Fatty Acid Desaturase Activities in Metabolic Syndrome and Cardiovascular Disease

Special Reference to Stearoyl-CoA-Desaturase and Biomarkers of Dietary Fat

EVA WARENSJÖ
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Abstract

The development of the metabolic syndrome (MetS) and cardiovascular diseases have been suggested to be influenced more by the quality than the amount of dietary fat. The FA composition of serum lipids may be used as biomarkers of dietary fat quality. FAs can, however, also be endogenously synthesized by lipogenic enzymes such as elongases and desaturases. Three desaturases are important in humans: Stearoyl-CoA-desaturase (SCD), Δ6-desaturase (D6D) and Δ5-desaturase (D5D) and surrogate measures of desaturase activities can be estimated as product-to-precursor FA ratios.

In this thesis, we demonstrated that high SCD, D6D and low D5D estimated activities predicted MetS 20 years later, as well as cardiovascular and total mortality during a maximum of 33.7 years. The relation between D5D and MetS was independent of lifestyle and BMI, while the relation between SCD, D6D and MetS was confounded by BMI. Serum proportions of palmitic (16:0), palmitoleic (16:1) and dihomo-γ-linoleic acids were higher and the serum proportion of linoleic acid (LA) lower at baseline in those individuals who developed MetS. Further, LA was inversely related to mortality, while palmitic, palmitoleic and dihomo-γ-linoleic acids were directly associated with mortality. We also demonstrated that a diet rich in saturated fat “induced” a similar serum FA pattern (including estimated desaturase activities) that was associated with MetS, cardiovascular disease and mortality. We also propose that the SCD ratio [16:1/16:0] might be a novel and useful marker of dietary saturated fat, at least in Western high-fat diets. Finally, genetic variations in the human SCD1 gene were linked to obesity and insulin sensitivity, results that agree with data in SCD1 deficient mice.

This thesis suggests that dietary fat quality and endogenous desaturation may play a role in the development of metabolic and cardiovascular diseases and the results support current dietary guidelines.

Keywords: Fatty acids, Dietary fat, Biomarker, Metabolic Syndrome, Mortality, Obesity, Insulin Sensitivity, Epidemiology, Estimated desaturase activities, Stearoyl-CoA-desaturase, delta-6-desaturase, delta-5-desaturase, Single Nucleotide Polymorphism, Dietary Intervention, Rapeseed oil, Saturated fat, SCD1

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The lipoprotein particle on the front cover was reprinted with the permission from PeproTech Inc..
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<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
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<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
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<tr>
<td>FA</td>
<td>Fatty acid</td>
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<td>SFA</td>
<td>Saturated fatty acid</td>
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<tr>
<td>MUFA</td>
<td>Monounsaturated fatty acid</td>
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<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
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<tr>
<td>LA</td>
<td>Linoleic acid</td>
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<td>ALA</td>
<td>α-linolenic acid</td>
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<td>TFA</td>
<td>Trans fatty acids</td>
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<tr>
<td>CLA</td>
<td>Conjugated Linoleic acids</td>
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<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>OA</td>
<td>Oleic acid</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
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<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>EPA</td>
<td>Eicosapentaenoic acid</td>
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<td>DHA</td>
<td>Docosahexaenoic acid</td>
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<td>NEFA</td>
<td>Non esterified fatty acid</td>
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<tr>
<td>CE</td>
<td>Cholesteryl esters</td>
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<td>PL</td>
<td>Phospholipids</td>
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<td>AA</td>
<td>Arachidonic acid</td>
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<tr>
<td>SCD</td>
<td>Stearoyl-CoenzymeA-desaturase</td>
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<tr>
<td>D6D</td>
<td>Δ⁶ desaturase</td>
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<tr>
<td>D5D</td>
<td>Δ⁵ desaturase</td>
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<tr>
<td>SREBP-1c</td>
<td>Sterol regulatory element binding protein-1c</td>
</tr>
<tr>
<td>PPAR</td>
<td>Peroxisome proliferator activated receptors</td>
</tr>
<tr>
<td>DHLA</td>
<td>Dihomo-γ-linolenic acid</td>
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<td>MetS</td>
<td>Metabolic syndrome</td>
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<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>WC</td>
<td>Waist circumference</td>
</tr>
<tr>
<td>RO-diet</td>
<td>Rapeseed oil rich diet</td>
</tr>
<tr>
<td>SAT-diet</td>
<td>Butter rich diet</td>
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<tr>
<td>HOMA</td>
<td>Homeostasis model insulin resistance</td>
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<tr>
<td>MAF</td>
<td>Minor allele frequency</td>
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<td>HWE</td>
<td>Hardy-Weinberg equilibrium</td>
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Introduction

Today the world is facing an obesity epidemic; over 1 billion adults are overweight and over 300 million of them are clinically obese. Many of these people are prone to suffer from obesity related metabolic diseases such as cardiovascular disease (CVD) and type 2 diabetes \(^1,^2\) and this is associated with premature death \(^3,^4\).

Fat is the most energy dense nutrient and provides a large portion of the total energy intake for most individuals. Fat is also needed for cell structure and function, intercellular communication and genetic transcription \(^5\). The development of obesity related metabolic diseases is influenced by the quantity and more so by the quality of fat in the diet \(^6,^7\).

**Dietary fatty acids**

Dietary fat is largely made up by triacylglycerol (TAG, “triglycerides”) consisting of three individual fatty acids (FAs), each linked by an ester bond to a glycerol backbone \(^8\). Individual FAs have different biological effects and physical properties due to chain length, the degree of saturation and isomeric form. When the quality (type) of dietary fat is discussed it is the FA composition of the fat that is considered. Most dietary FAs are uncomplicated in structure with a carboxyl group at one end and a methyl group at the other end of the carbon chain, but some FAs are branched \(^9\). The number of carbon atoms varies between 4 and 24 and are usually even numbered \(^5\). The most abundant FAs in the diet have 16 or 18 carbon atoms \(^8\) and oleic acid (18:1), palmitic acid (16:0), linoleic acid (18:2 n-6) and stearic acid (18:0) are by quantity the most important individual FA in the Swedish diet \(^10\). There are dietary FAs with an odd number of carbon atoms, e.g. pentadecanoic (15:0) and heptadecanoic acids (17:0), which are of ruminant origin \(^11\) and mostly found in dairy products.

There are three major groups of FA; saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and n-3 and n-6 polyunsaturated fatty acids (PUFA). The more double bonds a FA chain contains, the more unsaturated the FA is. More double bonds give the FA chain less regular shape, affecting the melting point. This gives unsaturated FA the ability to regulate metabolic processes in the cells; e.g. muscle cells with more unsaturated FAs in their membranes respond better to insulin \(^8\) since the membrane is more “fluid”.

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Two dietary PUFAs, linoleic acid (LA, 18:2 n-6) and α-linolenic acid (ALA, 18:3 n-3) cannot be endogenously synthesized in humans and are thus essential and must be provided with the diet. LA and ALA are precursors for eicosanoids, prostaglandins and leukotrienes, which are fast-acting effective and locally synthesized mediators which can acts as second messengers\textsuperscript{12}.

**Trans-fatty acids**
Lately much attention has been given to \textit{trans}-fatty acids (TFA), which are unsaturated fatty acids with the double bond in \textit{trans}-configuration, instead of the \textit{cis}-configuration (\textit{Figure 1}). Most TFAs are formed during the industrial process of partial hydrogenation when liquid fats are hardened and some TFAs are naturally formed in the rumen of dairy cows by an enzymatic reaction. TFAs stay solid even at room temperature and are more resistant to oxidation and spoilage. Natural TFAs are found in small amounts in some plants (pomegranates, peas and cabbage)\textsuperscript{13}. A special group of TFAs is the conjugated linoleic acids (CLA)\textsuperscript{14}.

\textbf{Figure 1. Different fatty acids with 18 carbon atoms; stearic acid, oleic acid and elaidic acid.}

**Dietary n-6/n-3 ratio**
There is a concern regarding a high dietary n-6/n-3 ratio, especially in the United States. A too high LA intake may lead to increased AA synthesis which is a precursor for pro-inflammatory and pro-thrombotic factors that may promote plaque formation. In the United States, this ratio is 10-12:1\textsuperscript{15} but the recommendation in the Nordic Nutrition Recommendations 2004 is
to keep this ratio between 3-9:1, but there is no consensus about the optimal n-6/n-3 ratio in the diet. The n-6/n-3 ratio in the Swedish diet is 5:1\textsuperscript{16}.

**Dietary fatty acids and cardiovascular disease**

A positive association between a diet high in SFAs and a negative association between a diet high in unsaturated fat, respectively, and the risk of CVD is documented \textsuperscript{17}, but not undisputed. In prospective studies within populations, it has often been difficult to demonstrate a positive relationship between intakes of SFA and coronary heart disease (CHD) \textsuperscript{12, 18, 19}, but cross-sectional studies have in general demonstrated a positive relationship between intake of SFA and CHD \textsuperscript{20}. Mechanisms that link dietary fatty acids and CVD have been related to effects on blood cholesterol levels \textsuperscript{20-22}, insulin resistance\textsuperscript{23}, inflammation and endothelial dysfunction \textsuperscript{24}.

SFAs are known to elevate low density lipoprotein (LDL) cholesterol, compared to carbohydrate. Myristic acid (14:0) is the most cholesterol-raising FA followed by lauric (12:0) and palmitic (16:0) acids. Stearic acid (18:0) is however neutral with respect to blood cholesterol levels \textsuperscript{21, 25}. LA is the most potent cholesterol lowering FA \textsuperscript{15} and MUFAs, mainly oleic acid (OA, 18:1) are known to lower LDL-cholesterol levels \textsuperscript{12}.

MUFAs may induce anti-inflammation \textsuperscript{12} and a MUFA rich diet improved insulin sensitivity, besides lowering plasma cholesterol and blood pressure in the KANWU-study \textsuperscript{26-28}. Substitution of saturated fat for unsaturated fat, mainly LA, reduced coronary heart disease events in several clinical studies (e.g. Finnish Mental Hospital Study \textsuperscript{29} and Los Angeles Veteran Hospital Study \textsuperscript{30}) but addition of a large amount of LA in a diet low in saturated fat did not prevent, in primary prevention, cardiovascular or all-cause mortality in the Minnesota Coronary Survey \textsuperscript{31} and some other studies (Reviewed in \textsuperscript{12, 18}). CVD mortality was considerable lower with a higher intake of PUFA and LA after 15 years of follow-up in a population based cohort study in Finland \textsuperscript{32}.

N-3 PUFAs are known to lower TAG levels, to induce anti-inflammation and may improve endothelial dysfunction, but are known to slightly elevate LDL-cholesterol \textsuperscript{33-35}. The effect of n-3 FA on insulin sensitivity is uncertain \textsuperscript{36}. A cardioprotective effect of n-3 FA from fish exists, especially for fatal events, but the relation to non-fatal events is not entirely clear \textsuperscript{37, 38}. ALA is probably not as potent as n-3 fish FAs, eicosapentaenoic (EPA) and docosahexaenoic (DHA) to reduce cardiovascular risk \textsuperscript{34}. However, ALA as part of a Mediterranean style diet, significantly reduced cardiac events in the Lyon Diet heart Study \textsuperscript{39} and in the Indo-Mediterranean Diet Heart Study \textsuperscript{40}. In the GISSI and DART trials n-3 fish FA as supplements or as part of a diet decreased cardiovascular mortality \textsuperscript{18, 41, 42}.
TFAs raise LDL-cholesterol, reduce high density lipoprotein (HDL) cholesterol and promote inflammation and endothelial dysfunction \(^\text{43}\) and may increase the risk of CHD \(^\text{44}\).

**Fatty acids as biomarkers**

It is known that dietary surveys often fail to adequately measure the intake of food and nutrients due to reporting bias and there is especially a problem with underreporting \(^\text{45}\). It is recognized that it is particularly difficult to assess the intake of fatty foods with traditional methods and it is known that the degree of underreporting of especially dietary fat varies with an increasing BMI among the reporters \(^\text{46-49}\). Instead, the use of biomarkers may provide an objective and more accurate way of estimating dietary intakes \(^\text{5,50}\).

FAs are transported in the blood in two ways; either as non esterified FA (NEFA) bound to albumin or in more complex structures known as lipoproteins (see front cover). Lipoproteins are made up by TAG, cholesteryl esters (CE), phospholipids (PL), cholesterol and apolipoproteins \(^\text{8}\).

The FA composition of the different fractions of the lipoprotein particle e.g. PL and CE, in platelets and erythrocyte membranes or other body

![Figure 2. Endogenous fatty acid synthesis](image)

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tissues, such as skeletal muscle and adipose tissue, can be used as biomarkers of dietary fat quality. This composition reflects the FA composition (fat quality) of the diet at different time points after eating. The FA composition in serum PL and CE reflects the habitual diet during the last days and weeks, respectively, while FAs of erythrocyte membranes and adipose tissue reflect the intake of those FA during the last months and year(s), respectively.\textsuperscript{5, 50-54}

FAs can be endogenously synthesized, elongated, desaturated or oxidized, which complicates the use of FA as biomarkers of dietary fat quality. SFAs can be synthesized \textit{de novo} from carbohydrates, but this synthesis is rare in people who consume a Western diet (high fat diet).\textsuperscript{55}

The correlation between dietary FA and plasma FA varies\textsuperscript{56} and the best biomarkers are FA that cannot be endogenously synthesized i.e. LA, ALA and TFA.\textsuperscript{5, 50} Tissue proportions of EPA and DHA may serve as biomarkers of fish intake\textsuperscript{50} and the proportions of 15:0 and 17:0 in serum and adipose tissue have been validated as reliable biomarkers of milk fat.\textsuperscript{57-59}

FA composition in tissues and lipid fractions is influenced by genetic variation, age, sex, smoking, physical activity\textsuperscript{50} and transport processes since different FAs compete for the incorporation in different lipid classes.\textsuperscript{60} Arachidonic acid (20:4 n-6, AA) is formed from LA and is the most important precursor for eicosanoids and is therefore tightly regulated in different membranes.\textsuperscript{5}

\textbf{Desaturases and elongases}

The degree of unsaturation of a FA is a major determinant of the melting point and thus of the fluidity of biological membranes. The enzymes responsible for the unsaturation are the delta (\(\Delta\)) desaturases. These enzymes are bound to the endoplasmic reticulum and introduce double bonds in specific positions of long chain FAs. Three \(\Delta\)-desaturases are important for human FA metabolism; \(\Delta^9\)-desaturase also referred to as stearoyl-CoA-desaturase (SCD), \(\Delta^6\)-desaturase (D6D) and \(\Delta^5\)-desaturase (D5D). Elongases are the products of the \textit{Elovll-6} (Elongation-of-very-long-chain-fatty-acids) genes. Elongases and desaturases work in concert to form long chain FAs in the endoplasmic reticulum.\textsuperscript{61, 62} The desaturation steps are believed to be rate limiting in the formation of FAs.

SCD introduces the first double bond at the 9, 10 position from the carboxyl end of the SFA and represent the last step in the formation of MUFAs. SCD catalyzes the desaturation of FAs with 12 to 19 carbon atoms\textsuperscript{63} and the preferential substrate is 18:0.\textsuperscript{64} D5D and D6D catalyze the synthesis of n-6 and n-3 long chain PUFAs. MUFAs are incorporated in membrane PL, adipose tissue TAG and CE. In addition, MUFAs can act as mediators in signal transduction and cellular differentiation.\textsuperscript{63, 65, 66} PUFAs are incorporated into
membrane PL, but are also needed for eicosanoid signaling, pinocytosis, ion channel modulation and regulation of gene expression. PUFAs regulate desaturases via transcription factors that bind in the promoter regions of the different desaturase genes. Sterol regulatory element binding protein-1c (SREBP-1c) and peroxisome proliferator activated receptors (PPARs) are thought to play key roles in this regulation. Moreover, in a recent study human subcutaneous adipose tissue SCD, D6D and D5D transcripts were significantly reduced in response to acute weight loss in moderately overweight men.

To study desaturase activities directly in sub-cellular microsomal (i.e. endoplasmic reticulum) fractions is complicated in humans and not practicable in clinical studies. To measure mRNA/protein expression of desaturases raise ethical concerns in humans, since the most relevant tissue to study is the liver. Surrogate measures of desaturase activities, estimated as FA ratios in serum CE and PL, have instead been used in the studies of this thesis.

LA and ALA compete for the same elongases and desaturases. Although elongases and desaturases have a preference for n-3 FA, a high dietary intake of LA may lead to a relative deficiency of ALA and may also reduce the endogenous conversion of ALA to long chain n-3 FA. Stearoyl-CoA-desaturase

The SCD-gene is situated on chromosome 10 and two iso-forms of the human SCD gene exists, SCD1 and SCD5. SCD1 is primarily expressed in the liver and adipose tissue, whereas SCD5 mainly is expressed in brain and pancreas. SCD1 share 85 % homology with murine SCD genes. SCD1 plays a major role in de novo synthesis of TAG, CE and wax esters and is needed for normal function of skin and eyelid. SCD is highly regulated and glucose, fructose, cholesterol, insulin, and estrogen are inducers, while PUFAs, alcohol and leptin are inhibitors of SCD-activity.

SCD has been proposed as a future therapeutic target for obesity and related morbidities. It is known that SCD knock-out mice have better insulin sensitivity, higher energy metabolism and are leaner than their wild-type littermates. In addition, genes involved in β-oxidation are up regulated and genes involved in lipid synthesis are down regulated in the knock-out mice. Moreover, mice lacking the SCD gene are deficient in hepatic TAG and CEs despite the presence of other enzymes responsible for the synthesis of TAG and CEs and supplementation with dietary 18:1 and 16:1 does not restore lipid levels. Attie et al reported that human plasma TAG levels were closely correlated to estimated plasma SCD activity (desaturation index). In addition, the desaturation index was validated in various mice strains as an in vivo marker of SCD activity in this study. The relative mRNA expression level of SCD1 was three-fold in skeletal muscle from extremely obese compared to lean subjects and this increased mRNA ex-
pression corresponded to changes in estimated SCD activity \textsuperscript{79}. Risérus et al reported that increased mRNA expression of SCD1 adipose tissue in humans corresponded to increased estimated SCD activity measured in serum \textsuperscript{80}. A recent study reported that SCD1 mediates prolipogenic effects of dietary saturated fat in mice \textsuperscript{81}.

**Biomarker fatty acids, estimated desaturase activities and metabolic and cardiovascular diseases**

FAs in serum CE and PL can be used as biomarkers of dietary fat quality and FA ratios as indicators of endogenous desaturation. Hence it is possible to use biomarker FAs and FA ratios (SCD, D5D and D6D) as the exposure variable in epidemiological studies of the association between dietary fat and disease outcome. A serum lipid FA composition characterized by high proportions of palmitic (16:0), palmitoleic (16:1) and dihomo-\(\gamma\)-linolenic (20:3 n-6, DHLA) acids and a low proportion of LA, has in epidemiologic studies been related to obesity \textsuperscript{82}, insulin resistance \textsuperscript{83}, CVD and type 2 diabetes \textsuperscript{84-86} and to individual components of the metabolic syndrome (MetS) \textsuperscript{87}. In a Finnish study, high serum proportions of LA decreased the risk of fasting glycemia and type 2 diabetes in middle-aged men \textsuperscript{88}. Harris et al reported in a recent meta analysis depressed levels of long chain n-3 FA (especially DHA) as a consistent marker of increased CHD risk \textsuperscript{37}. Moreover, estimated desaturase activities; higher SCD and D6D and lower D5D have been related to metabolic and cardiovascular diseases \textsuperscript{89}. In a lifestyle intervention study, an increase in estimated D5D and a decrease in estimated SCD and D6D activities were associated with an increase in insulin sensitivity, independent of lifestyle changes \textsuperscript{90}. Further, the estimated SCD-ratio \([16:1/16:0]\), as a measure of dietary saturated fat \textsuperscript{89}, was established as an independent predictor of directly measured insulin sensitivity over 20 years \textsuperscript{91}.

**Obesity and insulin resistance**

Obesity is a major risk factor for metabolic and cardiovascular diseases such as CVD and type 2 diabetes and is defined as the ratio between weight (kg) and height squared (m\(^2\), referred to as body mass index (BMI)). Overweight is defined in subjects with BMI >25 kg/m\(^2\) and obesity is defined in subjects with a BMI >30 kg/m\(^2\). About half of the Swedish population is overweight and 10 \% obese \textsuperscript{92}. Due to the current increase in childhood obesity the global life expectancy in the United States might decline for the first time in modern history \textsuperscript{93}. 
Metabolic changes associated with obesity, especially abdominal obesity, are generally linked to insulin resistance. Insulin resistance is a situation when normal concentrations of insulin produce a biological response which is insufficient and the regulation of glucose decay, which eventually might lead to type 2 diabetes. Insulin resistant individuals have hyperinsulinemia together with normoglycemia or hyperglycemia. Insulin sensitivity varies in healthy individuals, but obese individuals are very often insulin resistant. Insulin resistance develops because of genetic susceptibility, e.g. alterations in genes involved in the insulin signaling pathways, and lifestyle factors, such as intake of excess calories and decreased physical activity.

How is insulin resistance and obesity linked? Several mechanisms have been proposed. It is known that adipose tissue secrete NEFA into the circulation and elevated NEFA levels may induce many adverse metabolic and physiological effects such as insulin resistance, both in skeletal muscle and the liver. Adipose tissue act as an endocrine organ producing signals that may impair insulin sensitivity. Cytokines, such as the pro-inflammatory factors TNF-α and IL-6 are released from adipose tissue and have been proposed as candidates that would promote adverse effects but the studies are inconclusive. Adiponectin, an adipokine, is another candidate that seems to be involved in the link between obesity and insulin resistance. Adiponectin is known to increase FA oxidation in skeletal muscle either by directly regulating genes involved in FA oxidation or indirectly by stimulating PPARα expression. Adiponectin levels are reduced in obesity. Insulin resistance in skeletal muscle is coupled with accumulation of intramyocellular lipids and intracellular-signaling molecules such as ceramides and diacylglycerol.

**Metabolic syndrome**

The MetS denotes the clustering of several metabolic risk factors in one individual and usually includes disturbances in glucose and insulin metabolism, central obesity, dyslipidemia (high TAG levels, low HDL-cholesterol and high levels of small dense LDL-particles) and hypertension. Other disturbances often associated with MetS include impaired fibrinolysis and increased coagulation, signs of inflammation and endothelial dysfunction.

The concept of risk factor clustering was first introduced by Reaven in 1988 (although described as early as in the 1920s) and was referred to as Syndrome X. He meant that insulin resistance and compensatory hyperinsulinemia were the driving forces behind the other metabolic disturbances in syndrome X and that the syndrome was a risk factor for cardiovascular disease. Since then MetS has been a hot topic for researchers, but the definition and its clinical value, beyond the risk associated with its individual components, remains controversial.
There are several definitions available e.g. the definitions according to WHO\textsuperscript{106}, the European Group for the Study of Insulin Resistance (EGIR)\textsuperscript{107}, International Diabetes Federation (IDF)\textsuperscript{108} and the Adult Treatment Panel (ATP)\textsuperscript{III} of the National Cholesterol Education Program (NCEP)\textsuperscript{109}. The definitions are slightly different and thus depending on which definition you choose the prevalence of MetS will somewhat differ within the same study population\textsuperscript{110}. The most widely used definition is the one proposed by NCEP.

Studies have demonstrated that MetS predicts the risk to suffer and die from both CVD and type 2 diabetes but some studies have failed to confirm such a relationship\textsuperscript{111-115}. Many factors, such as lifestyle (physical activity and diet), genetic predisposition and the “thrifty phenotype”, have been suggested to be involved in the etiology of MetS\textsuperscript{99}. The pathophysiology behind the risk factor clustering is complex and unlikely to be caused by one single underlying factor\textsuperscript{102}, but increased adipose tissue (and especially abdominal obesity) with subsequent insulin resistance might be the primary mechanisms that drive the syndrome\textsuperscript{116,117}.

The prevalence of the MetS is increasing throughout the world (because of the current obesity epidemic) and increases with age and is higher in certain ethnic groups\textsuperscript{99,117}. In 60 year old men and women from Stockholm the prevalence of MetS was 13% among women and 19% among men (EGIR-definition)\textsuperscript{118} and in the Atherosclerosis and Insulin Resistance study from Gothenburg the prevalence was 16% among 58-year old men (WHO definition 1998)\textsuperscript{119}. In the US, in the Third National Health and Nutrition Examination Survey, it was estimated that roughly 24% out of a of total 8608 men and women had MetS, defined according to NCEP ATPIII\textsuperscript{110}. In Finland, in the Kuopio Ischemic Heart Disease Risk Factor Study, the prevalence of MetS varied between 9 and 14% (NCEP ATPIII or WHO 1998 definition) in 1209 men (42 to 60 years), if those with diabetes and CVD were excluded\textsuperscript{112}.

**Genetic factors and variation**

It is known that genetic factors in concert with the influences from the environment control and condition us to develop traits and diseases, including the different components of MetS such as insulin resistance, obesity and hypertension\textsuperscript{117,120}. Diet has a special place among the external influences that affects human health and is believed to be one of the most important risk modulators for several diseases with multifactorial pathogenesis\textsuperscript{121}.

In order to investigate genetic determinants of multifactorial diseases such as CVD, polymorphic genes likely to be involved in the pathogenesis of the disease/phenotype of interest are selected and then analyzed as candidates\textsuperscript{121}. The human genome holds the total genetic information and gives rise to mul-
ultiple tissues. This is due to differential gene expression of the genetic code (DNA) in different cell types. DNA is packaged as chromosomes and a human cell contains 22 pairs of autosomes and two sex chromosomes. The DNA consists of about three billion basepairs coding for approximately 30,000 genes. Although more than 99% of the human DNA sequence is the same across populations, these DNA differences can have great impact on how humans respond to the environment and predisposes us for different diseases. Genetic variations are fairly common and are relatively evenly distributed across the human genome. The most common type (>90%) of variation found in the genome is the Single Nucleotide Polymorphism (SNP). SNPs are variations in the DNA sequence when one single nucleotide is changed to another (e.g. AGGGCTAA to ATGGCTAA) and occurs both in coding and non-coding regions of the genome. SNPs do not cause disease but may determine the likelihood for a disease. In study III of this thesis SNPs in our candidate gene, SCD1, have been analyzed in association with risk factors for metabolic disease. The study is a so-called genetic association study, in which different genotypes (SNPs) in a study population are tested with statistical techniques in association to phenotypic variation in order to identify genetic variations that may underlie disease.
Aims

The overall aim of this thesis was to explore the serum proportions of individual FAs and estimated desaturase activities (SCD, D6D and D5D) as risk factors/markers for metabolic and cardiovascular diseases, to study genetic variations in the SCD1 gene and to investigate how dietary fat quality might influence estimated enzyme activities.

The specific aims were:

I To examine the effect of estimated desaturase activities and individual FAs in serum CE on the risk to develop the metabolic syndrome over 20 years.

II To examine the effect of estimated desaturase activities and serum FAs in serum cholesteryl esters, on cardiovascular and total mortality risk with a maximum follow-up time of 33.7 years.

III To examine cross-sectional associations between genetic variations in the human SCD1-gene, defined as SNPs and haplotypes, and markers of obesity; BMI and waist circumference (WC), insulin sensitivity and estimated SCD activity.

IV To compare the effects of two diets with different fat qualities, butter (SAT) vs. rapeseed oil (RO) diets, on FA composition and estimated desaturase activities in serum CE and PL.
Subjects and Methods

Study I, II and III were based on data from the Uppsala Longitudinal Study of Adult Men, ULSAM, and are observational in nature. Study IV is based on data collected during a strictly controlled dietary cross-over intervention study.

Study participants

I. 1558 men from the investigation at 50 years remained for analysis after the exclusion of 764 men in study I. Excluded were those who were hypertensive (supine diastolic BP >= 95 mmHg), were taking BP medication, had diabetes or were currently taking medication for hyperlipidaemia. 706 of these participated in the 70 years investigation, had complete FA data, did not have MetS at baseline and were included in the logistic regression analyses.

II. 2009 of the men who participated in the baseline investigation at 50 years had complete fatty acid data. Our analyses were carried out on this sample and on a healthy subsample (n=1558) excluding persons with previous myocardial infarction (n= 7), stroke (n= 3), cancer (n=7), diabetes (n= 104) and those using lipid lowering drugs (n= 21).

III. Out of the 1221 participants at the investigation at 70 years, 1143 men had a valid DNA sample for genotyping of the SCD1 gene and these men made up the study population.

IV. Twenty subjects with moderate hyperlipidaemia were recruited from an ongoing health survey at a local telephone company to participate in this study. Six females and fourteen males with a mean age of 50.9 ±10 (mean ± SD) years participated. They were all healthy and they did not take any drugs.
Estimation of desaturase activities

The desaturase activities were estimated in all four studies as a product to precursor ratio of individual FA in serum CE, and in study IV in PL as well, according to the following:

- \( \text{SCD} = \frac{16:1 \text{ (n-7)}}{16:0 \text{ (SCD-16)}} \) and \( \frac{18:1 \text{ (n-9)}}{18:0 \text{ (SCD-18)}} \)
- \( \text{D6D} = \frac{18:3 \text{ (n-6)}}{18:2 \text{ (n-6)}} \)
- \( \text{D5D} = \frac{20:4 \text{ (n-6)}}{20:3 \text{ (n-6)}} \)

D6D in PL was in study III estimated as \( \frac{20:3 \text{ (n-6)}}{18:2 \text{ (n-6)}} \), since the proportion of 18:3 (n-6) was too low for reliable quantification. It should be kept in mind that this ratio also measures the elongase activity but this step is regarded not to be rate-limiting (Figure 2). This ratio is thus considered to be a good estimate of the D6D activity.

The ULSAM- cohort

The Uppsala Longitudinal Study of Adult Men, ULSAM, is a population-based cohort study that started in Uppsala, Sweden, in 1970. All men born 1920-1924 living in Uppsala at that time were invited to participate. The participants were examined at baseline at age 50 and reinvestigated at age 60, 70, 77 and 82. The five investigations were extensive and carried out in similar manners; however the number of investigations differed between the ages and the 60 years investigation was the most limited. All investigations were carried out after an overnight fast. Only data from the 50 and 70 years investigations were used in the studies of this thesis. At baseline, 2841 men were invited and 82\% (2322) accepted to participate. At age 70 1681 men were invited and 73\% (1221) participated. 126

Figure 2. A schematic overview of the ULSAM-study. All men born 1920-24 and living in Uppsala in 1970 were invited to the baseline investigation at 50 years. For the 60, 70 and 77 years investigation all men alive and still living in Uppsala were invited to participate. For the 82-years investigation those who participated in the 70 and/or the 77 years investigation were invited.
Methods in ULSAM-based studies

Baseline investigations at 50 years
All investigations were carried out under standardized conditions and have been described in detail previously\textsuperscript{127, 128}. The investigations included a medical questionnaire and interview, as well as blood sampling, anthropometric measurements and blood pressures.

\textit{Anthropometry}
BMI was calculated as weight (kg) divided by height (m) squared and WC (cm) was measured midway between the lowest rib and the iliac crest in a supine position (measured only in a sub sample).

\textit{Blood Pressure}
Supine blood pressures were recorded after 10 minutes of rest in the right arm in a recumbent position with a Mercury manometer (Kifa Ercameter, wall-model). Systolic and diastolic blood pressures were read to the nearest 5 mmHg and the mean of two values was used in the analyses. Hypertension in study III was defined as a systolic blood pressure $\geq 140$ mmHg or diastolic blood pressure $\geq 90$ mmHg or a regular use of antihypertensive medication and used as a confounder in the Cox-regression.

\textit{Blood samples}
Blood samples were taken from the antecubital vein. Serum TAG and cholesterol concentrations were analyzed on a Technicon Auto Analyzer type II\textsuperscript{129}. HDL-cholesterol was assayed in the supernatant after precipitation with a sodium phosphotungstate and magnesium chloride solution\textsuperscript{130}. Serum fasting insulin were determined with the Phadebas Insulin test (Pharmacia, Uppsala, Sweden) based on radioimmunosorbent technique\textsuperscript{131}. Fasting blood glucose was measured by spectrophotometry using the glucose oxidaze method. Homeostasis model insulin resistance (HOMA) index was calculated as fasting insulin(mU/l) x fasting glucose(mmol/l) /22.5 according to Matthews\textsuperscript{132}.

\textit{Serum cholesteryl ester fatty acids}
The serum FA composition was analyzed as previously described\textsuperscript{133, 134}. The samples from the 50 years investigation had been stored in liquid nitrogen for about 15 years before analysis. Serum was extracted with a hexane-isopropanol solution (1+4) and CEs were separated from the extract by thin layer chromatography. Inter-esterification was carried out with acidic methanol at 85°C for two hours. Free cholesterol that had been liberated in the reaction was removed by aluminum oxide to avoid contamination of the
column. The percentage composition of methylated FAs from 14:0 to 22:6 was determined by gas chromatography (a 25 m NB-351 silica capillary column) with a flame ionization detector and helium as carrier gas. Every 25th sample was a serum control pool. The coefficient of variation varied between series analysis (n=35) from 2% (large peaks) to 10% (smaller peaks) and between successive gas chromatography runs 0.2-5% (n=17).

**Questionnaire and interview**
A self-administered questionnaire made according to Collen *et al*\(^{135}\) was used to collect information on various factor including physical activity, medical treatment and previous and current disease. Smoking data was obtained from an interview.

Investigations at 70 years
All investigations were carried out as previously described\(^{83,136}\).

**Anthropometric measurements**
BMI was calculated as weight (kg) divided by height (m) squared and waist circumference (cm) was measured midway between the lowest rib and the iliac crest in a supine position.

**Blood pressure**
Blood pressure was measured as described above.

**Blood samples**
Blood samples were taken from the antecubital vein. Serum cholesterol and TAG concentrations were analyzed by enzymatic techniques using IL Test Cholesterol Trinders's Method and IL Test Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). HDL particles were separated by precipitation with magnesium chloride/phosphotungstate. An oral glucose tolerance was carried out. 75 g glucose dissolved in 300 ml of water were given the subjects, and blood samples for plasma glucose and insulin were drawn immediately before, and 30, 60, 90, and 120 min after ingestion of glucose. Plasma glucose was measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany). The intra-individual coefficient of variation for fasting plasma glucose was 3.2%. Plasma insulin was assayed using an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim, Germany) performed in an ES300 automatic analyser (Boehringer Mannheim).
Serum cholesteryl ester fatty acids
The FAs were analyzed within one year of collection and in a similar way as at the 50 years investigation. However, the lipids were extracted in chloroform with 0.005% butylated hydroxy-toluene added as an antioxidant. After transmethylation the fatty acid methyl esters were separated by Gas Liquid Chromatography. The Gas Liquid Chromatograph system was made up by a Hewlett Packard (Avondale, PA, USA) GC 5890 with an automatic sampler 7671A and a 3392A integrator, and a 25 m Quadrex (New Haven, CT, USA) fused Silica capillary column (model OV-351). The FA were identified by comparing each peak’s retention time with the retention times of NuCheck Prep’s (Elysian, MN, USA) methyl standards (Gas Liquid Chromatograph reference standard GLC-68A).

Direct measurement of insulin sensitivity
Insulin sensitivity was determined by the golden standard euglycemic hyperinsulinemic clamp technique according to DeFronzo 137, slightly modified. Briefly, insulin was infused in a primary dose during the initial 10 minutes and then as a continuous infusion for 110 minutes. The infusion rate was 56 mU (instead of 40 mU) per minute per body surface area (meters squared) to achieve nearly complete suppression of hepatic glucose output 138. The level of plasma glucose was maintained at 5.1 mmol/L by glucose infusion. The total amount of glucose infused serves as the sensitivity to the existing plasma insulin concentrations. The glucose disposal (M (mg x kg\(^{-1}\) x min\(^{-1}\))) was calculated as the amount of glucose taken up during the last 60 minutes of the study. Tissue insulin sensitivity (amount of glucose metabolized per unit of plasma insulin) was also calculated as the insulin sensitivity index (M/I (mg x kg\(^{-1}\) x min\(^{-1}\) / 100 mgU/l)), where M is the glucose disposal and I is the mean insulin concentration during the clamp.

Questionnaire and interview
A questionnaire was used to obtain information on physical activity, medical treatment and previous and current disease. The medical questionnaire was based on the questionnaires previously used at 50 and 60 years investigations. During the clamp investigation, a nurse or a technician posed the question "Do you smoke?" and this forms the basis for the smoking variable.

Definition of the metabolic syndrome in study I
MetS was defined according to the definition of the National Cholesterol Educational Program (NCEP) Adult Treatment Panel III (ATP III) 109, 139. MetS was considered present when three or more of the following risk factors were met: Systolic blood pressure >=130 mmHg or diastolic blood pressure >=85mmHg, fasting plasma glucose >=6.1 mmol/l, TAG >=1.69 mmol/l, HDL-cholesterol <1.04 mmol/l or WC >102 cm. At the 50 years
investigation, WC was only recorded for those born in 1920 and 1921 (n=480) and to be able to define the obesity criteria a BMI cut-off was used instead. The BMI cut-off was 29.2 kg/m², which corresponded to a waist circumference >102 cm (Linear regression equation: $\text{BMI} [\text{kg/m}^2] = 0.2863 \times \text{WC} [\text{cm}] - 0.034$). This was derived from subjects with data on both BMI and WC. At the 70 years investigation, WC was measured for most men (n=1192) and was used to define the obesity criteria.

**Follow up and outcome measures in study II**

Follow-up was from the examination date for the baseline investigation in 1970-73 and to December 31st 2003, with a maximum of 33.7 years of follow up (median 30.7 years rendering 61518 and 58149 person years at risk in the total sample and the health subsample, respectively). Information regarding mortality was collected from the official Swedish Cause of Death registry held by the National Board of Health and Welfare (Socialstyrelsen) in Sweden. The register includes all Swedish citizens and the study participants were linked to the registry by means of a unique personal identification code. The registry uses the codes of the International Classification of Disease (ICD) and the outcomes in study III were defined a priori as cardiovascular mortality (ICD-9 codes 390-459, ICD-10 codes I00-I99, to comply with current European guidelines) and total mortality.

**DNA preparation, SNP selection and genotyping in study III**

The DNA preparation was carried out as previously described. Eleven SNPs were selected from the dbSNP database to be evenly distributed over a region including the SCD1 gene and about 10 kb upstream and 2 kb downstream of the gene. The SNPs were genotyped at the SNP technology platform at Uppsala University using a 12-plex GenomeLab SNPStream system (Beckman Coulter) using the $\chi^2$ distribution (p>0.05) for each assay. The remaining eight SNPs conferred to HWE. The overall genotype call rate was 96.7%, and the accuracy was 99.96% according to duplicate analysis of on average 40% (4723/11 870) of the genotypes.

**Statistical Analysis**

**Study I**

The statistical analyses were carried out using the software package STATA (version 6.0; STATA Corporation, TX, USA). Two tailed p-values and 95%
CI were given and $p<0.05$ considered significant. Continuous data are given as means ± SD. Smoking status was a categorical variable, where non-smoker=0, smoker=1 and ex-smoker=2 at 50 years and smoker=1 and non-smoker=0 at 70 years. Physical activity levels were graded 1 (lowest) to 4 (highest). The categorical variables were used as confounders in the multivariate logistic regression models.

**Distribution**
The normal distribution of continuous variables was examined with Shapiro-Wilk’s test. Variables non-normally distributed ($W<0.95$) were log-transformed.

**Basic Statistics**
Student’s t-test was used to test differences in means between baseline estimated desaturases, individual FAs and clinical characteristics in individuals with and without the MetS at age 70.

**Logistic regression analysis**
Logistic regression analysis was carried out to estimate the risk of having MetS at the age of 70 in relation to the estimated and standardized (SD=1, mean=0) desaturase activities at 50 years. Variables were standardized in order to be able to compare the effect of independent variables on the dependent variable. The logistic regression analysis was carried out univariate and multivariate. The following variables were included in the multivariate logistic regression models, either alone or in combination with each other: BMI, smoking behavior and/or physical activity.

Study II
The statistical analysis was carried out with STATA, version 8.2 (College Station, TX, USA)

**Distribution**
Continuous variables are presented as means (SD) or median (inter-quartile range) and categorical variables as number of individuals (%). The normal distribution of continuous variables was examined with Shapiro-Wilk’s test and variables non-normally distributed ($W<0.95$) were log-transformed (16:1, 18:0, 18:3 n-6, 20:5 n-3, 22:6 n-3, SCD and D6D).

**Cox proportional hazard analysis**
Independent variables- individual fatty acids (14:0 -22:6) and estimated desaturase activities (SCD, D6D and D5D) - were investigated in two ways: linear relations were investigated as the effect of 1 SD increments in con-
tinuous variables, and non-linear relations were examined using quartiles of the independent variables. Cox proportional hazard models were carried out to examine individual fatty acids and estimated desaturase activities in relation to cardiovascular and total mortality. The analysis was carried out both on the total study sample and on the healthy subsample and for each sample and endpoint an unadjusted and a multivariable-adjusted (smoking status, physical activity, BMI, total cholesterol and hypertension) model were examined. The proportional hazards assumption was checked using Schoenfeld’s test. Hazard ratios and two-tailed 95% CI are given.

Study III
The statistical analysis was carried out with STATA, version 8.2 (College Station, TX, USA) and the adjusted \( \alpha \)-level was calculated with a macro in SAS, version 8.0 (SAS Institute, Cary, NC, USA).

To account for possible false positive tests due to multiple testing an adjusted significance level using Meff correction \(^{144}\) was calculated. All test were two tailed and \( p \)-values were compared both with conventional significance level (\( p<0.05 \)) and the adjusted \( \alpha \)-level=0.0076.

**Distribution**
Continuous, normally distributed variables are presented as mean ± standard deviation (SD). Skewed variables (fasting insulin, fasting glucose and SCD-16) were log-transformed before analysis and are presented with geometric mean and 95% CIs.

**Linear regression models**
Associations between genotypes (SNPs and haplotypes) and phenotypes (insulin sensitivity, BMI and waist circumference and SCD-ratio), were analyzed in linear regression models. General (codominant, step-wise-test) and additive (trend test) genetic models, using the most common homozygous allele (for each SNP) and the non-carrier of a specific haplotype as reference level, were carried out.

Multivariable linear models adjusting, the SNP (rs7849) that showed the strongest association with insulin sensitivity, for BMI and WC were carried out. Concomitant co-morbidity and drug use (hypertension treatment, lipid medication, diabetes prevalence and variables based on questionnaire questions) as also adjusted for. To investigate whether the association between rs7849 and insulin sensitivity was modified by the level of obesity, the study population was stratified into tertiles of BMI and WC and a linear regression model was carried out on these strata, separately. The association between rs7849 and WC was also adjusted for insulin sensitivity.
Figure 3. Illustrates the pairwise linkage disequilibrium (LD) expressed as $r^2$ between the investigated Stearoyl-CoenzymeA-Desaturase Single Nucleotide Polymorphisms (SNPs) in the Haploview software setting. Shading represents magnitude and significance of the pairwise $r^2$. Black, $r^2 > 0.8$ (high $r^2$), dark grey, $r^2$ between 0.8 and 0.6, moderate grey, $r^2$ between 0.4 and 0.6 and white, $r^2 < 0.2$ (low $r^2$). The haplotype group was constructed by rs10883463, rs7849, rs2167444 and rs508384.

**Haplotype estimation**

Haplotypes were estimated using the software program PHASE \(^{145}\). One group of haplotypes was estimated by selecting four SNPs in strong linkage disequilibrium (LD) based on pairwise marker comparisons ($r^2$) in the Haploview software, version 3.2 \(^{146}\) (Figure 4). To account for uncertain phase in the haplotype estimations, only haplotypes with the probability of >0.98 were included in the statistical analysis.
Study IV; Study subjects, design, diets and methods

Study population
Six women and fourteen men were recruited from an ongoing health survey at a local company. They were all healthy, but slightly overweight and had moderate hyperlipidaemia. None of the participants took any drugs.

Study design
A two-period cross-over randomized study design was used. The intervention with each diet was three weeks with a wash-out period of four weeks in between. The subjects started either with a SAT-diet or a RO-diet. All were asked to maintain their usual physical activity and not to undertake any other lifestyle changes during and between the test periods.

Diets
The diets were prepared at a metabolic ward kitchen, where all participants picked up all meals they consumed during the test periods. The energy requirements of the participants were estimated to 30(126) and 35(147) kcal (KJ)/ kg body weight for women and men, respectively. The menu was planned as a seven day menu including breakfast, lunch and dinner with some snacks in between. Both diets were intended to contain equal amounts of macronutrients, besides the differences in FA composition. Both diets were prepared with the same foods, except for the quality of fat. The diet rich in butter fat included butter, high fat cheese and cream, while the RO diet was prepared with a special spread (margarine type) high in rapeseed oil, a liquid margarine made of rapeseed oil for cooking and pure rapeseed oil. The rapeseed oil contained no erucic acid. The FA compositions of the diets were analyzed according to methods earlier reported 133.

Investigations
All samples and anthropometric measurements were carried out after an overnight fast. Weight (kg) and height (m) was recorded and BMI was calculated as weight divided by height squared. TAG and cholesterol concentrations were measured in serum, using the IL Test Cholesterol Triander's method 181618-80 and IL Test Enzymatic-Colorimetric-Method 181709-00 for use in a Monarch 2000 centrifugal analyzer (Instrumentation Laboratory, Lexington, MA, USA.). The serum insulin concentrations were measured by an enzyme linked immunosorbent assay test from Boehringer Mannheim GmbH (Mannheim, Germany) and the blood glucose concentration by the glucose oxidase assay. The FA composition of CEs and PLs was determined by GLC as described previously 133, the same method used in the 70 years ULSAM investigation.
**Statistical analysis**

The statistical analyses were carried out with the software SAS, version 9.1 (SAS institute Inc., NC, USA). All test were two-tailed and $p<0.05$ considered significant.

The normality of the variables was tested with Shapiro Wilk’s test. Variables not normally distributed ($W<0.95$) were log-transformed before analysis. The cross-over design of the study, the scales and the distribution of the variables were taken into account. To test for differences in proportions of FAs and estimated desaturase activities between the two test diets an analysis of variance (ANOVA) model was carried out, in which factors for diet, patient and sequence of intervention was accounted for. A test for carry-over effects was carried out according to Jones and Kenwood. Least square means (LSM), which take imbalances between groups into account, formed the basis for all tests and estimates in the analyses; however data is presented as means ± SD.

**Ethics**

The studies were approved by the ethics committee at Uppsala University and all participants had given their informed consent.
Results and discussion

Study I

Baseline characteristics
All studied clinical characteristics; blood pressures, fasting blood glucose and insulin levels, BMI, WC, HDL-cholesterol, serum TAG and HOMA-index, were at baseline higher (except for HDL-cholesterol which was lower) in subjects who had developed MetS 20 years later.

Prevalence of the metabolic syndrome
The prevalence of MetS was relatively low at baseline (8.3%) but this was partly due to the fact that many individuals that could have been classified as having MetS at that time were excluded from the study. At the 70 years investigation the prevalence was higher and more as expected, 16.9%, when those classified as having the MetS at age 50 were excluded, and 19.8% when the entire study population was considered.

Baseline fatty acid profile and estimated desaturase activities
The difference in the relative proportions of individuals FA and estimated desaturase activities in cholesterol esters were as presented in Table 1 and in Figure 5 the difference in proportion (%) in the estimated desaturase activities in those with and without MetS at baseline is presented.

Longitudinal analysis
The risk of having MetS at age 70 (those having MetS at age 50 were excluded) was approximately 30 percent higher for each SD increase of the estimated SCD [OR= 1.29, 95%CI= 1.0; 1.60] and D6D [1.35, 1.10; 1.65] ratios, while 30 percent lower for each SD increase of the estimated D5D activity [0.71, 0.77; 0.87]. In the multivariate analyses the risk of developing MetS over 20 years disappeared for estimated SCD and D6D activity after adjustment for BMI alone and in combination with physical activity and smoking. The relationship between estimated D5D activity and MetS remained after adjustment for all confounders and the estimated activities of SCD and D6D.
Table 1. The difference in the relative amount of fatty acids and estimated desaturase activities in serum cholesterol esters at the age of 50, in those men who had (n=119) or had not (n=587) developed MS at age 70. The arrow indicates if the FA or ratio was higher (↑) or lower (↓) in subjects with MetS.

<table>
<thead>
<tr>
<th>Fatty acid (% of total FA) and fatty acid ratio</th>
<th>Subjects without MetS Mean ± SD</th>
<th>Subjects with MetS Mean ± SD</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0 (Myristic)</td>
<td>1.09 ± 0.24</td>
<td>1.15 ± 0.23</td>
<td>0.012 ↑</td>
</tr>
<tr>
<td>16:0 (Palmitic)</td>
<td>11.4 ± 0.93</td>
<td>11.7 ± 0.95</td>
<td>0.012 ↑</td>
</tr>
<tr>
<td>16:1 n-7 (Palmitoleic)</td>
<td>3.5 ± 1.1</td>
<td>3.8 ± 1.1</td>
<td>0.003 ↑</td>
</tr>
<tr>
<td>18:0 (Stearic)</td>
<td>1.14 ± 0.3</td>
<td>1.17 ± 0.3</td>
<td>0.34</td>
</tr>
<tr>
<td>18:1 n-9 (Oleic)</td>
<td>18.9 ± 2.5</td>
<td>19.4 ± 2.2</td>
<td>0.037 ↑</td>
</tr>
<tr>
<td>18:2 n-6 (Linoleic)</td>
<td>55.4 ± 4.7</td>
<td>54.0 ± 4.4</td>
<td>0.003 ↓</td>
</tr>
<tr>
<td>18:3 n-6 (γ-linolenic)</td>
<td>0.66 ± 0.16</td>
<td>0.73 ± 0.33</td>
<td>0.011 ↑</td>
</tr>
<tr>
<td>18:3 n-3 (α-linolenic)</td>
<td>0.66 ± 0.16</td>
<td>0.67 ± 0.19</td>
<td>0.27</td>
</tr>
<tr>
<td>20:3 n-6 (Dihomo-γ-linolenic)</td>
<td>0.54 ± 0.13</td>
<td>0.60 ± 0.15</td>
<td>0.000 ↑</td>
</tr>
<tr>
<td>20:4 n-6 (Arachidonic)</td>
<td>4.74 ± 0.94</td>
<td>4.79 ± 1.0</td>
<td>0.6</td>
</tr>
<tr>
<td>20:5 n-3 (Eicosapentaenoic)</td>
<td>1.30 ± 0.57</td>
<td>1.38 ± 0.74</td>
<td>0.28</td>
</tr>
<tr>
<td>22:6 n-3 (Docosahexaenoic)</td>
<td>0.70 ± 0.2</td>
<td>0.69 ± 0.2</td>
<td>0.31</td>
</tr>
<tr>
<td>Δ9↑6 [16:1 n-7/16:0]</td>
<td>0.31 ± 0.09</td>
<td>0.33 ± 0.09</td>
<td>0.02 ↑</td>
</tr>
<tr>
<td>Δ6 [18:3 (n-6)/18:2 (n-6)]</td>
<td>0.012 ± 6x10⁻³</td>
<td>0.014 ± 7x10⁻³</td>
<td>0.004 ↑</td>
</tr>
<tr>
<td>Δ5 [20:4 (n-6)/20:3 (n-6)]</td>
<td>9.0 ± 2.1</td>
<td>8.3 ± 2.1</td>
<td>0.001 ↓</td>
</tr>
</tbody>
</table>

a Those subjects having MetS at baseline were excluded in the analyses.

Discussion

In the present study the prevalence of MetS was low at baseline, 8.3% which is partly explained by the initial exclusions made. This is, however, in line with the results from a Finnish cohort consisting of 42-60 year old men. In that study, men with diabetes and CVD were excluded and the prevalence was between 9-14%, depending on the definition used 112. At the 70 years investigation the prevalence was higher, 16.9%, (those classified as having the MetS at age 50 were excluded), and 19.8% when the entire study population was considered, which accords with previous findings in Swedish men from Stockholm 118 and Gothenburg 119.

The serum lipid FA composition and the estimated desaturase ratios were already at baseline changed in those individuals that at the 70 years investigation were defined as having MetS. This changed FA composition was characterized by high proportions of palmitic (16:0), palmitoleic (16:1) and dihomo-γ-linolenic (20:3 n-6) acids and a low proportion of LA.
Figure 4. The difference in proportion (%) in the estimated desaturase activities at the age of 50 in those who were defined as having (MetS, n=119) or not having (Not MetS, n=587) the metabolic syndrome at age 70. To be able to fit all the information in a reader-friendly figure the SCD and D6D ratio was multiplied by 10 and 1000, respectively.

This means that a changed serum lipid FA composition with apparent high SCD and D6D and low D5D estimated activities might be an early sign of metabolic alterations preceding the risk factors clustering present in MetS. Also, the clinical characteristics (all of which can be associated with MetS) that were investigated in the present study had already started to deteriorate at baseline in those individuals who were defined as having MetS at the 70 years investigation. Thus, we can conclude that metabolic disturbances start early but it is not possible to say whether the changes in FA composition precedes or follows other disturbances from the results in this study.

In the logistic regression analysis, higher estimated activity of SCD and D6D and lower estimated D5D activity increased the risk of developing MetS over 20 years. The relationship between D5D and MetS was independent of BMI, smoking habit, physical activity and the estimated activities of SCD and D6D. The relationship between D6D and MetS was independent of smoking behavior, physical activity, as well as BMI in combination with smoking, whereas the effect of SCD activity was only independent of smoking. This suggests that the effect of D6D and D5D activity on the development of MetS is more or less independent of lifestyle factors, whereas the effect of SCD may be mediated via life style factors such as physical inactivity, diet or other factors that promote obesity. The associa-
tion between metabolic disease and life style is well known and the risk of diabetes in subjects with impaired glucose tolerance was reduced by 58% in a lifestyle intervention trial in Finland \textsuperscript{148} and moderate and vigorous leisure time physical activity was reported to decrease the risk of MetS by two-thirds in a cohort of middle-aged Finnish men \textsuperscript{149}. The findings in the present study further establish serum lipid FA composition and estimated SCD, D6D and D5D activities as risk factors/markers for the development of metabolic disease. However it remains unclear how desaturases are involved in the etiology of MetS.

Study II

**Baseline characteristics**

The prevalence of smoking was similar in both samples (51%), while hypertension was slightly more prevalent in the healthy subsample (43% vs. 41%). Five percent of the total study population had diabetes. Total cholesterol (6.9 mmol/l) and BMI (25 kg/m\textsuperscript{2}) did not differ between the two study samples.

**Mortality data**

During follow up from the examination date at baseline until December 31\textsuperscript{st} 2003, 1012 men died (rate 19.0/1000 person years at risk) and 461 from cardiovascular causes in the total sample and in the healthy subsample 931 men died (rate 18.5/1000 person years at risk) and 416 from cardiovascular causes. Due to the official hospital and cause-of-death registries in Sweden, there was no loss to follow up in this study.

**Cox proportional hazard analysis**

The results from the Cox analysis did not substantially differ in the total and the healthy subsample and the results discussed below are only from the analysis in the total sample.

One SD increase of the proportions of serum myristic, palmitic, palmitoleic, oleic, \(\gamma\)-linolenic, DHLA acids were associated with an increased risk of both cardiovascular and total mortality, while a high proportion of LA was associated with decreased mortality (table 2). The proportions of long chain n-3 fatty acids, ALA, EPA and DHA, as well as of stearic and arachidonic acids, were not associated with any mortality risk in the present study. For the majority of FA the associations followed the same pattern both for cardiovascular and total mortality and were most strongly associated with cardiovascular death. After adjustment for total cholesterol, BMI, smoking, physical activity and hypertension, the hazard ratios remained in the same ranges, but statistical significance was attenuated. The greatest risk was observed per 1 SD increment in the proportion of palmitoleic acid and the serum proportion of LA was most strongly and inversely associated with mortality.
The adjusted hazard ratio, taking risk factors into account, for each SD increase of SCD-16 was 1.15 (1.04-1.27), for D6D 1.12 (1.0-1.24) and for D5D 0.88 (0.80-0.98) for cardiovascular mortality. If we estimated SCD as the 18:1[n-9]/18:0 ratio, this ratio was only associated with an increased risk of total mortality. In the adjusted model, the hazard of cardiovascular death in quartile IV compared to the referent level was 60% higher for SCD and for D6D and D5D the hazard was 50% higher and 24% lower, respectively (Figure 6).

**Discussion**

This study is the first to relate indices of desaturase activities to mortality, and our results suggest a link between saturated fat and cardiovascular and total mortality and confirm a previous Finnish prospective study which linked high dietary and serum LA to decreased cardiovascular mortality.\(^8^8\)

**Desaturases**

The specific role of estimated desaturase activities in cardiovascular disease and subsequent mortality is presently largely unknown.

![Graph](image)

*Figure 5. The adjusted hazard ratio (95% CI) for cardiovascular mortality (total study sample) in 4th quartile (4thQ) compared to the referent level of estimated desaturase activity. Adjusted for total cholesterol, BMI, smoking, physical activity and hypertension.*

However, in the present study a predictive value, beyond classical cardiovascular risk factors, (including total cholesterol, BMI, smoking habit, physical activity and hypertension) was observed for estimated SCD and D6D activity. High D6D activity might lead to high serum proportions of
DHLA, which has been associated with insulin resistance and obesity. The observed decrease in mortality a high estimated D5D activity might be linked to improved insulin sensitivity.

**SCD and MUFA**

Each SD increase of estimated SCD activity and palmitoleic acid was associated with an approximate 30% mortality increase in the unadjusted model and in the adjusted model the increase was 15% and 20% for the SCD ratio and palmitoleic acid, respectively. High estimated SCD activity has previously been strongly associated with cardiovascular and metabolic risks, possibly mediated by increased lipogenesis. It is possible that high estimated SCD1 activity is secondary to high intake of saturated fats as reflected by increased serum proportions of palmitoleic acid and to some extent oleic acid.

Palmitoleic acid content in the diet is in general low and most palmitoleic acid in serum is thus derived from endogenous metabolism of palmitic acid catalyzed by SCD. Palmitoleic acid is known to raise LDL-cholesterol as much as palmitic acid, potentially relating high serum proportions of this FA to CVD.

Table 2. Standardized Cox proportional hazard ratios for fatty acids in serum cholesteryl esters and estimated desaturase activities (FA ratios) in relation to Cardiovascular mortality in the Total study sample (n= 2009).

<table>
<thead>
<tr>
<th>Fatty acid and estimated desaturase activities</th>
<th>Cardiovascular mortality</th>
<th>Number of events= 461</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude HR(95% CI)</td>
<td>Adjusteda HR(95% CI)</td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>1.16 (1.06-1.27)</td>
<td>1.12 (1.02-1.23)</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>1.25 (1.14-1.37)</td>
<td>1.15 (1.04-1.26)</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1)</td>
<td>1.32 (1.21-1.44)</td>
<td>1.18 (1.07-1.30)</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>1.07 (0.97-1.17)</td>
<td>1.04 (0.94-1.15)</td>
</tr>
<tr>
<td>Oleic acid (18:1)</td>
<td>1.29 (1.18-1.41)</td>
<td>1.18 (1.07-1.30)</td>
</tr>
<tr>
<td>Linoleic acid (18:2 n-6)</td>
<td>0.76 (0.70-0.83)</td>
<td>0.85 (0.78-0.94)</td>
</tr>
<tr>
<td>y-Linolenic acid (18:3 n-6)</td>
<td>1.15 (1.05-1.27)</td>
<td>1.09 (0.98-1.21)</td>
</tr>
<tr>
<td>a-Linolenic acid (18:3 n-3)</td>
<td>1.08 (0.99-1.18)</td>
<td>1.10 (1.0-1.21)</td>
</tr>
<tr>
<td>DH-γ-linolenic acid (20:3 n-6)</td>
<td>1.16 (1.07-1.27)</td>
<td>1.06 (0.96-1.18)</td>
</tr>
<tr>
<td>Arachidonic acid (20:4 n-6)</td>
<td>1.00 (0.92-1.10)</td>
<td>0.95 (0.86-1.05)</td>
</tr>
<tr>
<td>Eicosapentaenoic acid (20:5 n-3)</td>
<td>1.07 (0.98-1.17)</td>
<td>0.99 (0.90-1.09)</td>
</tr>
<tr>
<td>Docosahexaenoic acid (22:6 n-3)</td>
<td>0.97 (0.89-1.07)</td>
<td>0.92 (0.84-1.02)</td>
</tr>
<tr>
<td>SCD (16:1/16:0)</td>
<td>1.27 (1.16-1.39)</td>
<td>1.15 (1.04-1.27)</td>
</tr>
<tr>
<td>D6D (18:3 n-6 /18:2 n-6)</td>
<td>1.20 (1.10-1.32)</td>
<td>1.12 (1.0-1.24)</td>
</tr>
<tr>
<td>D5D (20:4 n-6/ 20:3-6)</td>
<td>0.84 (0.76-0.93)</td>
<td>0.88 (0.80-0.98)</td>
</tr>
</tbody>
</table>

DH indicates Dihomo, FA fatty acids. The adjusted model included total cholesterol, BMI, smoking, physical activity and hypertension. The arrows indicate if a high (↑) or a low (↓) proportion of the individual FAs or FA ratios was associated with mortality.
Figure 6. Nelson-Aalen plots of the cumulative hazard of cardiovascular mortality in the total study sample (n=2009) by above vs. below the median of the estimated (a) SCD (16:1/16:0), (b) D6D (16:3 n-6/18:2 n-6) and (c) D5D (20:4 n-6/20:3 n-6) indices in serum cholesteryl esters.
Oleic acid is possibly protective with regard to CVD \textsuperscript{12}, but was in the present study associated with increased mortality. However this might be caused by the fact that oleic acid was derived from the same dietary sources (dairy and meat products) as the SFAs when the samples were collected, as observed in a previous study from the United States \textsuperscript{56}.

\textit{Individual fatty acids}

High proportions of LA in serum CE were inversely related to cardiovascular and total mortality in the present study. A one-unit increment in the proportion LA was associated with a 15\% risk reduction of cardiovascular death, which is in line with previous findings\textsuperscript{32, 68, 153}.

The serum proportions of long chain n-3 FA; ALA, EPA and DHA, also known to partly reflect the dietary intakes\textsuperscript{51}, were not associated with mortality in the present study. This might seem surprising since n-3 FA have been associated with cardioprotective effects\textsuperscript{34, 35}, but this might be due to a relatively high n-3 content of the background diet in the present study population (between 1 and 2 grams/day).

Palmitic acid, but not stearic acid was significantly associated with increased mortality, especially cardiovascular mortality. The finding may agree with experimental data suggesting that palmitic acid have unique effects on several cellular function, such as apoptosis\textsuperscript{154}, endoplasmic reticulum stress\textsuperscript{155}, up-regulation of SCD-1\textsuperscript{81}, and may accumulate in lipid metabolites such as ceramide and diacylglycerol\textsuperscript{156, 157}.

The results in the present study are in accordance with the findings in study I and to previous observational studies relating estimated desaturase activities and individual FA to cardiovascular and metabolic diseases\textsuperscript{56, 68, 85, 86, 148, 158, 159} and as discussed in the background section of this thesis. In this study many associations between FA, estimated desaturase activities and mortality was independent of BMI and other risk factors for cardiovascular disease and this warrants further investigation.

\textbf{Study III}

\textbf{SNPs and phenotype associations}

Markers of obesity (WC and BMI) and insulin sensitivity (M-value, n=986) were associated with the same SNPs, but with effects in opposing directions. rs10883463, rs7849, rs2167444 and rs508384 were all negatively associated with markers of obesity and positively associated with the M-value. The most pronounced effects on both WC and M-value was observed for subjects with the CC allele in rs7849, which corresponded to 4 cm less WC and 23\% higher insulin sensitivity. The associations with BMI did not reach significance. If the M/I value was used instead of the M-value the $\beta$-coefficients and $p$-values were in the same range.
When the association between the CC-allele (rs7849) and the M-value was adjusted for markers of obesity, BMI attenuated the relationship ($\beta = 0.75, p=0.034$), while WC removed any significant effect ($\beta$-coefficient$=0.62, p=0.084$). Concomitant co-morbidity and drug use did not influence this association. The stratification on markers of obesity demonstrated that the association between rs7849 and insulin sensitivity was not associated with BMI. However, subjects in the lowest tertile of WC (85 ± 5 cm (mean ± SD)) homozygous for the rare allele of rs7849 had higher insulin sensitivity ($\beta = 1.21, p=0.023$). The estimated SCD activity (16:1 [n-7]/16:0, n=489) was associated to CC in rs3071 and to GG in rs3793767 but the effects were minor.

**Haplotypes and phenotype associations**

Three haplotypes (I, II and III) with a frequency of >0.05 were estimated. Subjects heterozygous (one copy) for haplotypes I and III were associated with higher obesity and lower obesity, respectively. The effect was the strongest between the haplotypes and WC, but the effect on BMI followed the same pattern, but did not reach significance. The direction of the association between the M-value and haplotypes I and III was opposite of that observed between obesity and haplotypes.

**Table 3. Information regarding the eleven genotyped Single nucleotide polymorphisms in the SCD-gene and associations + (positive) or – (negative) to the investigated phenotypes: M-value, Waist and SCD-ratio.**

<table>
<thead>
<tr>
<th>db SNP ID</th>
<th>Position$^a$</th>
<th>Role</th>
<th>MAF</th>
<th>Alleles</th>
<th>Associations to:</th>
<th>M-value</th>
<th>Waist</th>
<th>SCD-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs655120$^b$</td>
<td>-6376</td>
<td>Promotor</td>
<td>0.002</td>
<td>A/G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs612472$^b$</td>
<td>2863</td>
<td>Intron</td>
<td>0.015</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3870747</td>
<td>6417</td>
<td>Intron</td>
<td>0.069</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3071</td>
<td>7202</td>
<td>Intron, Exon/intron boundaries</td>
<td>0.342</td>
<td>A/C</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3793766</td>
<td>7981</td>
<td>Intron</td>
<td>0.072</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3793767</td>
<td>8484</td>
<td>Intron</td>
<td>0.393</td>
<td>A/G</td>
<td></td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>rs10883463</td>
<td>11692</td>
<td>Intron</td>
<td>0.068</td>
<td>C/T</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>rs7849</td>
<td>15341</td>
<td>Exon 6</td>
<td>0.165</td>
<td>C/T</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>rs2167444</td>
<td>17482</td>
<td>3’UTR</td>
<td>0.141</td>
<td>A/T</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>rs508384</td>
<td>17499</td>
<td>3’UTR</td>
<td>0.166</td>
<td>A/C</td>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>rs539480$^b$</td>
<td>18590</td>
<td>3’UTR</td>
<td>0.023</td>
<td>C/T</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Relative position from translation initiation site (ATG= +1)

$^b$ SNPs with MAF <5%

MAF minor allele frequency, Minor allele is bolded and in italics.
**Discussion**

In the present study, each added copy of the rare allele of rs10883463, rs7849, rs2167444 and rs508384 was associated with lower BMI and WC and improved insulin sensitivity. Being homozygous for the rare allele, compared to being homozygous for the common allele of rs7849, demonstrated the strongest effect on both insulin sensitivity ($\beta=1.19$, $p=0.007$) and WC ($\beta=-4.4$, $p=0.028$), corresponding to 23% higher insulin sensitivity and 4 cm less waist line. These results accord well with animal data; mice with a disrupted SCD1 gene have reduced adiposity, increased insulin sensitivity and are resistant to diet induced weight gain \(^{25}\).

The adjustment for and stratification on markers of obesity, that was carried out in the present study to find out whether the effect of rs7849 (CC) on insulin sensitivity was primary or secondary to obesity, indicated that the effect was secondary to abdominal obesity. This is in line with the results of study I, in which the estimated SCD activity was significantly associated with the development of MetS, but the association was lost after adjustment for obesity \(^{151}\). Also, the key function of SCD is to regulate lipogenesis, whereas effects on glucose metabolism are believed to be secondary \(^{74}\). Adjustment for concomitant co-morbidity and drug use did not influence the association between rs7849 and insulin sensitivity either. In the present study the associations between haplotypes and phenotypes indicated that the rare alleles (Haplotype III) were associated with lower adiposity and higher insulin sensitivity further supporting the findings in the present study.

The effects of the gene polymorphisms on estimated SCD-16 were small, unsure and borderline significant and a bit disappointing (Table 2), since the estimated SCD activity can be regarded as a surrogate measure of SCD activity \(^{89}\). The following has to be considered however:

1. FA composition was assessed in a sub-sample of the study population leading to decreased power.
2. Estimated SCD activity is not a true enzyme activity, but a surrogate measure.
3. SCD activity was estimated in serum CE and not in target tissues, i.e. adipose tissue, liver or skeletal muscle.

If the 18:1[n-9]/18:0 ratio was used in the analysis no significant associations were detected, in analogy with findings in study I and III. This might seem surprising since the favored substrate for SCD is 18:0 \(^{66}\). However, the lack of associations between SNPs in SCD and 18:1[n-9]/18:0 might be explained by the higher dietary content of 18:1, compared to 16:1. This would lead to a “dilution” of the index by exogenous 18:1 and thus result in a worse reflection of the desaturation by SCD, which might have weakened the statistical effects even more.

The present study may indicate that SCD-1 might be important for the development of obesity and consequently in the development of insulin re-
sistance. Thus it is possible that SCD1 might be a switch-point between obesity and insulin resistance. It is well known that metabolic diseases are associated with changes in intracellular lipid metabolism, which might lead to ectopic fat accumulation in e.g. liver and skeletal muscle. This involves the partitioning between *de novo* lipid synthesis and oxidation of lipids and SCD has been proposed as a key regulator of both these processes \(^74\). It is thus possible that high SCD activity during a prolonged time leads to an accumulation of lipids in tissues other than adipose, thus promoting lipotoxicity and insulin resistance, which further aggravates metabolic disturbances \(^97, 160\).

**Study IV**

*Fatty acid composition of the diets*

The fatty acid composition of the two test diets is presented in *Figure 8*.

<table>
<thead>
<tr>
<th>Table 4. <em>Baseline characteristics of study subjects</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline characteristics</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Body weight (kg)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
</tr>
<tr>
<td>Serum insulin (mU/l)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
</tr>
</tbody>
</table>

\(n=20\), except for insulin when \(n=19\)

*SD Standard deviation, IQR interquartile range*

**Clinical characteristics**

The subjects were moderately hyperlipidemic and on average slightly overweight at inclusion. Baseline characteristics are presented in Table 4. There was no difference in body weight before, after or between each diet period. The concentrations of serum cholesterol (5.59 and 6.65 mmol/l) and triglycerides (1.77 and 2.03 mmol/l) were significantly different \((p<0.001)\) after the RO- and SAT-diet, respectively.

**Fatty acid composition and estimated desaturase activities in serum lipid esters**

The relative proportions of the individual FA in serum CE and PL were at the end of the test periods as follows. The proportion of 16:0 and 18:0 (only in CE) as well as of 16:1 and 20:3 n-6 were all significantly higher on the SAT-diet while the proportions of LA and ALA were significantly higher after the RO- diet. Docosapentadecanoic acid (DPA, 22:5 n-3) in PL was significantly higher \((p<0.01)\) during the SAT-period. The milk fat biomarkers, 15:0 and 17:0 (only in CE), were significantly higher on the SAT-diet. The relative proportions of the estimated desaturase activities were as presented in Table 2. SCD-16 and D6D activities were significantly higher on the SAT-diet, while D5D significantly lower \((p<0.001 \text{ for all})\).
Figure 7. The top pie-chart (a) illustrates the calculated FA content (%) of the RO diet and the lower pie-chart (b) illustrates the calculated FA content (%) of the SAT-diet. LCFA Long chain fatty acids, MCFA medium chain fatty acid

Discussion

In this study we wanted to study how surrogate measures of desaturase activities were affected by dietary fat quality. This is the first study to report how the estimated activities of FA desaturases change after a strictly controlled intervention with test diets representing two fat qualities; one diet rich in saturated fat and the other rich in unsaturated fat, based on rapeseed oil. The estimated SCD-16 (only CE) and D6D activities were significantly higher while D5D significantly lower in serum CE and PL on the SAT-diet compared to the RO-diet (Table 5). The SAT-diet induced a FA composition, in the serum lipids, similar to that earlier reported in several epidemiologic studies to be associated with e.g. cardiovascular disease and type 2
diabetes and reported in study I and II to be associated with MetS and cardiovascular and total mortality.

The content of palmitic (16:0) and stearic (18:0) acids was twice as high in the SAT-diet, compared to the RO-diet. In spite of the large differences in SFA content between the two test diets, differed the proportion of SFA in serum lipids only slightly after the intervention periods.

Table 5. The relative proportions (% of total) of estimated desaturase activities in serum cholesteryl esters (CE) and phospholipids (PL) by diet group.

<table>
<thead>
<tr>
<th>Fatty acid ratio</th>
<th>SAT-diet</th>
<th>RO-diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CE*</td>
<td>PL*</td>
</tr>
<tr>
<td>SCD-16 (16:1n-7/16:0) X</td>
<td>0.34</td>
<td>0.027</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>SCD-18 (18:1n-9/18:0) X</td>
<td>21.2</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>D6D**</td>
<td>X</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.005</td>
</tr>
<tr>
<td>D5D (20:4n-6/20:3n-6) X</td>
<td>7.9</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Mean (X)± SD are presented, ** D6D-ratio was estimated in CE as 18:3n-6/18:2n-6 but in PL as 20:3n-6/18:2n-. *** P < 0.001 for difference between RO and SAT-diet.

This might indicate that SCD is up-regulated as a consequence of a high intake of SFA, probably in order to modify intracellular levels of SFA and maintain membrane integrity. When SCD is up-regulated more 16:0 and 18:0 will be desaturated, thereby increasing the estimated desaturation indices in serum and this is in line with an observation in mice. An alternative possibility is that a diet high in SFA will counteract the inhibitory effect of a more PUFA rich diet on the expression of SCD, thus increasing desaturation and the SCD-16 ratio.

We propose in this study that the SCD-16-ratio (at least in CE) might be regarded as a sensitive and useful marker of dietary SFA, at least in high-fat Western populations. The SCD-18 ratio is not a very good marker, since the high dietary intake of OA will “dilute” the endogenous synthesized OA and thus affect the ratio in serum. The proportion of 16:1 was similar in the two test diets and the higher SCD-16 ratio in serum CE and PL must be affected by an increased desaturation driven by the higher SFA-content of the SAT-diet. Other inducers of SCD e.g. insulin, cholesterol and glucose did not differ between the two diet-periods. However, there might be other factors involved in the up-regulation of SCD that we have not accounted for.
General Discussion

**Biomarker fatty acids and metabolic and cardiovascular diseases**

In this thesis a serum FA composition, characterized by high proportions of palmitic (16:0), palmitoleic (16:1) and DHLA (20:3 n-6) acids and a low proportion of LA together with high estimated SCD and D6D and low estimated D5D activities, was reported to predict MetS over 20 years (study I) and cardiovascular and total mortality, independent of total cholesterol, BMI, smoking habit, physical activity and hypertension with a maximum of 33.7 years of follow up (study II).

The estimated D5D activity predicted MetS independently of BMI, physical activity and smoking status, while the predictive value of the SCD [16:1/16:0] and D6D ratio was abolished by BMI. Study II suggested that endogenous FA desaturation might contribute to mortality risk since independent associations between estimated desaturase activity indices (high SCD and D6D and low D5D) and mortality were observed. In addition, the proportion of polyunsaturated fat, mainly LA, was inversely related to mortality, while FAs associated with a high intake of saturated fat, mirrored as high serum proportions of 16:0, 16:1 and 20:3 n-6, were directly associated with mortality. This is in analogy with previous studies.\(^{32,68,163}\)

The findings in study I and II corroborate previous studies investigating relations between biomarker FAs, estimated desaturase activities and metabolic and cardiovascular diseases. However, these studies add new information regarding estimated desaturase activities and the MetS and mortality in prospective settings with long follow-up. The findings also provide new information about the development of metabolic and cardiovascular diseases, since individual FAs and estimated desaturase indices were independently associated with the endpoints. The prevalence of the MetS is steadily climbing (as a consequence of the growing obesity epidemic) and cardiovascular diseases are the leading cause of death in the world.\(^{2,164}\) The FA composition and desaturase activities are modifiable by diet and this means that with the right type of dietary fat we may be able to prevent the progress of metabolic and cardiovascular diseases. However, the exact mechanisms behind these relationships are unknown and need to be further investigated.
Associations between FA composition and metabolic and cardiovascular risks have been reported in cross sectional and longitudinal epidemiological studies, conducted in different countries, age groups and in both men and women. Relations between low serum and tissue levels of LA and CHD have been reported since the 1950s and have been confirmed in later studies, but some studies have failed to demonstrate such a relationship. In Finland, subjects with low levels of LA and other PUFAs in serum lipids had greater risk to experience fatal or non-fatal MI and a higher risk of cardiovascular death. In another study from Finland, Salomaa et al reported serum CE proportions of 16:0, 16:1, 20:3 n-6, 18:3 n-6 and 20:4 to be higher and LA to be lower in serum CE in diabetics, compared to those with better glucose tolerance. In that study high estimated activity of SCD and D6D and low estimated activity of D5D were related to the degree of glucose intolerance. Correspondingly were the SCD[16:1/16:0] and D6D ratios positively, while the D5D ratio was inversely related to diabetes incidence in a recent Australian prospective case-cohort study. Lovejoy et al reported 14:0, 16:1 and 20:3 n-6 acids in serum CE and PL to be related to markers of insulin resistance in men and women in the US and a similar FA composition has been associated with the risk of diabetes in several populations.

In study IV, saturated fat was able to “induce” the same serum FA pattern (including estimated desaturase activities) that we previously established as a risk marker in the development of MetS (study I) and cardiovascular disease and subsequent mortality (study II). The combined results from study I, II and IV underline the potential importance of dietary fat quality in the development of MetS, CVD and total mortality. Taken together, these results support current dietary guidelines and imply that replacing saturated fat with polyunsaturated fat may be advised in the prevention of metabolic and cardiovascular diseases.

**Is the SCD [16:1/16:0] ratio a potential marker of saturated fat intake?**

In study IV we propose that the 16:1/16:0- ratio, especially in CE, might be regarded as a useful and sensitive marker of dietary SFA in Western high fat consuming populations. This marker could be potentially important and useful since it is widely recognized that dietary fat is difficult to assess with traditional dietary assessment methods. Why is it then better to use a ratio rather than an individual FA as a marker of SFA? Both the proportion of 16:1 and the 16:1/16:0 ratio have been related to metabolic and cardiovascular risks with similar strength, as observed in study II. However, we believe that the SCD-16 ratio better mirrors the physiological function (adaptation)
of the “dietary stress” that a high intake of saturated fat causes. 16:0 is not a good marker since dietary 16:0 is rapidly desaturated (fast and effectively) to 16:1 in the body. Also, there is some endogenous production of 16:0 from carbohydrate but this contribution is small from a Western high fat diet. The possibility to use the 16:1/16:0 ratio as a marker of dietary SFA should be further explored in future controlled studies investigating biomarkers in relation to dietary fat intake.

Should SCD be estimated as 16:1/16:0 or 18:1/18:0?

Estimated SCD activity can be estimated either as 18:1 (n-9)/18:0 or as 16:1 (n-7)/16:0. SCD is highly regulated by dietary, hormonal and other factors and regulates in turn the abundance of MUFA. The preferential substrate for SCD is 18:0 and one might wonder why the synthesis of the most abundant FA in the diet, oleic acid, needs to be so carefully regulated? SCD1 is involved in FA partitioning and there is a requirement of endogenously synthesized MUFA for the synthesis of TAG and cholesteryl esters in the liver. In addition, endogenously synthesized MUFA mediates the induction of de novo lipogenesis.

Both ratios have been associated with risks but in our prospective studies the 18:1 (n-9)/18:0 ratio did not predict risk. This might be explained by a much higher dietary proportion of 18:1 compared to 16:1 and this will “dilute” the endogenously synthesized OA. This dilution affects the ratio in serum and a high ratio may be more compatible with a healthy diet and therefore does not predict risk in the same way as the 16:1 (n-7)/16:0 ratio. A recent Australian study, the 16:1 (n-7)/16:0 ratio was positively related to, while the 18:1 (n-9)/18:0 inversely related to the incidence of diabetes, in line with this interpretation and the findings in our studies and to previous studies in ULSAM.

Genetic variations in the SCD1-gene

Study III of this thesis is one of the first studies completed in man investigating genetic variations in the SCD1 gene and phenotypes. Study III demonstrated that subjects who were homozygous for the rare allele of rs7849 had on average 23% higher insulin sensitivity and 4 cm less WC, compared to those who were homozygous for the common allele. A finding that could potentially be clinically relevant. These results accord well with animal data. We tried to disentangle whether the effect of the SCD1 gene variations was primarily on obesity or insulin sensitivity. Our results suggested that the effect was primary on obesity and secondary on insulin sensitivity and this is consistent with the temporal relationship between obesity and insulin resis-
It has previously been suggested that abdominal obesity is more genetically determined than general obesity \cite{169}, which the results in study III are in line with. The associations between the estimated SCD [16:1/16:0] activity and SNPs in the SCD1 gene were however a bit disappointing, but maybe not too surprising due to limited power in the analyses and since we did not measure true enzyme activities. It is possible that the FA desaturation by SCD1 is not under strong genetic control but more related to lifestyle (as suggested in study I). It is also possible that SCD1 affects metabolic processes through other mechanism than by regulating FA composition.

Our data suggests that genetic variations in the SCD1 gene might be related to obesity-induced phenotypes, as suggested in animal studies \cite{76,170}. However when interpreting data from a genetic association study one has to remember that these associations might be driven by LD \cite{171} to another functional polymorphism or to a nearby gene. In addition, we studied genotype-phenotype interactions in 70 year old men. This means that the influence of genetic effects on phenotypes might be less pronounced due to the healthy cohort effect and since we did not use a midlife sample (if we assume that the genetic effect would be more pronounced at a younger age).

\textbf{Changes in desaturase activities, Cause or consequence?}

It is tempting to speculate about cause and consequence when trying to interpret the results from the two prospective studies in this thesis. It is plausible that high SCD and D6D and low D5D activities lead to metabolic disturbances that later is manifested as MetS (study I) and cardiovascular disease (study II).

Diet, degree of physical activity, genetics or perinatal “imprinting” (epigenetic phenomenon \cite{79}) might cause changes in desaturase activities. This might lead to a deranged FA handling possibly influencing 1) ectopic fat accumulation (SCD) \cite{74}, 2) pro-inflammation and blood coagulation (D6D and D5D) \cite{172} or 3) membrane FA composition that have an effect on transport processes and receptor binding affinities \cite{173,174}. These mechanisms may in turn influence e.g. the development of insulin resistance and atherosclerosis that will later be manifested as MetS, type 2 diabetes or CVD. It is also possible that the causative pathway is directed in the opposite way; metabolic disturbances lead to adaptive changes in desaturating enzymes in order to cope with an aberrant situation.

Thus, high SCD activity might be good in certain situations in order to maintain membrane structures, cellular communications and to keep intracellular levels of SFA and other metabolites (e.g. ceramide) within the normal range. An experimental study suggested that the induction of SCD1 in obese
insulin-resistant phenotypes may be a consequence of insulin resistance (rather than the cause) and may be initiated to counterbalance further insulin resistance\(^ {175}\). However, in another study, over-expression of SCD1 in rats induced insulin resistance\(^ {176}\) and yet another study in rats, examining the role of SCD1 in the onset of diet-induced hepatic insulin resistance, demonstrated that a decrease in SCD1 activity per se improved insulin action in the liver\(^ {177}\).

Risérus et al studied effects of rosiglitazone (a PPAR\(\gamma\) receptor agonist and thus an insulin-sensitizing drug) on SCD1 mRNA expression in adipose tissue and estimated desaturase activities (SCD-16, D6D and D5D) in serum TAG\(^ {80}\). After rosiglitazone treatment SCD1 mRNA expression in adipose tissue had increased by 48\% and the estimated plasma SCD and D5D activities were increased, compared to placebo. The change in SCD-index was significantly correlated with the improvement in insulin sensitivity during the study. These results suggest that a high SCD expression might be benign when the body needs to activate \textit{de novo} lipogenesis in subcutaneous adipose tissue in order to decrease the FA burden from other organs and in fact ameliorate insulin sensitivity. In addition, this system probably needs to be carefully regulated to be able to turn down the SCD activity when there is no need for \textit{de novo} lipogenesis. Inflexibility of this system might lead to ectopic lipid deposition and metabolic disturbances manifested as e.g. lipotoxicity, which might predispose for MetS and CVD\(^ {160,178}\).

Hypothetically, a high intake of saturated fat (at the expense of unsaturated fat) may trigger SCD activity in order to inhibit accumulation of saturated fats, as observed in study IV. If this effect is persistent, including increased and persistent induction of SCD in other tissues, like abdominal fat depots, liver and skeletal muscle, this may be harmful. It is known that saturated fat may induce insulin resistance in humans\(^ {28}\) and this effect might be associated with changes in desaturase activities, as proposed in study IV. Mechanisms that link saturated fat intake to insulin resistance, include influences on membrane fluidity, changes in lipogenic gene transcription, the type of FA within TAG and direct interference with insulin signaling via accumulation of lipid metabolites such as ceramide\(^ {157}\).

**The D6D/D5D pathway and eicosanoid signaling**

AA is the major FA synthesized by the D6D/D5D pathway. Other important FAs synthesized by this pathway are DHLA, EPA and DHA. In many tissues AA is esterified at the sn-2 position of membrane PL and can be released from this position to participate in eicosanoid signaling. This release is stimulated by cell injury and other factors. AA is then enzymatically converted to eicosanoids\(^ {63}\). The eicosanoids is a group of closely related sub-
stances such as prostaglandins, thromboxanes, prostacyclins and leukotrienes that work as hormones and mediates local reactions such as inflammation and hemostasis and participates in the regulation of blood pressure, lipolysis and maintains the digestive tract epithelium. Eicosanoids are also derived from DHLA and EPA. Eicosanoids derived from AA and EPA can have opposite effects; e.g. leukotrienes derived from EPA have less pro-inflammatory properties, compared to those derived from AA \(^{63, 172}\). An increased synthesis of eicosanoids from AA at the expense of eicosanoids from the n-3 family has been implicated in thrombus formation and inflammatory processes \(^9\). An imbalance in the D6D/D5D pathway may thus be implicated in metabolic and cardiovascular diseases and may partly explain the results in study I and II.

A low proportion in serum lipids of LA has been related to the risk for type 2 diabetes \(^{85, 159, 165, 166}\) and has been attributed to a high intake of SFA and low intake of LA. An alternative explanation for a low LA in serum lipids could be that in the pre-diabetic state preceding manifest diabetes the high insulin levels might induce D6D thereby depleting LA and accumulating DHLA in serum. This is consistent with a high estimated D6D activity, that dietary LA was positively related to diabetes risk and that the risk was most pronounced in those with higher insulin levels, as observed by Hodge et al \(^{165}\). This has also been discussed by Wang et al \(^{85}\) and Vessby et al \(^{166}\). In these studies the associations between LA and diabetes were adjusted for baseline insulin levels and this did not affect the results, pointing at the first explanation to be the most adequate.

**How do estimated enzyme activities in plasma reflect corresponding activities in other tissues?**

Desaturase indices as evaluated in serum lipids probably reflect both hepatic and adipose tissue activities \(^{67}\). We used fasting serum lipid ester composition to estimate desaturase activities in all of our studies. This means that the systemic desaturase indices in our studies probably mainly reflect hepatic desaturation. This is very important to remember when interpreting data regarding estimated desaturase activities.

This also mean that, what is a true association between estimated serum desaturase activities (if mainly reflecting liver activities) and certain phenotypes might not be true for estimated desaturase activities in adipose tissue and the same phenotypes. In the physiological situation in Western societies with a relatively high dietary fat consumption, an increased SCD activity in adipose tissue may be positive and related to a normal adipo- and lipogenesis. Contrary, an increased SCD activity in the liver might indicate an unde-
sirable situation with an increased lipid load and need for increased desatur-
ination.

A recent study by Sjögren et al highlights the complexity regarding de-
saturase activities, estimated desaturase activities and insulin resistance. In
this study, subcutaneous mRNA expression levels of SCD, D6D and D5D
were analyzed in relation to adipose tissue desaturase indices and insulin
resistance. The expression of SCD but not D6D and D5D was related to the
estimated desaturase activities. Insulin resistant individuals had a higher
adipose tissue 18:1/18:0 but not 16:1/16:0 (although tightly correlated), com-
pared to insulin sensitive individuals. This relationship was independent of
WC and TAG. Since this was a cross-sectional study it is not possible to
say whether insulin resistance is the consequence or the cause of a high SCD
activity. It is interesting that the 18:1/18:0 but not 16:1/16:0 in subcutaneous
adipose tissue predict insulin resistance. This is in contrast to the results in
study I, in which SCD was estimated in serum CE. To bear in mind is that
oleic acid is the most abundant FA in adipose tissue (>50%) 63. It is thus
possible that this FA pool is not as sensitive to dietary and metabolic
changes, as other pools.

In the same study population as discussed above, the correlation be-
tween SCD[18:1/18:0] in serum PL and adipose tissue TAGs was not sig-
nificant meaning that the short- and long-term reflections of SCD-18 are not
analogous in this particular study population. In addition, the SCD-16 ratio
was well correlated between fractions and tissues (NEFA, serum PL and
adipose tissue TAG) (unpublished results).

**Estimated desaturase activities in different serum lipid fractions**

In this thesis, FAs in serum CE were measured in all studies, except in
study IV where the FA proportions were measured in both CE and PL. The
reason why we choose to study FAs in CE and not in any of the other lipid
fractions is that this was the only fraction originally available in the UL-
SAM-population. However, it is known that the proportion of FAs in all four
serum lipid fractions (TAG, NEFA, CE and PL) is affected similarly by a
dietary intervention; a diet high in LA will raise the amount of LA in all lipid
fractions 68. As observed in study IV, qualitative changes in the proportions
of individual FA in serum CE and PL due to the dietary interventions were
analogous. In TAG, NEFA and PL these changes will only take a few days
while changes in CE will take up to several weeks 68. Correlations between
FAs in the diet and in CE are moderate 56, 68, but usually stronger than for
those between FA of PLs and diet 68. In addition the short- and long-term
reliability (repeatability) was higher for most major FA in CE than in PL 180.
This suggests that especially CE may serve as a reasonably good biomarker of dietary fat quality, but PL may also be used \(^{181}\). Also, the method variability was low in our studies (like in other studies), suggesting that the changes in FA composition we observed in study IV was due to dietary changes and endogenous metabolism. It is possible that the CE fraction is better to choose when studying the SCD index (as indicated in study IV), and PL when studying D5D and D6D indices, considering the functions of the product FA \(^{63}\). However, this needs to be further explored in future intervention studies.

**Are estimated desaturase activities valid markers of desaturase activities?**

In all studies of this thesis surrogate measures of desaturase activities have been used. This is of course a limitation. Ideal would be to measure mRNA levels or protein expression of the desaturase genes. However, this is difficult to do in larger epidemiologic studies, but to some extent possible in smaller experimental studies (but not done in study IV). Nonetheless, there might be an ethical concern regarding the measurement in humans since the most relevant organ to study probably is the liver. Otherwise it is possible to measure desaturase expression in adipose tissue \(^{80}\) and skeletal muscle \(^{79}\). But we have to bear in mind that a high SCD activity might be good in adipose tissue during certain conditions but at the same time harmful in the liver.

To use an estimated desaturase activity as a surrogate measure of enzyme activity is widespread. It is known from studies using animal or *in-vitro* models that changes in SCD expression (mRNA or protein) is coupled with simultaneous changes in tissue FA ratios \(^{71, 78, 79, 157, 177}\) and the cellular activity of an SCD1 inhibitor was assessed by using the SCD index in human liver cells \(^{182}\). The relationship between D6D and D5D expression and FA ratios is not as widely investigated. However, it is known that SNPs and haplotypes in the FADS1 and FADS2 genes, that codes for D5D and D6D, respectively, are associated with the proportion of serum PL FAs belonging to the D5D and D6D pathways (*Figure 2*). The authors pointed out that their findings highlighted the importance of the desaturation pathways on n-6 and n-3 PUFA levels in PL and that this was under genetic control. The minor allele of several SNPs in these genes was associated with a decrease in the level of the desaturase product FA while the precursor FA was increased; indicating a decline in the desaturase activities (mRNA or protein) \(^{183}\). This finding implies that D5D and D6D indices of may serve as a fairly good estimate of actual desaturase activities.
Clinical utility and implications for public health

Biomarker FAs and estimated desaturase activities are independent risk factors/markers in the development of Mets and in cardiovascular disease and subsequent mortality. This may indicate that the FA composition marks a state of pre-metabolic abnormalities that could determine future risk. This could have clinical implications. However, biomarker FAs are relative measures and therefore not very useful for the clinical setting, since this would require a cut-off value in order to determine the risk. Also, the predictive values of biomarker FAs and estimated desaturase activities were obtained on the group level, which means that they may not predict risk on the individual level.

To measure FA in serum fractions is cumbersome and involves several steps before the results are obtained. One way of facilitating the measurement of FA could be to use whole blood samples as suggested by Baylin et al since this does not require separation of the different lipid fractions. From a public health perspective biomarker FAs and estimated desaturase activities are important since they are modifiable by diet and have been associated with health outcomes in different populations. The serum FA composition data itself could possibly be used as a preventive incentive to adhere to dietary recommendations. To use the 16:1/16:0 ratio as a marker of saturated fat intake may be potentially important in dietary surveys and intervention studies.

Epidemiological causation and considerations

Epidemiology uses quantitative methods to study diseases in different populations to provide information for prevention and control efforts in order to improve health.

The nature of a cohort study, such as the ULSAM study, is observational and causal inferences about the associations between FA biomarkers, desaturase activities and outcomes are hence difficult to make. However, prospective cohort studies generally provide the best epidemiological evidence (besides experimental studies) about causation and the most direct measurement of the risk of developing disease. Before judging causality one has to exclude the possibility that bias, chance or confounding generated the results. In our prospective studies (study I and II) differences in biomarker FA and desaturase activities were already manifest at baseline (in many times independent of confounding factors), fulfilling the temporal relationship for causality. FAs in serum and tissues and FA ratios might, however, be in the casual pathway where one factor leads to another factor until manifest disease (including factors that have not been measured). This could be plausible when considering the multifactorial nature of cardiovascular disease and
MetS. To judge causality in an epidemiological study one has to take many factors, besides the temporal relationship, into consideration; plausibility of the results, consistency with previous studies, dose-response relationships, strengths of the associations, the type of study design and if there are several lines of evidence that leads to the same conclusion\textsuperscript{185}.

The role of genetic association studies in the uncovering and description of genes causative to common multifactorial traits remains controversial. There has been a problem to confirm findings from one population in another population. Genetic effects of individual candidate genes on multifactorial traits are unlikely to be large and thus many studies have had limited power to detect true susceptibility effects. In order to overcome such problems candidate genes and SNPs must be carefully chosen and the study population should be homogenous and sufficiently large and the results replicated in other populations of similar ethnic origin. A case-control study design is preferred for genetic association studies\textsuperscript{125}. However, we decided to use a cross-sectional design using all cohort participants instead of a nested case-control design in order not to loose power. However, in the cross-sectional study it is not possible to say anything about the causality.

Study IV was a dietary cross-over intervention study which has the ability to prove causality\textsuperscript{185}, but selection of study participants, study power and duration influence the interpretation of the results from such a study.

\textit{Strengths and limitations}

\textit{ULSAM studies}

The strengths include that ULSAM is a large community based study sample with long follow up and with detailed phenotypic and genotypic characterization of the study subjects and minimal losses to follow-up. To our knowledge ULSAM is the largest study sample with the gold standard measurement of insulin sensitivity, the euglycemic insulin clamp technique\textsuperscript{137}. The clamp measurements were done in 95\% of those who participated in the 70 years investigation. The ULSAM population is homogenous (sex, age and geographically matched) and of the same ethnical background, decreasing the risk for population selection bias. Further, the analyses were specified \textit{a priori} and hypothesis-driven and adjustments for multiple testing were made in study II in order to reduce the risk for type I errors.

The most obvious limitation of the ULSAM population is that it only consisted of men of the same age and ethnical background, decreasing the possibility that the results can be generalized to other populations. When ULSAM was initiated in 1970 the general believe was that risk for cardiovascular disease was much greater in middle aged men than in women. Thus, only men were enrolled in several cohort studies including ULSAM.
To be able to detect effects of a genetic polymorphism in a genetic association study (Study III) the sample size needs to be sufficient but the present study population was not very large\textsuperscript{125}, which might have driven the results toward the null hypothesis. It has, however, been suggested by Hattersley and McCarthy that small studies are likely to overestimate the true effect size\textsuperscript{125}. The SNPs genotyped in study III were able to capture most of the genetic variability in the SCD gene (75%), but there is a possibility that we might miss genetic variation.

Participants in a cohort study are subject to investigations and this might lead to a “healthy cohort effect” in terms of health and risk of disease, compared to the general population. This should be kept in mind when using data from a cohort study. For our prospective studies we used data from the baseline investigation unlikely to have been affected by this effect.

There is a possibility that cardiovascular mortality was misclassified but the accuracy of the Swedish cause-of-death registry is known to be high\textsuperscript{186}.

Strengths of Study IV include the cross-over design and that the dietary intervention was strictly controlled and that all meals were provided to the participants. A limitation is that compliance was not measured in any other way than in changes in the serum FA composition, but effects on FA composition indicated that the participants kept to the intervention diet. Also, the study was not very large and results should be validated in a longer term and larger intervention study.

Last but not the least, in all four studies a limitation is the use of FA ratios as surrogate measures of enzyme activity. However, there are many studies that have indicated that changes in FA ratios, enzyme activities and mRNA expression follow each other, as discussed above.

\textit{Future directions}

In this thesis we have shed light on the complex relationships between dietary fat as mirrored in serum biomarker FAs, endogenous desaturation and the risk of metabolic and cardiovascular diseases. We have also investigated genetic variation in the SCD1 gene in association to obesity and insulin resistance, phenotypes that are the central drivers in the development of metabolic and cardiovascular diseases. In the intervention study we were able to demonstrate how estimated desaturase activities change in response to different fat qualities. These four studies contribute to the present knowledge and support current dietary guidelines but there are still questions to be answered. The estimated desaturase activities should be validated with in vivo measurements of mRNA/protein expression. Estimated desaturase activities should in a longer term dietary study be analyzed in associations to meta-
bolic changes, e.g. insulin sensitivity, to be able to verify changes in enzyme activities. The mechanisms behind the independent associations between estimated desaturase activities and metabolic and cardiovascular risks should be investigated in future studies. It is possible that we will combat the development of metabolic and cardiovascular diseases or at least stop the progression of them, with pharmacological interventions directed against desaturases\cite{170,172,182}. Pharmacological inhibitors of the SCD1 gene are already available and are currently tested in preclinical studies and others underway but the clinical utility have to be proven in the future.
Conclusions

In this thesis serum biomarker FAs and estimated SCD, Δ^6 and Δ^5 activities were explored in different settings. This thesis confirms and extends the role of serum biomarker FAs and estimated SCD, Δ^6 and Δ^5 activities as possible important players in metabolic and cardiovascular diseases and demonstrates that genetic variations in the SCD1 gene might be associated with obesity-induced phenotypes.

The estimated SCD, D6D and D5D activity predicted MetS over 20 years. The relation between D5D and MetS was independent of BMI, physical activity and smoking status, while the predictive value of the SCD [16:1/16:0] and D6D was mainly explained by BMI.

Endogenous FA desaturation might contribute to mortality risk on the group level since independent associations between estimated desaturase activity indices and mortality were observed. The proportion of LA in serum was inversely related to mortality, while FAs associated with a high intake of saturated fat (16:0, 16:1 and 20:3 n-6) were directly associated with mortality.

The rare allele of rs7849 of SCD1 demonstrated the strongest effect on both insulin sensitivity and waist circumference, corresponding to 23% higher insulin sensitivity and 4 cm less waist circumference.

Saturated fat was able to “induce” the same serum FA pattern (including estimated desaturase activities) that predicted the development of MetS and mortality risk. We also propose that the SCD ratio [16:1/16:0], especially in CE, might be regarded as a useful and sensitive marker of dietary SFA, in Western populations with a high-fat intake.

The combined results of this thesis emphasize the potential importance of dietary fat quality in the development of metabolic and cardiovascular diseases and support current dietary guidelines. Replacing saturated fat with polyunsaturated fat may be advised in the prevention of metabolic and cardiovascular diseases. However, this needs to be further evaluated in other observational studies and preferable formally tested in randomized controlled trials.
I dag är över en miljard människor i världen överviktiga (BMI >25 kg/m²) och av dessa är fler än 300 miljoner feta (BMI >30 kg/m²). Övervikt och fetma leder till ökad risk för sjukdomar såsom metabola syndromet (högt blodtryck, förändrade blodfetter, störd insulin- och glukosomsättning och fetma), hjärtkärlsjukdomar och typ 2 diabetes, och kan leda till för tidig död.

Förekomst av dessa sjukdomar kan till viss del tillskrivas fettet i kosten. För att utveckla dessa sjukdomar tycks typen (kvalitet) av fett i kosten större betydelse, än mängden. Fett är ett mycket viktigt näringsämne som behövs för att hålla membranstrukturer smidiga, för kommunikation mellan celler och reglering av genuttryck. Dessutom ger fett kroppen energi och bygger upp fettdepåer med många viktiga funktioner såsom isolering och produktion av hormonliknande substanser.

Fett i kosten består i huvudsak av triglycerider som i sin tur består av glycerol förbundet med tre fettsyror. Fettsyror kan vara mättade (t ex 16:0, palmitinsyra), enkelomättade (t ex 18:1, oljesyra; 16:1, palmitoljesyra) eller fleromättade (t ex 20:3 n-6, dihomo-γ-linolensyra). Mättnadsgraden bestäms av antalet dubbelbindningar i fettsyran och ju fler dubbelbindningar desto mjukare är fettsyran. Omättade fettsyror har egenskaper som gör att de kan reglera flera viktiga processer inne i cellen. Linolsyra (18:2 n-6) och α-linolensyra (18:3 n-3) är livsnödvändiga fettsyror som måste tillföras kroppen med kosten. Andra fettsyror kan kroppen själv bilda med hjälp av enzymer såsom desaturaser och elongaser.

Det är svårt att studera intaget av fett i kosten hos en grupp individer med hjälp av vanliga kostundersökningsmetoder eftersom risken är stor att personerna, medvetet eller omedvetet, inte rapporterar vad de egentligen ätit. Fettsyror i blodlipider eller vävnadsfraktioner kan användas som biologiska markörer, så kallade biomarkörer, för att objektivt kunna studera typen av fettsyror i kosten. Dessa biomarkörer kan sedan användas för att studera sjukdomsrisk i förhållande till kosten typ av fett.

I denna avhandling har jag studerat fettsyror i blod och fettsyrsjukdomar, som mått på desaturasaktivitet, i relation till 1) risken att utveckla det metabola syndromet under 20-års uppföljning (study I) och 2) risk för dödlighet under en uppföljning upp till 33,7 år (study II). Jag har dessutom studerat hur desaturasaktivitet i relation till de mättade fettsyror i blodlipider och vävnadsfraktioner.
desaturas (D5D)) och individuella fettsyror påverkas av två typer av kost där endast typen av fett skiljer sig. Vi jämförde effekter av en kost rik på mättat fett med en kost rik på omättat fett baserad på rapsolja (study IV). Slutligen studerades genetisk variation i SCD1 genen i relation till fetma, insulinkänslighet (hur bra kroppen svarar på insulins verkan) och SCD-kvoten (study III).


I denna avhandling visar jag att ett visst mönster av fettsyror i blod, höga proportioner av 16:0, 16:1 och 20:3 n-6 och låga proportioner av 18:2 n-6, samt hög SCD och D6D och låg D5D kvot, är kopplat till risk för att utveckla metabola syndromet och leder till ökad risk för dödlighet. Riskerna var oberoende av etablerade riskfaktorer för hjärtkärlsjukdom. Dessa resultat harmoniserar med tidigare resultat men ger ny information om fettsyror och desaturaskvoter i relation till metabola syndromet och dödlighet i prospektiva (framåtblickande) studier med lång uppföljningstid. Eftersom många av sambanden var oberoende av etablerade riskfaktorer kan detta tyda på att desaturas-enzyme tillsammans med typen av fett i kosten kan bidra till utvecklingen av metabola och hjärtkärl-sjukdomar på gruppnriv.

I koststudien, ”inducerades” det ovan nämnda mönstret av fettsyror och fettsyrakvoter av en kost rik på mättat fett. I koststudien lanseras även SCD-kvoten [16:1/16:0] som en möjlig biomarkör på mättat fett i kosten. Detta är potentialigt ett viktigt bidrag till kostundersökningar som ska verifieras i framtida studier.

Resultaten i dessa tre studier (I, II och IV) visar på betydelsen av rätt fett i kosten för att förhindra uppkomst av metabola och hjärtkärl-sjukdomar. Resultatet i denna avhandling överensstämmer med dagens kostrekommanderare som uppmanar oss att minska på mättat fett och att samtidigt öka intaget av omättat fett.

I studie IV visades att genetisk variation i SCD1 genen var kopplad till fetma och graden av insulinkänslighet, som överensstämmer med resultat från djurmodeller. Däremot var SCD-kvoten dåligt associerad till variationen i SCD1 genen. SCD enzymet ses som en möjlig kandidat för farmakologisk behandling för att förhindra/lindra utveckling av risken relaterat till fetma. Om detta är möjligt kommer framtiden att utvisa.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)