Sugar-Coated. The role of sugar intake and cardiovascular disease development in the context of nutritional recommendations

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2022

Document Version:
Publisher's PDF, also known as Version of record

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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 the 21st of April 2022 at 13:30 in Agardhsalen, Clinical Research Centre, Jan Waldenströms gata 35, Malmö.

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Organization LUND UNIVERSITY	**Document name** Doctoral dissertation
	Date of issue 21st April 2022
Author(s): Esther González-Padilla	Sponsoring organization

Title and subtitle: Sugar-coated: the role of sugar intake and cardiovascular disease development in the context of nutritional recommendations.

Abstract

Individual sugar consumption has increased over fifty-fold over the past two centuries. This sugar-coated environment has raised concerns regarding the possible pernicious health effects associated with a high sugar consumption. Rigorously investigating the association between sugar intake and disease development helps to inform evidence-based nutritional recommendations.

Micronutrient dilution is the displacement of nutrient-dense foods by the overconsumption of energy-dense and nutrient-poor foods. It has often been taken into consideration when nutritional recommendations are issued. In **Study 1**, we explored the association between added sugar intake and the intakes of nine micronutrients in two samples of the adult Swedish population: Riksmaten Adults (n = 1797) and the Malmö Diet and Cancer Study (n = 12,238). A general linear model revealed a significant negative linear association between added sugar intake and the intake of all micronutrients studied for both populations.

Cardiovascular disease continues to be one of the main causes of morbidity and mortality worldwide. Many cardiovascular diseases derive from an atherosclerotic process. Intima media thickness measured at the carotids is the first measurable sign of subclinical atherosclerosis. In **Study 2**, the intake of different types of sugars and sugar-rich foods and beverages was examined in association to intima media thickness. The intima media thickness was measured at the common carotid artery and the bifurcation of the carotids via ultrasound. We used a general linear model with multiple levels of adjustments for a sample of the Malmö Diet and Cancer Study – Cardiovascular Cohort (n = 5269). While no significant associations were found, a non-significant tendency to thicker intima media at the common carotid artery was observed for participants consuming the highest amount of added sugar.

Stroke is a cardiovascular disease characterised by the loss of brain function due to loss of blood flow, either by haemorrhage (haemorrhagic stroke) or blockage (ischaemic stroke). Dietary habits are closely related to the development of stroke. **Study 3** investigated the association between the adherence to healthy dietary patterns and incidence of total, ischaemic and haemorrhagic stroke. Two dietary scores were designed to measure adherence to the Swedish dietary guidelines and to the Mediterranean diet in the Malmö Diet and Cancer Study (n = 25,840). After a mean follow-up period of 19.5 years, 2579 cases of stroke were identified (80% ischaemic stroke). A Cox hazard regression revealed a protective effect against stroke for participants adhering to either of the dietary patterns. In regards to sugar intake, a protective effect against total and ischaemic stroke was found for participants adhering to soda intake recommendations from the Mediterranean diet. However, the opposite was found for participants adhering to the recommendations for sweets and pastries.

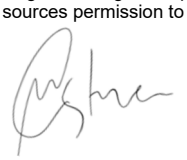
The study of genetics in nutrition has become an important tool to understand underlying pathways between diet and disease and inter-individual differences. **Study 4** explored the association between 114 single-nucleotide polymorphisms (previously linked with sugar consumption and preference), and the intakes of added and total sugar, as well as sugars with sweet taste. Our study of a sample of the Malmö Diet and Cancer Study (n = 22,794) revealed a Bonferroni-corrected significant association between three single-nucleotide polymorphisms in close proximity to the *FGF21* gene (rs838133, rs838145, and rs8103840) and the intakes of the sugars studied.

In summary, the link between sugar consumption, and micronutrient intake and genetic factors was shown in these studies. However, the link between sugar consumption and more complex outcomes like cardiovascular disease still needs to be studied further in order to provide accurate information for nutritional recommendations.

Key words: sugar intake, added sugar, free sugar, sugar-sweetened beverages, nutritional recommendations, micronutrient dilution, cardiovascular disease, atherosclerosis, intima media thickness, stroke, Swedish dietary guidelines, Mediterranean diet, genetics, *FGF21* gene, nutritional epidemiology

Classification system and/or index terms (if any)

Supplementary bibliographical information		**Language:** English
ISSN and key title 1652-8220 Doctoral dissertation series (Lund University, Faculty of Medicine)		**ISBN:** 978-91-8021-213-7
Recipient's notes	**Number of pages** 108	Price
	Security classification	

Signature  Date 2022-03-15

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Cover photo by Esther González-Padilla

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ISBN 978-91-8021-213-7
ISSN 1652-8220

Printed in Sweden by Media-Tryck, Lund University
Lund 2022

To my family, whether by blood or choice.
This could not have happened without any of you.

A mi familia, tanto de sangre como por elección.
Nada de esto habría sido posible sin ustedes.

Table of Contents

List of studies included in this thesis

This doctoral thesis is comprised by the following original studies:

1. **González-Padilla E**, A Dias J, Ramne S, Olsson K, Nälsén C, Sonestedt E. *Association between added sugar intake and micronutrient dilution: a cross-sectional study in two adult Swedish populations.* Nutr Metab (Lond). 2020 Feb 11;17:15. DOI: 10.1186/s12986-020-0428-6. PMID: 32071610; PMCID: PMC7011604. © González-Padilla et al.

2. **González-Padilla E**, Janzi S, Ramne S, Thuneland C, Borné Y, Sonestedt E. *Association between Sugar Intake and Intima Media Thickness as a Marker for Atherosclerosis: A Cross-Sectional Study in the Malmö Diet and Cancer Study (Sweden).* Nutrients. 2021 May 5;13(5):1555. DOI: 10.3390/nu13051555. PMID: 34063058; PMCID: PMC8147969. © González-Padilla et al.

3. **González-Padilla E**, Tao Z, Sánchez-Villegas A, Álvarez-Pérez J, Borné Y, Sonestedt E. *Association between adherence to Swedish dietary guidelines and Mediterranean diet and risk of stroke in a Swedish population*. Manuscript accepted in Nutrients (March 2022). © González-Padilla et al.

4. 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. PMID: 34836209; PMCID: PMC8622171. © Janzi et al.

List of studies not included in this thesis

- Ramne S, Alves Dias J, **González-Padilla E**, Olsson K, Lindahl B, Engström G, Ericson U, Johansson I, Sonestedt E. *Association between added sugar intake and mortality is nonlinear and dependent on sugar source in 2 Swedish population-based prospective cohorts.* Am J Clin Nutr. 2019 Feb 1;109(2):411-423. DOI: 10.1093/ajcn/nqy268. PMID: 30590448.
- Olsson K, Ramne S, **González-Padilla E**, Ericson U, Sonestedt E. *Associations of carbohydrates and carbohydrate-rich foods with incidence of type 2 diabetes.* Br J Nutr. 2021 Oct 14;126(7):1065-1075. DOI: 10.1017/S0007114520005140. Epub 2020 Dec 23. PMID: 33355062.
- 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. PMID: 33425973; PMCID: PMC7786303.

List of abbreviations

BMI: body mass index (kg/m^2).

***FGF21*:** *fibroblast growth factor 21*.

***FTO*:** *fat mass and obesity-associated* gene.

***GLUT2*:** *glucose transporter 2*.

GWAS: genome-wide association studies.

HDLc: high density lipoprotein cholesterol.

IMTbif: intima media thickness measured at the bifurcation of the carotids (mm).

IMTcca: intima media thickness measured at the common carotid artery (mm).

LDLc: low density lipoprotein cholesterol.

SNP: single-nucleotide polymorphism.

SSBs: sugar-sweetened beverages.

***TAS1R2*:** *taste 1 receptor member 2*.

***TAS1R3*:** *taste 1 receptor member 3*.

%E: percentage of non-alcoholic energy intake.

Abstract

Individual sugar consumption has increased over fifty-fold over the past two centuries. This sugar-coated environment has raised concerns regarding the possible pernicious health effects associated with a high sugar consumption. Rigorously investigating the association between sugar intake and disease development helps to inform evidence-based nutritional recommendations.

Micronutrient dilution is the displacement of nutrient-dense foods by the overconsumption of energy-dense and nutrient-poor foods. It has often been taken into consideration when nutritional recommendations are issued. In **Study 1**, we explored the association between added sugar intake and the intakes of nine micronutrients in two samples of the adult Swedish population: Riksmaten Adults (n = 1797) and the Malmö Diet and Cancer Study (n = 12,238). A general linear model revealed a significant negative linear association between added sugar intake and the intake of all micronutrients studied for both populations.

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In summary, the link between sugar consumption, and micronutrient intake and genetic factors was shown in these studies. However, the link between sugar consumption and more complex outcomes like cardiovascular disease still needs to be studied further in order to provide accurate information for nutritional recommendations.

A summary of the dietary sugars and outcomes studied, as well as the main results from the studies included in this doctoral thesis can be found in the visual abstract (**Figure 1**).

Keywords: sugar intake, added sugar, free sugar, sugar-sweetened beverages, nutritional recommendations, micronutrient dilution, cardiovascular disease, atherosclerosis, intima media thickness, stroke, Swedish dietary guidelines, Mediterranean diet, genetics, FGF21, nutritional epidemiology.

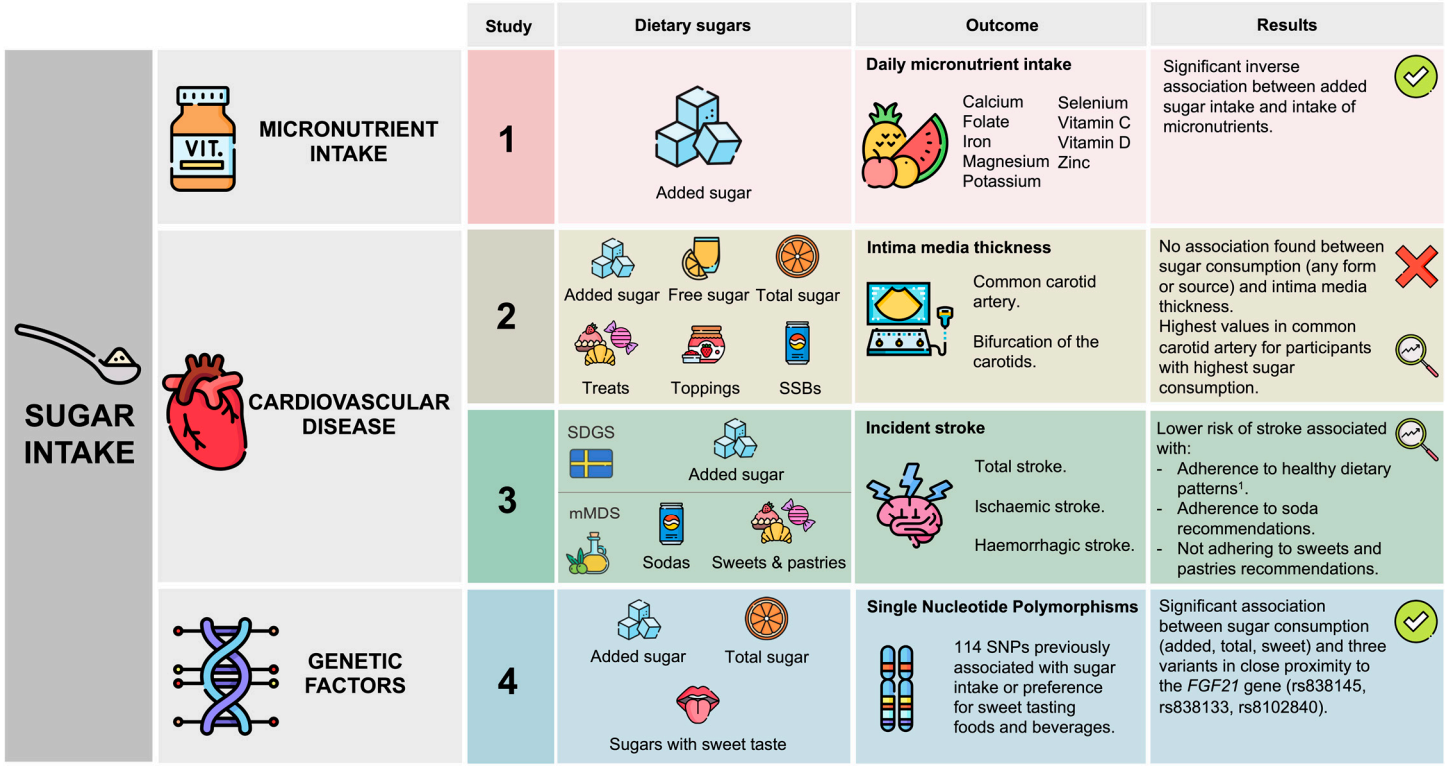


Figure 1. Visual abstract

[1]The association disappeared when adjusting for confounders but reappeared in sensitivity analyses. **SSBs**: sugar-sweetened beverages. **SDGS**: Swedish Dietary Guidelines Score. **mMDS**: modified Mediterranean Diet Score. **SNPs**: single-nucleotide polymorphisms. ***FGF21*: *fibroblast growth factor 21***. *Icons courtesy of Freepik via Flaticon*.

Introduction

"A spoonful of sugar helps the medicine go down" (Mary Poppins).

We are genetically predetermined to have a preference for sweet-tasting substances. This has been an evolutionary advantage for two reasons. First, breast milk is sweet, so a built-in preference for its taste will ensure that infants are well fed. But also, this preference has prevented us from eating poisonous substances, which are usually bitter (1).

However, in our current food system, this predetermination to like sweet products has become increasingly dangerous, as we are now living in what could be described as a sugar-coated environment. There are several contributing factors to this (2):

- *Sugar is ubiquitous and hiding.* Nowadays, sugar can be found in virtually any product in our fridges and pantries. When looking at the product label, we can find sugar either in plain sight or hiding under names that are not so easily recognised by the consumer, such as panela, dehydrated cane juice or maltodextrin. With the discovery of new sources for extracting sugar and the creation of alternative low-calorie sweeteners, the potential for hiding sugar in foods increases.
- *Too many types of sugars and sweeteners.* There are numerous classifications, denominations, and synonyms for sugar and sugar products that create confusion in the scientific community and the general public alike. There is a split opinion on what type of sugars should be used to issue recommendations, and there is still much that we do not know about the use of artificial and non-caloric sweeteners as substitutes for caloric sweeteners.
- *Liquid versus solid sugars*. Sugars in the form of sodas, fizzy drinks, and juices have become widely available worldwide. Liquid sugars pose a greater threat to our health as they are more easily absorbed than sugars in solid form. Additionally, they do not trigger satiety, and habitually they do not offer any additional nutritional benefits. Therefore, sugary drinks add to our overall energy intake without making us feel any fuller or adding any nutritional value.
- *Food industry involvement.* Food companies often try to distract the consumer from the staggering amount of sugar contained in their products

with misleading claims, such as “enriched with vitamins and minerals”, “light in calories”, or “low fat”. Additionally, food companies extensively research their recipes to reach what is known as the “bliss point”, the perfect level of sweetness to increase sales and consumption. Moreover, their marketing campaigns are becoming increasingly more aggressive, targeting at-risk populations (such as children and lower income families). Social media, celebrity influencers, sponsorships, and viral fads are often used to guarantee bigger sales.

- *Sugar is the “new normal”.* Sugar is a cheap and easily accessible commodity used in many products for different purposes, such as granting sweetness, preservation, fermentation, or masking other tastes. Thus, we have become accustomed to it and developed our “sweet tooth” to a point where it is virtually impossible to avoid sugar, whether from our diets or our environment.

As a result of all of these factors, it has become increasingly more difficult to identify sugars in food products, which in turn makes it all the more challenging to avoid them in our diets. Nutritional recommendations aim to set up a safety framework for our daily intake of nutrients including sugar. However, there is still a lack of consensus of where to set a healthy limit for sugar intake.

Background

An overview of the studies included in this doctoral thesis is depicted in **Figure 2**.

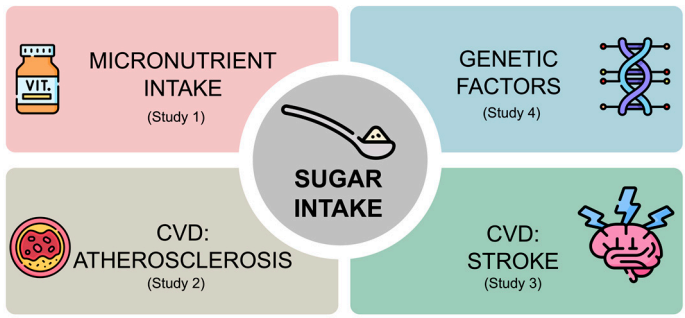


Figure 2. Project overview
Overview of studies included in this doctoral thesis. **CVD:** cardiovascular disease. *Icons courtesy of Freepik via Flaticon.*

Dietary sugars

Up to the 1970s, most sugars known to humans were extracted from either sugar cane or beets, and were presented in the form of the white, granulated, crystallised, sweet-tasting substance that is chemically referred to as sucrose and commonly known as table sugar. However, as food technology has advanced and nutritional trends have shifted, new forms and sources of sugar are now present in the food products that we buy and consume. For instance, human-made high fructose corn syrup has replaced sucrose as the main sweetener used for beverages in the United States, and the use of alternative plants, like agave, has replaced beets and sugar cane as the sole sources for sugars. Parallelly, the food industry has continuously tried to disguise the sugar in their products with names that can increase the confusion of the consumer. In order to avoid misunderstandings, let us go through some basic concepts that will be key to understanding the topics explored in this doctoral thesis.

Classification and types

Carbohydrates are molecules made of carbon, hydrogen, and oxygen. They are also the largest source of energy within our diets. Carbohydrates can be classified based on the complexity of their chemical structure into simple or complex carbohydrates. Simple carbohydrates, more commonly referred to as *sugars*, can be formed by a single molecule (monosaccharides) or by two molecules joined by covalent bonds (disaccharides). Complex carbohydrates refer to longer chains, named oligosaccharides (3-10 molecules) or polysaccharides (more than 10 molecules) (**Figure 3**) (3). For the purposes of this doctoral thesis, we will focus on simple carbohydrates or sugars exclusively.

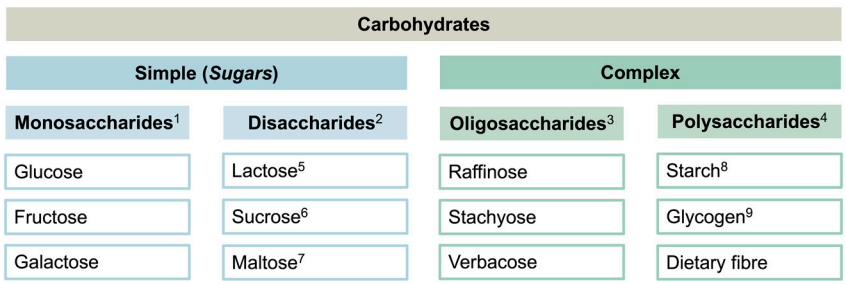


Figure 3. Classification of carbohydrates.
[1] Monosaccharides: 1 molecule. [2] Disaccharides: 2 molecules. [3] Oligosaccharides: 3-10 molecules. [4] Polysaccharides: more than 10 molecules. [5] Lactose: 1 glucose + 1 galactose. [6] Sucrose: 1 glucose + 1 fructose. [7] Maltose: 2 glucose. [8] Starch: glucose chain, vegetal origin. [9] Glycogen: glucose chain, animal origin.

Sugars can be classified based on how they are presented and consumed in our food and beverages. The most frequently used classification in nutritional recommendations and policies divides sugars into added, free, and total sugars (**Figure 4**). *Added sugar* is defined as the monosaccharides and disaccharides added to foods and beverages during processing, preparation, or at the table, as well as sugars consumed separately. In other words, added sugars are those sugars which do not occur naturally in foods and beverages. *Free sugar* includes all added sugars plus those sugars naturally present in honey, syrups, fruit and vegetable juices, and fruit and vegetable juice concentrates. Lastly, *total sugar* refers to all monosaccharides and disaccharides in our diet coming from any source, including those present in whole fruits and vegetables, and milk (4).

Figure 4. Definitions of added, free, and total sugar.
Definitions according to the European Food and Safety Authority Scientifc Opinion on Dietary sugars (4). *Icons courtesy of Freepik via Flaticon.*

These concepts might be more easily understood with a practical example. So, if, for instance, we eat an orange as a whole, the fructose contained in the fruit is considered total sugar. However, if we squeeze the orange and turn it into juice, that same fructose would now be classified as free sugar. But if we sprinkle some table sugar on either the juice or the whole orange, that extra sugar would be considered added sugar (**Figure 4**).

Consumption trends

Historically, sugar consumption has been increasing over the last few centuries but has seen a dramatic surge over the last few decades. In the United States, during the colonial period (circa 1750), the average person consumed around 1.8 kg of sugar per year, which translates to roughly one teaspoon of sugar per day (1 teaspoon is approximately 4 grams). This amount multiplied by five (9.1 kg per person/year) over the following century. Nowadays, the per capita consumption of sugar in the United States surpasses the 72kg per person and year, or approximately 50 teaspoons of sugar daily. That is 50 times more than in the 18th century (5). Similarly, soft drinks and sodas (or sugar-sweetened beverages) can now provide more than 40% of the sugars added to the diet of an average American. Daily soda consumption has increased five-fold between 1950 and 2000, rising to approximately 500ml per person per day (6). Similar trends in consumption have been observed in Europe, where sugar consumption represents between 15 and 25% of our energy intake, of which up to 17% is consumed in the form of added sugar (7).

Meanwhile, in Sweden, the latest national dietary survey (2010-2011) has reported a relative reduction in the consumption of sugar-sweetened beverages, pastries, and ice cream, but not in the consumption of sweets and chocolate (in comparison to the previous dietary survey, 1997-1998). Nowadays, up to 15% of the total energy intake of the average Swedish adult is estimated to come from sugary treats and snacks, as well as sugar-sweetened beverages. Sucrose intake (whether added or naturally occurring) is reported to come from different sources; mainly fruits and berries (15%), pastries (15%), sugar-sweetened beverages (13%), sweets and chocolate (8%), and desserts, marmalade and jam (8%) (8).

The problem does not only reside in the staggering rise in the consumption of sugars worldwide, but also in the fact that it has become virtually impossible to avoid them. Sugar has become a readily available commodity that can be used not only to confer sweet taste, but also to increase the palatability and mask the taste of other chemical products contained in our foods and beverages. Additionally, sugar is also a very cheap and 'natural' agent used for fermentation and preservation purposes. Thus, sugar has become ubiquitous and normalised in our everyday life and is no longer just a treat saved for special occasions.

Most of the sugar that we currently consume comes from easily identified sources such as fruits, pastries, sugar-sweetened beverages, and sweets (8). However, sugar can also be found in less obvious products such as bread, mayonnaise, tonic sodas, or pickling juice. Currently, sugar can be found in up to 70% of all packaged foods available at supermarkets and up to 80% of foods marketed as snacks (2). And yet, while sugar is contained in almost every ready-made product available to us, it is becoming increasingly more difficult to spot it. Sugar is often hidden under names that are more healthy-sounding (such as fruit juice concentrate or organic agave nectar) or under names that the consumer might not identify as sugars (such as maltodextrin or sorghum syrup). The list of sugar synonyms used by food industries comprises more than 200 names up to March 2020 (2).

Nutritional recommendations

Considering all the possible ways in which sugar can be classified, named, and consumed, it is no wonder that the scientific community has not agreed quite yet on a unified recommendation. Three main questions are still being debated in the context of designing nutritional recommendations and guidelines when it comes to sugar intake: 1) what type of sugar should be the focus, 2) where to set the threshold for safe intake, 3) which disease outcomes should be considered. As a result, there is an inconsistency in the existing international recommendations and country-level dietary guidelines in terms of sugar consumption.

Type of sugar

Current nutritional recommendations and dietary guidelines seem to be torn between focusing on added sugar or free sugar. On the one hand, added sugar is considered because these are sugars not naturally present in foods and beverages, therefore they add no nutritional value (other than caloric intake). On the other hand, free sugars, although they can be naturally found on foods and beverages, they are absorbed and metabolised faster than sugars presented with their fibrous capsule intact (in whole fruits and vegetables) causing acute effects in our body, especially when they are presented in liquid form (9).

Threshold

Many of the current nutritional recommendations, including the Nordic Nutrition Recommendations (2012) (10) and the World Health Organisation (11) have suggested that no more than 10% of our dietary energy intake should come from added or free sugars, respectively. However, the World Health Organisation also recommended a tentative further restriction to only 5% of energy intake to come from free sugars (11). This suggestion was supported by the Scientific Advisory Committee on Nutrition for the United Kingdom (12). However, the most recent Dietary Guidelines for Americans (2020-2025) rejected this advice and maintained a recommendation for added sugars of no more than 10% of the daily energy intake (13).

Disease outcomes

Many nutritional guidelines in the past have based their recommendations for sugar intake based on the effects that this can have on excess energy intake, dental caries, and nutrient displacement (or micronutrient dilution) (14). However, more current guidelines have placed a greater concern on the long-term effects of sugar consumption on chronic disease, pregnancy, and other conditions (4, 13).

Existing recommendations

A summary of a selection of current nutritional recommendations issued over the last decade is presented on **Table 1**.

Table 1.
Summary of existing nutritional recommendations.

Year	Guideline (reference)	Type of Sugar	Threshold	Outcome
2012	Nordic Nutritional Recommendations (10)	Added sugar	10% E	Excess energy intake/overweight Type 2 diabetes
2013	Australian Dietary Guidelines (15)	Added sugar	None[1]	Dental caries Excess energy intake/overweight Bone health
2015	World Health Organisation (11)	Free sugar	10% E[2] 5% E[3]	Dental caries Excess energy intake/overweight
2015	Scientific Advisory Committee on Nutrition (12)	Free sugar	5% E	Dental caries Excess energy intake/overweight Type 2 diabetes
2020	Dietary Guidelines for Americans (13)	Added sugar	10% E	Excess energy intake/overweight
2022	European Food Safety Authority (4)	Added and free sugar	None[4]	Chronic metabolic diseases[5] Dental caries Pregnancy-related endpoints[6]

[1] No quantitative recommendation, qualitative recommendation to limit the intake of foods and beverages containing added sugars. [2] Strong recommendation: the desirable effect outweight the undesirable consequences (16). [3]Conditional recommendation: less certainty about the balance between benefits and harms (16). [4] Not enough evidence to establish a threshold (4). [5] Chronic metabolic diseases: obesity, non-alcoholic fatty liver disease, type 2 diabetes mellitus, dyslipidaemias, hypertension, cardiovascular diseases, and gout (4). [6] Pregnancy-realted endpoints: gestational diabetes mellitus and birthweight-related endpoints (4). **%E:** percentage of energy intake.

As a result of the inconsistency of the existing recommendations, and at the request from five European countries (Denmark, Finland, Iceland, Norway, and Sweden), the European Food and Safety Authority delivered a scientific opinion on the tolerable upper intake level for dietary sugars. The tolerable upper intake level is defined as the maximum amount of continued dietary sugar consumption that is unlikely to develop adverse health effects in humans, it is not a recommendation per se but an estimation of a safety level of consumption (4). This consultation explored existing evidence on dietary sugar consumption (added, free, and total) on the basis of chronic metabolic diseases (obesity, non-alcoholic fatty liver disease, type 2 diabetes mellitus, dyslipidaemias, hypertension, cardiovascular diseases, and gout), pregnancy-related endpoints (gestational diabetes mellitus and birthweight-related endpoints), and dental caries (4).

Their findings, published in February 2022, concluded that no tolerable upper intake level could be established for the consumption of dietary sugars (total, free, or added). Therefore, the 10%E threshold could not be confirmed due to insufficient evidence from randomised controlled trials. Additionally, the health effects of added sugars versus free sugars could not be compared and were explored together However, the evidence collected for this scientific opinion pointed towards excess

energy intake and overweight to be the leading cause behind the effects of sugar consumption on chronic metabolic conditions (4).

While a causal relationship could be established between the consumption of added and free sugars and certain chronic metabolic conditions, the level of certainty for those associations varied from very low to moderate. For instance, the level of certainty behind the relationship found for obesity and dyslipidaemia was moderate (50-75% probability), for type 2 diabetes and liver disease was low (15-50% probability), and for hypertension was very low (<15% probability). However, no support was found in relation to cardiovascular diseases. Nonetheless, the evidence behind the association between cardiovascular disease and the consumption of sugar-sweetened beverages had a high level of certainty (75-100% probability) (4).

Ultimately, the European Food Safety Authority issued a recommendation to limit the intake of added and free sugars to be "as low as possible in the context of a nutritionally adequate diet" (4).

Understanding recommendations

In order to illustrate existing quantitative recommendations in practical terms, we can assume that the average diet of an adult consists of 2000 kcal/day. Considering that all carbohydrates (including sugars) yield 4 kcal per gram of dry weight, the daily intake of added or free sugars should not surpass 50 g/day (10%E) or 25 g/day (5%E). For instance, a can of soda (330 ml) can contain approximately 35g of sugar and a single blueberry muffin can contain up to 42g of sugar (**Figure 5**).

Figure 5. Sugar content of food items
Considering that one spoonful of sugar is approximately 4 grams, a can of soda can contain 8 ¾ teaspoons of sugar and a blueberry muffin can contain 10 ½ teaspoons of sugar, almost the full amount of sugar recommended per day.
Icons courtesy of Freepik via Flaticon.

Micronutrient intake

Micronutrient is the term used to jointly refer to vitamins and minerals. These are needed in our bodies in relatively small daily amounts, but they play key roles in our overall health. The importance of micronutrients dates all the way back to the 18th century, to one of the first recorded randomised trials in history. In this trial, James Lind (1716-1794) observed that the consumption of citrus fruits by sailors cured them of scurvy. These observations published in 1753, led to the discovery of vitamin C almost 200 years later, when scurvy was defined as vitamin C deficiency (3, 17).

Micronutrient deficiencies

Nowadays, micronutrients are still relevant to our health as they play key roles in our bodily functions. Therefore, micronutrient deficiencies can lead to dangerous health conditions. For instance, vitamin D plays an important role in bone health and calcium absorption; its deficiency can lead to bone fragility, rickets, and osteomalacia. Zinc deficiency can lead to a weakened immune system, selenium deficiency has been associated with degenerative diseases and muscle weakness, and folate is crucial during pregnancy to prevent neural tube defects. But micronutrients such as calcium, zinc, vitamin D and selenium also play an important role in cardiovascular health (18).

Micronutrient dilution

Micronutrient dilution is a phenomenon characterised by a decreased consumption of nutrient-dense foods (such as fruits and vegetables) as a result of an increased consumption of energy-dense foods (rich in fat and sugar but poor in nutrients) (19, 20). Micronutrient dilution is a widely studied phenomenon and numerous studies have found it to occur on several populations around the world (21-28). As a result, the displacement of nutrients has been a great concern in the development of past nutritional recommendations for dietary sugars (13, 29).

Dietary patterns

In later years, nutrition research has evolved from the study of single foods or nutrients to the study of dietary patterns, on the quest to evaluate the relationship between overall diet and disease. These approaches, however, are complementary to each other. Single nutrient studies can be a great source of information when trying to identify the biological mechanisms between diet and disease. Food item

studies are useful in identifying synergies between nutrients within the same diet component. Dietary pattern studies allow us to explore the overall influence of diet on the outcome of interest (17, 30).

There are certain advantages and limitations to the use of these approaches. Single nutrient studies might not be able to reflect the synergies, correlations, and interactions between nutrients. But these are well-captured in dietary pattern studies where foods can be studied within the context of the overall diet (17, 30). While subtle health effects of single foods might not be revealed in single nutrient studies; dietary patterns might capture the cumulative effects of the nutrients contained in various sources (30). However, the true health effect of a single food or nutrient can be masked in dietary pattern studies and is, therefore, much better captured in single nutrient studies (17). When associations are found in dietary pattern studies they can be more difficult to translate into nutrient-based recommendations (17). Yet, recommendations based on dietary patterns are much more easily understood by the consumer (and thus easier to adhere to) (17).

Diet and cardiovascular disease

For centuries the scientific community has been interested in the ways in which what we eat can affect our health. More recently, we have also started to wonder what role food plays in the prevention of chronic conditions, such as cardiovascular disease.

Cardiovascular disease is a term that comprises several pathological processes involving the heart and the vasculature. These can be divided into: coronary heart disease, cerebrovascular disease or stroke, peripheral artery disease, rheumatic heart disease, congenital heart disease, and deep vein thrombosis and pulmonary embolism (31). Cardiovascular disease is by far the largest contributor to the increasing figures of death by non-communicable diseases globally. The prevalence of cardiovascular disease has doubled between 1990 to 2019 (523 million cases), and the number of deaths attributable to cardiovascular disease has risen to almost 20 millions in 2019 alone (32).

Many of the risk factors for cardiovascular disease are common to all processes, despite their clinical differences. These factors include high blood pressure, elevated plasma glucose, high low-density lipoprotein cholesterol (LDLc), overweight and obesity, impaired kidney function, air pollution, tobacco use, low physical activity and an unhealthy diet (32).

In the context of the 2019 Global Burden of Disease report, an unhealthy diet was defined by the underconsumption of fruits, vegetables, legumes, whole grains, nuts and seeds, milk, fibre, calcium, omega-3 fatty acids from seafood, and

polyunsaturated fatty acids. But also by the overconsumption of red and processed meat, sugar-sweetened beverages, trans-fatty acids, and sodium. The primary consequence of this unhealthy diet is the development of cardiovascular diseases, accounting for almost 8 million annual deaths (32).

Sugar consumption, particularly in the form of sugar-sweetened beverages, has been studied in regards to many diseases and conditions. For instance, the relation between sugar consumption in any form and dental caries has been previously established (4, 11). However, the consumption of added sugars has also been suggested to be involved in the development of diet-related chronic conditions, including diabetes, obesity, cardiovascular disease, and many others (4). However, the evidence linking overall consumption of dietary sugars with cardiovascular disease development in particular seems to be still wanting.

In this doctoral thesis, a greater focus has been placed on two conditions classified as cardiovascular diseases, but pertaining mainly to the vasculature: atherosclerosis and stroke.

Atherosclerosis

Atherosclerosis is a chronic progressive condition that starts early on in life. It is characterised by the accumulation of fatty and fibrous materials on the walls of large arteries forming plaques. Many cardiovascular diseases, including heart disease and stroke, are a consequence of the atherosclerotic process. Thus, up to half of the deaths in Western countries can be attributed to atherosclerosis (33).

The thickening of arterial walls due to plaque formation, can lead to two different processes: 1) the narrowing of the lumen of the vessel to the point of occlusion, at which point the blood flow will cease leading to a lack of oxygenation of the tissues that the vessel irrigates, and 2) the dislodging of the plaque, which can travel through blood vessels until it impacts on vessels of lesser calibre causing a blockage of the blood flow to the tissues beyond it (33).

Depending on the level at which the occlusion happens we can observe different clinical syndromes. The most common, and most worrisome, are myocardial infarction when the coronary arteries (the vessels around the heart) are occluded, or stroke when the occlusion happens on the cerebral arteries (the vessels irrigating the brain) (33).

The measurement of the artery walls or intima media thickness of the carotids, through the use of ultrasound, is a well-established parameter to detect subclinical atherosclerosis. Furthermore, intima media thickness is not only the first measurable sign of atherosclerosis but can also act as an independent predictor for the development of several cardiovascular diseases (34-36).

Stroke

Stroke is defined by the loss of function in the brain due to a lack of blood flow to cerebral tissues. This loss of blood flow can occur due to haemorrhage (haemorrhagic stroke) or blockage of the arteries, by for instance an atherosclerotic plaque (ischaemic stroke). In 2019, over 6.5 million deaths could be attributed to stroke and over 12 million incident cases were recorded, of which 62.4% were ischaemic, and the rest were haemorrhagic in nature (27.9% intracerebral haemorrhage and 9.7% subarachnoid haemorrhage) (32).

Some risk factors associated with stroke incidence are non-modifiable, like age or sex. However, the majority of risk factors associated with stroke are subjective to modification, like dietary habits, among many others (37). Overall, dietary habits have been associated with many of the risk factors that can lead to the development of stroke either directly or indirectly. However, certain specific dietary patterns have also been directly linked to the prevention of stroke. For instance, adherence to a Mediterranean dietary pattern has been linked to a decreased risk of stroke (38) and a health-conscious dietary pattern was associated with lower risk of stroke for men in a Swedish population (39).

Genetic factors

The study of genetic factors has become an important instrument in the study of health and nutrition. The identification of genetic variations, like single-nucleotide polymorphisms (SNPs), can help us better understand inter-individual differences found in nutritional studies. Moreover, the use of genetics opens up the possibility to study the use of genetic markers to better understand the relationship between diet and disease, as well as to be able to classify outcomes based on genetic characteristics (17). With time, genetic factors might be also able to serve as objective biomarkers for intake of certain foods or nutrients.

Genetic association studies are used to identify associations between one or more polymorphisms and a phenotype (or trait), presented in the wider population in a similar manner (40). There are two main ways to identify single-nucleotide polymorphisms: 1) genome-wide association studies (GWAS), where the whole genome is explored without a preconceived assumption in order to uncover novel variants associated to a given outcome (41); and 2) candidate gene approach studies, where a selection of genes are explored based on known biological functions, phenotypes or traits of interest, or geographical proximity to phenotypes of interest within the chromosome (42).

Sugar and genetics

The genetics behind the variability in perception, preference, and consumption of sweet substances in humans still remains vastly understudied. Previous studies have reported an association between sweet-taste perception and preference with genetic variants within the sweet taste receptors *TAS1R2* and *TAS1R3* (43-45). Moreover, sugar consumption has been reported to be associated with the glucose transporter *GLUT2* (46, 47) and *TAS1R2* (43, 46, 48-52) but not *TAS1R3* (46, 50-53). However, all of these associations were found in studies with relatively small sample sizes and some of them even included mixed ethnicities (54). Nonetheless, large GWAS have reported an association between sugar consumption and genetic variants within the *FGF21* gene (54-58). Additionally, another large GWAS looking into perception, liking and consumption of sweet substances found a strong association between a genetic variant within the *FTO* gene and sugar consumption, but only suggestive associations for sweet perception (54).

Rationale

Sugar consumption has been increasing exponentially over the past two centuries. As a result, exploring the association between sugar consumption and chronic disease development is especially important in the context of informing nutritional recommendations.

The European Food Safety Authority's scientific opinion on dietary sugars (published in February 2022) (4), has placed a considerable focus on exploring the long-term health effects that sugar intake can have on chronic metabolic diseases. Yet, they could not establish a clear threshold beyond which the effects of sugar consumption on health would be evident. Meanwhile, cardiovascular disease continues to be one of the greatest causes of morbidity and mortality worldwide (32). Because of this, it is paramount to better understand the role that a high sugar consumption can have on cardiovascular health.

This doctoral thesis aims to contribute to the existing scientific evidence informing nutritional recommendations, by exploring the role of sugar consumption in association with micronutrient intake and cardiovascular disease (mainly vascular diseases: atherosclerosis and stroke), as well as possible genetic factors associated with a higher consumption of dietary sugars.

Aims

Overall aim

The overall aim of this doctoral thesis was to explore the role that sugar intake plays in the development of certain conditions that can lead to cardiovascular disease. Additionally, this doctoral thesis aimed to increase the pool of scientific evidence regarding sugar consumption, in particular added sugar, in order to better inform nutritional guidelines, recommendations, and policies aiming to regulate sugar consumption to prevent cardiovascular disease development. A summary of the topics studied in the doctoral thesis can be found in **Figure 6**.

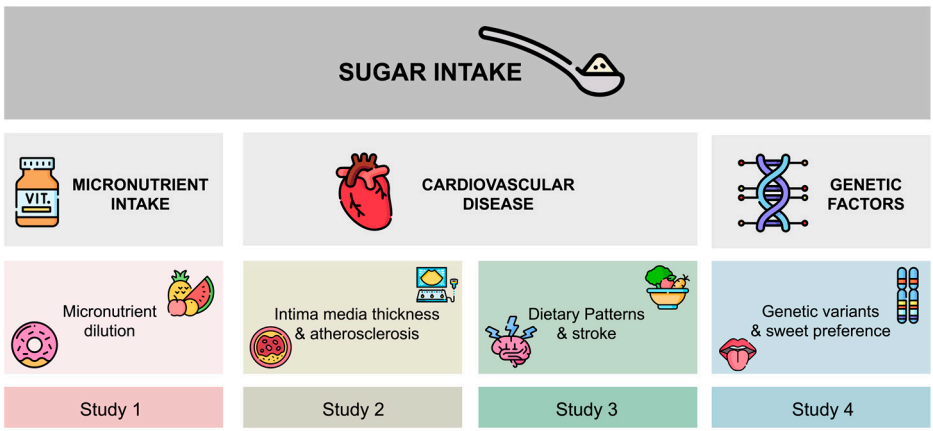


Figure 6. Project summary
Topics studied in this doctoral thesis. *Icons courtesy of Freepik via Flaticon.*

Specific aims

The specific aims of this doctoral thesis are as follows:

- To examine whether there is an association between the intake of added sugar and the intake of micronutrients (**Study 1**).
- To investigate the association between intake of different forms of sugar and sugar-rich foods and beverages and intima media thickness measured at the carotids (**Study 2**).
- To explore the association between two healthy dietary patterns (Swedish and Mediterranean) in relation to risk of total stroke and its subtypes (**Study 3**).
- To explore the association between the consumption of total sugar, added sugar, and sugars with sweet taste and a selection of single nucleotide polymorphisms previously associated with sugar intake and/or preference (**Study 4**).

Methods

Study design

A summary of the source population, exposures, outcomes, and study design has been recapitulated in **Table 2**.

Table 2.
Summary of the studies included in this thesis

Study	Population[1]	Exposures	Outcomes	Study Design
1	MDCS (n=12,238) Riksmaten Adults (n=1797)	Added sugar intake (%E).	Daily intake of 9 micronutrients [2] (mg/day, μg/day).	Cross-sectional
2	MDCS-CC (n= 5269)	Added sugar intake (%E). Free sugar intake (%E). Total sugar intake (%E). Treats (svg/week). Toppings (svg/week). SSBs (svg/week).	IMTbif (mm). IMTcca (mm).	Cross-sectional
3	MDCS (n= 25,840)	Swedish Dietary Guidelines Score. Modified Mediterranean Diet Score.	Incident total stroke. Incident ischaemic stroke. Incident haemorrhagic stroke.	Prospective cohort
4	MDCS (n= 22,794)	2 SNPs associated with *FGF21* gene. 8 top hit SNPs according to Hwang et al. (54) 104 SNPs previously identified with sugar intake and preference[3].	Added sugar intake (%E). Total sugar intake (%E). Sugars with sweet taste intake (%E) [4]. Other forms and sources of dietary sugars, and macronutrients [5].	Cross-sectional

[1] Number of participants in the final study sample after exclusions.[2] Calcium, folate, iron, magnesium, potassium, selenium, vitamin C, vitamin D, and zinc. [3] 104 SNPs include: 11 SNPs suggestively ($p < 10^{-5}$) associated with intake of sweets in the UK Biobank, 73 SNPs associated with perceived intensity and preference for sweet substances in twin studies, and 20 SNPs associated with sweet phenotypes via candidate gene approach (54).[4] All monosaccharides and sucrose. [5] Sucrose (%E), monosaccharides (%E), disaccharides (%E), sweets and chocolate (g/day), sugar-sweetened beverages (g/day), ice cream (g/day), pastries (g/day), total energy (kcal/day), carbohydrates (%E), fat (%E), and protein (%E). **MDCS:** Malmö Diet and Cancer Study; **%E:** percentage of non-alcoholic energy intake; **MDCS-CC:** Malmö Diet and Cancer Study-Cardiovascular Cohort; **svg/week:** servings per week; **IMTbif:** intima media thickness measured at the bifurcation of the carotids; **IMTcca:** intima media thickness measured at the common carotid artery, **SNPs:** single-nucleotide polymorphisms; ***FGF21*:** *fibroblast growth factor 21*.

Cross-sectional studies

Cross-sectional studies are often explained using the metaphor of a "snapshot" of the situation. This means that both exposure and outcome are measured at the same time. Cross-sectional studies are useful to evaluate whether there is an association between two variables: a disease or outcome of interest and a health-related characteristic, in a given population at a particular point in time. Cross-sectional studies are also a good exploration tool to measure prevalence of outcomes in relation to current exposure levels. However, as cross-sectional studies do not provide information about time, we cannot infer causality from them. These studies are widely used in public health for planning and exploring aetiologies (59). This type of study design was used in **Study 1** and **Study 2** to investigate whether there was an association between sugar intake and micronutrient dilution and intima media thickness, respectively. In **Study 4**, we made use of the results from a GWAS to replicate the associations between a selection of single-nucleotide polymorphisms identified by a previous study (54) and different forms of sugar intake.

Cohort studies

Cohort studies are considered "natural experiments" as no intervention is performed by the researcher. In these studies, we just observe how an exposure leads to an outcome (or not), on its own accord, and at its natural pace. Prospective cohort studies follow participants from the moment of exposure until the outcome of interest develops. These studies are good for measuring incidence rate or risk and they can offer a clear temporal line between exposure and outcome (59). **Study 3**, follows such study design. In it, we start observing the participants when they report their dietary intake (baseline examination) and follow them over time until the outcome appears, they drop out of the study (either through emigration from Sweden or death), or the study ended.

Study populations

Riksmaten Adults

The National Swedish Food Survey (2010-2011), also known as Riksmaten Adults, is the most recent dietary data collection among adults at a national level in Sweden. A total of 5000 invitations were sent to eligible participants based on the National Registry of Statistics Sweden. These invitations were sent with the intention of capturing a representative sample of the population in terms of sex, age group, and region. Participants of the Riksmaten Adults survey were all aged between 18 and 80 years old and were residing in Sweden at the time of data collection, which took

place between May 2010 and July 2011. Ultimately, 2268 individuals agreed to participate in Riksmaten Adults, of which 1797 completed the food diary (36% participation rate, 31% for men and 40% for women) (8).

Before any measurements were taken, all participants of Riksmaten Adults gave oral informed consent to participate in the study. They did so, after receiving information regarding the voluntary nature of the study and the possibility to withdraw from it at any given time (8). The participants reported their usual dietary intake by means of a four-day web-based food diary. In addition, they also filled a questionnaire including information regarding anthropometric measurements (including height and weight), as well as other lifestyle and socioeconomic factors. Both elements were filled out online but an interviewer was able to assist via telephone those participants who faced trouble accessing the website (8).

Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study is a population-based prospective study containing some of the most comprehensive dietary data collection performed in Sweden. The Malmö Diet and Cancer Study aimed to capture dietary habits from a cohort formed by all older adults residing in the city of Malmö (southern Sweden) at the time when the study took place. The data collection took place between March 1991 and October 1996. The participants of the Malmö Diet and Cancer Study included all men born between 1923 and 1945 and all women born between 1923 and 1950. These were invited to take part in the study passively (through the placement of public advertising) or actively (by means of a personal recruitment letter). Limited knowledge of the Swedish language or limited mental capacity were the only exclusion criteria for participation. The source population amounted to 74,138 individuals, of which 68,905 were considered eligible. Ultimately, 30,446 individuals completed at least one part of the baseline examination and were considered participants. Of these, 28,098 participants (11,063 men and 17,035 women) completed the dietary assessment (40.8% participation rate, 38.3% for men and 42.6% for women) (60, 61).

The data collection of the Malmö Diet and Cancer Study included a dietary assessment, a self-administered questionnaire gathering information regarding lifestyle and socioeconomic questions, and anthropometric measurements performed by trained personnel at the research site (including height and weight) (62). The Ethical Committee at Lund University approved the Malmö Diet and Cancer Study (LU51-90) and all participants signed a written informed consent before any measurements were taken (61, 62).

Malmö Diet and Cancer Study – Cardiovascular Cohort

The Malmö Diet and Cancer Study – Cardiovascular Cohort is a subcohort of the Malmö Diet and Cancer Study that aimed to better investigate the epidemiology of cardiovascular and carotid artery disease. Participation in the Malmö Diet and Cancer Study – Cardiovascular Cohort, built up on the data collected for the Malmö Diet and Cancer Study by adding further testing, which included the study of the carotid arteries and the collection of fasting blood samples (60).

Half of the participants that completed at least one part of the baseline examination of the Malmö Diet and Cancer Study between 1991 and 1994, were randomly selected to participate in the Cardiovascular Cohort. Ultimately, of the 12,445 participants that were recruited for the Malmö Diet and Cancer study between 1991 and 1994, 6103 were invited to become part of the Cardiovascular Cohort, of which 6057 had complete ultrasound data and 5711 had complete dietary data (60).

Sample selection for each paper

In order to build up the study samples needed for each study, further inclusion and exclusion criteria were taken into account (**Figure 7**).

In **Study 1**, the study sample comprised the 1797 participants from Riksmaten Adults with compete dietary data plus a total of 12,238 participants from the Malmö Diet and Cancer Study. In the Malmö Diet and Cancer Study, we excluded potential energy misreporters, and participants that had a shorter interview (45min) due to a change in the data collection method. Therefore, only those with the longer interview (60 minutes) were included.

In **Study 2**, the study sample originated from the 6103 participants of the Malmö Diet and Cancer Study – Cardiovascular Cohort. Individuals with complete ultrasound and dietary data were included, resulting in 5711 participants. After the exclusion of participants with a history of cardiovascular disease or diabetes, the study sample amounted to 5269 participants.

In **Study 3**, participants with complete dietary data from the Malmö Diet and Cancer Study were included. After the exclusion of participants with a history of cardiovascular events and/or diabetes, as well as of those with missing covariate information, the study sample resulted in 25,840 participants.

In **Study 4**, participants with complete dietary and anthropometric data from the Malmö Diet and Cancer Study were included and those born outside of Sweden or who had a history of diabetes were excluded, resulting in a study sample of 22,794 participants.

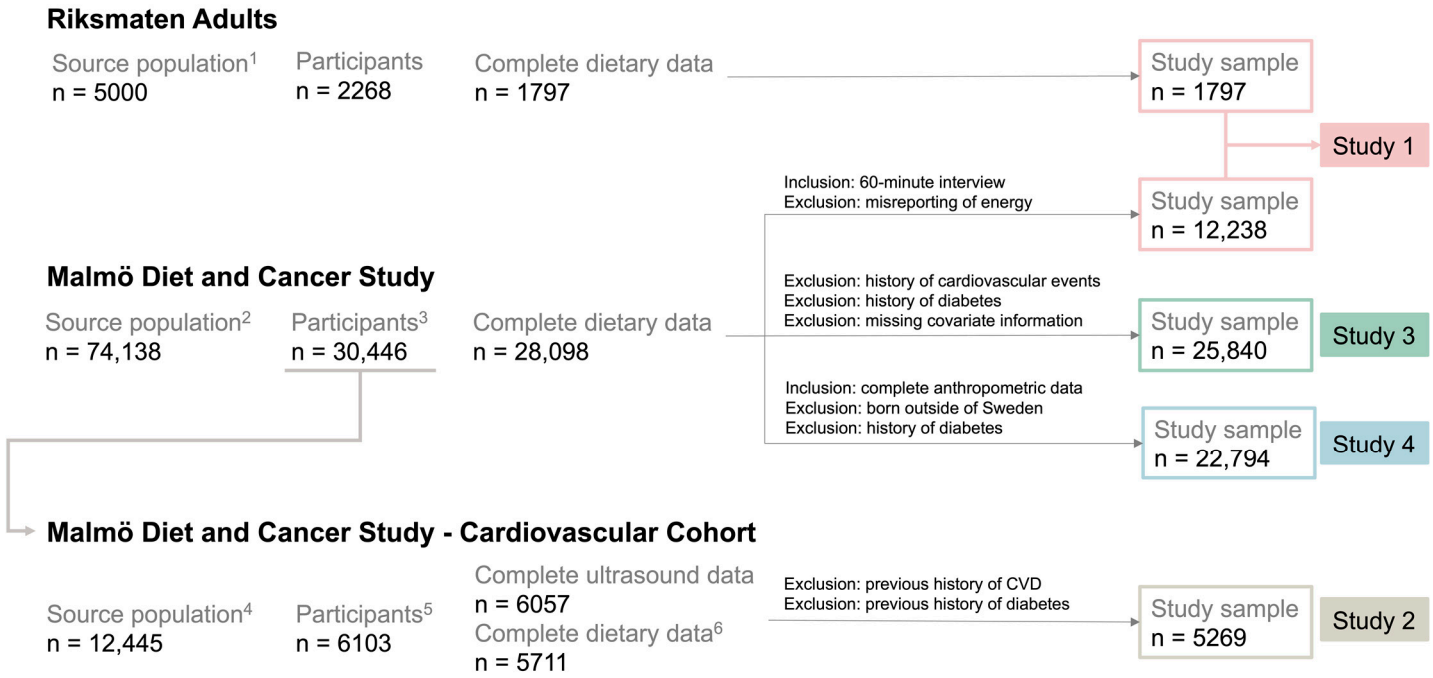


Figure 7. Sample selection of studies included in this thesis.
[1] Invitations sent. [2] Invitation by personal letter or public advertisement. Exclusion: limited knowledge of Swedish, reduced mental capacity. [3] Completed at least one part of the baseline examination. [4] Participants from the Malmö Diet and Cancer Study recruited between 1991-1994. [5] 50% of the participants from the Malmö Diet and Cancer Study recruited between 1991-1994 were invited for further analyses to make up the Cardiovascular Cohort. [6] Complete anthropometric, lifestyle, and dietary data.

Dietary data collection

Riksmaten Adults

The dietary data collection for Riksmaten Adults consisted of a four-day web-based food diary. Participants recorded on a website everything they had eaten or drunk during the four allotted days of data collection, day by day and meal by meal, including time and place in which the meal took place. All participants received a booklet with pictures to help them gauge portion sizes, a notebook where they described the foods consumed in as much detail as possible, and an information folder explaining how to navigate the website and register food intake on it.

In order to cover variation in dietary habits, the participants were randomly selected to start their food diary on different days during the week and the data collection was divided into four rounds allocated quarterly to also cover seasonal variation.

The Riksmaten website was linked to the national food composition database. However, when participants consumed foods that were not contained in this database, they were asked to choose the closest alternative or to register the ingredients separately. Information regarding cooking methods could also be registered on the website and adjustments were made for possible loss of nutrients depending on the preparation method. Average daily food intake was estimated based on the information gathered by the food diary and nutrient intakes were calculated using the national food composition database (8).

Malmö Diet and Cancer Study

The Malmö Diet and Cancer Study used a modified diet history method consisting of three tools. First, a seven-day food diary, used to record prepared meals (mostly lunch and dinner), cold drinks, and supplements. Second, a questionnaire was used to record food frequency (with the aid of a booklet with pictures to help gauge portion sizes) of 168 items consumed regularly that were not covered by the food diary (mostly breakfasts, snacks and hot drinks). Third, a 45- or 60-minute interview with trained personnel where further details regarding preparation and portion sizes of the items recorded in the food diary were discussed with the participant. During the interview, the research staff also assessed that there was no overlapping between the food diary and the food frequency questionnaire. The intakes recorded on these two methods were combined into an average daily intake (63, 64).

Once the diet history collection was complete, the data was introduced into a software in order to compare it with the Malmö Food and Nutrient Database which

was based on the Swedish Food Database PC KOST-93 (63-65). A validation study comparing the diet history method against 18 days of weighed food records, revealed a relatively high validity. Energy-adjusted Pearson correlation coefficients for men/women were reported for carbohydrates (0.66/0.70), protein (0.54/0.53), fat (0.64/0.69), fibre (0.74/0.69), and sucrose (0.60/0.74) (65, 66).

Assessment of main variables

Sugar intake variables

Added sugar intake was estimated by totalling the intake of monosaccharides (mainly glucose and fructose) and sucrose for each individual's whole diet and then subtracting the intake of monosaccharides and sucrose from the most common dietary sources of sugars (fruits and berries, fruit juice, vegetables). Added sugar intake was subsequently transformed into percentage of non-alcoholic energy intake (%E) and then stratified into six categories (less than 5%E, 5-7.5%E, 7.5-10%E, 10-15%E, 15-20%E and more than 20%). These thresholds were established in order to test against existing nutritional recommendations as well as to explore extreme intakes.

Free sugar was estimated similarly to the added sugar variable, but without subtracting the amount of monosaccharides and sucrose contained in fruit juices. The variable free sugar was also transformed into percentages of non-alcoholic energy intake and categorised in a similar fashion as added sugar (less than 5%E, 5-7.5%E, 7.5-10%E, 10-15%E, 15-20%E and more than 20%). These thresholds were also established in order to test against existing nutritional recommendations as well as to explore extreme intakes.

Total sugar was estimated by totalling the consumption of monosaccharides (mainly glucose, fructose and galactose) and disaccharides (mainly sucrose, lactose, and maltose) corresponding to all dietary sources. Similarly to added and free sugar intake, this variable was also transformed into percentages of non-alcoholic energy intake. However, this variable was divided into four categories (less than 15%E, 15-20%E, 20-25%E, and more than 25%E) to be able to explore low and high intakes while maintaining an adequate number of participants in each group.

In **Study 4**, we introduced the term *sugars with sweet taste* which corresponds to the sum of the intakes of all monosaccharides and sucrose either naturally occurring or added to foods and beverages.

Sugar-rich foods and beverages

In **Study 2**, we also aimed to examine the main sources of added sugars from the diet. To do so, we created the variables treats, toppings, and SSBs (which stands for sugar-sweetened beverages) (**Figure 8**).

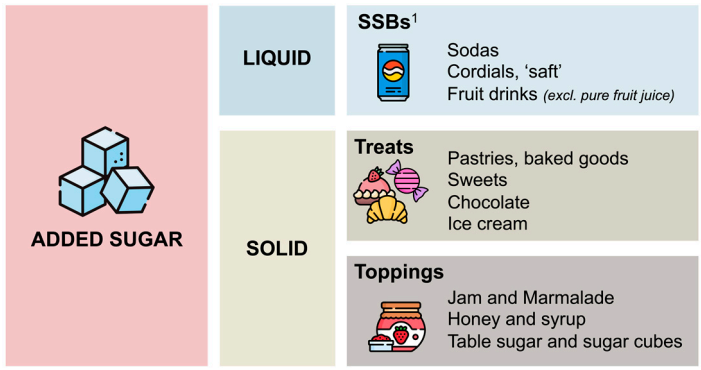


Figure 8. Classification of added sugar
[1] **SSB**: Sugar-sweetened beverages. *Icons courtesy of Freepik via Flaticon.*

Treats covered the consumption of food items with a generally higher energy density as they contain both a high content of fat and sugar. This variable included items such as pastries, sweets, chocolate and ice cream. Additionally, these foods are more easily binge-eaten or consumed in large quantities due to the balanced fat-sugar content (67, 68). *Toppings* covered the consumption of food items whose energy density stemmed mostly from their high sugar content. This variable included items such as table sugar, syrups, honey, and jams. These foods were not so commonly binge-eaten. *Sugar-Sweetened Beverages or SSBs* covered the liquid sources of added sugar, such as soft drinks and fruit drinks, but excluding pure fruit juice.

The intakes, collected in grams per day, were transformed into servings per week based on the average serving sizes according to the Swedish National Food Agency and food manufacturers. As a result, 60 grams of pastries (that is, cakes, pies, biscuits, and buns) equalled one serving, as did 60 grams of sweets and/or candy, and 60 grams of chocolate, respectively. For ice-cream (including sherbet), the serving size equalled 75 grams. A serving of table sugar (including syrups) was 10 grams and a serving of jam (including marmalade, applesauce, jelly and honey) was 20 grams. A serving of sugar-sweetened beverages was 280 grams. These variables were then categorised based on the number of servings per week 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 SSBs as ≤1, >1–3, >3–5, >5–8 and >8 servings/week (69).

Dietary scores

For **Study 3**, two dietary scores were created in order to explore the intake of sugar and sugar-sweetened foods and beverages within the context of healthy dietary patterns. One based on the Swedish dietary guidelines (Swedish Dietary Guidelines Score) and one based on the Mediterranean diet recommendations (Modified Mediterranean Diet Score).

Swedish Dietary Guidelines Score

This Swedish Dietary Guidelines Score was designed with the intention of reflecting a healthy dietary pattern as described by the Nordic Nutrition Recommendations 2012 (10) and the Swedish food based dietary guidelines (70).

The Swedish dietary guidelines score includes five diet components: 1) fibre intake (g/MJ of non-alcoholic energy intake), 2) fish and seafood intake (g/day of fish, shellfish, fish preserves, and other fish products), 3) fruit and vegetable intake (g/day of total intake of fruits, berries, vegetables, fruit juices, and vegetable juices), 4) added sugar intake (E%, estimated by totalling the intake of monosaccharides and sucrose from the whole diet and then subtracting the intake of monosaccharides and sucrose from the main sources of naturally occurring sugars; i.e., fruits, berries, vegetables, and fruit juices), and 5) red and processed meat intake (g/day of beef, pork, lamb, game, sausages, charcuteries, other red meat products) (**Table 3**).

In order to build the score, the following thresholds were established based on what would constitute a healthy diet as per the aforementioned nutritional recommendations and guidelines (10, 70): >2.4 g/MJ for fibre, > 300 g/week for fish and shellfish, > 400 g/day for fruit and vegetables, < 10% energy intake for added sugar, and < 500 g/week for red and processed meat (**Table 3**). The participants would then receive a score of one point if they adhered to these recommendations (range 0-5 points). Based on their individual scores, the participants would be classified into groups based on their level of adherence to the recommendations as low adherence (0-1 points), moderate adherence (2-3 points) or high adherence (4-5 points).

Table 3.
Construction of the Swedish Dietary Guidelines Score for the Malmö Diet and Cancer Study.

Diet component	Recommendation
Encouraged	
Fibre	> 2.4 g/MJ
Fish and seafood	> 300 g/week
Fruit and vegetable	> 400 g/day
Discouraged	
Added sugar	< 10% E
Red and processed meat	< 500 g/week

Modified Mediterranean Diet Score

The Modified Mediterranean Diet Score was designed with the intention of capturing elements of the Mediterranean Diet, as described by the PREDIMED (*Prevención con Dieta Mediterránea*, Prevention with Mediterranean Diet) Study (71). This score was based on the approved and validated (72) 14-item score designed for the PREDIMED and modified to adapt to the data available from the Malmö Diet and Cancer Study. Certain items present in the original Mediterranean diet adherence score could not be included in our score because these specific questions were not collected for the Malmö Diet and Cancer Study and certain adjustments had to be made in the diet components due to the way that the dietary data was collected for the Malmö Diet and Cancer Study.

The Modified Mediterranean Diet Score includes ten diet components: 1) fish and seafood intake (fish, shellfish, fish preserves, and other fish products), 2) fruit and berry intake (fruits, citrus fruits, berries, fruit juices, and citrus juices), 3) vegetable and legume intake (vegetables including legumes, and vegetable juice), 4) nuts and seeds intake (nuts, seeds, almond paste, and other nut products), 5) vegetable oils (olive oil, rapeseed oil, corn oil, sunflower seed oil, and other vegetable oils), 6) wine intake, 7) butter, cream, and margarine intake, 8) red and processed meat intake (beef, pork, lamb, game, sausages, charcuteries, and other meat products), 9) soda drinks intake (carbonated and non-carbonated sodas, whether caloric or non-caloric), and 10) sweets and pastries intake (biscuits, cakes, pies, other baked goods, sweets, and chocolate) (**Table 4**).

These intakes, collected in g/day, were transformed into servings per week, using the serving sizes estimated for the original Mediterranean Diet Adherence Screener used for the PREDIMED Study, described by Martínez-González et al. (73). In order to build the score, the thresholds were established based on what would constitute a Mediterranean Diet, as described in the original screener (72), although transformed into servings per week (**Table 4**). The participants would receive a score of one point if they adhered to these recommendations (range 0-10 points). Based on their individual scores, the participants would be classified into groups based on their level of adherence to the recommendations as low adherence (0-1 points), moderate adherence (2-4 points) or high adherence (5-10 points).

Table 4.
Construction of the modified Mediterranean Diet Score for the Malmö Diet and Cancer Study

Diet Component	Original Recommendation[1]	Serving size	Recommendation in servings/week
Encouraged			
Fish and seafood	≥3 svg/wk	Fish: 125g Seafood: 200g	≥3 svg/wk
Fruits and berries	≥3 units/day	100g[2]	≥21s vg/wk
Nuts and seeds	≥3 svg/wk	30g	≥3 svg/wk
Vegetables and legumes	Vegetables: ≥2 svg/d or ≥1 svg/d raw Legumes: ≥3svg/wk	300g[3]	≥7 svg/wk
Vegetable oils[4]	≥ 4 tablespoons/d	13.5g[5]	≥28 svg/wk
Wine	≥7 svg/week	100g	≥7 svg/wk
Discouraged			
Butter, margarine, cream	<1 svg/d	12g	<7 svg/wk
Red and processed meat	<1 svg/d	125g	<7 svg/wk
Soda	<1 svg/d	200g	<7 svg/wk
Sweets and pastries[6]	<3 svg/wk	Pastries: 50g Sweets, chocolate: 30g	<3 svg/wk

[1]Original recommendations in the Mediterranean Diet Adherence Screener, as stated by Martínez-González et al. (73). [2]The serving sizes for fruit and berry intake in the original Mediterranean Diet Adherence Screener were calculated based on their respective carbohydrate content, however we established a standard serving size of 100g to be used as an average portion size. [3]An average serving size of 300g was established for the pooled vegetable and legume intake to capture the differences in the recommendations established by the original Mediterranean Diet Adherence Screener. [4]Vegetable oils is a composite variable of vegetable oils to use in opposition to solid or animal fats for culinary purposes (the original Mediterranean Diet Adherenece Screener only considered olive oil for this item). [5]One tablespoon = 13.5g (72). [6]In the original Mediterranean Diet Adherence Screener, this category referred only to commercial or store-bought baked goods, however we were unable to separate home-made from store-bought pastries in the Malmö Diet and Cancer Study, both types are included in this category. **svg/wk:** servings per week.

Micronutrient intake

In **Study 1**, the daily intake of a selection of micronutrients was studied in association with added sugar intake. The estimation of the nutrient intake was obtained through the dietary data collection methods already described. The selection of micronutrients to be studied was based on several aspects: 1) the availability of information on both cohorts, 2) concerns for possible low levels or micronutrient deficiencies in the Nordic population expressed in the Nordic Nutrition Recommendations (10), 3) the involvement of said micronutrients in processes that may lead to disease when deficient, and 4) existing literature evaluating whether they were involved in micronutrient dilution (19-28). The final selection contained the daily intakes of calcium (mg/day), folate (µg/day), iron

(mg/day), magnesium (mg/day), potassium (mg/day), selenium (µg/day), vitamin C (mg/day), vitamin D (µg/day), and zinc (mg/day).

The daily micronutrient intake of the samples were also compared in relation to the Dietary Reference Values for the Nordic population, as established by the Nordic Nutrition Recommendations (10). *Average requirement* is defined as the level of a certain nutrient that is sufficient to fulfil the requirement of 50% of the population given a specific age and sex. *Recommended intake*, is defined as the level of a certain nutrient that is sufficient to fulfil the requirement of almost all healthy individuals of the population given a specific age and sex (8). In **Study 1**, sex and age specific values were obtained (**Table 5**).

Table 5.
Dietary Reference Values for the Nordic population according to the Nordic Nutrition Recommendations (10) for adult men and women.

	Average Requirement		Recommended Intake	
	Men	**Women**	**Men**	**Women**
Calcium	500 mg/day	500 mg/day	800 mg/day	800 mg/day
Folate	200 µg/day	200 µg/day	300 µg/day[1]	300 µg/day[1]
Iron	7 mg/day	10 mg/day[2] or 6 mg/day[3]	9 mg/day	15 mg/day[2] or 9 mg/day[3]
Magnesium	-	-	350 mg/day	280 mg/day
Potassium	-	-	3500mg/day	3100 mg/day
Selenium	35 µg/day	30 µg/day	60 µg/day	50 µg/day
Vitamin C	60 mg/day	50 mg/day	75 mg/day	75 mg/day
Vitamin D	7.5 µg/day	7.5 µg/day	10 µg/day[4]	10 µg/day[4]
Zinc	6 mg/day	6 mg/day	9 mg/day	7 mg/day

[1]Recommendation for adults aged 31 and older. [2]Premenopausal value (used for Riksmaten Adults participants). [3]Postmenopausal value (used for Malmö Diet and Cancer Study participants). [4]Recommendation for adults up to 74 years old.

Carotid artery measurements

Qualified sonographers were in charge of measuring the intima media thickness for the Malmö Diet and Cancer Study-Cardiovascular Cohort participants in **Study 2**. For this purpose, they performed a B-mode ultrasound (Acuson 128CT system, Mountain View, CA, USA) examination at the right carotid artery and took three images from the common carotid artery and three from the bifurcation of the carotids. Intima media thickness was measured over a one-centimetre segment, according to the leading-edge principle, off-line using the analysis programme Artery Measurement System (74, 75). All images were analysed. For the intima media thickness at the common carotid artery, the highest mean value was used, whereas the maximum value was used for the measurement at the bifurcation of the carotids (76).

Incidence of stroke

In **Study 3**, incidence of stroke and its subtypes were evaluated as endpoints. Stroke and the studied subtypes were defined based on the following codes of the 9th edition of the International Classification of Diseases (ICD-9): subarachnoid haemorrhage (ICD-9, 430), intracerebral haemorrhage (ICD-9, 431), occlusion of cerebral arteries (ICD-9, 434), acute but undefined cerebrovascular disease (ICD-9, 436). Incident cases of stroke were identified through the Stroma register and the hospital discharge register (77).

The participants from the Malmö Diet and Cancer Study that formed the study sample for **Study 3**, were followed until diagnosis of event, death, emigration from Sweden, or the end of the follow up period (31st December 2016). In our study, incident stroke cases were grouped into three variables: total stroke (a composite of all four subtypes of stroke previously defined), ischaemic stroke (occlusion of cerebral arteries), and haemorrhagic stroke (subarachnoid haemorrhage and intracerebral haemorrhage).

Genotyping and selection of SNPs

In **Study 4,** the blood samples obtained from Malmö Diet and Cancer Study participants were used for genotyping. For this purpose, the Illumina GSA v1 genotyping array was used. However, some single-nucleotide polymorphisms could not be genotyped directly and had to be imputed using the Haplotype Reference Consortium panel (78). The polymorphisms selected for analyses in this study, consisted mainly of two single-nucleotide polymorphisms located in close proximity to the *fibroblast growth factor 21* (*FGF21*) gene (rs838133 and rs838145) and the eight single-nucleotide polymorphisms identified by Hwang et al. (54) in a previous study to be the top hits in relation to total sugar intake in the UK biobank. Secondarily, we aimed to explore an additional 104 single-nucleotide polymorphisms of which, 11 were suggestively ($p < 10^{-5}$) associated with intake of sweets in the UK Biobank, 73 were associated with perceived intensity and preference for sweet substances in two populations of twins, and 20 were previously found to be associated with sweet phenotypes using the candidate gene approach and listed by Hwang et al. (54).

Assessment of covariates

Biological factors

The information regarding variables such as age and sex were gathered from the Swedish national registry for the participants from the Malmö Diet and Cancer Study and from Riksmaten Adults. However, while height and weight were measured onsite for the Malmö Diet and Cancer Study, those measurements were self-reported for the participants from Riksmaten Adults. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in metres squared (kg/m^2) (61, 79).

The collection of blood samples during the Malmö Diet and Cancer Study baseline examination, allowed for the ascertainment of several cardiometabolic risk factors. Including the measurements of circulating lipids that form a cardiovascular high risk profile: high triglycerides (defined as ≥1.7 mmol/L or triglyceride-lowering treatment), low high-density lipoprotein cholesterol (HDLc) (defined as < 1 mmol/L for men or <1.3 mmol/L for women) and high low-density lipoprotein cholesterol (LDLc) (defined as >4.1 mmol/L or lipid lowering treatment).

Blood pressure was also measured by a nurse during baseline examination and hypertension was defined as a systolic blood pressure of ≥130 mmHg or a diastolic blood pressure of ≥85 mmHg or the use of antihypertensive drugs, as per the American Heart Association's definition (80).

Socioeconomic and lifestyle factors

This information was collected from the questionnaire that formed part of both the Riskmaten Adults and the Malmö Diet and Cancer Study data collection (8, 62).

For participants from the Malmö Diet and Cancer Study, the questionnaire included variables such as smoking habits (never smoked, former smoker, current smoker) and educational level (elementary or less, primary and secondary school, upper secondary school, university education without a degree, and university education with a degree). Leisure-time physical activity was collected in the form of five pre-defined groups of metabolic equivalent tasks (MET) hours per week (<7.5, 7.5-15, 15-25, 25-50 and >50 MET hours/week) (81).

Alcohol consumption was, however, compiled from the information reported on the questionnaire as well as the food diary and classified into zero consumers and quintiles of sex-specific consumption (82).

Methodological variables

Other covariates with interest in terms of accounting for methodological aspects of the data collection process were also taken into consideration for the studies including the Malmö Diet and Cancer Study participants.

The season where the dietary data collection took place was recorded and adjusted for in order to account for differences in dietary intake throughout the year.

In September 1994, due to a shortening in the length of the interviews held with personnel (from 60 minutes to 45 minutes), a change in the way that dietary data was coded was introduced. After this change, the energy intake reported was slightly lower (63). This variable was adjusted for in **Study 3** and **Study 4**, but in **Study 1** we excluded participants recruited after September 1994, and kept only those that underwent a longer interview. In **Study 2**, adjusting or excluding participants based on this variable was not necessary, as all subjects from the Cardiovascular Cohort of the Malmö Diet and Cancer Study underwent a 60-minute interview (60).

Participants having drastically changed their diets during the year previous to their baseline examination were labelled "diet changers" and excluded in sensitivity analyses. Likewise, those participants whose reported energy intake was not in accordance to their energy expenditure levels were labelled "potential energy misreporters" and were also excluded in sensitivity analyses (83).

Statistical analyses

The statistical analyses for **Study 1**, **Study 2** and **Study 3** were performed using SPSS (IBM Statistics; New York, USA) version 24 (**Study 1**) and version 25 (**Study 2** and **Study 3**). The statistical analyses for **Study 4** were performed in R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria).

Statistical significance was established at $p < 0.05$ for **Study 1**, **Study 2** and **Study 3**. A Bonferroni corrected significance threshold was established at $p < 0.005$ for **Study 4** in order to correct for multiple testing. Variables not meeting normal distribution criteria were logarithmically transformed.

Main analyses

Study 1 and **Study 2** made use of linear models, with several levels of adjustment in the case of **Study 2**, to explore the relation between exposures (dietary sugars) and the outcomes (micronutrient intake and intima media thickness). In **Study 4**, we made use of a multiple linear regression model to estimate the effect sizes (presented as β/standard error of the estimate). A Cox proportional hazards regression was used

in **Study 3** to obtain hazard ratios for the two dietary indexes (exposures) and the incidence of different types of stroke (outcome). A summary of the main statistical analyses, the models of adjustment and the sensitivity analyses carried out for this doctoral thesis is presented on **Table 6**.

Linear regression models

General linear models are used to study the association between one or more exposures and an outcome (or dependent variable) that is continuous. This association is assumed to be linear in nature, meaning that observations tend to cluster around a straight line (59, 84).

Cox proportional hazards regression

Cox proportional models are used when we want to compare the rate of an outcome in a population (59). Cox regression builds a model that takes into account the time it takes for an individual to develop an event or outcome. All individuals, whether they develop the event or not, contribute to the analysis to estimate the probability or risk to develop said event. The results are expressed in hazard ratio (HR), the rate at which participants develop the event during the course of the study (84).

Sensitivity analyses

In **Study 2**, **Study 3**, and **Study 4**, sensitivity analyses were run in order to exclude participants whose dietary reporting might be considered unreliable. To do so, participants with drastic dietary changes on the year before data collection occurred were excluded. Additionally, participants whose balance between energy intake and expenditure did not match up were considered potential energy misreporters and were also excluded from sensitivity analyses (**Table 6**).

Additionally, in **Study 4**, the main model was run stratifying the population based on their body mass index (BMI $< 25 kg/m^2$ versus BMI $\geq 25 kg/m^2$), because some of the single-nucleotide polymorphisms under evaluation had been previously reported to have been associated with body mass index (**Table 6**).

Table 6.
Overview of statistical analyses and adjustment models for each study.

Study	Main Analyses	Model 1	Model 2	Model 3	Sensitivity Analyes
1	Linear regression	Age. Sex. BMI. Total energy intake.			
2	Linear regression	Age. Start date. Time between dietary data collection and IMT measurement. Season when dietary data collection took place.	(Model 1 +) Alcohol consumption. LTPA. Educational level. Smoking habits. BMI. Energy intake. Coffee intake. Meat intake. Fruit and vegetable intake. Fibre density. Saturated fat intake.	(Model 2 +) High triglycerides. High LDLc. Low HDLc. Hypertension.	Exclusion of diet changers and potential energy misreporters.
3	Cox hazards regression	Age. Sex. Interview method. Season when dietary data collection took place. Total energy intake.	(Model 1 +) Alcohol consumption. LTPA. Educational level. Smoking habits.	(Model 2 +) BMI.	Exclusion of diet changers and potential energy misreporters.
4	Linear regression	Age. Sex. Interview method. Total energy intake.			Exclusion of diet changers and potential energy misreporters. BMI-dependent associations (BMI<25 vs BMI≥25).

BMI: body mass index. **LTPA:** leisure time-physical activity. **LDLc:** Low-density lipoprotein cholesterol. **HDLc:** high-density lipoprotein cholesterol.

Ethical considerations

After reviewing all pertinent ethical considerations, the Ethical Committee at Lund University approved the Malmö diet and Cancer Study (LU-51-90). Additionally, all participants of the Malmö Diet and Cancer Study and Riksmaten Adults gave written and oral consent respectively after being thoroughly informed about the study and before any measurements were taken.

Results

Study 1

The aim of this study was to examine whether there is an association between the intake of added sugar and the intake of micronutrients to confirm the presence of micronutrient dilution in two samples of the adult Swedish population.

While Riksmaten Adults represented a smaller study sample (n=1797) and slightly younger population (mean: 48 years old) than the Malmö Diet and Cancer Study (n=12,238, mean age: 58 years old). Both populations presented a similar percentage of men (44% men in Riksmaten Adults, 45% men in Malmö Diet and Cancer Study) and BMI (25.5 kg/m^2 for Riksmaten Adults, 25.1 kg/m^2 for Malmö Diet and Cancer Study). The mean energy intake was higher for the population from the Malmö Diet and Cancer Study (2334 kcal/day) compared to those from Riksmaten (1903 kcal/day), as was the mean intake of added sugar (10.1%E for Malmö Diet and Cancer Study and 9.5%E for Riksmaten Adults).

Significant inverse associations were observed for added sugar intake and the intake of the nine studied micronutrients for the participants of the Riksmaten Adults and the Malmö Diet and Cancer Study. However, we were not able to identify an added sugar intake threshold beyond which there would be a marked micronutrient dilution effect. The largest decrease on micronutrient intake between high added sugar consumers (>20%E) compared to low added sugar consumers (<5%E) was found on vitamin D (38%), selenium (33%), folate (25%), and zinc (22%) for Riksmaten Adults participants (**Figure 9**); and on vitamin D (32%), selenium (32%), zinc (26%) and folate (25%) for Malmö Diet and Cancer Study participants (**Figure 10**).

Overall, micronutrient intakes in relation to dietary reference values (average requirement and recommended intake) were lower in Riksmaten Adults than in the Malmö Diet and Cancer Study. For instance, almost 80% of the men and 85% of the women in Riksmaten Adults presented vitamin D intakes below the average requirement. Similarly, almost 65% of the men and more than 70% of the women of the Malmö Diet and Cancer Study presented vitamin D intakes below the average requirements.

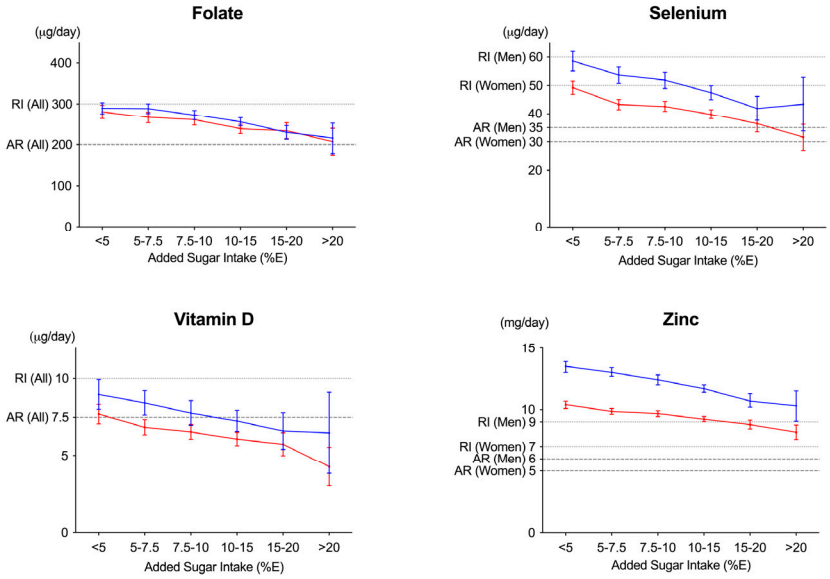


Figure 9. Micronutrient intake across added sugar intake groups for the participants from Riksmaten Adults.
Mean daily micronutrient intakes for men (blue) and women (red) from Riksmaten Adults are presented in relation to the dietary reference values (average requirement and recommended intake). **%E:** percentage of non-alcoholic energy intake. **AR:** average requirement. **RI:** recommended intake.

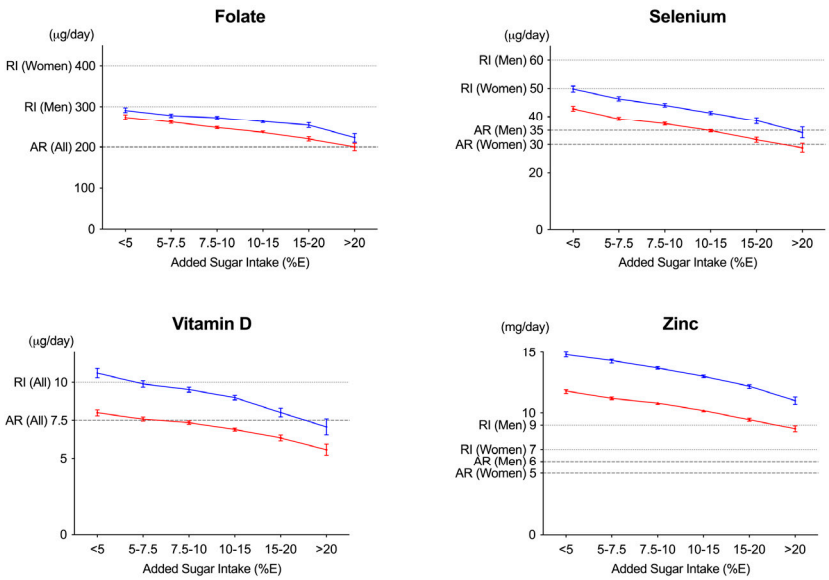


Figure 10. Micronutrient intake across added sugar intake groups for the participants from the Malmö Diet and Cancer Study.
Mean daily micronutrient intakes for men (blue) and women (red) from the Malmö Diet and Cancer Study are presented in relation to the dietary reference values (average requirement and recommended intake). **%E:** percentage of non-alcoholic energy intake. **AR:** average requirement. **RI:** recommended intake.

Study 2

The aim of this study was to investigate the association between the intake of different forms of sugar and sugar-rich foods and beverages and intima media thickness measured at the common carotid artery (IMTcca) and at the bifurcation of the carotids (IMTbif).

Our study sample was formed by participants of the Malmö Diet and Cancer Study – Cardiovascular Cohort (40% men) with a mean age of 57.3 years (for both men and women). However, men showed a slightly higher BMI (26.0 kg/m^2) than women (23.5 kg/m^2). Intima media thickness mean values were higher for men (IMTcca = 0.765mm, IMTbif = 1.540mm) than for women (IMTcca = 0.723mm, IMTbif = 1.397mm). Mean added sugar intake was around 10%E for both men and women, and the majority of participants reported consuming less than one serving of sugar-sweetened beverages per week.

No linear associations were found between all studied forms of sugar intake (added, free, total) or any sugar rich foods (treats, toppings) or beverages (SSBs) and intima media thickness measurements at either the common carotid artery or the bifurcation of the carotids for the participants of the Malmö Diet and Cancer Study – Cardiovascular Cohort (**Figure 11**). Similarly, these results remained when analysing men and women together.

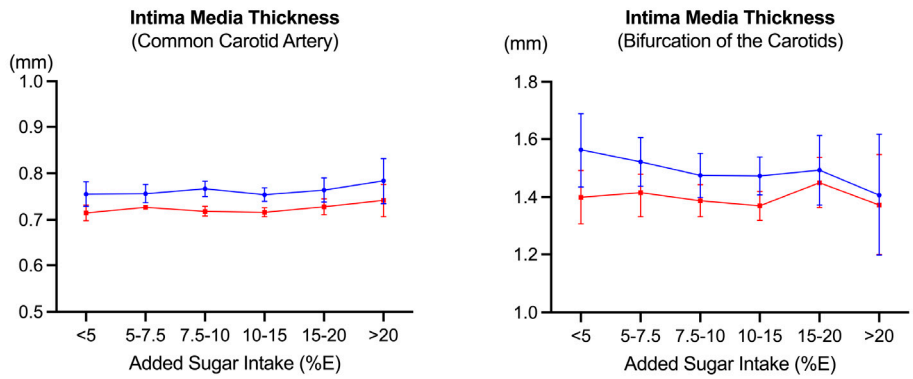


Figure 11. Association between added sugar intake and intima media thickness
Intima media thickness presented as mean values and 95% confidence intervals for men (blue) and women (red). Depicted is the main model: adjusted for age, start date, time between baseline and intima media thickness measurement, season, alcohol consumption, leisure-time physical activity, education, smoking habits, BMI, energy intake, coffee, meat, fruits and vegetables, fibre, and saturated fat. **p-trends:** IMTcca (men) = 0.811, IMTcca (women) = 0.985; IMTbif (men) = 0.203, IMTbif (women) = 0.402. **%E:** percentage of non-alcoholic energy intake.

When potential energy misreporters and diet changers were excluded in sensitivity analyses, the results remained unchanged for added sugar. However, the highest intima media thickness values measured at the common carotid artery were found

in men (0.791 mm) and women (0.738 mm) consuming >20%E from added sugar, when compared to those consuming < 20%E. Likewise, there was an apparent tendency found when the intima media thickness was measured at the bifurcation of the carotids for a higher measurements for men (1.385 mm) and women (1.384mm) consuming >20%E as opposed to those consuming 20%E or less from added sugar. However, none of these trends were statistically significant.

Study 3

The aim of this study was to explore the association between two healthy dietary patterns (Swedish and Mediterranean) in relation to the risk of stroke and its subtypes.

The sample of participants from the Malmö Diet and Cancer Study were aged between 44 and 74 years old, and their mean BMI was 25.6 kg/m^2. Only 2.7% of the participants adhered to all five items included in the Swedish Dietary Guidelines Score and none of them complied with the 10 items of the Modified Mediterranean Diet Score, with only 7.6% scoring five points or more (high adherence group). Additionally, 13.6% of participants did not meet any of the recommendations for the Swedish Dietary Guidelines Score and 15.0% did not meet any of the recommendations for the Modified Mediterranean Diet Score. The Spearman correlation coefficient between the two scores was 0.57. Those participants classified into the high adherence group of both scores were more likely to be female, had a university degree, and a higher physical activity level, and were less likely to be smokers.

The mean follow-up period of our study was 19.5 years, during which we observed 2579 cases of stroke (10%). From these cases, approximately 80% were classified as ischaemic stroke (2104 cases). In our study, we found an inverse association between the adherence to a healthy dietary pattern, whether defined by the Swedish dietary guidelines score (**Table 7**) or the modified Mediterranean diet score (**Table 8**), and the risk of total stroke and ischemic stroke in our basic model. However, this association disappeared in the multivariable model, and reappeared when diet changers and potential energy misreporters were excluded in sensitivity analysis. No significant associations were found in relation to the risk of haemorrhagic stroke.

Individual diet components for each score were also examined. Protective associations were found between all components of the Swedish Dietary Guidelines Dcore and the risk of total and ischaemic stroke. However, only dietary fibre showed a statistically significant association with total stroke risk in sensitivity analyses (hazards ratio = 0.89; 95% confidence interval: 0.79 – 1.00). In the Modified Mediterranean Diet Score, a protective effect against total stroke and especially

haemorrhagic stroke was found for participants consuming more than 100g of wine per day.

Looking more specifically at sugar consumption, in the Swedish Dietary Guidelines Score, sugar intake was captured under the variable added sugar intake. However, no significant association was found between adherence to the recommendations (added sugar <10%E) and the risk of stroke. In the Modified Mediterranean Diet Score, sugar consumption was captured with two variables, soda, and sweets and pastries intake. While remaining under 7 servings per week of sodas showed a protective effect against total and ischaemic stroke, consuming less than 3 servings per week of sweets and pastries showed the opposite effect in our population.

Table 7.
Association between Swedish Dietary Guidelines Score and risk of stroke (Hazards Ratio and 95% confidence intervals).

		Adherence to Swedish Dietary Guidelines Score			Per point	p-trend
		Low (0-1 points)	Medium (2-3 points)	High (4-5 points)		
n		10,642	11,849	3,349		
Years of follow-up		203,453	232,484	67,344		
Total stroke	Cases / cases per 1000 PY	1,101 / 5.41	1,189 / 5.11	289 / 4.29		
	Model 1	1.00	0.91 (0.84-0.99)	0.81 (0.71-0.93)	0.95 (0.92-0.98)	0.001*
	Model 2	1.00	0.97 (0.89-1.05)	0.89 (0.78-1.02)	0.98 (0.95-1.01)	0.13
	Model 3	1.00	0.96 (0.88-1.04)	0.89 (0.78-1.02)	0.97 (0.94-1.01)	0.10
	Model 3 (excl. misreporters)	1.00	0.94 (0.85-1.04)	0.87 (0.72-1.04)	0.96 (0.92-1.00)	0.04*
Ischaemic stroke	Cases / cases per 1000 PY	910 / 4.47	957 / 4.12	237 / 3.52		
	Model 1	1.00	0.89 (0.81-0.98)	0.81 (0.70-0.94)	0.94 (0.91-0.98)	0.001*
	Model 2	1.00	0.95 (0.86-1.04)	0.90 (0.78-1.05)	0.97 (0.94-1.01)	0.14
	Model 3	1.00	0.94 (0.86-1.03)	0.90 (0.77-1.04)	0.97 (0.94-1.01)	0.10
	Model 3 (excl. misreporters)	1.00	0.91 (0.81-1.02)	0.87 (0.72-1.07)	0.95 (0.91-1.00)	0.03*
Haemorrhagic stroke	Cases / cases per 1000 PY	172 / 0.85	209 / 0.90	51 / 0.78		
	Model 1	1.00	1.02 (0.83-1.25)	0.89 (0.64-1.22)	0.97 (0.90-1.05)	0.48
	Model 2	1.00	1.07 (0.87-1.32)	0.95 (0.69-1.31)	0.99 (0.92-1.07)	0.88
	Model 3	1.00	1.07 (0.87-1.32)	0.95 (0.69-1.31)	0.99 (0.92-1.07)	0.89
	Model 3 (excl. misreporters)	1.00	1.15 (0.90-1.48)	0.96 (0.62-1.48)	1.01 (0.91-1.11)	0.87

* Statistically significant p-trend ($p<0.05$). **PY:** person years. **Model 1:** adjusted for age, sex, method, season, energy intake. **Model 2:** adjusted for age, sex, method, season, energy intake, education, smoking, leisure-time physical activity, alcohol habits. **Model 3:** adjusted for age, sex, method, season, energy intake, education, smoking, leisure-time physical activity, alcohol habits, and BMI. **Model 3 excluding misreporters** (i.e., non-adequate reporters of energy) and those who indicated a substantial change in dietary habits in the past (35% of the study sample).

Table 8.
Association between Modified Mediterranean Diet Score and risk of stroke (hazards ratio and 95% confidence interval).

		Adherence to Modified Mediterranean Diet Score			Per point	p-trend
		Low (0-1 points)	Medium (2-4 points)	High (5-10 points)		
n		4,432	19,439	1,696		
Years of follow-up		84,834	378,907	39,542		
Total stroke	Cases / cases per 1000 PY	480 / 5.66	1,936 / 5.11	163 / 4.12		
	Model 1	1.00	0.90 (0.81-0.99)	0.84 (0.70-1.00)	0.96 (0.93-0.99)	0.008*
	Model 2	1.00	0.94 (0.84-1.04)	0.92 (0.76-1.11)	0.98 (0.94-1.01)	0.18
	Model 3	1.00	0.94 (0.85-1.04)	0.92 (0.76-1.11)	0.98 (0.94-1.01)	0.18
	Model 3 (excl. misreporters)	1.00	0.89 (0.78-1.00)	0.81 (0.63-1.05)	0.95 (0.91-0.99)	0.02*
Ischaemic stroke	Cases / cases per 1000 PY	396 / 4.67	1,576 / 4.16	132 / 3.34		
	Model 1	1.00	0.89 (0.79-1.00)	0.83 (0.68-1.02)	0.96 (0.93-1.00)	0.03*
	Model 2	1.00	0.93 (0.83-1.05)	0.92 (0.75-1.13)	0.98 (0.95-1.02)	0.37
	Model 3	1.00	0.94 (0.84-1.05)	0.93 (0.75-1.14)	0.98 (0.95-1.02)	0.38
	Model 3 (excl. misreporters)	1.00	0.87 (0.76-1.00)	0.79 (0.59-1.04)	0.95 (0.90-1.00)	0.03*
Haemorrhagic stroke	Cases / cases per 1000 PY	74 / 0.87	329 / 0.87	29 / 0.73		
	Model 1	1.00	0.96 (0.74-1.25)	0.90 (0.59-1.40)	0.94 (0.87-1.02)	0.12
	Model 2	1.00	1.00 (0.77-1.30)	0.97 (0.62-1.52)	0.95 (0.87-1.03)	0.24
	Model 3	1.00	1.00 (0.77-1.30)	0.97 (0.62-1.52)	0.95 (0.87-1.03)	0.24
	Model 3 (excl. misreporters)	1.00	1.02 (0.74-1.40)	1.07 (0.60-1.94)	0.96 (0.86-1.07)	0.47

* Statistically significant p-trend ($p<0.05$). **PY:** person years. **Model 1:** adjusted for age, sex, method, season, energy intake. **Model 2:** adjusted for age, sex, method, season, energy intake, education, smoking, leisure-time physical activity, alcohol habits. **Model 3:** adjusted for age, sex, method, season, energy intake, education, smoking, leisure-time physical activity, alcohol habits, and BMI. **Model 3 excluding misreporters** (i.e., non-adequate reporters of energy) and those who indicated a substantial change in dietary habits in the past (35% of study participants).

Study 4

The aim of this study was to explore the association between the consumption of total, added, and sweet-tasting sugars and a selection of single-nucleotide polymorphisms (SNPs) previously associated with sugar intake and/or preference.

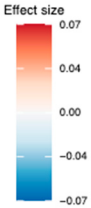
In this sample of the Malmö Diet and Cancer Study, the participants were mostly women (64.1% women) with a mean age of 58 years old, and a mean energy intake of 2,281 kcal/day. While the mean BMI for this population was 25.5 kg/m^2, about half of them (50.8%) had a BMI of 25 kg/m^2 or above. The mean daily intakes of total sugars for this population was 20.4%E, mean added sugar intake was 10.2%E, and mean intake of sugars with sweet taste (monosaccharides and sucrose) was 16.0%E.

We analysed the association between a total of 114 SNPs and the intakes of added sugar, total sugar and sugars with a sweet taste. Our study found various Bonferroni-corrected ($p < 0.005$) significant associations between the studied genetic variants and the three main outcomes of interest. The strongest relations were found for three SNPs within chromosome 19. These three SNPs were all located within (rs838133) or in close proximity (rs838145, rs8103840) to the *fibroblast growth factor 21* (*FGF21*) gene. The effect alleles (rs838145 G, rs838133 A, and rs8103840 C) were all found to be positively associated with the intakes of total sugar (β= 0.18, β = 0.22, and β = 0.20, respectively), added sugar (β = 0.13, β = 0.15,and β = 0.13, respectively), and sugars with sweet taste (β = 0.16, β = 0.22, and β = 0.20, respectively), where β represents an increase in percentage point of non-alcoholic energy intake per additional allele (**Table 9**). Another significant association was found for a genetic variant (rs60764613 G allele) on chromosome 18 within the *CTD-2015H3.1* gene and the intake of added sugar (**Table 9**).

While a SNP (rs11642841) within the *fat-mass and obesity associated* (*FTO*) gene was previously reported to have only GWAS-significant association with total sugar intake in the UK Biobank (54), we found no such association in our population. However, when our participants were stratified based on their BMI in sensitivity analyses, an association was discovered between added sugar and total sugar intake and the rs11642841 SNP for participants with a BMI $\geq$25kg/m^2. No further significant associations were found in our study, when replicating Hwang et al.'s (54) results, including for genes coding proteins involved in the sweet signalling pathway.

Table 9.
Association between the single-nucleotide polymorphisms (SNPs) and the intakes of total sugar, added sugar, and sugars with sweet taste. The colouring represents the effect size, measured as β/standard error of the estimate. Bold typeface p-values are Bonferroni-corrected significant p-values ($p < 0.005$).

SNP EA	Associated gene	Total sugar			Added sugar			Sugars with sweet taste[1]		
		β[2]	SE	p	β[2]	SE	p	β[2]	SE	p
rs11577403 A	PTPRF	0.04	0.05	0.42	0.01	0.04	0.82	0.04	0.05	0.40
rs7424551 G	AC073284.4	-0.02	0.05	0.71	0.03	0.04	0.45	-0.01	0.05	0.83
rs35267617 T	STK32A	-0.01	0.05	0.76	0.00	0.04	0.90	-0.04	0.05	0.34
rs6911544 A	RP3-335N17.2	0.08	0.06	0.22	0.07	0.05	0.20	0.08	0.06	0.19
rs 559904 A	POP5	0.08	0.05	0.13	0.01	0.04	0.74	0.06	0.05	0.21
rs 11642841 C	FTO	0.09	0.05	0.07	0.10	0.04	0.01	0.06	0.0.5	0.17
rs 60764613 G	CTD-2015H3.1	0.06	0.07	0.33	0.16	0.05	**2.89×10^{-3}**	0.12	0.06	0.06
rs838145 G	IZUMO1, FGF21	0.18	0.05	**2.32×10^{-4}**	0.13	0.04	**1.53×10^{-3}**	0.16	0.05	**6.52×10^{-4}**
rs8103840 C	FUT1, FGF21	0.20	0.05	**2.04×10^{-5}**	0.13	0.04	**4.85×10^{-4}**	0.20	0.04	**1.06×10^{-5}**
rs838133 A	FGF21	0.22	0.05	**2.42×10^{-6}**	0.15	0.04	**1.87×10^{-4}**	0.22	0.05	**6.82×10^{-7}**



[1] Sugars with sweet taste: all monosaccharides and sucrose. [2] β: represents an increase in percentage point of non-alcoholic energy intake per additional allele. **SNP:** single-nucleotide polymorphism. **EA:** effect allele. **SE:** standard error.

Discussion

Main findings and interpretation

Overall, this doctoral thesis aimed to explore the role that sugar intake, and more specifically added sugar, plays in the development of cardiovascular disease in the context of informing nutritional recommendations. Evidence-based nutritional recommendations can, in turn, inform policies aimed at regulating sugar consumption with the intention to prevent cardiovascular and other diseases.

The risk of micronutrient dilution has been an important factor considered in the development of many nutritional recommendations in the past. However, current nutritional guidelines are aiming to issue their recommendations based on long-term health effects and disease development. In the future, nutritional recommendations might need to address inter-individual variability based on, among others, genetic factors. As a result, this doctoral thesis explored the role of sugar intake (in different forms and from different dietary sources) in relation to: micronutrient intake (**Study 1**), atherosclerosis (**Study 2**), risk of stroke (**Study 3**), and genetic factors (**Study 4**).

Summary of main findings

In **Study 1**, we found that a higher intake of added sugar was cross-sectionally associated with a lower intake of micronutrients. Thus, confirming the existence of micronutrient dilution in our study populations. Additionally, in **Study 4**, we confirmed an association between sugar intake and three genetic variants in close proximity to the *fibroblast growth factor 21* gene on chromosome 19. This gene has been previously associated with sugar intake (85, 86).

However, this doctoral thesis has not been able to establish a significant relation between sugar intake and cardiovascular disease and its risk factors. In **Study 2**, we found no association between the consumption of different types of sugar and sugary foods and beverages and intima-media thickness (a marker for subclinical atherosclerosis). While the highest values of intima media thickness measured at the common carotid arteries were found in participants consuming more than 20%E from added sugar; the same could not be said about measurements at the bifurcation of the carotids. Nevertheless, neither of these findings were statistically significant.

In **Study 3**, a weak protective association against all forms of stroke was found for participants adhering to healthy dietary patterns (whether Swedish or Mediterranean) after adjustments. However, in sensitivity analyses, the association reappeared when excluding diet changers and potential energy misreporters. When exploring sugar consumption in particular, adhering to the Mediterranean diet recommendations for soda intake (<7 servings/week) had a protective effect against total and ischaemic stroke. Conversely, participants not adhering to the recommended <3 servings/ week of sweets and pastries (according to the Mediterranean diet), showed an increased risk of total and ischaemic stroke.

Our results within context

All in all, the evidence of the displacement of the consumption of nutritious foods by the overconsumption of energy-dense and nutrient-poor foods is remarkable. All across the globe, studies with different methodological approaches and population characteristics have shown that a higher intake of sugar seems to be associated with a lower intake of micronutrients (21-28). The amount of evidence backing the existence of micronutrient dilution, is most likely the reason why many nutritional recommendations have used this phenomenon as the gauging tool to issue recommendations for sugar intake in the past (87). However, nutritional recommendations based on micronutrient intake need to be country-specific due differences in food availability, dietary needs, dietary preferences, and cultural practices of their populations (20). Additionally, in some countries it is not uncommon to encounter products that are both high in sugar and fortified with micronutrients, such as sugary breakfast cereals or dairy-based sweet drinks (88). However, this is not the case in Sweden, where the fortification of unhealthy foods is very rare, which makes our finding from **Study 1** much more relevant.

The study of cardiovascular disease and sugar intake has rendered less clear results in the scientific literature. While studies in animal models have found that sucrose, fructose, and starch consumption can have atherogenic effects (89-91); studies in humans have not been able to prove the same effect. Previous studies have found no associations between intake of sugar-sweetened beverages and subclinical atherosclerosis (92), with the exception of one study in healthy Korean adult women which found an association between higher intakes of sugar-sweetened beverages and a higher coronary artery calcium (93). Another study found carbohydrate intake to be associated with atherosclerotic disease progression (measured via intima media thickness) in postmenopausal women, especially when their glycaemic index was high (94). However, a different study found no association between glycaemic index or glycaemic load and intima media thickness after a one-year follow up (95). In **Study 2**, we found a tendency for a higher intima media thickness measured at the common carotid arteries in participants with higher sugar intake. This coincides with another study in the same population that pointed towards a higher incidence

of cardiovascular disease in participants with the highest sugar intake (96). A high intake of sugar is usually associated with an overall unhealthier dietary pattern (low intake of fibre, whole grains, fruits, and vegetables) (39). Previous studies have found associations between adherence to healthier dietary patterns and lower intima media thickness (97-99). A systematic review of the existing evidence up to 2019, found that while there is a general support that healthier dietary patterns were associated with decreased intima media thickness, these associations were not significant (100).

Adherence to healthier dietary patterns can come with additional health benefits. Similarly to our results from **Study 3**, a Danish cohort study found that adhering to a healthy Nordic diet had a significant protective effect against ischaemic stroke (101) and adherence to Mediterranean diet has been associated to lower risk of cardiovascular disease (including stroke) (102). A recent study on the Malmö Diet and Cancer Study also found an independent association between a high quality diet and a lower risk of atherothrombotic (ischaemic) stroke (103). Both the Swedish dietary guidelines and the Mediterranean diet are mostly plant-based diets. Individual components of both dietary patterns have been linked to beneficial health effects. For example, fibre-rich diets have been linked to a reduction in cholesterol levels (104) and a protective effect against cardiovascular diseases in general (105), and stroke incidence in particular (**Study 3**). Additionally, consumption of fish, fruits, and vegetables also have shown a protective effect against cardiovascular disease (105).

In terms of sugar consumption and cardiovascular disease risk, a consumption of less than one soda (whether sugar- or artificially-sweetened) per day was associated with lower risk of stroke both in our study (**Study 3**). Currently, there are no existing randomised controlled trials exploring the association between the intake of sugar-sweetened beverages and cardiovascular disease endpoints. However, evidence from observational studies suggest a positive and causal association between sugar-sweetened beverage consumption and cardiovascular risk with a high level of certainty (4). Our finding of a protective effect against stroke in participants consuming more sweets and pastries than recommended has also been previously found in the Malmö Diet and Cancer Study (96). This particular finding could be explained by the strong *fika* culture that exists in Sweden. *Fika* is a social break from activity where people share a hot drink and a pastry or other type of snack. This custom is not necessarily associated with an unhealthy dietary pattern or other unhealthy behaviours. Moreover, it has been shown that individuals with a good social network have a lower risk of developing cardiovascular diseases (106). This could, therefore, explain the seemingly protective effect of not adhering to sweet and pastries recommendations. However, another plausible explanation behind this difference in stroke risk could stem for the metabolic differences of consuming sugars in solid (sweets and pastries) or liquid (soda) form. For instance, sugars consumed in liquid form are less satiating than those consumed in solid form (107)

and their digestion is much faster as they do not require mechanical digestion (108) and are not usually accompanied by any proteins or fats. Additionally, a high consumption of sugar-sweetened beverages is usually considered a marker for other unhealthy dietary and lifestyle habits (39). These results also coincide with the findings of the European Food Safety Authority, where the evidence backing the link between the consumption of sodas and cardiovascular risk is much more conclusive than the one found for solid sugars (4).

The field of genetics has become an increasingly useful tool in the study of food preference and intake. Particularly, in the study of sugar consumption previous GWAS have linked the *FGF21* locus with macronutrient intake (55, 58, 109, 110). The FGF21 hormone (coded by its homonymous gene) is released in response to sugar intake (85, 86), alcohol consumption (111), and diets deficient in protein (112, 113). This coincides with our findings in **Study 4**, linking genetic variants near the *FGF21* gene with high intakes of added and total sugar, and low intake of protein. However, studies in mice have found the effect of FGF21 to be primarily on carbohydrate rather than on protein intake, signalling that this finding could be the result of a substitution pattern (114). Additionally, our study also found a link between these genetic variants and sugars with sweet taste. The FGF21 hormone has also been related to the suppression for sweet preference and sugar intake via a negative feedback loop (115-117) in humans (118) and animal (119) models. The associations found in our study (**Study 4**) might also be partially associated with weight. In our main analyses we could not find a significant association with variants within the *FTO* gene (strongly associated with BMI (120)). However, when the sample was stratified based on BMI in sensitivity analyses, associations with genetic variants within the *FTO* gene (rs11642841) appeared, and most of the associations were found only on overweight and obese participants (BMI $\geq$ 25kg/m^2). However, neither our study nor Hwang et al. (54) could replicate any associations previously found in connection to the sweet taste receptor and sweet signal transduction genes (*TAS1R2* and *GNAT3*) (43, 56, 58, 121, 122).

Methodological considerations

Study design

Randomised controlled trials are the best way to explore causal relationships between exposure and outcome. However, experimental studies in nutrition research are not always feasible or even ethical. Additionally, there is the caveat of defining the control group. Should the control group be defined by avoiding a certain nutrient, a certain food, avoiding eating all together, or an *ad libitum* diet?

Moreover, adherence to the dietary intervention in long and costly randomised controlled trials is usually also an issue (59).

Instead, nutritional researchers usually rely on observational studies that could be restricted by confounding factors and other limitations. However, observational studies can offer information on a larger number of exposure variables than experimental studies. Observational studies are also relevant in the study of disease prevention and the factors involved in disease progression. The main limitation of these studies is that they have to run their natural course, without the full control of the researchers over other factors (59).

In this doctoral thesis, we have made use of a cross-sectional study design to ascertain the existence of association between two factors at a given time in a given population. The main limitation of this type of studies is that they do not offer information on causality as time is not considered as a variable in the design. However, by using a prospective cohort design in **Study 3**, we could ascertain the development of disease based on a given exposure over time. Although **Study 4** was cross-sectional in design, one could argue that a certain temporality could be assumed. Given how our genetic make-up (acting as the exposure) is determined at conception and the measurement of dietary habits (used as outcome) happened later in time (59).

Validity and bias

When gauging the association between an exposure and an outcome, we must also ascertain the validity of the obtained results. That is, we need to estimate whether the observed findings are true or not, also known as internal validity. To ensure internal validity of our results we must be able to exclude alternative explanations to the association found. First, we must eliminate bias (or systematic error), an error in the design of the study that can lead to incorrect associations. Second, we should also discard confounding, the intervention of a third variable (or confounder) between the exposure and the outcome. Lastly, we must be able to eliminate random error, the probability that the observed findings are a result of chance. Additionally, studies must also be susceptible of generalisation (external validity) to populations beyond the sample studied (59).

Bias is defined as a systematic error that could result in an inaccurate or incorrect association between exposure and outcome, deeming said association invalid (59). There are three main sources of bias in epidemiological studies: 1) selection bias, pertaining to how the study participants are selected, 2) information bias, pertaining to how relevant data is collected, and 3) confounding, pertaining to a third variable that is not the exposure or the outcome but which affects their association (123).

When there is a difference in the association between exposure and outcome between participants and non-participants, we are in the presence of selection bias

(123). In our study populations (Riksmaten Adults and Malmö Diet and Cancer Study), the participation rates were lower than desirable (36% and 41% respectively), this could pose a risk for generalisability of the results found in other populations. Participants in Riksmaten Adults had a higher level of education and annual income than non-participants (8). Participants and non-participants of the Malmö Diet and Cancer Study had similar socioeconomic characteristics but participants reported better perceived health than non-participants (61). Non-participants in both studies were more likely to be born outside of Sweden (8, 61). Selection bias due to loss of follow-up is particularly worrisome when the loss of follow-up differs between the groups. However, in our prospective cohort study with complete follow-up this was not an issue. Another issue in terms of generalisability, is the fact that both studies collected information from adults (Riksmaten Adults) or older adults (Malmö Diet and Cancer Study), neglecting the possibility to study the associations in younger populations.

When mistakes happen during the ascertainment of exposure, covariates, or outcomes that lead to differences in the accuracy of information obtained from the comparison groups, we are in the presence of information bias (59). In nutritional studies, this type of bias can occur in the form of dietary misreporting. Dietary misreporting occurs when the estimated energy intake reported by the study participant does not coincide with their energy expenditure (83). Misreporting of dietary intake can occur unconsciously, but more commonly it occurs when there is a factor of social desirability involved (59). Thus, misreporting is more commonly observed in participants with a higher body mass index and for energy-dense foods and snacks (124). To atone for this phenomenon, in our studies we have excluded potential misreporters in sensitivity analyses. In terms of outcome ascertainment, we have made use of medical records and registers that diminish the possibility of information bias.

When a third variable might explain all or part of the association found between exposure and outcome, we are in the presence of confounding (123). A confounder is a third variable connected to both exposure and outcome that could be masking the real association between them (59). In our studies, we have accounted for multiple possible confounders by including them in the adjustment models. However, even with all these measures in place we cannot completely rule out the presence of residual confounding. For instance, in **Study 3**, we have postulated that one of the possible alternative explanations behind the protective effect of a higher consumption of sweets and pastries against stroke could be explained by a Swedish cultural tradition (*fika*).

Diet as an exposure

Diet is a unique exposure for numerous reasons. Our diets are influenced by our individual choices, by the food environment and the availability of products, by cultural and religious practices, by personal preferences and characteristics, by socioeconomic factors, health status, and many more. As individuals, we eat differently from each other. But we also experience changes in our diets throughout our lives.

Each meal that an individual consumes contains infinite data about ingredients, nutrients, and additional chemicals or compounds (whether known or not). Plus, we consume several meals per day, every day, every year, for many years in our lives. Moreover, epidemiological studies collect all this information from hundreds or thousands of individuals. Additionally, it is often difficult to isolate the effect of a single nutrient over a health outcome due to the numerous interactions that might exist within the food matrix. Food matrix is a term used to refer to the differences in behaviour of nutrients when studied in laboratory conditions (isolation) versus when they form part of the food structure, accounting for effects that cannot be measured or are yet unknown (125).

As a way to compensate for this, the dietary data collection for the studies included in this doctoral thesis is comprehensive and extremely detailed. Additionally, we have explored sugar consumption by studying different types of sugar (added, free, and total), dietary sources of sugar in solid (treats and toppings) and liquid form (SSBs), and sugar consumption within healthy dietary patterns.

Dietary sugar sources

While there is no molecular difference in sugars contained in, for instance, fruits and vegetables, and those contained in sweets and sodas, the health effects associated with their consumption might be very different. For instance, when we consume fruits and vegetables, we are also consuming a copious amount of fibre and micronutrients. Furthermore, these naturally occurring sugars are more tightly bound to the food structure that delivers them. Food processing usually extracts the sugar contained or encapsulated in a dietary fibre structure, making them more easily available and more rapidly digested. A quick digestion of sugars (or carbohydrates) is usually linked with a higher glycaemic response, which is not recommended especially for subjects suffering from diabetes or other cardiometabolic conditions (126). This is yet another reason why we aimed to explore different sources of dietary sugars in the studies included in this thesis.

Dietary patterns

Dietary pattern studies can be classified based on two approaches. On the one hand, a hypothesis driven approach (named *a priori* dietary patterns), where there are pre-established conditions that define the dietary pattern and those are explored within the context of a population and an outcome. On the other hand, the dietary patterns can be data driven (named *a posteriori*), where the dietary pattern is defined based on the data itself without any preconceived hypothesis.

A posteriori dietary patterns are usually more susceptible to the subjectivity of the researchers who create the groups and might make it difficult to compare to the study of other populations or outcomes (17). However, *a priori* dietary patterns are usually constructed using indexes or scores (like the ones studied in **Study 3**) to be able to measure a specific dietary pattern, for instance the Mediterranean Diet, or to gauge whether the participants follow certain nutritional recommendations. A limitation of these types of scores is that they tend to attach an equal weight of importance to each of the components, which are also usually defined as dichotomous variables ("adheres" versus "does not adhere") instead of exploring a range of consumption (127).

As previously stated in this thesis, it is important to account for possible synergies and interactions by studying dietary patterns as well as single nutrients. Additionally, dietary patterns allow us to explore the overall effect of diet on a given outcome (17, 30).

Strengths and limitations

Strengths

Overall, the studies contained in this doctoral thesis presented numerous methodological strengths. To start with, both the Malmö Diet and Cancer Study and the Riksmaten Adults had collected detailed information from large populations. Additionally, the Malmö Diet and Cancer Study has a long follow up period that permits the identification of diseases with a longer development time. Moreover, both datasets collected information regarding dietary habits, anthropometric, socioeconomic, and lifestyle factors, as well as other possible confounding factors. The data collection was performed using validated methods (66, 128-130). The use of a food diary as part of the dietary data collection method decreased the possibility of recall bias, as the meals were reported one at a time. The Malmö Diet and Cancer Study remains to this day one of the most comprehensive dietary data collections in Sweden. Riksmaten Adults, on the other hand, is the most recent source of dietary information on adults on a national level. A relevant advantage of the Malmö Diet and Cancer Study is the existence of information regarding misreporting and changes in diet.

Other strengths of our studies include the use of internationally approved definitions for added, free, and total sugar, making it easier to compare our results with other studies. The use of established thresholds of sugar intake, as opposed to percentiles, also allows for easier comparison with other studies. Moreover, the cut-offs established (especially those for added sugar and free sugar) permit to explore the already existing nutritional recommendations for sugar intake as well as extreme intake values. Additionally, the extensive study of several sugar types and sources, including added sugar and sugar-sweetened beverages, is an advantage as they are the target of many studies and nutritional recommendations alike (10, 11, 131).

The use of not one, but two large population-based cohorts for **Study 1**, allowed us to explore dietary habits covering over two decades. Moreover, finding similar trends in both cohorts pointed towards the perpetuation of a tendency towards micronutrient dilution in the Swedish adult population. Another strength of this study was the use of dietary reference values for micronutrient intake specific to the Nordic countries, as these thresholds might differ from other regions (20). An increase in intima media thickness is the first measurable sign of atherosclerosis, and the use of ultrasounds is the gold standard for said measurement (132). Therefore, our choice of marker for subclinical atherosclerosis is a strength of **Study 2**. Additionally, the almost complete follow-up rate of **Study 3**, granted a reduced potential for selection bias due to systematic loss to follow-up. To our knowledge, **Study 2** is one of the first ones to explore the association between intima medica thickness and sugar intake in humans, particularly one of the first to investigate added sugar consumption. Likewise, **Study 4** is also one of the first to explore different forms of sugar intake (including added sugar) and dietary sources of sugar in relation to genetic variants. While we had no information on sweet preference for the design of **Study 4**, the possibility to measure sugars with sweet taste as a proxy for them was also a strength of our study.

Limitations

One of the biggest limitations of both Riksmaten Adults and Malmö Diet and Cancer Study is that dietary data collection occurred only once at baseline, and therefore changes in dietary habits could not be evaluated. Another issue is the reliance on self-reported dietary data. However, the possibility to ascertain potential energy misreporting and major changes in diet in the year prior to the baseline examination in the Malmö Diet and Cancer Study allowed us to adjust for or exclude participants with a less reliable reporting or a less stable dietary habits. Unfortunately, this information was not available for participants in Riksmaten Adults.

Furthermore, the fact that both cohorts studied only adults and older adults and mostly Swedish-born citizens, could threaten the generalisability of the findings to other populations of different age and ancestry. Another factor threatening generalisability could be linked to cultural differences and food availability when it comes to adopting a Mediterranean-style diet in the Nordic region in **Study 3** (133).

In our study, the adherence to the modified Mediterranean diet score was very low with no participants fulfilling all ten criteria of the score and very few reaching nine or eight points. It is also worth remarking that the data collection for the Malmö Diet and Cancer Study occurred in the early 1990s and could, as a result, not be reflective of the current dietary consumption patterns.

In general, participants of the Malmö Diet and Cancer Study were more likely to be healthier in terms of cardiovascular events than non-participants (61) and other populations (134). As a result, in **Study 2**, one possible explanation for the lack of association between intima media thickness and sugar consumption in our study, could be that the Malmö Diet and Cancer Study population was "too healthy". Indeed the mean values of intima media thickness measured at the common carotid artery were way below the pathological threshold of 0.9mm (135) with a mean of 0.759 mm (standard deviation: 0.17) for men and 0.179mm (standard deviation: 0.13) for women. An additional limitation of **Study 2** was the impossibility to investigate the progression of atherosclerotic disease or to establish an intima media thickness gradient due to its cross-sectional study design.

The dietary outcomes under scrutiny in **Study 4** are most likely polygenic traits, influenced by multiple single-nucleotide polymorphisms some of which might have low effects that were not captured in our study. Moreover, while the study population was much larger than previous studies looking at associations between sugar consumption and genetic variants (43-53), we cannot rule out that it still might not have been large enough to identify single-nucleotide polymorphisms with smaller effect sizes in our study.

An additional limitation of the data is the lack of information on artificially sweetened beverages and on the consumption of non-caloric sweeteners. This information could be crucial when exploring the effects of sweet-taste preference and consumption independently form caloric intake. Lastly, even with all the measures taken in order to avoid biases, errors, and confounding; residual confounding cannot be ruled out completely.

Public health implications

Nutritional recommendations

Cardiovascular disease is still one of the leading causes of death and morbidity worldwide (31). Therefore, understanding the role that sugar consumption plays in the development of cardiovascular and other diseases is crucial in order to set accurate and reliable nutritional recommendations. Until now, nutritional recommendations have based their suggestions for sugar intake primarily on the

basis of three outcomes: excess energy intake, dental caries, and nutrient displacement (14). However, the European Food and Safety Authority has set out to explore disease outcomes beyond these, including cardiovascular disease among others (4).

The need for a unified front in terms of nutritional recommendations, goes beyond the outcomes potentially prompted by a high sugar consumption. The types of sugar considered and where to set the upper intake level are still being debated within the scientific community. There is a disagreement on whether the focus should be placed on added sugars because they are not naturally present in foods and beverages, or in free sugars because they are more readily available. Likewise, various thresholds have been recommended (primarily, <10%E, and <5%E) but no global consensus has been reached (4, 10-13). It seems, however, that the main source of these disagreement has been the lack of undeniable evidence as to where and how to establish those thresholds of consumption.

In the latest European Food and Safety consultation on dietary sugars, the panel of experts agreed that there is enough evidence confirming the association between sugar intake and several disease outcomes, including obesity, liver disease, type 2 diabetes, and cardiovascular disease among others. However, it was not possible for them to establish a tolerable upper intake level (i.e. the maximum amount of a nutrient than can be consumed over a long period of time without health consequences) or even a safe level of intake for dietary sugars. This is because the association between sugar intake and disease seems to be linear, meaning the higher the intake of sugars, the higher the risk of disease. Therefore, their recommendation is to reduce the consumption of dietary sugars to the minimum possible, in the context of a healthy and nutritionally adequate diet (4).

However, more research is needed in order to inform future nutritional recommendations regarding outcomes not covered in the European Food and Safety Authority's consultation.

Policies regulating sugar consumption

As the concern for the pernicious health effects linked to a high sugar consumption rises, so does the need for policies to regulate sugar intake. The World Health Organisation issued a report in 2017 (131) highlighting the need to reduce sugar intake and the importance of using policy to do so. The report addresses six interconnected insights into this issue: 1) the need to create disincentives for manufacturers and retailers to add sugar to manufactured foods and drinks, 2) the need to level the playing field between industry and public health stakeholders with the use of policies, 3) the need to reduce the public's demand for sugary products, 4) the need to encourage substitution of sugar-dense products for healthier options, 5) the need to oversee pernicious substitution patterns, and 6) the need, on the long

run, to promote reformulation strategies to be able to transform the sugar-coated environment. The report also mentions existing measures such as clearer labelling, portion reduction, reformulation strategies, product placement, marketing, packaging, and advertising, among others. However, a special mention should be granted to the introduction of fiscal measures, such as sugar taxation.

In the same year, the World Health Organisation also issued a separate document explaining why it is important to establish and promote the use of fiscal measures to reduce sugar consumption (136). Among others, the document highlights the effectiveness of taxation in the reduction of the consumption of sugar-sweetened beverages, the monetary savings for healthcare systems, the benefits derived from the reinvestment of the collected revenue into public health strategies, and the greater effect of said policies in low-income consumers and at-risk groups. Sugar taxes, up to date, have been introduced in more than 50 countries. However, Sweden is still debating whether this would be a viable policy for its population (137).

Industry involvement

The food, sugar, and beverage industries have also played a role in the development of our current sugar-coated environment. Food marketing has been identified as one of the main influences on individual food choice and preference (138).

The most frequently marketed food categories fall under the umbrella of what is considered unhealthy. That is, fast food, sugar-sweetened beverages, chocolate and confectionery, salty snacks, sweet baked goods and snacks, breakfast cereals, dairy products and desserts. Companies usually make use of tactics that include celebrity endorsements, competitions, games, gifts, and many more that make it attractive particularly to children. Another strategy used is the inclusion of misleading health claims (“enriched with vitamins and minerals” , or “low in fat”) in the packaging to trick consumers into believing they are purchasing healthier products. Such aggressive marketing strategies influence the public’s beliefs and attitudes towards food, nutrition, and health. As a result, this influence exerted by heavy marketing of unhealthy products can prompt changes in food behaviours and ultimately have serious repercussions in the health of children and adults alike (138).

A separate matter is the influence of the industry in the scientific field. While we have no reason to believe that all researchers with private funding from the food industry would present biased or inaccurate results. Industry-funded research is far more likely to come to conclusions that are favourable for the industry sponsoring said research. This occurs with such staggeringly high frequency, that it is difficult to not develop a suspicious attitude towards industry influence, particularly in the field of nutrition research. For instance, a beverage company exerted extreme influence on shaping obesity policies in order to create a favourable environment for the sales of their products (139).

Conclusion

This doctoral thesis examined the role that sugar intake can play in the development of cardiovascular disease, within the context of informing nutritional recommendations. To do so, four studies have been performed in a sample of the adult Swedish population, obtaining the following results:

1. The association between added sugar intake and the intake of nine micronutrients was found to be linear and inverse. In other words, a higher intake of added sugar was linked with a lower intake of micronutrients. Similar trends were found in two samples of Swedish adults, which points towards the existence of micronutrient dilution for over two decades.
2. No associations were found between intima media thickness measured at the carotids and the intakes of several types of sugars or with the intakes of sugar-rich foods and beverages. However, a tendency to a higher intima media thickness measured at the common carotid artery was found for participants with the highest sugar intake.
3. A weak protective association against stroke risk was found for participants adhering to healthy dietary patterns, whether they were following the Swedish dietary guidelines or a Mediterranean-like diet. Adhering to the Mediterranean diet recommendations for soda intake (<7 servings/week) was also associated with a protective effect against total and ischaemic stroke. However, adhering to the Mediterranean diet recommendations for sweets and pastries (<3 servings/week) was associated with a higher risk of total and ischaemic stroke.
4. Three single-nucleotide polymorphisms (rs838145, rs838133, and rs8102840) in close proximity to the *fibroblast growth factor 21* gene in chromosome 19 were significantly associated with the intakes of total sugar, added sugar, and sugars with sweet taste.

In summary, this thesis has encountered significant associations between sugar intake and both micronutrient intake and genetic variants. However, the evidence linking sugar consumption with cardiovascular disease development seems less conclusive and requires further study.

Future Perspectives

As technology and research tools continue to evolve, we might be able to access new ways to measure and explore diet as an exposure in the future. The development of more user-friendly methods to collect dietary data (such as online platforms, remote testing, and the use of apps) will help participation rates and the possibility to make multiple measurements of exposures, outcomes, and covariates during the follow-up period. This might also facilitate the possibility to conduct longer randomised controlled trials to explore causal links between sugar consumption and disease development.

The emergence of new fields including the study of the gut microbiome, proteomics, and metabolomics, as well as genetics might open up the possibility to identify objective biomarkers for sugar intake. The discovery of novel genetic markers associated with sugar consumption would also allow for the use of Mendelian randomisation studies to explore associations with disease. In these studies, the participants are classified based on a specific genetic factor that is used as a proxy for exposure (in our case, sugar consumption). Thus, the findings of Mendelian randomisation studies offer stronger causal claims than conventional observational studies (40).

The field of personalised nutrition has also been gaining interest from the scientific community and the general public alike. This field steers away from the "one size fits all" approach of our current nutritional recommendations and promotes instead the use of more individualised advice. Understanding inter-individual differences in relation to nutrition and health might prove crucial in the design of nutritional recommendations in the future.

Current policies aiming to curb sugar consumption, like sugar taxes, have focused their efforts into taxing mostly caloric sweeteners. However, it is paramount to fully understand the repercussions of the substitution patterns that these policies might be triggering. Therefore, further insight into the health effects of non-caloric and artificial sweeteners is still needed. Furthermore, stricter policies to regulate aggressive marketing strategies and misleading labelling of foods and beverages with a high content of sugar will also be needed in order to improve adherence to healthier dietary habits.

Popular science summary

Living in a sugar-coated world

We are presently living in a sugar-coated environment. Nowadays, sugar is easily accessible, cheap, and present in up to three out of every four products that we can buy in supermarkets. As a result, the individual consumption of sugar worldwide has increased fifty-fold over the past 200 years. Just as sugar intake has increased, so has the concern about how it might impact our health. While the connection between overall diet and disease has been explored widely, there are still unanswered questions regarding sugar consumption specifically.

What is sugar?

We tend to interchangeably use the terms carbohydrates and sugar, but this is a common misconception. While all sugars are carbohydrates, not all carbohydrates are sugars. Sugar represents the simplest form of carbohydrates, consisting only of one or two molecules. They are soluble in water and are present (naturally or added) in foods and drinks granting them their sweet taste. However, sugars can also be used as part of other processes, such as preservation (like in jams) or fermentation (like in wine). Sugars are naturally present in fruits, vegetables, or milk; and are usually added to pastries, sweets, or sodas, among others.

Which sugars should we be worried about?

Dietary sugars are usually classified into total sugars, free sugars, and added sugars. Added sugars are those simple carbohydrates that are not naturally present in foods and beverages. These sugars have been, as the name indicates, added to foods during processing, manufacturing, cooking, or consumption. The definition of free sugars includes all added sugars plus sugars naturally present in honeys and syrups, as well as in fruit and vegetable juices and juice concentrates. Lastly, total sugars is a term used to refer to all sugars contained in the diet, whether they are added, free, or present in whole fruits and vegetables, or in dairy products.

Sugars consumed in either added or free form are generally considered more harmful for our health. Added sugars are not naturally present in foods and beverages so they only add to the caloric intake without any additional value. While the free sugars from, for instance, natural juices might still provide vitamins and

minerals, these have less fibre and nutritional benefits than consuming the fruit whole.

Moreover, sugars that are consumed in liquid form present two additional issues. First, they do not make us feel as full, so we end up consuming more than we really need, adding to our overall caloric intake. Second, as they do not require digestion, they are more easily absorbed causing more acute effects in the body.

How much sugar should I eat?

Answering this question is more difficult than one might think. Even the scientists behind national nutritional recommendations (such as the Nordic Nutrition Recommendations, the Dietary Guidelines for Americans, and Public Health England, to name a few) and international organisations (such as the World Health Organisation) have not managed to reach consensus. To this day, there is still a disagreement as to which type of sugar to focus on (added or free), where to set up the threshold for safe consumption, or which health outcomes to use as a basis for recommendations.

As a result, the European Food and Safety Authority set out to investigate sugar consumption in relation to pregnancy-related conditions, dental caries, and chronic metabolic disease, which include pathologies of the heart and the blood vessels (cardiovascular disease), diabetes, and obesity, among others. The European Food and Safety Authority concluded that while there is in fact an association between sugar consumption and the development of chronic metabolic diseases, there is not enough scientific evidence to establish a safe consumption threshold, particularly when related to cardiovascular disease. Nonetheless, their overall recommendation is to reduce our consumption of added and free sugars to a minimum within the limits of a nutritionally adequate diet.

Let them eat cake?

My research interest over the past few years focused on increasing the amount of evidence exploring the link between sugar consumption and disease development (particularly cardiovascular disease) in the context of nutritional recommendations. Therefore, as part of my doctoral thesis, I have conducted four studies on sugar consumption, with different factors connected to disease development in a large sample of the adult Swedish population.

For years, many nutritional recommendations for sugar intake used the risk of causing micronutrient dilution as a basis for their advice. Micronutrient dilution is characterised by a decreased consumption of nutrient-dense foods (like fruits and vegetables) due to an increased consumption of energy-dense foods (like processed snacks and sodas), which are high in fats and sugars and low in vitamins and minerals. My first study confirmed that the higher the intake of added sugars in the

participants, the more likely they were to have a low intake of vitamins and minerals. Found in two independent samples of Swedish adults, these results pointed towards the continued existence of micronutrient dilution for over two decades.

Cardiovascular disease remains one of the greatest causes of disease and mortality worldwide. Therefore, understanding the dietary risk factors for cardiovascular outcomes is important in order to establish nutritional recommendations. Many cardiovascular diseases evolve from a phenomenon called atherosclerosis. Atherosclerosis is a process by which fatty materials accumulate on the walls of the arteries reducing their diameter, which decreases blood flow to organs. This accumulation can also form plaques that can dislodge and cause other pathologies.

My second study, investigated the association between several forms of sugar consumption and a marker of atherosclerosis called intima media thickness. This marker measures the thickness of the wall of the carotid arteries and can predict the possibility of developing cardiovascular diseases later on in life. However, this study could not find any conclusive link between sugar consumption and intima media thickness. While participants with a higher sugar consumption tended to have thicker artery walls, this finding would need to be studied further to confirm an association.

One of the most dangerous presentations of cardiovascular disease is stroke. Stroke is defined as a loss of brain function due to lack of sufficient blood flow. This lack of flow can occur if there is bleeding (haemorrhagic stroke) or if the blood vessels are blocked (ischaemic stroke). Stroke, like many other cardiovascular diseases, can be influenced by modifiable lifestyle factors, such as diet. My third study, analysed the risk of developing stroke within the context of dietary patterns considered healthy. Two healthy dietary patterns were chosen for such effect: following the Swedish dietary guidelines, and following a Mediterranean-style diet. In this study, a protective effect against stroke was found for individuals who better followed the recommendations of either of these healthy dietary patterns. For sugar in particular, a protective effect against stroke (particularly for ischaemic) was found in individuals consuming less than 200ml of soda per day, as recommended by the Mediterranean diet. Interestingly, a protective effect against stroke was also found for individuals consuming more than the three servings of sweets and pastries per week that the Mediterranean diet recommended. This contradictory finding could be explained by two possible scenarios. First, the deep-rooted Swedish tradition of *fika* (a break from activity where a hot beverage is consumed usually accompanied by a pastry). *Fika* breaks are not necessarily associated to an overall unhealthy diet or other unhealthy behaviours. Plus, the social element involved in this tradition has also proven to have beneficial health effects. Second, the fact that sugars in liquid form (like sodas) have a much more acute effect on our body than those consumed in solid form (like sweets and pastries).

Lately, scientists around the world have started to point towards the need for individualised advice when it comes to nutrition. As a result, the study of genetic markers in nutritional research has been on the rise to help understand the inherent differences between individuals. The first step towards understanding these disparities is to identify genetic variants that can be traced to the intake of specific foods or nutrients. In my fourth study, the association between genetic factors and sugar consumption was investigated. A connection was found between a genetic region in chromosome 19, linked to the *fibroblast growth factor 21* gene, and the intake of sugar. This particular gene is known to code a hormone that has been previously linked to both intake and preference for sweet foods.

Informing nutritional recommendations on a regional, national, or international level is just the tip of the iceberg. Evidence-based dietary guidelines are usually the stepping stone for advising public health policies and strategies aiming to improve the health of millions. All in all, it seems that the study of sugar consumption still presents us more questions than answers. Solving our sugar-coated environment requires a multidisciplinary approach much more complex than what I could have covered during my time as a doctoral student. However, we should not stop wondering: should we have our cake, or should we eat it?

Although my doctoral thesis has been able to establish links between sugar consumption and both micronutrient dilution and genetic factors, the connection between sugar intake and more complex conditions, like cardiovascular disease, grants further study. While the overall scientific evidence is still inconclusive in its details, we should nonetheless aim to follow this simple advice: *eat less sugar, you are sweet enough already*.

Resumen de divulgación científica

Viviendo en un mundo glaseado

Actualmente vivimos en un mundo glaseado, cubierto de azúcar por todos lados. Hoy, el azúcar es una comodidad por su fácil acceso. Es barato y está presente, aproximadamente en casi tres de cada cuatro de los productos que podemos obtener en el supermercado. Como resultado, el consumo global de azúcar se ha visto incrementado por 50 en los dos últimos siglos. Y en la misma medida que el consumo de azúcares ha aumentado, también lo ha hecho nuestra preocupación por los posibles efectos secundarios que esto pueda repercutir en nuestra salud. Mientras la conexión entre nuestra dieta habitual y el desarrollo de enfermedades ha sido estudiado extensamente; en lo que se refiere al consumo de azúcares, aún hay algunas preguntas que debemos resolver.

¿Qué entendemos por azúcar?

De forma general tendemos a usar los términos carbohidratos y azúcar de forma intercambiable. Sin embargo, esto es un malentendido bastante común, porque, aunque todos los azúcares son carbohidratos, no todos los carbohidratos son azúcares. Los azúcares son el tipo de carbohidratos más sencillo, formados por una o dos moléculas, solamente. Los azúcares son solubles en agua y se encuentran, de manera natural o añadida, en comidas y bebidas, confiriéndoles un sabor dulce. Hay que tener en cuenta que, los azúcares, también pueden ser usados como factores en procesos de conservación (como en las mermeladas) y de fermentación (como en el vino). Los azúcares se pueden encontrar de manera natural en frutas, verduras y productos lácteos, y suelen ser añadidos a dulces, pasteles y refrescos, entre otros.

¿Qué tipos de azúcar deberían preocuparnos?

Los azúcares contenidos en la dieta suelen clasificarse en añadidos, libres y totales. Azúcares añadidos son esos carbohidratos sencillos que no están presentes en los alimentos y bebidas de forma natural. Éstos, como su nombre indica, se han añadido a los alimentos durante el procesado, manufacturación, preparación o a la hora de consumirlos. La definición de azúcares libres incluye todos los azúcares añadidos además de los azúcares naturalmente presentes en miel y siropes, y en zumos y concentrados de zumo de frutas y verduras. Por último, azúcar total es un término empleado para referirse a todos los azúcares de la dieta, ya sean añadidos, libres, o presentes en frutas, verduras y productos lácteos.

Por normal general, los azúcares consumidos en forma de azúcares añadidos o libres tienden a considerarse más perjudiciales para la salud. Los azúcares añadidos no se encuentran en los alimentos y bebidas de forma natural, por lo tanto sólo añaden aporte calórico sin ningún otro valor nutricional. Aunque los azúcares libres presentes en, por ejemplo, zumos de frutas nos aportan una fuente de vitaminas y minerales, éstos contienen menos fibra y beneficios nutricionales que consumir la fruta entera.

Además, los azúcares consumidos en forma líquida presentan dos problemas adicionales. Primero, no nos hacen sentir saciados con lo que consumimos más de lo que realmente necesitamos, añadiendo a nuestra ingesta calórica total. Segundo, al no necesitar de digestión, son absorbidos más fácilmente provocando efectos más agudos en nuestro organismo.

¿Cuánto azúcar debo consumir?

La respuesta a esta pregunta es más difícil de lo que uno pueda pensar. Ni siquiera los científicos encargados de diseñar los consejos nutricionales a nivel nacional (como por ejemplo: las Recomendaciones Nutricionales para los países Nórdicos, las Guías Alimentarias para Norteamericanos, o las Guías del Instituto de Salud Pública del Reino Unido, por nombrar algunas); ni los encargados de diseñarlas a nivel internacional (como la Organización Mundial de la Salud) han sido capaces de llegar a un consenso. Hoy en día, no hay un acuerdo sobre qué tipo de azúcar debe ser especificado (añadido o libre), dónde establecer el límite de consumo, o qué enfermedades usar como base para establecer nuevas guías alimentarias.

Como resultado la Autoridad Europea de Seguridad Alimentaria (EFSA) ha investigado posibles conexiones entre el consumo de azúcares y el desarrollo de condiciones asociadas al embarazo, la caries dental y las enfermedades metabólicas crónicas, que incluyen patologías del corazón y los vasos sanguíneos (enfermedades cardiovasculares), diabetes y obesidad, entre otras. La EFSA concluyó que aunque existe una asociación entre el consumo de azúcares y el desarrollo de enfermedades metabólicas crónicas, no hay suficiente evidencia científica para establecer un límite de consumo seguro de azúcares. Aún así, su recomendación general es reducir al mínimo posible el consumo de azúcares añadidos y libres, dentro de los límites de una dieta adecuada nutricionalmente.

¿Dejadlos que coman pasteles?

El interés principal de mi proyecto de investigación durante los últimos años ha estado centrado en añadir, a la evidencia existente explorando la conexión entre el consumo de azúcares y el desarrollo de enfermedades (sobre todo cardiovasculares), en el contexto de las guías alimentarias. Por lo tanto, como parte de mi tesis doctoral, he llevado a cabo cuatro estudios sobre el consumo de azúcares y diferentes factores

conectados al desarrollo de enfermedades en una muestra de gran tamaño de la población adulta en Suecia.

Durante años, muchas guías alimentarias utilizaron el riesgo de dilución de micronutrientes como base para sus consejos. La dilución de micronutrientes es un fenómeno caracterizado por la disminución del consumo de alimentos nutritivos, como las frutas y las verduras, debido a un exceso de consumo de alimentos altos en energía, como los snacks procesados y los refrescos, que poseen un alto contenido en grasas y azúcares y bajo contenido en vitaminas y minerales. Mi primer estudio, confirmó que cuanto más alto era el consumo de azúcar en los participantes del estudio, mayor era la probabilidad de que el consumo de vitaminas y minerales fuera bajo. Estos resultados, encontrados en dos muestras independientes de adultos en Suecia, apuntan al mantenimiento de esta tendencia a la dilución de micronutrientes durante más de dos décadas.

Las enfermedades cardiovasculares siguen siendo una de las principales causas de enfermedad y muerte en la población a nivel mundial. Por lo tanto, es importante entender los factores dietéticos asociados con riesgo cardiovascular para poder informar guías alimentarias. Muchas enfermedades cardiovasculares son producto de la evolución de un fenómeno conocido como aterosclerosis. La aterosclerosis es un proceso por el cual materiales grasos se acumulan en las paredes de las arterias reduciendo su diámetro (lo cual reduce el flujo sanguíneo a los órganos) y formando placas (que pueden desprenderse causando otras patologías).

Mi segundo estudio investigó la asociación entre el consumo de distintos tipos de azúcares y un marcador de aterosclerosis llamado grosor de la íntima-media carotídea. Este marcador mide el espesor de la pared arterial de las arterias carótidas y puede predecir la posibilidad de desarrollar enfermedades cardiovasculares en el futuro. Sin embargo, este estudio no fue capaz de encontrar una asociación concluyente entre ninguna de las formas de consumo de azúcares y el grosor de la íntima-media carotídea. Aunque los participantes con un mayor consumo de azúcares presentaban una tendencia a tener paredes arteriales más gruesas, este hallazgo debe ser estudiado con más detalle para poder confirmar una asociación.

Uno de los cuadros clínicos más peligrosos de enfermedad cardiovascular es el accidente cerebrovascular, el cual se define como una pérdida de función cerebral producida por un insuficiente flujo sanguíneo. Esta pérdida de flujo sanguíneo puede ser el producto de una hemorragia (derrame cerebral) o de un bloqueo de los vasos sanguíneos (accidente cerebrovascular isquémico). Los accidentes cerebrovasculares, como muchas otras enfermedades vasculares, están influenciados por estilos de vida modificables, incluyendo los hábitos alimenticios. Mi tercer estudio, analizó el riesgo de desarrollar un accidente cerebrovascular en el contexto de patrones alimentarios saludables. Dos patrones alimentarios saludables fueron elegidos a tal efecto: el seguimiento de las guías alimentarias suecas y seguir una dieta estilo Mediterránea. En este estudio, encontramos un

efecto preventivo contra todos los tipos de accidente cerebrovascular en los individuos con mayor adherencia a cualquiera de los dos patrones alimentarios saludables. En el caso del consumo de azúcares, este efecto preventivo se encontraba (sobre todo para accidente cerebrovascular isquémico) en los participantes que, siguiendo las recomendaciones de la dieta Mediterránea, consumían menos de 200 ml diarios de refrescos. Curiosamente, un efecto preventivo también fue encontrado para participantes que consumían más de las tres porciones semanales de dulces y repostería que recomienda la dieta Mediterránea. Este resultado contradictorio, puede ser explicado por dos posibles fenómenos. Primero, la arraigada tradición sueca de la *fika* (una pausa social en la actividad diaria para el consumo de una bebida caliente normalmente acompañada de repostería). La *fika* sueca no suele asociarse con una dieta generalmente poco saludable ni con otros comportamientos poco saludables. Además la naturaleza social de este fenómeno también tiene beneficios comprobados para la salud. Segundo, el hecho de que los azúcares consumidos en forma líquida (como en los refrescos) tiene un efecto más agudo en nuestro organismo que los consumidos en forma sólida (como los dulces y pasteles).

Recientemente, científicos por todo el mundo han empezado a apuntar a la necesidad de conceder un consejo nutricional más individualizado. Como resultado, el estudio de marcadores genéticos en investigación nutricional se ha elevado como una de las maneras de entender las diferencias inherentes entre individuos. Los primeros pasos para la comprensión de estos mecanismos pasan por la identificación de variantes genéticas que puedan ser trazadas al consumo de alimentos o nutrientes específicos. En mi cuarto estudio, investigamos la asociación entre marcadores genéticos y el consumo de azúcares. Como resultado, encontramos una conexión entre una región genética en el cromosoma 19, asociada al gen del *factor de crecimiento fibroblástico 21*, y el consumo de azúcares. Este gen en particular es conocido por codificar una hormona que se ha asociado previamente tanto con el consumo como la preferencia por los productos azucarados.

Informar guías alimentarias a nivel regional, nacional o internacional es solo la punta del iceberg. Las guías alimentarias basadas en la evidencia son el primer paso para aconsejar políticas y estrategias de Salud Pública que pueden mejorar la salud de millones. En resumen, parece que el estudio del consumo de azúcares aún nos plantea más preguntas que respuestas. Y aunque el problema de resolver nuestro entorno glaseado requiere un abordaje multidisciplinar mucho más complejo de lo que podría abarcar durante mis estudios doctorales, no deberíamos dejar de preguntarnos si sería conveniente o no quitarle el caramelo al niño.

Siendo mi tesis doctoral capaz de corroborar una conexión entre el consumo de azúcares tanto con la dilución de micronutrientes como con factores genéticos, la conexión entre el consumo de azúcares y condiciones complejas, como la enfermedad cardiovascular, requiere más investigación. Y aunque la evidencia científica generalizada aún es poco concluyente en cuanto a los detalles; no haremos mal si seguimos este simple consejo: "*come menos azúcar, ya eres lo bastante dulce*".

Populärvetenskaplig sammanfattning

Att leva i en söt värld

Vi lever i en söt värld. Nuförtiden är socker lättillgängligt, billigt och finns i upp till tre av fyra produkter som kan köpas i mataffärer. Som ett resultat har konsumtionen av socker över hela världen ökat femtiofaldigt de senaste 200 åren. I takt med att sockerintaget har ökat har även vår oro för hur det påverkar vår hälsa ökat. Trots att sambanden mellan kost i allmänhet och kostrelaterade sjukdomar har utforskats brett, finns fortfarande många obesvarade frågor om just sockerkonsumtion. De första frågorna som vi måste ställa oss är: Vad är socker? Vilka sockerarter bör vi vara orolig för? Och hur mycket kan vi äta utan att bli sjuka?

Vad är socker?

Att kolhydrater och socker är samma sak är en vanlig missuppfattning. Trots att alla socker är kolhydrater, är inte alla kolhydrater socker. Socker utgör den enklaste formen av kolhydrater, och består av en eller två molekyler. Det är vattenlösligt och finns (naturligt eller tillsatt) i livsmedel och drycker och ger dem en söt smak. Socker kan också användas i konserverings- eller jäsningsprocesser. Dessa enkla kolhydrater, eller sockerarter, finns i bland annat frukt och grönsaker, bakverk och godis, läsk och mjölk. Men alla sockerarter har inte samma effekt på vår hälsa, och mycket beror på hur vi äter dem.

Vilka sockerarter bör vi vara oroliga för?

Socker klassificeras vanligtvis i totalt socker, fritt socker och tillsatt socker. Tillsatt socker är de sockerarter som inte finns naturligt i mat och dryck. Dessa sockerarter har, som namnet indikerar, tillsatts vid tillverkning av livsmedel eller till den färdiga maten. Definitionen för fritt socker inkluderar alla tillsatta sockerarter samt sockerarter som finns i honung och sirap, såväl som naturligt förekommande sockerarter i frukt och grönsaker som gjorts om till juice eller juicekoncentrat. Slutligen syftar termen totala sockerarter på alla sockerarter som finns i kosten, oavsett om de är tillsatta, fria eller finns naturligt förekommande i till exempel frukt, grönsaker eller mejeriprodukter. Socker som konsumeras i antingen tillsatt eller fri

form anses generellt vara mer skadligt för vår hälsa i och med att de vanligtvis inte kommer tillsammans med kostfibrer eller näringsämnen.

Hur mycket socker kan vi äta utan att bli sjuka?

Att svara på denna fråga är svårare än man kan tro. Så pass svårt att inte ens forskarna bakom nationella eller regionala näringsrekommendationer (som näringsrekommendationerna i Norden, USA, och England) och internationella organisationer (som Världshälsoorganisationen) har lyckats nå konsensus. Det råder även oenighet om vilken typ av socker man ska fokusera på (tillsatt eller fritt), var man ska sätta gränsen (10% eller 5% av energiintaget) eller vilka hälsoaspekter som ska ligga till grund för rekommendationerna.

Som ett resultat av detta beslöt sig den europeiska myndigheten för livsmedels säkerhet för att undersöka saken närmare och gå igenom all genomförd forskning på sockerkonsumtion i relation till metabola sjukdomar (inklusive hjärt- och kärlsjukdomar, typ 2-diabetes och fetma), graviditetsrelaterade tillstånd, och karies. De fann relativt tydliga samband mellan intag av tillsatt socker och fetma och försämrade blodfetter, men kunde dessvärre inte dra några definitiva slutsatser för hjärt-kärlsjukdom. Trots att det finns stöd för ett faktiskt samband mellan sockerkonsumtion och utveckling av vissa metabola sjukdomar kunde deras granskning inte fastställa en säker övre gräns av sockerintag. Deras rekommendation är att vi bör hålla vår konsumtion av tillsatt och fritt socker så låg som möjligt.

Låt dem äta tårta?

Mitt forskningsintresse under de senaste åren har fokuserats på att öka mängden evidens kring sambandet mellan sockerkonsumtion och sjukdomsutveckling (särskilt hjärt-kärlsjukdom). Därför har jag som en del av min doktorsavhandling genomfört fyra studier som undersöker sambandet mellan sockerkonsumtion och riskfaktorer kopplade till sjukdomsutveckling i ett stort urval av den vuxna svenska befolkningen.

I många år har flera näringsrekommendationer för sockerintag baserats på risken för utspädning av mikronäringsämnen (dvs vitaminer och mineraler) i kosten. Utspädning av mikronäringsämnen i kosten är ett fenomen där det finns en minskad konsumtion av näringstäta livsmedel (som frukt och grönsaker) på grund av en ökad konsumtion av energitäta livsmedel (som chips och sötade drycker) som innehåller mycket fett och socker men inte så mycket vitaminer och mineraler. Min första studie bekräftade att ju högre intag av tillsatt socker var hos våra deltagare, desto mer sannolikt var det att de hade ett lågt intag av vitaminer och mineraler.

Hjärt-kärlsjukdom är ett samlingsbegrepp för sjukdomar i hjärta och blodkärl (som hjärtinfarkt och stroke) och är den vanligaste dödsorsaken i världen. Det är viktigt att undersöka vilka kostfaktorer som påverkar risken att drabbas av dessa sjukdomar

och utifrån det sätta näringsrekommendationer. Många hjärt-kärlsjukdomar utvecklas till följd av ateroskleros. Ateroskleros, eller åderförkalkning, är en process där fett ansamlas på blodkärlens väggar. Detta gör att blodkärlens diameter minskar och i sin tur blodflödet till organen. De plack som bildas i blodkärlens väggar kan lossna och orsaka hjärt-kärlsjukdomar.

Min andra studie syftade till att utforska sambandet mellan flera former av sockerkonsumtion och en markör för åderförkalkning som kallas intima-media tjocklek. Markören mäter tjockleken på väggen av halspulsådern och kan förutsäga risken att utveckla hjärt-kärlsjukdom senare i livet. Denna studie kunde dock inte hitta någon signifikant koppling mellan sockerkonsumtion och intima-media tjocklek. Även om det verkade finnas en trend för tjockare artärväggar hos de individer som konsumerade mest socker, skulle dessa resultat behöva utforskas i större studier eller hos mindre friska individer för att bekräfta sambandet.

En av de allvarligaste hjärt-kärlsjukdomarna är stroke (slaganfall). Stroke kännetecknas av en förlust av funktioner i hjärnan till följd av otillräckligt blodflöde. Otillräckligt blodflöde kan uppstå på grund av blödning (hemorragisk stroke) eller på grund av blockerade blodkärl (ischemisk stroke). Stroke, liksom många andra hjärt-kärlsjukdomar, kan påverkas av livsstilsfaktorer såsom kost. Min tredje studie syftade till att utforska hur hälsosamma kostmönster påverkar risken att utveckla stroke. Två hälsosamma kostmönster valdes ut i denna studie: att följa de svenska kostråden och att följa en medelhavskost. Båda dessa kostmönster inkluderar en rekommendation att konsumera mindre socker och sockerrika livsmedel och drycker. I denna studie fann vi en skyddande effekt mot stroke för de individer som följer någon av dessa hälsosamma kostmönster. Vi fann även en skyddande effekt mot stroke hos individer som höll sig till rekommendationerna om att konsumera mindre än ett glas läsk per dag. Intressant nog hittades en skyddande effekt mot stroke även för individer som konsumerade mer godis och bakverk än vad som rekommenderas i en medelhavskost. I vår svenska befolkning skulle detta motsägelsefulla fynd kunna förklaras av den djupt rotade traditionen av fika. Fikapauser är inte nödvändigtvis förknippade med en allmänt ohälsosam kost eller andra ohälsosamma beteenden och kan förklara varför en högre konsumtion av bakverk inte nödvändigtvis är förknippad med högre risk för stroke hos svenska individer.

På senare tid har forskare runt om i världen börjat peka på behovet av individuella råd när det gäller kost. Som ett resultat har studierna av genetiska markörer inom näringsforskning ökat för att hjälpa till att förstå de biologiska skillnaderna mellan individer. Det första steget mot att förstå dessa mekanismer är att identifiera genetiska varianter som kan spåras till intag av specifika livsmedel eller näringsämnen. I min fjärde studie undersöktes sambandet mellan genetiska faktorer och sockerkonsumtion. Som ett resultat fann man ett samband mellan en genetisk

variation i *FGF21*-genen på kromosom 19 och intaget av socker. Denna speciella gen kodar för ett hormon som tidigare har kopplats till både intag och preferens för söt mat.

Att komma fram till näringsrekommendationer är bara toppen av isberget. Evidensbaserade kostråd är vanligtvis språngbrädan för att ge råd om strategier som syftar till att förbättra hälsan för miljoner människor. Sammantaget verkar det som att studier av sockerkonsumtion fortfarande ger oss fler frågor än svar. Och trots att utmaningen med att förstå sockerintag och dess effekter är mycket mer komplex än vad jag kunde ha föreställt mig under min tid som doktorand, bör vi inte sluta undra: ska vi behålla vår tårta, eller ska vi äta den?

Även om min doktorsavhandling har kunnat fastställa samband mellan sockerkonsumtion och både intag av vitaminer och mineraler och genetiska faktorer, skulle sambandet mellan sockerintag och mer komplexa tillstånd, som hjärt-kärlsjukdomar, behöva ytterligare studier. Vetenskapliga studier över hela världen verkar dock peka på följande slutsats: *ät mindre socker, vi är redan söta nog*.

Acknowledgements

> "It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of light, it was the season of darkness, it was the spring of hope, it was the winter of despair."
>
> *Charles Dickens, A Tale of Two Cities*

A journey of 1,300 days feels short when accompanied by amazing, inspiring, caring, loving, adventurous, supportive, crazy, weird, magnificent people. I am lucky enough to have been surrounded by many of such individuals throughout the past three and a half years. A simple "thank you" doesn't quite cover the amount of gratitude I have for each and every one of you so, here comes the long version. Brace yourselves! It is bound to be a long journey… and it is in Spanglish!

The Family

Primero que nada, quiero, no sólo agradecer, sino también dedicar este libro a mi familia. Esta tesis doctoral es la culminación de años de estudio, esfuerzo, sacrificio, sudor y lágrimas, y nada de esto habría sido posible sin el apoyo y cariño constante de mis seres más queridos:

Mis padres, **Julián** y **Manoli**, que me inculcaron la importancia de nunca dejar de aprender cosas nuevas y me ayudaron a comprender que con empeño, constancia y trabajo duro podía conseguir lo que me propusiera. Y ¡vaya si me he propuesto cosas… y las he conseguido! Gracias por enseñarme a ser amable, bondadosa y paciente, pero sobre todo a ser perseverante y a no olvidarme de mis metas. Gracias por el cariño y el apoyo, y por dejarme surcar mi propio camino, aunque fueran estelas en el mar. Gracias por confiar en que a través del afecto y la educación que me han dado sabría llegar a buen puerto. ¡Los quiero infinito! Y este libro, más que para ninguna otra persona, es para ustedes.

Mis abuelos, **Chanita 'Luna'**, **Amalia**, **Miguel** y **José**, aunque ninguno de ellos podrá leer este libro, porque son estas cuatro personas los pilares sobre los que he construido el futuro que ahora se me presenta. Y es por ellos y para ellos que me he convertido en la persona que soy. Gracias… por todo.

A mi tía **Yayo**, mi tío **Pepe 'Jean Claude'** y mi prima **Ana**. Porque nunca dudaron en acogerme como una más y brindarme techo y cobijo cada vez que necesité un retiro junto al mar con un excelente servicio gourmet.

No woman is an island and my family would not be complete without mentioning my three fierce sisters (from other misters):

Noe, mi primera (y única) wifey. ¿Qué persona pseudo-cuerda no querría tenerte como amiga? Gracias por las aventuras, los viajes, las charlas, las botellas de vino compartidas. Gracias por los consejos, los bailoteos y los poemas de amanecida. Gracias por caminar conmigo, por apoyarme, escucharme, entenderme (a veces) y aceptarme (siempre). Gracias por empujarme a seguir mi corazón más allá de la cordura y vivir siempre *Sin miedo al olvido.* Te quiero con locura. ¿Empezamos ya a planear la siguiente locura?

Joana, the yin to my yan, the reason to my madness, the ultimate companion, and the best housemate! Life would have been so dull without you in it. Thank you for the fried camembert and the trashing sessions at Malmöfestivalen, for the nights of pizza and rosé in the balcony, for inventing the *tablecloth or curtain?* game, for all the memories we have shared, and the ones that are still ahead of us. Thank you for being the rock and the lighthouse that I needed in stormy weather. Words cannot express how much I love, admire, and respect you as a scientist, as a friend, as a mentor, as a colleague, as a flatmate, as a travel companion… You will forever be my first 'hubby'! Thanks for joining me in the madness of this journey and for screaming with me through the ups and downs of this rollercoaster ride that is getting a PhD.

Iram, my sweet neighbourino. One could not wish for a kinder neighbour or a better friend than you. I am constantly inspired by your wisdom, your kindness, and your resilience. I am truly blessed to count you amongst my Friends (with capital F). Every moment I share with you instantly becomes a precious memory that I will cherish forever. Here's to many more dinner parties, brunches, walkies in the park, Netflix binges, and to always having a safe space to vent whatever is clouding our minds. Thanks for your friendship, your guidance, and support. I love you millions.

The scientists

It takes a (scientific) village to raise a researcher child. So, as I sit here ready to release my scientific toddler (after almost four years, I can hardly call it a 'baby' anymore) into the world, I want to thank the "villagers" that helped me get to this point:

It has been an honour and a privilege to have been guided into PhD delivery by not one but three amazing female scientists. My supervisor, **Emily Sonestedt**, thank you for taking a chance on me and for giving me the opportunity to continue to play with science. I have learned so much through this process, I will be forever grateful for your support and guidance. My co-supervisors, **Yan Borné** and **Emma**

Ahlqvist, thank you for all your help and input, and for coming to the rescue when I was in need of epidemiological advice.

To all current and former members of the *Team Carbs*. But especially to **Suzanne Janzi**, **Mary Farrell**, **Kjell Olsson**, **Stina Ramne**, and **Anna Stubbendorf**. The 'sweetest' band of colleagues and fellow lovers of sugary research! It's been great to share with you wonderful science, PowerPoint tricks, contraband chocolate, post-lunch walks, conference dancing, online fikas, and a never-ending supply of sugary puns! Thank you for sharing this journey with me.

Suneela Zaigham, thank you for always putting a smile on my face with your beautiful accent and sunny disposition. I look forward to more red-lip dinners and more conversations about science, life, or whatever you want... truly, I just want to hear you talk.

Stanley Ulijaszek, thank you for making my childhood dream come true. My experience at University of Oxford would have never been possible without your kindness and your guidance. I am so glad that I got to sit at the Magic Table and learn from you, and share stories, cupcakes, and cheese platters. Thank you for helping me find a home away from home at the UBVO. I could say thank you for a million years, and it would still not quite cover how grateful I am for your support.

Paul Franks, COVID winters in Sweden would have been a lot duller without our walk & talks. Thanks a million for all your support, your guidance, your crazy near-death stories, the game nights with the GAME unit, the picnics in nature… But most of all, thank you for your friendship through thick and thin (and lockdown).

Karen Watson, it is not often that you get to meet the giants. Thank you for taking me under your wing and for teaching me how to walk the line towards creating demand for health.

Angelo Modica, thank you for helping me fight tunnel vision and allowing me to see the bright future ahead by helping me step out of the box.

To all my corridor neighbours: **Louise Brunkwall** (thank you for your help in the halftime), **Sophie Hellstrand** (thank you for always wearing a smile to the office), **Ulrika Ericsson**, **Stanley Teleka**, **Tanja Stocks**, **Marju Orho-Mellander**, and many, many more… thank you for the shared moments, for your valuable expertise, for a friendly chit-chat, for the fun times at the retreats… You have all been part of this journey with me too. Also, a special thanks to **Gunilla Hughes Wulkan**, for organising all those Friday breakfasts.

A mis 'vitaminitas' (**Sabrina García-Santana**, **Ana Mirallave-Pescador**, **Marival Groba-Marco** y **Elisa González-Rodríguez**) y a **Manuel Sosa** y **José Sarmiento** de la Unidad Metabólica Ósea. Ustedes fueron testigos de mis primeros pasos en investigación y miren hasta dónde me han llevado.

A mis compis del Grupo de Nutrición del IUIBS: **Lluís Serra-Majem**, **Judith Pérez**, **Jacqueline Álvarez Pérez**, **Almudena Sánchez Villegas**, **Cristina Ruano**, **Antonio Rodríguez**, **Tamara Casañas**, **Romi García**, **Adriana Ortiz**, y muchos más que seguro me dejo atrás. Gracias por los buenos ratos pasados charlando sobre ciencia, nutrición y Dieta Mediterránea entre risa y risa.

Lastly, to all my **collaborators** and **co-authors**, and to all the staff and participants of the **Malmö Diet and Cancer Study** and **Riksmaten Adults**.

The extracurriculars

MPHers

The first people that filled my Swedish social agenda were, in fact NOT Swedish. I have to thank you all for your friendship and support through the ups and downs, the lows and highs, the DALYs and the QALYs…

Kasper Frondelius, thank you for always listening except when I told you not to get into medschool, for going to GC more times than I do but always without me (let's fix that soon, shall we?), for sharing walks, glogg (or the idea of it), culinary experiments, and so many wonderful times and memories. I can now reset my clock to keep a countdown to our trip to Osteria Francescana. Love you!

Zainab Babukerkhail, my 'boo' I am so thankful for you, your friendship, your kindness, your wisdom beyond your years, our brunches, and conversations. No matter how far apart we have beem geographically, you will always have a special place in my heart. I love you!

Hugo Fitipaldi & **Camila Freitas**, thank you for the game nights, the shared stories, and for always being there for me, whether to help me with a work project or to teach me how to (try to) not kill my plant. Thank you for always being open to listening to me trash about (insert random topic here) and for finding the way to agree with me so I don't feel crazy. I love you both.

Moe Zogheib (and family), 'babe', thank you for allowing me to spoil your children, for hosting movie nights, for the cheesecakes at Pronto, and for all the pizza nights. I miss having you this side of the pond! Come back already!

Noel Wu & Caro, thank you for adding that little spark of crazy and sweet that every MPH gathering needed. I miss you both.

Hugo Pomares, qué alegría poder compartir conversaciones contigo en español de vez en cuando para asegurarme de que no me he olvidado de mi lengua materna (aunque ahora cuando visito a mis padres hablo con acento mexicano). Memories of Bohemian Rhapsody and Sweet Caroline will stay with me forever now.

And to **Jack Palmieri**, **Keeva Duffey**, **Daniel Coral, Debra Carroll-Beight**, **Tanya Nysted**, and many others, thank you for adding colour and fun to the madness of navigating life in Sweden.

Student organisations: DPLU & MDR

'Why yes, I will join that committee!', 'absolutely, I can help with that event', 'no worries, I am happy to volunteer for that task'. Chances are, if you've met me you know that the little overachiever and ultimate joiner in me has said those things more than once, which leads me to my next categories of friends:

DPLU Young Researcher Network: there is no such thing as too many pizza nights! You wonderful gang! What a treat it has been to have shared with you the madness of event organising with you all whether in-person or online. If I could I would do it all again.

Claire Lyons, you absolute legend! Thank you for sharing the burden of chairing the network, thank you for the initiative, the follow-through, the emails, and especially spam-mail, the pictures of sunsets, the elevator selfies, the ducks, and the brunches… always the brunches! Now that this is over, we can start planning more foodie adventures. Thank you for your friendship.

Klinsmann Carolo, another superstar, so glad to have been able to get to know you and share with you the madness of living in general, and my successes on wordle in particular. Life is better knowing that I can count you among my friends.

Gad Hatem, I am not sure how much you belong in this category, you little ghost of camless-zoom's past! But since we got to become friends through DPLU… I am thankful for you whether you turn your camera on or not. I am happy we got to become friends and talk about anything that worries us or makes us happy. Thank you for always leaving the door of your office open so I could come and gossip whenever I was on my way to printing or refilling my tea.

And to everyone else at the network: **Julia Nilsson**, **Franzi Kopietz**, **Alex Hamilton**, **Alex Karagianoupoulos**, **Sabrina Ruhrmann**, **Joao Da Cunha**, **Tania Singh**… for making the past few years a heck of a lot more fun.

Special thanks to **Karl Bacos** and **Ulrika Blom-Nilsson** and the rest of the DPLU board for giving us a seat at the table and letting us make a contribution that mattered.

The MDR Team: bureaucracy has never been so fun as when surrounded by this bunch. This is high a compliment coming from a Spanish person! (if you get it, you get it… if not, text me. I'll draw you a sketch). Being part of MDR has been one of the most rewarding and enjoyable parts of becoming a PhD. I honestly cannot think of happier times had around a meeting table or at a zoom call talking about "work-ish" things! Getting to know you and work with you has been an absolute privilege.

Shelby Shrigley, thank you for welcoming me into the MDR fold with open arms, and for bringing little fluffy Stevie into my life. Thank you for the advice, the support, the teas and brunches, and for your friendship. I can't wait to see what the future brings us, starting with a trip to Edinburgh.

Kreema James, we became friends before we even met each other in person! What a crazy year to start up a committee, a social media brand, and a newsletter. I cannot think of a better person to share 3-hour long zoom calls fixing Santa hats on MDR pets while we talked about life together. I am truly lucky to count you amongst my friends.

Sakshi Vats, the sweetest things come in small packages. I am happy that the we are in each other's lives and I hope that we will continue to be for many years to come!

Vasiliki Pantazopoulou, I am and will always be in awe of you: smart, funny, accomplished, adventurer, and kind above all. Thank you for sharing pandemic-mania with me. Now let's tour Greece together!

Gjendine Voss, I am so glad we got to know each other, I could always count on your smile to brighten my day and on your outspokenness and wisdom to fight for our rights. Bringing Katie along with you never hurt either.

Sanjay Thompson, life can't be all that bad if you have a Sanjay to brighten your day! Your outgoing personality and the never-fading smile on your face will haunt me (in a good way) for the rest of my days. Thank you for bringing all those dance videos into my Instagram feed and for all the amazing work and ideas that you have brought into the communications office.

Vibha Kumra-Ahnlide, the sweet voice of reason in the chaos of COVID-MDR! I am so glad that you got us all to safety during the year of madness that was 2020.

Richard Dehyle, I am glad that my trashing of American politics got you into my life. You still owe me a tap-dance duet! Don't think I will forget.

And to **Christopher Godina**, **Sabine Konings**, **Kajsa Brolin**, **Divya Bali**… and all the wonderful people that have been involved in MDR over the past few years, all the ones that paved the way for us before I joined, and all the future ones that will continue to fight for our rights… thank you!

The Oxford troupe

How amazing is it to get to live your childhood dream of going to Oxford and find yourself there with the bonus surprise of being surrounded by incredibly amazing, talented, funny, smart people? I'll tell you: pretty amazing indeed!

The **St. Theosevia** people: **Fuchsia**, **Megan**, **Teddy**, **Alberto**, **Freddy**, **Julia**, **Luana** and **Adam**. Thank you for making me feel at home during my time in Oxford. I loved sharing dinners, co-working all-nighters, jaffa cakes, mulled wine, mince pies, gossip at the kitchen table, jokes about the oven (and other appliances), tapas nights, and the COVID-madness of 2021 with all of you. Thank you for all the laughs. I miss you greatly and I hope to see you again soon (calling dibs on the guest room for the summer already).

Dr Milan, thank you for pushing me to find adventures in everyday things, for showing me the city at night, for dragging me up the stairs of the Eiffel Tower, for inspiring me to care more for the world we live in, for sharing with me the wild experience that was COVID-quarantine, for the muddy hikes (yes, even for those), and for getting me back on a bike! But most of all thank you for all your support, kindness, craziness, and honesty. I look forward to many more years of friendship.

Sabine & Luke, thank you for making use of your network to help my homelessness. Thank you for the pizza, the pub crawls, and the classiest Halloween gathering I have ever gone to. Looking forward to seeing a lot more of you two in the future!

The people at **The Vicarage**, and the Vicarage extended family, thank you for so many delicious brunches and kitchen dancing! I hope you are taking care of Bilbo and Frodo.

Susana Kolb, thank you for our Spanglish conversations, the craziness of fresher's fair, and the pub fun. Tell **Carlos**, I am team Oxford, I don't think Cambridge can beat us.

Alexandra Shipley, thank you for your help in the project. We still have to go get those cupcakes from Barefoot, I haven't forgotten.

To **Kate Atherton**, the **UBVO**, and the **Anthropology** department for adopting me for a term and making this experience really a dream come true.

The Inklings and the Narnians

Category is: bookstagram realness! The absolute mess that was 2020 (and 2021, and 2022…) would have been too horrible to bear without the magic of Instagram book clubs! It all started "in a hole in the ground" with **James**, **Noe 'Pip'**, **Drew**, **Ryan**, **Lorianne**, **Grace**, **Emily**, **Clara**, **Khatra**, and **Tori**… but soon we added the likes of **Carla**, **Laura**, **Pauline**, **Sophie-Elise**, **Becca**, **Sally**, **Kristy** and many more chest-hair enthusiasts (you know what you did). I owe this little band of book nerds most of my sanity (and joy) during the infinite lockdowns and beyond. Here's to more zoomcktail parties and literary shag-marry-kill feuds! I love you all from the bottom of my ink-soaked heart.

Special shoutout to Rohan and Sunaina, the cutest baby twins the bookstagram has seen. Also, to all your attention-craving pets who were constantly interrupting the (I'm sure) clever thoughts we were all sharing on our meetings in the cutest way possible.

PS: I already started saving for our literary retirement plan.

The extras

Not all the friends that I have met throughout the past four years could be neatly packed into the categories above, so here is a disaster-drawer of wonderful people that have walked this path with me, or waited patiently for me to finish it so I can go back to being social again.

Graciela & Erik, thank you for the dinners, the walks in Las Canteras, and for reminding me how good it feels to sit in the sun. I look forward to visiting you both! Y pensar que todo esto empezó por las carreras para coger la 11 y no perdernos El Informal hace más de 20 años…! Gracias por ser mi amiga "más vieja" (en tiempo, no en edad).

Yanira, que lo que Medicina y una botella de buen vino ha unido no lo separe el hombre. Gracias por estar ahí siempre, tenemos pendiente otra visita al Triciclo en

cuanto esté de vuelta por Las Palmas, espero que nos estén guardando el sitio de "abonadas".

Ray, gracias por ser mi Marvel-buddy y aguantar estoicamente mientras suspiraba por Capi o Loki o ambos. Gracias por estar ahí para hablar de boberías, para compartir chistes super frikis y memes inapropiados pase el tiempo que pase.

Merci Reyes y **Bea Candela**, anda que no ha llovido poco desde aquellos años en el Pérez. Gracias por seguir a mi lado pese a todo. Y ahora que ya hemos terminado con todas las tesis, podemos celebrarlo con otra ronda de cócteles.

Lua Rangel, thank you for always being up for a quick catch-up, a walk, or a fika. I like how no matter how much time passes by, it is always easy to talk to you about anything going on in my life. Thank you for listening! And thank you, **Andreas**, for putting up with us and cooking us dinner more than once.

Viktor, thank you for all the fun Copenhagen foodie tours. Tonkin soon?

To the **Lindy Hop and Swing dance** community in Malmö/Lund, for teaching me how to dance through life quick-quick, but also slow… slow. I will keep rock stepping and swinging out wherever I go!

Walter Quiroga, I am so glad that we have managed to remain in touch after all this years, and that we continued to celebrate each other's successes through all this time. Thank you!

The "oopsies"

Now, keep in mind that a PhD is a long journey and that I was beyond stressed and sleep-deprived when I wrote this during my 8th consecutive weekend of work without rest. So, if by a horrible series of unfortunate events I have forgotten your name, please accept my apologies and kindly write your name here: _____.

If, however, you have become important to me or my project after the book was sent to print and want to join in on the fun. You can write your name here instead: _____.

That's all folks!

Stay tuned for more, but not better (because it is impossible!)

Thank you all!

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Printed by Media-Tryck, Lund 2022 NORDIC SWAN ECOLABEL 3041 0903

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ESTHER GONZÁLEZ-PADILLA holds a Medical Doctor diploma from the University of Las Palmas de Gran Canaria (Spain). In parallel to her Medical studies, Esther completed an internship in the United States and obtained research experience in the Bone Metabolism Unit and the Nutrition Group within the University of Las Palmas de Gran Canaria. In 2018, she graduated with a MSc in Public Health (MPH) from Lund University, and started her PhD studies within the field of nutritional epidemiology. Her main interests lie within nutrition, preventive medicine, public health, food and health policies, and science communication. This doctoral thesis is mainly focused on the intersection between sugar consumption and cardiovascular disease prevention under the lens of nutritional recommendations.

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FACULTY OF MEDICINE

Department of Clinical Sciences, Malmö

Lund University, Faculty of Medicine
Doctoral Dissertation Series 2022:52
ISBN 978-91-8021-213-7
ISSN 1652-8220