 1.18 (1.09, 1.28) | 4.6E-5* |
| FUR | 4,253 | 1.78 (1.64, 1.92) | 2.8E-46* | 1.54 (1.42, 1.68) | 4.9E-24* | 1.30 (1.20, 1.42) | 2.2E-9* |
| CHI3L1 | 4,370 | 1.22 (1.13, 1.31) | 6.9E-8* | 1.17 (1.09, 1.26) | 2.1E-5* | 1.17 (1.09, 1.26) | 1.5E-5* |

Plasma proteins are standardized. Cox proportional hazards regressions were adjusted for age, sex, education, smoking, alcohol and leisure-time physical activity (and BMI and FPG in the additional models). *Significant after Bonferroni correction, $P = 0.05/16 = 0.003$.

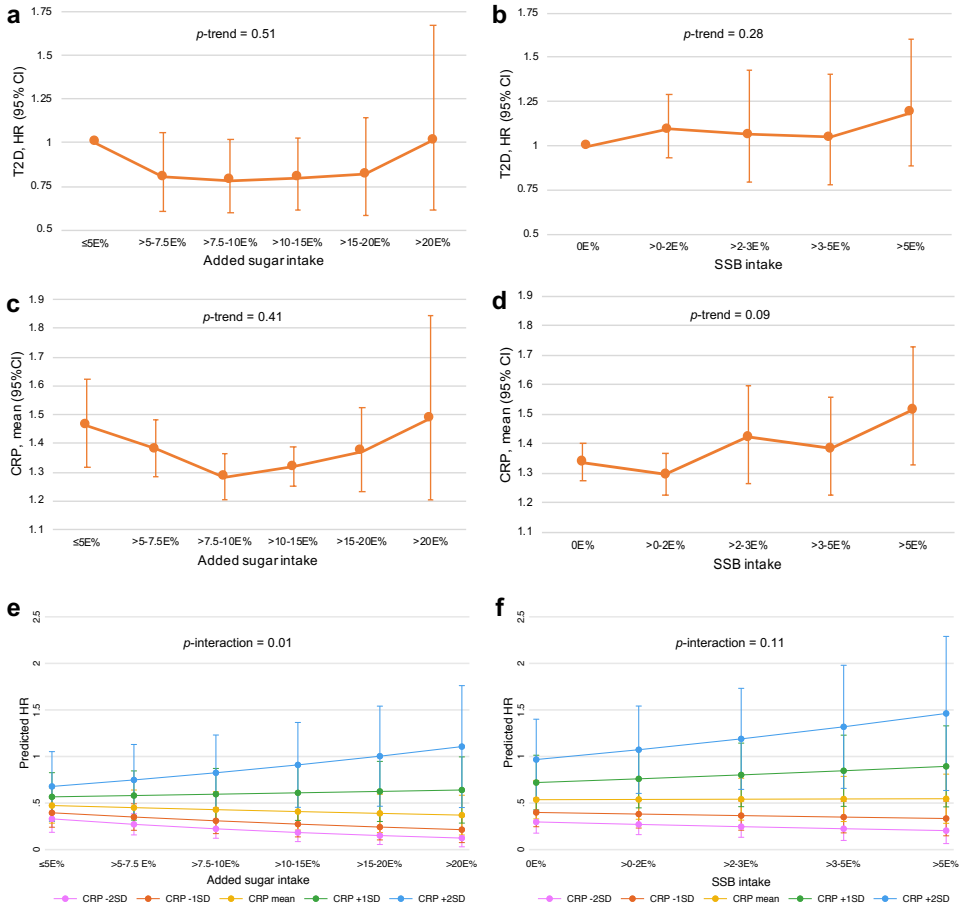


**Figure 12.** (a) Association between added sugar intake and T2D; (b) Association between SSB intake and T2D; (c) Association between added sugar intake and CRP; (d) Association between SSB intake and CRP; (e) Interaction between added sugar intake and CRP on T2D risk; (f) Interaction between SSB intake and CRP on T2D risk. Cox proportional hazards regressions and linear regressions were adjusted for age, sex, season, screening date, total energy intake, education, smoking, alcohol and leisure-time physical activity. CRP was studied as log-transformed and the predicted marginal means of CRP levels were exponentiated back for presentation.

# Discussion

## Main findings

From the five studies included in this thesis, it is still difficult to conclude a clear increased cardiometabolic risk with high total intake of added sugar, whereas a much clearer picture of an increased cardiometabolic risk with high intake of SSBs has been shown. Although increased cardiometabolic risk frequently has been observed in the absolute highest added sugar intake category in the MDC, the associations between added sugar intake and all-cause mortality, cardiovascular mortality, T2D incidence and cardiometabolic risk markers such as CRP have taken on a U-shaped form, which complicates the interpretations. For SSBs, on the other hand, intake has been shown to be significantly and linearly associated with increased all-cause mortality, a T2D-related plasma proteomic signature, a higher Firmicutes:Bacteroidetes ratio and a lower abundance of the genus *Lachnobacterium*.

The urinary sucrose and fructose biomarker from overnight urine samples was significantly correlated with self-reported added sugar intake in a healthy population. This biomarker from overnight urine samples supported self-reported intake assessment of added sugar intake, and in combination associated with higher BMI, waist circumference, systolic blood pressure and lower HDL cholesterol among women. The 24-h sucrose and fructose biomarker should, however, likely be used with caution in individuals with metabolic impairment, as we observed elevated fructose excretion and reduced correlations with self-reported total sugar intake in individuals with prediabetes compared to previous observations in healthy individuals.

## Agreement with previous studies

The findings that SSB intake is much more clearly associated with cardiometabolic disease risk than the total intake of added sugars are in line with previous research (165). The associations observed between added sugar and SSB intake and the composition of the gut microbiota and the plasma proteome have not been previously reported in published research, as these research questions have not been studied before. Considering the findings with the urinary sucrose and fructose biomarker, the

correlations between self-reported sugar intake and the overnight urinary sugar biomarker are comparable, but at the lower end, to those found with the 24-h urinary sugar biomarkers (130, 131). In individuals with prediabetes, the sucrose and fructose biomarker has not been studied before, but the finding that urinary fructose excretion may be elevated resembles findings in patients with diabetes in whom both circulating and urinary fructose have been found to be elevated (166).

# Other findings from the MDC

During the many years that the MDC cohort has been studied, much knowledge on diet and disease links has been provided. The findings from this thesis together with additional findings in the MDC regarding sugar and SSB intake present an even larger picture of the role of sugar intake in cardiometabolic risk.

Very high intake of added sugar (>20E%) has been observed to be associated with an increased incidence of coronary events and stroke (although, no significant linear trend was observed), while high consumption of SSBs was associated with increased stroke incidence. In contrast, while in accordance with the results on mortality (Paper I), for the intake of treats, the highest risk of coronary events, stroke and atrial fibrillation was seen at the lowest consumption level (99). Likewise, higher sucrose intake has been associated with a higher incidence of coronary events, while also showing tendencies for a U-shaped association (98). Additionally, disaccharide intake has been associated with an atherogenic lipoprotein profile, characterized by smaller LDL particles, lower HDL cholesterol and higher triglycerides (167), while no association with any variety of sugar intake was associated with intima media thickness as a measure of atherosclerosis (168).

No association between quintiles of added sugar intake and T2D incidence was observed after full covariate adjustment in the full MDC (91), similar to as in the MDC-CC shown in Paper V (although, no tendency for a U-shape was observed). Furthermore, the same study also demonstrated that the intake of monosaccharides was inversely associated with T2D incidence, while intake of disaccharides was positively associated (91). In addition, it was observed that the positive association between SSB intake and T2D incidence was attenuated after BMI adjustment, while a prior study of the full MDC found a robust (following BMI adjustment) association between SSB intake and T2D incidence when SSB intake was modeled in three categories instead of four (169).

Furthermore, several relations between genetic variation, the intake of sugars and SSBs and cardiometabolic risk have been studied in the MDC. It has been observed that the association between SSB intake and BMI is slightly intensified in individuals with

genetically increased obesity risk assessed using a genetic risk score based on 32 single-nucleotide polymorphisms (SNPs) that are associated with BMI (170). Similarly, this was also observed for the association between SSB intake and T2D incidence based on a genetic risk score including 48 T2D-associated SNPs (169). Furthermore, a positive association between the intake of sucrose and sweets and the incidence CVD may be limited to those with low genetic susceptibility for high triglyceride levels (97).

## The U-shaped associations

The U-shaped associations seen in this thesis between added sugar intake and all-cause mortality, cardiovascular mortality and T2D incidence, as well as some cardiometabolic risk markers such as CRP, in Papers I and V are difficult to explain but are likely in some part due to dietary misreporting and reversed causation. Hence, these results are likely a consequence of study design limitations and d not provide a reason for why it would be harmful to omit added sugar from one's diet.

### Dietary misreporting

The issue of dietary misreporting that is already outlined on page 36 is likely present in the results of this thesis, as it is reasonable that those who are self-aware of being at increased cardiometabolic risk, for example, individuals who are obese, dyslipidemic or hypertensive, have reported a lower intake of added sugar than their true consumption (112, 114). This misreporting could have been done completely unconsciously, or also to some degree as a conscious action. This results in that a larger proportion of individuals at high risk being categorized into the lowest intake group, driving up the mean cardiometabolic risk in this group. However, in Papers I and V, after exclusion of individuals who were classified as low energy reporters, the increased cardiometabolic risk in the lowest added sugar intake group remained in the MDC and MDC-CC, while in the NSHDS, the exclusion of low energy reporters attenuated the slightly elevated all-cause mortality seen in the lowest added sugar intake group (Paper 1).

It is primarily for this reason that there is an ongoing search for objective nutritional biomarkers to be able to study diet-disease associations free from the bias of dietary misreporting. It is warranted to study the association between the overnight urine sugar biomarker in the MOS and the incidence of T2D, CVD and mortality to evaluate whether the use of this objective marker, rather than only self-reported added sugar intake, would yield a more linear and non-U-shaped association. This must, however, be a project for the future, since sufficient follow-up data are not yet available in the MOS.

### Reverse causation

Nonetheless, those at higher cardiometabolic risk not only could have reported lower added sugar intake than what was true but also could have actually actively reduced their sugar intake in an attempt to improve their health. This would have a similar effect as underreporting, with more individuals at high risk being categorized in the lowest intake group, but because of their changed dietary habits rather than erroneous reporting, meaning that the dietary measurement is not incorrect but not representative of long-term intake. Hence, the elevated cardiometabolic risk precedes the low consumption of added sugar and not the other way around, reversing the direction of causality.

The same phenomenon could also go in the opposite direction – individuals with a healthy weight who have never had struggled with it may not avoid foods and beverages high in added sugar because it is not necessary for them (171). Hence, individuals with rather low cardiometabolic risk, attributable to genetics or other lifestyle factors, may be categorized in the higher added sugar intake categories.

## Differences between sugar sources

The clear difference that is demonstrated between the total intake of added sugars and the intake of SSBs in relation to cardiometabolic risk is somewhat difficult to explain. SSBs are solely water, added sugar, and some flavorings and coloring; hence, sucrose or high-fructose corn syrup are basically the only nutritious ingredient in SSBs. So why is there is such a consistent discrepancy? First of all, a difference such as this could theoretically be visible only if the SSB intake relative to the total added sugar intake of the studied population is rather low, whereas if the majority of added sugar comes from SSBs, the two different variables would be more similar and a more similar association would be observed. As estimated in Paper V, an average of only 10% (median <1%) of the added sugar intake came from SSBs in the MDC-CC.

Nevertheless, reasons why a difference was observed are likely in part due to some actual differences between SSBs and other sources of added sugar. SSBs are always in a liquid form, they are in general slightly acidic, they are often carbonated, some of them contain caffeine and caramel coloring (especially cola beverages), and they are generally served in cans or bottles of standardized sizes.

The liquid state of SSBs makes them less satiating than solid sugary foods (172). Studies have shown that energy from sugars consumed in liquid form is not compensated for in a subsequent meal as much as the energy from sugars consumed in a solid state (173), resulting in an increased energy intake over time. When consumed as a liquid, the digestion process is also much faster, as no chewing or other mechanical digestion in

the upper gastrointestinal tract is necessary. The gastric emptying time is drastically reduced in liquid compared to solid foods (174); hence, in SSBs, the sugars are rapidly ready for enzymatic hydrolyzation in the small intestine, resulting in a faster and steeper blood glucose and insulin response than for solid sugary foods (175). Unrelated to the liquid form of SSBs, the fact that SSBs are purely sugars, with no fat or protein to slow down the digestion, results in a rapid metabolic response. Digestion is also believed to be sped up because the acidic state of SSBs may contribute to the hydrolyzation of sucrose into glucose and fructose already in the can or bottle (176), resulting in a similar nutritional content as SSBs sweetened with high-fructose corn syrup. However, it is not known whether there is a meaningful difference between the ingestion of intact sucrose or free fructose and glucose (high-fructose corn syrup) for cardiometabolic health, although, recent mouse studies may indicate a less pronounced risk for intact sucrose intake (177).

Furthermore, the addition of caffeine to some of the most common SSBs also contributes to its taste and likely also increases the risk of addiction-like consumption (178). Caramel colourings in cola beverages are rich in advanced glycation end-products, which potentially also contributes to increased cardiometabolic risk (179, 180). The actual carbonation of beverages could perhaps contribute to increasing appetite and the risk of weight gain (181).

Additionally, in the setting of epidemiological studies where we are primarily reliant on self-reported intake, a difference may appear between SSBs and the total intake of added sugar because SSBs are generally consumed in standardized portion sizes of 33 cl cans or 50 cl bottles. This may facilitate more accurate self-reporting of SSB intake than of the total added sugar (which may enter our diet in various ways and at various moments throughout a day), independent of which dietary assessment method is used. With more accurate dietary assessment, it is more likely that we will find an association with health that conforms with reality.

On the contrary, the negative associations observed between intake of treats (solid sugar foods) and mortality in MDC and NSHDS (Paper I) is not easily explained considering that such foods generally are also high in saturated fats or trans fats (e.g., pastries, deserts, ice cream, chocolate etc), which also contributes to a higher energy density. However, a possible contributing explanation could be the age and origin of these populations. The participants in the MDC and NSHDS were middle-aged during the baseline data collections in 1991-96 and were almost homogeneously Swedish. Hence, eating a daily pastry or cookie would not be unusual in this population, as this is, or especially was, a large part of the Swedish food culture. Therefore, a high intake of treats would not necessarily be accompanied by other poor dietary or lifestyle choices in this population, as compared to a high intake of SSBs, which likely would be accompanied by other poor dietary or lifestyle choices in this population. High intake of SSBs can often be considered a marker of unhealthy dietary habits and lifestyle in

general (182), which has been shown to be the case in both the MDC (182, 183) and the MOS (184). This is why studying a single nutrient or food can be problematic in nutrition research, as a single dietary component cannot determine health or disease (185, 186). A diet low in sugar can still be unhealthy and a diet with a relatively high sugar content can still be healthy, dependent on all other dietary components.

Nevertheless, high intake of SSBs is often also correlated with a lower socioeconomic status and, therefore, generally coexist with various other risk factors that generally belong to a lower socioeconomic status. The frequently observed risks with high SSB intake may therefore be exacerbated by such coexisting socioeconomic risk factors, and the actual contribution from the intake of SSBs is difficult to disentangle. However, one must be very careful when extrapolating such assumptions. For example, a Chinese study showed that SSB intake was associated with higher socioeconomic status (187). Hence, the link between SSB intake and lower socioeconomic status may be applicable only in "Western" populations.

## The urinary sucrose & fructose biomarker

The results of Papers II and III indicate that the urinary sucrose and fructose biomarker in overnight urine samples is likely useful to complement self-reported sugar intake data, but that the 24-h urinary sucrose and fructose biomarker might not perform optimally and should be used with caution in individuals with prediabetes. In contrast to 24-h urinary sucrose and fructose excretion, which is believed to give the most complete measure of sugar intake from the past 24 h (minimum), it is still unclear what exact time frame sucrose and fructose excretion from an overnight urine sample reflects. It has been shown that the first fasting morning urine voids are lower in sucrose and fructose than those following meals during the day (123). Therefore, it is reasonably an advantage to evaluate overnight urine samples (which in addition to the first morning urine includes any voids during the night) rather than only morning urine samples, as this may better capture the consumption from the day before. However, such a hypothesis needs to be evaluated. Nevertheless, considering that the errors of self-reported intake of sugar and the potential errors of using overnight urine samples rather than 24-h sucrose and fructose excretion are completely unrelated, we hypothesize that their combination has the possibility to better reflect the true sugar intake.

The findings indicating that fructose excretion may be elevated and that 24-h urinary sucrose and fructose excretion was only modestly correlated with self-reported total sugar intake in individuals with prediabetes (Paper III) are not surprising, as a few previous studies have indicated that circulating and urinary levels of fructose may be elevated in individuals with diabetes (166, 188) or predict future T2D incidence (189), even though insulin does not actively control plasma fructose levels. However, there are

surprisingly few studies that have evaluated fructose concentrations in patients with diabetes, considering the hypothesis of a contributing role of high fructose intake in T2D development. The elevated plasma concentrations of fructose in diabetic patients have been hypothesized to in part originate from increased endogenous fructose production via the polyol pathway (166), which first converts glucose to sorbitol using the enzyme aldose reductase and then converts sorbitol to fructose using the enzyme sorbitol dehydrogenase (190). The polyol pathway is induced in a state when glucose concentrations are elevated to a degree that regular glucose metabolism is saturated, such as in prediabetes (190). We also speculate that renal reabsorption of fructose could be altered in a prediabetic state, similar to the excessive glucose excretions observed in untreated diabetes. Although the SGLT2 and SGLT1 transporters have primary responsibility for glucose renal reabsorption and fructose reabsorption is limited to SGLT4 and SGLT5 (191-195), fructose reabsorption can speculatively be inhibited if plasma glucose levels are high enough to saturate normal glucose reabsorption (as seen in the case with SGLT4 and 1,5-anhydroglucitol (192, 196)), resulting in higher urinary fructose excretion. Nevertheless, in Paper III, we did not make a proper comparison between healthy individuals and individuals with prediabetes, and we have only compared the excreted amounts in individuals with prediabetes with reported excretion levels in previous studies of healthy individuals. Therefore, we cannot rule out that the higher fructose excretion in Paper III could be due to differences in laboratory analyses or other study-specific differences, rather than due to differences in metabolic health, even though we have mechanistic support to suggest such a difference. Clearly, fructose and sucrose metabolism must be further studied in individuals with prediabetes to fully understand the results in Paper III, and the actual mechanisms underlying intact sucrose absorption must be studied in any population to fully understand the mechanisms we rely on when using this biomarker.

In Paper II, we primarily evaluated the biomarker in comparison to added sugar intake, as we observed the strongest correlations with added sugar and because added sugar is generally more important in public health terms. In Paper III, however, we studied total sugar intake, mainly because of the limitations of the food database. Whether this biomarker should be used as a biomarker of total or added sugar intake has been discussed. An early feeding study showed that the 24-h biomarker is better correlated with the intake of extrinsic sugars (added) than that of intrinsic sugars (naturally occurring) (197). Another feeding study observed that added sugar intake explained more of the variation in the excreted sucrose and fructose than total sugar intake, and based on this concluded that the biomarker appears to be a better biomarker of added sugar intake than of total sugar intake (127). However, a very recent and large feeding study showed that both added sugars and naturally occurring sugars were significant determinants of the 24-h biomarker. Hence, the authors argue that this biomarker cannot be a biomarker for only added sugar intake, although the correlation might be higher with added sugar than total sugar (129). In fact, urinary sucrose and fructose

cannot be a perfect biomarker of either added or total sugar intake. The naturally occurring sucrose and fructose in fruits and vegetables will contribute to urinary excretions, while on the other hand, components of total sugars, such as lactose, maltose, glucose and galactose, are not measured by the biomarker. Reasonably, in a Nordic setting, where dairy intake is generally rather high, this discrepancy between the biomarker and total sugar intake may be particularly high.

The recommended application of the urinary sucrose and fructose biomarkers is to use it to calibrate self-reported sugar intake; however, we considered this an unsuitable approach in the two studies of this thesis. According to Tasevska (124), biomarker calibration equations can only be used with 24-h urine samples but not spot urine samples (or in the case of Paper II, overnight urine samples). Furthermore, since our results from Paper III indicated that the urinary excretion of sucrose and fructose may not accurately reflect sugar intake in individuals with prediabetes, using those excretion values to calibrate reported sugar intake would not be appropriate.

## The gut microbiota

The role of the gut microbiota in obesity and cardiometabolic disease risk has been a popular research topic over the past one or two decades. The first research within this area focused on trying to identify simplified measures of the microbiota that were important for health. Indeed, early on, it was found that the ratio between the abundances of the two most abundant phyla in our gut microbiome, Firmicutes and Bacteroidetes, was elevated in individuals with obesity as compared to lean individuals (198) and that microbiome diversity was reduced in obese individuals (199). Both of these findings have been replicated in many later studies in both humans and animals. However, while reduced microbiome diversity and richness in obese individuals is now more or less established as a fact (58, 200), the role of the Firmicutes:Bacteroidetes ratio in obesity still remains debated (201, 202). Furthermore, what starts to become clearer when the microbiome analyses can consider more details is that a high abundance of butyrate-producing bacteria may serve to protect for obesity and cardiometabolic disease (58, 200, 203). However, studying microbiotic alterations in individuals with cardiometabolic disease is challenging because we know that the microbiota is largely affected by common drugs such as metformin and statins, which are heavily used in populations with cardiometabolic disease (58). In prediabetic individuals, on the other hand, who are likely not as heavily medicated, a reduced abundance of butyrate-producing bacteria and especially the species *Akkermancia muciniphila* has been observed (204, 205). However, a major issue in microbiome research is that we have very limited knowledge of what actually characterizes a healthy microbiome, other than that a sufficient amount of richness and diversity should be present.

Although the consequences of a high sugar intake on the oral microbiome are well understood, the actual potential for our sugar intake to affect the colonic microbiota is less straightforward. Sugars are generally hydrolyzed and absorbed in the small intestine and do not reach the colon. The exception is that fructose absorption is not always complete when large amounts are consumed within a short time frame (39). The increased fructose substrate available in the colonic microbiota following high sugar consumption may affect our gut microbiota (60). According to a review by Di Rienzi et al., there are three main potential mechanisms that are responsible for these potential microbiotic alterations (206). Gut bacteria may adjust their transcriptional protein and metabolite profiles to better fit the altered environment to improve the use of the available substrate (transcriptional changes). This may also lead to alterations in the composition of the microbiota, where the bacteria that are better fitted to the environment increase in abundance (compositional changes). Last, genetic adaptation may occur in order for bacteria to better adapt to the environment, which can possibly be detected at the strain level (genetic changes) (206). These theories are extracted from findings in rodent studies, but there is a complete lack of human studies confirming these theories. Furthermore, various hypotheses exist on how a high fructose intake could reduce gut barrier function also exist, which could constitute an important link between the gut microbiota and cardiometabolic health (207).

Very few studies have analyzed the associations between the intake of sugar and sugar-rich foods and beverages and the gut microbiota in humans. Two studies have observed associations between SSB intake and reduced alpha diversity (208, 209). The larger of these studies (n=3409) also observed an inverse association between the intake of pastries and the genus *Akkermansia* and a genus classified in the Christensenellaceae family among individuals with a microbiota enriched in the genus *Bacteroides* (209). Another recent study found various significant correlations between the intake of sugary drinks, sucrose and fructose and various gut bacteria, but there was minimal agreement between these associations; e.g., many of the bacteria that were correlated with the intake of sugary drinks were correlated in the opposite direction with the intake of fructose, making the accuracy of these findings questionable (210). In addition, in a very small cohort of only 52 overweight adolescents, a high intake of fructose was associated with a reduced abundance of *Eubacterium eligens* and *Streptococcus thermophilus*, while no associations were observed with total or added sugar intake (211). This is in contrast to the positive nominal associations observed in Paper IV between added sugar intake and the genus *Streptococcus* and between SSB intake and the genus *Eubacterium*.

Among the bacterial genera that were identified to be nominally associated positively with added sugar or SSB intake in Paper IV, *Eubacterium ventriosum* and *Roseburia intestinalis* have been observed to be positively associated with obesity, *Prevotella copri* has been associated with T2D, and various species of *Streptococcus* have been positively

associated with cardiometabolic disease, and especially metabolic liver disease (58). None of the other genera that was nominally identified to associate with added sugar and SSB intake in Paper IV is currently central in the discussion of the role of the gut microbiota for cardiometabolic diseases, and certainly not the only genera that remained associated with SSB intake after multiple testing correction, *Lachnobactierum*, which has been very scarcely studied previously. What is known about the *Lachnobacterium* genera is that it can ferment sugars to primarily lactic acid, and small amounts of butyrate and acetate (212). Nevertheless, the finding in Paper IV showing a positive association between SSB intake and the Firmicutes:Bacteroidetes ratio gives an indication of a higher cardiometabolic risk in association with SSB intake, but once again not with added sugar intake, as a high Firmicutes:Bacteroidetes ratio can be considered commensurate with an obesity-related gut microbiota.

## The plasma proteome

By studying the plasma proteome, new potential mechanisms for disease etiology or progression can be identified, which can pave the way for future treatment and drug development (139).

In this thesis, the plasma proteins potentially linking added sugar and SSB intake with T2D were studied. No previous research has explored a large array of plasma proteins in relation to added sugar or SSB intake. Out of the six identified proteins associated with both SSB intake and T2D, a role of HGF in the development of insulin resistance and T2D has already been described (213). Circulating furin has previously been found to be associated with higher T2D incidence in the MDC-CC (214) and, contrastingly, furin has been shown to be essential for β-cell function (215). Furin has also been suggested to contribute to atherosclerosis development (216). CHI3L1, also known as YKL-40, is known to be a marker of CVD (217, 218), and may perhaps be particularly involved in the vascular complications of T2D (219). Circulating levels of tPA have also previously been found to be associated with CVD incidence (220), and is well known to be involved in thrombolysis is it for long has been used as treatment for a variety of thrombotic conditions (221). Furthermore, resistin, which was found to be associated with higher added sugar intake and T2D, has also been observed to associate with CVD in previous studies (222), while CD40L, which was negatively associated with added sugar intake but positively associated with T2D, has been suggested to mediate the link between inflammation and CVD (223, 224). The inverse association observed between added sugar intake and CD40L in Paper V, may once again indicate the U-shaped association between added sugar intake and cardiometabolic risk.

On the other hand, just as an example, the protein growth differentiation factor 15 (GDF15), which has consistently been recognized to be associated with T2D and CVD

in proteome-wide association studies (222, 225, 226), as well as in the MDC-CC (227), was not found to be associated with added sugar or SSB intake in Paper V. The same can be said about several other proteins. This may indicate either that added sugar and SSB intake are not that strongly involved in the development of cardiometabolic disease after all or at least that they likely are not involved in the development via a mechanism involving GDF15. Hence, looking within the plasma proteome may provide us extended knowledge through which pathways the intake of added sugar and SSBs may, or may not, affect cardiometabolic risk, and future research is needed to further investigate the potential actual effect of added sugar and SSB intake and the various proteins identified in Paper V.

## Methodological strengths and limitations

An important strength of this thesis work is the large size of the MDC and NSHDS cohorts. However, these cohorts are rather old, and the collected dietary data may not be representative of today's dietary habits. This limitation is important and may hamper generalization. However, especially in the study of added sugar intake, it might actually be an advantage that the cohort is not too recent. It is reasonable to suggest that underreporting of added sugar intake specifically would be more pronounced today than 25 years ago because of a higher public awareness of the possible effects of a high sugar intake currently. When the MDC and NSHDS were conducted, it is plausible that fat intake was predominantly underreported, considering findings from a Danish study in which the authors concluded that higher underreporting of fat occurred in 1993-94 than in 1987-88, likely as a consequence of increased awareness (228). The same problem may be present for added sugar instead of fat in today's studies, hypothetically such as in the more recent MOS and PREVIEW. The potential underreporting of primarily fat in the MDC may also be a factor contributing to the observed increased mortality in the lowest treat intake category in Paper I, as treats are generally high in both sugar and fat. Furthermore, the dietary data collection in the MDC was very advanced and thorough, as it combined a 7-day food diary, an FFQ and a dietary interview in what can be considered a modified diet history method.

The MOS has the advantage of being more recent and having deeper data collection including urine and fecal samples, that has been highly used in the work of this thesis. However, thus far, we have been limited to conducting only cross-sectional analyses in the MOS, since the follow-up yet is not long enough to study incidence outcomes.

As in most cohort studies, the generalizability of the results can be questioned due to selective participation. It is well established that cohort populations in general are not perfectly representative, as study participants tend to be healthier than the source population (health-conscious individuals are generally more interested in participating

in clinical studies). This is likely true in all studied cohorts and has been fully mapped out in the MDC, showing that MDC participants had lower cancer incidence and mortality than nonparticipants (229).

In most studies of this thesis, we have estimated the intake of added sugars by assuming that added sugars are only in the form of sucrose, fructose, glucose or galactose (sucrose + monosaccharides), and that fruits, berries, vegetables and juices are the only natural sources of these particular sugars. An assumption is certainly just an assumption, and may not always hold completely true. The main limitation of this estimation comes from the foods that may contain naturally occurring sugars from fruits and vegetables, but as a whole, they would not be classified as fruits or vegetables, such as jams, marmalades, fruit pies and fruit yogurts. The natural sucrose and monosaccharides in such foods have not been subtracted in this estimation; however, those amounts are generally small in comparison to the amounts of added sugars in such foods. We also estimated the average sugar content in entire food groups (fruits and berries, vegetables, and juices) when we subtracted the naturally occurring sugars, but in fact, the sugar content varied greatly between foods within these food groups. Additionally, all this is based on measurements of varying quality of sucrose and monosaccharide contents in foods from food composition databases.

The PREVIEW study is a very resourceful RCT considering its length, sample size and amount of data collected. However, the general advantages of RCTs in comparison to observational studies were not utilized in this particular study (Paper III), as this investigation was basically an observational study within a randomized weight loss study. Nevertheless, the fact that there was no difference in effects between the intervention groups made it easier to study the entire study population jointly (151). Additionally, the fact that the adherence to the interventions has been observed to not be perfect is actually not is not a major problem in Paper III, considering that the examined exposure (sugar intake) was not actually a part of the intervention diets. Limited adherence could instead have provided enough variation in sugar intake to enable the comparisons made in Paper III.

A major limitation in regard to the investigations of the urinary sucrose and fructose biomarker is that we had no ability to properly validate the biomarkers in any of the studies (Paper II and III). We could study only correlations between the biomarker values and self-reported sugar intake. Therefore, we cannot conclude much more than that the correlation coefficients using overnight urine samples ire similar, but in the lower range, to those from previous observations of 24-h urine samples, and that the correlation coefficients are somewhat lower when using 24-h excretions in a population with prediabetes than previous observations in healthy individuals.

Another limitation worth noting is that this thesis has focused on the role of sugar in adults and not in children and adolescents. The role of sugar in cardiometabolic risk in children found in the literature might differ from that portrayed in adults in this thesis.

Overall, all papers in this thesis are limited by their observational study design, which limits any conclusions being drawn about the potential causality of the associations. Although the regression analyses were adjusted for potential confounding factors, there is likely still residual confounding introducing some bias.

# Conclusions

Taken together, the findings presented in this thesis contributes to the understanding of the potential association between sugar intake and cardiometabolic risk by investigating new plausible physiological pathways for such a potential association and evaluating a biomarker for objectively measuring sugar intake to improve future investigations of such a potential association. To conclude, on the basis of the findings of this entire thesis, the following can be stated:

1. The total intake of added sugars showed a U-shaped association with all-cause and cardiovascular mortality, T2D incidence and CRP, and was not linearly associated with any aspect of the gut microbiota or T2D-related plasma proteins.
2. The intake of SSBs was consistently associated with higher cardiometabolic risk, as it was observed to be linearly associated with higher all-cause mortality, a plausible obesity-related gut microbiota composition and a T2D-related proteomic plasma profile.
3. The urinary sucrose and fructose biomarker correlated modestly with self-reported added sugar intake and is likely a useful complement to self-reported sugar intake even when measured in overnight urine samples, rather than in 24-h urine samples, as previously validated. The composite measure of the overnight urinary sucrose and fructose and self-reported sugar intake associated with adverse cardiometabolic health in women.
4. The 24-h urinary sucrose and fructose biomarker was weakly correlated with self-reported total sugar intake and the fructose excretion appeared elevated in individuals with prediabetes. This biomarker should be used with caution to estimate sugar intake in prediabetic populations, as impaired metabolic status might distort the relationship between ingested and excreted sucrose and fructose.

Unlike SSB intake, the total intake of added sugar remains not clearly associated with an increased cardiometabolic risk. However, the implication is still that a general reduction in added sugar intake is encouraged despite the U-shaped associations observed, considering the study design limitations and that there are no known benefits to consuming added sugar.

# Public health perspective

Nevertheless, does it matter whether we have sufficient evidence in regards to the effects of added sugar intake on health? Even if we do not have sufficient evidence, we can make the general recommendation to reduce added sugar intake, because there are no known benefits to consume added sugar. Well, the answer is that we need a solid evidence base to be able to:

1. Set evidence-based recommendations. *If nutritional recommendations are not supported by evidence, they will be received with scepticism and plausibly insufficient adherence.*
2. Counterbalance the lobbying from the food industry. *Currently, if there is even the smallest loophole in the evidence, those with a conflict of interest will take advantage of it.*
3. Set the correct public health strategies. *For example, should we have a tax on all sugar or only on SSBs? How much should the tax be? Should all added sugars in food products be replaced with low-calorie sweeteners?*

## Sugar intake recommendations

An important implication of nutrition research is to form the basis of nutritional recommendations. Therefore, a central question is as follows: What sugar intake recommendation should we make?

However, should the sugar recommendations be based solely on the evidence we have of effects of certain levels of sugar intake on the risk of diseases and/or their risk factors? Alternatively, should the recommendations be based on theoretical reasoning and modeling, such as, *if we reduce sugar consumption further, we have room for more nutritiously dense foods within our energy needs and the population will gain less weight and be healthier*?

It appears as if such a discrepancy of what ground to base the sugar recommendations on may have recently been given a practical example. In the recently published DGA 2020, the US Departments of Agriculture (USDA) and Health and Human Services (HHS) set the recommended maximum intake of added sugars to the same as previous years, 10E% (20), although the Dietary Guidelines Advisory Committee, which was

assigned to examine the evidence on specific nutrition and public health topics and provide independent scientific advice to the USDA and HHS, had recommended a reduction of the recommendation to 6E% (230). The Dietary Guidelines Advisory Committee based their lowered recommendation on that "evidence suggests that adverse effects of added sugars, particularly from SSB, may contribute to unhealthy weight gain and obesity-related health outcomes" and "less than 6E% from added sugars is more consistent with a dietary pattern that is nutritionally adequate while avoiding excess energy intake from added sugars than is a pattern with less than 10E% from added sugars" (230). The USDA and HHS decided against this recommendation and to keep the 10E% recommendation from 2015 on the basis of "evidence of detrimental effects of added sugar on a variety of health outcomes" (231). The evidence that the USDA and HHS refers to here is likely in part from the systematic review on added sugar intake and the risk of CVD (T2D or measures of glycemic control were not evaluated) conducted by the Dietary Guidelines Advisory Committee within the preparatory work for the update of these dietary guidelines (232). They concluded that there was insufficient evidence for all studied outcomes except for cardiovascular mortality, where the evidence was limited for increased risk. Further in line with the results of this thesis, the conclusion of this systematic review encourages future research to "Distinguish between food and beverage sources of added sugars when conducting intervention or assessing exposure" (232). From my perspective, it appears as if the Dietary Guidelines Advisory Committee supported their advice primarily based on theoretical modeling, but the recommendation set by the USDA and HHS seems to rather be based on evidence on health outcomes, i.e., as if the two different bodies had not agreed on what should lay the groundwork for the recommendations.

The findings of this thesis are in agreement with the USDA and HHS conclusion, that the hard evidence for a lower added sugar recommendation than 10E% is not solid. However, there are, theoretically, no potential reasons for why a population would not benefit further from reducing the added sugar intake below 10E% to, for example, 6E%. As stated, added sugar does not contribute with anything valuable, other than perhaps pleasure and joy (which maybe should not be neglected) there are no losses of reducing intake further. I believe the population could benefit from a reduced added sugar intake, in spite of the many U-shaped curves that have been produced of the associations between added sugar intake and cardiometabolic risk in this thesis, for reasons that have been described in the chapter named *The U-shaped Associations* (page 90). Therefore, here the question comes down to the following: should the recommendations actually be only evidence-based? According to a recently published perspective by members of the Dietary Guidelines Advisory Committee, the recommendation set by the USDA constitutes "a missed opportunity to send a stronger message about the value of reducing of added sugars" (231).

The EFSA is currently conducting a new review with the aim of setting a tolerable upper intake level for total sugar, added sugar and/or free sugar, which is to be determined by identifying a specified level of exposure where the probability of an adverse effect is elevated (233). A draft of the EFSA statement is currently out for public consultation (as of July 22, 2021), and the preliminary conclusion is that the available data did not allow the setting of a tolerable upper intake level, but that the intake of added sugar should be as low as possible (234). Furthermore, the NNR are currently being updated to be published in 2022 and it will be interesting to see what added sugar intake recommendation will be agreed upon for the Nordic countries.

## Food industry lobbying

The sugar research field likely ranks as one of the top areas in which the food industry has taken the liberty to skew the published research. Large companies in the food industry, such as the Coca-Cola Company or PepsiCo, are very active in supporting research. Likewise, just as often we are faced with lobbying from the other end of the spectra, from those who have benefits to gain from carbohydrates and sugars being blamed as the main culprit for poor public health. The involvement of the food industry in research has skewed the evidence in the pool of published literature to their advantage (235). It has been shown that published systematic reviews investigating the relationship between SSB intake and body weight were less likely to conclude a harmful effect if they were sponsored by the food industry (236, 237). It is terrifying to see how far the industry is willing to go to make money to the detriment of the health of the population. Public (and planetary) health would seemingly be given better chances if the entire food industry system were collaborating with the primary focus on improving health (and the environment), rather than fighting over profit. Unfortunately, profit is also necessary for maintaining healthy populations – a perfect catch-22 situation.

## Taxation policies

One popular strategy to reduce the consumption of added sugars is to additionally tax foods and beverages high in added sugars. In recent years, close to 50 countries or states (USA) have introduced a tax on sugars or SSBs in one way or another. The most common strategy is to focus on SSBs specifically because we have the most solid evidence of harmful effects for SSBs. However, other strategies exist, where, for example, all foods high in added sugar or energy-dense foods are taxed (238). Since 2017, the WHO has officially encouraged taxes on SSBs to be implemented (239). It is only very recently that enough SSB taxes have been implemented long enough to enable proper evaluation of the effect that these SSB taxes have had. In general, the SSB taxes have been shown to be effective, but unless the taxation is carefully designed,

substitution behaviors may distort the effects of these taxes if there are possibilities for replacement by other nontaxed sugary foods and beverages, or for other purchases somehow exempt from taxation (240).

The Nordic countries have a long tradition in taxing unhealthy foods, among which Sweden stands out for never having had a tax on SSBs; however, the Swedish Cancer Foundation is currently raising the question of introducing an SSB tax in Sweden (241).

### Low-calorie sweeteners

The substitution principle in dietary research have been introduced in the *Introduction* of this thesis (page 35), meaning that if consumption is reduced of one food group or nutrient, it ought to be replaced by something else. Therefore, a discussion of reductions in added sugar consumption would be incomplete without the consideration of what may be consumed in its place. An obvious alternative is low-calorie sweeteners.

Low-calorie sweeteners, and more specifically artificial sweeteners, are believed by some in the general public to be as harmful as consumption of added sugars. Surely, some epidemiological investigations have found associations between the intake of ASBs and an increased risk of T2D (242, 243), CVD (244, 245) and mortality (246, 247). However, a clear problem in all of these studies is the likelihood of reversed causation (242), which has been discussed in *The U-shaped Associations* chapter (page 90). If one consumes high amounts of low-calorie sweeteners, it is likely that this is a consequence of an already increased cardiometabolic risk, i.e., it is a dietary choice to try reduce one's risk. Hence, a positive association between low-calorie sweetener intake and cardiometabolic risk is possibly not due to causation, but rather reverse causation. Epidemiological substitution models have indicated that the substitution of SSBs with artificially sweetened beverages is associated with reduced body weight (248), T2D (249, 250) and CVD (251), while other investigations have not been able to draw such conclusions (252). In existing experimental studies, however, there is no support for suggesting that low-calorie sweeteners can increase the risk for cardiometabolic diseases (253-255). However, large and comprehensive studies are lacking, just as for added sugars. Furthermore, a common assumption that is often made but requires reconsidering within the research on low-calories sweeteners is that various low-calorie sweeteners can be treated equally and studied jointly; in fact, they may be very different and very differently associated with disease (256).

A dilemma worth mentioning is found in the example of the recently adopted SSB tax in the UK and the new food labeling regulations in Chile. The SSB tax in the UK differ slightly from the majority of such public health actions, as it was primarily designed to

generate changes in the food industry rather than changes in consumer behavior (257). The new regulations in Chile have also resulted in major changes in the food supply by the food industry (258). In both the UK and Chile, significant reductions in added sugar content in the food supply have occurred due to a country-wide substitution with low-calorie sweeteners. Currently, in the UK, the majority of sodas contain a maximum of 5 g sugar per 100 g, and the remaining is sweetened with low-calorie sweeteners to remain below the threshold sugar content for increased taxation. Consequently, UK inhabitants no longer have the right to choose for themselves whether to consume sugar-sweetened or artificially sweetened beverages (they can of course always choose to drink water, but if people were willing to choose this option, this discussion would not be necessary). The same issue exists in Chile, where the new regulations have been described as "a threat to consumers' free choice" (259). These wide actions contrast a policy statement by the British Dietetic Association, which states that the available artificial sweeteners are safe to consume and may assist in the management of conditions such as obesity and diabetes mellitus, but a tailored individualized approach is required, and recommendations should be given on a case-by-case basis (260). Only a few evaluations of the effects of the new regulations in the UK have been published thus far, and they indicate that there has not been a reduction in SSB intake, but a reduced sugar intake (261). To my knowledge, no proper evaluation of the effects on the consumption of low-calorie sweeteners in the UK has been published yet.

For reasons such as this, it is truly valuable to know the true harms resulting from high added sugar intake. I think that the use of low-calorie sweeteners can be beneficial for curbing the public health issue of obesity; however, if individuals do not have the right to choose between eating something that we have some evidence may not be beneficial or something that is even less studied, we might be moving too fast. In my opinion, only solid evidence of actual harmful effects from added sugars (which we, nonetheless, have for SSBs specifically) can support these public health actions, and the argument that the intake of added sugar provides nothing beneficial, only empty calories, is not enough to support actions of unavoidable substitution with food ingredients that are even less studied than added sugars.

# Future perspectives

To understand the role of sugar intake in cardiometabolic risk, more studies are needed. Well-planned and well-conducted long RCTs are warranted to ultimately answer the question of whether sugar intake causes cardiometabolic disease. Preferably these studies would include both isocaloric and ad libitum trials of various doses, as well as trials differentiating between solid and liquid sources of sugar. Future trials are also needed to determine whether various low-calorie sweeteners are actually a better option for long-term consumption. These trials will be very difficult, but not impossible, and I believe they will be worth the effort.

The urinary sucrose and fructose biomarker has great potential to improve epidemiological research on sugar intake. However, proper validation studies of the use of this biomarker in non-24-h samples are needed, as well as studies clarifying the relation between sugar intake and circulating and urinary sucrose and fructose in individuals with prediabetes, diabetes and other specific population groups. This means conducting well-controlled feeding studies of various sugar doses so that proper dose-response relationships can be determined. For the purpose of improving epidemiological studies of sugar intake and cardiometabolic risk, studies from the USA and other places around the world where the added sugars primarily come from sugar cane and corn (rather than sugar beets, as in Europe) have the advantage that they can study stable carbon isotope ratios as biomarkers of sugar intake in a large variety of biological samples (262-264), as these carbon isotope ratios theoretically should not be affected by a metabolically impaired status. Another new but promising alternative to objectively measure sugar intake is to study the oral microbiome (265), but this methodology requires further research before it can be utilized to improve the research on sugar intake and cardiometabolic risk.

To enable improved future epidemiological studies that utilize biomarker-measured sugar intake and to obtain a deeper mechanistic understanding by investigating not only the gut microbiome and the plasma proteome but also the metabolome, transcriptome and much more, future cohort studies must be designed to collect samples of blood, urine and feces at several timepoints. Future cohort studies should also invest in performing multiple dietary collections, to increase sensitivity to the ever-changing trends in society today.

Ultimately, to fully understand the role of sugar intake in health, we must also steer the research focus toward understanding the determinants of our sugar intake. For example, genetic variation may influence our sweet taste sensitivity (the *TAS1R3* and *TAS1R2* genes (266, 267)) and preference (the *FGF21* gene (268)), and the hormonal and neural influence on our preference for sweet taste is still to be fully understood. Further research exploring this is crucial to an understanding of why sugar intake can be difficult to control for some people, but not for others, so that one day we can potentially tackle this issue with more tailored and personalized dietary advice. The identification of genetic variants that determine our sugar intake is also of value for Mendelian randomization studies to investigate the causal role of our sugar intake in cardiometabolic disease risk. More ideal would be the identification of genetic variants that are associated with objectively measured sugar intake, rather than relying on self-reported sugar intake.

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This PhD thesis focused on epidemiologically studying the role of sugar intake in the risk of cardiometabolic diseases. In detail, differences between the total of intake added sugar and intake of different added sugar sources were investigated, the objective urinary sucrose and fructose biomarkers of sugar intake were evaluated and new physiological pathways in which sugar intake could possibly affect cardiometabolic risk, such as via the gut microbiota and the plasma proteome, were explored.

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