Sugar intake and cardiovascular disease risk: With an emphasis on genetic and metabolomic biomarkers of sugar intake

Janzi, Suzanne

2025

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With an emphasis on genetic and metabolomic biomarkers of sugar intake

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LUND
UNIVERSITY

DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Medicine at Lund University to be publicly defended on 30th of May at 13.00 in Agardhsalen, Clinical Research Center, Jan Waldenströms gata 35, Malmö

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Organization: LUND UNIVERSITY

Document name: Doctoral Dissertation **Date of issue** May 30th 2025

Author(s): Suzanne Janzi **Sponsoring organization:**

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Aim: The aim of this thesis was to investigate the associations between added and free sugar intake, as well as intake of various sugar-sweetened foods and beverages, and cardiovascular disease risk. Further, this thesis aimed to identify genetic and plasma metabolite markers of sugar intake to be used as objective markers of sugar intake.

Method: Prospective associations between added and free sugar intake, sugar-sweetened foods and beverages, and cardiovascular disease incidence were studied in the Malmö Diet and Cancer study (n=25,877), and the Swedish mammography cohort combined with the cohort of Swedish men (n=69,705). Genetic markers of sugar intake were studied in the Malmö Diet and Cancer study (n=25,660) and the UK biobank (n=141,837), including both a replication study and genome-wide association studies. Finally, plasma metabolite profiles of sugar intake were identified in the Malmö Diet and Cancer study (n=830).

Results: High sugar intake was linked to higher ischemic stroke risk, but the highest risk for most studied outcomes was found in the lowest sugar intake group. Sugar-sweetened beverage intake consistently showed increased cardiovascular disease risk, while treats showed a negative association. Genetic variants in the *FTO* gene, an intergenic region on chromosome 18, and near the *FGF21* gene on chromosome 19 are linked to sugar intake, with only the *FGF21* associations being independent of education, smoking, and BMI. Previously identified genetic variants in sweet-taste receptor and glucose transporter genes were not replicated. Finally, distinct metabolite profiles for various categories of habitual consumption of sugar and sugar-sweetened foods and beverages were characterized.

Conclusion: Based on the findings of this thesis, it is still difficult to conclude a clear increased risk of cardiovascular disease incidence associated with added or free sugar intake as the shape and directions of the associations vary between different cardiovascular diseases. The results did however consistently show positive linear associations between sugar-sweetened beverage intake and cardiovascular disease risk, while conversely, showing negative linear associations for intake of treats and cardiovascular disease risk. Future evaluation of these associations can be aided using the genetic and metabolomic markers of sugar intake identified in this thesis, but they should be validated in other populations first.

Key words: Added sugar intake, dietary sugar intake, cardiovascular disease, metabolomics, genomics, sugar biomarkers

Language English **Number of pages**: 99

ISSN: 1652-8220 **ISBN:** 978-91-8021-719-4

Signature Date 2025-04-15

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ISBN 978-91-8021-719-4
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2025

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Abstract

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Populärvetenskaplig sammanfattning

Till skillnad från den bild som ofta uppmålas i media är det faktiskt inte en självklarhet att konsumtion av socker är skadlig för hjärt- och kärlhälsa. Faktum är att inte särskilt många studier har undersökt sambandet, och de fåtal som gjorts visar motstridiga resultat. Dock vet vi att konsumtion av läsk och andra sockersötade drycker har skadliga effekter på vår hälsa. Frågan kvarstår om sambanden man sett för sockersötade drycker gäller även för andra källor av tillsatt socker, samt för sockerintag i allmänhet. Det är dock svårt att få en korrekt bild av personers sockerkonsumtion, samt att skilja påverkan av själva sockret i en kost från andra kost- och livsstilsfaktorer. Detta leder till att vi behöver använda förbättrade metoder för att studera sambanden mellan sockerintag och risk att insjukna i hjärt- och kärlsjukdom. I den här avhandlingen studerar vi sambanden mellan intag av socker och olika sockersötade livsmedel, och utvecklar sätt att förstå sambanden bättre med hjälp av genetik och metaboliter.

I avhandlingens studie av tre svenska kohorter med totalt nästan 100,000 deltagare visade sig sambanden mellan sockerintag och hjärt- och kärlsjukdom skilja sig mellan olika sockersötade livsmedel: Sockersötade drycker tycks öka risken medan högre intag av sötsaker som kakor, choklad, och glass var kopplat till lägre risk att utveckla hjärt- och kärlsjukdom. Att intag av sötsaker var kopplat till lägre risk för hjärt- och kärlsjukdom skulle kunna förklaras av den svenska fika-traditionen. Fika, som ofta involverar sociala samlingar och konsumtion av sötsaker, kan fungera som en indikator på ett aktivt socialt liv. Ett rikt socialt liv har visats skydda mot ett flertal sjukdomar, och det är möjligt att fika agerar som en markör för dessa sociala faktorer. För tillsatt socker i allmänhet tycks sambanden skilja sig beroende på vilken hjärt- och kärlsjukdom vi tittar på. Tillsatt socker var kopplat till en ökad risk av ischemisk stroke och bukaortaaneurysm medan den största risken för de flesta hjärt- och kärlsjukdomarna fanns hos de med lägst sockerintag. Dessa resultat är svårtolkade och svårförklarade, och kan förklaras av annat än sanna direkta effekter. Exempelvis kan sambanden påverkas av andra faktorer som BMI, samt felrapportering av kostintag till följd av att studiedeltagare ofta underskattar sitt matintag, särskilt när det gäller mindre hälsosamma livsmedel som är rika på socker. Därför är objektiva mätningar av sockerintag nödvändiga för att förbättra forskningen och ge insyn kring huruvida dessa effekter beror på själva sockerintaget.

Vi undersökte den genetiska bakgrunden till högre sockerintag i en studie från Sverige och en studie från Storbritannien, med totalt nästan 170,000 deltagare, och använde resultaten för att studera genetiska samband mellan sockerintag och hjärt- och kärlsjukdom. I den här studien försökte vi isolera genetiska varianter som är direkt kopplade till sockerintag och inte genom exempelvis BMI eller diverse livsstilsfaktorer. Resultaten pekar mot att högre intag av fritt socker faktiskt är kopplat till en högre risk att drabbas av ischemisk stroke, och ger en indikation på en möjlig mekanism bakom sambandet eftersom det också var kopplat till lägre nivåer av det goda HDL kolesterolet och högre nivåer av triglycerider. Vi tog även fram en profil av blodmetaboliter som är kopplad till ett högre sockerintag.

Sammanfattningsvis tyder fynden i denna avhandling på att ett högre intag av tillsatt socker kan öka risken för hjärt- och kärlsjukdomar. Sambanden varierar avsevärt mellan olika sockersötade livsmedel, där sockersötade drycker är den källan till tillsatt socker som ökar risken för hjärt- och kärlsjukdomar mest. Slutligen karaktäriserade vi högkonsumenter av socker med avseende på genetik och metaboliter, som skulle kunna användas som objektiva markörer för sockerintag om de kan valideras i andra populationer.

Abbreviations

BMI	Body Mass Index
CI	Confidence interval
COSM	Cohort of Swedish Men
CVD	Cardiovascular disease
E%	Percentage of energy intake
EFSA	European Food Safety Authority
FGF21	Fibroblast growth factor 21
FTO	Fat Mass and Obesity-Related Gene
HDL	High-density lipoprotein
HR	Hazard ratio
HRC	Haplotype Reference Consortium panel
ICD	International Classification of Diseases
LDL	Low-density lipoprotein
MAF	Minor allele frequency
MDC	Malmö Diet and Cancer study
MDC-CC	Malmö Diet and Cancer study cardiovascular cohort
NNR	Nordic Nutrition Recommendations
SMC	Swedish Mammography Cohort
SCAPIS	The Swedish CArdioPulmonary bioImage Study
SD	Standard deviation
SIMPLER	Swedish Infrastructure for Medical Population-Based Life-Course and Environmental Research
SNP	Single-nucleotide polymorphism
SSB	Sugar-sweetened beverages
UKB	UK biobank
WHO	World Health Organization

Papers included in thesis

Study 1 **Janzi S**, Ramne S, González-Padilla E, Johnson L, Sonestedt E. Associations Between Added Sugar Intake and Risk of Four Different Cardiovascular Diseases in a Swedish Population-Based Prospective Cohort Study. Front Nutr. 2020 Dec 23;7:603653. doi: 10.3389/fnut.2020.603653.

Study 2 **Janzi S**, González-Padilla E, Ramne S, Bergwall S, Borné Y, Sonestedt E. Added sugar intake and its associations with incidence of seven different cardiovascular diseases in 69,705 Swedish men and women. Front Public Health. 2024 Dec 9;12:1452085. doi: 10.3389/fpubh.2024.1452085.

Study 3 **Janzi S**, González-Padilla E, Najafi K, Ramne S, Ahlqvist E, Borné Y, Sonestedt E. Single Nucleotide Polymorphisms in Close Proximity to the *Fibroblast Growth Factor 21 (FGF21)* Gene Found to Be Associated with Sugar Intake in a Swedish Population. Nutrients. 2021 Nov 5;13(11):3954. doi: 10.3390/nu13113954.

Study 4 **Janzi S**, Kou M, Ramne S, Stubbendorff A, Borné Y, Qi L, Sonestedt E. Identifying genetic variants associated with sugar intake and appraising the genetic correlations with cardiovascular outcomes. (manuscript)

Study 5 **Janzi S**, Johansson OF, Borné Y, Sonestedt E. Plasma metabolite profiles of habitual sugar consumption in a Swedish cohort. (manuscript)

Papers not included in thesis

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Acknowledgements

First and foremost, I wish to thank my supervisors **Emily** and **Yan** for guiding me through this journey. I could not have asked for better mentors! I also wish to thank my former and current colleagues **Anna**, **Kjell**, **Esther**, **Stina**, **Mary**, and **Joana** for all the great insights and inspiration you've sent my way. It is amazing having a team to share my love for nutrition research with.

I also wish to thank my family for all your support and encouragement. **Mum** and **dad**, thank you for believing in my abilities long before I ever did. Thank you to my siblings **Magda, Akram, Adam**, and "the kids", **Elsa, Ellen, Alex**, and **Julia**, as well as my extended family **Thomas**, **Camilla**, and **Julia**. You're the best!

David and **Hilma**, words feel superfluous. You're my everything and part of all that I do and achieve. I love you!

Finally, I want to thank my coming daughter **Alina**. I feel so blessed having had you with me during the last part of this doctoral journey, always keeping me company and encouraging me with your gentle little kicks. See you soon my lovely girl, I can't wait to embark on the next journey with you in my arms.

Background

Dietary sugars

What are sugars? Sugars typically refer to the smallest components of carbohydrates. Sugars consisting of only one sugar-molecule (e.g., glucose, fructose, and galactose) are called monosaccharides, whereas sugars that consist of two paired sugar molecules (e.g., sucrose, lactose, and maltose) are called disaccharides (**Figure 1**). Different sugars vary in their sweetness and how they are metabolized (1-3). Monosaccharides and disaccharides are commonly referred to as simple carbohydrates, whereas polysaccharides, or complex carbohydrates, can be made up of hundreds or thousands of sugar molecules which cause them to have vastly different properties. When discussing dietary sugar intake, we typically refer to intake of the simple carbohydrates (i.e., sugars).

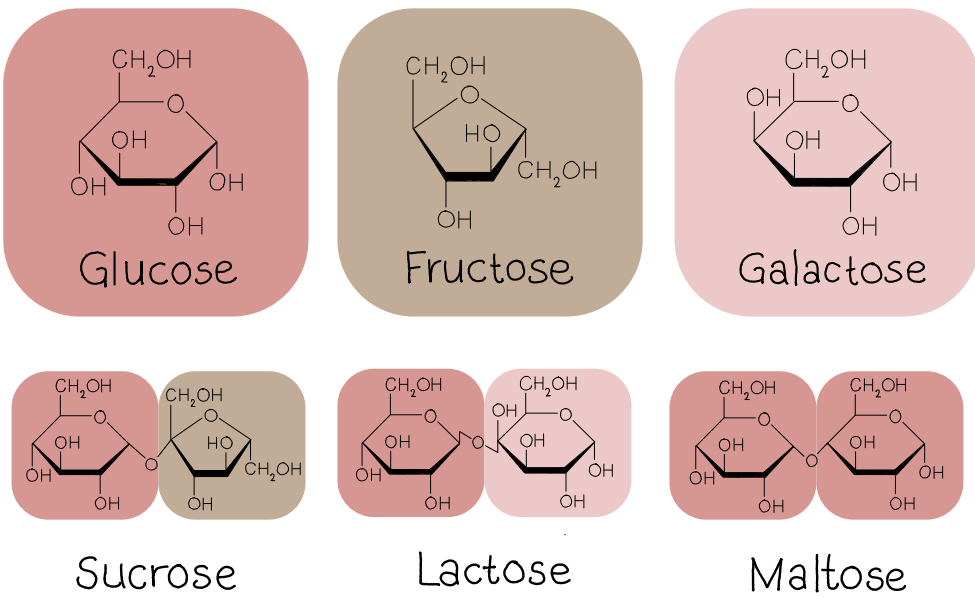


Figure 1. Most common simple carbohydrates (i.e., sugars).

Dietary sugar subgroups

In foods, the composition of dietary sugars varies, thus affecting their taste, consumption patterns, and physiological effects. *Total sugar* intake refers to all sugars in the diet, coming from any source. *Added sugar* comprises all refined mono- and disaccharides that are used as such or added during manufacturing or cooking processes. *Free sugar* varies in its definition, but the European Food safety Authority (EFSA) defines it as including all added sugars as well as naturally occurring sugars in honey, syrups, fruit juices, and fruit juice concentrates (1, 4). Many guidelines and recommendations for sugar intake are based on added or free sugar intake, and the available body of evidence of adverse health effects tend to be stronger for added and free sugar intake than for total sugar intake. *Sweet-tasting sugars* is not an established term but something that was studied in this thesis, including all monosaccharides as well as sucrose, coming from any source (**Figure 2**). Sucrose is a disaccharide which is commonly used as an added sugar but also occurs naturally in fruit and vegetables. Unlike other disaccharides with a less sweet taste (such as lactose) (3), sucrose consumption is more likely to be influenced by personal factors such as sweet taste sensitivity and preference.

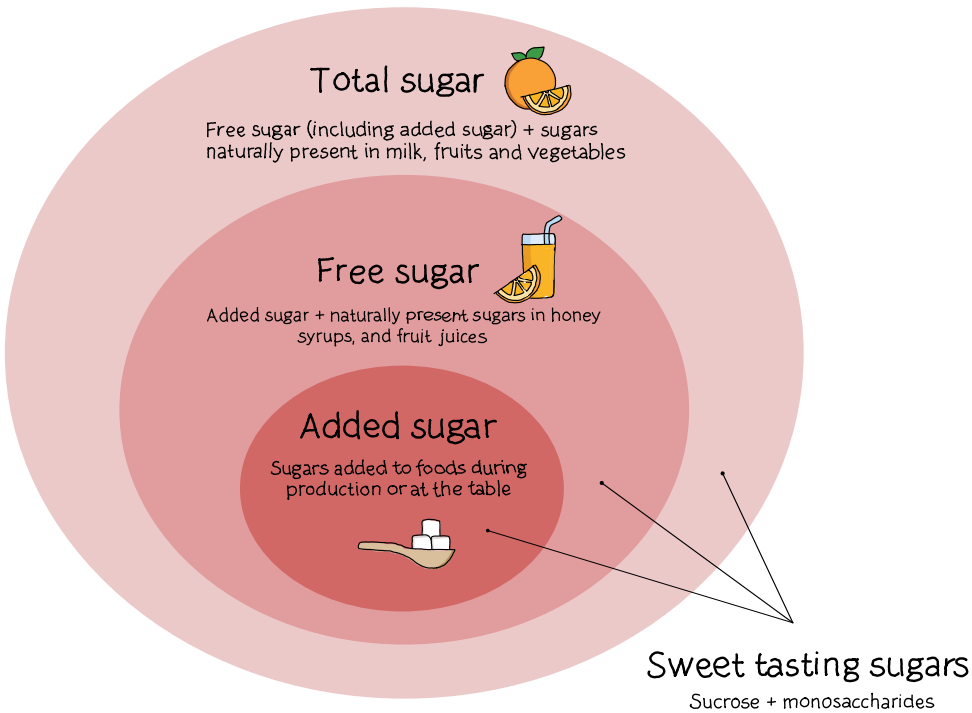


Figure 2. Definitions of different sugar subgroups.

Sugar-sweetened foods and beverages

Sugars have different properties, and the same is true for different sources of added sugar. Prior research has suggested that the format in which added sugars are "packaged" affects consumption patterns and physiological effects. For example, there seems to be a difference in the metabolism of solid and liquid sugars, where liquid sugars are believed to give lower satiety than solid sugars, facilitating overconsumption (5). This thesis has a focus on three groups of sugar-sweetened foods and beverages: treats, toppings, and sugar-sweetened beverages (**Figure 3**). *Treats* include chocolate, sweets, ice cream, pastries, and cakes. One could hypothesise that these foods are more likely to be consumed in a social context and during special occasions, and might be consumed in large quantities due to the balanced fat-sugar content (6, 7). *Toppings* include table sugar, honey, jams, and marmalades. Toppings are typically consumed as a condiment to various other foods, thus the overall food matrix associated with toppings intake may to an extent reflect consumption of those foods. One could also hypothesize that the risk of overconsuming toppings may be lower than for treats. *Sugar-sweetened beverages* include all sweetened sodas and fruit drinks except for pure fruit juices. A lot of research on added sugar intake has been carried out specifically on sugar-sweetened beverage intake rather than overall added sugar or free sugar intake or other sources added sugar.

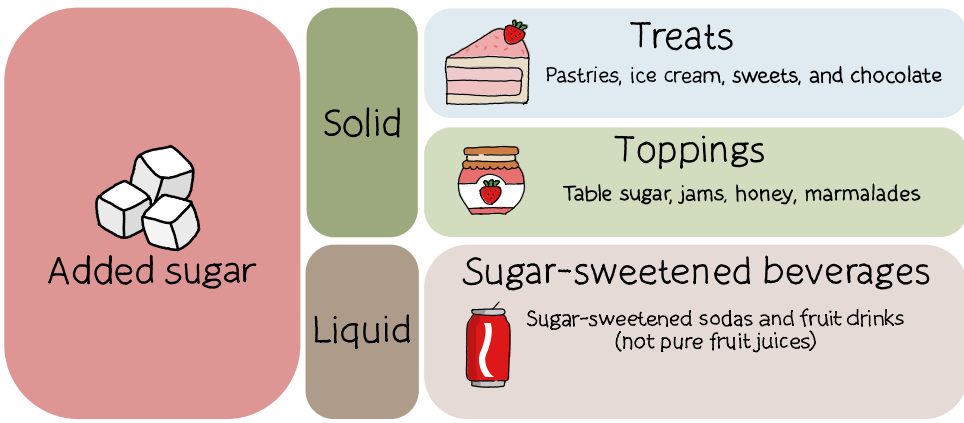


Figure 3. Different sources of added sugar studied in this thesis.

Sugar consumption trends

Consumption of sugar in Europe increased from the 1700s and in particular in the 1800s following the industrialization of food production when for example the process to extract sugar from sugar beets instead of sugar cane was developed. The largest elevation in sugar consumptin in Europe, as well as North America, occurred between the 1970s and 2000, and the increase in North America was characterized by increased use of high-fructose corn syrup (8). In Sweden, the total consumption of sugar (including all refined sugars and syrups) has generally decreased since year 1995, but an increase can be seen after 2020, from 37 kg/per capita in 2020 to 43 kg/per capita in 2023 (**Figure 4**).

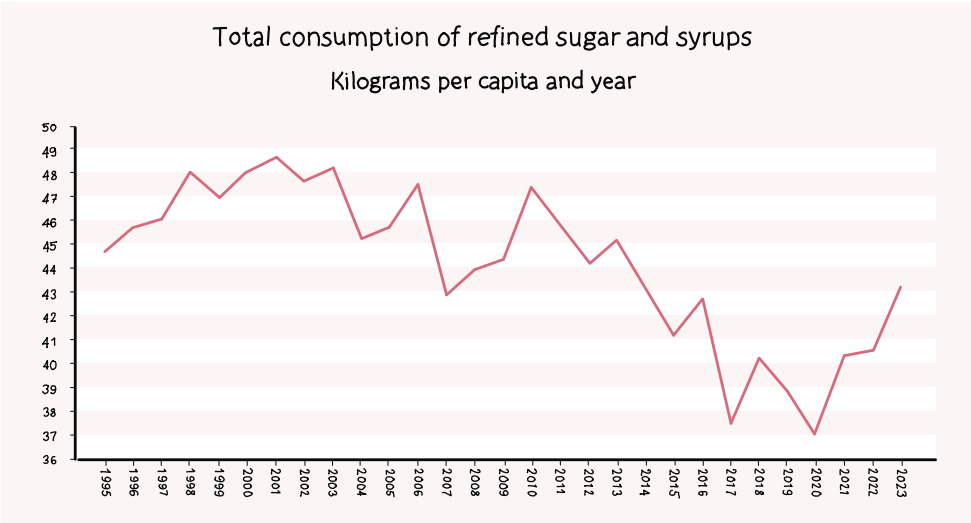


Figure 4. Total consumption of refined sugar and syrups in Sweden per capita and year between the years 1995-2023. Data source: Jordbruksverket (9).

In European countries, the primary sources of added and free sugars include confectionery, table sugar, honey, syrups, and water-based sweet desserts, followed by beverages such as sugar-sweetened beverages and fruit juices (10, 11). Data from a Swedish nation-wide survey from 2010-2011, showed that an estimated 24% of the mean added sugar intake among adults came from sugar-sweetened beverages, followed by 23% coming from table sugar and similar, confectionery and water-based sweet desserts (10, 12).

In Sweden the consumption trends over time vary between different sugar-sweetened foods, where for example a fairly steady increase has been seen for chocolate and sweets between 1960-2023, whereas the consumption trends of marmalades and jams, and ice cream have been less clear. For pastries and cakes, the consumption decreased from 1960-1985, and then increased 1985-2023 (9) (**Figure 5**).

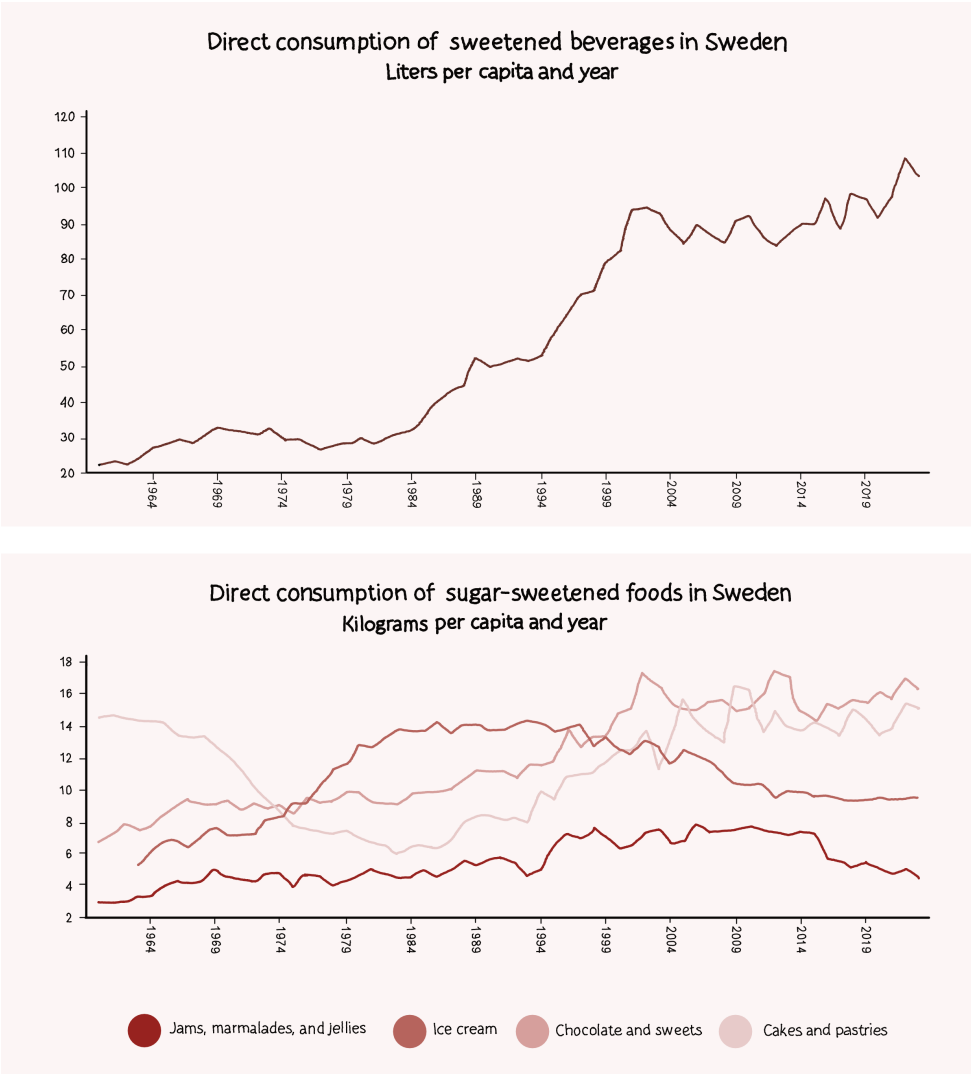


Figure 5. Direct consumption of sweetened beverages and sugar-sweetened foods in Sweden per capita and year between the years 1960-2023. Data source: Jordbruksverket (9).

Sugar intake recommendations

Sugar intake guidelines vary between different authoritative bodies globally, both in terms of recommendation thresholds and the basis of the guidelines, with some being based primarily on micronutrient dilution (i.e., the displacement of nutrient-dense food intake by overconsumption of energy-dense foods low in nutrients), while others are based mainly on the established risks of caries and overweight associated with sugar intake (10). Further, there is currently no consensus regarding what types of sugars should be focused on in research or dietary guidelines (4). The Nordic Nutrition Recommendations (NNR) recommend limiting free sugar and added sugar intake to less than 10% of energy intake (E%), due to micronutrient dilution the increased risks of developing chronic metabolic diseases such as obesity, dyslipidaemia, and dental caries associated with high free sugar intake (13, 14). The American dietary guidelines make a similar recommendation for added sugar intake based on the risk micronutrient dilution as well as increased risks of overweight and obesity as well as related chronic metabolic diseases (15). The World Health Organization (WHO) makes a similar recommendation for free sugar intake based on the risk of dental caries and overweight, and further suggests reducing the intake to less than 5 E% for additional benefits for dental health (16).

Table 1. A summary of some of the current nutritional advice issued during the past decade.

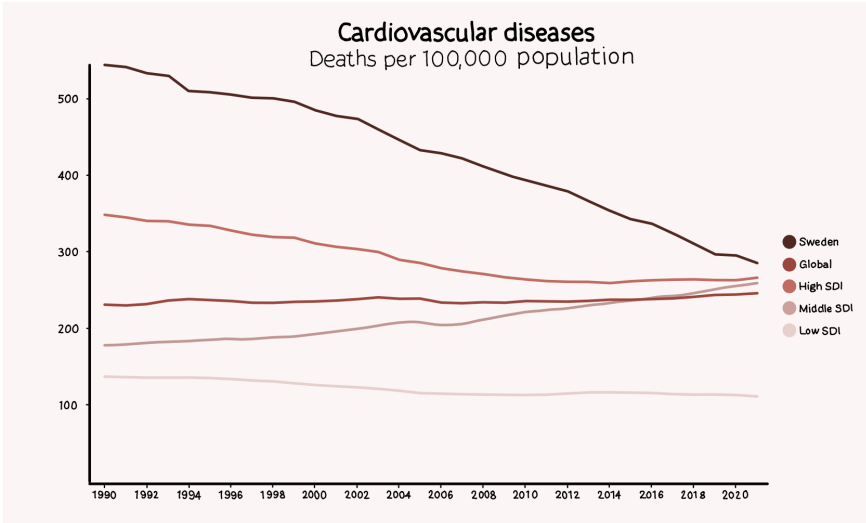
Organization	Year	Sugar type	Recommendation	Motivation
Nordic Nutrition Recommendations	2023	Free sugar and added sugar	<10 E%	Micronutrient dilution, chonic metabolic diseases and dental caries
Dietary Guidelines for Americans	2020	Added sugar	<10 E%	Micronutrient dilution, chronic metabolic diseases and overweight
World Health Organization	2015	Free sugar	<10 E%/<5 E%	Overweight and dental caries

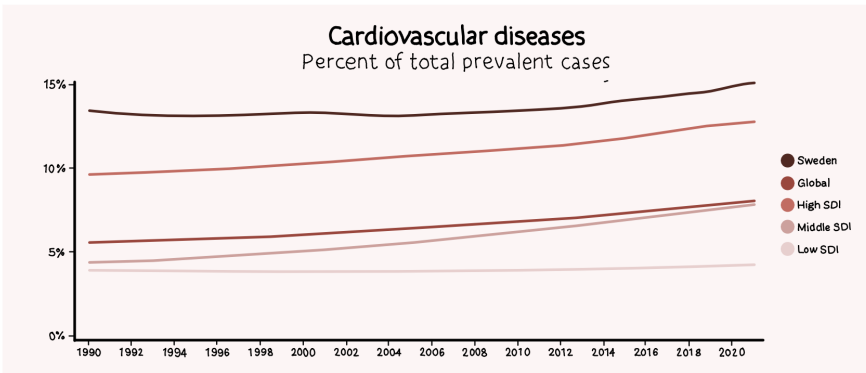
E%: Percentage of energy intake.

To guide evidence-based nutritional advice, five European Nordic countries requested the EFSA Panel on Nutrition, Novel Foods and Food Allergens to deliver a scientific opinion on the tolerable upper intake level of dietary sugar intake based on available data on chronic metabolic diseases, pregnancy-related endpoints, and dental caries. The EFSA scientific opinion was published in 2022, in which it concludes that an upper level of intake for sugar could not be defined but recommends keeping the sugar intake “as low as possible” (10). This was because EFSA could not find evidence for a safe level of sugar intake for the risk of dental caries or chronic metabolic diseases. The EFSA further notes that the relationship between sugar intake and chronic metabolic disease risk could not be adequately explored at intake levels below 10 E% (10).

Cardiovascular disease

Cardiovascular disease encompasses a range of conditions affecting the heart and vascular system. It is currently the leading cause of death worldwide, primarily due to stroke and myocardial infarction (17). Remarkably, it is estimated that up to 90% of all cardiovascular disease cases are preventable, with dietary risk factors contributing to approximately 50% of cardiovascular disease related deaths (18). The trends in cardiovascular disease mortality are currently improving in Sweden, which can be attributable to improvements in health care and of risk factors (19). The prevalence of cardiovascular disease does however not show the same trend, which could have to do with improvements in health care allowing individuals to live with cardiovascular disease for longer, but it also highlights the need for primary prevention to combat the increasing burden on the health care system, as it is currently estimated that cardiovascular disease accounts for 11% of the European union's total healthcare expenditure (20) (**Figure 6**).





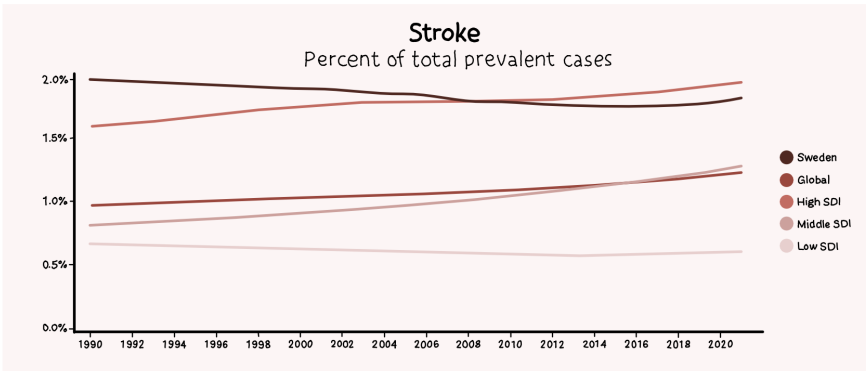


Figure 6. Mortality and prevalence of cardiovascular disease, as well as prevalence of stroke in Sweden, globally, and for countries with different socio-demographic indices. SDI: Socio-demographic index. Data obtained from the Global Burden of Disease (GBD) Compare Viz Hub, Institute of Health Metrics and Evaluation (21).

Understanding and addressing risk factors for cardiovascular disease is crucial for improving public health strategies aimed at preventing cardiovascular diseases. General risk factors for cardiovascular disease include obesity, a poor diet, high blood pressure, smoking, dyslipidaemia, diabetes mellitus, and excessive alcohol consumption (19). However, different cardiovascular diseases have diverse risk factors and aetiologies. For instance, hypertension, dyslipidaemia, diabetes mellitus, and obesity are major contributors to ischemic stroke and myocardial infarction, while for example aortic stenosis may be more influenced by factors such as congenital heart defects. Despite the varied risk factors and causes, many cardiovascular diseases share atherosclerosis as a common underlying process (**Table 2**). Atherosclerosis is an inflammatory disease characterized by the buildup of oxidized fat, low-density lipoprotein (LDL) cholesterol, macrophage cells, and various other particles in the arterial walls, forming atheromatous plaque (22). The plaque narrows and stiffens arteries, obstructing blood and oxygen circulation, and if the plaque ruptures, it can cause a blood clot. The blood clot can travel through the vascular system and give rise to a blockage of oxygen delivery, also known as ischemia. A myocardial infarction (i.e. heart attack) refers to ischemia occurring in the heart, while ischemic stroke refers to ischemia occurring in the brain.

Key dietary risk factors for cardiovascular disease include a high intake of saturated fat, trans fat, and sodium, alongside a low intake of fruits, vegetables, and whole grains. For example, a decreased risk of stroke has been linked to diets rich in fruits, vegetables, legumes, whole grains, and lean protein sources, and limiting processed foods, trans-fats, and sugar sweetened beverages (23, 24). Contrary to popular belief, there is limited research on sugar intake as a risk factor for cardiovascular disease.

Table 2. Overview of the cardiovascular diseases studied in this thesis.

Cardiovascular disease	Description	Aetiology	Risk factors
 **Ischemic stroke**	Blocked blood flow to the brain	Atherosclerosis, cardioembolism, small vessel disease	Hypertension, dyslipidaemia, diabetes mellitus, obesity, atrial fibrillation, smoking, sedentary lifestyle, age, family history
 **Haemorrhagic stroke**	Bleeding in the brain	Aneurysms, arteriovenous malformations, hypertension, cerebral amyloid angiopathy, vascular malformations	Head trauma, hypertension, anticoagulant use, age, family history
 **Myocardial infarction**	Blocked blood flow to the heart	Atherosclerosis, coronary artery spasm, coronary embolism, coronary artery dissection	Hypertension, dyslipidaemia, smoking, diabetes mellitus, obesity, sedentary lifestyle, age, family history
 **Heart failure**	Impairment in the heart's ability to fill with and pump blood	Coronary artery disease, hypertension, cardiomyopathy, valvular heart disease	Coronary artery disease, hypertension, diabetes mellitus, obesity, smoking, previous myocardial infarction, age, family history
 **Aortic stenosis**	A narrowing of the aortic valve, obstructing blood flow	Calcific degeneration, congenital heart defects, rheumatic heart disease	Hypertension, dyslipidaemia, diabetes mellitus, chronic kidney disease, age
 **Atrial fibrillation**	Irregular and often rapid heart rate	Hypertension, heart disease, hyperthyroidism, excessive alcohol consumption	Hypertension, obesity, diabetes mellitus, heart disease, age, family history
 **Abdominal aortic aneurysm**	Enlargement of the abdominal aorta which may rupture	Atherosclerosis, infection or inflammation	Hypertension, smoking, male gender, atherosclerosis, age, family history

The associations between sugar intake and cardiovascular disease risk

The positive and causal relationship between sugar-sweetened beverages and risk of cardiovascular disease is well-established (10). In contrast, very few studies have investigated whether overall sugar intake and other dietary sources of free sugar are linked to cardiovascular disease risk (**Figure 7, Table 3**).

Figure 7. Dose-response meta-analysis on the relationship between the intake of sugar-sweetened beverages and risk of CVD. CVD: Cardiovascular disease. Source: EFSA Panel on Nutrition, Novel Foods and Food Allergens (10).

Evidence from randomized controlled trials

According to a recent systematic review from the EFSA, there is evidence suggesting a positive and causal link between the consumption of added and free sugars and the risk of certain chronic metabolic diseases including obesity, dyslipidaemia, hypertension, and type 2 diabetes mellitus (10). These conclusions are drawn from randomized controlled trials that examined the effects of high versus

low sugar intake on surrogate disease endpoints such as body weight, fasting glucose, fasting triglycerides, and systolic blood pressure. However, due to the limited number of studies available, no conclusions could be drawn regarding the relationship between added and free sugar intake and cardiovascular disease risk. Similar conclusions were drawn in a Cochrane review published in 2022, which found no randomized controlled trials on the relationship between added sugar intake and cardiovascular disease risk but reported minimal effects of added sugar intake on total cholesterol levels, triglycerides, and both systolic and diastolic blood pressure (25).

Evidence from observational studies

According to the EFSA, the available body of evidence from prospective cohort studies do not support a positive relationship between the intake of dietary sugars (total, added, or free) and cardiovascular disease or other chronic metabolic diseases (10). As shown in **Table 3**, out of the eleven observational studies that have investigated the association between sugar intake and cardiovascular disease, only four studies reported positive associations (26-29). The sugar types that were associated with cardiovascular disease risk were free sugar, added sugar, and sucrose. In terms of sugar-sweetened foods and beverages, two of the studies reported positive associations between sugar-sweetened beverages and cardiovascular disease risk. One study reported mostly non-linear associations between intake of toppings and treats and cardiovascular disease risk (29).

Table 3. Overview of observational studies investigating the associations between sugar intake and cardiovascular disease risk.

Study	N	Exposure	Outcome	Result
Liu, 2000 (30)	75,521	Sucrose	CHD	
		Fructose	CHD	
Beulens, 2007 (31)	15,714	Mono- and disaccharides	CVD	
Sieri, 2010 (32)	44,132	Sugar, not specified	CHD	
Burger, 2011 (33)	19,608	Sugar, not specified	CHD	
		Sugar, not specified	Stroke	
Bernstein, 2012 (34)	127,456	SSBs	Stroke	
Eshak, 2012 (35)	18,875	SSBs (men)	IHD	
		SSBs (men)	Stroke	
	20,911	SSBs (women)	IHD	
		SSBs (women)	Stroke	
Sieri, 2013 (36)	44,099	Sugar, not specified	Stroke	
Sonestedt, 2015 (37)	26,445	Sucrose	CVD	
		Cookies and cakes	CVD	
		Sugar and sweets	CVD	
		SSBs	CVD	
Warfa, 2016 (26)	26,190	Sucrose	Coronary events	
		Sweets	Coronary events	
		Chocolate	Coronary events	

		Table sugar and jam	Coronary events	
		Fruit juice	Coronary events	
		SSBs	Coronary events	
		Cakes and pastried	Coronary events	
Pase, 2017 (38)	2,888	SSBs	Stroke	
Tasevska, 2018 (39)	82,254	Total sugar	CVD	
		Total sugar	CHD	
		Total sugar	Stroke	
Keller, 2020 (40)	284,289	SSBs	Coronary events	
Pacheco, 2020 (41)	106,178	SSBs	CVD	
Yang, 2022 (27)	109,034	Added sugar	CVD	
		Added sugar	CHD	
		Added sugar	Heart failure	
		Added sugar	Stroke	
		SSBs	CVD	
		SSBs	CHD	
		SSBs	Heart failure	
		SSBs	Stroke	
Kelly, 2023 (28)	110,497	Total sugar	CVD	
		Total sugar	IHD	
		Total sugar	Stroke	
		Free sugar	CVD	
		Free sugar	IHD	
		Free sugar	Stroke	
Schaefer, 2024 (29)	176,352	Free sugar	CVD	
		Free sugar	IHD	
		Free sugar	Stroke	
		Soda/fruit drinks	CVD	
		Soda/fruit drinks	IHD	
		Soda/fruit drinks	Stroke	
		Treats	CVD	
		Treats	IHD	
		Treats	Stroke	
		Toppings	CVD	
		Toppings	IHD	
		Toppings	Stroke	

CVD: Cardiovascular disease. IHD: Ischemic heart disease. CHD: Coronary heart disease. SSBs: Sugar-sweetened beverages.

Positive association

Non-linear association

No association

Why it is difficult to study the health effects of sugar intake

Studying the health effects of dietary intake presents a unique set of challenges that make it a complex area of research (42). Diet is inherently multifaceted, encompassing a wide variety of foods and nutrients within the food matrix. This complexity makes it difficult to isolate the effects of individual dietary components. This, together with the study design challenges commonly encountered in nutritional epidemiology, and how tightly linked dietary habits are with other lifestyle factors, results in it being difficult to study the health effects of sugar intake.

Randomized controlled trials not feasible

Randomized controlled trials are considered the gold standard in clinical research due to their ability to minimize bias and establish causality. However, conducting randomized controlled trials to study the long-term health effects of sugar intake may be impractical and unethical. Long-term dietary interventions require participants to adhere to specific diets for extended periods, and in the case of studying cardiovascular disease incidence, it could require decades. Another aspect is that it may be unethical to expose participants to potentially harmful diets for extended periods for the sake of research. This often results in researchers having to rely on surrogate disease endpoints, such as blood pressure and lipid profiles. Ultimately, observational studies are often required to infer whether dietary factors are associated with actual disease incidence (43).

Shortcomings of estimating sugar intake

Accurate dietary assessment is a significant challenge in nutritional research. Traditional dietary assessment methods such as food-frequency questionnaires and dietary recalls rely on self-reported data, which can be prone to inaccuracies due to memory lapses, social desirability bias, and intentional misreporting (44). This may prove particularly important for dietary intakes most commonly misreported due to social desirability, such as for example sugar-sweetened foods and beverages (45). Underreporting or overreporting of sugar consumption can lead to inaccurate data which skews the results and makes it difficult to draw reliable conclusions about the health effects of sugar. This issue is compounded by the fact that sugar is present in many foods, sometimes in hidden forms, making it even harder to accurately report and estimate sugar intake.

Estimating sugar intake is further made difficult by the fact that different dietary assessment methods capture diet in different ways. For example, food-frequency questionnaires tend to be better at capturing habitual diet and intake of various seasonal foods but may not be suitable for accurately capturing current dietary intake or absolute intakes. 24-hour dietary recalls may more accurately capture

current dietary intakes and weighted food records may be better at capturing absolute intakes, but these two methods are less likely to accurately capture habitual diet unless repeated several times (46). Finally, estimating the sugar intake in the diet based on the dietary assessment data requires linking to food composition databases which often give average nutrient values of foods (e.g., chocolate chip cookies in general rather than for the specific variety that the individual consumed). The methods of nutrient analysis and calculation may also vary between different food composition databases (47).

Complexity of diet as an exposure

Diet is a multifaceted exposure that includes a wide array of nutrients and food components. People consume sugar in various forms and in combination with other foods, making it difficult to isolate its specific effects. Moreover, dietary patterns vary widely among individuals and populations, influenced by cultural, economic, and personal preferences. This complexity makes it hard to attribute health outcomes to sugar intake alone without considering the broader context.

Handling of BMI

The health effects of sugar intake can be direct or indirect, further complicating the analysis. For example, the health effects may be mediated by body mass index (BMI) and not be caused by the sugar itself. It is well-established that high BMI is a cardiovascular disease risk factor. It is however not entirely clear what role BMI plays in the potential associations between sugar intake and cardiovascular disease risk and, consequently, how to incorporate BMI in statistical analyses. It is possible that BMI acts as a confounder between sugar intake and cardiovascular disease risk as higher BMI may result in individuals either changing their sugar intake, and/or impact their self-reporting of sugar intake (48). It is however also possible that BMI acts as a mediator for the association between sugar intake and cardiovascular disease risk as higher sugar intakes may cause an energy surplus, causing higher BMI, which in turn might increase cardiovascular disease risk. Consequently, the interpretation of the results of the associations between sugar intake and cardiovascular disease risk might be influenced by how BMI has been accounted for in studies.

Nutritional biomarkers

Nutritional biomarkers can act as proxies for dietary intake, and could be used in combination with self-reported intakes to increase the accuracy of research findings regarding diet-disease associations (49). According to the Biomarkers Definitions Working Group, biomarkers are defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic

processes or pharmacologic responses to a therapeutic intervention (50). Different classifications have been suggested for nutritional biomarkers depending on whether they are a marker of nutritional exposure (for example sugar intake) or nutritional status (such as levels of nutrients in tissues). Nutritional biomarkers could further be classified depending on their temporality, i,e., whether they reflect diet during the past hours or days (short-term) or weeks to years (medium- and long-term). Generally, biomarkers measured in urine, plasma or serum reflect short-term intakes well, whereas measurements of biomarkers in adipose tissue, or in hair, nails, or teeth, are more often employed as medium- to long-term biomarkers (51).

For some dietary exposures, very strong biomarkers have been identified. For example, use of urinary nitrogen was proposed as a biomarker of protein intake as early as 1980 and is still used today (49). For other dietary exposures, however, identification of biomarkers is not as straightforward, largely due to how they are metabolized in the body. For these dietary exposures, and to better be able to take inter-individual differences in response to diet into account, omics-based biomarkers have become increasingly interesting. In recent years, high-throughput technologies have revolutionized the study of diet and health by enabling comprehensive characterization of large populations (52). In this thesis, the primary objective of utilizing omics data was to identify objective markers of sugar intake, specifically genomic and metabolomic biomarkers. This approach is particularly valuable as it can reduce the impact of some common pitfalls of the traditional nutritional epidemiology approaches, such as dietary misreporting. The insights gained from these analyses can further enhance our understanding of the determinants of sugar intake, such as the case for genetic markers of sugar intake, as well as of potential mechanisms linking sugar intake to various disease outcomes by for example revealing metabolic pathways affected by sugar intake, helping to identify potential targets for intervention.

Previously suggested biomarkers of sugar intake

The carbon stable isotope ^{13}C is a potential biomarker for cane sugar and high fructose corn syrup intake, as these sugars come from C_4 plants (including molasses and brown and powdered cane sugar). Studies show that non-fasting plasma ^{13}C measurements correlate with recent cane sugar or high fructose corn syrup consumption (i.e., in the previous meal) ($R^2 = 0.90$) (53), and ^{13}C measurements from fingerstick blood samples were correlated with sugar-sweetened beverage intake ($r = 0.35$) and added sugar intake ($r = 0.37$) and were indicated to be a more accurate measure over a longer time period (54). Limitations of this biomarker include that it does not cover C_3 plants like beet sugar, a substantial sugar source in Sweden, as well as maple syrup and honey, and the biomarker can additionally be influenced by corn and animal protein intake (55). Finally, research is needed to more exactly determine the intake period reflected in the isotope.

Urinary sucrose, fructose, and combined sucrose/fructose have been indicated to be able to detect changes in sugar intake and classify individuals as high or low sugar consumers, with studies indicating strong associations with sugar consumption (R^2 = 0.86, 0.80, and 0.89, respectively) (56). However, other studies show moderate agreement with self-reported sugar intake (r = 0.2-0.3) and emphasize that it should be used with caution when studying individuals with metabolic impairment (57, 58). Limitations of these urinary markers of sugar intake further include that they only reflect short-term intake and require collection of urine samples. Further research is needed to develop biomarkers for habitual sugar intake (55).

Genetic biomarkers of sugar intake

Genomics encompasses the study of gene expression, interactions among genes, and their impact on overall biology and health. The candidate gene approach began to be used in genetic studies in the 1980s. Using the candidate gene approach means studying specific genes, usually with known biological functions, that are thought to be associated with a particular phenotype. The associations between genetic variants within the candidate gene and the phenotype of interested are then studied (59).

During the 2000s, Genome-wide association studies (GWAS) were developed and constitute a pivotal tool in this field as they are conducted without any presuppositions regarding genes of interest but rather scan the entire genome. They involve the analysis of single-nucleotide polymorphisms (SNPs), which are the most common type of genetic variation among humans. This approach further allows SNPs to serve as objective markers of sugar intake, facilitating the study of its associations with disease outcomes, ultimately mitigating some of the biases inherent in the self-reported dietary data used in many traditional nutritional studies. Identifying direct genetic determinants of dietary intake can further be used to identify causal associations between the dietary intake and a clinically relevant outcome using Mendelian randomization if the instrumental variable (i.e., the genetic determinant) meets three core assumptions (60):

(1) It must be reproducibly and strongly associated with the exposure.

(2) It must not be associated with confounders.

(3) It is only associated with the outcome through the exposure.

The heritability of perception of and liking for sweet taste has been reported to range from low to moderate (h^2 = *0.23–0.40*) (53, 61-63), however, the genetics responsible for variation in sweet taste perception, preference, and particularly intake of sugar remain largely unexplored. Several sweet taste receptor genes, and a glucose transporter gene, have previously been indicated to be associated with sweet taste phenotypes or sugar consumption in studies using the candidate gene

approach. These studies had small sample sizes, however, and the findings have not been replicated in larger study samples (64). Further, the few GWASs that have been conducted on sugar intake have looked at total sugar intake, but no other subgroups of sugar intake, and they also require replication in other cohorts (64, 65).

Metabolomics

Metabolomics involves the comprehensive analysis of metabolites in biological samples like blood or urine. These metabolites can reflect the intake of specific foods or nutrients. Furthermore, metabolomics can detect endogenous compounds, which are substances naturally produced within the body. These compounds are vital for numerous physiological processes and offer valuable insights into the body's metabolic state and overall health. One use of metabolomics is to identify metabolite profiles of dietary intakes, which can be used to validate self-reported dietary data, identify dietary patterns associated with health outcomes, and explore the metabolic effects of different diets. Metabolite profiles have for example been used in studies investigating the associations between different plant-based diets, dairy consumption, and the risk of type-2 diabetes, revealing novel relationships between metabolites, diet, and health (66, 67). Currently, there are no well-established metabolites that reflect long-term sugar intake, and metabolite profiles of different sugar intakes are lacking.

Aims

The overarching aims of this thesis were to study the associations between sugar intake and cardiovascular disease risk and to identify genetic and metabolomic markers of sugar intake. The specific aims of the included studies in the thesis were to:

Study 1

- Study the associations between added sugar intake as well as different sugar-sweetened foods and beverages and incidence of four different cardiovascular diseases in a cohort from southern Sweden.

Study 2

- Study the associations between added sugar intake as well as different sugar-sweetened foods and beverages and incidence of seven different cardiovascular diseases in two cohorts from central Sweden.
- Investigate the role of BMI in the associations between added sugar intake and cardiovascular disease risk.

Study 3

- Explore the associations between intake of total, added, and sweet-tasting sugars and a selection of SNPs previously associated with sugar intake, preference, and/or sweet taste sensitivity
- Investigate the role of BMI in the associations between genetic variants and sugar intake.

Study 4

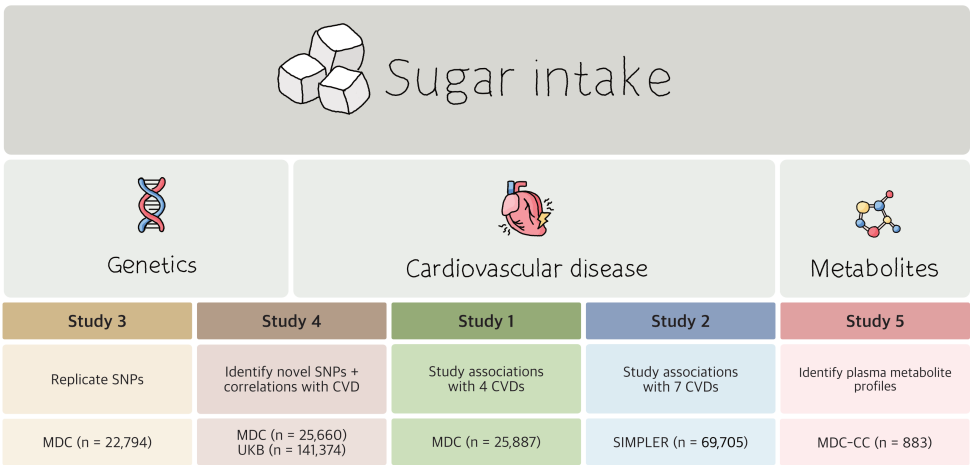
- Identify novel genetic variants associated with free sugar intake and sweet-tasting sugar intake using GWAS.

- Investigate whether the associations identified between genetic variants and sugar intake were independent of BMI and various lifestyle factors.
- Study the genetic correlations between sugar intake and cardiovascular outcomes.

Study 5

- Identify plasma metabolite profiles for subgroups of sugar intake and sugar-sweetened foods and beverages.

Figure 8. Overview of the aims of the studies included in this thesis.



MDC: The Malmö Diet and Cancer study. UKB: UK Biobank. SIMPLER: The Swedish Infrastructure for Medical Population-Based Life-Course and Environmental Research cohorts. MDC-CC: The Malmö Diet and Cancer cardiovascular cohort.

Methods

Study populations

This thesis work has been conducted in several different cohorts (**Table 4**):

Study 1: The Malmö Diet and Cancer study (MDC)

Study 2: The Swedish Infrastructure for Medical Population-Based Life-Course and Environmental Research (SIMPLER) cohorts

Study 3: The MDC

Study 4: The MDC and The UK biobank (UKB)

Study 5: The Malmö Diet and Cancer study cardiovascular cohort (MDC-CC)

Table 4. Overview of the study designs of the studies included in this thesis.

Study	Population (n)	Study design	Exposure	Outcome
1	MDC (25,877)	Prospective cohort	Added sugar (E%) Treats (serv/wk) Toppings (serv/wk) SSBs (serv/wk)	Incident stroke Incident coronary events Incident atrial fibrillation Incident aortic stenosis
2	SIMPLER (69,705)	Prospective cohort	Added sugar (E%) Treats (serv/wk) Toppings (serv/wk) SSBs (serv/wk)	Incident ischemic stroke Incident hemorrhagic stroke Incident heart failure Incident myocardial infarction Incident atrial fibrillation Incident aortic stenosis Incident abdominal aortic aneurysm
3	MDC (22,794)	Cross-sectional	101 SNPs	Total sugar (E%) Added sugar (E%) Sweet-tasting sugars (E%)
4	MDC (25,660) UKB (141,837)	Cross-sectional	~ 7.5M SNPs	Free sugar (E%) Sweet-tasting sugar (E%)
5	MDC-CC (830)	Cross-sectional	Total sugar (E%) Free sugar (E%) Monosaccharides (E%) Treats (g/day) Toppings (g/day) SSBs (g/day)	992 plasma metabolites

SNP: Single-nucleotide polymorphism. MDC: Malmö Diet and Cancer study. UKB: UK biobank. SIMPLER: The Swedish Infrastructure for Medical Population-Based Life-Course and Environmental Research. SCAPIS: The Swedish CArdioPulmonary bioImage Study. SSBs: Sugar-sweetened beverages. CVD: Cardiovascular disease. E%: Percentage of energy intake.

The Malmö Diet and Cancer study (MDC)

In **study 1**, **study 3**, and **study 4,** the study populations included the MDC. The MDC is a population-based prospective cohort study in southern Sweden. From 1991 to 1996, invitations were sent via mail and distributed in public spaces as part of recruitment efforts. All men born between 1923 and 1945, and women born between 1923 and 1950, in Malmö were invited to participate; the only exclusion criteria being mental impairment and inadequate proficiency in the Swedish language. Of the source population consisting of 74,138 individuals, 28,098 individuals completed the baseline examinations as well as the dietary assessment. The baseline examinations included a self-administered questionnaire containing details on diet, lifestyle, socioeconomic factors, as well as anthropometry and blood sample collection performed by trained personnel (68). Ethical approval for the

MDC was granted by the Regional Ethical Review Board in Lund, Sweden (LU 51-90, LU 2012/762), and prior to participation, each participant provided written informed consent.

In **study 1** we further excluded individuals with a history of aortic stenosis, atrial fibrillation, stroke, coronary events, and diabetes mellitus at baseline, resulting in a study sample of 25,877 individuals. In **study 3** we excluded participants lacking relevant genetic information, individuals who were born outside of Sweden, as well as individuals with diabetes mellitus at baseline, resulting in a study sample of 22,794 individuals. In **study 4** we excluded individuals with diabetes mellitus at baseline, lacking genetic information, individuals with a call rate <95%, inbreeding coefficient <-0.2 or 0.2<, individuals with sex-mismatch, a second-degree relatedness or higher within the sample based on identity by descent sharing calculations. The participants were further restricted to individuals of European descent using the first two principal components, resulting in a study sample of 25,660 individuals.

The SIMPLER cohorts

In **study 2**, the study population consisted of participants from the SIMPLER cohorts. The SIMPLER cohorts include female participants from the Swedish Mammography Cohort (SMC) and male participants from the Cohort of Swedish Men (COSM), both of which are population–based prospective cohort studies in Central Sweden. Invitations were sent by mail to all women without cancer born between 1914-1948 living in Uppsala county and Västmanland county, and to all men born between 1918-1952 living in Örebro county and Västmanland county (69).

Participants of SMC and COSM completed similar questionnaires on diet, health, and lifestyle in 1987-1990 (SMC only), 1997, 2008, and 2009. In 1997, a total of 88,077 (39,227 women and 48,850 men) individuals participated. A total of 47,918 (19,598 women and 22,729 men) participants responded to the 2009 questionnaire. After baseline exclusions, a total of 69,705 individuals (32,934 women and 36,771 men) remained for analysis of the 1997 data, and 42,327 individuals (19,598 women and 22,729 men) remained for analysis of the 2009 data. The present study was approved by The Swedish Ethical Review Authority (dnr 2019-03986), and questionnaire completion was considered to convey informed consent.

As SMC originally only included women without cancer, participants in both cohorts with cancer recorded in Swedish registers prior to the baseline were excluded from **study 2**. Further exclusions were made of those with prevalent cardiovascular disease or self-reported diabetes at baseline, death prior to January 1st, 1998, missing or incorrect ID, and those deemed to have had extreme energy

intakes (defined as being outside of three standard deviations (SDs) below and above the $\log_e$-transformed mean energy intake).

The UK biobank (UKB)

In **study 4**, the study population included participants from the UKB. The UKB is a population-based prospective cohort study conducted in the United Kingdom, including over 500,000 participants aged 40–69 years who were recruited between the years 2006 and 2010. At recruitment, data on socio-demographic characteristics, lifestyle factors, diet, anthropometry, and biological samples were collected (70). A total of 149,873 individuals provided both dietary and genetic information. All participants provided written informed consent prior to participating, and the UKB received ethical approval from the National Health Service North West Multi-Centre Research Ethics Committee (11/NW/0382).

For **study 4**, participants were restricted to individuals of European ancestry, as defined by an in-house k-means cluster analysis performed using the first 4 principal components, and individuals with prevalent diabetes at baseline were excluded. After exclusions, 141,437 participants from the UKB remained for further analyses.

The Malmö Diet and Cancer-Cardiovascular Cohort (MDC-CC)

In **study 5**, the study population consisted of participants from the MDC-CC. The MDC-CC is a subcohort of the MDC selected to study the epidemiology of carotid artery atherosclerosis. During the years 1991 to 1994, 6,103 randomly selected MDC participants were invited to undergo a second examination. Fasting plasma samples were obtained in 5,540 individuals (71).

In **study 5**, we included 928 MDC-CC participants for which metabolomics profiling had been carried out. We excluded individuals without information about the studied exposure variables and covariates, resulting in a study sample of 883 individuals.

Dietary assessment

The MDC (including MDC-CC)

The MDC used a modified diet history method consisting of three parts. First, the participant completed a seven-day food diary in which they recorded intake of cooked meals, cold beverages, and supplements during seven consecutive days. Second, a 168-item food-frequency questionnaire was filled out with the aid of a

booklet with pictures to help with estimation of portion sizes. The food-frequency questionnaire was designed to cover regularly consumed foods that were not included in the food diary (i.e. breakfasts, snacks, and hot beverages). Third, a 45- or 60-minute interview was conducted by trained personnel to gather details about food preparation methods and portion sizes of the foods listed in the food diary. During the interview, the personnel made sure that there was no overlap between the information in the food diary and in the food-frequency questionnaire (72).

The recorded dietary intakes were aggregated into an average daily consumption using the Malmö Food and Nutrient Database which was based on the Swedish Food Database (72, 73). An 18-day weighted food record was used to validate the diet history method, and the results showed a relatively high ranking validity. Energy-adjusted Pearson correlation coefficients for men/women were reported for intake of carbohydrates (0.66/0.70), protein (0.54/0.53), fat (0.64/0.69), fiber (0.74/0.69), and sucrose (0.60/0.74) (74).

The SIMPLER cohorts

In 1997, baseline dietary data was collected using a 96-item semi-quantitative food-frequency questionnaire, which assessed the average consumption of a variety of foods and drinks during the previous year. Participants indicated their consumption frequency from a range of eight or nine options for each food and drink item, with portion sizes being either predefined or reported by the participants themselves (e.g., for alcohol consumption). Consumption frequencies for beverages like sweetened beverages, tea, and coffee, as well as for sugar and honey, were determined through open-ended questions about the number of daily or weekly servings consumed during the previous year (75).

A decade later, in 2009, participants from the COSM and SMC cohorts were given a 132-item food-frequency questionnaire to update their dietary information. This expanded questionnaire included new food items to better reflect the dietary habits prevalent in 2009 compared to 1997. Nutrient intakes were estimated by multiplying the consumption frequency of each food item with the nutrient content of age-specific portion sizes for that food and nutrient values were derived from the Swedish Food Composition Database (76).

The dietary survey from 1997 was validated using fourteen 24-hour recalls with 248 men from central Sweden, who were randomly chosen from the Swedish population register. This validation showed a Spearman rank correlation coefficient of 0.70 for sucrose (75). Additionally, a separate validation study involving 129 women from the SMC was conducted using four one-week dietary records spaced three to four months apart. This study reported correlation coefficients of 0.6 for sweetened beverages, 0.5 for jams and marmalades, and 0.4 for sweets (A. Wolk, unpublished observations, 1992).

The UKB

The dietary assessment in UKB used in this thesis was performed using a 24-hour dietary recall collected at baseline and on up to four additional occasions, with an average interval of 6 months between recalls. The dietary data were gathered using the validated Oxford WebQ online 24-hour dietary recall questionnaire, where participants reported their consumption of approximately 200 commonly consumed foods in the previous 24 hours, along with the quantities consumed (77). The dietary assessment method was validated against biomarkers for protein, potassium, and total sugar intake (urinary sucrose and fructose concentrations), as well as total energy expenditure estimated by accelerometery. The correlation coefficients were 0.40 for protein, 0.34 for potassium, and 0.33 for total sugar intake. For nutrient density, the correlation was 0.27 for total sugar intake when using one 24-hour dietary recall, and 0.40 when using five dietary recalls (78). The sugar intakes used in the UKB were the average intakes across all available assessments for each participant, with the mean number of 24-hours recalls provided for UKB participants being two. The UK McCance and Widdowson's The Composition of Foods 6th edition was used to calculate the nutrient data (79).

Estimation of sugar intake

Added sugar, free sugar, and sweet-tasting sugar

Added sugar was investigated in **study 1**, **study 2**, and **study 3**, whereas free sugar intake was investigated in **study 4** and **study 5.** *Added sugar* intake was estimated by adding the intake of all monosaccharides and sucrose from each participant's whole diet and then subtracting the intake of naturally occurring monosaccharides and sucrose in the diet (mainly coming from fruits and berries, fruit juice, and vegetables). In the MDC and the SIMPLER cohorts, *free sugar* intake was estimated similarly to the added sugar variable, but without subtracting the monosaccharides and sucrose naturally present in fruit juices. In the UKB, the participants' free sugar intakes were estimated using the method proposed by Wanselius et al. (80). In **study 3** and **study 4**, *sweet-tasting sugars* were studied, which included all monosaccharides and sucrose, both added and naturally occurring in foods. The participants' estimated added sugar, free sugar, and sweet-tasting sugar intakes were subsequently converted into percentages of non-alcoholic energy intakes and stratified into six categories: ≤5 E%, >5-7.5 E%, >7.5-10 E%, >10-15 E%, >15-20 E%, and >20 E%. The categories were selected to allow the study of a wide range of sugar intake, including those commonly used in nutritional recommendations as well as extreme intakes.

Sugar-sweetened foods and beverages

In **study 1, 2, 3,** and **5**, various common sources of added sugar were studied. The sugar-sweetened foods and beverages were divided into categories of *treats* (pastries, ice cream, sweets, and chocolate), *toppings* (table sugar, honey, jams, and marmalades), and *sugar-sweetened beverages* (all sugar-sweetened sodas and fruit drinks but not pure fruit juices). In **study 2**, *artificially sweetened beverages* were studied as well. In **study 3** and **study 5**, the consumed amounts of sugar-sweetened foods and beverages were analysed as grams per day, whereas in **study 1** and **2**, the consumed amounts of sugar-sweetened foods and beverages were recoded to servings/week based on average serving sizes according to the Swedish National Food Agency's food database and information from manufacturers, and divided into categories as follows: *Treats* as ≤2, >2–5, >5–8, >8–14 and >14 servings/week; *Toppings* as ≤2, >2–7, >7–14, >14–28 and >28 servings/week, and *sugar-sweetened beverages* as ≤1, >1–3, >3–5, >5–8 and >8 servings/week. The categories for the sugar-sweetened foods and beverages were set based on categories previously used in two other Swedish cohorts carried out around the same time period as the baseline of COSM and SMC, and were determined by examining the restricted cubic spline curves of associations between added sugar intake and total mortality (81).

Genotyping

Study 3

For **study 3**, SNPs that had previously been linked to sugar-related phenotypes were selected from the MDC data. In MDC, blood samples obtained from the participants were used for genotyping, which was carried out using the Illumina GSA v1 genotyping array. Some SNPs could not be genotyped directly but were imputed using the using the Michigan Imputation Server with the Haplotype Reference Consortium panel (HRC) (82). The SNPs included in **study 3** were eight of the top hits from a previous GWAS on total sugar intake from the UK biobank (64), two SNPs adjacent to the fibroblast growth factor 21 (*FGF21*) gene, 73 SNPs associated with the perceived intensity and preference of various sweet substances in GWAS (64), and 20 SNPs that were previously identified using the candidate-gene approach in association with sweet phenotypes (83-85). After removal of duplicates, a total of 101 SNPs were included for further investigation.

Study 4

In the MDC, genotyping of DNA from blood samples was performed using the Illumina GSA v1 genotyping array. Additional SNPs were imputed using the

Michigan Imputation Server with the HRC panel (82). Exclusions were carried out for variants with minor allele frequencies (MAF) <1%, missingness >1%, Hardy-Weinberg equilibrium <1E-15, and non-autosomal variants. After exclusions, 7,622,353 SNPs remained for further analyses.

In the UKB, the full data release contained the cohort of successfully genotyped samples (n=488,377), with 49,979 individuals genotyped using the UK BiLEVE array and 438,398 using the UKB axiom array. Before phasing, multiallelic SNPs and those with MAF ≤1% were removed. Phasing of genotype data was performed using a modified version of the SHAPEIT2 algorithm. Genotype imputation to a reference set combining the UK10K haplotype and the HRC reference panels was performed using IMPUTE2 algorithms (70). The analyses were limited to autosomal variants within the HRC site list using a graded filtering method with varying imputation quality for different allele frequencies, resulting in rarer genetic variants being required to have a higher imputation quality INFO score, i.e., the ratio between the observed and expected statistical information (INFO >0.3 for MAF >3%; INFO >0.6 for MAF 1-3%; INFO >0.8 for MAF 0.5-1%; INFO >0.9 for MAF 0.1-0.5%). The MAF and INFO scores were recalculated on an in-house derived 'European' subset. Genotyped variants were filtered, excluding variants with MAF <1%, missingness >1.5%, Hardy-Weinberg equilibrium <1E-4, and non-autosomal variants. After exclusions, 7,402,703 SNPs remained for further analyses.

Plasma metabolite assessment

For **study 5**, data on plasma metabolites from the MDC-CC were used. Metabolomics profiling was conducted for a random sample of 928 MDC-CC participants. Venous fasting blood samples were collected from the participants at enrolment, and plasma was stored at -80°C until metabolomic analysis. A total of 1,372 biochemicals were quantified using the Metabolon Platform (Morrisville, NC, USA) through non-targeted relative quantitative liquid chromatography-tandem mass spectrometry (LC-MS/MS). This analysis identified 835 named metabolites, 268 unnamed metabolites, and 269 xenobiotics. All metabolites except for the xenobiotics with >75% missing values were excluded, while missing values for xenobiotics were imputed with 0. Metabolite values beyond ±5 SD away from the mean of that specific metabolite were recoded to the ±5 SD threshold value. After exclusions, a total of 992 metabolites were available for further analyses. All metabolites were normalized by transformation using the natural log to achieve a normal distribution of the data. Finally, the elastic net regression models generally benefit from standardization because it helps with the convergence of the optimization algorithm and ensures that the regularization terms are applied consistently across features. Thus, all values were standardized by converting them to Z-scores.

Cardiovascular disease outcomes

Incidence outcomes

Endpoints for **study 1** and **study 2** were ascertained using the Swedish National Inpatient Register and the Cause of Death Register, in accordance with the International Classification of Diseases 9th revision (ICD-9) and the ICD-10. These registries include all Swedish residents, and there was therefore minimal loss to follow-up during registry linkage.

In **study 1**, the studied outcomes were stroke (subarachnoid or intracerebral hemorrhage, occlusion of cerebral arteries or other acute cerebrovascular disease), coronary events (myocardial infarction, other forms of ischemic heart disease or angina pectoris), atrial fibrillation (atrial fibrillation or flutter events), and aortic stenosis. The studied endpoints were stroke (ICD-9 codes 430, 431, 434, 436), coronary events (ICD-9 codes 169 410-414), atrial fibrillation (ICD-9 code 427 or code 4273 in the Cause of Death Register), and aortic stenosis (ICD-9 code 424.1). The Swedish National Inpatient Register has previously been demonstrated to have high diagnostic validity, with positive predictive values over 90% for the studied outcomes (86).

In **study 2**, the studied outcomes and their ICD-10 codes were ischemic stroke (I63), hemorrhagic stroke (I61 and I60), myocardial infarction (I21), heart failure (I50 and I11.0), atrial fibrillation (I48), aortic valve stenosis (I35.0 and I35.2), and abdominal aortic aneurysm (I71.3 and I71.4). Because lifestyle factors have been shown to have a greater impact on ischemic stroke incidence than on hemorrhagic stroke incidence (35), ischemic stroke cases and hemorrhagic stroke cases were studied separately.

Cardiovascular outcomes from GWAS

To study the genetic correlations between sugar intake and cardiovascular disease risk in **study 4**, summary statistics from publicly available GWAS on included cardiovascular diseases and cardiovascular risk markers were used. Specifically, data from GWASs on ischemic stroke (87), heart failure (88), atrial fibrillation (89), aortic aneurysm (90), coronary artery disease (91), triglycerides (92), low-density lipoprotein cholesterol (92), and high-density lipoprotein cholesterol (92) were used.

Statistical analyses

Most statistical analyses in this thesis were conducted using R (93). Generally, two-sided P-values below 0.05 were considered statistically significant, but in cases where many variables were studied (as in **study 3**, **study 4**, and **study 5**), lower P-value thresholds were set using Bonferroni correction to correct for multiple testing. The distributions of the studied variables were studied using histograms, and severely skewed variables were log-transformed.

Survival analyses

Cox proportional hazards regression models were used to study the associations between sugar intake and cardiovascular disease incidence in **study 1** and **study 2**, using time of follow-up (date from baseline examinations to date of event, emigration, or end of follow-up, whichever occurred first) as the time variable. The participants of **study 1** were followed up until December 31st, 2016, and the participants of **study 2** were followed up until December 31st, 2019. A set of different models of covariate adjustments were used, including a basic adjustment model (age, sex, energy intake, and cohort-specific methodological variables), an extended model (with additional adjustment for lifestyle factors such as smoking, alcohol consumption, physical activity, and education), an extended model with additional inclusion of BMI, and a final model with additional adjustment for dietary covariates. The specific covariates were selected based on possible associations with sugar intake and cardiovascular disease risk, explored using directed acyclic graphs.

Restricted cubic splines

In **study 2**, the fully adjusted Cox proportional hazards regression models were studied further using restricted cubic splines to visualize the dose-response curves. Using the restricted cubic splines, the free sugar variable was split into segments at predetermined "knots", and single polynomials were then fitted into each segment. According to Harrell's recommended percentiles, the knots were placed at the 5th, 27.5th, 50th, 72.5th, and 95th percentiles of free sugar intake (94).

Linear regression

In **study 3**, linear regression models were used to study the associations between SNPs and various types of sugar intake, with sugar-sweetened foods and beverages being log-transformed as they were not normally distributed. The SNPs were coded as 0, 1, and 2, with 2 being homozygous for the effect allele. The model was adjusted for age, sex, method (45- or 60-minute dietary interviews), and total energy intake

(kcal per day). 10 SNPs were included as primary exposures; thus, the Bonferroni-corrected significance threshold was set to P<0.005.

Analysis of genomic data

In **study 4**, genomic data from the UKB and the MDC were analysed to:

1. Identify SNPs associated with various subtypes of sugar intake.
2. Appraise SNPs with direct effects on sugar intake.
3. Study the genetic correlations between sugar intake and various cardiovascular outcomes.

Mixed model association tests

Mixed model association tests were used to study the genome-wide associations between SNPs and various sugar intake subtypes as they can account for both population stratification and cryptic relatedness and can achieve increased statistical power by jointly modeling all genotyped markers. The GWAS was conducted using the BOLT-LMM algorithm (95), which assumes a Bayesian mixture-of-normals prior for the random effect attributed to SNPs other than the one being tested, generalizing the standard infinitesimal mixed model used by other mixed model association methods. This helps both with avoiding confounding as well as optimising power compared with similar linear mixed models. The BOLT-LMM algorithm is furthermore increasingly powerful in relation to the size of the cohort, making it ideal for large cohort GWASs. The mixed model association tests were adjusted for age, sex, and total energy intake (kcal/day) in the UKB, and additionally for diet assessment method (45- or 60-hour diet assessment interview) in the MDC. Suggestive significance and GWAS significance thresholds were set to P=1E-5 and P=5E-8, respectively.

GWAS meta-analysis

Meta-analyses of the GWAS summary statistics from the MDC and the UKB for each phenotype were conducted using the Genome-Wide Association Meta-Analysis (GWAMA) software (96). We performed genomic control on summary statistics to account for population structure across the studies. To obtain a more comprehensive understanding of the data, we conducted both fixed effects and random effects meta-analyses to account for variability in effect sizes between studies.

Estimation of genetic variants' direct effects on sugar intake

We used Bayesian GWAS (bGWAS) to estimate the direct effects of genetic variants on sugar intake in order to gain a better understanding of which SNPs influence sugar intake directly rather than indirectly through other lifestyle factors.

In bGWAS, estimates for genetic variants are generated by incorporating prior information and observed data to model the relationship between genetic variants and the phenotype of interest (sugar intake in this case) (97). In this study, prior knowledge from existing GWAS on lifestyle factors from various populations was incorporated to refine the estimates of the genetic effects (98). Using the bGWAS R-package (99), the GWAS data from the fixed effects meta-analyses of free sugar intake and sweet-tasting sugar intake were corrected for potential confounders using prior GWAS on BMI (100), education (years of schooling) (101), and smoking (having ever/never been a regular smoker) (102). Education was corrected for as a proxy for socioeconomic status, and smoking was corrected for since it has been linked to changes in sweet taste perception (103) and is a known risk factor for cardiovascular disease (104).

Genetic correlation

To study the genetic correlations between sugar intake and cardiovascular outcomes, uncorrected GWAS summary statistics as well as corrected estimates from the bGWAS analyses were analyzed using a bivariate linkage disequilibrium-score regression model in the ldsc software (105, 106). The studied outcomes used for the genetic correlation were taken from publicly available GWAS (87-92). Three models were used for the genetic correlation analyses: Model 1 was the uncorrected fixed effects GWAS on the sugar phenotype, model 2 was corrected for BMI, and model 3 was corrected for BMI, education, and smoking.

Analysis of metabolomic data

For **study 5**, metabolite profiles of intake of sugar subtypes and sugar-sweetened foods and beverages were identified using elastic net regression (ENR) models. Participants were assigned to either a testing or training set using a 30/70% split using a pseudo-random split to maintain the data distribution. In the training set, an elastic net regression model with a tenfold cross-validation was utilized to establish a relation between sugar intake and the metabolites. The trained model was then applied to the testing set to be able to calculate a metabolite profile score for sugar. The metabolite profile score was calculated as the weighted sum of the identified metabolites, with weights corresponding to the ENR coefficients. A leave-one-out approach was used when computing the metabolite profile score in the training dataset to prevent overfitting. This methodology aligns with those used to create metabolomic profile scores indicative of dietary intake in other studies (67, 107, 108). We computed the Spearman correlation coefficients between the sugar metabolite profile scores and the sugar intake variables, as well as other foods, to examine how specific the score was to the studied exposure variable.

We further evaluated the associations between the metabolites that were identified in the ENR model and sugar intake using linear regression models. The covariates

adjusted for in these analyses included age, sex, season, total energy intake, leisure-time physical activity, alcohol consumption, smoking status, education, fiber intake, coffee, and BMI. A Bonferroni corrected P-value (0.05 divided by the number of metabolites) indicated the statistical significance threshold (5E-5).

Sensitivity analyses and handling of BMI

In **study 1**, sensitivity analyses were conducted excluding potential energy misreporters, identified using Black's revised Goldberg method (109), as well as individuals who had self-reported to have had made drastic diet changes prior to the baseline assessments. Further, to take comorbidities into account, an additional sensitivity analysis was conducted by studying only the first reported diagnosis for each participant. In this analysis, subjects who had experienced an incident event of another cardiovascular disease prior or diabetes mellitus (ICD-9 codes 150.0–150.9 or ICD-10 codes E10–E14) prior to diagnosis of the specific outcome of interest in the analysis were excluded.

In **study 2**, the interactions between sex and added sugar intake, as well as BMI and added sugar intake, were examined by incorporating them as continuous interaction terms in the main model for each outcome. For the associations with statistically significant interactions, stratified analyses were conducted. To take comorbidity into account, a sensitivity analysis was conducted in which all participants with incidence of any of the other studied cardiovascular diseases prior to incidence of the outcome of interest were excluded from the analysis. Further, a sensitivity analysis was conducted to examine whether the results varied between different methods of energy adjustment (110). For added sugar, the residual method was used alongside the nutrient density method (that was used in the main analyses). For sugar-sweetened foods and beverages, both the nutrient density method and the residual method were employed in addition to the standard multivariate method (that was used in the main analyses). The energy-adjusted intakes using the nutrient density method and the residual method were standardized to the study population's mean energy intake. Additionally, the associations for sugar-sweetened beverage and artificially sweetened beverage intake were studied separately among the 42,327 participants who had answered the 2009 dietary assessment. To study how the addition of the 2009 diet assessment affected the risk estimates in the main analyses for added sugar intake, a sensitivity analysis using only the 1997 baseline dietary data was also conducted. Further, to reduce bias by reverse causality, a sensitivity analysis was conducted where cases of the studied outcome that occurred within the first 3 years of the follow-up period were excluded. Finally, to investigate how including missing data may have affected the results for added sugar, we conducted a sensitivity analysis in which only participants with complete data for all sugar-sweetened foods and beverages and categorical covariates were included. Similarly, we conducted sensitivity analyses for the sugar-sweetened foods and

beverages, including only participants with complete data for the respective variables and categorical covariates.

In **study 3,** sensitivity analyses were conducted excluding potential energy misreporters, identified using Black's revised Goldberg method, as well as individuals who had self-reported to have had made drastic diet changes prior to the baseline assessments. As some of the genetic variants examined in this study have previously been suggested to be associated with BMI or have BMI as an effect modifier (83, 111-113), the associations in individuals with BMI < 25 and ≥25 were studied separately. Finally, an analysis that excluded current smokers and those with missing information on smoking was conducted since it has been suggested that smokers might have impaired taste sensitivity, which might affect the associations with for example the sweet-taste receptor genes.

As previously mentioned, in **study 4**, the GWAS data from the meta-analyses of free sugar intake and sweet-tasting sugar intake were corrected for potential confounders using prior GWAS on BMI, education, and smoking. Education was corrected for as a proxy for socioeconomic status, and smoking was corrected for since it has been linked to changes in sweet taste perception (103) and is an known risk factor for cardiovascular disease (104). GWASs were also conducted in participants with BMI 18.5-25 kg/m^2 and >25 kg/m^2 separately to further study the impact of BMI on the associations between sugar intake and genetic variants.

In **study 5**, potential energy misreports, identified using Black's revised Goldberg method (109), were excluded as a sensitivity analysis.

Results

Study 1

The aim of **study 1** was to investigate the associations between added sugar intake as well as different groups of sugar-sweetened foods and beverages and incidence of four different cardiovascular diseases (stroke, coronary events, atrial fibrillation and aortic stenosis). In this sample of 25,877 individuals from the MDC, of which 62.4% were female, the mean age was 57.8 years and the mean added sugar intake was 10.1 E%. During a mean follow-up of 19.5 years there were 2,580 stroke cases, 2,840 coronary events, 4,241 atrial fibrillation cases and 669 aortic stenosis cases. Individuals with high added sugar intake were more likely to be male, older, and with lower BMI than individuals with low added sugar intakes. Lower added sugar consumers tended to be overrepresented when it came to potential energy misreporting (primarily underreporting) and prior drastic diet changes, while they were generally more physically active and had a higher education level than those with higher added sugar intakes.

Added sugar intake and cardiovascular disease risk

A U-shaped association was found between added sugar intake and risk of incident stroke; consumers in the 7.5–10 E% group had the lowest risk, while increased risks were observed among the lowest (HR: 1.14; 95% CI: 0.97–1.34) and highest (HR: 1.31; 95% CI: 1.03–1.66) intake groups. For coronary events, increased risks were found among those with added sugar intakes above 20 E% compared to the lowest intake group (HR: 1.39; 95% CI: 1.09–1.78). The lowest added sugar intake group had the highest risk of atrial fibrillation, with the lowest risk found among intakes of 10–15 E% (HR: 0.85; 95% CI: 0.75–0.96). Similar findings were made for aortic stenosis, albeit not statistically significant, where lower risks of incident aortic stenosis were observed among intakes of 7.5–10 E% (HR: 0.75; 95% CI: 0.56–1.03) and 15–20 E% (HR: 0.69; 95% CI: 0.47–1.02) compared to the lowest intake group (**Figure 9**).

Sugar-sweetened foods and beverages and cardiovascular disease risk

The lowest intake category of treats (≤2 servings/week) were found to have the highest risk of all studied outcomes. No associations were found between intake of toppings and any of the studied outcomes. For sugar-sweetened beverage intake, an increased risk of stroke was observed in the highest intake category (>8 servings/week) compared to the lowest intake category (<1 serving/week) (HR: 1.19; 95% CI: 1.01–1.40), while no associations were found for any of the other studied outcomes (**Figure 9**).

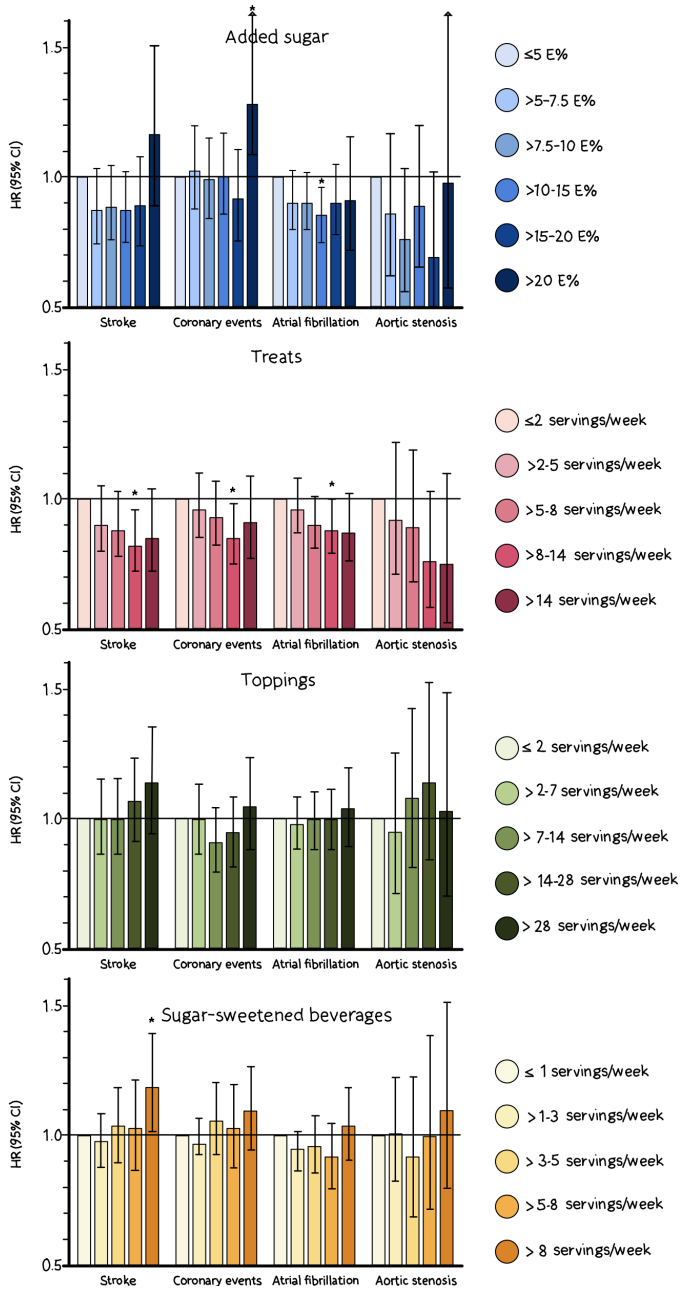


Figure 9. The associations between sugar intake variables and cardiovascular disease risk. The associations were investigated using multivariable Cox proportional hazards regressions adjusted for age, sex, season of dietary assessment, diet method, energy intake, smoking status, educational level, leisure-time physical activity, alcohol consumption, BMI, and dietary habits including intake of processed meat, coffee, saturated fatty acids, and fiber density. HR: Hazard ratio. CI: Confidence interval. E%: Energy percentage. BMI: Body Mass Index.

Study 2

The aim of **study 2** was to study the associations between added sugar intake and incidence of seven different cardiovascular diseases (ischemic stroke, hemorrhagic stroke, myocardial infarction, heart failure, aortic stenosis, atrial fibrillation, and abdominal aortic aneurysm). In this sample of 69,705 individuals from the SIMPLER cohorts, of which 47,2% were female, the mean added sugar intake was 9.1 E% at baseline. Higher added sugar consumers were more likely to be male, have higher exercise levels and have lower education levels than lower added sugar consumers. Furthermore, those consuming high amounts of added sugar were generally older, had higher energy intakes, and had higher intakes of toppings and sweetened beverages. The intake of treats was more evenly distributed across the added sugar intake groups than the other sugar-sweetened foods and beverages. During the follow-up period, 25,739 participants were diagnosed with at least one cardiovascular disease, including 6,912 cases of ischemic stroke, 1,664 cases of hemorrhagic stroke, 6,635 cases of myocardial infarction, 10,090 cases of heart failure, 1,872 cases of aortic stenosis, 13,167 cases of atrial fibrillation, and 1,575 cases of abdominal aortic aneurysm.

Added sugar intake and cardiovascular disease risk

Indications of positive linear associations (P_{trend} <0.01) were found between added sugar intake and risk of ischemic stroke and abdominal aortic aneurysm in the main model. For abdominal aortic aneurysm, a 31% (95% CI: 5-65%) higher risk of abdominal aortic aneurysm was found for added sugar intakes of >20E%, and for ischemic stroke, a 9% (95% CI: 0-19%) higher risk was found for intakes of >15-20 E%, compared to the lowest intake category of ≤5 E%. For most the outcomes, however, the highest risks were found in the lowest intake category and with the lowest risks being found among those with low- to moderate added sugar intakes. Compared to the lowest intake category (≤5 E%), added sugar intake of >5-7.5 E% was linked to statistically significant lower risks of ischemic stroke (8% (95% CI: 2-13%)), myocardial infarction (5% (95% CI: 0-11%)), heart failure (9% (95% CI: 5-13%)), aortic stenosis (9% (95% CI: 0-18%)), and atrial fibrillation (7% (95% CI: 3-11%)). Furthermore, compared to added sugar intakes of ≤5 E%, lower risks of heart failure and atrial fibrillation were found for intakes of >7.5-10 E% (6% (95% CI: 1-10%), and 4% (95% CI: 0-8%), respectively), as well as of heart failure, atrial fibrillation, and aortic stenosis for intakes of >10-15 E% (5% (95% CI: 0-10%), 4% (95% CI: 0-8%), and 17% (95% CI: 7-26%), respectively). No associations were found between added sugar intake and hemorrhagic stroke risk (**Figure 9**).

Stratification by BMI

When stratifying the study participants by BMI 18.5-25 kg/m^2 and 25 kg/m^2, higher added sugar intake was associated with higher risks of abdominal aortic aneurysm and ischemic stroke in individuals with BMI >25 kg/m^2, while higher added sugar intake was associated with a higher risk of heart failure in individuals with BMI 18.5-25 kg/m^2.

Sugar-sweetened foods and sweetened beverages and cardiovascular disease risk

Negative linear associations were found between intake of treats and all outcomes (P_{trend} <0.01), and between intake of toppings and heart failure, and aortic stenosis. A positive linear association was found between intake of toppings and risk of abdominal aortic aneurysm (P_{trend} <0.01), and a 34% (95% CI: 18-51%) higher risk of abdominal aortic aneurysm was found for the highest intake category of toppings (>28 servings/week) compared to the lowest intake category. For aortic stenosis, 16% (95% CI: 5-25%), 20% (95% CI: 9-29%), and 15% (95% CI: 3-25%) lower risks were found for toppings intakes of >7-14, >14-28, and >28 servings/week, respectively, compared to the lowest intake category (**Figure 10**).

For sweetened beverages, positive linear associations were found for ischemic stroke, heart failure, atrial fibrillation, and abdominal aortic aneurysm (P_{trend} <0.001). Intake of >8 servings per week of sweetened beverages was associated with a 19% (95% CI: 11-27%) higher risk of ischemic stroke, an 18% (95% CI: 11-24%) higher risk of heart failure, an 11% (95% CI: 6-17%) higher risk of atrial fibrillation, and a 31% (95% CI: 15-50%) higher risk of abdominal aortic aneurysm (**Figure 10**).

When studying intake of sugar-sweetened beverages and artificially sweetened beverages separately in 42,327 of the participants, positive linear associations were observed between intake of artificially sweetened beverages and risk of ischemic stroke (P_{trend} < 0.01) and heart failure (P_{trend} < 0.01), whereas intake of sugar-sweetened beverages was not associated with cardiovascular disease risk (**Figure 10**).

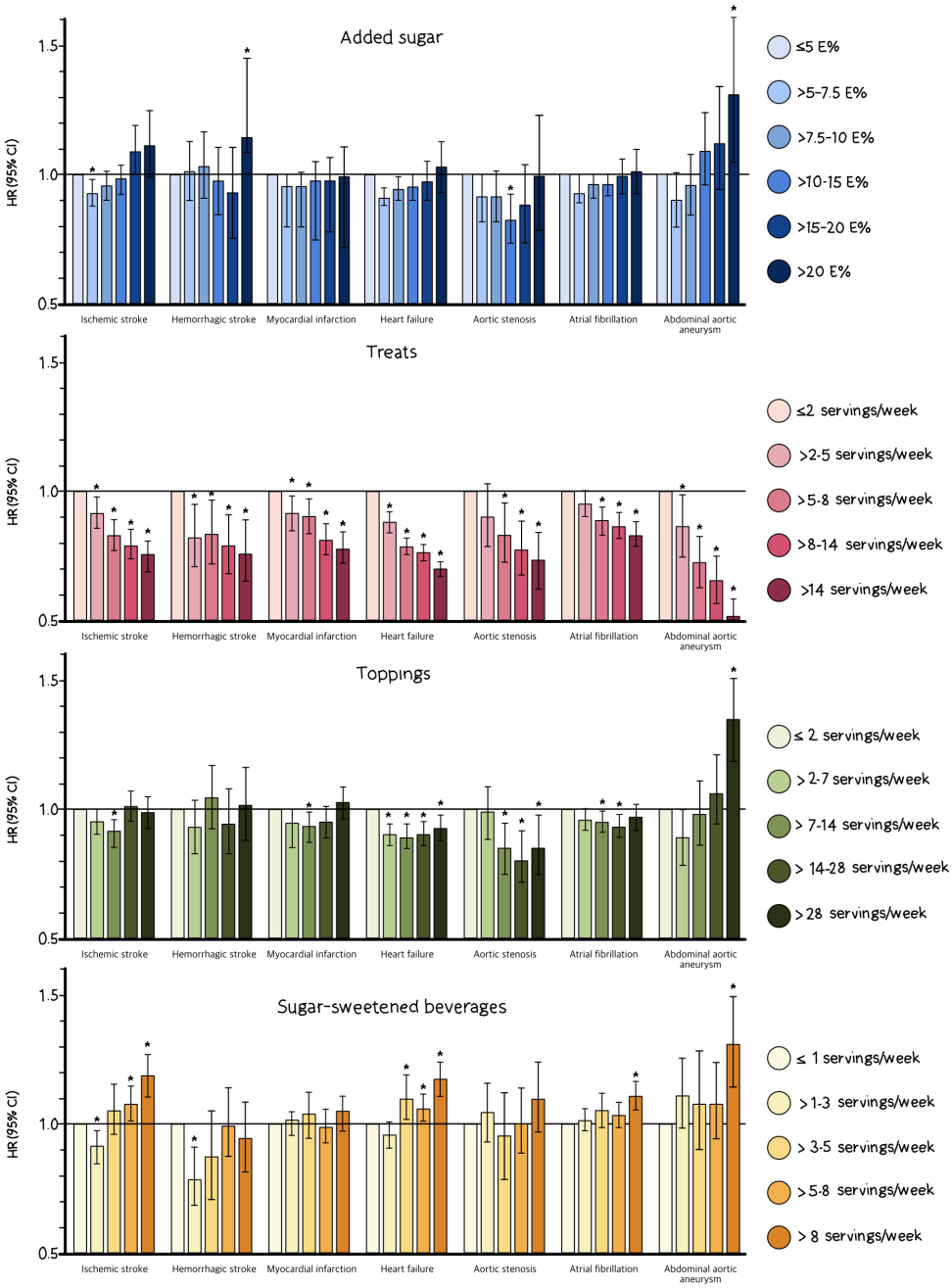


Figure 10. Associations between sugar intake variables and cardiovascular disease risk. The associations were investigated using multivariable Cox proportional hazards regressions adjusted for age, sex, total energy intake, smoking status, educational level, alcohol consumption, walking/bicycling, exercise, BMI, intake of processed meat, coffee, saturated fatty acids, and fiber intake. HR: Hazard ratio. CI: Confidence interval. E%: Energy percentage. BMI: Body Mass Index.

Study 3

The aim of **study 3** was to explore the associations between intake of total, added, and sweet-tasting sugars and a selection of SNPs previously associated with sugar intake, preference, and/or sweet taste sensitivity. In this sample of the MDC, 64.1% of the participants were women, had a mean age of 58 years, and a mean BMI of 25.5 kg/m^2. The mean daily intake of total sugars for this population was 20.4 E%, the mean added sugar intake was 10.2 E%, and the mean intake of sweet-tasting sugars was 16.0 E%.

Replication of SNPs associated with sugar phenotypes

We analysed the association between a total of 101 SNPs and intakes of added sugar, total sugar and sweet-tasting sugars. The main SNPs of interest were 8 SNPs identified in previous GWAS on total sugar intake, as well as 2 SNPs in the *FGF21* gene. Our study found various Bonferroni-corrected significant associations between the studied SNPs and the three main outcomes of interest. The strongest associations were found for three SNPs within chromosome 19, all located within (rs838133) or in close proximity (rs838145, rs8103840) to the *FGF21* gene. Another significant association was found between the rs60764613 within the CTD-2015H3.1 gene and the intake of added sugar. No associations with SNPs previously identified using the candidate gene approach and intake of total, added, or sweet-tasting sugars reached the Bonferroni-corrected significance threshold (**Figure 11**).

Stratification by BMI

The associations between the studied genetic variants and sugar intake for participants with BMI ≥25 kg/m^2 (50.8% of the population) were generally stronger than those with BMI <25 kg/m^2. Among participants with a BMI ≥25 kg/m^2, the associations between rs11642841 within the FTO gene and total sugar, added sugar, and sweet-tasting sugars were strengthened compared to the main results. Indications of interactions between BMI and rs11642841 on total sugar, added sugar, and sweet-tasting sugars were found ($p = 0.04$ for all outcomes). For BMI < 25 kg/m^2, only a few associations met the Bonferroni-corrected threshold of significance, such as the association between rs838133 with an intake of sweet-tasting sugars.

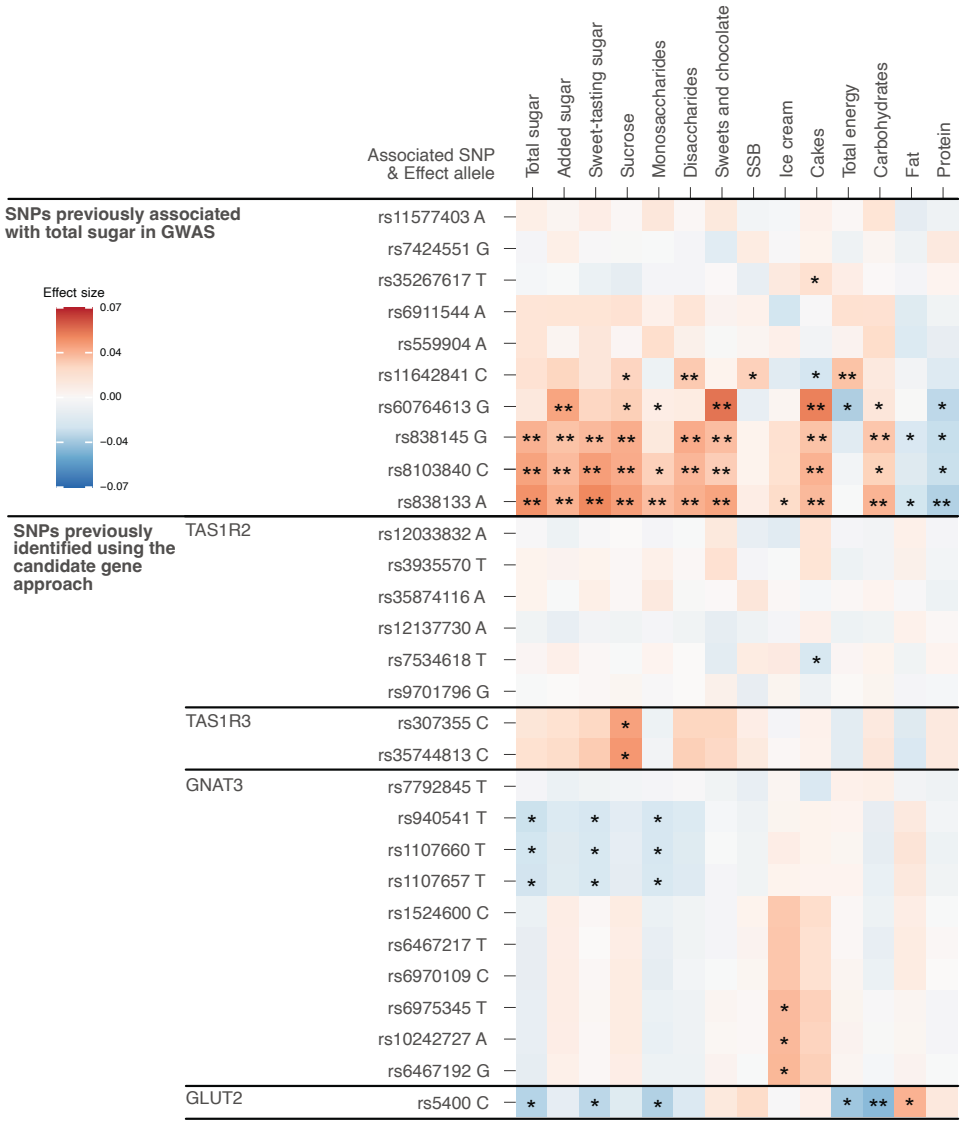


Figure 11. Associations between the 10 primary SNPs as well as SNPs previously identified using the candidate gene approach and total sugar, added sugar, and sweet-tasting sugar intake. The analyses were adjusted for age, sex, method (45- or 60-min dietary interviews), and total energy intake (kcal/day). The effect sizes are presented as β/SEE. TAS1R2: Taste receptor type 1 member 2 gene. TAS1R3: Taste receptor type 1 member 3 gene. GNAT3: G protein subunit alpha transducin 3. GLUT2: Glucose transporter 2 gene. SEE: Standard error of the estimate.

* $p < 0.05$, ** $p < 0.005$.

Study 4

The aim of **study 4** was to identify novel genetic variants associated with free sugar intake and sweet-tasting sugar intake in the MDC and the UKB, to investigate whether the associations were independent of BMI and various lifestyle factors, and to study the genetic correlations between sugar intake and cardiovascular outcomes. The MDC study included 25,660 participants, 61% of whom were female, with an average age of 58 years. The mean free sugar intake was 11 E% and the mean sweet-tasting sugar intake was 16 E%. In the UKB study, there were 141,437 participants, 62% of whom were female, with an average age of 56 years. The mean free sugar intake was 13 E% and the mean sweet-tasting sugar intake was 22 E%.

GWAS results

For free sugar intake, no GWAS-significant SNPs were identified in the MDC, but in UKB the lead SNPs were found on chromosome 3, 16, and 19. When meta-analysing the GWAS-results from the MDC and the UKB for free sugar intake, the lead SNPs for free sugar were identified in the *FTO* gene on chromosome 16, and near the *FGF21* gene on chromosome 19 (**Figure 12**).

For sweet-tasting sugar intake, no GWAS-significant SNPs were identified in the MDC, but in the UKB the lead SNPs were found on chromosome 1, 5, 15, and 19. When meta-analysing the GWAS-results from the MDC and the UKB, the lead SNPs for sweet-tasting sugar intake were found in the *FTO* gene on chromosome 16 and near the *FGF21* gene genes on chromosome 19 (**Figure 12**).

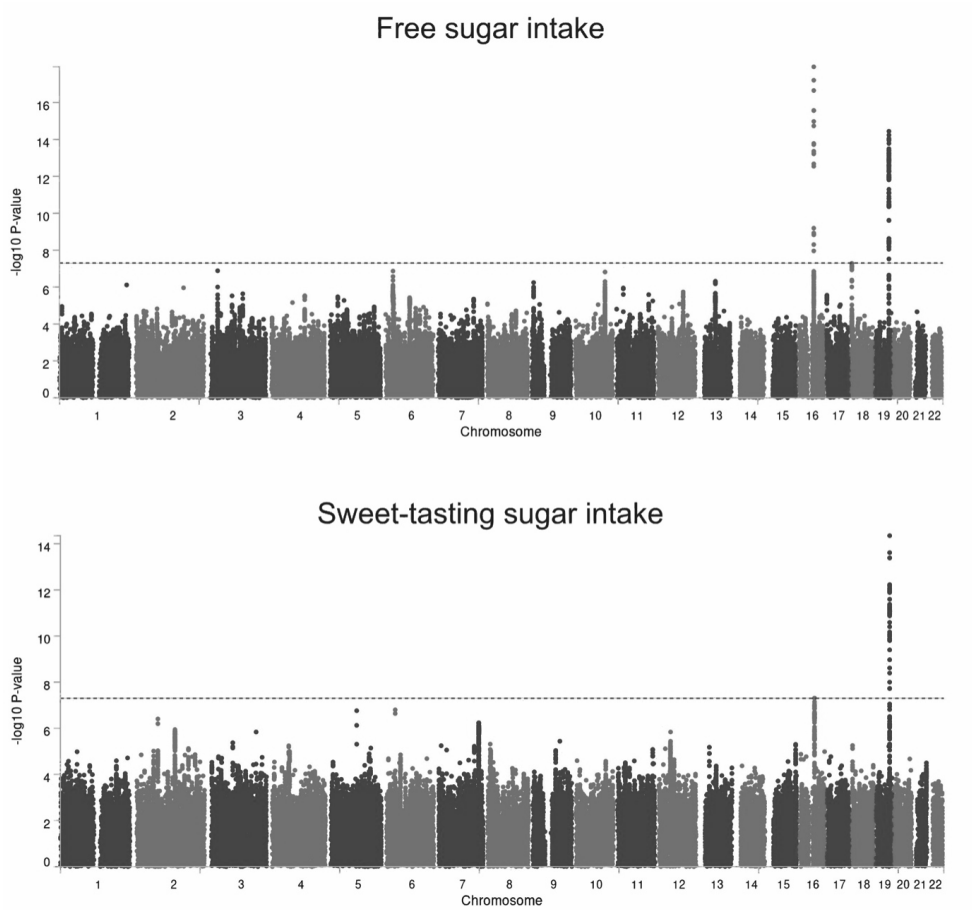


Figure 12. Manhattan plots of the meta-analyzed GWAS results from the UKB and the MDC for intake of free sugar and sweet-tasting sugars. GWAS: Genome-wide association study. MDC: Malmö Diet and Cancer study. UKB: UK Biobank.

Stratification by BMI

When stratifying the GWAS analyses based on BMI 18.5-25 kg/m^2 or >25 kg/m^2, no GWAS significant associations were found for sweet-tasting sugar intake among individuals with BMI 18.5-25 kg/m^2 in either cohort. For free sugar intake among individuals with BMI 18.5-25 kg/m^2 in the UKB, the lead SNP was found in the *FTO* gene on chromosome 16. Among individuals from the UKB with BMI ≥25 kg/m^2, the lead SNPs were found in the *FTO* gene for free sugar intake and near the *FGF21* gene for sweet-tasting sugar intake. When meta-analyzing the GWAS-results of individuals with BMI ≥25 kg/m^2, the lead SNPs for free sugar intake was found in the *FTO* gene, and near the *FGF21* gene on chromosome 19. The lead SNP

for sweet-tasting sugar intake was found near the *FGF21* gene on chromosome 19. No GWAS significant associations were found among individuals with BMI 18.5-25 kg/m^2 in the meta-analyzed GWAS-results.

bGWAS and genetic correlation

When adjusting the free sugar GWAS results for BMI, the associations with chromosome 18 SNPs and *FTO* SNPs were weakened, with further reduction observed after additional correction for education and smoking. The associations with *FGF21*-adjacent SNPs remained unchanged after correcting the free sugar GWAS. Adjusting the sweet-tasting sugar GWAS statistics for BMI also weakened the associations with *FTO* SNPs, and further adjustments for education and smoking did not alter these associations (**figure 13)**.

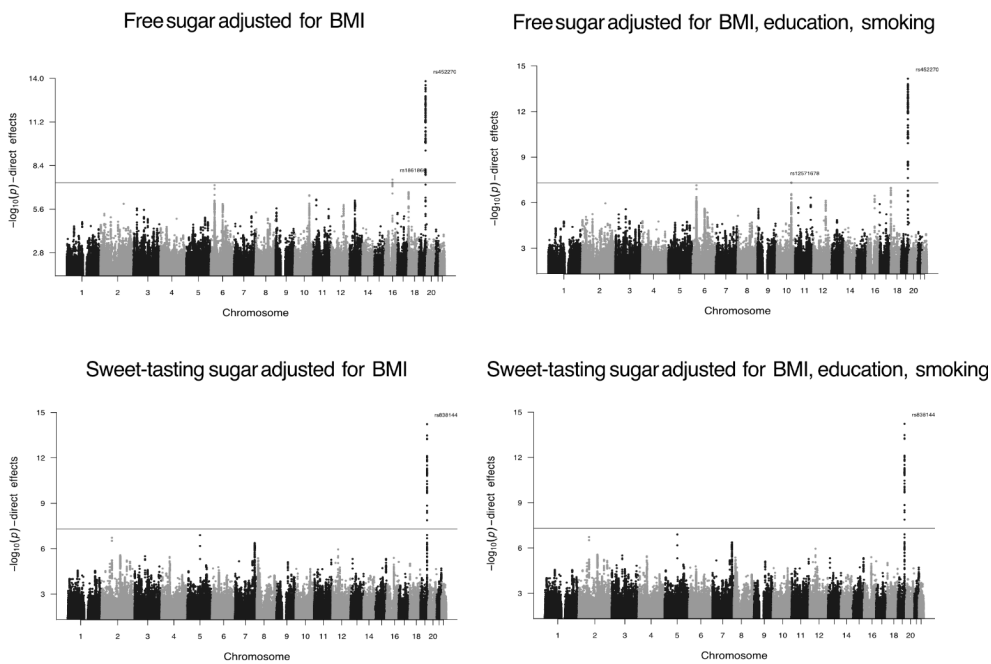


Figure 13. Manhattan plots of the bGWAS of the fixed effects meta-analyzed results of free sugar intake and sweet-tasting sugar intake from UKB and MDC, adjusted for BMI (left) and BMI, education, and smoking (right). BMI: Body Mass Index.

The genetic links between uncorrected free sugar and sweet-tasting sugar intake and cardiovascular outcomes were either insignificant or seemed favorable (i.e., indicated lower cardiovascular risks). However, after adjusting the free sugar data for BMI, several genetic links became significant and indicated negative cardiovascular outcomes. Specifically, after adjusting for BMI, free sugar intake was negatively linked to HDL cholesterol levels and positively linked to triglyceride levels, ischemic stroke risk, and atrial fibrillation. Conversely, it was negatively linked to heart failure risk. Further adjustments for education and smoking strengthened these links.

Similarly, after adjusting the sweet-tasting sugar data for BMI, sweet-tasting sugar intake was negatively linked to HDL cholesterol levels and positively linked to triglyceride levels, ischemic stroke risk, and atrial fibrillation. It was also negatively linked to heart failure risk. These links remained after further adjustments for education and smoking (**Table 5**).

Table 5. Correlations between genetically predicted free sugar intake, sweet-tasting sugar intake, and various cardiovascular outcomes.

	Free sugar intake						**Sweet-tasting sugar intake**					
	Model 1		Model 2		Model 3		Model 1		Model 2		Model 3	
	r_g	P	r_g	P	r_g	P	r_g	P	r_g	P	r_g	P
HDL	0.02	0.50	-0.05	0.05	-0.07	0.03	-0.03	0.30	-0.06	0.04	-0.06	0.04
LDL	-0.05	0.13	0.02	0.55	0.03	0.43	-0.04	0.6	0.04	0.29	0.04	0.29
logTG	-0.03	0.55	0.10	0.002	0.11	0.002	0.05	0.18	0.10	0.01	0.10	0.005
IS	-0.20	0.005	0.24	<0.001	0.26	<0.001	0.01	0.86	0.22	<0.001	0.22	<0.001
HF	-0.08	0.27	-0.19	0.001	-0.24	<0.001	-0.04	0.51	-0.20	0.001	-0.20	0.001
AF	0.01	0.95	0.12	0.003	0.15	<0.001	-0.01	0.97	0.11	0.005	0.11	0.005
AA	-0.14	0.12	-0.01	0.81	-0.02	0.83	-0.02	0.84	0.05	0.46	0.05	0.46
CAD	-0.08	0.06	0.04	0.29	0.05	0.24	0.01	0.81	0.06	0.06	0.06	0.06

Model 1: Uncorrected. Model 2: Corrected for prior GWAS on BMI. Model 3: Corrected for prior GWAS on BMI, education, and smoking. r_g: Genetic correlation. HDL: High-density lipoprotein. LDL: Low-density lipoprotein. logTG: Log-transformed triglyceride levels. IS: Ischemic stroke. HF: Heart failure. AF: Atrial fibrillation. AA: Aortic aneurysm. CAD: Coronary artery disease.

Study 5

The study included 883 participants, 50% of whom were female, with an average age of 57.9 years, a BMI of 26.0, a mean total sugar intake of 20.1 E%, a mean free sugar intake of 11.0 E%, and a mean monosaccharide intake of 7.4 E%.

Metabolite profiles

Following ENR modelling, the metabolite profiles included 60 metabolites for total sugar, 134 for free sugar, and 71 for monosaccharides. In total, 277 unique metabolites were identified across all sugar intake categories, with only 6 metabolites common to all sugar exposures. For sugar-sweetened food and beverage categories, 423 unique metabolites were identified, predominantly from sugar-sweetened beverages, with only 6 metabolites being common to all exposures (**Figure 14**). Lipid metabolites, primarily fatty acids, sphingomyelins, and those related to fatty acid metabolism, made up the largest proportion of metabolites for most exposures. Fatty acids were one of the most common sub-pathways among the metabolites identified for nearly all exposures.

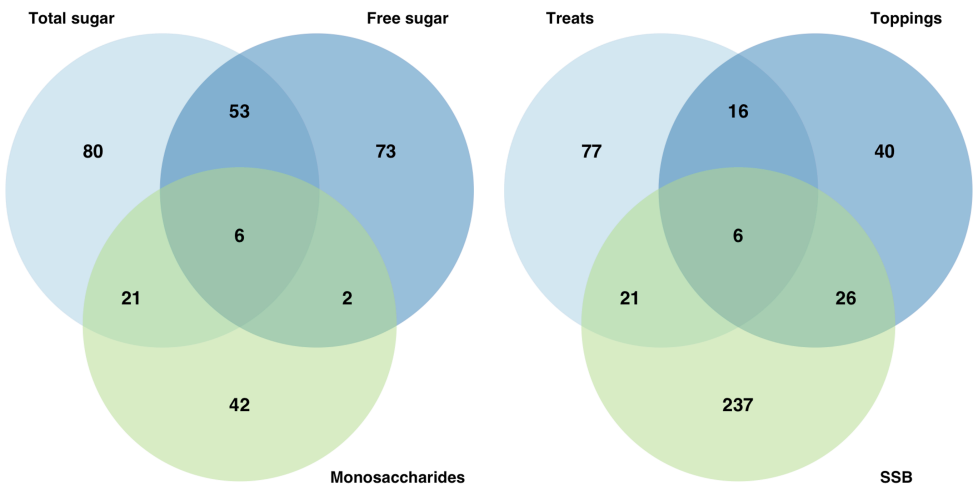


Figure 14. Venn diagram of metabolites included in the identified metabolite profiles for various sugar subtypes and sugar-sweetened foods and beverages. SSB: Sugar-sweetened beverages.

The calculated metabolite profiles score for all sugar intake categories were statistically significantly associated with their dietary sugar intake ($P<0.0001$), respectively, for both the training and testing set and for the overall metabolite profile. Monosaccharide intake ($r_{train}=0.61$, $r_{test}=0.58$, $r_{total}=0.60$) had the strongest correlation out of all the exposures, followed by total sugar ($r_{train}=0.69$, $r_{test}=0.37$,

r_{total}=0.60). The free sugar score was the lowest for the testing set (r = 0.23) but comparable to the other models for the training set (r = 0.63) and total (r = 0.52). The correlation coefficients for the testing sets for the sugar-sweetened foods and beverages were generally lower than for the sugar intake categories, being 0.18 for treats, 0.23 for toppings, and 0.05 for sugar-sweetened beverages.

Some of the metabolite profile scores were correlated with other dietary intakes aside from the exposure of interest. For example, the correlation coefficient between the monosaccharide profile score and fiber density was 0.50 (**Figure 15**).

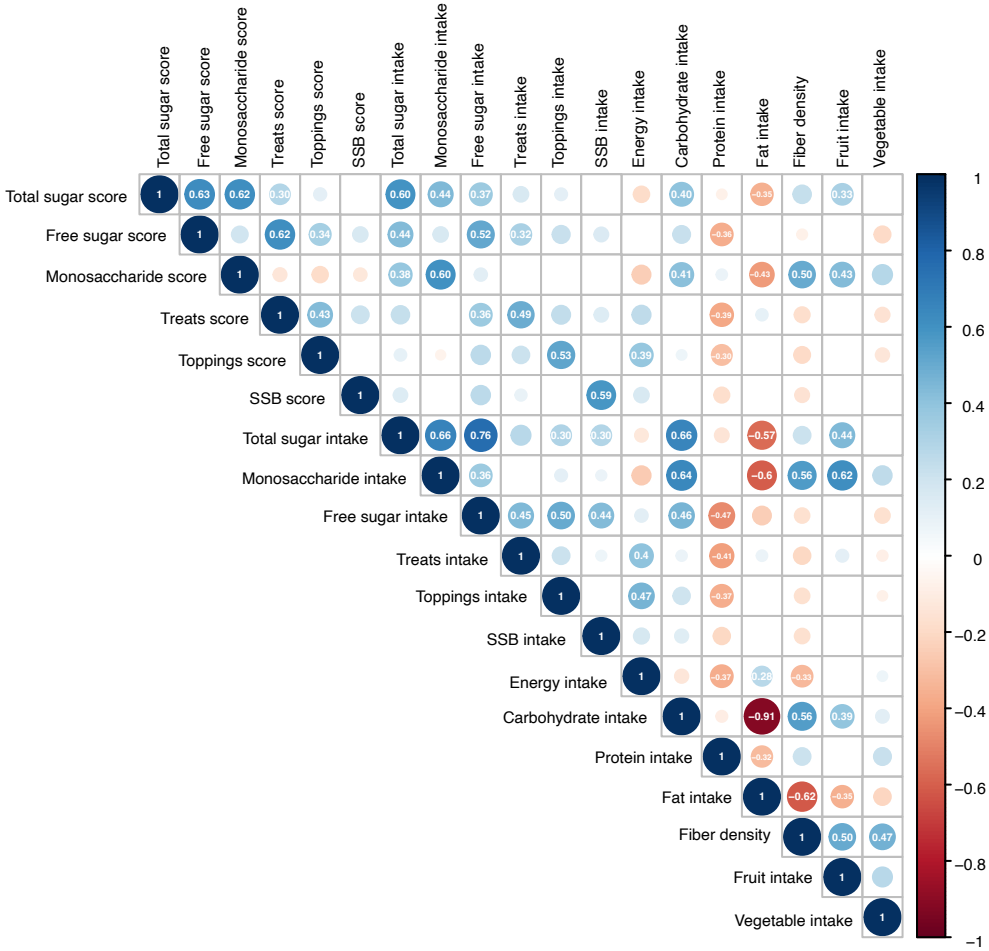


Figure 15. Heatmap showing the correlation coefficients between the identified metabolite profile score and various dietary intakes.

Metabolites associated with sugar subtypes

Total sugar intake

The distribution of pathways among the 160 metabolites identified for total sugar intake consisted of 32% lipid metabolites, 18% amino acids, 10% xenobiotics, and 22% unknown metabolites, among others (**Figure 16**). Out of the 160 metabolites, 8 metabolites reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model, including two out of the ten top metabolites.

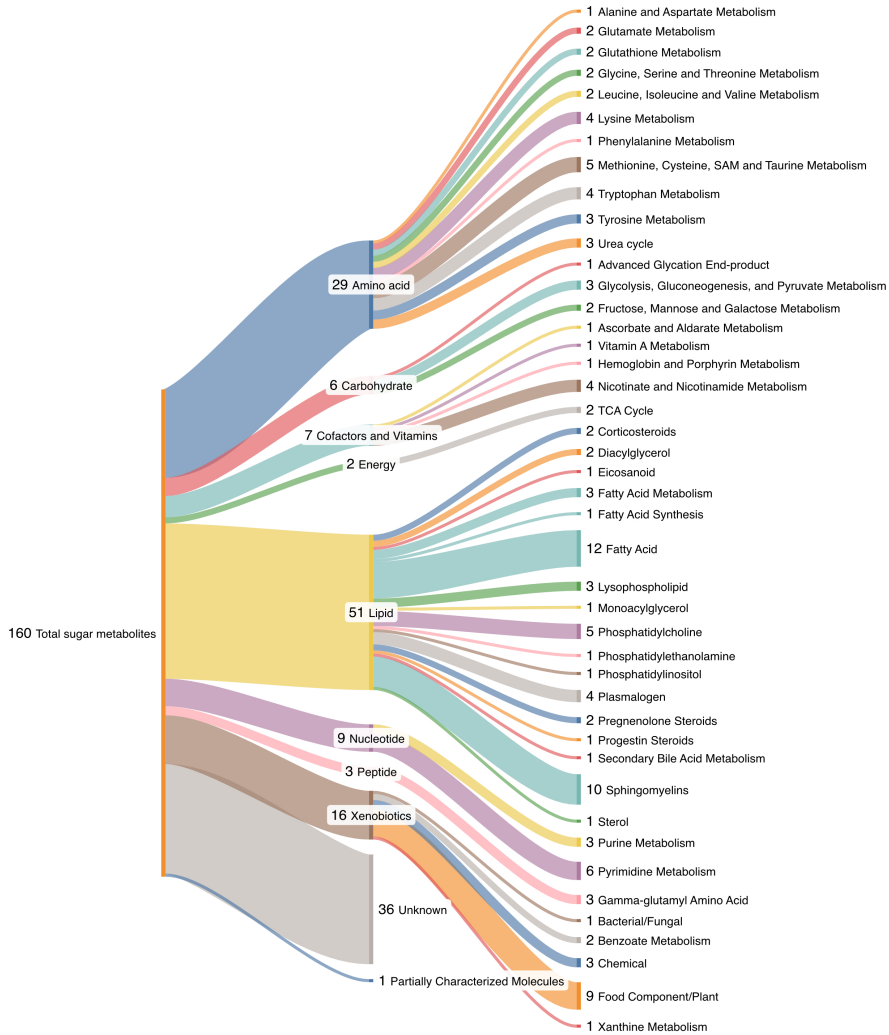


Figure 16. Overview of the pathways of the metabolites included in the total sugar metabolite profile.

Free sugar intake

The distribution of pathways among the 134 metabolites identified for free sugar intake consisted of 31% lipid metabolites, 22% amino acids, 10% xenobiotics, and 16% unknown metabolites, among others (**Figure 17**). Out of the 134 metabolites, 8 metabolites reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model, including four out of the ten top metabolites.

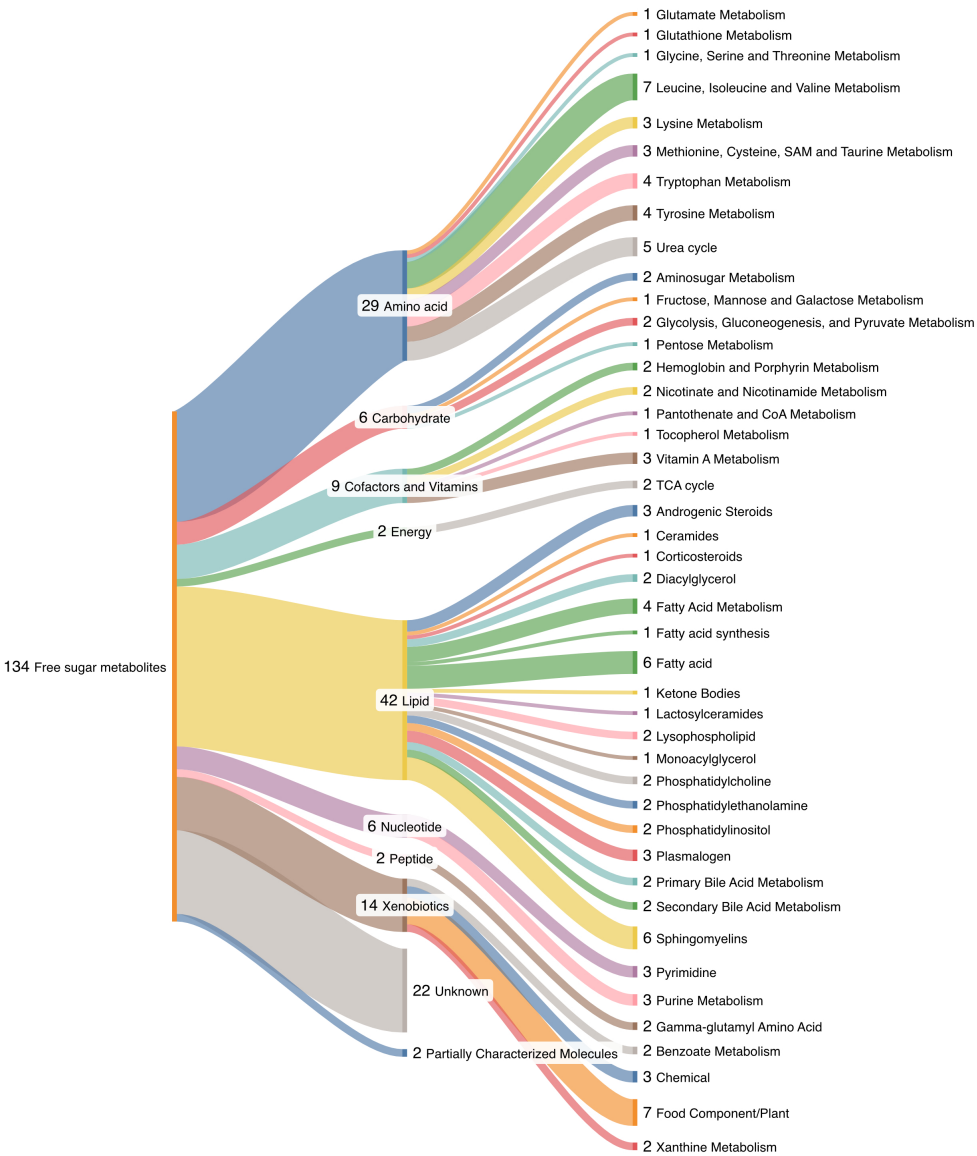


Figure 17. Overview of the pathways of the metabolites included in the free sugar metabolite profile.

Monosaccharide intake

The distribution of pathways among the 71 metabolites identified for monosaccharide intake consisted of 34% lipid metabolites, 16% amino acids, 16% xenobiotics, and 30% unknown metabolites, among others (**Figure 18**). Out of the 71 metabolites, 9 metabolites reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model, including four out of the ten top metabolites.

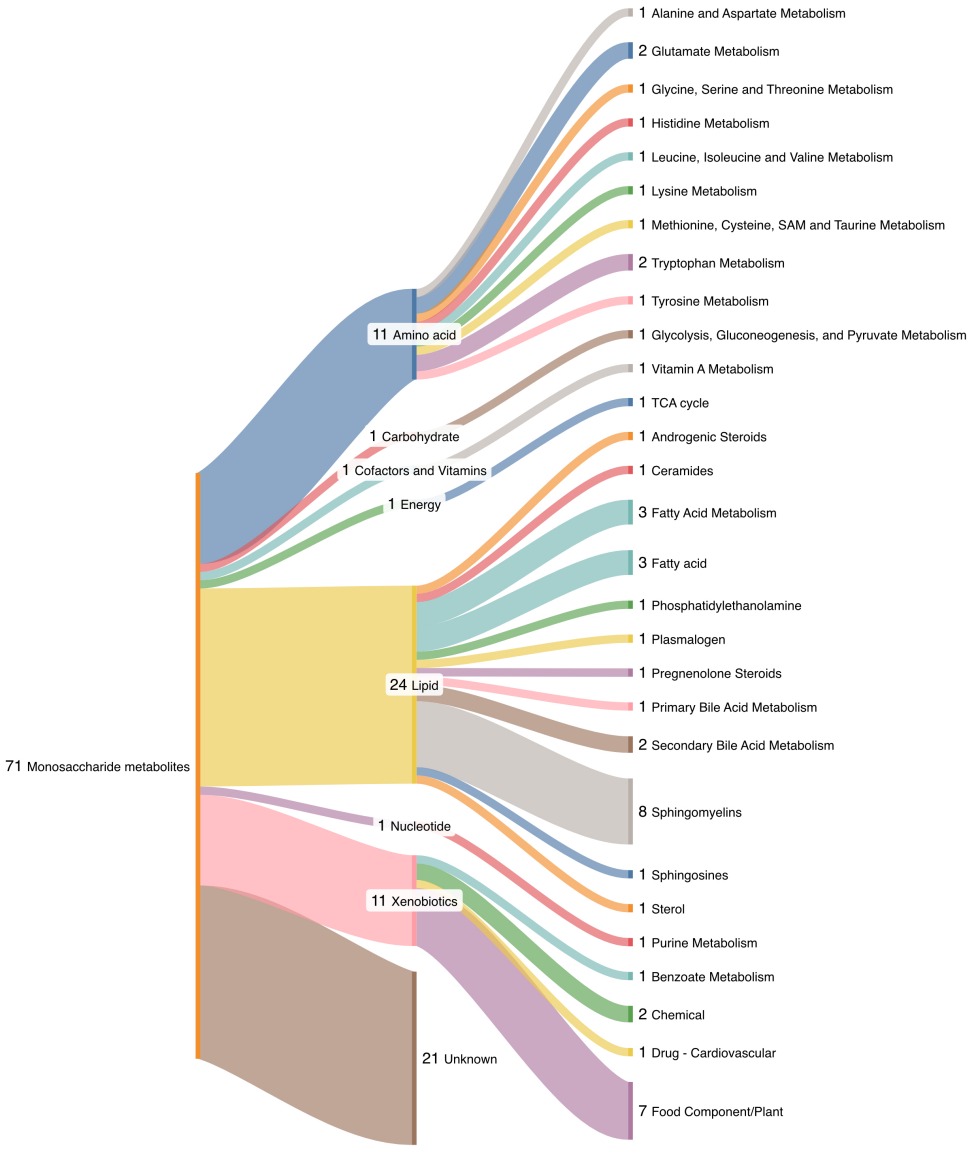


Figure 18. Overview of the pathways of the metabolites included in the monosaccharide metabolite profile.

Metabolites associated with sugar-sweetened foods and beverages

Treats intake

The distribution among the 119 metabolites identified for treats intake consisted of 36% lipid metabolites, 16% amino acids, 10% xenobiotics, and 22% unknown metabolites, among others (**Figure 19**). Out of the 119 metabolites, two metabolites reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model, including none of the ten top metabolites.

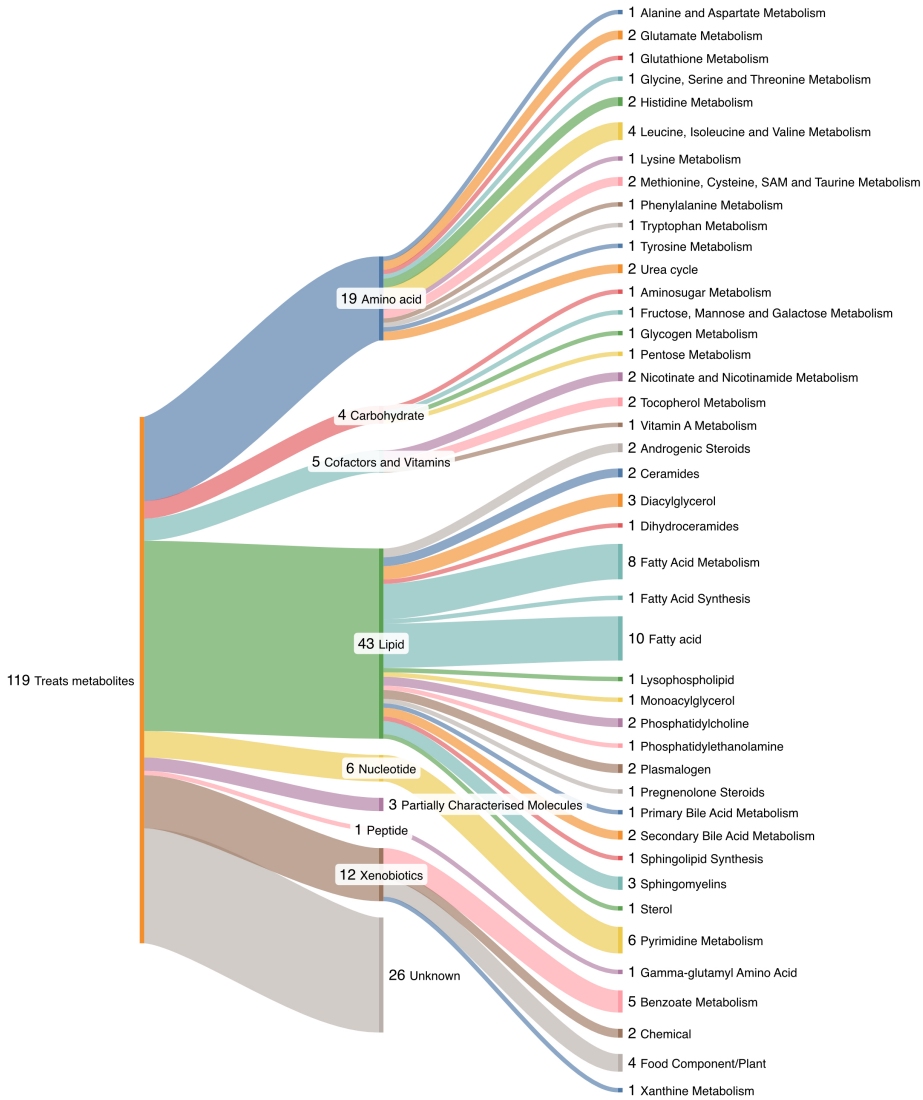


Figure 19. Overview of the pathways of the metabolites included in the treats metabolite profile.

Toppings intake

The distribution among the 87 metabolites identified for toppings intake consisted of 37% lipid metabolites, 17% amino acids, 8% xenobiotics, and 24% unknown metabolites, among others (**Figure 20**). Out of the 87 metabolites, one metabolite, which was included in the top ten metabolites, reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model.

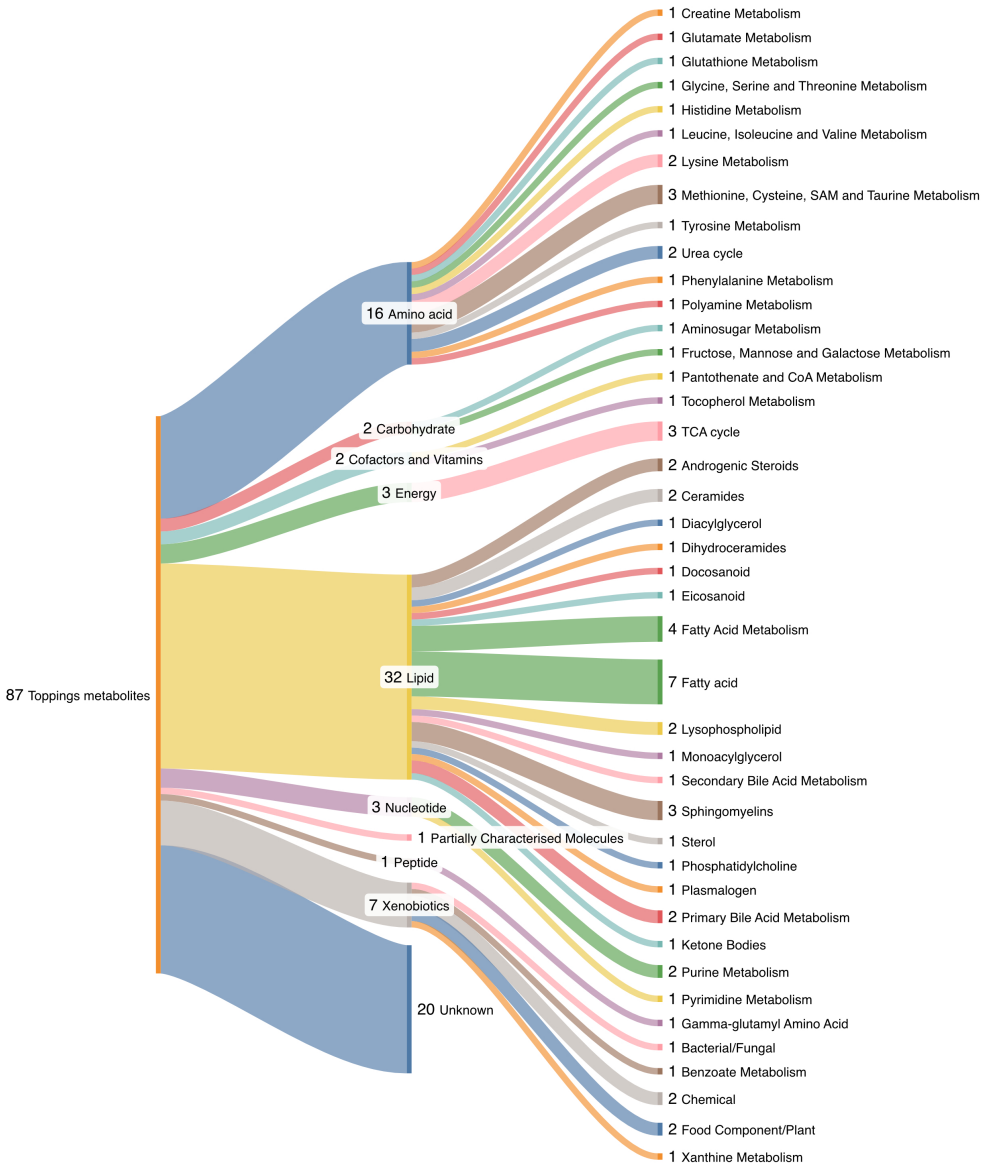


Figure 20. Overview of the pathways of the metabolites included in the toppings metabolite profile.

Sugar-sweetened beverage intake

The distribution among the 289 metabolites identified for sugar-sweetened beverages intake consisted of 28% lipid metabolites, 24% amino acids, 12% xenobiotics, and 23% unknown metabolites, among others (**Figure 21**). Out of the 289 metabolites, no metabolites reached the Bonferroni-corrected significance threshold in the fully adjusted linear regression model.

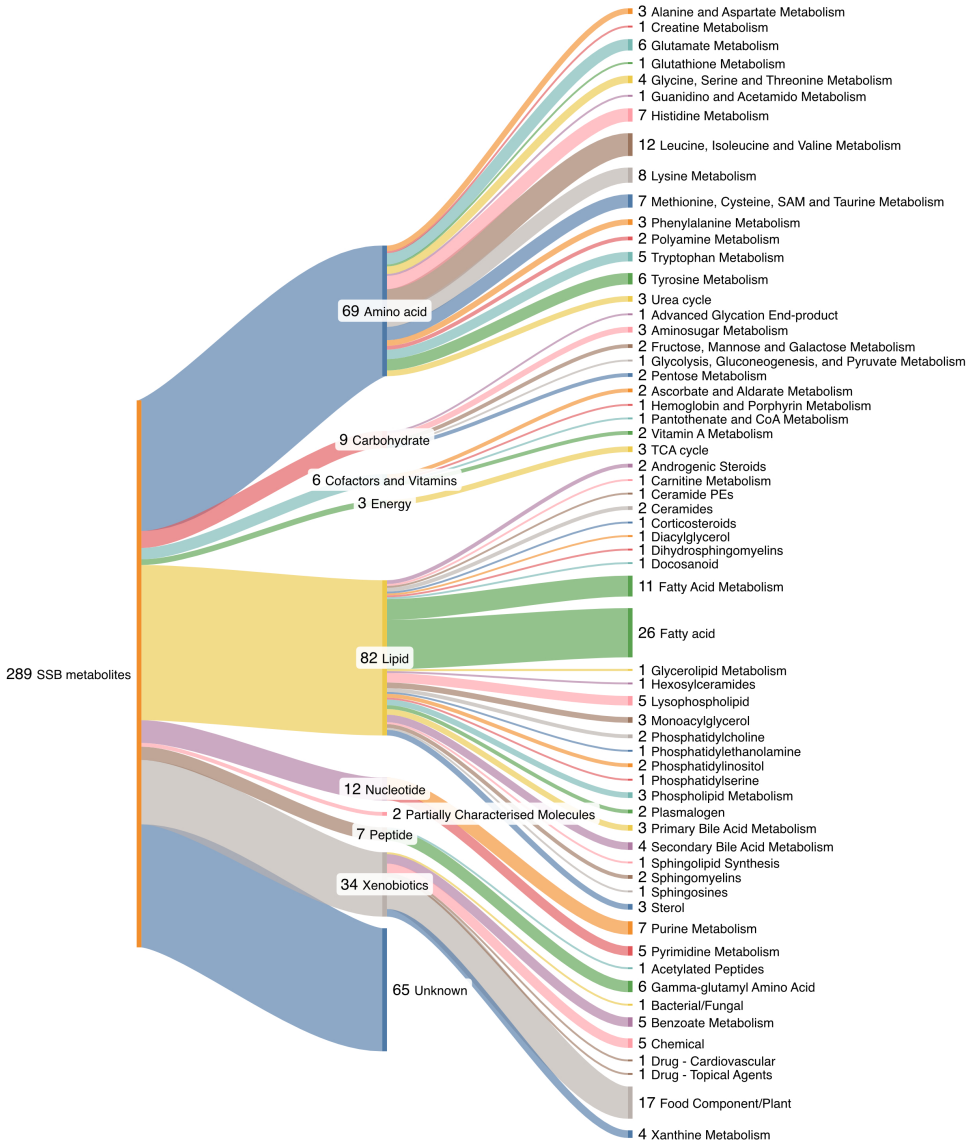


Figure 21. Overview of the pathways of the metabolites included in the SSB metabolite profile. SSB: sugar-sweetened beverages.

Discussion

Summary of the main findings

This doctoral thesis aimed to explore the role that sugar intake in different forms and from different dietary sources play in the development of cardiovascular disease. In **study 1**, high added sugar intakes (>20 E%) were associated with increased risks of incident coronary events, U-shaped associations were found for incident stroke, and the lowest added sugar intake category (<5 E%) was indicated to have the highest risk of atrial fibrillation and aortic stenosis. In **study 2**, statistically significant positive linear associations were found between added sugar intake and ischemic stroke and abdominal aortic aneurysm risks, but the highest risk of most of the studied outcomes were found in the lowest added sugar intake category. No associations were found between added sugar intake and hemorrhagic stroke risk. The non-linear associations between added sugar intake and atrial fibrillation and aortic stenosis were comparable between **study 1** and **study 2**, with the highest relative risk found in the lowest intake category for both outcomes. The findings in both studies further consistently show positive linear associations between sugar-sweetened beverage intake and cardiovascular disease risk, while conversely, showing negative linear associations for intake of treats and cardiovascular disease risk.

In **study 4**, the genetic correlations between free sugar intake, sweet-tasting sugar intake and cardiovascular disease risk and cardiovascular disease risk markers were studied. For both exposures, positive genetic correlations were observed with ischemic stroke risk and atrial fibrillation risk, while negative genetic correlations were found with heart failure risk. For the cardiovascular disease risk markers, positive genetic correlations were found with log-transformed triglyceride levels, while negative genetic correlations were found with HDL levels.

This thesis further aimed to study markers of sugar intake by replicating genetic markers identified in previous studies (**study 3**) and identifying novel genetic markers (**study 4**) of sugar intake, and by identifying metabolite profiles of sugar intake (**study 5**). Previously identified associations with genetic variants in sweet-taste receptor genes and glucose transporter genes could not be replicated in this thesis. The genetic variants previously identified in the *FTO* gene to be associated with total sugar intake in the UKB were not replicated in the MDC. In **study 4**, GWAS-significant associations were found with SNPs in the *FTO* gene (in the UKB

and meta-analyzed results), in an intergenic region on chromosome 18, and near the *FGF21* gene on chromosome 19. Only the associations with genetic variants in the *FGF21* gene were independent of lifestyle factors such as education, smoking, and BMI. Finally, metabolite profiles of different groups of sugar intake from different dietary sources were identified and they could, if replicated in other populations, potentially be suitable as biomarkers of habitual sugar intake.

The results in context of the existing literature

Sugar intake and cardiovascular disease risk

Few studies have investigated the associations between added sugar intake and incidence of specific cardiovascular disease. Results from a study on 109,034 women from the Women's Health Initiative show increased risks of total cardiovascular disease and coronary heart disease associated with higher added sugar intake, while no associations were found with incident heart failure, total stroke, ischemic stroke, nor hemorrhagic stroke (27). A study of 110,497 participants from the UK biobank investigated the associations between free sugar intake and cardiovascular disease risk, showing positive associations with total cardiovascular disease, total stroke, and ischemic heart disease per 5 E% increase of free sugar intake (28). It should be noted that these studies, and **study 1** and **study 2**, have been conducted in different populations (UK and US, while our studies were conducted in Sweden), investigating different exposures (free sugar or added sugar), using different dietary assessment methods (repeated 24-hour dietary recall in the UK biobank, a modified diet history method in MDC, and food-frequency questionnaires in SIMPLER and Women's Health Initiative), and different cutoffs used for the categorization of sugar intake, as well as different exclusion criteria and covariates adjusted for. All these differences could affect the results.

Although consumption of <5 E% of added sugar was associated with a higher risk of several cardiovascular diseases in **study 1** and **study 2**, it is important to highlight that there are no well-established biological mechanistic explanations of why low added sugar intake would increase cardiovascular disease risk. Results from The Australian Health Survey 2011–2012 (weighted n=6,150) found that added sugar intakes below 5 E% were linked to lower micronutrient intakes, and it is possible that added sugar might be replaced by other nutrients among this low added sugar intake group, such as saturated fats, both of which could negatively impact cardiovascular health (114). Conversely, studies from two Swedish populations (n = 12,238 and n = 1,797) showed an inverse relationship between added sugar intake and micronutrient intake, challenging the idea that lower added sugar intakes are linked to lower micronutrient intakes (115). The findings might rather have to do,

at least in some part, with dietary misreporting and reversed causation. Overall, the findings of **study 1** and **study 2** support the added sugar recommendations given in dietary guidelines and recommendations (<10 E%) (13, 15, 16) but do not support lowering the recommendations to <5 E%.

Finally, the genetic correlations identified between free sugar intake and sweet-tasting sugar intake and cardiovascular disease risk and risk markers in **study 4** are largely in line with the associations found in **study 1** and **study 2**, showing correlations with higher risks of some cardiovascular diseases and an unfavorable impact on cardiovascular disease risk markers such as higher log-transformed triglyceride levels and lower HDL levels. The findings for the risk markers are in line with previous randomized controlled trials indicating positive and causal relationships between free sugar intake and dyslipidaemia, with a moderate level of certainty of evidence (10, 25). The negative genetic correlations found with heart failure do however not have support in existing literature on phenotypic links between sugar intake and heart failure risk (27).

Sugar-sweetened food and beverage intake and cardiovascular disease risk

In **study 1** and **study 2**, the associations between cardiovascular disease risk and various groups of sugar-sweetened foods and beverages were studied. As previously mentioned, the adverse cardiovascular health effects of sugar-sweetened beverage intake are well-documented (10), but few studies have investigated other sources of added sugar. The findings for sugar-sweetened beverages in **study 1** and **study 2** are in line with previous studies consistently showing positive linear associations with cardiovascular disease risk (10). The results from treats and toppings intake were also largely consistent between **study 1** and **study 2**. A study on 176,352 participants from the UK biobank reported positive linear associations between free sugar intake from beverages and overall cardiovascular disease risk, ischemic heart disease, and stroke, while free sugar from treats (pastries, candies, chocolate, ice cream, sweetened yoghurt) and toppings (table sugar, jam, honey, syrup, peanut butter, chocolate/nut spread, stewed/cooked fruit) displayed U-shaped associations with overall cardiovascular disease risk, ischemic heart disease risk, and especially stroke risk (29).

The distinct difference observed between the total intake of added sugars and the intake of sugar-sweetened beverages in relation to cardiovascular disease risk is challenging to explain. Specifically, since sugar-sweetened beverages generally consist of water, added sugar, and some flavorings and coloring, sucrose or high-fructose corn syrup are the primary nutritious ingredients. One explanation for this discrepancy is that the sugar-sweetened beverage consumptions in the studied populations are relatively low, with many zero-consumers (~45% in both the MDC

and the SIMPLER cohorts). Another possible explanation for the relatively clear associations found between sugar-sweetened beverage intake and cardiovascular disease risk compared to overall added sugar intake could be due to it being easier to estimate and self-report. Sugar-sweetened beverages tend to come packaged in standardized portion sizes (33 cl cans or 50 cl bottles), whereas added sugar may be more difficult to estimate as it enters our diet in various different ways and may be hidden in many products under more healthy-sounding names or under names that the consumer might not identify as sugars (such as fruit juice concentrate or maltodextrin). With a more accurate dietary assessment, it is more likely that an association with cardiovascular disease risk is identified in analyses if there truly is one, than if the dietary assessment is inaccurate.

There are likely some inherent differences between sugar-sweetened beverages and other sources of added and free sugar that can help explain the differences observed in the findings for different sugar-sweetened foods and beverages in this thesis. The liquid state of sugar-sweetened beverages makes them less satiating than solid added sugar, leading to insufficient compensatory reduction of caloric intake, and potentially increased energy intake over time (116, 117). The digestion process for liquid is faster since no chewing or other mechanical digestion in the upper gastrointestinal tract is required. Further, gastric emptying time is significantly reduced for liquids compared to solids, allowing sugars in sugar-sweetened beverages to be rapidly ready for enzymatic hydrolysis in the small intestine, resulting in a quicker and steeper blood glucose and insulin response than solid sugary foods (118). Additionally, sugar-sweetened beverages consist solely of sugars, with no fat, protein, or fiber to slow down digestion, leading to a rapid metabolic response (119). The acidic nature of sugar-sweetened beverages may also contribute to the hydrolysis of sucrose into glucose and fructose already in the can or bottle. Moreover, the presence of caffeine in some of the most common sugar-sweetened beverages enhances their taste and the carbonation in these beverages could potentially also increase appetite and the risk of weight gain (120).

The negative associations found between treats and cardiovascular disease risk in **study 1** and **study 2** are however more difficult to explain. Treats such as chocolate, ice cream, and pastries are usually high in added sugar and saturated fats, contributing to a higher energy density and a lower micronutrient density of the diet. One possible explanation for the negative associations between intake of treats and cardiovascular disease risk is the social tradition of “fika” in Sweden, where people get together with friends, relatives, or coworkers for a break with coffee and pastries (121, 122). Therefore, it is possible that consuming treats is a common aspect of many Swedish people's daily routines and is not necessarily associated with overall poor dietary or lifestyle habits but may rather indicate social engagement. Conversely, literature suggests that consumption of sweetened beverages is linked to less healthy dietary and lifestyle patterns (123, 124). This highlights the challenges of focusing on a single nutrient or food in nutrition research, as single

dietary components do not typically dictate health or disease but are part of dietary patterns (125, 126). A diet low in sugar can still be unhealthy, while a diet with relatively high sugar content can be healthy, depending on the overall dietary and lifestyle context.

The genetics of sugar consumption

In **study 3** and **study 4**, the genetic background of sugar intake was studied. In **study 3**, only four out of the 101 studied SNPs could be replicated in the MDC. These SNPs included three SNPs within or near the *FGF21* gene, which were associated with total sugar, added sugar, and sweet-tasting sugar intake, and one SNP within the *CTD-2015H3.1* gene which was associated with added sugar intake. Unlike the findings reported by Hwang et al. (64), our main analyses did not reveal any significant associations with the rs11642841 variant within the *FTO* gene. However, when we stratified our sample by BMI, we found a link between rs11642841 and both total and added sugar intakes in participants with a BMI of 25 kg/m² or higher. In **study 4**, GWAS-significant associations were found with SNPs in the *FTO* gene (in the UKB and the meta-analyzed results, but not in the MDC), in an intergenic region on chromosome 18, and in a region near the *FGF21* gene on chromosome 19.

The findings for the *FGF21*-adjacent SNPs were in line with previous studies that have identified multiple variants within the *FGF21* locus associated with macronutrient intake (64, 127-129). The hormone FGF21 is thought to signal to the central nervous system to suppress the preference for sweet tastes and sugar consumption through a negative feedback mechanism, aiming to balance macronutrient intake (130-132). Studies have shown that FGF21 analogues can suppress sweet taste preference in animal models (133), and similar effects have been suggested in humans through antibody-mediated activation of the FGF21 receptor complex (134). While there is substantial evidence that FGF21 can decrease sugar intake in mice, emerging research indicates this might be a secondary effect related to FGF21's influence on protein intake, though findings are mixed (135, 136). The FGF21 hormone is secreted in response to sugar and alcohol intake, as well as low-protein diets, indicating pleiotropic effects of the FGF21 hormone (130-132, 134-136). Due to these pleiotropic effects of FGF21, the genetic variants identified do not meet the assumptions of Mendelian randomization (60) and could thus not be used as instrumental variables to study causal effects between sugar intake and cardiovascular disease risk.

In **study 4**, strong associations were found between free sugar intake and SNPs within the *FTO* gene. However, it should be noted that the associations between *FTO* SNPs and sugar intake were only found in the UKB and the meta-analyzed results, and not in the MDC. The *FTO* gene has been consistently associated with overweight and obesity in both humans and mice, although the exact mechanisms

are not fully understood (113). The *FTO* risk alleles linked to sugar intake in this study have either been previously associated with lower BMI or are in linkage disequilibrium with variants that are (113). The genetic correlation between sugar intake and BMI aligns with previous findings, showing negative genetic and phenotypic correlations between relative total sugar intake and BMI, waist circumference, and waist-hip ratio (128). One suggested explanation is related to physical activity, as positive genetic correlations have also been reported between total sugar intake and physical activity levels. It is possible that individuals with a predisposition to be more physically active may consume more sugar as a convenient energy source for exercise (128). A report from the EFSA indicated a positive and causal relationship between free sugar intake ad libitum and obesity, but the available body of evidence does not support a positive relationship between free sugar intake in isocaloric exchange with other macronutrients and risk of obesity (10). Reporting bias could also influence the associations, with individuals with higher BMI potentially underreporting sugar intake. This warrants further investigation, and future studies should evaluate whether these results can be replicated in other cohorts outside of the UKB.

Metabolite profiles for sugar consumption

In **study 5**, we identified multi-metabolite profiles associated with habitual intake of total sugar, free sugar, and monosaccharides, as well as various sugar-sweetened foods and beverages. Although metabolite profiles of sugar intake have not previously been identified, the magnitude of the association strengths of the metabolite profiles with the self-reported sugar intakes ($r = 0.40$-0.69) was comparable to previous findings using ENR to identify metabolite profiles related to different plant-based diets ($r=0.33$-0.45) (67), and the Mediterranean diet ($r=0.24$-0.37) (108). The correlation coefficients for the testing sets for the sugar-sweetened foods and beverages were however generally lower than for the sugar intake categories. This could be explained by the number of non-consumers of sugar-sweetened foods and beverages being relatively high, especially for sugar-sweetened beverage intake. The high number of non-consumers likely affected the quality of the identified metabolite profiles for treats, toppings, and sugar-sweetened beverages.

The metabolites included in the different sugar intake metabolite profiles were quite distinct, with only 6 out of 252 metabolites being shared between total sugar, free sugar, and monosaccharide intake. This is notable since the examined categories of sugar intake share common intermediate endpoints in digestion and metabolism, specifically the monosaccharides glucose, fructose, and galactose. The fact that the sugar intakes share common intermediate endpoints in digestion and metabolism but still had very few metabolites in common could indicate that the consumption of total sugar, free sugar, and monosaccharides do in fact elicit different metabolite

responses, or that multiple metabolite profiles can represent the same response. Another possible explanation is that the metabolite profiles do not specifically reflect intake of the different sugar intake categories but rather the overall dietary pattern associated with higher intake of total sugar, free sugar, and monosaccharides, respectively. For example, the monosaccharide metabolite profile score was correlated with fiber density and fruit intake while the other sugar categories were not. It is also important to keep in mind that the identified plasma metabolite profiles reflect the general metabolic homeostasis resulting from the complex interactions between factors influencing metabolism. In addition to diet, other factors that influence plasma metabolites include general health status, genetic variability, and microbiome, among other factors (137-141), leading to further variation in the plasma metabolites measured. If the obtained metabolite score profiles can be validated in other cohorts, they could be part in forming new biomarkers for estimation of sugar consumption.

Methodological considerations

Study design

While randomized controlled trials are generally the best way to explore causal relationships between exposures and outcomes, experimental studies are not always feasible in nutrition research, especially when it comes to disease outcomes that can take years or even decades to develop. Additionally, maintaining adherence to the dietary intervention often poses a challenge in lengthy and expensive randomized controlled trials (142). Instead, nutritional researchers often have to rely on observational studies, which are prone to confounding factors and other limitations. There are however many strengths of observational studies, such as being able to study large numbers of exposure and outcome variables, and being more cost-effective than large experimental studies.

In this thesis, two types of observational studies were used: Prospective cohort studies (in **study 1** and **study 2**) and cross-sectional studies (in **study 3**, **study 4**, and **study 5**). Cross-sectional studies are designed to study the associations between variables at a given time in a given population, a snapshot in time, one could say. This study design is suitable for studying non-modifiable variables, such as genetics. As a person's genetic makeup will remain the same throughout the course of their lifetime, a snapshot in time is sufficient to study it. While a person's genetic makeup won't vary, their diet may in fact vary over time. In cross-sectional studies, we consequently have to assume that we've accurately captured the study participants' habitual diets and that their dietary habits remain stable over time. However, with large enough study samples, this limitation may play a smaller role, and we could

still be able to see patterns in how genetics affect dietary intake, assuming that the variation is random among the population. Further, cross-sectional studies don't allow us to draw conclusions about temporality, meaning that, for example, we can't determine if the dietary exposure in fact precedes the measured metabolomics. This is a major limitation of this study design. Prospective cohort studies have the advantage of following the participants over time, which allows us to study disease outcomes that require a long follow-up period. They also allow us to draw conclusions about temporality, and further allow us, as in **study 2**, to get a better idea of the participants' dietary habits over time by conducting repeated dietary assessments during the follow-up period.

Overall, all studies included in this thesis are limited by their observational design, which restricts the ability to infer causal relationships from the associations observed. Despite adjusting the regression analyses for potential confounding variables, residual confounding likely remains, introducing some bias.

Validity of the findings

To determine the validity of the results obtained, we need to assess whether the findings are accurate, known as internal validity. Ensuring internal validity involves ruling out alternative explanations for the observed association (142). Further, we need to consider the generalizability, or the external validity, of the findings.

Internal validity

Some of the main factors that could negatively impact the internal validity of the findings of this thesis include dietary misclassification, reverse causation, and confounding. Misclassification occurs when study variables are incorrectly measured or classified. In nutritional studies, it often occurs in the form of dietary misreporting. The issue of dietary misreporting previously described in this thesis is likely present in the results, partly due to social desirability bias and difficulties in estimating sugar intake. Further, individuals who are aware of their increased cardiovascular disease risk, such as for example individuals with obesity, dyslipidaemia, or hypertension, tend to report lower sugar than their true consumption (48, 143). This was indicated to be true in the studied populations as well, and individuals with higher BMI were also overrepresented as estimated energy under reporters. This could result in a larger proportion of high-risk individuals being categorized into the lowest intake group, driving up the mean cardiovascular disease risk in this group. However, in **study 1**, after exclusion of individuals who were classified as misreporters of energy intake, the tendency of increased cardiovascular disease risk in the lowest added sugar intake group remained.

Another possibility is that individuals at higher cardiovascular risk due to for example a high BMI might not have misreported their added or free sugar intake but rather actively reduced their sugar intake to improve their health. This would result in the classification of sugar consumers mimicking underreporting, resulting in more high-risk individuals being classified in the lowest intake group due to their altered dietary habits rather than inaccurate reporting. Therefore, elevated cardiovascular disease risk precedes low added sugar consumption, reversing the causality direction. Conversely, individuals with low perceived cardiovascular risk or individuals of a healthy weight may not avoid sugar-sweetened foods and beverages because they don't feel a need to (144), and may therefore be categorized in higher added sugar intake groups.

Finally, confounding occurs when there is a third variable connected to both exposure and outcome that could be masking the real association between them, thus affecting the interval validity of the findings. In the studies included in this thesis, we have considered various potential confounders by incorporating them into the adjustment models. Despite these precautions, we cannot eliminate the risk of residual confounding. For example, in **study 1** and **study 2**, we suggested that one possible alternative explanation for the negative associations between higher consumption of treats and cardiovascular disease risk might be attributed to the Swedish cultural tradition of fika. This is something that should consequently be investigated in more detail in future studies. Further, sodium intake might be an important confounder for the associations between sugar intake and cardiovascular disease risk, but the dietary assessment methods used in the included studies did not have detailed enough information about the participants' sodium intakes to be able to include that variable as a confounder in the adjustment models.

External validity

One factor that could affect the generalizability, or external validity, of the findings is that the study participants may not be representative of the general population. For example, participants of the MDC had similar socioeconomic characteristics as non-participants but reported better perceived health and were more likely to be born in Sweden than non-participants (145). Another aspect that can affect the generalisability of the results is the fact that the studies included in this thesis are conducted in adults or older adults and the results might thus not be appropriate for extrapolation to younger populations. Similarly, most of the studies in this thesis are carried out in Swedish populations. Dietary habits may vary considerably between different populations, an example of which is the distinct Swedish cultural tradition of fika, which may cause the findings to not be generalizable to other populations. **Study 4** was conducted in a Swedish and a UK population, but the analyses were restricted to individuals of European ancestry. Therefore, while those results might

be extrapolated to other European populations, they can likely not be generalized to individuals of other ancestries.

Strengths and limitations

Strengths

The studies included in this thesis have several methodological strengths. The MDC, the UKB, and the SIMPLER cohorts are all large cohort studies with detailed information about dietary habits and anthropometric, socioeconomic, and lifestyle factors. Further, the follow-up period for the MDC and the SIMPLER cohorts are long enough to allow identification of diseases with long development time. Another strength is that all included cohorts used validated data collection methods. Particularly, The Malmö Diet and Cancer Study remains to this day one of the most comprehensive dietary data collections in Sweden, and it also contains information about potential energy misreporting and prior drastic dietary changes. The SIMPLER cohorts have the advantage of having carried out two dietary assessment methods, in 1997 and then in 2009, allowing for updated dietary information to be incorporated into the analyses. The UKB has the advantage that the 24-hour recall was repeated several times for some participants which may help in estimating the participants' habitual dietary intakes.

Limitations

One of the biggest limitations of the MDC is that the dietary data collection was carried out only once at baseline and therefore changes in dietary habits could not be evaluated. Additionally, the cohort is rather old, and the collected dietary data may not be representative of today's dietary habits. This could however also be an advantage as it is likely that underreporting of added sugar and free sugar intake specifically would be more pronounced today than 30 years ago because the public awareness of the possible adverse health effects of a high sugar intake have increased since then.

A major limitation of **study 5** is that the blood samples used for analyzing metabolites were collected at only one point in time. Had metabolites been analyzed several times, what exposure the metabolites reflected would have been easier to tease out. Some metabolites may reflect more short-term exposures while other metabolites reflect more long-term exposures. We also have to make the assumption in this study that the participants' reported dietary intakes are representative of their habitual diets and that they remain stable over time. Finally, as previously mentioned, a major limitation of the studies included in this thesis is residual confounding.

Conclusions

This doctoral thesis investigated the role that sugar intake in different forms and from different dietary sources play in the development of cardiovascular disease, and identified genetic markers and metabolite profiles of sugar intake that have the potential to be used as objective markers of sugar intake in future studies. Taken together, the findings of the five studies included in this thesis indicate the following:

1. The associations between added and free sugar intake and cardiovascular disease risk are difficult to conclude as the shapes and directions of the associations vary across different cardiovascular diseases. Overall, high added and free sugar intake was associated with ischemic stroke risk but the highest risks for most outcomes were found in the lowest added and free sugar intake categories.
2. The associations between sugar-sweetened foods and beverages and cardiovascular disease risk vary depending on the cardiovascular disease and on the sugar source, with sugar-sweetened beverages consistently being positively associated with cardiovascular disease risk while the results indicate negative associations between intake of treats and cardiovascular disease risk.
3. Free sugar intake and sweet-tasting sugar intake is associated with genetic variants in the *FTO* gene (in the UKB but not in the MDC), an intergenic region on chromosome 18, and near the *FGF21* gene on chromosome 19. Only the associations with the *FGF21*-adjacent genetic variants were independent of education, smoking, and BMI. Previously identified genetic variants in sweet-taste receptor genes and glucose transporter genes, as well as those in the *FTO* gene, could not be replicated in the MDC.
4. Distinct metabolite profiles for habitual consumption of total sugar, free sugar, and monosaccharides, as well as various sugar-sweetened foods and beverages were characterized.

In summary, this thesis contributes valuable insights into the complex relationships between sugar intake and cardiovascular disease risk. Replication in other populations is however required, especially since the findings for treats may be culturally specific to Sweden. Further, the identified markers of sugar intake require further study before they can be used as biomarkers of sugar intake in other settings.

Public health perspective

Sugar intake recommendations

One reason for why there are some discrepancies between sugar intake recommendations is that some organizations or authoritative bodies vary in their basis of the recommendations. Some base their recommendations solely on the evidence of the associations between sugar intake and disease risk and risk factors of disease. Others also consider theoretical reasoning and modelling, such as the idea that reducing sugar consumption allows for more nutritious foods within our energy needs, leading to healthier populations.

An example of this dichotomy is evident in the development of the Dietary Guidelines for Americans, 2020-2025. The US Departments of Agriculture (USDA) and Health and Human Services (HHS) maintained the recommended maximum intake of added sugars at 10 E%, the same as previous years (15). However, the Dietary Guidelines Advisory Committee, which examines evidence on specific nutrition and public health topics to provide independent scientific advice, recommended reducing the intake to 6% (146). The committee's recommendation was based on evidence suggesting that added sugars, particularly from sugar-sweetened beverages, may contribute to unhealthy weight gain and obesity-related health outcomes. They argued that less than 6% from added sugars is more consistent with a nutritionally adequate dietary pattern while avoiding excess energy intake from added sugars. Despite this, the USDA and HHS decided to retain the 10 E% recommendation on the basis of evidence of detrimental effects of added sugar on various health outcomes (147, 148).

The findings of this thesis align with the USDA and HHS conclusion that there is insufficient solid evidence to support a recommendation for added sugar intake below 10 E%. However, theoretically, there are no reasons why reducing added sugar intake further, to 6% of total energy, for example, would not benefit the population. Since added sugar does not provide any valuable nutrients, there are no nutritional drawbacks to further reducing intake.

Sugar consumption policies

In 2017, the WHO released a report emphasizing the necessity of reducing sugar intake and the role of policy in achieving this goal (149). The report outlines six key insights:

(1) The importance of discouraging manufacturers and retailers from adding sugar to processed foods and beverages.

(2) The need to balance the interests of industry and public health stakeholders through policy measures.

(3) The necessity of decreasing public demand for sugary products.

(4) The encouragement of substituting sugar-rich products with healthier alternatives.

(5) The oversight of harmful substitution patterns.

(6) The long-term promotion of reformulation strategies to transform the sugar-laden environment.

The report also discusses existing measures such as improved labelling, portion control, reformulation strategies, product placement, marketing, packaging, and advertising. Notably, it highlights the introduction of fiscal measures like sugar taxation. Additionally, the WHO published another document in the same year, which underscores the effectiveness of taxation in reducing the consumption of sugar-sweetened beverages, the financial savings for healthcare systems, the benefits of reinvesting the collected revenue into public health initiatives, and the greater impact of these policies on low-income consumers and at-risk groups (150). To date, sugar taxes have been implemented in nearly 120 countries (151), though Sweden is still considering whether this policy would be suitable for its population.

Food industry involvement

It has been suggested that the sweetened-beverage industry has employed several tactics to oppose the policies to decrease sugar-sweetened beverage intake by sugar-sweetened beverage taxation. For example, it was reported that the industry pledged to donate $10 million to the Children's Hospital of Philadelphia if the city council voted down the sugar tax that was being considered at the time, and that Coca-Cola donated $3 million to establish fitness programs in Chicago after a soda tax was proposed for the city (152). Another strategy employed has been suggested to be sowing doubt and distorting the available body of evidence linking sugar-sweetened beverages to adverse health outcomes. Studies show that systematic reviews on the relationship between sugar-sweetened beverage intake and body weight are less likely to report harmful effects if sponsored by the food industry (153-155). Although not all industry-funded research is biased, it frequently concludes

favorably for the sponsoring industry, and this high frequency of favorable outcomes raises suspicion about industry influence in nutrition research. This has an unfortunate consequence in contributing to public scepticism about the validity of all nutrition studies and the evidence-based recommendations from authoritative bodies.

Non-nutritive sweeteners

The substitution principle in dietary research suggests that reducing the consumption of one food group or nutrient should be accompanied by the replacement with another. Therefore, in discussing reductions in added or free sugar consumption, it is essential to consider what might replace it. A common alternative is artificial sweeteners, or non-nutritive sweeteners. Some people believe that non-nutritive sweeteners are as harmful as added sugars, but the fact is that the topic of the health effects of non-nutritive sweeteners is even less studied than the health effects of added or free sugar consumption. Epidemiological studies have found associations between the intake of artificially sweetened beverages and increased risks of type 2 diabetes, cardiovascular disease, and mortality (156-159). However, these studies often face the issue of reverse causation. High consumption of non-nutritive sweeteners may result from an already increased cardiovascular disease risk, making it a dietary choice to reduce risk. Epidemiological substitution models suggest that replacing sugar-sweetened beverages with artificially sweetened beverages is associated with reduced body weight, type 2 diabetes, and cardiovascular disease risk, and existing experimental studies do not support the idea that non-nutritive sweeteners increase cardiovascular disease risk. Finally, the assumption that all non-nutritive sweeteners can be treated equally and studied together needs reconsideration, as they may differ significantly in their molecular structure and health effects.

Currently, we need to determine if there is enough evidence to support policies aimed at reducing added or free sugar intake, considering that these sugars might be replaced with less-studied non-nutritive sweeteners. In my opinion, these policies are justified for sugar-sweetened beverage intake, however I believe that more research is needed to understand the health effects of overall added or free sugar intake and other dietary sources of these sugars before including them in taxation strategies.

Future perspective

As technology and research tools advance, we may discover new methods to assess and investigate diet as an exposure. The creation of more user-friendly approaches for gathering dietary data, such as online platforms, apps, and artificial intelligence based methods will enhance participation rates and enable multiple measurements of exposures, outcomes, and covariates throughout the follow-up period (160-162). These modern data collection methods could also increase the feasibility and cost-effectiveness of randomized controlled trials, which could pave the way for longer randomized controlled trials to examine causal relationships between sugar consumption and disease development.

To enhance future epidemiological studies that measure sugar intake through biomarkers and to gain a deeper mechanistic understanding by examining the metabolome, but also gut microbiota, the proteome, transcriptome, and more, future cohort studies should be designed to collect blood, urine, and fecal samples at multiple time points. Additionally, these prospective studies should invest in conducting multiple dietary assessments to better capture the dynamic dietary trends in today's society.

Lately, the field of precision nutrition is attracting increasing attention from both the scientific community and the general public. Unlike the traditional "one size fits all" approach to nutritional guidelines, this field advocates for tailored advice based on the needs of individuals or small subgroups of populations (163, 164). Utilizing the above-mentioned high-throughput technologies to better understand inter-individual differences in dietary responses could be essential for developing future nutritional recommendations and interventions to improve public health.

Finally, there is strength in numbers in research. More studies investigating the impact of sugar intake on cardiovascular disease risk are needed. This likely requires longer randomized controlled trials as well as observational studies utilizing novel biomarkers to get an understanding of whether higher sugar intakes cause cardiovascular disease and to understand the mechanisms behind any potential links. To do this, promising biomarkers of sugar intake first need to be validated in studies on individuals of different age groups, geographical areas, and ancestries.

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Printed by Media-Tryck, Lund 2025 NORDIC SWAN ECOLABEL 3041 0903

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Department of Clinical Sciences Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2025:66
ISBN 978-91-8021-719-4
ISSN 1652-8220