Interaction between dietary factors and genetic risk for lipoprotein traits and cardiovascular disease

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Interaction between dietary factors and genetic risk for lipoprotein traits and cardiovascular disease

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Title and subtitle: Interaction between dietary factors and genetic risk of lipoprotein traits and cardiovascular disease

In previous studies, a high quality diet has been associated with a reduced risk of cardiovascular disease (CVD) compared to a low diet quality, and specific "healthy" diet components, such as polyunsaturated fatty acids (PUFAs), have been hypothesized to reduce the risk of CVD. However, results from epidemiological studies have been conflicting. This may be due to individuals having varied genetic profiles that are differentially associated with CVD. In genome-wide association studies (GWAS), genetic variations in the fatty acid desaturase gene (*FADS1*), which encodes the FADS1 enzyme, have been associated with blood lipid and cholesterol concentrations, enzyme activity and concentrations of long-chain PUFAs.

The aim of this doctoral thesis was to examine the interaction between a common genetic variant of *FADS1* and the intake of dietary fatty acids with respect to cholesterol concentrations and CVD risk. We also examined whether an overall genetic risk for dyslipidemia can be modified by diet quality and whether diet quality can increase the risk of dyslipidemia and CVD.

We used the population-based Malmö Diet and Cancer study (n=28,098, 61% women) that included baseline examinations that were conducted between1991 and 1996. The participants' dietary intakes, lifestyle factors, and body compositions were examined, and blood samples were taken. A diet quality index based on the Swedish nutrition recommendations was used to assess diet quality. Incident cases of CVD were identified from registers.

Our results showed that intake of long-chain omega-3 (ω-3) PUFAs can modify the associated effects of *FADS1* genetic variations on LDL-C concentrations. The association between *FADS1* and reduced LDL-C was observed only among participants who had the lowest intakes of long-chain ω-3 PUFAs. However, genetic variations in *FADS1* had little effect on the association between dietary PUFA intake and CVD risk. We also observed that a high quality diet that reflects the Swedish nutrition recommendations might attenuate the association between genetic risk for high LDL-C and increased risk of ischemic stroke compared to a low quality diet. Furthermore, the risk of developing dyslipidemia over 16 years of follow-up was lower in participants who consumed higher quality diets than those who consumed lower quality diets.

In conclusion, our results suggest that it is important to consider gene-diet interactions to understand the etiology of CVD.

Key words: Diet, polyunsaturated fatty acids, diet quality index, fatty acid desaturase, blood lipids, lipoproteins, polymorphisms, genetic risk score, gene-diet interaction, cohort, epidemiology, cardiovascular disease

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Contents

List of papers

This doctoral thesis is based on the following original papers;

I. **Hellstrand S**, Sonestedt E, Ericson U, Gullberg B, Wirfält E, Hedblad B, Orho-Melander M: Intake levels of dietary long-chain PUFAs modify the association between genetic variation in *FADS* and LDL-C. *J Lipid Res* 2012. **53**(6):1183-9.*

II. **Hellstrand S**, Ericson U, Gullberg B, Hedblad B, Orho-Melander M, Sonestedt E: Genetic variation in *FADS1* has little effect on the association between dietary PUFA intake and cardiovascular disease. *J Nutr* 2014. **144**(9): 1356-63.

III. **Hellstrand S**, Ericson U, Schulz C-A, Drake I, Gullberg B, Hedblad B, Engström G, Orho-Melander M, Sonestedt E: Genetic susceptibility to dyslipidemia and incidence of cardiovascular disease depending on a diet quality index in the Malmö Diet and Cancer cohort. Manuscript.

IV. Sonestedt E, **Hellstrand S**, Drake I, Schulz C-A, Ericson U, Hlebowicz J, Gullberg B, Hedblad B, Engström G, Orho-Melander M: Diet quality and change in standard lipids during 16 years of follow-up and its interaction with genetic risk for dyslipidemia. Manuscript.

Abstract

In previous studies, a high quality diet has been associated with a reduced risk of cardiovascular disease (CVD) compared to a low diet quality, and specific "healthy" diet components, such as polyunsaturated fatty acids (PUFAs), have been hypothesized to reduce the risk of CVD. However, results from epidemiological studies have been conflicting. This may be due to individuals having varied genetic profiles. In genome-wide association studies (GWAS), genetic variations in the fatty acid desaturase gene (*FADS1*), which encodes the FADS1 enzyme, have been associated with blood lipid and cholesterol concentrations, enzyme activity and concentrations of long-chain PUFAs.

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We used the population-based Malmö Diet and Cancer study (n=28 098, 61% women) that included baseline examinations that were conducted between1991 and 1996. The participants' dietary intakes, lifestyle factors, and body compositions were examined, and blood samples were taken. A diet quality index based on the Swedish nutrition recommendations was used to assess diet quality. Incident cases of CVD were identified from registers.

Our results showed that intake of long-chain omega-3 (ω-3) PUFAs can modify the associated effects of *FADS1* genetic variations on LDL-C concentrations. The association between *FADS1* and reduced LDL-C was observed only among participants who had the lowest intakes of long-chain ω-3 PUFAs. However, genetic variations in *FADS1* had little effect on the association between dietary PUFA intake and CVD risk. We also observed that a high quality diet that reflects the Swedish nutrition recommendations might attenuate the association between genetic risk for high LDL-C and increased risk of ischemic stroke compared to a low quality diet. Furthermore, the risk of developing dyslipidemia over 16 years of follow-up was lower in participants who consumed higher quality diets than those who consumed lower quality diets.

In conclusion, our results suggest that it is important to consider gene-diet interactions to understand the etiology of CVD.

Sammanfattning (in Swedish)

Sambanden mellan kostens sammansättning och risk för hjärt-kärlsjukdom är i många fall oklara. En kost rik på fleromättade fettsyror har ansetts kunna ge skydd mot hjärt-kärlsjukdom, men resultaten från olika studier är motstridiga. En anledning till detta kan vara för att man inte har tagit hänsyn till de genetiska skillnader som finns mellan individer.

Omega-3 och omega-6 är namn på de två viktigaste typerna av fleromättat fett, och de har olika funktion i kroppen. Omega-3 finns framför allt i fet fisk, ägg, rapsolja och valnötter, medan omega-6 framför allt finns i majsolja, solrosolja och rapsolja.

Enzymet delta-5-fettsyradesaturas har en viktig funktion i kroppen som är att omvandla de kortare fleromättade fettsyrorna till lång-kedjiga fleromättade fettsyror. Man har sett en lägre aktivitet av enzymet hos de personer som bär på en variant av genen (*FADS1*), som kodar för enzymet. I studier har man sett att detta medför en lägre koncentration i blodet av de lång-kedjiga fleromättade fettsyrorna hos dessa personer. Denna genetiska variant har även, i studier där hela människans genuppsättning undersöks, kopplats till blodfetts- och kolesterolnivåer. Nivån av blodfetter och kolesterol är kopplade till risk för hjärt-kärlsjukdom.

I detta doktorandprojekt har ett eventuellt samspel mellan en vanligt förekommande variant i genen *FADS1* och kostens innehåll av fleromättade fettsyror och hur detta påverkar blodfetts- och kolesterolnivåer och risk för hjärt-kärlsjukdom studerats. Vi har använt oss av data från Malmö Kost-Cancer-studien där 28,098 personer (61% kvinnor) undersöktes mellan åren 1991-1996. Deltagarna lämnade blodprover och deras matvanor, livsstilsfaktorer och kroppssammansättning undersöktes. Uppgifter om deltagarnas insjuknande i hjärt-kärlsjukdom under de 16 år som vi har följt dem kommer från olika register.

Våra resultat (arbete 1) visar att intaget av lång-kedjiga omega-3 fettsyror (de s.k. fiskfettsyrorna) kan modifiera sambandet mellan genetisk variation i *FADS1* och LDL-kolesterolnivåer. LDL-kolesterol brukar kallas det onda kolesterolet, till skillnad från HDL-kolesterol (det goda kolesterolet), eftersom höga nivåer av LDL-kolesterol i blodet ökar risken för hjärt-kärlsjukdom. Samband mellan den genetiska varianten (dvs. varianten som har visat sig ge lägre enzymaktivitet) och

lägre LDL-kolesterol sågs endast hos deltagarna med det lägsta intaget av lång-kedjiga omega-3 fettsyror. Vår slutsats är att det är viktigt att ta hänsyn till kostens innehåll av fleromättade fettsyror när man undersöker sambandet mellan varianter i *FADS1* och blodfetts- och kolesterolnivåer. I arbete 2 visar våra resultat inget tydligt samband mellan kostens innehåll av fleromättade fettsyror och risken för hjärt-kärlsjukdom. Däremot fann vi att hos de med *FADS1*-varianten, var en hög intagskvot mellan alfa-linolensyra (omega-3) och linolsyra (omega-6) kopplad till lägre risk för hjärt-kärlsjukdom. Eftersom *FADS1*-varianten innebär lägre aktivitet av enzymet FADS1 och därmed lägre omvandling av alfa-linolensyra och linolsyra till lång-kedjiga fettsyror, tyder dessa resultat på att de höga nivåer av alfa-linolensyra som uppkommer i kroppen hos individer med *FADS1*-varianten, som samtidigt har en kost med stort intag av alfa-linolensyra, skulle kunna vara skyddande mot hjärt-kärlsjukdom.

Vi har i denna avhandling även undersökt om kostens totala kvalité påverkar sambandet mellan genetisk risk för blodfettsrubbningar på risken för att utveckla hjärt-kärlsjukdom. För att spegla den totala kostkvalitén använde vi ett kostindex utvecklat efter näringsrekommendationerna och kostråden i Sverige. I detta kostindex fick deltagarna poäng (0 till 6) efter hur väl deras kostvanor följde rekommendationerna för mättat fett, fleromättat fett, fisk, socker, frukt och grönsaker, och fibrer. För att fånga den genetiska risken för blodfettsrubbningar använde vi ett genetiskt riskscore (80 genetiska varianter kopplade till blodfetter och kolesterol). Vi fann i arbete 3 att kostkvalitén kan modifiera sambandet mellan genetisk risk för högt LDL-kolesterol och ischemisk stroke genom att risken för stroke var lägre hos de individer som hade bättre följsamhet till kostrekommendationerna. I arbete 4 visar våra resultat att risken för att få höga triglycerider och LDL-kolesterol över 16 års uppföljningstid var lägre hos deltagare som i större utsträckning följde kostrekommendationerna. Vidare fann vi att en låg kostkvalité visade samband med minskade HDL-kolesterolnivåer under uppföljningen enbart hos personer med låg genetisk risk.

Sammantaget tyder studierna på att det är viktigt att ta hänsyn till hur kosten samverkar med gener för att förstå mekanismerna bakom hjärt-kärlsjukdom.

Abbreviations

AA	Arachidonic acid
ALA	α-linolenic acid
ALP	Atherogenic lipoprotein phenotype
ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B-100 and B-48
ApoC	Apolipoprotein C
ApoE	Apolipoprotein E
BMI	Body mass index
BMR	Basal metabolic rate
CE	Coronary event
CI	Confidence interval
CHD	Coronary heart disease
CNV	Copy-number variation
CVD	Cardiovascular disease
DALY	Disability-adjusted life-year
DASH	Dietary Approach to Stop Hypertension
DHA	Docosahexanoic acid
DNA	Deoxyribonucleic acid
DPA	Docosapentanoic acid
E%	Energy percentage
Elovl1-6	Elongation-of-very-long-chain-fatty-acids 1-6
EPA	Eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition
FADS	Fatty acid desaturase
FFQ	Food frequency questionnaire
GLA	γ-linolenic acid
GRS	Genetic risk score
GWAS	Genome-wide association study
HDL-C	High-density lipoprotein-cholesterol
HR	Hazard ratio
HWE	Hardy-Weinberg equilibrium
IARC	International Agency for Research on Cancer
ICD	International Classification of Disease
IDL-C	Intermediate-density lipoprotein-cholesterol
kb	Kilo base-pair

LA	Linoleic acid
LD	Linkage disequilibrium
LDL-C	Low-density lipoprotein-cholesterol
Lp(a)	Lipoprotein (a)
MDC	Malmö Diet and Cancer
MDC-CC	Malmö Diet and Cancer Cardiovascular Cohort
mg/dL	milligram/deciliter
mmHg	millimeter mercury
mmol/L	millimol/liter
mRNA	messenger Ribonucleic acid
MUFA	Monounsaturated fatty acids
NCBI	National Center for Biotechnology Information
nmol/L	nanomol/L
OR	Odds ratio
pmol/L	picomol/liter
PPAR	Peroxisome proliferator activated receptors
PUFA	Polyunsaturated fatty acids
SD	Standard deviation
SE	Standard error
SFA	Saturated fatty acids
SNP	Single nucleotide polymorphism
TG	Triglycerides
VLDL-C	Very low-density lipoprotein-cholesterol
WHO	World Health Organization

1. Introduction

Cardiovascular disease (CVD) is the most common cause of death and disability in the world today (1). Additionally, CVD due to atherosclerosis is the main cause of pre-mature death and disability-adjusted life-years (DALYs) in Europe, and it is also increasing in many developing countries (2). In Sweden, even though a decline of CVD within the population has been observed in recent years, it still remains the most common cause of death (3) and continues to impart a heavy burden on both society and the health care system (1).

Serum concentrations of lipids and lipoproteins are strongly associated with the development of CVD. The causes of dyslipidemia and CVD are multifactorial, and many of the risk factors are preventable or modifiable. Such modifiable risk factors include tobacco smoking, lack of physical activity, poor dietary habits, high blood pressure, psychosocial stress, diabetes, overweight and obesity. There are additional, non-modifiable risk factors of CVD, including age, sex, family history and genetic factors (1, 4).

Genome-wide association studies (GWAS) have pinpointed a number of gene regions that contribute to multifactorial diseases, including those associated with lipid- and lipoprotein traits and CVD. Each risk variant contributes to only a small fraction of the observed differences in lipid and lipoprotein concentrations, but by combining genetic variants from several loci, a genetic risk score (GRS) can be created. A previous study indicated that calculating a GRS that encompassed nine separate, validated single nucleotide polymorphisms (SNPs) that were associated with either high low-density lipoprotein-cholesterol (LDL-C) or low high-density lipoprotein-cholesterol (HDL-C) improved the assessment of CVD risk (5).

There have been contradictory findings in epidemiological studies on the association between dietary intake and CVD risk which may, at least partially, be explained by genetic differences among individuals and by the many gene-diet interactions that have not been well characterized to date. This doctoral thesis challenges the question whether dietary intake interacts with genetic factors on the lipid and lipoprotein concentrations and on CVD risk.

2. Background

2.1 Cardiovascular disease

CVD is defined by a broad spectrum of diseases of the heart, the brain vasculature and of vessel walls (1). Non-atherosclerotic causes of CVD include congenital heart disease, rheumatic heart disease, cardiomyopathies and cardiac arrhythmias (1). CVDs that are caused by atherosclerosis include coronary heart disease (CHD) and myocardial infarction (e.g., heart attack), which in this thesis is referred to as coronary events (CE), cerebrovascular disease (e.g., stroke) and diseases of the aorta and arteries, including hypertension and peripheral vascular disease (1, 6, 7). Atherosclerosis is the most common cause of CVD (4) and we have therefore focused on atherosclerotic forms of CVD that affect the heart and brain in this thesis.

Atherosclerotic disease

The term "atherosclerosis" is defined from the Greek words "athero" (gruel/paste) and "sclerosis" (hardness). Atherosclerosis is characterized by chronic inflammation of blood vessel walls that develops over many years. The process of inflammation is activated by free radicals, particularly reactive oxygen species, which initiate the accumulation and oxidation of LDL-C (8). When oxidized LDL-C comes into contact with a vessel wall, the permeability of the endothelium is altered, allowing oxidized LDL-C to pass through it (9). This induces a reaction from the body´s immune system with increased expression of chemokines and leukocyte adhesion molecules (10), which subsequently attracts leukocytes to an affected site (11). Macrophages are then recruited to clear the oxidized LDL-C; however, a continuous in-flow of more LDL-C leads to a state of chronic inflammation and finally cell death in the blood vessel wall. When this situation occurs, macrophages and other immune cells will produce pro-thrombotic factors, such as cytokines, chemokines and proteases, that will lead to further progression of atherosclerosis (6). If an atherosclerotic plaque occurs within one of the heart's coronary arteries, which are the blood vessels that supply cardiac muscle with blood and oxygen, it can lead to irregular cardiac rhythm and, in the worst-case scenario, sudden death. This state is called myocardial infarction (i.e., a coronary

event) and is most often associated with severe chest pain. The pain is caused by insufficient blood circulation and constriction of the coronary arteries.

The most common cause of ischemic stroke is a blood clot that stops blood flow to the brain. If atherosclerosis is present, blood clots can enter arteries in the brain from various locations in the body (4).

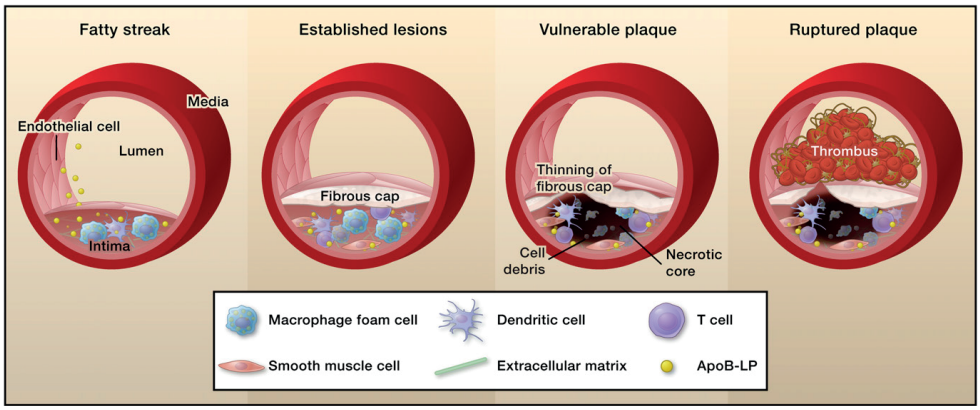

Figure 1. Progression of atherosclerosis
Reproduced with permission from the publisher (12).

Descriptive epidemiology

Although mortality caused by CVD has decreased significantly in recent decades in Sweden, and although both the onset of CVD and CVD-associated mortality are being shown to occur at increasingly higher ages, CVD is still the leading cause of death in the population. During the years 2009 to 2011, there were on average 15,815 and 10,607 new cases of coronary events per year, among men and women, respectively, in the adult (older than 20 years) Swedish population. In the same age group and over the same time period, there were 12,899 and 12,885 new cases of stroke per year in men and women, respectively (3).

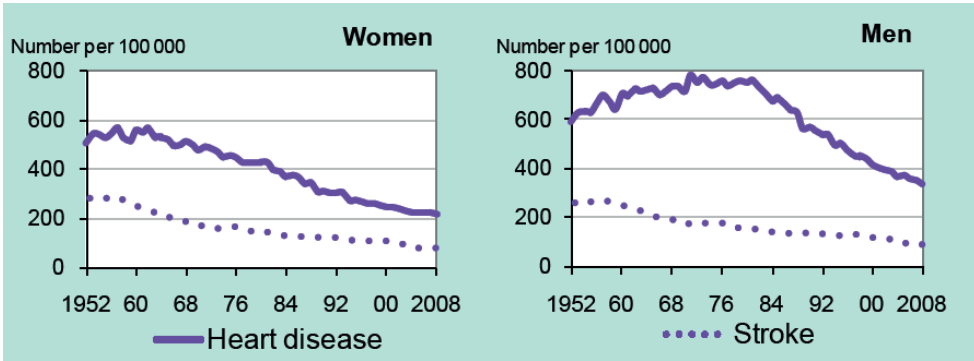

Figure 2. Mortality associated with coronary events and strokes in Sweden
Number of coronary events and strokes per 100,000 inhabitants, shown in men and women seperately, from 1952-2008. Standardized by age. Reproduced with permission from the publishers (4).

A study by Björck *et al*. examined the risk factors and treatments that had the greatest impact on decreasing CVD-associated mortality in Sweden between 1986 and 2002. It was found that the most important factor was decreasing total cholesterol, which explained almost 40% of the observed decrease in CVD mortality. Reducing tobacco smoking accounted for approximately 10% of the decrease, whereas medical treatments accounted for 36% (13).

Coronary events are approximately 40% more prevalent in Sweden compared to Sothern Europe. In addition to a north-south gradient, there is also a west-east gradient of coronary event or stroke mortality, especially among men in Eastern Europe, who are respectively ten and six times more likely to experience these events than men in France (4).

Stroke mortality in Sweden is relatively low compared to countries in Eastern Europe, such as Russia and Latvia, where it is five times higher (4).

2.2 Risk factors for CVD

Lipids and lipoproteins

Lipids are fats, oils, waxes, certain vitamins, hormones and a majority of the non-protein components of cell membranes. Lipids are members of a diverse group of hydrophobic compounds that have many biological functions, such as acting as structural components of cell membranes, energy storage sources and serving as important intermediates in numerous signaling pathways (14, 15). Triglycerides (TG) consist of three fatty acids that are linked through an ester bond to a glycerol

backbone. Lipids such as TG and cholesterol are not soluble in water and are therefore bound to various proteins, called apolipoproteins, which enables them to be transported in blood. TG and esterified cholesterol comprise the cores of lipoproteins, and their surfaces are composed of free cholesterol, phospholipids, and apolipoproteins (14).

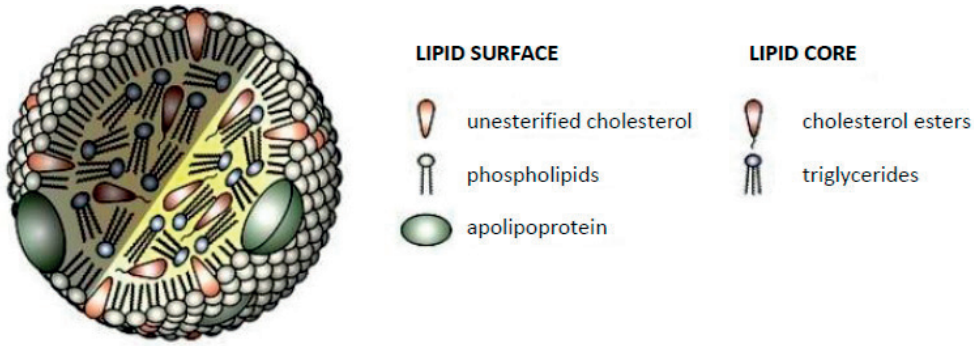

Figure 3. Lipoprotein particle
The lipoprotein core consists of cholesteryl esters and triglycerides surrounded by a membrane composed of unesterified cholesterol, phospholipids and apolipoproteins.

Cholesterol is a sterol and has numerous roles: it serves as a component of cell membranes and as a precursor for steroid hormones, vitamin D and bile acids. The most important dietary sources of cholesterol are meat, offal, eggs and dairy products. However, only around 25% of circulating cholesterol originates from the diet while approximately 75% of it is derived from endogenous synthesis in the body (14).

There are three major classes of lipoproteins that transport TG and cholesterol around the body: LDL, HDL, and very low-density lipoprotein (VLDL). Lipoproteins are produced by the liver and can be divided into different subfractions (e.g., small, medium and large), which are suggested to have different roles in the pathogenesis of atherosclerosis (16, 17).

LDL-C comprises approximately 60-70% of total cholesterol in serum (15, 18). Apolipoprotein B-100 (ApoB) acts as a carrier protein for LDL-C in the bloodstream. Subfractions of LDL, such as small, dense LDL particles, have previously been recognized as the most atherogenic of the lipoproteins and are included in the definition of the atherogenic lipoprotein phenotype (ALP) (19).

HDL-C compromises approximately 20-30% of total serum cholesterol, and its main carrier protein is apolipoprotein A1 (ApoA1) (15). The primary role of HDL-C is to carry excess cholesterol from tissue back to the liver for recycling or secretion.

VLDL consists mainly of TG. The major apolipoproteins for VLDL include ApoB, different ApoCs and ApoE. Because VLDL is a precursor of LDL, it has also been suggested to promote atherosclerosis (15). Both the intermediate-density lipoprotein (IDL), which is a particle that is produced during the conversion of VLDL to LDL, and lipoprotein (a) (Lp(a)) have also been suggested to be involved in the development of atherosclerosis (20, 21).

Because ApoB and ApoA1 concentrations respectively correspond to LDL-C and HDL-C concentrations, either the concentrations of ApoB or the ratio between ApoB and ApoA1 can be used as alternative measures of lipid and lipoprotein concentrations in the blood. For example, the INTERHEART study observed that an elevated ApoB to ApoA1 ratio was one of the most important risk factors of coronary events (22).

Chylomicrons lipoproteins are very rich in TG. When dietary fat is digested in the intestine, chylomicrons are produced to enable the transport of TG in the bloodstream. The apolipoproteins associated with chylomicrons are the same as for VLDL, except that ApoB-48 is present instead of ApoB-100 (15). Chylomicrons are not believed to be atherogenic, but high concentrations of these lipoproteins can cause pancreatitis (23).

Dyslipidemia

Dyslipidemia increases the risk of atherosclerosis and CVD (1, 4). Lipid metabolism can be disturbed in numerous ways that can change the functions or concentrations of lipids and lipoproteins and thereby impact the development of CVD. The most common definition of dyslipidemia is elevated total cholesterol, LDL-C and TG in addition to decreased HDL-C. The Adult Treatment Panel III criteria for the metabolic syndrome were used to identify individuals with high plasma high LDL-C, TG and low HDL-C as follows (15):

- LDL-C >4.1 mmol/L (160 mg/dL)
- Triglycerides ≥1.7 mmol/L (150 mg/dL)
- HDL-C <1.0 mmol/L (40 mg/dL) men; <1.3 mmol/L (50 mg/dL) women

Patient-centered recommendations for the management of dyslipidemia have highlighted non-HDL-C and LDL-C as primary targets (18). Non-HDL-C refers to all potential, currently known atherogenic particles (i.e., LDL-C, VLDL-C, VLDL-C remnants, IDL-C, Lp(a) and chylomicron remnants). Remnants are particles that have left behind the majority of their TGs in different tissues throughout the body and are therefore enriched with cholesterol. Additionally, epidemiological studies have indicated that non-HDL-C is a stronger risk factor for morbidity and mortality of CVD caused by atherosclerosis than is LDL-C (24). However, evidence from numerous randomized controlled trials has demonstrated that reducing total cholesterol and LDL-C are efficient in prevention of CVD (25);

therefore, total cholesterol and LDL-C currently receive the majority of attention in efforts that are aimed at the prevention of CVD (15). Each 1.0 mmol/L (40 mg/dL) reduction of LDL-C has been associated with a 22% reduction in CVD mortality and morbidity (26). In addition to traditional dyslipidemia, a more specific atherogenic risk profile has recently become clear, which includes increased concentrations of small, dense LDL-C particles and TG and reduced HDL-C concentrations; this profile is the hallmark of the so-called ALP (19). In the Malmö Diet and Cancer (MDC) study, small and medium LDL-C particles were shown a have a positive association with CVD, whereas concentrations of large LDL-C particles were not significantly associated with CVD (27). However, thus far, the evidence on the effectiveness of changing this lipid and lipoprotein pattern to reduce CVD risk has not been entirely convincing; rather, it should be observed as providing optimal target for CVD prevention (28).

Additionally, several randomized clinical trials have evaluated the benefit of reducing LDL-C and raising HDL-C concentrations for the tertiary prevention of coronary events (29-31). However, none of these studies have successfully shown that raising HDL-C has a protective effect on CVD. Conversely, studies and trials that have been conducted on patients with hypercholesterolemia have strongly indicated that therapies designed to reduce LDL-C also reduce the risk of coronary events (29, 32), which is in agreement with results from observational studies (33).

Blood pressure

High blood pressure (hypertension) is often one of the first symptoms of atherosclerosis and can further damage the blood vessels and in particular, increase the risk of stroke (4). Hypertension is defined by the World Health Organization (WHO) as a systolic pressure of ≥140 mmHg and/or a diastolic pressure of ≥90 mmHg (1). The causes of high blood pressure are the same as for atherosclerosis: tobacco smoking, low physical activity, unhealthy dietary habits, including high salt (sodium chloride) and high alcohol consumption, psychosocial stress, diabetes, overweight, obesity and genetic factors. Mildly elevated blood pressure can be treated with lifestyle and dietary interventions, but more aggressive cases of hypertension require anti-hypertensive medication (4, 23).

Age and sex

Age and sex are non-modifiable risk factors for CVD. In both men and women, the risk of CVD increases as age increases, and age is one of the most important risk factors of CVD. At any given LDL-C concentration, an older person has a higher risk of experiencing a coronary event than a younger person (34). Men who are >45-years old and women who are >55-years old have an especially increased

risk of CVD. Most commonly, coronary events occur in individuals at age 65 years and older (15). On average, older persons have a greater degree of atherosclerosis in their arteries than younger persons, and the increasing risk of a coronary event that is associated with increasing age reflects the accumulation of atherogenic risk factors, both known and unknown (15). Male sex is associated with a higher risk of coronary event at any given age compared to women (34). Women are 10-15 years behind men in terms of coronary event risk, and reasons behind this discrepancy are not fully understood. In may partially be explained by faster progression of dyslipidemia and hypertension in men (15). Another possible reason could be that estrogen imparts a protective effect on women before they reach menopause and that they have a better "natural defense" because of their higher HDL-C concentrations.

Dietary factors and CVD

For many years, studies have examined associations between dietary factors and CVD risk. A systemic review by Mente *et al*. of 146 prospective population-based studies and 43 intervention studies that were published between 1950 and 2007, showed that dietary patterns are more strongly associated with CVD than individual food items or nutrients (35).

One dietary pattern that has been developed with the goal of preventing of CVD is the Dietary Approach to Stop Hypertension (DASH) diet (36). This diet advocates a high intake of fruit and vegetables, whole-grains, legumes and low fat dairy products and a reduced intake of salt and sugar-sweetened beverages. Several intervention studies have demonstrated associations between the DASH-diet and decreases in blood pressure and blood lipid and lipoprotein concentrations (36-38). The reductions in total cholesterol and LDL-C were greater than the reduction in HDL-C (37). In the Nurses' Health Study that was conducted in the USA, 88,000 women were categorized into five groups based on their levels of adherence to the DASH-diet (39). Over a course of 24 years of follow-up, women who most strongly adhered to the DASH-diet were shown to have a 24% lower risk of both fetal and non-fetal coronary events as well as an 18% lower risk of stroke. These women also had the highest intakes of dietary fiber and the lowest intakes of saturated fatty acid (SFA) and trans-fat (39).

In the large, multi-centered INTERHEART study, associations between dietary patterns and coronary event and stroke were examined in cases and controls. In an INTERHEART study of coronary events that was conducted across 52 countries, it was observed that a dietary pattern characterized by high intake of fruit and vegetables was inversely associated with coronary event risk (40). Additionally, participants who had dietary patterns characterized by high intakes of fried food, salty snacks, eggs and meat were at an increased risk of coronary event compared

to participants with low intakes of these foods (40). In a separate INTERHEART study that was conducted across 22 countries, O'Donnell *et al*. observed an elevated risk of stroke in participants with high intakes of fried food, salty snacks and red meat. Alternatively, a high intake of fruit and fish were associated with a reduced risk of stroke (41). Collectively, these two INTERHEART studies concluded that reduced blood pressure, increased physical activity and improved dietary patterns would most likely reduce the risk of coronary events and stroke in all parts of the world (42).

How specific dietary components affect CVD risk is thought to be mediated, at least in part, by their effects on lipid and lipoprotein metabolism. Several, although not all, clinical trials that had participants replace SFAs with polyunsaturated fatty acids (PUFAs) showed a reduction in coronary events (43). Additionally, long-chain PUFAs, such as arachidonic acids (C20:4ω-6, AA) and eicosapentanonic acids (C20:5ω-3, EPA), are precursors for inflammatory molecules (i.e., eicosanoids) (44, 45), and a high concentration of long-chain PUFAs has been associated with a lower prevalence of both metabolic syndrome and CVD (44, 46). To reduce the risk of CVD, it has therefore been suggested that the intake of SFAs and trans-fats should be replaced with PUFAs, especially ω-3 PUFAs (47, 48). However, the link between the intake of dietary fat and the risk of CVD still remains controversial and unclear and this may, at least partially, be explained by genetic differences between individuals.

Swedish studies

A Swedish prospective cohort study that included 24,000 middle-aged and elderly women demonstrated that a diet rich in fruits and vegetables, whole-grains and fish, and with moderate alcohol consumption, may reduce the risk of coronary events (49). This study also observed that women who, in addition to having a high quality diet, were also physically active, weight stable and did not smoke had a 92% lower risk of coronary events during 6 years of follow-up. Only one third of the participating women had high intakes of fruit and vegetables, whole-grains and fish. The results also indicated that the combination of a high quality diet, physical activity, normal weight (in particular lack of abdominal obesity) and non-smoking status may prevent up to 75% of coronary events in this population. The authors concluded that there are many possible ways of lowering the risk for coronary events by making positive changes to diet and lifestyle (49).

Previous results from the Malmö Diet and Cancer (MDC) study have shown that participants with high fiber intakes (50) and high quality diets had a reduced incidence of CVD compared to those with low quality diets (51). Diet quality was examined using a diet quality index based on the Swedish nutrition recommendations and Swedish dietary guidelines. This diet quality index was created by combining six dietary components to an index of 0-6 points. Each component provided one point if the criteria in parenthesis was fulfilled by the

participant: contribution to non-alcohol energy percentage (E%) from SFA (≤14 E%), PUFA (5-10 E%), fish and shellfish (≥300 g/week), dietary fiber (≥2.4 g/MJ), fruit and vegetables (≥400 g/day) and sucrose (≤10 E%) (52). Previously, this diet quality index has been more strongly associated with CVD than the individual dietary components that it includes (51), illustrating the importance of examining the whole diet when studying disease risk.

Dietary recommendations to prevent CVD

Diet and several other lifestyle characteristics are very important factors that affect blood lipid and lipoprotein concentrations and therefore, individuals with dyslipidemia are recommended to increase their physical activity and decrease their body weight. The American Association of Clinical Endocrinologists Guidelines for the management of dyslipidemia and the prevention of atherosclerosis recommends a calorie-restricted diet based on vegetables and fruits, grains (one third as whole-grains), fish and low fat meats (25). These guidelines also suggest a decreased intake of SFAs, trans-fats and cholesterol. These fats are found in high amounts in high fat dairy products, high fat meat products and sweets and snacks and therefore minimal consumption of these food items is recommended. Cessation of smoking is also included in the recommendations (25). In Sweden, the dietary recommendations for individuals at risk of CVD or with previous CVD are in line with the general dietary recommendations (14). However, these recommendations are not always sufficient for achieving target blood lipid and lipoprotein concentrations and therefore statins, fibrates and nicotine acid drugs are commonly used for treating dyslipidemia (15).

Dietary fatty acids

Fat is the most energy-rich macronutrient, and dietary fat provides the body with essential fatty acids and fat-soluble vitamins. The biological effects caused by fatty acids correlate with chain-length, degree of saturation and isomeric form. When discussing fat quality, it is the composition of fatty acids that is being considered. Internal exposure to fatty acids is determined by a combination of fatty acid intake and the efficiency of the enzymes that metabolize these fatty acids, which is genetically determined. Dietary fats include TG, phospholipids and cholesterol, the latter two of which can also be synthesized in the body (14).

Fatty acids are structured as a carbon chain with a methyl group at one end and a carboxyl group at the other end, but some fatty acids are also branched. The carbon atoms in the carbon chain are usually even numbered and typically range from 4 to 24. The most common fatty acids in food include 16 to 18 carbon atoms

(53), and in the Swedish diet they include palmitic (C16:0), oleic (C18:1), stearic (C18:0) and linoleic acid (LA, C18:2ω-6) (54).

The three main groups of dietary fatty acids are SFAs, monounsaturated fatty acids (MUFA) and PUFAs. PUFAs are divided into two main families of ω-3 and ω-6 PUFAs. The more double bonds that a fatty acid chain contains, the more unsaturated it is considered to be. Double bonds impart a less regular and therefore more flexible structure to fatty acids, which affects their melting points and their ability to regulate metabolic processes within the cell (53). The positions of the double bonds are usually calculated and named starting from the methyl-containing end of the carbon chain (ω or n). MUFAs have only one double bond, whereas PUFAs have 2 to 6 double bonds (14).

In Sweden, the mean total fat intake of the population remained stable from 1997 to 2011 (34 E%), although a slight decrease in the consumption of SFAs, from 14 E% to 13 E% and an increase in the consumption of PUFAs, from 4.7 E% to 5.6 E%, has been observed (54, 55). The main sources of fat in the Swedish diet include 1) spreads, butter, and oils, 2) milk and milk products and 3) meat and meat products. The main sources of SFAs are high-fat dairy products, meat products, cakes and sweet bakery products (14).

Essential fatty acids

There are two dietary fatty acids that are essential and cannot be synthesized endogenously in humans, i.e., LA and α-linolenic acid (ALA, C18:3ω-3) and thus they must be supplied through diet (14). LA and ALA have important roles in the body. They can act as second-messengers in intracellular signaling pathways and are precursors for inflammatory molecules such as eicosanoids (e.g., prostaglandins and leukotrienes) (56). Dietary PUFAs mainly consist of LA and a lower proportion of ALA (57). Dietary sources of LA include vegetable oils, such as corn and sunflower oil, and food products made with such oils. ALA is found in vegetable oils, including rapeseed and flax-seed oil, walnuts and green plants (54).

Long-chain PUFAs

Long-chain PUFAs such as EPA (C20:5ω-3) and AA (C20:4ω-6) and their derivatives, the eicosanoids, are important ligands of peroxisome proliferator-activated receptors (PPARs), which serve as transcription factors for several genes that are involved in lipid and glucose metabolism, inflammation and wound healing (44, 45, 58, 59). In general, eicosanoids that are produced from AA are suggested to be more actively involved in inflammatory processes compared to eicosanoids that are produced from EPA, which are instead suggested to have anti-

inflammatory properties (53, 60). In line with this hypothesis, several previously conducted case-control and case-cohort studies found either no association or only a slightly increased risk of coronary events when AA concentrations were elevated in adipose tissue, suggesting that excess AA may be pro-atherosclerotic (61-63).

In men and post-menopausal women, approximately 5% of ALA is converted into EPA, and even less is converted into docosahexanonic acid (C22:6ω-3, DHA) (64-66). Additionally, the human body is able to retroconvert DHA into EPA (67). Although both EPA and DHA have important roles in the body, earlier findings have indicated that an adequate supply of DHA may be able to compensate for both EPA and DHA; however, EPA cannot compensate for DHA (9).

In the majority of diets, the intake of long-chain PUFAs is much lower than the intake of LA and ALA. Although the conversion rate of essential fatty acids into long-chain PUFAs is low, bodily requirements for long-chain ω-3 and ω-6 PUFAs can usually be met with dietary intake of LA and ALA (14). Fatty fish that live in cold waters, such as mackerel, salmon, herring, sardines, anchovy and tuna, are the main dietary sources of long-chain ω-3 PUFAs, EPA and DHA. These fatty fishes contain more than 8 grams of fat per 100 grams of body weight and are very good dietary sources of long-chain ω-3 PUFAs (14). Dietary intake of LA and ALA is recommended to be 3% of the total consumed energy (E%), whereas ALA should contribute to at least 0.5 E% and approximately 200 mg/day of DHA should be consumed, according to the Nordic nutrition recommendations (14). The ideal ratio between ω-6 and ω-3 PUFAs has been suggested to be 4:1 (53); however, well-documented evidence of what the exact ideal ratio is between these PUFAs is still missing. Recent results from a dietary assessment study in an adult population in Sweden indicated that the current average ratio is approximately 4:1 (54). However, many modern diets have an average ratio around of approximately 10:1 (53).

It is also possible that a high intake of PUFAs may induce negative effects, such as increased lipid peroxidation and bleeding tendency, and impaired immune function (68). The upper level of PUFA consumption has been recommended to not exceed 10 E% (14, 57, 68).

Fatty acids and desaturases

LA and ALA need similar enzymes (elongases and desaturases) to be converted into more bioactive long-chain PUFAs. Elongases elongate the fatty acid carbon chain and are encoded by *Elovl1-6* (Elongation-of-very-long-chain-fatty-acids) genes. Delta (Δ) desaturases are responsible for introducing a new double bond at a specific position in the carbon chain, resulting in a fatty acid becoming more unsaturated. Elongases and desaturases work together to form long-chain fatty acids in the endoplasmic reticulum of the cell (69, 70). Humans have three main

desaturases: Δ-9 desaturase (stearoyl-CoA-desaturase) (70), Δ-6 desaturase (FADS2) and Δ-5 desaturase (FADS1) (44, 46). The *FADS1* and *FADS2* genes encode for their respective desaturases in the conversion of PUFAs, i.e., ALA (C18:3ω-3) into EPA (C20:5ω-3) and LA (C18:2ω-6) into AA (C20:4ω-6). The same genetic variant in the *FADS* locus that has been found to be associated with lipid and lipoprotein concentrations has also been shown to be strongly associated with the concentrations of the above metabolites, as well as with their relative ratios (71). PUFAs can also activate PPARs via their metabolites, the eicosanoids (44, 45), which can regulate the transcription of genes that are directly involved in for example, HDL-C production (72). Although elongases and desaturases both have preferences for ω-3 fatty acids, a higher dietary intake of LA may create a deficiency of long-chain ω-3 PUFAs because ALA and LA compete for the same enzymes.

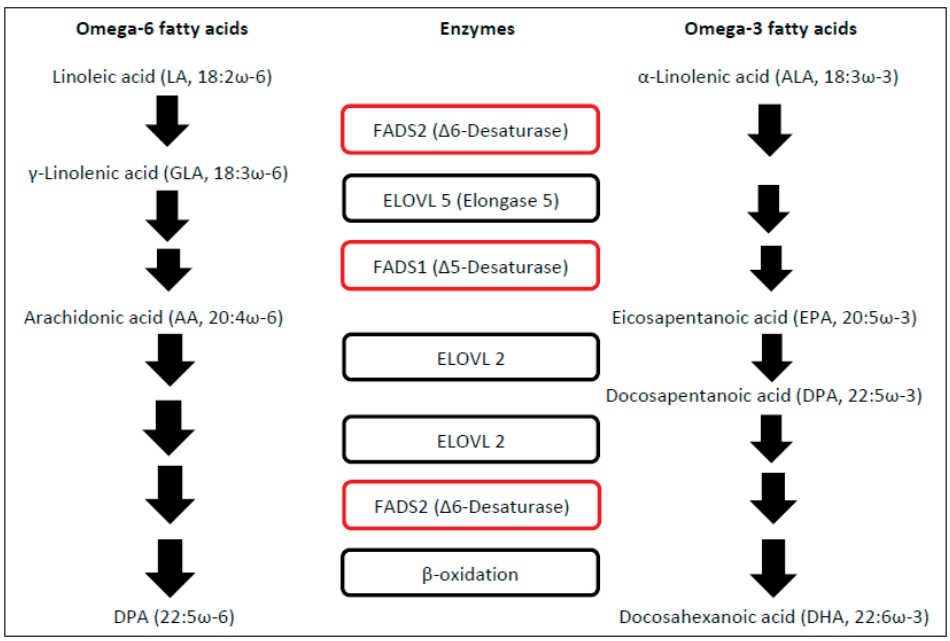

Figure 4. Metabolic pathways of ω-6 and ω-3 PUFAs

Other lifestyle factors

Smoking

Smoking is a strong independent risk factor for CVD, and the cessation of smoking is an important facet of CVD prevention programs (4, 15, 23, 25). Especially in individuals with pronounced atherosclerosis, smoking is very

dangerous because it accelerates plaque development and may lead to plaque rupture (23, 25). Smoking has been shown in many studies to decrease HDL-C concentrations and to increase both TG and the ratio between LDL-C and HDL-C; therefore, the cessation of smoking has been observed to significantly increase HDL-C already after less than 30 days (25). Smoking has declined in Sweden over the last decades (4), but it is still common in specific societal groups such as in persons of low socio-economic status (1, 23).

Overweight and obesity

Overweight and obesity are growing health problems in the world today. Both conditions associate with several risk factors for CVD, such as hypertension, type 2 diabetes and dyslipidemia (1, 4), hence, the independent roles of overweight and obesity on CVD risk is uncertain (15). About half of all men and one third of all women in Sweden in the 16-84 years age range are overweight or obese, according to self-reported data (4). Many prospective observational studies have shown an association between overweight and obesity and CVD (1). The body mass index (BMI) cut-offs for adults are <18.5 kg/m^2 to be considered underweight, 18.5-24.9 kg/m^2 for normal weight, 25-29.9 kg/m^2 for overweight and ≥ 30 kg/m^2 to be considered obese, according to the WHO (73). In addition to BMI, body fat distribution has been suggested to play a crucial role in CVD risk, and waist or waist-to-hip circumferences serve as useful indicators of abdominal fat (23). Abdominal fat is important because it surrounds the intestines and produces hormones that are active in appetite, glucose, lipid, and insulin regulation (4). The most commonly used threshold in Europe for waist circumference is ≤ 94 cm in men and ≤ 80 cm in women (73), but these cut-offs may vary with ethnicity (23). It should be noted that individuals with high BMI may have large muscle mass without being overweight, and for these individuals waist circumference will be a more accurate measure of adiposity than BMI (4). There is currently not enough evidence to substitute BMI with measurements of waist circumference in routine public health surveillance or in clinical practice (23).

Physical inactivity

Lack of physical activity is a major risk factor for CVD (1, 15, 23). The beneficial mechanisms behind why regular physical activity decreases the risk of CVD are not completely understood, but are probably, at least partly, explained by intermediate risk factors, including the following: weight loss, glycemic control, improved blood pressure, lipid profile, and insulin sensitivity (1, 15). Many observational studies have indicated a dose-response association between increased physical activity and decreased CVD. However, physically active individuals also tend to have healthier food habits and smoke less, although in studies controlling for these potential confounders an inverse association between physical activity and CVD was still observed (23).

Alcohol

Harmful alcohol consumption can damage the heart, increase the risk of stroke and induce cardiac arrhythmia (1). Observational studies have revealed that a J-shaped curve describes the association between alcohol consumption and total mortality, wherein reduced alcohol consumption is linked with lower mortality and increased alcohol consumption is linked with higher mortality. The decreased total mortality is primarily related to a reduced CVD mortality (15). A protective role of low to moderate alcohol consumption has been suggested in middle-aged and older individuals, but thus far there is not a sufficient amount of convincing evidence to advise people who do not drink alcohol to do so or to prevent CVD because the negative effects of alcohol consumption can outweigh the potential positive effects (4, 15).

Diabetes

Diabetes increases the risk of CVD by two- to three-fold (1). In Sweden, approximately 365,000 individuals have been diagnosed with diabetes, of which 40,000 have type 1 diabetes (4). Thus, the majority of data refers to type 2 diabetes, even though persons with type 1 diabetes also have an increased risk of CVD (25). Type 2 diabetes is more common in men than in women, and many patients have been suffering for several years before getting diagnosed with it (4). Type 2 diabetes is defined by impaired glucose tolerance and fasting glycemia, and early detection of diabetes is central to preventing future CVD events (1). In people with diabetes, approximately 60% of all mortality is caused by CVD (1, 23, 25). Hyperglycemia contributes to both micro- and macrovascular complications, which may in turn lead to diseases such as retinopathy, nephropathy and neuropathy. Additionally, it is common for a person with diabetes to have other risk factors for CVD, such as central obesity, hypertension and dyslipidemia (23). People with diabetes also have poorer recovery after a CVD event (1). Recently, it has been stressed that LDL-C commonly remains at normal concentrations or is even slightly elevated in individuals with diabetes. Rather, high TG and low HDL-C are the main contributors to diabetic dyslipidemia (23). More research is needed to evaluate the effectiveness of reducing LDL-C in preventing CVD in people with diabetes.

Family history of CVD

Family history of CVD is an important risk factor (15), despite that it is commonly overlooked during individual risk assessment of CVD (25). Among first-degree relatives, siblings to patients with premature CVD have the highest risk of developing CVD, probably because they not only share similar genetic factors but also social environments and other lifestyle exposures. Many of these risk factors are, at least in part, modifiable. The younger an individual is at the onset of CVD and the greater the number of affected family members is, the higher the risk is for

the relatives of the individual. Additionally, many risk factors are under genetic control but the genetic factors thus far identified involved in blood pressure, lipid metabolism and obesity only explain a minor part of the increased risk of CVD seen in these families. Regardless, numerous case-control and case-cohort studies have shown that a history of CVD is independently associated with a higher risk (15). Because family history is generally easy to assess by direct questioning it should be used more often in the primary prevention of CVD (25).

Psychosocial stress

Psychosocial stress holds a close connected to increased risk of CVD (4). Psychosocial stressors are related to factors such as low income, stress at work and in family life, poor housing conditions, a lack of social networks and support from close relationships, depression, anxiety, labor market insecurity and feelings of helplessness related to work. These factors act as barriers for treatment adherence and healthy lifestyle changes, and several of them have also been recognized to be directly involved in the pathogenesis of CVD (23). Stress activates biological mechanisms that act via the nervous system and hormonal processes that influence reactions involved in abdominal obesity, inflammation processes and disturbances in blood vessel walls. Unfortunately, changes in the blood vessel wall tend to reduce elasticity and induce fat storage, which are among the first steps in the development of atherosclerosis. Additionally, disturbances to the vessel wall may reduce insulin sensitivity and increase blood pressure, glucose concentrations and the tendency of platelets to aggregate, increasing the risk of blood clots (4).

Genetics

Deoxyribonucleic acid (DNA) is located within the nucleus of every cell in the body and consists of four different types of nucleotides: adenine (A), thymine (T), cytosine (C) and guanine (G). In every individual, these nucleotides are organized in a specific sequence, which enables the heritability of genetic information. Protein encoding genes are comprised of nucleotides and include coding regions (exons) or non-coding regions (introns) (74, 75). The human genome consists of approximately 30,000 to 40,000 protein-coding genes (76) but these genes can express many more gene products due to alternative splicing of most of the genes.

The human genome consists of approximately 3 billion base pairs, which are organized into 22 autosomal chromosome pairs and 2 sex-specific chromosomes. An entire sequence of a human genome was first published in 2001. Approximately 99.9% of a given human genetic sequence is shared among the population, and single nucleotide polymorphisms (SNPs) are the most common type of polymorphism in the genome. Together with the International HapMap project, at least 30 million common SNPs, with allele frequencies of ≥1% (77, 78),

are known today. In addition to these SNPs, there are also a large number of rare SNP variants (allele frequency ≤0.1%) (79).

Figure 5. Single nucleotide polymorphism (SNP)
A SNP occurs when one nucleotide in a DNA sequence is substituted for another nucleotide. For the majority of SNPs, there are three different genotypes, which are illustrated above i.e., homozygous for the C-allele (CC), heterozygous (CT) and homozygous for the T-allele (TT).

Depending on where a SNP is located within a genome, it may or may not be functional. A SNP that is located within a coding region of a protein coding gene can change the amino acid sequence of the protein product of the gene; this type of SNP is called a non-synonymous SNP. Conversely, if the amino acid sequence remains the same, the SNP would instead be called a synonymous SNP. A non-coding SNP is a SNP that is located outside of the coding region of a protein coding gene, and it can alter the expression of one or several genes by affecting functional elements such as promoters, silencers and enhancers (80). Further, SNPs can be located in genes that encode small RNA molecules or regions that regulate expression of these genes. Finally, SNPs can affect the methylation of DNA sequence thus affecting epigenetic regulation.

Other sequence variations are structural variations in DNA, such as: deletions (i.e., the loss of DNA sequence), insertions (i.e., addition of one or more nucleotide base pair into DNA sequence) or microsatellites, a short repeated nucleotide sequences of DNA (2-5 base pair is repeated), and copy-number variations (CNVs), a large region of DNA that can vary in copy-number due to deleting or duplicating in a genome.

Linkage disequilibrium

A non-random association between two alleles at two or more loci is referred to as linkage disequilibrium (LD) (76). LD occurs because genetic information is inherited as haplotypes, which leads to some combinations of alleles occurring more frequently than others. Due to recombination hot spots, recombination typically occurs outside of a haplotype and therefore an association between two markers declines as the distance between them increases. Statistical measurements of LD include D´ and r^2 which can range from 0 to 1. A value of 0 describes no association between loci, and a value of 1 describes loci that are in complete dependency (81). The D reflects the chance ordering of the alleles at each locus which can be important in the comparison of LD between the same loci genotyped in different populations (e.g., cases and controls). The r^2 is the squared correlation,

where r scales D by the standard deviations (SD) of the allele frequencies at two loci (82).

Hardy-Weinberg equilibrium

In genetic studies it is very important that allele and genotype frequencies are constant in the population, i.e., that they follow the Hardy-Weinberg equilibrium (HWE). If HWE is not present, it is most likely because of systematic genotyping errors (83). However, there are other causes of deviation from HWE that are important to be aware of, such as inbreeding (non-random mating), relatedness among the participants of the study population, novel mutations, selective migration and a mortality bias.

Genetics of dyslipidemia and CVD

Family history is an important risk factor for CVD and one important part of the heritability of the disease is due to genetics. The heritability of coronary event is estimated to be in the range of 40-60% (84, 85). The heritability estimates for different lipid and lipoprotein traits range from 28-78% and for blood pressure from 50- 70% (86, 87). Studies of stroke heritability have thus far not been very successful, which may at least partly be due to the different subgroups of this disease, even in its ischemic forms. The strongest risk factor for stroke is hypertension and it has been estimated that hypertension accounts for 25-33% of the heritability of stroke (87). A Swedish twin study observed that premature death due to coronary event in one of the twins was associated with almost a 4-fold increased risk of coronary event mortality among dizygotic and an 8-fold increased risk among monozygotic male twins. Female twins had a 2.5-fold increased risk in dizygotic and a 15-fold increased risk among monozygotic twins (88). These findings highlight the importance of the genetic component of coronary events.

Early findings from linkage studies in families examining genetics and the risk of CVD demonstrated that the risk of CVD was mainly attributed to monogenic rare forms of dyslipidemia. Individuals with the monogenetic disease familial hypercholesterolemia have an inherited increased LDL-C concentration associated with an increased risk of premature coronary event. The autosomal dominant forms of familial hypercholesterolemia include mutations in the hepatic LDL-C receptor gene (*LDLR*) (89), ApoB gene (*APOB*) (90) and the proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) (91). Other mutations in the *PCSK9*, *APOB* and angiopoietin-like 3 (*ANGPTL3*) genes have been liked to familial hypobetalipoproteinemia, a condition with low LDL-C concentrations and low risk of coronary event (32, 90, 92). However, linkage studies have been rather unsuccessful in identifying common variants for CVD.

Genome-wide association studies

GWAS is a powerful experimental approach for gene mapping. These studies evaluate SNP markers across the human genome to identify genetic regions that are statistically associated with quantitative or qualitative traits in samples of unrelated individuals. In GWAS, in order to detect SNP markers with small associated effects on multifactorial diseases or traits, a genome-wide scan (often including one million SNPs) must to be performed on a very large number of individuals. When selecting SNPs for GWAS, LD is very useful because it can reduce the number of SNPs that require genotyping to capture genetic variation. Regardless, due to the large number of tests that are required, GWAS analyses must be corrected according to Bonferroni correction for one million tests to avoid the risk of false positive hits. Thus, in GWAS, a *P*-value of $5x10^{-8}$ represents significant associations (93, 94). For qualitative traits, the analysis is performed comparing healthy control individuals and cases with a disease to identify specific genetic variants which are more common or rare in cases with disease as compared to controls. For quantitative traits, SNP markers are used to determine whether a given SNP shows a linear association with a selected trait.

GWAS analyses have pinpointed a large number of gene regions that contribute to lipid and lipoprotein traits and CVD; in 2010, a very large meta-analysis of several GWASs (with >100,000 individuals) reported 95loci that significantly associated with lipid and lipoprotein concentrations (95). These 95 loci explain 25-30% of the genetic variance (representing 10-12% of the total variance) that is related to an individual's lipid and lipoprotein concentrations. However, each risk variant contributes to only a small fraction of the difference in lipid and lipoprotein concentrations. By combining the variants of several genes, genetic risk scores (GRS) can be created, which can improve CVD risk assessment (5, 96). For example, individuals in the highest quartile of GRS that combined validated loci were 13 times more likely to have high LDL-C concentrations than individuals in the lowest quartile of GRS (95). At the present time, 185 common variants have been found in 157 loci that are associated with lipid and lipoprotein concentrations (71, 97-99). A total of 54 of these variants associated with total cholesterol, including 37 with LDL-C, 55 with HDL-C and 24 with TG. However, these loci have very low effect sizes, and adding these new loci to a GRS would probably not improve the risk prediction of CVD to an appreciable extent. Several genetic risk variants and loci that affect lipid and lipoprotein concentrations have unknown functions in lipid and lipoprotein metabolism, and novel mechanisms based on the newly identified CVD susceptibility genes have been described (100).

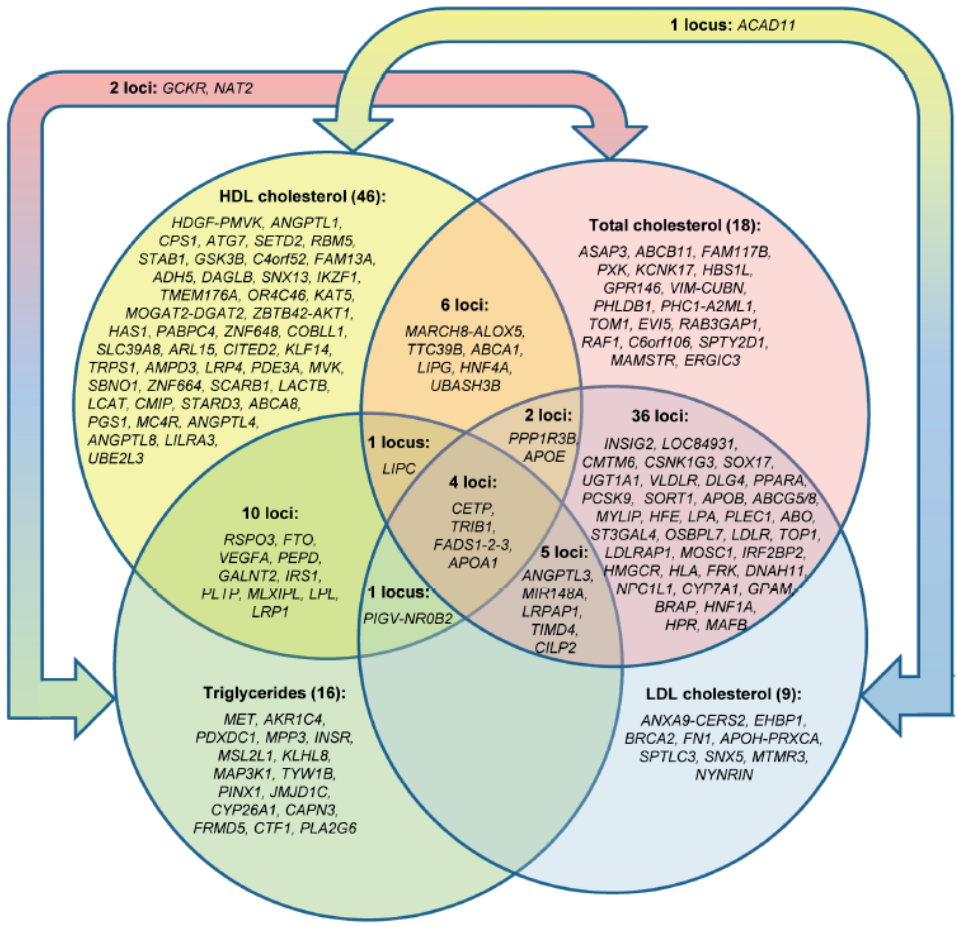

Figure 6. Lipid and lipoprotein associated loci
In parentheses after the trait names are the number of primary loci that are associated with a given trait, and the locus name is listed below in italics. Loci that are associated with two or more traits are shown in the appropriate sections. Reproduced with permission from the publishers (97).

Plasma LDL-C and TG have been confirmed to be causally linked to coronary events in Mendelian randomization studies (101-103) but thus far evidence of a causal relevance of HDL-C is lacking (29, 101-103). For example, a recent Mendelian randomization study by Voight *et al*. indicated that a genetic score of 13 LDL-C increasing alleles increased the risk of coronary event, whereas a genetic score of 14 HDL-C increasing alleles did not decrease the risk of coronary event (103). The authors concluded that genetic mechanisms that increase HDL-C do not automatically reduce the risk of coronary event.

Genetic variations in the FADS gene cluster

Schaffer *et al*. 2006 demonstrated that common genetic variations in *FADS1* and *FADS2,* which reside within a cluster of three fatty acid desaturase genes (*FADS1-FADS2-FADS3*) on 11q12-13.1, were strongly correlated with fatty acid composition in serum phospholipids as well as with the concentration of several PUFAs that act as direct precursors for inflammatory eicosanoids such as AA (104). The rs174547 (T/C) SNP in the *FADS* gene cluster has been associated with circulating concentrations of total cholesterol, LDL-C, HDL-C, and TG as well as with fasting glucose concentrations in GWAS (95, 99). Expression data have demonstrated an association between the SNPs and hepatic mRNA expression of *FADS1* and *FADS3* (99). The rs174547 (T/C) SNP is in complete LD (r^2=1) with rs174546 (C/T), and rs174546 is also in very high LD (r^2=0.99) with other SNPs in this locus, such as rs174537 (G/T), according to HapMap CEU. The *FADS* gene cluster comprises 91.9 kilo base pairs (kb), and these three genes have similar exon and intron organization, suggesting that a duplication of this gene region occurred at some point during evolution (105, 106). Within these 91.1 kb, 500 SNPs have been registered in the National Center for Biotechnology Information (NCBI) database.

bp position	SNP	rs695867	rs4246215	rs174541	rs174545	rs174546	*rs174547*	rs174548	rs174549	rs174550	rs174555	rs174556
61793816	rs695867	rs695867										
61796827	rs4246215	0.084	rs4246215									
61798436	rs174541	0.092	1.000	rs174541								
61801834	rs174545	0.104	0.965	0.931	rs174545							
61802358	rs174546	0.092	0.867	0.865	1.000	rs174546						
61803311	*rs174547*	0.089	0.870	0.871	1.000	1.000	*rs174547*					
61803876	rs174548	-	0.692	0.700	0.800	0.794	0.798	rs174548				
61803910	rs174549	-	0.700	0.679	0.774	0.813	0.817	0.981	rs174549			
61804006	rs174550	0.092	0.885	0.873	1.000	1.000	1.000	0.791	0.800	rs174550		
61812288	rs174555	-	0.705	0.687	0.791	0.813	0.813	0.981	1.000	0.817	rs174555	
61813163	rs174556	-	0.705	0.710	0.789	0.813	0.812	0.981	1.000	0.815	1.000	rs174556

Figure 7. Linkage disequilibrium (r^2) for rs174546, rs174547 and other SNPs located on chromosome 11. Position 61793311-61813310 in population HapMap CEU.

The associations between the *FADS* locus SNPs and PUFA concentrations in blood are very strong: homozygous individuals of the minor allele had 27% lower plasma concentrations of AA compared to homozygous individuals of the major allele. The SNP accounted for as much as 18.6% of the additive variance in AA concentrations (71). Homozygous carriers of the minor allele of *FADS1* may have a lower gene transcription and expression of *FADS1* (71, 104). If enzymatic activity is also reduced in individuals who are homozygous for the minor allele, this may decrease the efficiency of the fatty acid desaturation step that is initiated by Δ-5 desaturase, LA (C18:2ω-6) and ALA (C18:3ω-3) to modify AA (C20:4ω-6) and EPA (C20:5ω-3).

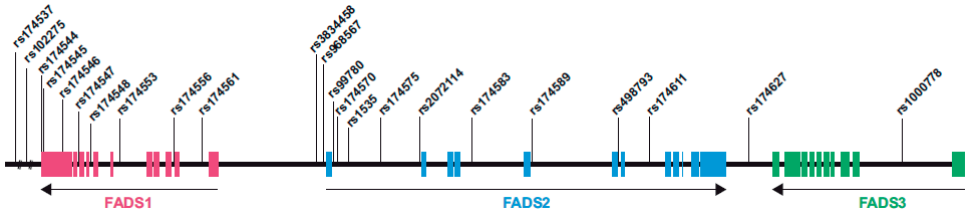

Figure 8. Schematic of the *FADS* gene cluster on chromosome 11.
Introns and exons are indicated, as are the SNPs with the statistically strongest association to fatty acid and lipid and lipoprotein concentrations. Reproduced with permission of the publishers (44).

Gene-environment interactions

Gene-environment interaction studies aim to describe how genetic and environmental factors work together to influence disease risk that cannot be explained by their separate marginal effects (107, 108). This concept is useful for evaluating multifactorial diseases in which environmental and genetic factors, and the thus far less understood interactions between them may importantly contribute. Gene-environment interactions can be described as changes to the association between a genetic variant and a disease or trait in the presence of a particular environmental exposure, or vice versa, such that the effect size of the genetic association is modified by the environmental exposure, or that the associated effect of the environmental exposure is modified by the genetic variant, or both (109). Interactions in epidemiology can be defined in different ways; statistical interaction, quantitative interaction, qualitative interaction, public health synergy and biological interaction, and the meaning of interaction depends on the context (108).

In gene-diet interaction studies (110), environmental exposure (diet) is difficult to measure accurately because it consists of numerous and varied components that are usually highly correlated with each other. Additionally, an individual's dietary intake may vary over time. It is therefore difficult to isolate the associated effects of individual dietary components. Thus, the dietary assessment method is crucial in all diet studies.

It is currently acknowledged that complex diseases probably arose as a result of intricate interplay between genetic and environmental factors, often including diet. Obtaining a better understanding of gene-diet interactions and how they might modulate the effects of the underlying genetic factors could reveal the potential mechanisms underlying a given disease or trait, which could be useful in clinical practice. However, although large observational cohort studies (111) and randomized intervention trials (112) have provided some insights into gene-diet interactions, there remains a need for further research and more widespread replication of results in this field. As an example from GWAS findings, the

chromosome 9p21 locus is the strongest and most validated CVD susceptible locus known thus far. Variation in this locus has been tested for interactions with environmental factors such as dietary patterns, physical activity and smoking in case-control and cohort studies (113). In this study, the increased risk of coronary event by the risk allele of 9p21 was attenuated among participants with by a prudent diet score. The interaction was mainly driven by the raw vegetable component of the prudent dietary pattern. However, no interactions with physical activity and smoking were observed (113). A more recent study in the MDC cohort demonstrated that the risk of coronary event by the 9p21 variant was attenuated among smokers (114).

Studies examining interactions between diet and genetic variation on lipid and lipoprotein concentrations are limited, and the majority of them have only focused on a small number of genetic variants (115, 116). As discussed above, recent GWASs have identified numerous loci that contribute to dyslipidemia and CVD risk (95, 98, 99, 117), and a very large meta-analysis of several GWASs (with >100,000 individuals) reported 95 significantly associated loci (95). Therefore, in this thesis, we employed these 95 validated loci to create GRSs for high TG, LDL-C and low HDL-C to examine the interaction between diet quality and GRSs on CVD incidence and change of lipid and lipoprotein concentrations by time.

3. Aims

Several pertinent reasons compelled us to use the MDC study to facilitate our own study of the interaction between dietary factors and genetic risk in dyslipidemia and CVD. First, the MDC study contains very detailed dietary information, which provides us with an opportunity to examine associations between diet and disease. Additionally, the MDC study included data on lifestyle factors and anthropometric measurements that are important to take into consideration as they may confound associations between diet and disease. Second, DNA is available from stored blood samples, which enables the genotyping of specific polymorphisms to account for genetic predisposition for dyslipidemia and CVD, in addition to gene-diet interactions. Furthermore, previous results from the MDC cohort have shown that participants with higher fiber intake (50) and a high quality diet have a reduced incidence of CVD (51) compared to those with a low quality diet. However, these studies have not taken into account genetic variation between individuals. Therefore, we aimed to further examine whether genetic factors modify associations between dietary factors, in particular PUFAs and diet quality, on blood lipid and lipoprotein concentrations and the incidence of CVD.

Overall aim

The overall aim of this doctoral thesis was to examine whether dietary intake, in particular diet quality and the intake of PUFAs, interacts with genetic factors on the lipid and lipoprotein concentrations and on CVD risk.

Specific aims

1. To study whether dietary intake of PUFAs interacts with *FADS1* genotype on TG, LDL-C and HDL-C concentrations (Paper I)
2. To study whether dietary intake of PUFAs interacts with *FADS1* genotype on CVD risk (Paper II)
3. To study whether a dietary quality index interacts with genetic risk for dyslipidemia on the risk of CVD, by combining the validated genetic variants into genetic risk scores (Paper III)
4. To study whether a dietary quality index is associated with changes in TG, LDL-C and HDL-C concentrations over a mean time of 16 years of follow-up (Paper IV)
5. To study whether a dietary quality index interacts with genetic risk for dyslipidemia on the change of TG, LDL-C and HDL-C concentrations over a mean time of 16 years of follow-up (Paper IV)

4. Participants and methods

4.1 The Malmö Diet and Cancer study

The MDC study is an urban population-based prospective cohort from the Southern Sweden including 30,447 individuals with baseline data collection conducted throughout the years 1991-96 (118). The main objective of the MDC study was to examine if diets high in total energy and fat, but low in vitamins and fiber increase the risk of cancers such as, breast, colon, rectum, pancreas, prostate, ovary and endometrium. The MDC study was planned by the Swedish Cancer Society and the International Agency for Research on Cancer (IARC) and the Faculty of Medicine, Lund University, Sweden (118).

In March 1991, all men and women born between 1926 and 1945 (119) and living in Malmö, the third largest city in Sweden which had about 250,000 citizens in the 1990's, were invited via personal letters, advertisements in newspapers and public places. However, in 1994, the recruitment was extended to all men born 1923-1945 and all women born 1923-1950 creating a source population of 74,138 individuals. The only compensation for participation was gifts such as T-shirts, pens and plastic bags.

The participants visited the MDC study screening center twice. During the first visit, groups of 6-8 participants were instructed by trained project staff for how to register meals in the menu book and the diet questionnaire and the extensive questionnaire covering lifestyle and socioeconomic factors including; 1) use of tobacco, 2) alcohol consumption, 3) leisure time physical activity, 4) sleeping habits, 5) education, 6) previous and current occupation (including physical and psychological conditions at work), 7) country of birth, 8) social network and support, 9) previous and current diseases, 10) medication and diet supplement use, 11) food habit changes in the past, 12) diseases among close relatives, 13) oral contraceptive use, and 14) reproductive factors (age at menarche, age at menopause, parity, breast feeding and miscarriage). All questionnaires were completed at home.

Nurses drew non-fasting blood samples, registered blood pressure and made anthropometric measurements (weight, height, waist and hip circumference, lean body mass and body fat mass). At the second visit, approximately two weeks after

the first visit, the participants were interviewed by trained dietary interviewers to complete the diet history and to check the socioeconomic questionnaire.

Out of the source population of 74,138 individuals, 1,975 persons were excluded due to limited Swedish language skills and mental incapacity. Additionally, 17 persons could not be identified, 3,017 died or moved before they received the first invitation letter and 224 persons died before they completed the baseline examination. Totally, 68,905 individuals were classified as eligible and when the recruitment closed in October 1996, 28,098 participants (11,063 men and 17,035 women) had completed the baseline examination regarding collection of lifestyle factors, dietary intake and anthropometrics which gave a participation rate of approximately 40% (120). Of those, 5,082 joined spontaneously from community advertisements and 23,016 were recruited by invitation letter. All individuals provided a written informed consent and the ethics committee of Lund University approved the MDC study protocols (LU 51–90).

The MDC study is together with 26 other prospective studies from 10 European countries participating in the European Prospective Investigation into Cancer and Nutrition (EPIC), which includes about 500,000 people (121). EPIC is organized by IARC, WHO, Lyon, France (122).

The Malmö Diet and Cancer study – Cardiovascular Cohort

Among MDC study participants recruited from November 1991 to February 1994 (n=12,445), a random 50% (n=6,103) was invited for an additional visit (after a mean 0.7 years) to further participate in a carotid artery disease study, the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC). Participants underwent a review of their medical history, a physical examination and a laboratory assessment of cardiovascular risk factors, such measurements including collection of overnight fasting blood for determination of fasting glucose and fasting serum lipid and lipoprotein concentrations (5, 118, 120). In total, 5,533 individuals have data on time between baseline- and follow-up examinations (mean 0.8 years, range -1.0 to 2.9 years).

During the years 2007 to 2012 (after an average of 16 years of follow-up, range 13-20 years) participants in this sub-cohort were invited to participate in a re-examination including analyses of fasting blood lipids and lipoproteins using the same methods as during the baseline, a questionnaire on lifestyle factors, and anthropometric measurements. In total 4,924 participants who were still alive and had not emigrated, were invited for the re-examination of whom 3,734 individuals attended.

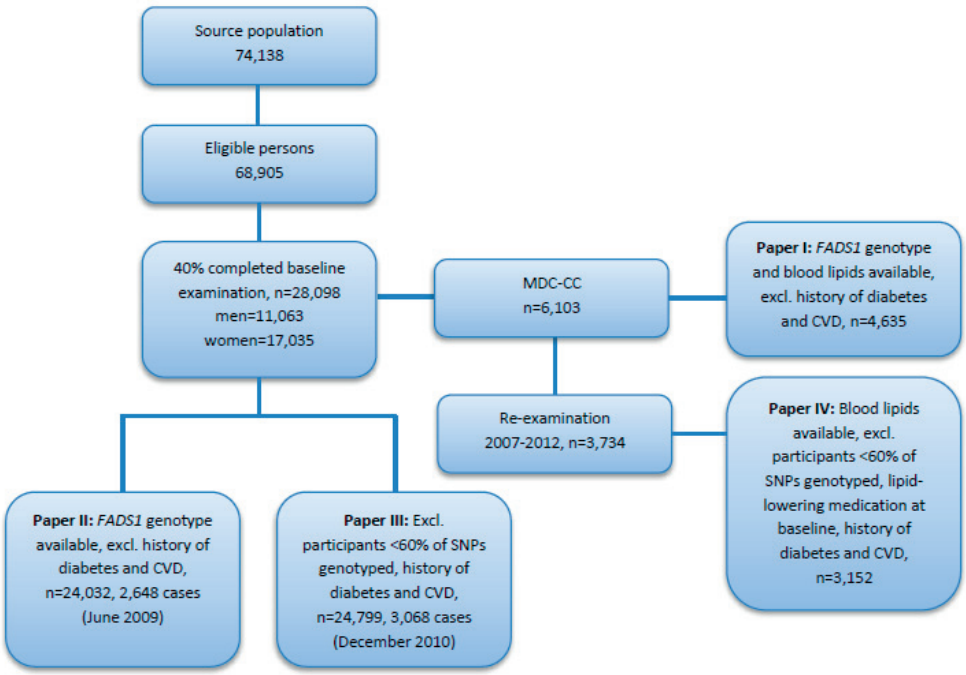

Figure 9. Study populations in paper I-IV

The Biobank

The blood was stored from each participant in different fractions; 10 ml was used for the serum sample (stored at -80ºC) and 30 ml was used to purify mononuclear leucocytes (-140ºC), granoulocytes (-80ºC), erythrocytes (-80ºC) and plasma (-80ºC). In the quality control program, the instrument variability, purity of the blood cell fraction and quality of the stored blood fraction are presented (123, 124).

4.2 Dietary assessment method

Dietary intake was measured by a modified diet history methodology by combining a 168-item dietary questionnaire, a 7-day menu book and a 1-h diet history interview, specifically designed for the MDC study (125). The 168-item dietary questionnaire covered food items regularly consumed during the past year, not covered by the menu book. The participants were asked to fill in the frequency of food intake and estimate the usual portion sizes using a booklet containing 48 photographs. Each set of photographs showed four different portion sizes of a

dish. The 7-day menu book covered meals that usually vary from day to day such as cooked lunch and dinner meals as well as cold beverages (including alcoholic beverages), medications, natural remedies and dietary supplements used by the participants. The questionnaire and menu book were filled out at home. About two weeks after the first visit at the study center, the participants were interviewed, for approximately one hour, about their food preparation practices, detailed food choices, e.g., type of bread and fat, and portion sizes (using a more extensive booklet of photos) of the food reported in the menu book. The trained interviewers checked the menu book and questionnaire for notably high reported intakes and for overlapping information. Furthermore, the total consumption of broader food groups such as bread, crisp bread, fruits and vegetables, was checked for reasonable values since it is easy to over-report one's intake when many different food items constitute a broader food group. In total 17 trained diet interviewers performed the interviews during the baseline examination period. The average daily food intake (grams per day) was calculated based on the information from the menu book, interview and questionnaire, and converted into nutrient and energy intakes by using the MDC Food and Nutrient Database, developed from the PC KOST-93 of the Swedish National Food Administration (125).

The dietary method was chosen to capture the total diet, with a special focus on total fat in an elderly urban population in Sweden. The eating habits in this population were expected to be fairly regular and commonly include cooked sit-down meals.

Validity and reproducibility

The relative validity of the modified diet history method used in the MDC study was examined in 105 women and 101 men, 50-69 years old and residents in Malmö, 1984-85. As the reference method a total of 18 days of weighed food records was collected during 3 days, every second month during one year to get the seasonal variation, as well as weekdays and weekends equally represented. The energy-adjusted Pearson correlations for men and women were between 0.50 and 0.80 for most of the food groups (126, 127) (**Table1**).

Reproducibility of the dietary assessment method was examined approximately one year after the first diet assessment point on 126 men and 115 women, 50-69 years old and residents in Malmö. Energy-adjusted Pearson correlations in men and women were between 60 and 80 for most of the food groups (128) (**Table 1**).

Dietary variables

PUFA variables (Paper I and II)

We constructed seven PUFA intake variables (including supplements); 1) ALA (18:3ω-3); 2) long-chain ω-3 PUFAs (EPA [20:5ω-3], docosapentanoic acid [DPA, 22:5ω-3] and DHA, [22:6ω-3]); 3) total ω-3 PUFAs (ALA, EPA, DPA and DHA); 4) LA (C18:2ω-6); 5) total ω-6 PUFAs (LA, γ-linoleic acid [GLA, 18:3n-6] and AA [20:4ω-6]); 6) ALA to LA ratio (ALA/LA), and 7) total ω-3 to total ω-6 PUFAs ratio (ω-3/ω-6 PUFAs). PUFA intakes were expressed as percentage of non-alcohol energy (E%) (Paper I) or by regressing the PUFA intakes on total energy intake (residual model) (Paper II). The participants were divided into tertiles and quintiles depending on their PUFA intakes (E%) (Paper I) and residual ranking (Paper II), respectively.

Diet quality index (Paper III and IV)

A diet quality index has been developed in the MDC to assess the adherence to the Swedish nutrition recommendations and the Swedish dietary guidelines issued in 2005 by Drake *et al.* (52). However, a revised version of the recommendations is under consideration at this time. The main points when developing the diet quality index were; 1) information on nutrients and dietary components had to be available in the MDC database; 2) the index was constructed to reflect overall diet quality by selecting dietary components that have previously been suggested to be associated with chronic diseases; and 3) the included components had to have low correlation with each other.

The diet quality index includes six dietary components; contribution to non-alcohol E% from 1) SFAs; 2) PUFAs (E%); 3) fish and shellfish (g/week); 4) dietary fiber (g/MJ); 5) fruit and vegetables (g/day); and 6) sucrose (E%). Cut-offs were set according to the Swedish nutrition recommendations from 2005: SFAs ≤14 E%, PUFAs 5-10 E%, fish and shellfish ≥300 g/week, fiber ≥2.4 g/MJ, fruit and vegetables ≥400 g/day and sucrose ≤10 E%. However, only 2% of the participants had an intake below the recommended level (≤10 E%) for SFAs; therefore, the cut-off for SFAs was increased to ≤14 E% (i.e., one SD increase). The fruit and vegetable component cut-off was lowered to ≥400 g/day instead of the original ≥500 g/day because fruit juices were excluded. One point was given to the participants for each dietary component that reached the recommended intake level, and zero points were given if they were not within the recommended range (52). A total score was created by summing the points, anddivided into three categories: low (0-1 points), medium (2-4 points) and high (5-6 points).

Table 1. Relative validity and reproducibility of the MDC dietary assessment method
Nutrients and components selected due to relevance in this thesis.

	Relative validity (men/women)[a]	Reproducibility (men/women)[b]
ALA (18:3ω-3)	0.22/0.58	0.40/0.58
LA (18:2ω-6)	0.23/0.68	0.72/0.74
AA (20:4ω-6)	0.55/0.44	0.82/0.58
EPA (20:5ω-3)	0.24/0.38	0.72/0.49
DPA (22:5ω-3)	0.37/0.40	0.77/0.60
DHA (22:6ω-3)	0.20/0.27	0.71/0.47
SFAs	0.56/0.68	0.64/0.62
PUFAs	0.26/0.64	0.68/0.70
Fish	0.35/0.70	0.78/0.22
Fiber	0.74/0.69	0.66/0.70
Fruits	0.60/0.77	0.80/0.81
Vegetables	0.65/0.53	0.71/0.76
Sucrose	0.60/0.74	0.78/0.46

a) Energy-adjusted Pearson correlation coefficients between daily intakes estimated by the MDC method and the reference method (18 days weight food record).

b) Energy-adjusted Pearson correlation coefficients between daily intakes estimated by the MDC method at the baseline and after 12 months.

Methodological variables

The season of the dietary interview was noted: winter (Dec–Feb), spring (Mar–May), summer (Jun–Aug) or autumn (Sep–Nov) because dietary food intake may vary with season. In September 1994, the routines for coding dietary data were slightly altered in order to shorten the interview time. The changes included standardized (instead of individualized) portion sizes for a few food items and standardized (instead of individualized) recipes for a few dishes. The diet assessment method version variable indicates whether the data were collected before or after the 1st of September 1994. This change did not appear to have any major influence on the ranking of participants (126).

4.3 Lifestyle and background variables

Age and sex

Age and sex were identified from each individual's person-identification number. In Sweden, each person is assigned a personal 10-digit number at birth: six digits indicate the birth date and one indicates the sex.

Anthropometric measurements

Participants were wearing light clothing and no shoes, using a balance-beam scale for weight (kg) and a fixed stadiometer for height (cm) conducted by trained project staff. Weight was measured to the nearest 0.1 kg. Thereafter body mass index (BMI) was calculated as weight in kilograms divided by square of height in meters (kg/m^2) from the direct measurements. Waist (midway between the lowest rib marginal and iliac crest) and hip circumferences (horizontally at the level of greatest lateral extension of the hip) were also measured by the trained staff at the first study visit.

Smoking

Three categories of smoking status were used: current (including irregular smoking), former and never smokers.

Alcohol

Participants were divided into five categories based on their alcohol habits (Paper I, II and III). Participants reporting no alcohol consumption during the last year in the questionnaire, who were also zero-reporters of alcohol in the 7-day menu book, were categorized as zero-consumers of alcohol. We divided the other study participants into categories (low, moderate, high and very high) based on their alcohol consumption (grams per day) with different cut-offs according to gender. The cut-off levels for females were 5, 10, and 20 grams of alcohol per day and the cut-off levels for males were 10, 20 and 40 grams of alcohol per day. Besides that, alcohol consumption was categorized into six groups (Paper IV). Participants were divided into gender-specific quintiles with zero-consumers as a separate category.

Education

Education categories were based on the type of education attained and the participants were divided into five categories: elementary or less, primary and secondary, upper secondary, further education without a degree, and university degree. Retired and unemployed participants were categorized according their position before retirement or unemployment.

Physical activity

Physical activity levels during leisure were calculated from a list of 17 pre-defined activities and 1 open activity option in the questionnaire that were adapted from the Minnesota Leisure Time Physical Activity Instrument (129, 130). The participants were asked to estimate the number of minutes per week, and for each of the four seasons, they spent on each activity. The time spent on each activity was multiplied with an intensity factor, creating a leisure time physical activity score. The score was moderately correlated to accelerometer measurements (131). Leisure time physical activity score was then divided into quintiles, with the same cut-offs for both genders.

Use of medication

At the baseline examination, information about use of anti-diabetes medication, lipid-lowering medication and hypertension medication were self-reported in the questionnaire and menu book. The information was obtained from two open-ended questions; 1) "Which prescription drugs and non-prescription drugs do you use on regular basis?" in the questionnaire and 2) the open-ended item for listing drug use in the 7-days menu book, both recorded at home. All pharmacologic agents reported in the personal diary or the questionnaire was classified according to the 1997 version of the Anatomic Therapeutic Chemical classification system.

At follow-up (Paper IV), use of lipid-lowering medication (LDL-lowering medication [Crestor, Lipitor, Pravachol, Zocord or Ezetrol] and used fibrates (Lopid) were self-reported in a questionnaire. Correction for lipid-lowering medication at follow-up were performed by adding a constant to the respective lipid and lipoprotein concentrations (statins: +0.208 mmol/L for triglycerides, -0.060 for HDL-C and +1.290 for LDL-C; fibrates: +0.645 for triglycerides, -0.153 for HDL-C and +1.037 for LDL-C) (132).

The Adult Treatment Panel III criteria for the metabolic syndrome was used to identify individuals with high plasma TG concentrations (≥1.7 mmol/L and/or triglyceride lowering treatment) and low HDL-C (using corrected values; <1.0

mmol/L for men and <1.3 mmol/L for women) (133). High LDL-C was defined as above 4.1 mmol/L and/or lipid lowering treatment (15).

Energy misreporters and food habit change in the past

Misreporters of energy intake were identified by comparing the individually estimated physical activity level (PAL) expressed as the energy expenditure divided by the basal metabolic rate (BMR) with energy intake divided by BMR (134). Individuals were defined as misreporters when the ratio of the reported energy intake to BMR was outside the 95% confidence limits of the calculated PAL (i.e., under- and over-reporters). Information regarding dietary change in the past (yes/no) was based on the question "Have you substantially changed your eating habits because of illness or for other reasons?". The dietary habits of participants reporting dietary changes in the past may reflect only a short period of their lives and may therefore have less influence on the development of chronic disease.

4.4 Laboratory analyses

Lipids and lipoproteins

Lipid and lipoprotein concentrations at the baseline examination and follow-up were analyzed with the same laboratory methods at the Department of Clinical Chemistry at the Skåne University Hospital in Malmö. Blood concentrations of fasting TG and total cholesterol were measured on a DAX 48 automatic analyzer using reagents and calibrators from the supplier of the instrument (Bayer AB, Göteborg, Sweden). HDL-C was determined by the same procedure as used for total cholesterol but after precipitation of LDL and VLDL with dextran sulphate. LDL-C concentration was calculated with the Friedewald formula: LDL-C = total cholesterol – HDL-C – (TGs/2.2) (120). To individuals with a TG concentration of more than 400 mg/dL (4.5 mmol/L), LDL-C was defined as missing (5).

Concentrations of lipoprotein subfractions at baseline were measured with the ion mobility method (135, 136). HDL particles were divided into small (76.5-105.0 Å) and large (105.0-145.0 Å) subfractions. LDL particles were divided into very small (LDL 3b, 4a, 4b and 4c; 180.0-204.9 Å), small (LDL 3a, 204.9-214.1 Å), medium (LDL 2b; 214.1-224.6 Å), and large (LDL 2a and 1; 224.6-233.3 Å) subfractions. IDL particles were divided into small (233.3-250.0 Å) and large (250.0-296.0 Å) subfractions. VLDL particles were divided into small (296.0-

335.0 Å), medium (335.0-424.0 Å), and large (424.0-547.0 Å) subfractions. Particle diameter of the major LDL peak was also determined.

Genotyping and the construction of genetic risk scores

The genotyping of the *FADS1* rs174547 (T/C) (Paper I) and *FADS1* rs174546 (C/T) (Paper II) was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry on the Sequenom Mass-ARRAY platform (San Diego, CA) at the Clinical Research Center, Malmö, Sweden.

Genotyping of rs174547 was successful in 5,806 (96%) of the 6,055 participants (Paper I) with DNA available and the SNP was in HWE (P=0.92). In addition, 5,490 of the 6,055 participants, of whom we still have DNA available, were additionally genotyped by the TaqMan allelic discrimination on ABI 7900 with a concordance rate for the two methods of 99.2%.

Genotyping of rs174546 was successful in 27,615 (96%) of the 28,768 participants (Paper II) with DNA available and the SNP was in HWE (P=1.00).

Genetic susceptibility to dyslipidemia (Paper III and IV) was estimated by combining the validated genetic variants reported in the GWAS meta-analysis by Teslovich *et al.* (95). All SNPs (n=91) that reached the GWAS significance level (i.e., $P<5x10^{-8}$) for TG, LDL-C or HDL-C were genotyped except *LPA* rs1084651, *JMJD1C* rs10761731 and *NPC1L1* rs217386 because of difficulties in genotyping or lack of proxies available. Genotyping was performed by Sequenom MassARRAY (Sequenom, San Diego, CA, USA) or Taqman allelic discrimination on an ABI 7900 (Applied Biosystems, Foster City, CA, USA). Thereafter, the SNPs with a success rate of less than 90% (i.e., *COBLL1* rs10195252, *KLF14* rs4731702, *PLEC1* rs11136341 and *ABCA8* rs4148008) and those with HWE *P*-values less than 0.00057 (0.05/87) (i.e., *ANGPTL3* rs2131925, *TYW1B* rs13238203, *SCARB1* rs838880, *OSBPL7* rs7206971, *LILRA3* rs386000, *PLTP* rs6065906 and *MOSC1* rs2642442) were excluded. Weighted GRSs were constructed using PLINK (version 1.07) for TG (26 SNPs), LDL-C (32 SNPs) and HDL-C (41 SNPs) by multiplying the effect size (i.e., β-coefficients) found in the meta-analysis (95) by the number of risk alleles and then summing the products. The respective GRSs explained 7.3% of the variance in LDL-C, 5.7% of the variance in HDL-C and 4.7% of the variance in TG.

4.5 Ascertainments of CVD cases

Incidence of cardiovascular events (i.e., coronary events and stroke as the first event) is continuously monitored by data-linkage with the Swedish Hospital Discharge register (137) and the Swedish Cause of Death register (138). Stroke events are also extracted from the local stroke register in Malmö (STROMA) (139). A coronary event was defined on the basis of codes 410–414 (fatal or non-fatal myocardial infarction or death due to ischemic heart disease) in the International Classification of Diseases, 9th Revision (ICD-9). Ischemic stroke was defined on the basis of code 434 (ICD-9) and diagnosed when computed tomography, magnetic resonance imaging or autopsy could verify the infarction and/or exclude hemorrhage and non-vascular disease. A stroke was classified as unspecified if neither imaging nor autopsy was performed. Hemorrhagic or non-specific stroke cases (ICD-9 codes 430, 431 and 436) were excluded because these subtypes of stroke do not have the same underlying risk factors as ischemic stroke. The National Tax Board provided information on vital status and emigration. The participants contributed person-time from their date of enrollment until their first CVD event, death, emigration from Sweden or the end of follow-up (i.e., June 30st, 2009 [Paper II] or December 31st 2010 [Paper III]).

4.6 Statistical methods

For all statistical analyses, Statistical Package for the Social Sciences (SPSS) 18.0/20.0/22.0 software for Windows (IBM corporation, Armonk, NY, USA) were used. Statistical significance was set at α=0.05 (i.e., P<0.05) and all P-values are 2-sided.

Statistical power

Statistical power was calculated using Quanto Software Version 2.1.4 (available at http://biostats.usc.edu/software). For paper I, we assumed a population risk at 10%, a genetic main effect (odds ratio) of 1.01 per *FADS1* allele (33% allele frequency and an additive genetic model), and an environmental main effect (odds ratio) of 1.01 per fatty acid intake quintile (Paper II); with 80% power and α=0.05 (2-sided) we are able to detect an interaction odds ratio of 1.02.

Paper I

Assuming an additive model, associations between baseline characteristics, including blood lipid and lipoprotein concentrations, and the *FADS1* rs174547 (T/C) genotype categories were investigated by using general linear model adjusted for age and sex (basic analysis) and thereafter age, sex and BMI. This was done because BMI can be regarded as a mediating factor between *FADS1* genotype and blood lipid and lipoprotein concentrations. All continuous variables except age were logarithmically (ln) transformed to achieve normal distribution when testing for trend across *FADS1* genotype categories and interaction between *FADS1* genotype categories and tertiles of dietary intake levels of PUFA on LDL-C, HDL-C and TG; before transformation, a very small amount (0.001 g) was added to ω-3 PUFA intake to handle zero intakes.

Interaction between *FADS1* genotype and tertiles of dietary PUFA intakes on serum lipid and lipoprotein concentrations were studied by introducing a multiplicative factor of genotypes and diet tertiles as continuous variables in addition to these main factors as separate variables. The interaction analyses were adjusted for potential confounders: age, sex, BMI, season of diet collection, smoking, leisure time physical activity, alcohol consumption, and total energy intake (including alcohol). Separate categories for smoking, alcohol consumption, and leisure-time physical activity were constructed for the participants with missing data to avoid exclusion of these individuals in the analyses.

In sensitivity analyses, potential misreporters of energy were excluded.

Paper II

The differences in baseline characteristics between the *FADS1* rs174546 (C/T) genotype categories, cases and non-cases of CVD, and between the lowest and highest PUFA intake quintiles were tested using chi-square analyses for categorical variables and with general linear model for continuous variables. All continuous variables except age were ln-transformed to achieve normal distribution when testing differences of means between the lowest and highest PUFA intake quintiles, cases and non-cases and for trend across *FADS1* genotype categories adjusted for age and sex, assuming an additive model. Before transformation, a very small amount (0.001 g) was added to ω-3 PUFA intakes to handle zero intakes. Spearman's bivariate correlation coefficient was used to examine associations between PUFAs and macronutrient intakes. Cox proportional hazard regression (hazard ratios [HR], 95% confidence interval [CI]) was used to examine association between *FADS1* rs174546 and incident CVD as well as PUFA intakes and incident CVD, with the lowest quintile as reference. Years of follow-up was used as the underlying time variable. To examine the trends across

quintiles, PUFA quintile variables were handled as continuous variables. The basic model was adjusted for age, sex, diet assessment method version, season of diet collection and total energy intake. Thereafter we included smoking, alcohol consumption, leisure time physical activity, education and BMI in the multivariate analysis. Separate categories for smoking, alcohol consumption, and leisure-time physical activity were constructed for the participants with missing data to avoid exclusion of these individuals in the analyses. These variables were selected from the literature for being known risk factors for CVD and for being associated with dietary PUFA intakes in this study. We also performed the multivariate model excluding BMI since it might be an intermediate between dietary habits and disease. In addition we adjusted for intakes of fiber and SFAs as these dietary factors are suspected to be associated with eating pattern and with incidence of CVD (50). We also performed the analyses adjusting for use of hypertension medication.

The association between PUFA intakes and incident coronary event and ischemic stroke was also analyzed separately. Interaction between PUFA intakes and *FADS1* genotype on incidence of CVD were examined by introducing a multiplicative factor of genotypes and PUFA quintiles as continuous variables in addition to these main factors as separate variables in a multivariate-adjusted model. To further examine the interactions between PUFA intakes and *FADS1* genotype categories on CVD risk we performed the analyses in strata of PUFA intakes. All analyses were also examined separately in men and women due to gender differences in food selection and reporting as well as biological differences. Formal tests for interaction by sex were also performed.

We also performed the analyses excluding cases that were diagnosed within two years after baseline examination.

In sensitivity analyses, we excluded participants reporting dietary changes in the past, because they may have unstable food habits, and potential misreporters of energy.

Paper III

Differences between baseline characteristics across the diet quality categories (i.e., low, medium and high) and GRSs per SD were tested in men and women separately using chi-square analyses for categorical variables and general linear models adjusted for age for continuous variables. All continuous variables except age were ln-transformed to achieve a normal distribution when testing for trends across the diet quality index (0 to 6) and GRSs (continuous); before transformation, a very small amount (i.e., 0.001 g) was added to fish and shellfish and fruit and vegetables intakes to handle zero intakes. Linear regression was used to examine the association between the GRSs and TG, LDL-C and HDL-C

concentrations at baseline. For analyses of the *P* for trend and variance explained, we used ln-transformed TG, LDL-C and HDL-C. A Cox proportional hazard regression was used to examine associations between GRSs and incident CVD, coronary events and ischemic stroke, adjusted for age and sex and with years of follow-up as the underlying time variable. Additionally, we examined these associations in strata of diet quality categories (i.e., low, medium and high) adjusted for age and sex and both with and without BMI because BMI can be regarded as a mediating factor. The combined diet index was the main dietary variable, but we also examined the associations between the GRSs and CVD with adherence to each component separately.

The influence of the interactions between the diet quality index and the three GRSs on CVD were examined by introducing multiplicative factors of GRSs and diet quality index as continuous variables, in addition to the main factors as separate variables, in a multivariate model adjusted for age, sex, dietary assessment method version, season of diet collection, total energy intake, smoking, alcohol consumption, leisure time physical activity, education and BMI. These variables were selected from the literature for being known risk factors for CVD (15, 23) and for being associated with dietary intakes in this study. Because many of the SNPs included in the GRSs have pleiotropic effects the analyses for each GRS were adjusted for the other two GRSs. To further interpret the statistically significant interactions between GRSs and the diet quality index on CVD risk, we examined associations with diet quality index (both as a continuous and categorical variable) in tertiles of GRSs. The first diet index category was used as the reference. All analyses were also carried out separately in men and women due to gender differences in food selection and reporting as well as biological differences. Formal tests for interactions by sex were also performed.

In sensitivity analyses, we excluded individuals reporting dietary changes in the past and potential misreporters of energy intake.

Paper IV

General Linear Model was used to estimate the association between diet quality index and clinical risk factors at baseline adjusted for age, sex (if applicable), season of diet collection, total energy intake, education, smoking, leisure time physical activity, alcohol consumption and waist circumference. We also examined the associations with blood lipid and lipoprotein concentrations at follow-up (with additional adjustments for follow-up time) and change in concentrations (delta-values) during the follow-up (with additional adjustments for follow-up time, and baseline lipid and lipoprotein concentrations). Change in waist circumference and smoking status during follow-up was included as covariates in an additional model with the analyses of lipid and lipoprotein change during the

follow-up. Trends across diet index categories were tested with diet quality index as continuous variable (0 to 6) and ln-transformed clinical risk factors. The combined diet quality index was the main exposure, but we also examined the associations with adherence to each individual component separately. Logistic regression was used to examine the association between diet quality index categories and risk of developing high TG, LDL-C or, low HDL-C. These analyses were adjusted for age, sex (if applicable), season of diet collection, total energy intake, education, smoking, leisure time physical activity, alcohol consumption, and waist circumference. Change in waist circumference and smoking status during follow-up was included as covariates in an additional model. All analyses were also performed for men and women separately due to difference in dietary habits and blood lipid and lipoprotein concentrations between men and women.

Interaction analyses between diet quality index and the three GRSs on baseline and change in blood lipids and lipoproteins were performed using continuous variables adjusted for sex, age, season of diet collection, total energy intake, education, smoking, leisure time physical activity, alcohol consumption and waist circumference; analyses with changes during follow-up were also adjusted for follow-up time and baseline lipid and lipoprotein concentrations.

In sensitivity analyses, we excluded potential misreporters of energy intake and participants reporting dietary changes in the past.

5. Results

Paper I

The aim of this study was to examine whether dietary intakes of PUFAs interact with *FADS1* rs174547 (T/C) genotype on TG, LDL-C and HDL-C concentrations (Aim 1). In this study we included 4,635 participants (60% women, age 45-68 years) from the MDC-CC.

FADS1 genotype and TG, LDL-C and HDL-C

We observed that each C-allele of *FADS1* rs174547 (allele frequency 34%) was associated with 0.05 mmol/L (i.e., 1.9 mg/dl) lower LDL-C concentration (*P*-trend=0.03, **Table 2**), but not with HDL-C or TG concentrations (*P*-trend=1.00 and *P*-trend=0.10, respectively) in the basic analysis (i.e., adjusted for age and sex). BMI was significantly associated with *FADS1* genotype and when BMI was included as a covariate in the model, a significant association of 0.02 mmol/L (i.e., 1.8 mg/dl) higher TG concentration per C-allele was observed (*P*-trend=0.04). Similar to the basic analysis, the association with LDL-C concentration remained significant (*P*-trend=0.047), and no association with HDL-C concentration was detected (*P*-trend=0.52) after adjusting for BMI.

We observed a significant interaction between the *FADS1* genotype and long-chain ω-3 PUFAs on LDL-C concentrations (*P*=0.01) (**Table 3**). The minor C-allele was significantly associated with lower LDL-C among individuals with low intakes of long-chain ω-3 PUFAs (≤0.14 E%, *P*<0.001), but not among those with medium (0.14-0.28 E%) or high (>0.28 E%) intakes (*P*=0.98 and *P*=0.86, respectively).

Table 2. Characteristics of the Malmö Diet and Cancer–Cardiovascular Cohort participants by *FADS1* rs174547 (T/C) genotype[a]

Characteristics	All (n=4,635)	T/T (n=2,054)	T/C (n=2,056)	C/C (n=525)	*P*-trend[c]
Women, n (%)	2795 (60.3)	1227 (59.7)	1240 (60.3)	328 (62.5)	0.52
Age (yr)	57.7 (52.3-62.6)	57.6 (52.2-62.3)	57.7 (52.2-62.6)	58.0 (52.2-62.7)	0.54
BMI (kg/m^2)	25.1 (22.8-27.7)	25.3 (23.0-27.8)	25.0 (22.9-27.3)	24.8 (22.7-27.3)	0.047
Fasting glucose (mmol/L)	4.9 (4.6-5.3)	4.9 (4.6-5.3)	4.9 (4.6-5.3)	4.9 (4.6-5.2)	0.44
LDL cholesterol (mmol/L)	4.10 (3.5-4.8)	4.10 (3.5-4.8)	4.10 (3.5-4.8)	4.00 (3.4-4.7)	0.03
HDL cholesterol (mmol/L)	1.35 (1.1-1.6)	1.36 (1.1-1.6)	1.36 (1.1-1.6)	1.36 (1.1-1.6)	1.00
TG (mmol/L)	1.14 (0.9-1.6)	1.13 (0.9-1.5)	1.13 (0.9-1.6)	1.18 (0.9-1.6)	0.10

a) Data is median (inter-quartile range) or number (%), if not otherwise indicated.

b) Participants (n) included; sex, age, diet variables and LDL-C, HDL-C and TG (n=4,635); BMI (n=4,633); fasting glucose (n=4,624).

c) ln-transformed, adjusted for age and sex.

Table 3. Association between *FADS1* rs174547 (T/C) for each additional C-allele and blood LDL-C, HDL-C and TG in strata of diet intakes among 4,635 participants

Dietary intakes (E%)	LDL-C			HDL-C			TG		
	β[a]	*P*[b]	*P*-int[c]	β[a]	*P*[b]	*P*-int[c]	β[a]	*P*[b]	*P*-int[c]
ALA			0.94			0.55			0.47
Low (≤0.65)	-0.034	0.39		-0.003	0.81		0.024	0.37	
Medium (0.65-0.80)	-0.078	0.03		0.003	0.98		0.028	0.29	
High (≥0.80)	-0.021	0.55		0.009	0.71		0.013	0.89	
LC ω-3 PUFAs			0.01			0.53			0.70
Low (≤0.14)	-0.138	<0.001		-0.006	0.43		0.025	0.30	
Medium (0.14-0.28)	0.001	0.98		0.007	0.69		0.020	0.53	
High (≥0.28)	-0.007	0.86		0.004	0.95		0.025	0.49	
ω-3 PUFAs			0.38			0.78			0.87
Low (≤0.86)	-0.071	0.05		0.004	0.67		0.006	0.91	
Medium (0.86-1.09)	-0.049	0.24		0.005	0.93		0.037	0.17	
High (≥1.09)	-0.013	0.62		0.001	0.78		0.024	0.55	
ω-6 PUFAs			0.35			0.90			0.90
Low (≤4.35)	-0.052	0.16		0.002	0.89		0.013	0.53	
Medium (4.35-5.48)	-0.074	0.04		0.004	0.97		0.025	0.33	
High (≥5.48)	-0.007	0.83		0.002	0.86		0.030	0.53	
ALA/LA			0.49			0.03			0.15
Low (≤0.14)	-0.022	0.51		-0.020	0.07		0.041	0.17	
Medium (0.14-0.16)	-0.055	0.12		0.015	0.35		0.040	0.25	
High (≥0.16)	-0.059	0.12		0.014	0.35		-0.013	0.78	
ω-3/ω-6 PUFAs			0.73			0.26			0.16
Low (≤0.17)	-0.056	0.11		-0.002	0.58		0.035	0.30	
Medium (0.17-0.22)	-0.018	0.77		-0.007	0.67		0.043	0.10	
High (≥0.22)	-0.064	0.05		0.015	0.38		-0.007	0.64	

a) β (effect size) = difference in lipid and lipoprotein concentration for each additional C-allele.

b) *P*-trend, adjusted for age and sex.

c) *P*-interaction, adjusted for age, sex, season, alcohol consumption, smoking, leisure time physical activity, BMI and total energy intake.

When examining within genotype categories, the high long-chain ω-3 PUFA intakes associated significantly with higher LDL-C concentration among the CC-genotype- ($P<0.001$) and TC-genotype carriers ($P=0.04$) but not among TT-genotype carriers ($P=0.17$) (**Figure 10**). In addition, there was a significant interaction between the *FADS1* genotype categories and ALA/LA intakes on HDL-C concentration ($P=0.03$) despite lack of significant associations between the *FADS1* genotypes and HDL-C concentration in any of the ALA/LA tertiles. However, we observed significant associations between ALA/LA and HDL-C concentration among CC- ($P=0.046$) and TC-genotype carriers ($P=0.02$) but not among those with the TT-genotype ($P=0.96$) (**Figure 11**). No significant

interactions were observed between the *FADS1* genotype categories and any of the different PUFA intake levels on TG concentration (**Table 3**). In sensitivity analyses, when excluding suspected misreporters of energy (19.3% of the study sample), the results remained substantially the same.

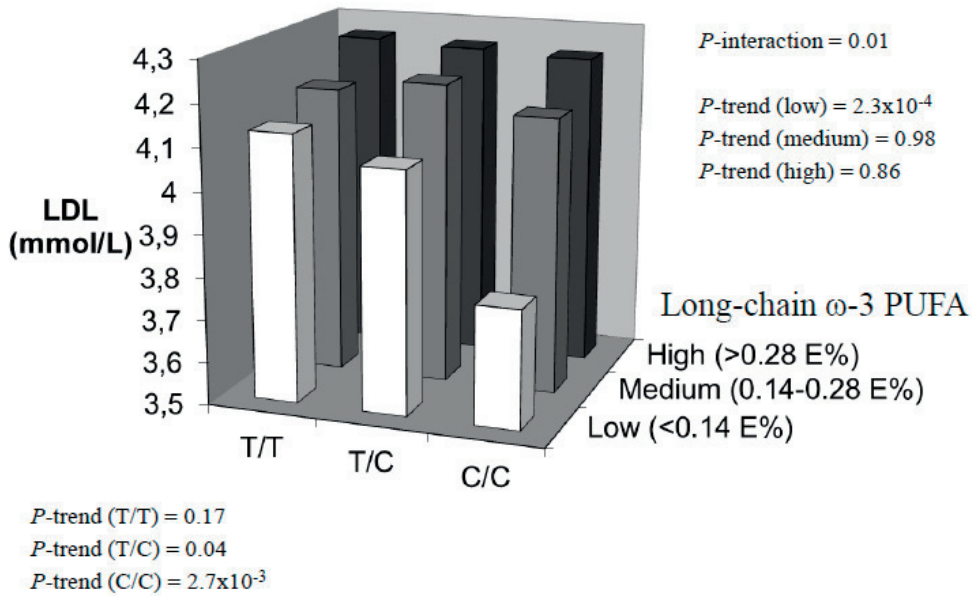

Figure 10. **Association between *FADS1* rs174547 (T/C) and LDL-C in strata of long-chain ω-3 PUFAs among 4,635 individuals**. The CC-genotype was associated with 0.14 mmol/L lower LDL-C concentration among individuals with low intakes of long-chain ω-3 PUFAs (≤0.14 E%, P=2.3x10^{-4}), but not among those with medium (0.14-0.28 E%) or high (>0.28 E%) intakes (P=0.98 and P=0.86, respectively). A low intake of long-chain ω-3 PUFAs was associated with 0.20 mmol/L and 0.06 mmol/L lower LDL-C concentration among individuals with CC-genotype (P=2.7x10^{-3}) and TC-genotype (P=0.04) but not among those with TT genotype (P=0.17).

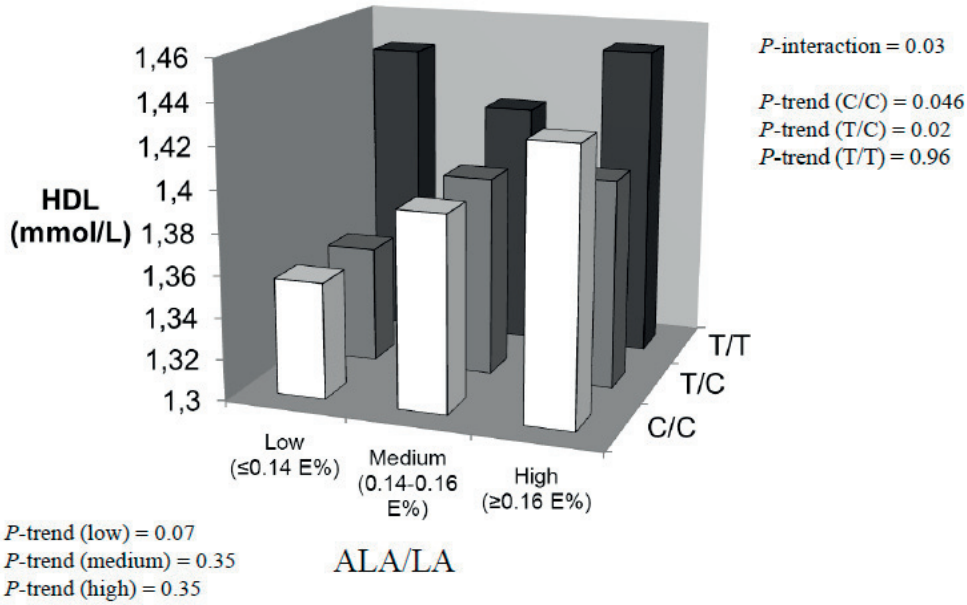

Figure 11. Association between ALA/LA and HDL-C in strata of *FADS1* rs174547 (T/C) among 4,635 participants. A high ALA/LA was associated with 0.04 mmol/L and 0.02 mmol/L higher HDL-C concentration among individuals with CC-genotype (*P*=0.046) and TC-genotype (*P*=0.02) but not among those with TT-genotype (*P*=0.96). There was no association between the CC-genotype and HDL-C in individuals with low, medium or high ALA/LA (*P*=0.07, *P*=0.35 and *P*=0.35, respectively).

Paper II

The aim of this study was to examine whether dietary intakes of PUFAs interact with *FADS1* rs174546 (C/T) genotype on the risk of CVD (Aim 2). We included 24,032 participants (62% women, age 44-74 years) from the MDC cohort in this study. During a mean follow-up time of 14 years (330,774 person-years) we identified 2,648 CVD cases.

PUFA intakes, *FADS1* genotype and CVD risk

As expected, men had a higher incidence of CVD and the CVD cases had higher age and BMI compared to those who did not develop CVD. The distribution of *FADS1* genotypes did not differ between incident CVD cases and non-cases (*P*=0.44). We did not observe any statistically significant association between any of the PUFA intakes and incident CVD neither in the basic analysis model (i.e., adjusted for age and sex, *P*-trend≥0.29) nor in the multivariate analysis (*P*-

trend≥0.28, **Table 4**). Excluding BMI or including intakes of fiber and SFAs in the multivariate analyses did not change the results substantially. Furthermore, *FADS1* rs174546 (C/T) was not associated with CVD risk (HR per allele=0.99, 95% CI: 0.93-1.04, *P*-trend=0.61). A borderline interaction was observed between the intake ratio of ALA/LA and *FADS1* genotype on CVD incidence (*P*=0.06) (**Table 5**).

Table 4. Hazard ratios of incident cardiovascular disease by PUFA quintiles and per 1 E% increase of PUFA intakes among 24,032 participants in the Malmö Diet and Cancer cohort[a]

Dietary PUFAs	PUFA intake quintiles					
	1 (n=4806)	2 (n=4807)	3 (n=4806)	4 (n=4807)	5 (n=4806)	Per 1 E% increase of PUFA intakes[b]
ALA						
Median (E%)	0.52[2]	0.63	0.72	0.82	0.99	
HR (95% CI)	1	0.93 (0.82-1.06)	1.04 (0.92-1.17)	0.97 (0.85-1.09)	0.98 (0.87-1.11)	1.07 (0.89-1.29)
LC ω-3 PUFAs						
Median (E%)	0.07	0.13	0.19	0.30	0.53	
HR (95% CI)	1	0.96 (0.85-1.10)	1.01 (0.89-1.15)	1.00 (0.88-1.13)	1.00 (0.88-1.14)	0.97 (0.82-1.16)
ω-3 PUFAs						
Median (E%)	0.68	0.83	0.96	1.10	1.37	1.02 (0.90-1.15)
HR (95% CI)	1	0.97 (0.85-1.10)	1.02 (0.90-1-15)	1.05 (0.93-1.19)	1.00 (0.88-1.13)	
LA						
Median (E%)	3.26	4.05	4.73	5.49	6.80	1.01 (0.99-1.04)
HR (95% CI)	1	1.15 (1.02-1.30)	1.08 (0.96-1.22)	0.99 (0.88-1.13)	1.15 (1.02-1.30)	
ω-6 PUFAs						
Median (E%)	3.32	4.11	4.79	5.55	6.86	1.01 (0.99-1.04)
HR (95% CI)	1	1.15 (1.02-1.30)	1.09 (0.96-1.23)	0.99 (0.87-1.12)	1.16 (1.03-1.31)	
ALA/LA						
Median (E%)	0.12	0.14	0.15	0.17	0.21	0.96 (0.88-1.05)
HR (95% CI)	1	0.90 (0.80-1.02)	0.94 (0.83-1.06)	0.96 (0.84-1.08)	0.92 (0.81-1.04)	
ω-3/ω-6 PUFAs						
Median (E%)	0.14	0.17	0.19	0.23	0.30	0.97 (0.92-1.02)
HR (95% CI)	1	1.01 (0.89-1.15)	1.01 (0.89-1.14)	1.01 (0.89-1.15)	0.95 (0.83-1.07)	

a) Cox proportional hazard regression was used to calculate HRs (95% CI) for each quintile of PUFA intakes with the lowest quintile as reference and $P<0.05$. Multivariate model were adjusted for age, sex, BMI, diet assessment method version, season, total energy intake, alcohol intake, leisure time physical activity, education and smoking.

b) Per unit increase is 0.1 for ratios of PUFAs.

Table 5. Hazard ratios for per 1 E% increase of PUFA intakes in strata of *FADS1* rs174546 (C/T) genotype on incidence of total cardiovascular disease, coronary event and ischemic stroke among 24,032 participants in the Malmö Diet and Cancer cohort[a]

	CC	CT	TT	*P*-interaction[b]
Total CVD				
ALA	1.21 (0.92-1.59)	1.03 (0.77-1.38)	0.85 (0.47-1.55)	0.22 (0.07)[c]
LC ω-3 PUFAs	0.93 (0.72-1.20)	1.02 (0.78-1.33)	1.21 (0.69-2.13)	0.93 (0.73)[c]
Total ω-3 PUFAs	1.04 (0.87-1.26)	1.02 (0.84-1.24)	1.02 (0.68-1.53)	0.64 (0.50)[c]
LA	1.03 (0.99-1.07)	1.00 (0.96-1.04)	1.04 (0.96-1.13)	0.74 (0.88)[c]
Total ω-6 PUFAs	1.03 (0.99-1.07)	1.00 (0.96-1.04)	1.04 (0.96-1.13)	0.75 (0.90)[c]
ALA/LA	0.97 (0.85-1.11)	1.01 (0.88-1.16)	0.76 (0.58-1.00)	0.06 (0.04)[c]
ω-3/ω-6 PUFAs	0.96 (0.89-1.04)	1.00 (0.92-1.08)	0.91 (0.77-1.08)	0.27 (0.34)[c]
Coronary event				
ALA	1.22 (0.85-1.74)	1.12 (0.78-1.62)	1.56 (0.78-3.13)	0.86
LC ω-3 PUFAs	1.02 (0.73-1.41)	0.95 (0.67-1.36)	0.74 (0.34-1.61)	0.48
Total ω-3 PUFAs	1.10 (0.87-1.39)	1.02 (0.80-1.32)	1.09 (0.65-1.82)	0.64
LA	1.03 (0.98-1.08)	0.98 (0.93-1.03)	1.11 (1.00-1.23)	0.31
Total ω-6 PUFAs	1.03 (0.98-1.08)	0.98 (0.93-1.03)	1.11 (1.00-1.23)	0.31
ALA/LA	0.96 (0.81-1.15)	1.09 (0.92-1.30)	0.83 (0.59-1.17)	0.30
ω-3/ω-6 PUFAs	0.98 (0.89-1.08)	1.02 (0.92-1.13)	0.85 (0.68-1.06)	0.26
Ischemic stroke				
ALA	1.21 (0.79-1.85)	0.93 (0.59-1.47)	0.27 (0.09-0.77)	0.03
LC ω-3 PUFAs	0.81 (0.53-1.22)	1.11 (0.74-1.67)	2.21 (0.99-4.96)	0.47
Total ω-3 PUFAs	0.97 (0.73-1.30)	1.02 (0.76-1.38)	0.91 (0.47-1.75)	0.88
LA	1.03 (0.97-1.09)	1.02 (0.96-1.09)	0.94 (0.81-1.08)	0.48
Total ω-6 PUFAs	1.03 (0.97-1.09)	1.03 (0.96-1.09)	0.94 (0.82-1.08)	0.47
ALA/LA	0.97 (0.80-1.19)	0.90 (0.72-1.12)	0.65 (0.41-1.04)	0.08
ω-3/ ω-6 PUFAs	0.92 (0.82-1.04)	0.97 (0.85-1.10)	1.00 (0.77-1.29)	0.73

a) Values are HRs (95% CIs). Cox proportional hazard regression model was used to calculate the HR per each quintile of PUFA intakes with the lowest quintile as reference and per-unit increase of PUFA intakes (1 E% for PUFA intakes and 0.1 for PUFA ratios), $P<0.05$. Multivariate model were adjusted for age, sex, BMI, diet assessment method version, season, total energy intake, alcohol intake, leisure time physical activity, education and smoking.

b) Quintile of PUFA intakes×genotype.

c) Values in parentheses are *P*-values for sensitivity analysis. Sensitivity analyses excluded those reporting dietary change in the past and potential misreporters of energy, n=15,538.

ALA/LA was only inversely associated with CVD risk among the TT-genotype carriers of *FADS1* rs174546 (HR for quintile 5 vs. quintile 1=0.72, 95% CI: 0.50-1.04, *P*-trend=0.049) (**Figure 12**). The above reported results remained essentially the same when including intakes of fiber, SFAs or use of anti-hypertensive medication in the multivariate model.

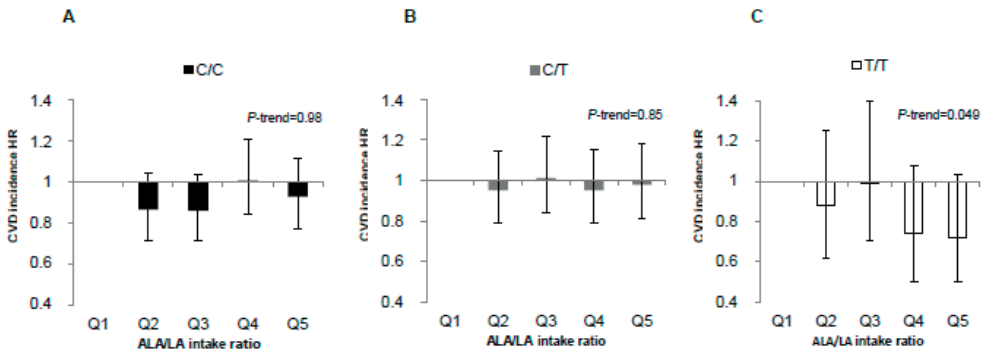

Figure 12. Association between ALA/LA intake ratio and incidence of CVD according to *FADS1* rs174546 genotype CC (A), TC (B) and TT (C) among 24,032 participants in the Malmö Diet and Cancer cohort. Cox proportional hazard regression was used to calculate HR for each quintile of PUFA intakes with the lowest quintile as reference. Multivariate model were adjusted for age, sex, BMI, diet assessment method version, season, total energy intake, alcohol consumption, leisure time physical activity, education and smoking. The median ALA/LA intake ratio and n per quintile; quintile 1=0.12, n=4,806; quintile 2=0.14, n=4,807; quintile 3=0.15, n=4,806; quintile 4=0.17, n=4,807; quintile 5=0.21, n=4,806.

We also examined the associations separately for coronary events and ischemic stroke and we observed a significant interaction between ALA and *FADS1* genotype on risk of ischemic stroke incidence (*P*=0.03) where ALA was inversely associated with risk of ischemic stroke only among TT-genotype carriers (HR for quintile 5 vs. quintile 1=0.50, 95% CI: 0.27-0.94, *P*-trend=0.02). Additionally, we observed a borderline interaction between ALA/LA and *FADS1* genotype on ischemic stroke incidence (*P*=0.08). However, there was no significant association between the ALA/LA intakes and ischemic stroke incidence among the TT-genotype carriers (*P*-trend=0.17).

In sensitivity analyses we excluded participants reporting dietary changes in the past and those being suspected misreporters of energy (35% of the study sample). The findings after this exclusion were in line with previous results; however, the interaction between ALA/LA intake and *FADS1* genotype on CVD incidence was slightly strengthened and statistically significant (*P*=0.04, **Table 5**).

Paper III

In this paper, our aim was to examine whether a diet quality index interacts with genetic risk for dyslipidemia on the risk of CVD, by combining the validated gene variants into genetic risk scores (Aim 3). We included 24,799 participants (62% women, age 44-74 years) from the MDC cohort in this study. During a mean follow-up of 15 years (369,996 person-years), 3,068 CVD cases were identified.

Baseline characteristics

GRSs were composed of 26 SNPs for TG, 32 SNPs for LDL-C and 41 SNPs for HDL-C. The GRSs explained 7.3, 4.7 and 5.7%, respectively, of the variance in the traits. We also tested for associations between baseline characteristics and the GRSs in cases in which we observed several statistically significant associations; for example, all the GRSs were significantly associated with higher dietary fiber intakes and lower SFA intakes. These associations were in the same direction but attenuated when excluding diet changers in the past and potential misreporters of energy.

Genetic risk for dyslipidemia and CVD

GRSLDL-C (P=5x10^{-6}) and GRSHDL-C (P=0.02) but not GRSTG (P=0.08) were significantly associated with an increased risk of CVD after adjusting for age and sex (**Table 6**). GRSLDL-C (P=1.5x10^{-4}) and GRSTG (P=0.01) but not GRSHDL-C *(*P=0.18) were significantly associated with an increased risk of coronary events. Additionally, GRSLDL-C (P=0.02) and GRSHDL-C (P=0.04) but not GRSTG (P=0.59) were associated with an increased risk of ischemic stroke.

When we adjusted for pleiotrophy (i.e., adding the other two GRSs to the statistical model), the risk estimates observed above remained approximately the same although the P-values were slightly changed (**Table 6**).

Table 6. Hazard ratios (95% CI) of incident total cardiovascular disease, coronary event and ischemic stroke per 1 SD increase of the genetic risk scores among 9,383 men and 15,416 women in the Malmö Diet and Cancer[a]

GRSs	Total CVD		Coronary event		Ischemic stroke	
	Cases n_{men}= 1,759/n_{women}=1,309		Cases n_{men}= 1,129/n_{women}=685		Cases n_{men}=630/n_{women}=624	
	Model 1[b]	Model 2[c]	Model 1	Model 2	Model 1	Model 2
GRS_{LDL-C}						
All	1.09 (1.05-1.13)	1.08 (1.04-1.12)	1.09 (1.04-1.15)	1.08 (1.03-1.14)	1.07 (1.01-1.13)	1.07 (1.01-1.14)
Men	1.08 (1.03-1.13)	1.07 (1.02-1.12)	1.08 (1.02-1.14)	1.07 (1.01-1.14)	1.07 (0.99-1.16)	1.07 (0.99-1.16)
Women	1.10 (1.04-1.16)	1.09 (1.03-1.15)	1.13 (1.05-1.22)	1.11 (1.03-1.20)	1.07 (0.99-1.16)	1.07 (0.99-1.16)
GRS_{HDL-C}						
All	1.05 (1.01-1.08)	1.03 (1.00-1.07)	1.03 (0.99-1.08)	1.00 (0.95-1.05)	1.06 (1.00-1.12)	1.08 (1.01-1.14)
Men	1.03 (0.98-1.08)	1.01 (0.96-1.06)	1.01 (0.95-1.07)	0.97 (0.91-1.04)	1.06 (0.98-1.15)	1.08 (0.99-1.18)
Women	1.07 (1.01-1.13)	1.06 (1.00-1.13)	1.08 (1.00-1.16)	1.05 (0.97-1.14)	1.06 (0.98-1.14)	1.07 (0.98-1.17)
GRS_{TG}						
All	1.03 (1.00-1.07)	1.00 (0.96-1.04)	1.07 (1.02-1.12)	1.05 (1.00-1.10)	0.99 (0.93-1.04)	0.94 (0.88-1.00)
Men	1.03 (0.99-1.08)	1.02 (0.96-1.07)	1.06 (1.00-1.13)	1.06 (1.00-1.14)	0.98 (0.91-1.06)	0.94 (0.86-1.02)
Women	1.03 (0.98-1.09)	0.98 (0.93-1.04)	1.07 (0.99-1.15)	1.02 (0.94-1.11)	0.99 (0.91-1.07)	0.94 (0.86-1.03)

a) Cox proportional hazard regression model.

b) Model 1 is adjusted for age and sex.

c) Model 2 is adjusted for age, sex and the two GRSs simultaneously.

Interaction with diet quality index

No significant interaction between the GRSs and diet quality was observed regarding the incidence of CVD ($GRS_{LDL\text{-}C}$ *P*=0.39, $GRS_{HDL\text{-}C}$ *P*=0.85, and GRS_{TG} *P*=0.86) (**Table 7**). When coronary and ischemic stroke events were examined separately, we observed a significant interaction between $GRS_{LDL\text{-}C}$ and diet quality index on ischemic stroke incidence (*P*=0.01). When the analysis was stratified by dietary quality categories, a high compared to a low diet quality attenuated the association between $GRS_{LDL\text{-}C}$ and the risk of incident ischemic stroke (HR per SD, HRl_{ow}=1.08 [0.95-1.24], *P*=0.26; HR_{medium}=1.10 [1.03-1.17], *P*=0.01; HR_{high}=0.93 [0.79-1.10], *P*=0.40 (**Table 7**). When we examined the association between the diet quality index and the incidence of ischemic stroke in tertiles of $GRS_{LDL\text{-}C}$, we observed only a significant inverse association between the diet quality index and the incidence of ischemic stroke among the participants in the highest tertile of $GRS_{LDL\text{-}C}$ (HR for highest vs. lowest diet quality=0.64, [0.44-0.95], *P*-trend=0.001) (**Figure 13**). Adding BMI to the statistical model did not markedly change the results.

Table 7. Hazard ratios per 1 SD increase of the genetic risk scores in strata of the diet quality index on incidence of total cardiovascular disease, coronary event and ischemic stroke among 24,799 participants in the Malmö Diet and Cancer cohort[a]

	Diet quality index			*P*-interaction[b]
	Low	Medium	High	
	n=3,360/530	n=15,538/2,186	n=2,833/352	
	HR (95% CI)	HR (95% CI)	HR (95% CI)	
Total CVD				
$GRS_{LDL\text{-}C}$	1.11 (1.02-1.21)	1.09 (1.04-1.14)	1.07 (0.96-1.19)	0.39 (0.86)[c]
$GRS_{HDL\text{-}C}$	1.08 (0.99-1.18)	1.03 (0.99-1.07)	1.10 (0.99-1.22)	0.85 (0.58)[c]
GRS_{TG}	1.02 (0.93-1.11)	1.03 (0.99-1.08)	1.05 (0.95-1.17)	0.86 (0.20)[c]
Coronary event	Cases n=313	Cases n=1,285	Cases n=216	
$GRS_{LDL\text{-}C}$	1.13 (1.01-1.26)	1.08 (1.02-1.14)	1.15 (1.01-1.32)	0.33 (0.08)[c]
$GRS_{HDL\text{-}C}$	1.02 (0.91-1.14)	1.03 (0.97-1.08)	1.11 (0.97-1.27)	0.35 (0.78)[c]
GRS_{TG}	1.06 (0.95-1.19)	1.06 (1.01-1.12)	1.09 (0.95-1.25)	0.78 (0.23)[c]
Ischemic stroke	Cases n=217	Cases n=901	Cases n=136	
$GRS_{LDL\text{-}C}$	1.08 (0.95-1.24)	1.10 (1.03-1.17)	0.93 (0.79-1.10)	0.01 (0.07)[c]
$GRS_{HDL\text{-}C}$	1.16 (1.02-1.33)	1.04 (0.97-1.11)	1.07 (0.91-1.26)	0.18 (0.21)[c]
GRS_{TG}	0.96 (0.84-1.10)	0.99 (0.93-1.06)	0.99 (0.83-1.17)	0.98 (0.59)[c]

a) Cox proportional hazard regression was used to calculate HRs (95% CI) per 1 SD increase of the GRSs, *P*<0.05, adjusted for age and sex.

b) *P*-interactions (GRSs×diet quality index as continuous variables) were adjusted for age, sex, BMI, diet assessment method version, season, total energy intake, alcohol consumption, leisure time physical activity, education and smoking.

c) *P*-values in parentheses are sensitivity analyses excluding those reporting dietary changes in the past and potential misreporters of energy, n=16,030.

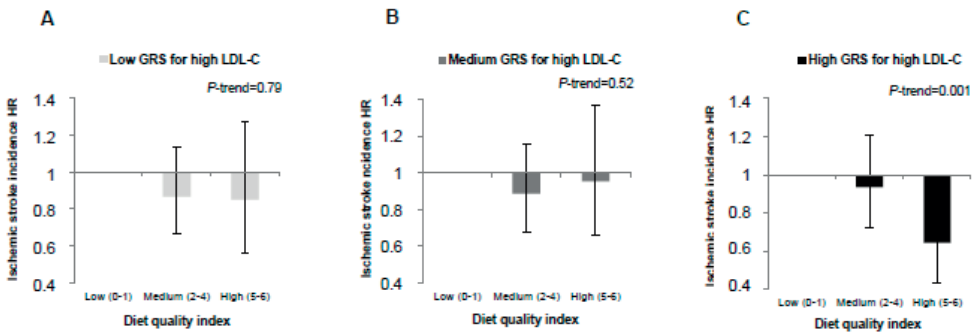

Figure 13. Association between the diet quality index and incidence of ischemic stroke according to tertiles of $GRS_{LDL\text{-}C}$, low (A), medium (B) and high (C) among 24,799 participants in the Malmö Diet and Cancer cohort. A Cox proportional hazard regression was used to calculate HRs for each diet quality category with the lowest category as a reference. Multivariate models were adjusted for age, sex, BMI, diet assessment method version, season, total energy intake, alcohol consumption, leisure time physical activity, education and smoking. In tertiles of $GRS_{LDL\text{-}C}$ (non-cases/cases) of ischemic stroke; low n=7,293/395; medium n=7,272/415; high n=7,166/444.

Thereafter, we examined the interaction between the GRSs and each diet index component on the incidence of CVD, coronary events and ischemic stroke. There was no significant interaction between any of the diet index components and the GRSs on incidence of CVD or coronary events. However, we observed a significant interaction between $GRS_{LDL\text{-}C}$ and fruit and vegetable intake (P=0.01) on ischemic stroke incidence.

In sensitivity analyses we excluded individuals reporting dietary changes in the past and those with suspected misreporting of energy (35% of study sample), the results reported above remained substantially the same. Overall, the results did not markedly change when adding the two GRSs simultaneously to the statistical model.

Paper IV

The two aims of this study were to examine whether a diet quality index associates with change in TG, LDL-C and HDL-C concentrations (in this paper referred to as standard blood lipids) during 16 years of follow–up (Aim 4), and if a diet quality index interacts with genetic risk for dyslipidemia on the change in standard blood lipids during 16 years of follow–up (Aim 5). We included 3,152 participants (61% women, age 46-68 years) from the MDC cohort who had standard lipid concentrations measured both at the baseline examination (i.e., 1992-1994) and after an average of 16 years of follow-up.

Baseline characteristics

The diet quality index was positively associated with age, but not with BMI. In addition, we observed lower frequency of smokers and individuals with low education and higher frequency of individuals with high physical activity in high compared to low diet quality index groups. At follow-up, 28% of the participants were using lipid-lowering medication; however, it was not more common in any of the diet index groups. During the 16 years of follow-up, the average TG and LDL-C concentration had decreased both with and without correction for lipid-lowering medication. The average HDL-C concentration was approximately the same at baseline and follow-up.

During the same time, the average BMI had increased from 25.3 (SD: ± 3.6) kg/m^2 to 26.7 ± 4.3 kg/m^2 and waist circumference from 82 ± 12 cm to 92 ± 12 cm. The frequency of smokers was 23% at baseline compared with 9% at follow-up.

Diet quality index and blood lipids and lipoproteins

The diet quality index was only marginally associated with blood lipid and lipoprotein concentrations when cross-sectionally analyzed at baseline. However, among individuals with a high diet quality we generally observed a better lipoprotein profile (i.e., higher HDL-C, large LDL, lower medium and large VLDL in analyses with men and women combined, compared to those with low diet quality.

The associations between diet quality index and blood lipid and lipoprotein concentrations were rather strong even after 16 years of follow-up (**Table 8**). After excluding diet changers in the past and misreporters, all associations between diet quality index and blood lipids and lipoproteins were statistically significant (*P*-values for TG=0.008; HDL-C=0.006; and LDL-C=0.03).

Table 8: Standard blood lipids at follow-up and change in standard lipids during follow-up (means and 95% CI) by categories of the diet quality index in 1,222 men and 1,930 women in the Malmö Diet and Cancer cohort

		Low (0-1)	Medium (2-4)	High (5-6)	*P*-trend
TG at follow-up[a]	All	1.19 (1.13, 1.24)	1.12 (1.09, 1.15)	1.15 (1.10, 1.19)	0.07 (**0.008**)[b]
	Men	1.22 (1.13, 1.31)	1.11 (1.06, 1.16)	1.18 (1.10, 1.27)	0.22 (**0.04**)
	Women	1.15 (1.09, 1.22)	1.12 (1.08, 1.15)	1.12 (1.06, 1.17)	0.17 (0.08)
HDL-C at follow-up	All	1.38 (1.34, 1.42)	1.42 (1.40, 1.44)	1.41 (1.38, 1.45)	0.10 (**0.006**)
	Men	1.16 (1.10, 1.22)	1.24 (1.20, 1.27)	1.19 (1.13, 1,25)	0.52 (0.07)
	Women	1.52 (1.46, 1.58)	1.54 (1.51, 1.57)	1.55 (1.51, 1.60)	0.11 (**0.04**)
LDL-C at follow-up	All	3.65 (3.57, 3.74)	3.67 (3.62, 3.71)	3.61 (3.54, 3.68)	0.30 (**0.03**)
	Men	3.49 (3.36, 3.62)	3.54 (3.47, 3.61)	3.43 (3.31, 3.55)	0.20 (0.08)
	Women	3.75 (3.64, 3.87)	3.74 (3.68, 3.80)	3.71 (3.62, 3.81)	0.80 (0.18)
ΔTG[c]	All	-0.09 (-0.14, -0.04)	-0.13 (-0.16, -0.11)	-0.11 (-0.15, -0.07)	0.11 (**0.03**)
	Men	-0.20 (-0.28, -0.12)	-0.26 (-0.31, -0.22)	-0.22 (-0.30, -0.14)	0.42 (0.07)
	Women	-0.02 (-0.08, 0.04)	-0.06 (-0.09, -0.03)	-0.05 (-0.09, 0.002)	0.14 (0.28)
ΔHDL-C	All	-0.01 (-0.04, 0.02)	0.01 (-0.004, 0.03)	-0.003 (-0.03, 0.02)	0.43 (0.18)
	Men	-0.04 (-0.08, 0.01)	0.01 (-0.01, 0.04)	-0.01 (-0.06, 0.03)	0.49 (0.11)
	Women	0.01 (-0.03, 0.06)	0.02 (-0.01, 0.04)	0.01 (-0.03. 0.04)	0.59 (0.57)
ΔLDL-C	All	-0.48 (-0.55, -0.41)	-0.44 (-0.48, -0.40)	-0.54 (-0.60, -0.48)	**0.047 (0.02)**
	Men	-0.66 (-0.77, -0.55)	-0.59 (-0.65, -0.53)	-0.70 (-0.81, -0.60)	0.23 (0.31)
	Women	-0.37 (-0.46, -0.27)	-0.35 (-0.40, -0.30)	-0.43 (-0.51, -0.35)	0.14 (0.06)

a) Standard blood lipids at follow-up were adjusted for the following variables collected at baseline: sex, season of diet collection, energy intake, smoking, education, leisure time physical activity, alcohol consumption, waist circumference, and follow-up time.

b) In parenthesis: results excluding individuals reporting dietary changes in the past and misreporters of energy, n=2,001.

c) Change in standard blood lipids during follow-up was also adjusted for ln-transformed baseline lipid and lipoprotein concentrations.

Diet quality and standard blood lipids

When longitudinally analyzed, we found no significant associations between diet quality index and change in standard blood lipids during follow-up (delta-values) (**Table 8**). However, after excluding diet changers and misreporters, those with a high diet quality had a more pronounced decrease in TG (*P*=0.03) and LDL-C (*P*=0.02) concentrations compared to those with low diet quality (**Table 8**). Adjusting for change in waist circumference and smoking habits during follow-up slightly attenuated these associations (*P*=0.10 and 0.03 for TG and LDL-C, respectively).

Diet quality index was inversely associated with risk of developing high TG and LDL-C. Among those with normal TG concentrations at baseline (82% of the participants), 11% had developed hypertriglyceridemia in the low diet quality group and 6% in the high diet quality group. The risk of developing hypertriglyceridemia during follow-up were 46% lower (95% CI: 5-69%) among those with a high compared with a low diet quality (**Table 9**). The risk estimates were stronger after excluding diet changers and misreporters. However, adjusting for waist circumference and smoking did not affect the results.

Table 9: Risk of developing dyslipidemia according to the diet quality index among 3,152 participants in the Malmö Diet and Cancer cohort[a]

	Sample size (cases)	Incident cases in low/medium/high index groups	Low (0-1)	Medium (2-4)	High (5-6)	*P*-trend
High TG	2587 (168)	11%/6%/6%	1.00	**0.57 (0.37-0.88)**	**0.54 (0.31-0.95)**	**0.02 (0.02)[b]**
Low HDL-C	2330 (380)	16%/17%/17%	1.00	1.03 (0.71-1.48)	1.07 (0.69-1.65)	0.93 (0.20)
High LDL-C	1686 (876)	54%/53%/48%	1.00	0.94 (0.69-1.28)	0.75 (0.51-1.09)	**0.03 (0.03)**

a) Logistic regression was used to estimate ORs with 95% CI adjusted for age, sex, season of diet collection, energy intake, smoking, education, leisure-time physical activity, alcohol consumption, waist circumference.

b) *P*-values in parenthesis: results excluding individuals reporting dietary changes in the past and misreporters of energy, n=2,001.

Dietary components and standard blood lipids

Those with a low intake of sucrose had lower TG and higher HDL-C concentrations at baseline compared with those that did not reach the recommendation for sugar. We also observed this difference between the groups with HDL-C at follow-up (*P*=0.0001), but not for TG concentrations (*P*=0.18). Those that reached the dietary fiber recommendation showed stronger decrease in

TG levels during follow-up (on average -0.15 mmol/L) compared with those with a lower fiber intake (on average -0.11 mmol/L). After excluding misreporters and diet changers in the past, these findings were still statistically significant and in general, stronger associations were observed.

Interaction with genetic risk for dyslipidemia

We found a significant interaction between diet quality index and GRS for low HDL-C on change in HDL-C during follow-up (P=0.04) (**Table 10**). Specifically, we found an association between diet quality and HDL-C change during follow-up among those with lower genetic risk for low HDL-C but not among those with higher genetic risk; among those with low genetic risk for low HDL-C, low diet quality was associated with decreased HDL-C during follow-up.

Table 10: Interaction between the diet quality index and the genetic risk scores on baseline and changes in standard lipids during 16 years of follow-up in the Malmö Diet and Cancer cohort[a]

		Diet quality index			*P*-trend	*P*-int
		Low (0-1)	Medium (2-4)	High (5-6)		
LDL-C	Low GRS_{LDL}	4.04 (3.90, 4.18)	3.89 (3.81, 3.96)	3.83 (3.80, 4.05)	0.16 (0.09)[b]	0.20 (0.52)[b]
	High GRS_{LDL}	4.28 (4.13, 4.43)	4.30 (4.23, 4.37)	4.37 (4.25, 4.49)	0.10 (0.77)	
Δ-LDL-C	Low GRS_{LDL}	-0.39 (-0.48, -0.30)	-0.36 (-0.41, -0.31)	-0.45 (-0.53, -0.36)	0.15 (0.15)	0.33 (0.29)
	High GRS_{LDL}	-0.59 (-0.70, -0.47)	-0.53 (-0.59, -0.48)	-0.64 (-0.73, -0.56)	0.16 (0.07)	
HDL-C	Low GRS_{HDL}	1.43 (1.39, 1.48)	1.45 (1.43, 1.48)	1.45 (1.41, 1.49)	0.26 (0.27)	0.99 (0.66)
	High GRS_{HDL}	1.31 (1.26, 1.36)	1.35 (1.26, 1.36)	1.38 (1.34, 1.42)	0.04 (0.001)	
Δ-HDL-C	Low GRS_{HDL}	-0.04 (-0.09, <0.01)	0.02 (-0.002, 0.05)	0.01 (-0.03, 0.05)	0.05 (0.02)	0.04 (0.06)
	High GRS_{HDL}	0.03 (-0.01, 0.08)	0.01 (-0.02, 0.03)	-0.01 (-0.05, 0.03)	0.35 (0.66)	
TG	Low GRS_{TG}	1.24 (1.17, 1.32)	1.20 (1.16, 1.24)	1.14 (1.08, 1.21)	0.07 (0.01)	0.46 (0.55)
	High GRS_{TG}	1.38 (1.29, 1.47)	1.33 (1.28, 1.37)	1.38 (1.30, 1.45)	0.97 (0.41)	
Δ-TG	Low GRS_{TG}	-0.12 (-0.18, -0.06)	-0.15 (-0.18, -0.12)	-0.12 (-0.17, -0.06)	0.88 (0.43)	0.19 (0.94)
	High GRS_{TG}	-0.05 (-0.12, 0.03)	-0.12 (-0.16, -0.08)	-0.10 (-0.17, -0.04)	0.06 (0.03)	

a) Genetic risk scores were split by the median values. The interactions were examined with continuous variables of diet categories and genetic risk scores, adjusted for age, sex, season of diet collection, energy intake, waist circumference, smoking, alcohol consumption, leisure-time physical activity, education.

b) *P*-values in parenthesis: results excluding individuals reporting dietary changes in the past and misreporters of energy, n=2,001.

6. Discussion

6.1 Methodological considerations

There are several limitations that affect observational studies, the most notable of which are reverse causation, confounding and selection bias. In the field of nutritional epidemiology, we study the association between an exposure (diet) and a disease/trait in free-living individuals who assign themselves to different exposure levels. It is almost impossible to study the effect of long-term dietary intake on disease development in a controlled setting and therefore observational of studies are irreplaceable. However, it must to be highlighted that random and systematic errors (bias) are of high concern in observational studies. The impact of errors and biases can be reduced by using an appropriate study design and data collection method as well as by taking them into account when analyzing data.

Study design

The MDC study has a prospective study design that assures that the exposure is measured before the diagnosis of disease. This design has several advantages compared to a retrospective study design, in which information about exposure is collected after diagnosis of disease. Additionally, a cohort study design follows participants who are free of disease either until diagnosis or until the follow-up period ends. In contrast, disease status cannot affect the reported exposure (diet), which can be a significant limitation in retrospective case-control studies.

CVD status can affect biomarkers such as blood lipid and lipoprotein concentrations and their subfractions concentrations. Additionally, the diagnosis of a disease such as CVD or diabetes may influence the patient's dietary intake and lifestyle habits and therefore affect these biomarkers. Because participants with a known history of CVD or diabetes were excluded from the study population, the biomarkers that were measured in the remaining healthy participants should not have been affected by their diagnosis.

An individual's genotype remains the same throughout life, and CVD diagnosis, diet and other lifestyle factors cannot influence the genotype. However, when examining gene-environment interactions it is important with prospective data regarding the exposure.

Selection bias

If participants are not randomized, as in observational epidemiological studies, different selection biases may occur. For example, the participation rate in the MDC study was rather low, being approximately 40% (120). This might have contributed to a selection bias of the study participants (i.e., people who participated may have been healthier than those who did not participate, a so-called "healthy workers effect"). This also might affect the generalizability of our results in addition to the exposure levels. However, the MDC study had a high internal validity, meaning that the methods that were used measured what they were supposed to measure, which is very important when examining associations between diet and disease. We are interested in the etiology of CVD, and the association between exposure and disease is not affected by low participation rate.

A questionnaire was sent to each of the individuals who were identified as part of the source population of the MDC study. This questionnaire had a high response rate (75%) and showed that no general health benefits were attained with respect to socio-demographic structure, obesity or smoking habits in participants of the MDC study compared to non-participants. However, the MDC study participants did report a higher degree of social participation and better subjective health than did non-participants (140).

Additionally, participants that were included in the follow-up (Paper IV) had to be alive 16 years after the baseline examination and were required to visit the study center again for the re-examination. Participants who were included in the baseline examination but who were not present at re-examination were more often smokers, had higher BMI and blood pressure, and had more often diabetes (141). This selective survival may contribute to fewer individuals with severe dyslipidemia being included in the study sample than expected, introducing a more narrow range in blood lipid and lipoprotein concentrations, and probably also in dietary intake, and therefore reducing the likelihood of observing differences between diet quality and risk of dyslipidemia.

Measurement errors and misclassification of dietary intakes

All methods of assessing dietary intake are prone to errors, and there is no golden standard for measuring dietary intake, meaning that methods of high relative validity may have the same errors as the reference method, which in the MDC study was an a 18-day weighted food record (142). Random errors in diet measurements may occur when dietary intakes are inaccurately measured. Large day-to-day variation in dietary intakes makes it difficult to estimate the mean daily intake, whereas random measurement errors in regard to long-term dietary intake may occur. Systematic errors (bias) may occur when a dietary assessment method

fails to cover frequently consumed foods that are more commonly consumed in specific group, which may lead to an underestimation of the dietary intakes. Additionally, people may under- or over-report certain foods systematically (142).

Misclassification may be equivalent for all groups (non-differential misclassification) or may vary between groups (differential misclassification). A low magnitude of random error (non-differential misclassification) provides high precision, whereas low systematic error (differential misclassification) provides high validity of the measured exposure. In prospective studies, measurement errors are typically randomly distributed in regard to disease (142). If measurement errors are correlated to true dietary intake or are found to influence specific groups of individuals more than others, the result is a differential misclassification, which can either attenuate or strengthen diet-disease associations.

The MDC study was originally designed to examine the association between diets that are high in fat and low in fiber, vitamins and minerals, and cancer (118). The modified dietary history method that was used in the MDC study combined a dietary questionnaire, food diary, and dietary interview, and the dietary data that was produced in the MDC is generally of high relative validity (127) and reproducibility (128). However, the relative validity of several of the PUFAs are still rather low, especially for long-chain ω-3 PUFAs in men (energy-adjusted correlation coefficients: 0.24 for EPA, 0.37 for DPA, and 0.20 for DHA), which is a weakness of our studies. One potential reason for this low validity may be an infrequent consumption of fatty fish (a major source of long-chain ω-3 PUFAs) among many of the study participants. Because the consumption of during main meals was only registered over a course of 7 consecutive days, and because fish is likely to be consumed relatively infrequently, misclassification might be a problem.

We also used a diet quality index to estimate overall diet quality. However, the diet quality index itself could be a limitation. The diet quality index has previously been shown to reflect overall diet quality and to sufficiently rank participants in the MDC cohort into low, medium, or high quality diets based on their intake of a wide range of foods and nutrients and may thus be more predictive of disease risk than individual foods or nutrients (51). However, reducing dietary habits, which are a very complex, into a diet quality index constructed by adding a few dichotomous diet variables may be a disadvantage. Additionally, dietary patterns are likely to vary according to social and cultural backgrounds; therefore, it is necessary to replicate our results in other populations.

Unfortunately, we only have dietary data from the baseline examination and therefore we do not have any information about change in dietary habits that might have occurred during follow-up. However, previous studies have shown that additional measurements add only a small effect (143, 144). Additionally, because the participants in the MDC study were already middle-aged at the baseline

examination during the 90's, they are more likely to have well-established food habits compared to a younger population. Additionally, we observed that participants with low intakes of sucrose had higher HDL-C concentrations, both at baseline and after 16 years of follow-up, compared to those with high sucrose intakes (Paper IV), which might indicate that food habits are rather stable in this population.

The available data on changes in dietary habits that occurred in the past is an advantage of the MDC cohort, despite that it was self-reported. For example, we observed that the effect of the interaction between ALA/LA intake and *FADS1* genotype on CVD incidence was slightly strengthened and statistically significant when excluding participants who reported past changes of dietary habits as well as those who potentially misreported energy intake. Additionally, associations between diet quality index and TG, LDL-C and HDL-C concentrations at follow-up were only observed after excluding misreporters of energy and participants who indicated unstable food habits.

A potential reason for the more pronounced associations that were observed between dietary intake and TG, LDL-C and HDL-C concentrations and between these factors and CVD risk when excluding participants who reported past changes to dietary habits may be that the unstable food habits of these participants resulted in a misclassification of dietary exposure in relation to disease development. For example, a participant's past dietary habits may be related to disease development as opposed to their reported dietary intake during the study, resulting in a reversed association. This information may explain, at least in part, why many observational studies produce null findings even when animal studies and ecological studies indicate strong dietary associations.

Furthermore, changes in dietary habits may also correlate with other confounding factors. In the MDC cohort, participants who had changed their dietary habits in the past were more likely to be obese than non-changers (145), and obesity is a risk factor for several conditions that are associated with CVD, such as hypertension, type 2 diabetes and dyslipidemia (1, 4). Obesity has also been shown to be related with under-reporting of dietary intake when using self-reported dietary data (146). However, self-reported dietary changes in obese individuals might indicate an attempt to lose weight, which is usually associated with restricted consumption of food. Such changes may also include other lifestyle factors, such as engaging in new healthy behaviors. Therefore, the indicated dietary changes may not necessary reflect a real change.

Misreporting of energy intake has been examined in relation to other characteristics of the MDC cohort. Almost 18% of the women and 12% of the men were classified as low-energy reporters, whereas 2.8% of the women and 3.5% of the men were classified as high-energy reporters. In both genders, high BMI, large waist circumference, low education and a blue-collar profession were all

significantly associated with low-energy reporting. High-energy reporting was significantly associated with participants who had low BMI, lived alone and were current smokers. These results indicate the importance of including energy adjustments to reduce the influence of errors in risk assessment (134).

Energy adjustments

Diet variables were energy-adjusted either based on nutrient density (E%) or by the residual method. It is very important to energy-adjust dietary intake because the majority of nutrients are positively correlated with total energy intake. Absolute intakes of energy, and therefore nutrients, are mainly determined by body size and physical activity level. If total energy intake is associated with but not a direct cause of disease, it may confound associations. In the field of nutritional epidemiology, we are mainly interested in relative intakes (i.e., diet composition, and not absolute amounts) with regard to disease risk. Energy-adjusted nutrient variables provide a measure of nutrient intakes that is not correlated with total energy intake. Additionally, energy adjustment is recommended to reduce the impact of measurement errors associated with reported intakes (147).

Blood lipids and lipoproteins

Misclassification of lipid and lipoprotein concentrations may occur due to laboratory errors. Additionally, selection bias may occur because participants with fasting TG >400 mg/dL (4.5 mmol/L) were excluded because Fridewald's formula is not capable of calculating LDL-C in individuals with very high TG concentrations. Additionally, although similar methods were used to measure lipid and lipoprotein concentrations at baseline and follow-up, it is always important to consider the precision of measurements that are taken at baseline vs. re-examination.

We excluded participants who reported the use of lipid-lowering medication at baseline (Paper I and IV). We found that the use of lipid-lowering medication was more commonly reported at follow-up (2007-2012) than at baseline (1992-1994) (Paper IV). Using an established formula, we have therefore corrected the TG, LDL-C and HDL-C concentrations that were measured at follow-up among participants who reported using lipid-lowering medication (132), although it should be noted that misclassification may still occur.

Genetics

There are many challenges in performing gene-environmental studies and interpreting the results. The main obstacles include the requirement of a very large sample size, obtain a valid assessment of environmental exposures, and the differences between study samples (107-109, 148). A primary advantage associated with large prospective studies compared to case-control studies is that exposures and risk factors are assessed prior to the onset of disease, which minimizes the biases that are common in case-control studies, such as recall bias and stratification bias (109). However, a limitation of prospective studies is that the environmental variable (exposure) that is being examined usually varies over time. This variable may also be affected by the duration of exposure or age at exposure. In large prospective studies, it is usually only feasible to measure environmental exposure once; therefore, the assessment of exposure is limited (108).

For quality control purposes, the genotyping of the included SNPs was checked by several different methods. First, we examined the HWE to detect signs indicative of genotyping errors or confounding due to population admixture. The HWE cut-off for excluding SNPs was $P<0.05$. We noted no significant deviation from HWE for either rs174547, $P=0.92$ (Paper I) or rs174546, $P=1.00$ (Paper II). However, when constructing the GRSs, 7 SNPs with HWE *P*-values of less than 0.00057 (0.05/87) were excluded as a result of correction for multiple testing, which enables us to assure that the allelic frequencies of the included SNPs were correct. Second, genotyping was found to be successful in 96% of the participants (Paper I and II), and the concordance rate between two different genotyping methods was 99.2% (Paper I), which is considered to be high. However, 4 SNPs with success rates of less than 90% were excluded from the GRSs (Paper III and IV) to minimize the risk of genotyping errors in the data.

Population stratification occurs when the frequency of the minor allele differs within a study population, i.e., when the participants have different ethnicity, which might incorrectly introduce an association between the minor allele and an outcome (Type 1 (α) error, false positive association). Type 2 (β) error relates to false negative associations. In the MDC cohort, population stratification is not considered to be a major confounder because the majority of the participants were either from Sweden or from countries in geographic proximity to Sweden (118). The population substructure has been examined with the Eigenstrat software among individuals with GWAS data and the very few outliers indicate minor problem with population stratification in the MDC cohort.

We examined the association between genetic susceptibility to dyslipidemia and risk of CVD (Paper III), as well as the association between diet quality and risk of dyslipidemia, over 16 years of follow-up (Paper IV) by combining 80 validated genetic variants associated with TG, LDL-C and HDL-C concentrations. These variants have been suggested to account for approximately 25-30% of the genetic variance that is related to these standard lipid concentrations (95) and can therefore be used as a marker of genetic susceptibility to dyslipidemia in Caucasians. However, several of the SNPs that were included in the GRSs have demonstrated pleiotropic effects associated with several of the standard lipid traits that were assessed, which could bias our results. We chose to include these SNPs to avoid weakening the effects of the GRSs and therefore we corrected for pleiotropic associations in our statistical models. However, the included genetic variants are located in or near genes that are involved in varying, and in many cases unknown, mechanisms and pathways of lipid and lipoprotein metabolism, which may attenuate their interactions when they are included in GRSs.

The translation of our findings into clinical practice may be limited, as each variant that was studied had only a very small effect and would therefore not be useful for predictive purposes. Moreover, the implicated variants themselves are rarely themselves the causal variants; rather, they are linked to the true causal variants, and identification of the latter usually warrants a great deal of additional work.

Misclassification of other lifestyle factors

Lifestyle factors such as smoking, leisure time physical activity, alcohol consumption, education, socioeconomic circumstances, current medication, diet and supplement use, and history of disease are all self-reported and are therefore prone to measurement errors. Additionally, this information was only collected at baseline. A major strength of the MDC study is that weight, height, and waist and hip circumferences were measured in a standardized way by trained project staff, making this study less prone to errors compared to studies using self-reported anthropometrics. BMI was calculated from direct measurements of weight and height.

Potential misreporters of energy intake were identified by comparing reported energy intake with total energy expenditure (estimated from the calculated basal metabolic rate and self-reports of leisure time physical activity, work activity, household work, and sleep hours, which together form the so-called PAL). Leisure time physical activity was estimated using a very extensive method that several participants found difficult to complete. Because self-reported leisure time physical activity level was used to estimate PAL, there is a risk that misreporting

and bias might influence the results and that this might contribute to misclassification of the participants' status.

Misclassification of disease status

Information regarding the prevalence and incidence of CVD was retrieved through several different national and local registers. There is an almost complete follow-up of the MDC participants and a detailed ascertainment and verification of CVD diagnosis (137, 139, 149, 150). We included only ischemic stroke events in the analyses. We were able to verify and extract ischemic stroke cases through the local stroke register in Malmö (STROMA), that was created to prospectively follow-up stroke incidence, recurrence and fatality rates (139). Continuous validation against hospital diagnosis showed well over 90% of all stroke cases in Malmö to have been included. Ischemic stroke was diagnosed when computed tomography, magnetic resonance imaging or autopsy could verify the infarction and/or exclude hemorrhage, and non-vascular disease.

We performed sensitivity analyses to exclude participants who had been diagnosed with CVD within 2 years after baseline examination (Paper II) to assure that preclinical CVD did not influence the observed diet-disease associations. However, because CVD does not have a latency period as similar to diseases such as cancer, we will certainly not introduce a large bias, and we therefore did not perform this sensitivity analysis in paper III.

Self-reported diabetes diagnosis or the use of glucose-lowering medication was used to identify participants with diabetes, and these participants were excluded in our analyses. Type 2 diabetes has rarely of an acute clinical onset; consequently undiagnosed cases in this study population are likely. However, participants that did not report diabetes or the use of glucose-lowering medication were not likely to be aware of their diabetes status and therefore it should not have any impact on their dietary habits, which otherwise could attenuate diet-disease associations.

Confounding factors

A confounding factor has to be associated both with the exposure and the outcome separately. It should not be affected by the exposure or the disease and especially not be in the causal chain, i.e. intermediate factor. Selection of confounders using statistical procedures based on significance levels might be misleading (151). Using casual diagrams, so called directed acyclic graphs (DAGs), linking variables by arrows representing direct causal effects, is an established method to identify variables that must be measured and controlled (152).

Dietary habits are usually correlated with other lifestyle factors which could influence the associations and interactions between diet, genetics and lipid and lipoprotein traits/CVD. We selected covariates based on a biological model from previous knowledge about risk factors for CVD or variables associated with lifestyle and food habits (i.e., age, sex, smoking, alcohol consumption, leisure time physical activity, BMI and educational status). Consequently, access to information on different lifestyle and socioeconomic factors are of great importance. Since the baseline examinations included anthropometric measurements and an extensive questionnaire about lifestyle factors, we managed to adjust for several potential confounders.

Fatty fish is not only the main source of long-chain ω-3 PUFAs, it also an important dietary source for vitamin D (14). The role of vitamin D status in the development of atherosclerosis and CVD are not clear but growing evidence suggests that vitamin D deficiency associates with hypertension, inflammation and insulin sensitivity (153). However, the minor allele of *FADS1* genotype was only observed to associate with lower LDL-C in the lowest tertile of long-chain ω-3 PUFAs (which could reflect a low fish intake). Additionally we did not observe any associations between any of the dietary PUFAs and CVD risk which indicated that vitamin D status should not be an important confounder in our studies.

We did not account for psychosocial stress in the included studies and there is no information about family history of CVD in MDC. However, we adjusted for educational status which can be used as a marker for other lifestyle factors and, at least in past, may reflect the psychosocial stress since higher education likely correlates with higher socioeconomic status and therefore less psychosocial stress. Family history of CVD is partly due to the inherited genetic risk. By examining the genetic risk for dyslipidemia in relation to CVD (Paper III) risk and standard lipid change (Paper IV) we were able to account for some of the family history of CVD.

Additionally we excluded users of lipid lowering medication (Paper I and IV) at baseline which may attenuate the associations between diet and lipid and lipoprotein concentrations since these individuals may be at a high genetic risk of dyslipidemia.

When examining the association between genetic risk for dyslipidemia and CVD, we only adjusted for age and sex since lifestyle factors are generally not able to influence the genotype (154).

Residual confounding

Residual confounding may occur due to poorly measured confounders. Lack of repeated measurements as well as measurement error of lifestyle and socioeconomic factors such as smoking, leisure time physical activity, alcohol consumption, and education, may lead to residual confounding even if these factors are adjusted for in the statistical analyses. Additionally, a high diet quality index might indicate an overall healthy lifestyle and therefore correlate with other factors important for CVD risk and thus, residual confounding can still occur.

Other unmeasured variables may also confound the associations between diet and lipid and lipoprotein concentrations/CVD, because unknown risk factors could covary with diet. Additionally, categorizing may in some cases be to crude and could bias the associations in either direction.

Interaction

The association between an exposure and the outcome may depend on other factors, a so called effect modifier. An effect modification is usually a synonym for interaction in statistical models. For a biological interaction there must be a causal interaction. The stable factors (e.g., genetics) are usually considered as the effect modifiers from a public health perspective.

We examined the interactions observed in this thesis by assessing heterogeneity of effect, where the interpretation is based on another factor increasing or decreasing susceptibility to develop the disease/trait. In other words, that there is a stronger association in subgroups of individuals that are exposed to a particular factor compared to other individuals. This means that the slopes of the associations are not parallel when the interaction is present. For example, we observed an interaction between *FADS1* genotype and intake of long-chain ω-3 PUFAs on LDL-C concentrations (Paper I). The association between *FADS1* and lower LDL-C were observed only among participants with the lowest intake of long-chain ω-3 PUFAs. That is, the associated effect of genetic variation in *FADS1*on LDL-C was modified by the dietary intake levels of long-chain ω-3 PUFAs.

A confounder may also be an effect modifier. In that case, it is not correct to adjust for this factor.

Statistical considerations

In all studies we examined associations with a prior hypothesis based on a proposed biological mechanism. However, there is an increasing risk of chance findings with increasing number of statistical tests performed. When many hypothesis-free tests for statistical significance are preformed, such as in GWAS, the significance level has to be much lower, to correct for multiple testing. We performed multiple tests and thus some of the observed significant associations and interactions could be due to chance. It is therefore important to replicate our results in well-powered studies with high quality dietary data.

In the first study, we examined the association between a single exposure factor, i.e., *FADS1* polymorphism rs174547 (T/C), and TG, LDL-C and HDL-C concentrations (Paper I). However, several PUFA variables were used as affect modifiers for these associations. Although, the PUFA variables are correlated, and it is therefore not correct to adjust for multiple testing using the more conservative Bonferroni correction, some results might have occurred by chance.

In the second study, we examined 7 PUFA variables and CVD (Paper II) as well as the interaction between these PUFAs and *FADS1* polymorphism rs174546 (C/T) on CVD, and some results may have occurred by chance. However, we did not observe any statistically significant association between any of the PUFA intakes and incident CVD neither in the basic analysis model nor in the multivariate analysis. Excluding BMI or including intakes of fiber and SFAs in the multivariate analyses did not change the results substantially. Anyhow, we observed an association between high ALA/LA intake ratio and decreased risk of CVD only among individuals homozygote for the minor allele that has been associated with lower Δ-5 FADS activity. However, this interaction between ALA/LA and *FADS1* genotype on CVD only became significant when excluding diet changers in the past and thus, this finding needs to be replicated.

In both the first and the second study we assumed an additive model for testing association between the *FADS1* polymorphisms and TG, LDL-C and HDL-C concentrations/CVD. In an additive model each additional risk allele increases the response, mean phenotype level or HR of the disease, by the same amount. Another way for testing association between polymorphisms and an outcome is the dominant model. Hence, the heterozygote and one of the homozygote genotypes are treated as a single category. This dichotomization of the genotypes forces heterozygotes to have the same risk or mean phenotype as one of the homozygotes (154).

In the third study, we examined numerous associations and interactions between GRSs for high TG, LDL-C and low HDL-C, diet quality and its components, and CVD (Paper III). Although we tried to reduce the number of tests by combining

the SNPs into GRSs, instead of analyzing every individual SNP, some findings might have occurred by chance. Since several of the SNPs included in the GRSs have pleotropic effects we performed the analyses both with and without the other GRSs to the statistical model. However, this did not change our results markedly.

In the last study, we examined the association between diet quality index and standard lipid concentration and change after 16 years of follow-up (Paper IV). Therefore, we examined several outcomes, i.e., blood lipids and lipoproteins, and hence some results might have occurred by chance. However, we noted that the results were in line with our main hypothesis that a high compared to low diet quality was associated with less risk of developing dyslipidemia at follow-up.

In the first three studies we performed the analyses both with and without BMI in the basic model because BMI can be considered to be a mediating factor both between genes and TG, LDL-C and HDL-C and CVD as well as between diet and CVD and lipid and lipoprotein concentrations. However, waist circumference can be a better measurement for fat tissue distribution and therefore we used waist circumference instead of BMI in the last study.

7. Findings and interpretations

In this doctoral thesis we examined whether PUFAs intakes and overall diet quality associate with an impaired lipoprotein profile and CVD risk, and if these associations depend on genetic predisposition for dyslipidemia. The major challenge and opportunity of this thesis was to make use of the genetic information and their interactions with dietary factors to further understand predisposition to dyslipidemia and risk of CVD. Our results will hopefully contribute to better prevention possibilities and more personalized prevention strategies and are therefore, very important in terms of public health.

FADS1 genotype, PUFAs and TG, LDL-C and HDL-C (Paper I)

Several studies, in a number of cohorts with different ethnic backgrounds and age groups, have found that genetic variation in the *FADS* gene cluster can modify desaturase function. Additionally, common genetic variations in the *FADS* gene cluster have been shown to account for up to 28.5% of the variability in PUFAs and long-chain PUFA concentrations in human tissues (104, 155). This demonstrates that blood and tissue concentrations of the essential fatty acids LA and ALA and of their biologically active long-chain PUFA derivatives, the eicosanoids, are influenced not only by diet, but to a large extent also by genetic variation.

Similar to our results, several other studies have found an association between the minor allele of rs174547 (or a SNP in high allelic association) and decreased LDL-C concentrations (71, 72, 156-158). However, there are still inconsistent associations between genetic variation of *FADS1* genotype and TG, LDL-C and HDL-C concentrations. For example, Nakayama *et al*. reported different associations in two genetically similar Asian populations. In a Japanese population, the minor allele of rs174547 was associated with lower HDL-C and higher TG concentrations. However, in a Mongolian population the minor allele of rs174547 was found to associate with decreased LDL-C, but not with HDL-C or TG (159). It was suggested that differences in dietary fish intake between Japanese and Mongolians could provide an explanation of the dissimilar results. Accordingly, Japanese participants had higher plasma concentrations of ω-3 PUFA and a higher ω-3/ω-6 PUFA compared to Mongolians (160). In our study, the minor allele of rs174547 was associated with lower LDL-C concentration only

in the lowest intake tertile of long-chain ω-3 PUFA. As the dietary source of long-chain fatty acids mainly comes from fatty fish, our finding is in agreement with Nakayama *et al*.'s suggestion that among individuals with low fish intake, in our study represented by those in the lowest intake tertile of long-chain ω-3 PUFAs, the association between the *FADS1* genotype categories and LDL-C concentrations may be accentuated.

Additionally, we observed that the intake of ALA/LA may modify the associated effect of *FADS1* genotype on HDL-C concentrations. The potential mechanism for the association between high ALA/LA intake and higher HDL-C among the minor allele carriers could be that the minor allele, which has been associated with a lower desaturase activity (161) may affect the availability of long-chain ω-3 PUFAs differently between the genotypes. The metabolic derivatives, eicosanoids, of these long-chain ω-3 PUFAs are potent activators of PPAR (44, 45) which can regulate the expression of genes directly involved in HDL-C production (72). Therefore, our result may reflect a limited long-chain PUFA availability among rs174547 minor allele carriers and thereby, an association between HDL-C concentrations and ALA/LA intake.

When interpreting the results it is important to consider that the discrepancy between different studies may be due to different population characteristics, covariates included in the statistical model, or the differences in medical treatment on lipids and blood pressure, along with other potential CVD risk factors (e.g., dietary intakes) that are not fully controlled for. However, our findings suggest that it is important to examine associations between *FADS1* genotype and TG, LDL-C and HDL-C concentrations in strata of different PUFA intakes. We suggest that analyses of *FADS1* gene variants should be included into all large cohort and intervention studies addressing biological effects of PUFAs and long-chain PUFAs in order to consider these important confounders, and to enhance study sensitivity and precision.

Dietary PUFAs, *FADS1* genotype and CVD (Paper II)

In agreement with other studies (46, 162, 163) we did not observe any association between the *FADS1* genotype and CVD risk when the analysis was not stratified by dietary intakes. However, our results suggest that individuals with genetically elevated ALA and LA concentrations together with a higher dietary intake of ALA/LA or ALA may reach higher tissue concentrations of ALA which may have a protecting effect against CVD. However, it is not clear if ALA has an independent role in cardiovascular health or if the potential beneficial effect is driven by the conversion to long-chain ω-3 PUFAs (164, 165).

Further on, our findings suggest that individuals with higher ALA intake in combination with genetically higher ALA availability may have a protective effect

against ischemic stroke. In line with our results, earlier studies have indicated that insufficient intake and tissue concentrations of ω-3 PUFAs increase the risk of stroke (166, 167). Although, in particular, EPA and DHA intake have been associated with neurovascular-, cell membrane- and tissue functions (168, 169), ALA, as well as ALA/LA intake ratio, have also been recognized to be important for these functions (164, 165, 169, 170). Whether the interaction on stroke incidence and lack of interaction on coronary event may reflect differences in risk factors and pathophysiology of these cardiovascular endpoints, or is simply a statistical power issue, cannot be answered in the current study. Other studies need to replicate our findings to answer this question.

In our previous study (Paper I), we observed that the minor allele of *FADS1* rs174547 (T/C) (a SNP in high allelic association with rs174546) was associated with lower LDL-C only among participants in the lowest tertile of long-chain ω-3 PUFA intakes. However, the present study did not show any significant interactions between the intake of long-chain ω-3 PUFAs and *FADS1* genotype on CVD incidence. An explanation to the inconsistency of the results may be that other PUFAs, such as ALA and LA (before the Δ-5 FADS desaturation step), are more dependent on the *FADS1* genotype to affect the long term risk of CVD. For example, one benefit for ALA as compared to long-chain ω-3 PUFAs is that ALA can compete with LA in the first elongation-desaturation step whereas the long-chain ω-3 PUFAs only compete with the long-chain ω-6 PUFAs in the late steps of eicosanoid metabolism. Thus, in contrast to long-chain ω-3 PUFAs, ALA can prevent accumulation of long-chain ω-6 PUFAs (171).

It is common with an increased dietary intake of ω-6 fatty acids in relation to ω-3 fatty acids in countries with a westernized diet (53). This may create an unbalance between the ω-3 and ω-6 PUFAs because these fatty acids are depending on the same enzymatic pathway. Since the PUFAs and their derivatives, the eicosanoids, are important ligands of PPARs, which are transcription factors for several genes involved in the lipid and glucose metabolism and inflammation (44, 45, 58, 59), an unbalance between the ω-3 and ω-6 PUFAs may therefore affect these metabolic systems which may lead to a progress in disease development of e.g., atherosclerosis.

GRSs, diet quality index and CVD (Paper III)

The $GRS_{LDL\text{-}C}$ was significantly associated with an increased risk of CVD and coronary events. This is in line with a number of Mendelian randomization studies (101-103). Additionally, numerous potential genetic risk alleles for stroke have been reported; however, the genetic evidence is thus far not conclusive (172), and studies examining the association between $GRS_{LDL\text{-}C}$ and stroke are missing. We observed that a high compared to low quality diet attenuated the association

between GRS_{LDL-C} and the increased risk of incident ischemic stroke. Although GRS_{LDL-C} was tended to be associated with an increased risk in the low and medium diet quality groups, no such tendency was observed in the high diet quality group. However, the non-significant trend among the participants in the high and low diet quality groups might, at least in part, be explained by a lower number of individuals in these groups (n_{low}=3,890, n_{medium}=17,724 and n_{high}=3,185). When we divided the GRS_{LDL-C} into tertiles, we observed that a high diet quality was inversely associated with ischemic stroke incidence among individuals in the highest tertile. The observed interaction between GRS_{LDL-C} and the diet quality index on ischemic stroke seems to mainly be driven by fruit and vegetable intakes in men and fat quality (i.e., SFAs and PUFAs) in women, although no significant heterogeneities regarding these interactions were observed between the sexes.

Additionally, we observed significant associations between several baseline characteristics, such as higher dietary fiber, lower SFA intakes, and high GRSs, are not easy to explain. These findings may indicate that those individuals with high genetic risk for dyslipidemia are likely to be aware of their dietary habits. An explanation may be that these individuals have experienced different health problems previously or that they are aware of their increased risk of dyslipidemia and CVD (e.g., family history of CVD or that these individuals have performed a lab test) because their dietary intakes are in line with the dietary advice for prevention of dyslipidemia and CVD. In the future we will also need to take into account if the participant's themselves have performed a genetic test because that could confound the associations. An interpretation of this observation is that both dietary intakes and genetics are important to consider when examining associations between genetic risk for dyslipidemia and CVD.

The dietary assessment method used in the MDC study was specifically designed to measure intakes of vegetables, fiber and fat (118) and the relative validity of the method is generally high. The high validity may contribute to the ability to observe significant interactions between GRS_{LDL-C} and fruit and vegetable intakes affecting ischemic stroke incidence. The high relative validity of PUFA intakes in women (0.64) compared to men (0.26) might explain why we observed a nominal interaction between GRS_{LDL-C} and PUFA intake affecting ischemic stroke in women but not in men.

In conclusion, we found no convincing evidence that dietary quality modifies the association between the GRSs for TG, LDL-C and HDL-C and CVD risk. Further studies may need to consider different mechanisms and pathways for genetic variants associated with dyslipidemia separately to clarify the interaction between GRSs and diet quality on CVD risk. In addition, to examine the modifying effect of diet quality, further studies are needed to examine whether any specific dietary factors may modify the associations between genetic susceptibility to dyslipidemia and the incidence of CVD.

Diet quality and standard lipid change (Paper IV)

We found that the participants with high adherence to the Swedish nutrition recommendation had higher HDL-C concentrations at baseline and lower risk of developing high TG and LDL-C during follow-up. A few studies have examined the association between diet quality and incident dyslipidemia but the results are inconclusive. For example, a study in France found that an increased diet quality score was associated with a lower 6-year risk of developing the metabolic syndrome (173), and in the Framingham Heart Study they found that a high diet quality score was associated with lower TG and higher HDL-C after 7 years of follow-up (174).

Those with lower intakes of sucrose had lower TG and higher HDL-C concentrations at baseline compared to those with high sucrose intakes. After 16 years we could still see a significant difference in HDL-C concentrations between those that followed the recommendation for sucrose at baseline and those that were not within the recommended range. This observation may indicate that the food habits are rather stable in this population. We observed associations between the diet quality index and standard blood lipids at follow-up only after excluding misreporters and those indicating unstable food habits. Those individuals might have changed their diet during follow-up, including misclassification of the dietary exposure, and therefore limited the possibility to see any association in this group. This observation highlights the importance of excluding misreporters in the analyses and to ask the participants about their stability of food habits, especially when you only have the food habits collected once.

Fiber was the only included diet component that significantly associated with change in standard blood lipids. The relative validity of the diet method used in the MDC study is generally high. However, the dietary instrument used in the MDC study was specifically developed to estimating fiber intake in this middle-aged population. Although we have adjusted for several confounders, including change in waist circumference and smoking habits during follow-up, it might be that the diet quality index correlates with other factors that are of importance for the change in standard blood lipid concentrations.

Diet quality, GRSs and standard lipid change (Paper IV)

We combined 80 validated genetic variants associated with standard blood lipid concentrations to examine if genetic susceptibility to dyslipidemia modifies the association between a diet quality index and standard blood lipid concentrations both cross-sectional and longitudinally. By taking genetic risk for low HDL-C into account we found a decrease in HDL-C during follow-up with low diet quality only among those with low genetic risk for low HDL-C. However, overall we

found no strong evidence that genetic risk for dyslipidemia modified the association between the diet quality index and change in standard blood lipids. One study has previously found an attenuated LDL (especially small LDL) lowering effect of a weight-loss lifestyle intervention among those with a high genetic risk score composed of 32 lipid-associated SNPs (175). The genetic variants included in the score are associated with different mechanisms of lipid and lipoprotein metabolism and it might therefore be important to examine genetic variants affecting specific mechanisms and pathways separately, to address whether genetic susceptibility affecting specific mechanisms or pathways would modify the associations. Within the MDC cohort we have previously observed that *APOE* rs4420638 was associated with change in total cholesterol, and *TRIB1* rs2954029 and *APOA1* rs6589564 were associated with change in TG levels (176).

The diet quality index indicating adherence to the Swedish nutrition recommendations and dietary guidelines has previously shown a protective association with CVD risk. Our findings indicate that this association may partly be explained through changes in standard blood lipids. We found no strong evidence that genetic risk for dyslipidemia modified the association between the diet quality index and change in standard blood lipids.

8. Conclusions

Taken together, this thesis examined interactions between the dietary intake of PUFAs, diet quality, and genetic variants on blood lipid and lipoprotein concentrations and the incidence of CVD in the MDC cohort. Our results suggest that:

1. Dietary intake levels of long-chain ω-3 PUFAs modify the associated effect of genetic variation in *FADS1* on LDL-C concentrations. The association between *FADS1* and lower LDL-C was observed only among participants with the lowest intake of long-chain ω-3 PUFAs.
2. Genetic variation in *FADS1* had little effect on the association between dietary PUFA intake and CVD risk. However, for the 11% of the study population that is homozygous for the allele that associates with lower Δ-5 FADS activity, a high ALA and ALA/LA intake ratio may be preferable for the prevention of CVD and ischemic stroke.
3. We observed that a high quality diet may attenuate the association between genetic risk for high LDL-C and increased risk of ischemic stroke compared to a low quality diet.
4. The risk of developing dyslipidemia during 16 years of follow-up was lower in participants with higher quality diets compared to those with lower quality diets.
5. In general, our exclusion of potential misreporters of energy and of participants who reported unstable food habits in the past strengthened our results. This observation highlights the importance excluding potential misreporters of energy when performing analyses and of asking participants about the stability of their dietary habits, especially in cases in which dietary habits were only collected once.

In conclusion, our results suggest that it is important to consider gene-diet interactions to understand the etiology of CVD.

9. Future challenges

"Livet är icke, det bygges"

- *Eddan*

Observational studies have received many criticisms in the past years; randomized controlled trials are still the golden standard to examine causal inference. However, Mendelian randomization, a recently developed study design has opened up new possibilities for epidemiological research. This method can be used to examine whether there is a causal link between a biomarker and an outcome by examining gene markers influencing biomarker concentration. Because our genetic make-up remains the same over the course of our lives, it is not affected by lifestyle factors. Therefore, this method enables us to avoid the influence of confounders that otherwise must always be considered when examining associations between an exposure and outcome in observational studies. However, this Mendelian Randomization method only works if the gene of interest does not cause pleotropic effects on other traits. Additionally, the causal association between the genetic marker and the outcome must go through the biomarker.

Another challenge in the field of nutritional epidemiology is the collection of accurate dietary information. Self-reported dietary data include measurement errors and confounding by other dietary and life-style factors. However, in most cases, common diseases, such as CVD, type 2 diabetes, cancer and obesity, take many years to develop and therefore participants must be followed for many years, which render a randomized control design in-feasible. To improve the reliability of dietary data, valid biomarkers would be useful. For example, dietary fat intake and fat quality can be reflected by measuring the compositions of fatty acids in serum, erythrocytes and adipose tissue. Because circulating fatty acids depends heavily on both the level of dietary intakes and genetic variation in *FADS1*, it is important to also measure fatty acids in blood to further interpret our findings regarding the interaction between *FADS1* genotype and long-chain ω-3 PUFA intake on blood lipid and lipoprotein concentrations and CVD risk.

In future epidemiological observational studies, it would be interesting to account for other factors that are relevant to disease development, such as gut microbiota and the heritability of lifestyle factors within families.

Another imminent question is how to handle the increasing amount of genetic information that is accessible. Genetic tests are already available on the market, but they have been criticized because of difficulties in understanding how to interpret the results. Issues surrounding the ethics behind enabling individuals to perform genetic testing without providing medical consultation or follow-up are becoming more prominent. Furthermore, a rising question is "How much knowledge is required before we can use information about gene-diet interactions in prevention of diseases?".

Recent studies have shown that many people are interested in obtaining personalized dietary recommendations based on their genetic information. Dietary advice that is based on genetics likely feels more trustworthy than advice stemming from other recommendations and therefore it may increase an individual's motivation to try it (177). However, at this point in time, a genetic testing company will only provide results from genetic polymorphisms in a very little number of genes. Therefore, the following question remains: "Will a genetic test give us more knowledge than we already have?".

At the present time, it is still too early to provide dietary advice based on an individual's genetic profile. As more knowledge is gained regarding how lifestyle and dietary factors interact with our genes and on the biological mechanisms behind these interactions, as well as how these interactions can affect each other, it may one day be possible to develop personalized dietary recommendations and prevention strategies for disease.

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