Dietary Fatty Acids and Inflammation

Observational and Interventional Studies

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Dissertation presented at Uppsala University to be publicly examined in Sal IV, Universitetshuset, Biskopsgatan 3, Uppsala. Wednesday, September 14, 2011 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English.

Abstract

Dietary fat quality influences the risk of type 2 diabetes and cardiovascular disease. A low-grade inflammation is suggested to contribute to the disease development, often accompanied by obesity. Whereas n-3 polyunsaturated fatty acids (PUFA) have been considered anti-inflammatory, n-6 PUFA have been proposed to act pro-inflammatory. Saturated fatty acids (SFA) act pro-inflammatory in vitro.

This thesis aimed to investigate effects of different fatty acids on low-grade inflammation in observational and interventional studies. In Paper I and II, fatty acid composition in serum cholesterol esters was used as objective marker of dietary fat quality and related to serum C-reactive protein (CRP) and other circulating inflammatory markers in two population-based cohorts, conducted in middle-aged men and elderly men and women, respectively. In Paper III and IV, the impact of diets differing in fat quality on inflammation and oxidative stress was investigated in randomised controlled studies, in subjects with metabolic syndrome and abdominal obesity.

In Paper I and II, a low proportion of linoleic acid (18:2 n-6) in serum was associated with higher CRP concentrations, indicating that a low intake of vegetable fats may be related to low-grade inflammation. High CRP concentrations were also associated with high proportions of palmitoleic (16:1) and oleic (18:1) acids and high stearoyl coenzymeA desaturase index, possibly reflecting altered fat metabolism and/or high SFA intake in this population. When comparing two high-fat diets rich in either saturated or monounsaturated fat, and two low-fat diets with or without long-chain n-3 PUFA supplementation during 12 weeks (Paper III), no differences in inflammation or oxidative stress markers were observed. Moreover, a 10-week intervention (Paper IV) with high linoleic acid intake showed no adverse effects on inflammation or oxidative stress. Instead, interleukin-1 receptor antagonist and tumor necrosis factor receptor-2 decreased after linoleic acid intake compared with a diet high in SFA.

The results in this thesis indicate that dietary n-6 PUFA found in vegetable fats is associated with lower inflammation marker levels, and to some extent reduces systemic inflammation when compared with SFA. Supplementation of n-3 PUFA did not exert any systemic anti-inflammatory effects, maybe due to a relatively low dose.

Keywords: Dietary fat, Fatty acids, Serum fatty acid composition, Linoleic acid, Stearoyl coenzymeA desaturase, SCD-1, Inflammation, C-reactive protein, Oxidative stress, Lipid peroxidation, Isoprostanes, Prostaglandins, Obesity, Epidemiology, Dietary intervention, Metabolic syndrome

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ISSN 1651-6206 0346-5462
ISBN 978-91-554-8112-4
urn:nbn:se:uu:diva-156074 (http://urn.kb.se/resolve?urn=nbn:se:uu:diva-156074)
List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.


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Contents

Introduction..........................................................................................................................9
Fatty acids ..................................................................................................................................9
Dietary sources of fatty acids .................................................................................................10
Desaturases ..........................................................................................................................12
Serum fatty acids as biomarkers of dietary fat ........................................................................13
Inflammation .........................................................................................................................14
Oxidative stress and lipid peroxidation ..................................................................................16
Inflammation and oxidative stress in obesity, type 2 diabetes and cardiovascular disease ..................................................................................................................17
Fatty acids, inflammation and oxidative stress .......................................................................18
Rationale for this thesis ..........................................................................................................18

Aims .........................................................................................................................................19

Subjects and Methods ........................................................................................................20
Paper I – Serum fatty acids and CRP (ULSAM) ...................................................................20
  The ULSAM cohort ...............................................................................................................20
  Participants ..........................................................................................................................21
  Methods ..............................................................................................................................21
Paper II – Serum fatty acids and inflammation (PIVUS) .........................................................22
  The PIVUS cohort ...............................................................................................................22
  Participants ..........................................................................................................................22
  Methods ..............................................................................................................................23
Paper III – Intervention with n-3, SFA and MUFA ................................................................23
  Participants ..........................................................................................................................23
  Intervention ........................................................................................................................24
  Methods ..............................................................................................................................25
Paper IV – Intervention with n-6 and SFA ...........................................................................25
  Participants ..........................................................................................................................25
  Intervention ........................................................................................................................25
  Methods ..............................................................................................................................26
Assessment of fatty acid composition ..................................................................................27
Assessment of inflammation ...................................................................................................28
Assessment of oxidative stress and lipid peroxidation .........................................................29
Statistics ...................................................................................................................................29
  Paper I and II ......................................................................................................................29
Abbreviations

BMI  Body mass index
CRP  C-reactive protein
COX  Cyclooxygenase
HDL  High density lipoprotein
HOMA-IR  Homeostasis model assessment of insulin resistance
HMUFA  High-fat diet rich in monounsaturated fat
HSFA  High-fat diet rich in saturated fat
JNK  JUN N-terminal kinase
IKK  Inhibitor of nuclear factor-κB kinase
IL  Interleukin
IL-1Ra  Interleukin-1 receptor antagonist
LA-diet  Diet rich in linoleic acid (18:2 n-6)
LDL  Low density lipoprotein
LFHCC  Low-fat high-complex carbohydrate diet, oleic acid supplement
LFHCCn-3  Low-fat high-complex carbohydrate diet, n-3 supplement
MCP-1  Monocyte chemoattractant protein-1
MUFA  Monounsaturated fatty acid
NF-κB  Nuclear factor-κB
PGF$_{2\alpha}$  Prostaglandin F$_{2\alpha}$
PIVUS  Prospective Investigation of the Vasculature in Uppsala Seniors
PPAR  Peroxisome proliferator activated receptor
PUFA  Polyunsaturated fatty acid
SCD-1  Stearoyl coenzymeA desaturase-1
SFA  Saturated fatty acid
SFA-diet  Diet rich in saturated fat
ICAM-1  Intercellular adhesion molecule-1
SREBP  Sterol regulatory element binding protein
VCAM-1  Vascular cell adhesion molecule-1
TFA  Trans fatty acid
TLR  Toll-like receptor
TNF-α  Tumor necrosis factor-α
sTNF-R  Soluble tumor necrosis factor receptor
ULSAM  Uppsala Longitudinal Study of Adult Men
VLDL  Very low density lipoprotein
Introduction

Fatty acids

Fatty acids are the building blocks of lipids. Fatty acids do not only function as key components in energy storage; they are also incorporated as structural components of cell membranes and are precursors in the eicosanoid production. Fatty acids can also regulate gene expression by for example interaction with the transcription factors peroxisome proliferator activated receptors (PPAR) and sterol regulatory element binding proteins (SREBP). The fatty acid is composed by a carbon backbone with a carboxyl group at one end and a methyl group at the other end. The nomenclature is derived from the number of carbon atoms, the number of double bonds and the position of the first double bond from the methyl terminal. The fatty acids that are most common in the diet are composed of even numbers of carbon atoms, with 16 and 18 carbons being most frequent. Saturated fatty acids (SFA) lack double bonds whereas monounsaturated fatty acids (MUFA) have one and polyunsaturated fatty acids (PUFA) contain two or more double bonds. The more double bonds a fatty acid has, the more unsaturated it is. Further, more double bonds give the fatty acid a less regular shape and thereby decreases its melting point. Due to the double bonds, unsaturated fatty acids are more chemically reactive than the more stable SFA. The reactivity increases with increasing number of double bonds. The double bonds of the most abundant dietary unsaturated fatty acids are in the cis configuration, which means that the hydrogen atoms attached to the double bond are located on the same side. In the body, fatty acids can be converted to longer and more unsaturated fatty acids. The unsaturated fatty acids are classified into three main families; n-3, n-6 and n-9, due to which carbon atom from the methyl end where the first double bond is attached. These fatty acid families cannot be interconverted. SFA, and indirectly n-9 fatty acids, can, in addition to dietary intake, also be produced by endogenous synthesis from carbohydrates. Due to lack of enzymes essential for desaturation at carbon atoms 3 and 6, the n-3 and n-6 families cannot be produced in the body. Thus, the parent fatty acids in these families (i.e. α-linolenic acid [18:3 n-3] and linoleic acid [18:2 n-6], respectively) are essential and can only be derived from the diet.

Triacylglycerols are the body’s major energy store and also the major form of dietary fat (94%). They are composed of glycerol and three fatty acids.
Other forms of dietary fat are cholesterol (1%) and phospholipids (5%). Non-esterified fatty acids or “free fatty acids” are circulating in plasma bound to albumin and are released in adipose tissue lipolysis. Triacylglycerols and cholesterol are transported by lipoprotein particles. These particles have a hydrophobic lipid core consisting of triacylglycerols and cholesterol esters, and a hydrophilic surface of phospholipids and free cholesterol. Each lipoprotein particle is associated with one or more apolipoproteins. The lipoproteins differ in lipid and protein composition and size and are classified according to their density. The larger triacylglycerol-rich chylomicrons and very low density lipoproteins (VLDL) are mainly involved in delivery of triacylglycerols to tissues. The smaller low density lipoproteins (LDL) and high density lipoproteins (HDL) are more involved in the regulation of the cellular cholesterol content. LDL particles deliver cholesterol to the cells whereas HDL particles remove cholesterol and transport it to the liver for excretion³.

![Figure 1. The lipoprotein particle](image)

**Dietary sources of fatty acids**

Fat contributes to approximately 34% of the energy intake in the Swedish diet⁵. The main sources of fat are 1) margarines, butter, and oils, 2) milk and milk products, and 3) meat and meat products⁶,⁷. The quantitatively most important individual fatty acids are palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2 n-6) and stearic acid (18:0)⁵.
Around 14% energy of the average Swedish diet is saturated fat\textsuperscript{5,7}, with hard margarines, meat and dairy products as the main sources\textsuperscript{5}. Palmitic acid (16:0) is the most common SFA\textsuperscript{4}. It occurs in most fats but the main sources in Sweden are meat, butter and other dairy products\textsuperscript{5}. Stearic acid (18:0) is also widely distributed in most fats and oils\textsuperscript{4}.

MUFA composes 12% energy of the dietary intake and is presented in most fats\textsuperscript{6}. In Sweden, meat products, edible fat and dairy products are still the main sources of MUFA\textsuperscript{5,7}. The most common MUFA is oleic acid (18:1) which is present in high amounts in olive oil and rapeseed oil. Palmitoleic acid (16:1) is a minor MUFA component in most fats (<1-2%), but higher amounts are present in macadamian oil (22%) and marine oils (10%)\textsuperscript{4}.

The main dietary sources of PUFA are soft margarines and vegetable oils\textsuperscript{6}. N-6 fatty acids are the major dietary PUFA (approximately 3.7% energy, compared with 0.8% energy n-3). The n-6/n-3 ratio in Sweden is 5:1\textsuperscript{5}. Of the n-6 PUFA, the essential fatty acid linoleic acid (18:2 n-6) accounts for about 90%\textsuperscript{8}. In Sweden, the intake of linoleic acid is approximately 9 g/day whereas arachidonic acid intake is about 0.1 g/day\textsuperscript{5}. Linoleic acid is present in almost all types of fat but the most important sources are vegetable oils, especially sunflower and soybean oil. Typical sources for arachidonic acid (20:4 n-6) are animal fats, liver, egg and fish. The fatty acids \(\gamma\)-linolenic acid (18:3 n-6) and dihomo-\(\gamma\)-linolenic acid (20:3 n-6) are rare in the diet, but are present in evening primrose oil and in very small amounts in animal fat, respectively. The essential parent n-3 PUFA, \(\alpha\)-linolenic acid is present in very high concentrations in flaxseed oil (55%) but occurs also in other vegetable fats, especially rapeseed and soybean oil. Important dietary sources for the very long-chain n-3 PUFA eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) are fish, particularly oily fish such as salmon, herring and mackerel\textsuperscript{4}.

Trans fatty acids (TFA) are unsaturated fatty acids with the double bonds in \textit{trans} configuration, i.e. the hydrogen atoms attached to the double bond are located on the opposite sides. They are naturally produced in the stomach of ruminants and occur in small amounts in ruminant products and dairy fats. TFA are also produced during industrial partial hydrogenation\textsuperscript{1,4}. The most important TFA in ruminant fats and partial hydrogenated vegetable oils are the \textit{trans}-18:1 isomers\textsuperscript{4}. The dietary intake of TFA in Sweden is approximately 2-3 g/day, corresponding to 1% energy\textsuperscript{5,7}. Dairy products contribute to the largest part\textsuperscript{7}. 
Desaturases

In the body, fatty acids can be converted to longer and more unsaturated fatty acids by elongation and desaturation. The elongation is catalysed by enzymes called elongases incorporating carbon atoms in the fatty acid backbone, whereas double bonds are formed by enzymes called desaturases. The Δ-desaturases insert double bonds at a specific position from the carboxyl end of the fatty acid chain. There are three known desaturases in humans; stearoyl coenzymeA desaturase (SCD, also called Δ9-desaturase), Δ5- and Δ6-desaturases. SCD catalyses the last step in the synthesis of MUFA from SFA, e.g. oleic acid from stearic acid and palmitoleic acid from palmitic acid, whereas the Δ5- and Δ6-desaturases participate in the conversion of PUFA into more desaturated forms. The desaturases are localised in the membrane of the endoplasmic reticulum. In humans, two isoforms of SCD have been identified; SCD-1 and SCD-5. SCD-1 is expressed in several tissues with the highest levels in adipose tissue and liver. SCD-5 is mainly detected in brain and pancreas. Δ5- and Δ6-desaturases are widely expressed in human tissues, with the highest concentrations found in the liver. The activity of the three desaturases have been shown to be suppressed by dietary PUFA mainly via two transcription factors; SREBP-1c and PPAR-α. SCD-1 expression is also regulated by other dietary, hormonal and environmental factors such as glucose, fructose, cholesterol, insulin, temperature, and thiazolidinediones.
Serum fatty acids as biomarkers of dietary fat

Fat intake is highly difficult to assess through traditional recording methods. Firstly, it is difficult for an individual to recognise and quantify fat. Secondly, the reporting is associated with measurement errors. These include difficulties in identifying fat sources, assessing portion size and coding errors associated with database values of food composition. Underreporting of fat consumption is another problem, which is more frequent among overweight individuals. Subjects are also known to alter their usual diet, consciously or unconsciously, during the recording period.

Instead of dietary recording, analyses of fatty acid composition in serum and tissues can be used as an objective biomarker of the quality of the dietary fat intake. However, fatty acid composition in serum does not exactly reflect the dietary fatty acid intake due to utilization of the fatty acids before reaching storage, selective fatty acid absorption to tissues and endogenous fatty acid metabolism. Fatty acid composition is also affected by the “background diet”, i.e. the regular food intake, as well as by the genetic disposition. The accuracy of the relation between dietary intake of a specific fatty acid and the proportion in serum varies for different fatty acids. Essential fatty acids (i.e. α-linolenic acid and linoleic acid), odd-chain fatty acids (15:0 and 17:0) and long-chain n-3 fatty acids are better correlated to their dietary intakes, whereas SFA and MUFA are in general weaker biomarkers. It is

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Figure 3. Endogenous fatty acid metabolism
also important to keep in mind when considering fatty acid composition in serum and tissues that the measurement gives the relative amount of the fatty acid, i.e. it is based on the percentage that an individual fatty acid contributes to the total fatty acids and not the absolute amount. Thus, increased intake of a specific fatty acid lowers the relative percentage of another fatty acid even though its intake may be unaltered. Fatty acid composition reflects the dietary intake at different time aspects depending on in which body and tissue compartment it is measured. When measured in fractions of the lipoprotein particle in serum (i.e. cholesterol esters or phospholipids), the fatty acid composition reflects the habitual diet during the preceding days and weeks. Fatty acid composition in erythrocyte membranes and adipose tissue mirrors the diet during the last months and year(s), respectively. Fatty acid composition in total serum lipids is also used, although such measurement has its limitations as compared with fatty acids assessed in lipid fractions. For example different lipid fractions have distinct fatty acid compositions. Change in concentrations of lipoproteins could therefore potentially affect the total serum fatty acid composition.

**Inflammation**

The inflammatory process has a profound role in health and disease. It serves as a defence against invasion of foreign material, but excessive or prolonged inflammation leads to diseases. Inflammation is classically divided into acute or chronic. Whereas recruitment of polymorphonuclear leukocytes characterizes the acute inflammation, the chronic inflammation is accompanied by lymphocytes and macrophages. The immune system can be divided into the innate and the adaptive systems. The innate system is quickly mobilized and has a phagocytic capacity, but can only recognize a limited amount of structures, whereas the adaptive immunity can be specific to an almost infinite amount of molecular structures such as bacterial polysaccharides. The inflammatory process is driven and controlled by several mediators, such as cytokines, acute phase proteins and eicosanoids.

Cytokines are small proteins acting as messengers between the cells in the inflammatory system. Apart from recruiting and activating immune cells into and in damaged tissue, cytokines can also modulate metabolism by promoting lipolysis and protein production, such as acute phase proteins, from the liver. Interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) induce several acute and chronic inflammatory responses by inducing expression of a variety of genes and protein synthesis. To the IL-1 family belongs IL-1α, IL-1β and IL-1 receptor antagonist (IL-1Ra), all binding to the same receptors. Thus, IL-1Ra acts as a natural antagonist to IL-1α and IL-1β. TNF-α is mainly produced by macrophages and monocytes. TNF-α binds to two types
of receptors; TNF-R1 and TNF-R2. Among others, TNF-α stimulates monocytes and macrophages to secrete IL-1 and IL-6. IL-6 has a broad variety of biological activities, including induction of acute phase proteins in the liver and acting as a differentiation factor for B and T cells\textsuperscript{17,20}. IL-6 is produced by a wide range of cell types but the main source is macrophages and monocytes\textsuperscript{20}.

Acute phase proteins are produced mainly by hepatocytes upon stimulation by cytokines. Some of these proteins, such as C-reactive protein (CRP), can increase thousand-fold within a couple of days. The production by the liver is mainly triggered by IL-1 and IL-6\textsuperscript{17,20}. The main biological function of CRP is to eliminate pathogens and dead cells by recruitment of phagocytic cells and the complement system. Inflammation with serum concentrations of CRP below 10 mg/l is often defined as a low-grade inflammation\textsuperscript{20}. The current thesis aims to only explore the association between low-grade systemic inflammation and dietary fatty acids, whereas acute inflammation or local inflammatory processes are not directly investigated.

Eicosanoids are an umbrella term for inflammatory mediators such as prostaglandins, thromboxanes and leukotrienes. These compounds are produced from fatty acids with a 20-carbon chain located in the cell membranes, which is partly a consequence of our dietary fat intake. Fatty acids in the cell membranes are liberated by the enzyme phospholipase A2, making it available for prostaglandin synthesis\textsuperscript{17}. One of the major prostaglandins, prostaglandin F\textsubscript{2α} (PGF\textsubscript{2α}), is produced from arachidonic acid in a process catalysed by the enzyme cyclooxygenase (COX). However, due to its short half-life, the PGF\textsubscript{2α}-metabolite 15-keto-13,14-dihydro-PGF\textsubscript{2α} (15-keto-dihydro-PGF\textsubscript{2α}) is often used as a marker of COX-dependent inflammation. 15-keto-dihydro-PGF\textsubscript{2α} is known to act as a vaso- and bronchoconstrictor, and a smooth muscle stimulatory compound\textsuperscript{21}.

Adhesion molecules are involved in inflammation by directing circulating leukocytes to the endothelium and facilitate leukocyte migration into the sites of tissue inflammation. E-selectin is expressed on endothelial cells after inflammatory stimuli, P-selectin is expressed on both endothelial cells and platelets, and L-selectin is located on activated leukocytes. Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are recruited to the surface of activated endothelial cells but are also expressed on other cell types such as smooth muscle cells and monocytes\textsuperscript{17}. 

Oxidative stress and lipid peroxidation

Oxidative stress occurs when there is a severe disturbance in the free radical-antioxidant balance in favor of the former. A free radical is a species containing unpaired electrons but is capable of independent existence. Free radicals are produced as by-products during many biochemical processes, but also by activated immune cells and by electromagnetic radiation. Free radicals are highly reactive molecules and are therefore also very short-lived. Direct measurement is for that reason difficult, instead products of the oxidative stress reaction are measured.

One consequence of free radicals is the oxidation of lipids, so called lipid peroxidation. Lipid peroxidation can also be induced by a non-radical reaction with for example oxygen as the oxidant. Both these reactions are non-enzymatic. Furthermore, lipid peroxidation can also be mediated by enzymes such as COX. This enzyme-mediated lipid peroxidation generates prostaglandin and thromboxane precursors. PUFA are more prone to damage by free radical attacks, at least in vitro, due to the fact that the double bonds weaken the carbon-hydrogen bond at the adjacent carbon atom.

Isoprostanes are prostaglandin-like compounds but unlike the COX-dependent prostaglandin production, isoprostane formation is catalyzed by free radicals. Isoprostanes may however also be produced via the COX-pathway but the formation in vivo is minimal. 8-Iso-prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) is a major F$_2$-isoprostane. It is produced by free radical oxidation of arachidonic acid in the cell membranes. F$_2$-isoprostanes have been shown to be reliable biomarkers of oxidative stress in vivo. Biological functions of 8-iso-PGF$_{2\alpha}$ are for example vasoconstriction, platelet activation and COX activation. In this thesis, urinary 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ are used as markers of free radical triggered and COX-mediated lipid peroxidation, respectively (Figure 4).

![Figure 4. Formation of isoprostanes and prostaglandins from arachidonic acid in cell membrane due to lipid peroxidation](image-url)
Inflammation and oxidative stress in obesity, type 2 diabetes and cardiovascular disease

A low-grade inflammatory state is often observed concomitant with obesity and increased fat mass. This inflammation has been proposed to partake in the pathogenesis of insulin resistance and type 2 diabetes and also of cardiovascular disease. The fat accumulation during obesity is accompanied by infiltration of monocytes and macrophages into the adipose tissue. These cells, but also the adipocytes themselves, excrete inflammatory mediators into the circulation. Thus, obesity and associated metabolic pathologies are linked to a low-grade inflammation characterised by increased acute phase reactants, abnormal cytokine production and activation of inflammatory signalling pathways.

Type 2 diabetes is predicted by elevated CRP levels and other markers of inflammation such as IL-6, IL-1Ra, and soluble (s) TNF-R2. The risk of diabetes is also associated with circulating adhesion molecules. There are many proposed triggers of inflammatory responses in the fat tissue e.g. hypoxia and adipocyte cell death due to the expansion of adipose tissue during obesity. Moreover, induction of inhibitor of nuclear factor-κB kinase (IKK) and JUN N-terminal kinase (JNK) pathways by signalling via e.g. Toll-like receptors (TLR) is suggested. Both IKK and JNK can impair insulin signalling by phosphorylating insulin receptor substrate-1 (IRS-1). IKK is also able to activate nuclear factor-κB (NF-κB) by phosphorylating its inhibitor and thus stimulate production of inflammatory mediators such as TNF-α and IL-6. An inflammatory role in diabetes is further supported by clinical trials observing improved glycemic control and beta-cell function after administration of anti-inflammatory agents, i.e. IL-1Ra and salsalate (NF-κB pathway inhibition).

Elevated CRP levels also predict risk of coronary heart disease, but it is unclear whether low-grade inflammation reflected by elevated CRP levels is pathogenic in itself or only a disease marker. Statin treatment in the randomised controlled JUPITER-trial reduced CRP levels and also the risk for major cardiovascular events in persons with CRP ≥2 mg/l but “normal” LDL-cholesterol levels. Moreover, patients with autoimmune diseases, e.g. rheumatoid arthritis, have increased risk for coronary heart disease. Other markers of inflammation has also been related to future risk for coronary heart disease. Inflammation has been shown to affect several phases of the atherosclerotic process, such as influencing the fragility of the fibrous cap.

Isoprostanes are elevated in individuals with type 2 diabetes and in individuals with coronary heart disease. Whether oxidative stress is a conse-
quence of pathological processes such as chronic hyperglycemia or rather a cause is still under debate. Obesity induces endoplasmatic reticulum stress which activates inflammatory signalling and thereby contributes to insulin resistance\textsuperscript{29}. Also production of reactive oxygen species is induced during obesity leading to enhanced inflammation\textsuperscript{29}.

**Fatty acids, inflammation and oxidative stress**

In the earlier literature, n-3 PUFA have usually been described as anti-inflammatory, whereas n-6 PUFA have been considered as pro-inflammatory\textsuperscript{63}. Such view appears too simplified and remains to be proven in humans as accumulating findings suggest a more complicated role of different PUFA\textsuperscript{64-66}. The proposed mechanism behind a pro-inflammatory effect of linoleic acid is an increased conversion into arachidonic acid and an increased incorporation of arachidonic acid into the cell membrane phospholipids. The endogenous conversion of n-3 and n-6 PUFA is competitive and catalyzed by the same enzymes. Thus, an increased conversion of arachidonic acid may be at the expense of long-chain n-3 PUFA production causing a decreased production of these less inflammatory eicosanoids\textsuperscript{63,67}. There are only few studies in humans indicating a pro-inflammatory effect of linoleic acid, why further investigations within this area are needed. PUFA are also more prone to oxidation than SFA and MUFA due to the higher amount of double bonds\textsuperscript{23,25}, but the clinical implications of an altered lipid peroxidation by a modified fat intake are still unclear. \textit{In vitro} studies indicate that SFA may promote inflammation by inducing gene products including interleukins and COX\textsuperscript{68}. Therefore, human studies investigating potential pro-inflammatory capacity of SFA are warranted.

**Rationale for this thesis**

Dietary fat quality, rather than fat quantity, appears to be more relevant for the development of coronary heart disease and metabolic disorders\textsuperscript{69-71}. Dietary fatty acids have been shown to alter insulin sensitivity\textsuperscript{72,73}, blood lipids\textsuperscript{74-76} and the risk for type 2 diabetes\textsuperscript{77,78} and cardiovascular disease\textsuperscript{70,79,80}. The role of different fatty acids in low-grade inflammation in humans clearly needs further investigations. In addition, the link between fatty acid desaturase activities and inflammation is unclear. Given the potential key role of low-grade inflammation in the aetiology of several major diseases it is thus relevant to further investigate if different fatty acids and desaturase indices may affect systemic inflammation as well as oxidative stress in vivo.
Aims

Overall aim:

The overall aim of this thesis was to investigate if dietary fat quality may influence low-grade inflammation in humans. This aim is investigated by both observational and controlled interventional studies.

Specific aims:

- To investigate the longitudinal association between serum fatty acid composition and desaturase indices in men at age 50 and CRP levels 20 years later (Paper I).

- To examine the cross-sectional relationship between serum fatty acid composition and desaturase indices and several markers of inflammation and endothelial function in 70 year old men and women (Paper II).

- To investigate the effects of dietary fat modification on markers of inflammation and oxidative stress in subjects with the metabolic syndrome. In a randomised controlled study four diets were compared; two high-fat diets (38% energy) rich in either SFA or MUFA, and two low-fat (28% energy), high-complex carbohydrate diets with or without very long-chain n-3 PUFA supplementation (Paper III).

- To in a randomised controlled study investigate the effects of a diet high in either SFA or n-6 PUFA (i.e. linoleic acid, 18:2 n-6) on inflammation and oxidative stress in subjects with abdominal obesity (Paper IV).
Subjects and Methods

Both Paper I and II included observational studies, by use of two independent cohorts. Paper I was based on data from the population-based cohort Uppsala Longitudinal Study of Adult Men (ULSAM). In Paper II, data from the population-based cohort Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) were used. In Paper III and IV data were derived from two randomised controlled dietary interventions; the LIPGENE study (III) and the HEPFAT study (IV), respectively.

![Figure 5. Design of the studies included in the thesis](FA comp., serum fatty acid composition.)

Paper I – Serum fatty acids and CRP (ULSAM)

The ULSAM cohort

ULSAM (http://pubcare.uu.se/ULSAM/) is a population-based cohort study that started in Uppsala, Sweden, in 1970. All men born between 1920 and 1924 and living in Uppsala County were invited to participate. Of the 2841 invited men, 2322 (82%) chose to participate. The baseline survey at age 50 was carried out between September 1970 and September 1973. The men were reinvestigated at the ages of 60, 70, 77, 82 and 88 years. At age 70, all participants in the baseline investigation were invited to reinvestigation (including non-participants at age 60). During the 20-year follow-up, 422 indi-
viduals died and 219 had moved out of the county. The survey was carried out between August 1991 and May 1995. Participation rate was 73% (1221 of 1681).

Participants

Out of the original population-based cohort (n=2322), 767 men were included in the study. 1020 individuals participating in the ULSAM study had measures of serum cholesterol ester fatty acid composition at age 50 and CRP at age 70. Exclusion criteria were diabetes (fasting blood glucose ≥6.1 mmol/l), cardiovascular disease (defined by ICD-8 codes 401-443) or malignancy at baseline, usage of lipid-lowering medicine or glucocorticoids at age 50 or 70, and serum CRP concentrations >10 mg/l at age 70.

Methods

Investigations at age 50

All measurements were performed under standardised conditions and have been described in detail previously. The survey included blood sampling, anthropometric measurements and blood pressure as well as a medical questionnaire and interview. Blood samples were drawn from an antecubital vein after an overnight fast. Height was measured without shoes to the nearest whole cm. Weight was measured in undershorts to the nearest whole kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared. Blood glucose was determined by spectrophotometry with the glucose oxidase method. Serum insulin concentration was measured with the Phadebas Insulin Test (Pharmacia AB, Uppsala, Sweden), based on radio-immunosorbent technique. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR) and calculated as (fasting insulin [mU/l]*fasting glucose [mmol/l])/22.5. Erythrocyte sedimentation rate was determined by Westergren’s method. Fatty acid composition was measured in serum cholesterol esters (see below). Desaturase activities were estimated according to the following fatty acid product-to-precursor ratios in serum: Δ5-desaturase: 20:4 n-6/20:3 n-6, Δ6-desaturase: 18:3 n-6/18:2 n-6, and SCD-1: 16:1/16:0. The samples were stored in liquid nitrogen for about 15 years before fatty acid analysis.

A self-administered questionnaire was made according to Collen et al and used to assess information about lifestyle, diseases and medical treatment among others. Smoking habits were followed up by an interview. Physical activity was defined as sedentary, moderate, regular or athletic and smoking habits as smoker or non-smoker.
Investigations at age 70
The investigation was performed in the same manner as at baseline and as previously described\textsuperscript{87,88}. High-sensitivity CRP was measured in serum as described below. Insulin sensitivity was measured by euglycaemic hyperinsulinaemic clamp technique according to DeFronzo et al\textsuperscript{89}, slightly modified. An infusion rate of 56 mU/min per body surface area (m\textsuperscript{2}) was used instead of 40. Insulin was infused in a primary dose for the first 10 minutes and then as a continuous infusion for 110 minutes to maintain steady state hyperinsulaemia. Plasma glucose level was maintained during the clamp study by measuring plasma glucose every 5 minutes and adjusting the infusion rate of a 20% glucose solution. Target plasma glucose level was 5.1 mmol/l. The glucose disposal (M, mg/kg body weight/min) was calculated as the amount of glucose taken up during the last 60 minutes of the clamp.

Two self-administered questionnaires were used. One concerned general and medical background and was based on the questionnaires previously used at the investigations at age 50 and 60. The other concerned living conditions. Dietary intake, including alcohol, was assessed by a 7-day dietary record. The food record used was a pre-coded menu book prepared and used by the National Food Administration\textsuperscript{90}. Prior to the assessment, a dietician gave oral instruction on how to perform the dietary registration.

Paper II – Serum fatty acids and inflammation (PIVUS)
The PIVUS cohort
The population-based cohort PIVUS (www.medsci.uu.se/pivus/pivus.htm) was carried out between April 2001 and June 2004 in Uppsala, Sweden. All persons aged 70 and living in the community of Uppsala were eligible. 2025 individuals were randomly invited within one month of their 70\textsuperscript{th} birthday in order to standardise for age, and 1016 (50\%) participated. As the participation rate was only 50\%, an evaluation of cardiovascular disorders and medications was carried out in 100 consecutive non-participants in order to obtain information about differences in cardiovascular health. The prevalence of cardiovascular drug intake, ischemic heart disease, statin use and insulin treatment were similar to those in the investigated sample, while the prevalence of diabetes, congestive heart failure and stroke tended to be higher among the non-participants\textsuperscript{91}.

Participants
The study population consisted of 264 participants from the PIVUS baseline investigation. Of the 2025 participants in PIVUS, fatty acid composition in
serum cholesterol esters was assessed in 273 randomly selected participants at the age of 70 years. Exclusion criterion was CRP concentrations >10 mg/l (n=9).

Methods
The survey was performed after an overnight fast as previously described. BMI was calculated by weight (kg) divided by height (m) squared. Medical history, regular medication and smoking habits were assessed by a questionnaire. Physical activity was divided into light and hard exercise and classified as number of activities for at least 30 minutes per week. This was assessed by asking the participants how many times per week he/she performed light (e.g. walking, gardening) or strenuous exercise (e.g. running, swimming), respectively for at least 30 minutes. Fatty acid composition was assessed in serum cholesterol esters (see below). SCD-1 activity was estimated by serum 16:1/16:0 ratio. Circulating levels of several inflammatory markers (i.e. CRP, IL-2, IL-6, IL-8, TNF-α, interferon-γ, monocyte chemoattractant protein-1 [MCP-1]) and soluble adhesion molecules (ICAM-1, VCAM-1, E-selectin, P-selectin and L-selectin) were assessed.

Paper III – Intervention with n-3, SFA and MUFA
Participants
Paper III is based on the LIPGENE study, a 12-week dietary intervention trial with a randomised parallel design. Participants were 486 volunteers with the metabolic syndrome. The study was conducted during spring 2005 and spring 2006 in eight European countries; Republic of Ireland, United Kingdom, Norway, France, the Netherlands, Spain, Poland and Sweden. Study design (Figure 6) and recruitment strategies have been described previously. Inclusion criteria were age 35–70 years, BMI 20–40 kg/m² and the metabolic syndrome defined by three or more of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP-III), slightly modified to aid the recruitment process. The criteria were; fasting plasma glucose 5.5–7.0 mmol/l, serum triacylglycerols ≥1.5 mmol/l, serum HDL-cholesterol <1.0 mmol/l (men) or <1.3 mmol/l (women), waist circumference >102 cm (men) and >88 cm (women), and systolic blood pressure ≥130 mmHg, diastolic blood pressure ≥85 mmHg or prescribed hypertension treatment.
Intervention

Participants were randomised to one of four isoenergetic diets differing in fat quantity and quality. The randomisation was performed centrally and stratified for age, sex and fasting plasma glucose concentration. The diets were two high-fat diets (38% energy) and two low-fat (28% energy), high-complex carbohydrate diets. Of the high-fat diets, one was rich in saturated fat (HSFA; 16% SFA, 12% MUFA, 6% PUFA) and the other one was rich in monounsaturated fat (HMUFA; 8% SFA, 20% MUFA, 6% PUFA). The low-fat, high-complex carbohydrate diets (8% SFA, 11% MUFA, 6% PUFA) were either supplemented with 1.24 g/day very long-chain n-3 PUFA (LFHCCn-3) (Marinol® C-38, the ratio between eicosapentaenoic and docosahexaenoic acids was 1.4:1) or 1 g/day high-oleic acid sunflower oil (LFHCC). To attain the dietary targets, a food exchange model was used as previously described. The amount of exchangeable fat in the average UK diet was calculated as the sum of fat provided by added fats (spreads and oils), milk, cheese, biscuits, cakes, buns and pastries. A 3-day weighed dietary record and food frequency questionnaire was performed to assess habitual dietary intake. These were used as basis in the individually advice given by nutritionists about food choices for the allocated diet. Study foods including spreads, cooking oils, mayonnaises, baking fats and biscuits were also...

Figure 6. Flowchart for the LIPGENE study.

PGF$_{2\alpha}$; 15-keto-dihydro-PGF$_{2\alpha}$. 
provided by Unilever Bestfoods (The Netherlands). Dietary records were performed during the intervention to monitor compliance. Energy intake was adjusted if body weight change exceeded 2 kg. Physical activity, alcohol consumption and smoking habits were not altered during the intervention.

Methods
Before and after intervention all subjects completed a health and lifestyle questionnaire. Anthropometric and biochemical measurements were performed according to standardised protocols. Fasting morning urine was collected by the participants and brought to the clinic. Blood pressure was assessed by an automatic blood pressure device. Blood samples were taken after 12 hours fasting. Serum CRP, urinary 15-keto-dihydro-PGF$_{2\alpha}$ and urinary 8-iso-PGF$_{2\alpha}$ were assessed as markers of inflammation and oxidative stress. Fatty acid composition was measured in total plasma lipids as described below. Plasma triacylglycerols, HDL-cholesterol and glucose were analysed with an ILAB 600 clinical chemical analyser by enzymatic colorimetric kits (Instrumentation Laboratory, Warrington, UK).

Paper IV – Intervention with n-6 and SFA
Participants
Paper IV is based on the HEPFAT study, a randomised, 10-week dietary intervention trial with parallel groups. Sixty-seven abdominally obese individuals were included in the study which was conducted in Uppsala, Sweden, between February 2009 and April 2010. Inclusion criteria were age 30 to 65 years and a sagittal abdominal diameter $>25$ cm or waist circumference $>88$ cm (women) or $>102$ cm (men). Exclusion criteria were diagnosed liver disease, type 1 diabetes, insulin-dependent type 2 diabetes, history of serious cardiovascular event, BMI $>40$ kg/m$^2$, high alcohol intake, internal metal or electronic device disturbing magnetic resonance imaging, claustrophobia or anomalous results in blood analyses. After screening of 84 individuals by questionnaire and clinical examination, 67 were eligible (Figure 7).

Intervention
Participants were randomised to one of two diets; either a sunflower oil-based diet high in n-6 PUFA (linoleic acid, 18:2 n-6) (LA-diet) or a dairy-based diet high in saturated fat (SFA-diet). The randomisation was stratified according to gender and performed in blocks of four. The participants were instructed (unblinded) to change their dietary fat quality without altering their total fat intake. They also received food items (scones, margarine or
butter, and, in the LA-group, sunflower oil and sunflower seeds). Based on weight and gender, participants on the LA-diet were instructed to consume amounts of given food items corresponding to 15% energy linoleic acid. The scones contained the same ingredients except for type of fat (one tablespoon sunflower oil or butter per piece). All participants were instructed not to change their intakes of protein, carbohydrates, fish, sugar and alcohol, as well as physical activity or weight during the study.

Figure 7. Flowchart for the HEPFAT study

*The diseases were known before enrolment (heart valve disorder and chronic obstructive pulmonary disease).

Methods

Clinical and laboratory examinations were performed at baseline (visit 1), after 5 weeks (visit 2), and after 10 weeks (visit 3). All visits took place in the morning after an overnight fast. Visit 1 and 3 started with liver and body fat assessment by magnetic resonance technique, followed by anthropometry, blood pressure measurement, blood sampling, oral glucose tolerance test, adipose tissue biopsy and total body fat measurement by air displacement technique (BOD POD®). Visit 2 included anthropometry and blood sampling. Body weight was measured in underwear to the nearest 0.1 kg. Dietary intake was assessed by 3-day weighed dietary registration (two weekdays and one weekend day) before randomisation and between visit 2 and 3. Blood samples were drawn from an antecubital vein with Vacutainer tubes. Glucose, triacylglycerols, cholesterol, apolipoproteins, alanine aminotransferase, γ-glutamyltransferase and CRP were measured in plasma and insulin.
was measured in serum according to standard laboratory procedures at Uppsala University Hospital. Other inflammatory markers (IL-1Ra, IL-1β, IL-6, IL-10, sTNF-R2) were assessed in plasma. Fatty acid composition as a measure of compliance was assessed in serum cholesterol esters (see below). SCD-1 activity was estimated by serum 16:1/16:0 ratio. Fasting morning urine was collected by the participants the same morning as visit 1 and 3 and brought to the clinic for analysis of 8-iso-PGF$_2\alpha$ and 15-keto-dihydro-PGF$_2\alpha$. 15-keto-dihydro-PGF$_2\alpha$ was only measured in compliant participants (see definition below).

Subcutaneous adipose tissue biopsies were obtained from the abdominal fat pad by needle aspiration under local anaesthesia. The obtained fat tissue was washed with physical saline and directly frozen on dry ice covered with ethanol and stored in -70°C. Adipose tissue mRNA expression was assessed in compliant participants (see definition below) by quantitative realtime-PCR (iCycler IQ, Bio-Rad Laboratories) using a comparative threshold cycle (Ct) method. Ct values were normalized to the reference genes LDL receptor-related protein 10 (LRP10) or 18S, according to the formula $2^{ΔCt\text{-target gene}}/2^{ΔCt\text{-reference gene}}$=arbitrary units. Selected target genes were the following; TNF-α, IL-6, MCP-1, CD14, adiponectin, SCD, PPAR-γ, fatty acid synthase, carnitine palmitoyl transferase-1, acetyl-CoA carboxylase β, and acyl-CoA dehydrogenase.

**Assessment of fatty acid composition**

Fatty acid composition was measured in serum cholesterol esters in Paper I, II and IV, and assessed in total plasma in Paper III. Fatty acid composition in serum cholesterol esters was assessed as previously described$^{95,96}$. Hexane-isoprostanol solution (Paper I) or methanol (Paper II and IV) was added to serum for lipid extraction. Cholesterol esters were separated by thin-layer chromatography before inter-esterification with acidic methanol at 85°C for two hours. To avoid contamination of the gas liquid chromatography column, free cholesterol liberated in the reaction was removed by an aluminium oxide column. The percentage composition of methylated fatty acids (14:0 to 22:6) was determined by gas chromatography with a flame ionisation detector and helium as carrier gas. Divergences between the papers were that a 25-m NM-351 silica capillary column and a Hewlett-Packard system, consisting of GC 5830A, capillary injection system 18835B, operating terminal and integrator 18850A and auto-sampler 7671A was used in Paper I, whereas a 30-m glass capillary column coated with Thermo TR-FAME (Thermo Electron Corporation, USA) and an Agilent Technologies system consisting of model GLC 6890N, autosampler 7683 and Agilent ChemStation was used in Paper II and IV. Moreover, the temperature was pro-
grammed to 180-215°C in Paper I and to 150-260°C in Paper II and IV. The fatty acids were identified by comparing each peak’s retention time with fatty acid methyl ester standard Nu Check Prep (Elysian, MN, USA). Fatty acids are presented as the relative percentage of the total quantified fatty acids.

In Paper III, fatty acid composition was measured in plasma as previously described. Fatty acids were extracted from plasma and transmethylated with a boron trifluoride-methanol solution. The methylated fatty acids were determined by gas chromatography on a Perkin-Elmer Autosystem XL (Perkin-Elmer, Paris, France) and a Shimadzu GC2010 (Shimadzu, Kyoto, Japan), with hydrogen and helium as carrier gas, respectively. The samples were randomly distributed between the machines. Temperature was programmed to 215-260°C. The retention times of the fatty acids were compared with fatty acid methyl ester standard SUPELCO (Saint Quentin Fallavier, France). Fatty acids are presented as the relative sum of the fatty acids analysed.

**Assessment of Inflammation**

CRP was measured by high-sensitive immunoassays in all papers, but with different reagents. In immunoassays, specific antibodies bind to the molecule of interest (the antigen). An analytical reagent associated with a detectable label, such as enzymes, latex or radioactive elements, produces a measurable signal in response to a specific binding. The response can then be detected by changes in for example absorbance. In Paper I, CRP was measured in serum by latex-enhanced reagent (Dade Behring, Deerfield, IL, USA) by a Behring BN ProSpec analyser. In Paper II, serum concentrations of CRP were assessed by particle enhance immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20 autoanalyser (Thermo Clinical Labsystems, Espoo, Finland). In Paper III, CRP was measured in serum by enzyme-linked immunosorbent assay (ELISA), with mouse anti-CRP as capture antibody, goat anti-CRP conjugated with peroxidase as detection antibody and tetramethylbenzidine as substrate (BioCheck Inc., CA, US). In Paper IV, CRP was measured in plasma by latex-enhanced reagent (CRP Vario, Abbot) with an Architect instrument (Abbott Diagnostics, IL, USA). Functional sensitivity limits were 0.1 mg/l (Paper II, III) and 0.2 mg/l (Paper I, IV). Different high-sensitive CRP methods have been shown to be closely correlated \( (r>0.90)^{98} \).

In study II and IV, other inflammatory markers than CRP were also investigated. In Paper II, inflammation markers (IL-2, IL-6, IL-8, TNF-α, interferon-γ, MCP-1) and soluble adhesion molecules (ICAM-1, VCAM-1 and
selectins) were analysed on the Evidence® array biochip analyser (Randox Laboratories, Ltd., Crumlin, UK). Intra- and inter-assay CV for most cytokines were <10%\(^9\). The sensitivity was as follows; IL-2: 4.1 pg/ml, IL-6: 0.3 pg/ml, IL-8: 1.5 pg/ml, TNF-α: 1.8 pg/ml, ICAM-1: 18.6 ng/ml, VCAM-1: 3.1 ng/ml, E-selectin: 3.1 ng/ml, P-selectin: 11.2 ng/ml, L-selectin: 32.8 ng/ml, interferon-γ: 1.8 pg/ml, and MCP-1: 19.4 pg/ml. Also IL-1α, IL-1β, IL-4 and IL-10 were included in the Evidence® array biochip cytokine panel, but were found to have insufficient sensitivity for measurements in the present sample and were therefore not evaluated. In Paper IV, plasma concentrations of IL-1β, IL-6, IL-10, IL-1Ra and sTNF-R2 were assessed by ELISA (R&D systems Quantikine®). The sensitivity was as follows; IL-1β: 0.057 pg/ml, IL-6: 0.039 pg/ml, IL-10: <0.5 pg/ml, IL-1Ra: 6.26 pg/ml, sTNF-R2: 0.6 pg/ml.

Assessment of oxidative stress and lipid peroxidation

In Paper III and IV, urinary 8-iso-PGF\(_{2α}\) and 15-keto-dihydro-PGF\(_{2α}\) were used as markers of free radical-induced and COX-mediated oxidation of arachidonic acid, respectively. Concentrations of urinary 8-iso-PGF\(_{2α}\)^{100} and 15-keto-dihydro-PGF\(_{2α}\)^{101} were assessed by radioimmunoassay and adjusted for urinary creatinine levels. Creatinine was analysed by an IL™ Test (Monarch, Amherst, NH, USA) or a Konelab 20 instrument (Thermo Clinical Lab Systems, Thermo Electron Corporation, Vantaa, Finland) in Paper III and IV, respectively. The intra-assay coefficient of variation was 12.2-14.5%. Detection limit was approximately 23 pmol/l (8-iso-PGF\(_{2α}\)) and 45 pmol/l (15-keto-dihydro-PGF\(_{2α}\)).

Statistics

Baseline characteristics are described as means ± standard deviation. Nonnormally distributed variables are described as median and interquartile range. The distribution of continuous variables was examined by Shapiro-Wilk W test. Non-normally distributed variables (W<0.95) were logarithmically transformed and if not attaining normality, non-parametrical tests were used. P<0.05 was considered as statistically significant, except for in Paper II where p<0.01 was used to reduce the risk for type-1 errors due to multiple testing.

Paper I and II

In the observational studies (Paper I and II), the univariat association between fatty acid composition and inflammation markers was investigated by
correlation (Pearson’s and Spearman’s rank test) and linear regression analyses. In Paper I, three multivariat models were used; one adjusting for BMI, physical activity, smoking and erythrocyte sedimentation rate at age 50 and alcohol consumption at age 70, and two models additionally adjusting for insulin resistance (either determined by the HOMA-IR index at baseline or by euglycemic clamp, M-value, at follow-up). In Paper II, adjustments were made for BMI, smoking habits, alcohol consumption, physical activity and use of lipid lowering drugs.

Paper III

In Paper III, changes (concentration_{follow-up}-concentration_{baseline}) in inflammatory and oxidative stress markers between the diet groups were investigated by Kruskal-Wallis test. Within-group differences were investigated by Wilcoxon signed rank test. To avoid elevated CRP due to acute infections, subjects with CRP concentrations ≥10 mg/l at either baseline (n=60) or end of study (n=49) were excluded in post-hoc analysis (HSFA: n=18, HMUFA: n=16, LFHCC: n=22, LFHCCn-3: n=19). Subgroup analyses were performed according to gender and smoking habits. Two subgroup analyses were also performed dividing the participants according to the total fat intake (above or below the median). One was based on fat intake at baseline and the other one was based on fat intake in each diet group at follow-up. The change in major plasma fatty acids were associated with changes in oxidative stress and inflammation markers by Spearman’s rank correlation.

Paper IV

One participant was excluded from the statistical analyses regarding cytokines due to extensive haemolysis. The diet groups were compared by two-sided t-test or Wilcoxon rank sum test. Adjustments for baseline values were made by ANCOVA or a residual method. Post-hoc analyses based on compliance were also performed (LA-diet: n=27, SFA-diet: n=19). Compliance was defined according to changes in serum cholesterol ester fatty acid composition, i.e. change in linoleic acid >0.0% during LA-diet and linoleic acid <0.0% during SFA-diet. To avoid that acute infections affected the results, analyses excluding individuals with CRP levels ≥10.0 mg/l (n=9) at either baseline or follow-up were also performed.

Ethics and clinical trial registration

All studies were approved by the Ethics Committee of the University of Uppsala. For the LIPGENE trial approval was obtained from the local ethics committees of all eight study sites. The participants gave informed consent
before entering the study. The LIPGENE study was registered at US National Library of Medicine Clinical Trial (NCT00429195) and the HEPFAT study at ClinicalTrials.gov (NCT01038102).
Results

Paper I

Mean BMI at baseline was 24.7 ± 2.9 kg/m². The median of CRP concentration in the population at age 70 was 1.9 (0.9-3.8) mg/l. The proportions of the individual fatty acids in serum cholesterol esters at baseline are shown in Figure 8.

![Figure 8. Proportion of fatty acids in serum cholesterol esters at age 50 in ULSAM Variables are presented as mean ± standard deviation.](image)

Results from the univariat analyses are presented in Table 1. The proportions of palmitoleic (16:1), oleic (18:1) and γ-linolenic (18:3 n-6) acids were positively correlated to CRP concentrations 20 years later, whereas linoleic acid (18:2 n-6) was inversely related. Indices of Δ6-desaturase and SCD-1, but not Δ5-desaturase, were positively related to CRP levels. As judged by the plots the regressions were modest (Figure 9).

When adjusting for BMI, smoking, physical activity, erythrocyte sedimentation rate and alcohol consumption, the associations with CRP remained for palmitoleic, oleic and linoleic acids as well as for Δ6-desaturase and SCD-1. After further adjustments for insulin resistance, MUFA, linoleic acid and SCD-1 were related to CRP concentrations. The results did not change substantially when excluding subjects with cardiovascular disease or non-steroidal anti-inflammatory drug use at age 70.
Table 1. Correlation between serum fatty acid composition at age 50 and C-reactive protein at age 70 in the ULSAM cohort

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>r</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myristic acid 14:0</td>
<td>0.03</td>
<td>0.47</td>
</tr>
<tr>
<td>Palmitic acid 16:0</td>
<td>0.05</td>
<td>0.13</td>
</tr>
<tr>
<td>Palmitoleic acid 16:1</td>
<td>0.13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Stearic acid 18:0</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>Oleic acid 18:1</td>
<td>0.20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Linoleic acid 18:2 n-6</td>
<td>-0.18</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>γ-linolenic acid 18:3 n-6</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td>α-linolenic acid 18:3 n-3</td>
<td>0.003</td>
<td>0.94</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid 20:3 n-6</td>
<td>0.06</td>
<td>0.09</td>
</tr>
<tr>
<td>Arachidonic acid 20:4 n-6</td>
<td>0.03</td>
<td>0.46</td>
</tr>
<tr>
<td>Eicosapentaenoic acid 20:5 n-3</td>
<td>0.06</td>
<td>0.08</td>
</tr>
<tr>
<td>Docosahexaenoic acid 22:6 n-3</td>
<td>-0.02</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Desaturase indices

| Δ5-desaturase 20:4 n-6/20:3 n-6 | -0.06 | 0.11  |
| Δ6-desaturase 18:3 n-6/18:2 n-6 | 0.11  | <0.01 |
| Stearoyl coenzymeA desaturase-1 16:1/16:0 | 0.13  | <0.01 |

r, Pearson’s correlation coefficient.

Figure 9. Association between linoleic acid, SCD-1 index and C-reactive protein in the ULSAM cohort
Bars indicate median within each quartile.
Paper II

Of the 264 subjects included, 117 (44%) were women. Mean BMI was 26.8 ± 4.0 kg/m² and median of CRP was 1.9 (0.9-3.0) mg/l. The relative percentages of fatty acids analysed are presented in Figure 10.

Figure 10. Proportion of fatty acids in serum cholesterol esters in PIVUS Variables are presented as mean ± standard deviation.

Fatty acid composition was associated with concentrations of CRP and E-selectin (Table 2), whereas no associations were found between the other markers for inflammation and endothelial function (i.e. IL-2, IL-6, IL-8, TNF-α, VCAM-1, ICAM-1, P-selectin, L-selectin, interferon-γ and MCP-1).

Table 2. Correlation between serum fatty acid composition, C-reactive protein and E-selectin in the PIVUS cohort

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>C-reactive protein</th>
<th>E-selectin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
</tr>
<tr>
<td>Myristic acid</td>
<td>14:0</td>
<td>-0.002</td>
</tr>
<tr>
<td>Palmitic acid</td>
<td>16:0</td>
<td>0.03</td>
</tr>
<tr>
<td>Palmitoleic acid</td>
<td>16:1</td>
<td>0.22</td>
</tr>
<tr>
<td>Stearic acid</td>
<td>18:0</td>
<td>-0.03</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>18:1</td>
<td>0.18</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>18:2 n-6</td>
<td>-0.19</td>
</tr>
<tr>
<td>γ-linolenic acid</td>
<td>18:3 n-6</td>
<td>0.13</td>
</tr>
<tr>
<td>α-linolenic acid</td>
<td>18:3 n-3</td>
<td>-0.004</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid</td>
<td>20:3 n-6</td>
<td>0.16</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>20:4 n-6</td>
<td>0.08</td>
</tr>
<tr>
<td>Eicosapentaenoic acid</td>
<td>20:5 n-3</td>
<td>0.02</td>
</tr>
<tr>
<td>Docosahexaenoic acid</td>
<td>22:6 n-3</td>
<td>-0.06</td>
</tr>
</tbody>
</table>

Desaturase index
Stearoyl coenzymeA desaturase-1 16:1/16:0 0.22 <0.01 0.14 0.02

r, Pearson’s correlation coefficient.
After adjusting for obesity, lifestyle factors (smoking, physical activity, alcohol consumption) and lipid-lowering therapy, palmitoleic acid \((p=0.003)\) and SCD-1 index \((p=0.001)\) remained related to CRP concentrations. None of the other inflammatory markers were significantly associated with fatty acid composition in multivariat analysis.

\[\text{Figure 11. Association between linoleic acid, SCD-1 index and C-reactive protein in the PIVUS cohort.} \]
\[\text{Bars indicate median within each quartile.}\]
Paper III

Four hundred-seventeen participants (79%) completed the LIPGENE study. Measures of CRP, 15-keto-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ concentrations at both baseline and follow-up were assessed in 415, 408 and 409 participants, respectively. Baseline characteristics for individuals completing the intervention are presented in Table 3.

<table>
<thead>
<tr>
<th></th>
<th>HSFA (n=100)</th>
<th>HMUFA (n=111)</th>
<th>LFHCC (n=106)</th>
<th>LFHCCn-3 (n=100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>55 ± 9</td>
<td>55 ± 9</td>
<td>55 ± 9</td>
<td>55 ± 10</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>54 (54%)</td>
<td>65 (59%)</td>
<td>59 (56%)</td>
<td>54 (54%)</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>31.9 ± 4.5</td>
<td>32.4 ± 4.3</td>
<td>32.5 ± 4.2</td>
<td>32.4 ± 4.2</td>
</tr>
<tr>
<td>WC, cm</td>
<td>105 (98–113)</td>
<td>106 (98–115)</td>
<td>105 (99–114)</td>
<td>107 (100–112)</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>5.8 (5.5-6.4)</td>
<td>5.7 (5.4-6.3)</td>
<td>5.8 (5.4-6.3)</td>
<td>5.8 (5.5-6.4)</td>
</tr>
<tr>
<td>TAG, mmol/l</td>
<td>1.6 (1.2-2.3)</td>
<td>1.6 (1.2-2.2)</td>
<td>1.6 (1.1-2.2)</td>
<td>1.6 (1.3-2.0)</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.0 (0.9-1.2)</td>
<td>1.1 (0.9-1.2)</td>
<td>1.1 (0.9-1.3)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>138 ± 15</td>
<td>139 ± 16</td>
<td>138 ± 15</td>
<td>138 ± 15</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>86 ± 9</td>
<td>86 ± 10</td>
<td>85 ± 9</td>
<td>86 ± 9</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>3.6 (2.3-7.3)</td>
<td>3.7 (1.8-7.0)</td>
<td>3.9 (2.1-7.6)</td>
<td>4.4 (2.0-6.7)</td>
</tr>
<tr>
<td>15-keto-PGF$_{2\alpha}$</td>
<td>0.16 (0.12-0.19)</td>
<td>0.17 (0.14-0.21)</td>
<td>0.15 (0.12-0.18)</td>
<td>0.15 (0.12-0.20)</td>
</tr>
<tr>
<td>8-iso-PGF$_{2\alpha}$</td>
<td>0.43 (0.36-0.54)</td>
<td>0.46 (0.38-0.57)</td>
<td>0.44 (0.37-0.53)</td>
<td>0.45 (0.38-0.54)</td>
</tr>
</tbody>
</table>

WC, waist circumference; TAG, triacylglycerol; HDL-C, high density lipoprotein cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure; 15-keto-PGF$_{2\alpha}$, 15-keto-13,14-dihydro-prostaglandin F$_{2\alpha}$ (nmol/mmol creatinine); 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$ (nmol/mmol creatinine).

Dietary intake at end of study is shown in Figure 12. At the end of the study, the HMUFA group had higher proportions of oleic acid in plasma compared with the HSFA and LFHCCn-3 groups (p=0.0008 and p=0.005, respectively). There was also a trend towards a higher proportion of oleic acid in the HMUFA group compared with the LFHCC group (p=0.06). With regard to the n-3 PUFA, the LFHCCn-3 group had significantly higher proportions of eicosapentaenoic acid (20:5 n-3, p=0.0001), docosapentaenoic acid (22:5 n-3, p=0.05) and docosahexaenoic acid (22:6 n-3, p<0.003) compared with the other groups, except for docosahexaenoic acid which did not differ significantly between the two low-fat diets.
Concentrations of CRP, 15-keto-dihydro-PGF$_{2\alpha}$ and 8-iso-PGF$_{2\alpha}$ did not differ between the diet groups at baseline (p>0.07). These investigated inflammatory and oxidative stress markers were not altered during the intervention and no differences in changes were observed between the diet groups (CRP: p=0.97, 15-keto-dihydro-PGF$_{2\alpha}$: p=0.45, 8-iso-PGF$_{2\alpha}$: p=0.83) (Figure 13). Results were not altered in post-hoc analyses based on smoking status or sex. Furthermore, no significant differences were observed after classifying subjects according to their habitual fat intake. Participants were also divided as above or below the median of total fat intake in each diet group, respectively (medians; HSFA: 39.8% energy, HMUFA: 39.5% energy, LFHCC: 29.2% energy, LFHCCn-3: 28.2% energy). Subjects with a fat intake above the median in the LFHCCn-3 group had increased concentrations of 8-iso-PGF$_{2\alpha}$ (p=0.05) during the intervention whereas those with a fat intake above median in the HMUFA group had decreased their concentrations of 15-keto-dihydro-PGF$_{2\alpha}$ (p=0.02).
Figure 13. Box plot of changes in inflammatory and oxidative stress markers during the LIPGENE study

The boxes represent 25th, 50th and 75th percentiles. The whiskers correspond to the lower and upper adjacent values from 10th and 90th percentiles, respectively. Higher and lower values are presented as dots. Change, concentration (follow-up) - concentration (baseline).

*C-reactive protein levels ≥10 mg/l are excluded.
†One extreme value (8.4 nmol/mmol creatinine) is excluded.

The changes in concentrations of CRP and 15-keto-dihydro-PGF2α during the intervention were not related to changes in plasma fatty acids whereas the change in 8-iso-PGF2α was negatively related to changes in docosapentaenoic and docosahexaenoic acids (r=-0.15, p=0.003 and r=-0.13, p=0.007, respectively).
Paper IV

Sixty-one participants (91%) completed the HEPFAT study. Baseline characteristics are presented in Table 4. Weight changes during the intervention were 0.4 ± 1.4 kg (LA-diet) and 0.8 ± 1.6 kg (SFA-diet).

Table 4. Baseline characteristics for individuals completing the HEPFAT study

<table>
<thead>
<tr>
<th></th>
<th>LA-diet (n=32)</th>
<th>SFA-diet (n=29)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>57 (51-63)</td>
<td>56 (50-64)</td>
</tr>
<tr>
<td>Female, n(%)</td>
<td>21 (66%)</td>
<td>19 (66%)</td>
</tr>
<tr>
<td>Diabetes, n(%)</td>
<td>4 (13%)</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>30.3 ± 3.7</td>
<td>31.3 ± 3.9</td>
</tr>
<tr>
<td>WC, cm</td>
<td>104 (99-111)</td>
<td>107 (101-120)</td>
</tr>
<tr>
<td>fP-Glucose, mmol/l</td>
<td>5.3 (4.7-5.8)</td>
<td>5.4 (5.2-6.3)</td>
</tr>
<tr>
<td>fP-TAG, mmol/l</td>
<td>1.51 ± 0.70</td>
<td>1.44 ± 0.66</td>
</tr>
<tr>
<td>P-HDL-C, mmol/l</td>
<td>1.3 (1.1-1.5)</td>
<td>1.4 (1.2-1.6)</td>
</tr>
</tbody>
</table>

WC, waist circumference; TAG, triacylglycerol; HDL-C, high density lipoprotein cholesterol. 
*Diabetes was defined as previous diagnosis, fasting glucose ≥7.0 mmol/l, or glucose ≥11.0 mmol/l 2 hours after oral glucose tolerance test.

Figure 14. Dietary fat intake during the HEPFAT study

Energy intake was 8593 ± 2151 kJ (2054 ± 514 kcal) (LA-diet) and 8142 ± 2064 kJ (1946 ± 493 kcal) (SFA-diet) at baseline and increased by 577 ± 1890 kJ (140 ± 452 kcal) (LA-diet) and 942 ± 2129 kJ (225 ± 508 kcal) (SFA-diet) during the intervention but did not differ between the groups (p=0.59). Dietary fat composition during intervention is presented in Figure 14. The intake of linoleic acid corresponded to 14 ± 3% energy in the LA-group and 4 ± 1% energy in the SFA-group. Saturated fat intake was 10 ± 3% energy in the LA-group and 21 ± 5% energy in the SFA-group. The intervention had considerable effects on fatty acid composition in serum cholesterol esters. The proportion of linoleic acid increased from baseline by 11% in the LA-group. The mainly endogenously synthesized n-6, γ-linolenic (18:3 n-6), dihomo-γ-linolenic (20:3 n-6) and arachidonic (20:4 n-6) acids,
did not differ between the diets (p>0.36). Proportions of α-linolenic (18:3 n-3) and eicosapentaenoic acids (p≤0.003), but not docosahexaenoic acid (p=0.97), were higher after SFA-diet. SCD-1 index, palmitoleic and oleic acids decreased after LA-diet compared with SFA-diet (p≤0.0004).

Table 5. Concentrations of inflammatory and oxidative stress markers at baseline and their changes during the HEPFAT study

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LA-diet</td>
<td>SFA-diet</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>1.7 (1.3-3.5)</td>
<td>1.7 (0.9-3.4)</td>
</tr>
<tr>
<td>IL-1β, ng/l</td>
<td>0.12 (0.12-0.16)</td>
<td>0.14 (0.12-0.28)</td>
</tr>
<tr>
<td>IL-6, ng/l</td>
<td>1.78 (1.16-2.25)</td>
<td>1.60 (0.99-2.26)</td>
</tr>
<tr>
<td>IL-10, ng/l</td>
<td>0.77 (0.77-1.41)</td>
<td>0.88 (0.77-1.72)</td>
</tr>
<tr>
<td>IL-1Ra, ng/l</td>
<td>289 (208-497)</td>
<td>264 (210-350)</td>
</tr>
<tr>
<td>sTNF-R2, ng/l</td>
<td>2116 (2009-2501)</td>
<td>2107 (1898-2554)</td>
</tr>
<tr>
<td>15-keto-PGF2α†</td>
<td>0.15 (0.11-0.18)</td>
<td>0.15 (0.12-0.18)</td>
</tr>
<tr>
<td>8-Iso-PGF2α</td>
<td>0.56 ± 0.20</td>
<td>0.60 ± 0.18</td>
</tr>
</tbody>
</table>

Data are described as median (Q1-Q3). Change, concentration(follow-up)-concentration(baseline); 15-keto-PGF2α, 15-keto-13,14-dihydro-prostaglandin F2α (nmol/mmol creatinine); 8-iso-PGF2α, 8-iso-prostaglandin F2α (nmol/mmol creatinine).

*Significant difference between the diet groups, p<0.05.
†Assessed only in compliant participants (LA-diet: n=27, SFA-diet: n=19).

Plasma concentrations of IL-1Ra (p=0.02) and sTNF-R2 (p=0.005) were affected by the intervention whereas no changes were observed for the other inflammation markers (p>0.35) (Table 5). Similar results were assessed when excluding individuals with CRP levels >10 mg/l at baseline or follow-up. During LA-diet, IL-1Ra and sTNF-R2 decreased from baseline with 2% and 5%, respectively, whereas they increased during SFA-diet with 12% and 5%, respectively) (Figure 15). These results remained when adjusting for energy and total fat intake. Moreover, no changes were observed with regard to lipid peroxidation markers in urine (p>0.45) (Table 5). No effects on adipose tissue mRNA expression were observed for the investigated genes, i.e. genes regulating inflammation (MCP-1, TNF-α, IL-6, CD14, adiponectin) and fat metabolism (SCD, PPAR-γ, fatty acid synthase, carnitine palmitoyl transferase-1, acetyl-CoA carboxylase β, acyl-CoA dehydrogenase).
Figure 15. Box plot of changes in significantly affected inflammatory markers (i.e. plasma IL-1Ra and sTNF-R2) in the HEPFAT study. The boxes represent 25th, 50th and 75th percentiles. The whiskers correspond to the lower and upper adjacent values from 10th and 90th percentiles, respectively. Higher and lower values are presented as dots. Change, measure(follow-up)-measure(baseline). *C-reactive protein levels ≥10 mg/l are excluded.

Figure 16. Regressions of the association between changes in serum fatty acid composition and IL-1Ra and sTNF-R2 in the HEPFAT study. Change, concentration(follow-up)-concentration(baseline).
In this thesis, the impact of dietary fat quality on low-grade inflammation was investigated, both from an observational and an interventional perspective. In the observational studies, palmitoleic acid (16:1), oleic acid (18:1) and SCD-1 index in serum were positively associated with serum CRP levels, independently of obesity and lifestyle factors, whereas linoleic acid (18:2 n-6) was inversely related to CRP. When investigating the inflammatory effects of fat more directly in clinical trials but with a shorter perspective (10-12 weeks), the effects were small or absent. When comparing a high intake of SFA with a high MUFA-intake, no differences were observed. Neither did a low dose of very long-chain n-3 PUFA lower systemic inflammation. However, when compared with a SFA-rich diet, a high intake of linoleic acid reduced plasma levels of IL1-Ra and sTNF-R2, rather indicating certain anti-inflammatory effects in contrast to the pro-inflammatory properties previously described. Moreover, the current studies do not support increased oxidative stress or COX-mediated lipid peroxidation after increased intake of neither n-6 nor n-3 PUFA.

Fatty acids and inflammation in observational studies (Paper I and II)

The major finding of Paper I was that linoleic acid content in serum cholesterol esters at age 50 was inversely related to CRP levels at 70 years of age. Since linoleic acid in plasma reflects the dietary intake well, a diet high in vegetable fat may explain the link with lower CRP levels. It should be mentioned that there is a possibility that the subjects could have changed their diet during the 20 years whereafter CRP was measured. However, the fatty acid composition in serum cholesterol esters corresponded well between the ages of 50 and 70 supporting the result. Moreover, linoleic acid in serum cholesterol esters at age 70 was related to CRP in a subpopulation of ULSAM (n=320). The proportion of linoleic acid in total plasma, serum and erythrocyte membrane has been related to lower levels of circulating CRP in other studies, whereas some did not observe this association. However, when measuring total n-6 PUFA, an inverse association is observed with CRP, IL-1Ra, IL-6 and TNF-α. In Paper I
and II, we found no significant associations between n-3 fatty acids and inflammation. In contrast, other studies show negative relations between plasma n-3 PUFA and inflammatory markers\textsuperscript{105,106,108-111} whereas SFA is positively related\textsuperscript{105,106,109,112}. In studies investigating dietary intake by food frequency questionnaire, n-3 PUFA is inversely related to circulating inflammatory markers\textsuperscript{113-115}. Interestingly in one previous study no association was observed between dietary linoleic acid and inflammation, whereas individuals with the highest intake of both long-chain n-3 PUFA and linoleic acid had the lowest levels of sTNF receptors\textsuperscript{115}. In another study, TNF-\(\alpha\) was elevated in individuals with a high SFA and TFA intake, whereas sTNF-receptors were higher in those with a low PUFA intake, especially n-3 PUFA\textsuperscript{116}.

The findings of Paper II confirmed the association between serum fatty acid composition and CRP. Moreover, there was a correlation with E-selectin but not with the other inflammatory markers or adhesion molecules. These findings may indicate that the latter are weaker markers of systemic inflammation. CRP and E-selectin may have a greater sensitivity in detection and lower variability than the other markers. This could favour CRP and E-selectin as outcome variables with regard to epidemiological studies. When compared with IL-6, ICAM-1 and serum amyloid A, CRP was in fact a more robust marker of cardiovascular risk\textsuperscript{51}. Moreover, plasma concentrations of several cytokines are low and near the detection limits of enzyme immuno-assays. Their use as biomarkers may therefore be limited\textsuperscript{117}. Further, it cannot be excluded that there may be significant associations between fatty acid composition and various inflammatory markers in larger populations also including younger subjects.

The scattering in the plots of the regressions between linoleic acid and CRP, and between SCD-1 index and CRP were modest. Therefore the results should be interpreted with caution. However, results from Paper I and Paper II are in accordance showing similar associations. Also, large population-based cohorts include a diversity of individuals often resulting in broader distribution of variables.

Fatty acids, inflammation and oxidative stress in interventional studies (Paper III and IV)

In the LIPGENE study reported in Paper III, no difference in CRP levels or oxidative stress markers were induced by changing the dietary content of MUFA or SFA. Neither were there any effects observed with regard to other inflammatory markers (IL-6, TNF-\(\alpha\)) as reported elsewhere\textsuperscript{93}. However,
when investigating postprandial effects in the LIPGENE study, the diet high in MUFA seemed to improve endothelial function and reduced postprandial plasma ICAM-1 levels\textsuperscript{118} whereas fasting levels of ICAM-1 were not changed\textsuperscript{93}. High MUFA-diet also improves postprandial oxidative stress response\textsuperscript{119} whereas fasting levels do not appear to be affected by MUFA\textsuperscript{120-122}. However, adherence to a Mediterranean diet for a longer time period reduced inflammatory markers\textsuperscript{123}. But the intervention in that trial included changes in several nutrients such as fibres, PUFA and MUFA which complicates drawing conclusions on the role of dietary fat quality alone. After a fatty meal rich in SFA, the observed postprandial procoagulant state is more pronounced\textsuperscript{124}. TFA may increase inflammation\textsuperscript{125-128}, COX-mediated lipid peroxidation\textsuperscript{126,129} as well as free-radical induced lipid peroxidation\textsuperscript{126,129,130}.

Controlled trials investigating the effects of n-3 PUFA on CRP are inconclusive\textsuperscript{131}. No anti-inflammatory or anti-oxidative stress effects of long-chain n-3 PUFA were observed in the LIPGENE study. In contrast, n-3 supplementation in the KANWU study decreased 8-iso-PGF\textsubscript{2a} but did not affect 15-keto-dihydro-PGF\textsubscript{2a}\textsuperscript{132}. However, the supplementation dose was twice as high in the KANWU study as in the LIPGENE study (i.e. 2.4 g/day and 1.2 g/day, respectively). In other controlled trials, long-chain n-3 PUFA decreases inflammation\textsuperscript{133-135} and oxidative stress\textsuperscript{136-138} whereas others fail to show this effect\textsuperscript{139-145}. Potential anti-inflammatory properties of the essential n-3 fatty acid, \(\alpha\)-linolenic acid, have also been discussed\textsuperscript{146-150}, possibly via conversion to eicosapentaenoic acid\textsuperscript{146}. In Paper II there was an inverse relation between serum \(\alpha\)-linolenic acid and E-selectin levels, but no associations were observed with other inflammation markers. Inconsistencies between interventional studies may depend on differences in background diet\textsuperscript{151} and inflammation status among populations\textsuperscript{152}. Divergences may also depend on that various supplementation doses and/or relative contents of fat types in the diets used\textsuperscript{152,153}. Further, due to the variation of immune function parameters, many human studies are underpowered\textsuperscript{152}. Also, n-3 PUFA has a greater impact on inflammatory response among elderly individuals, which is why study population age may be one factor for divergences between studies\textsuperscript{152}. Moreover, genotype may affect the response to dietary fat intake\textsuperscript{19,154}, something we did not investigate in this thesis.

In Paper IV, we observed no indications of increased inflammation and lipid peroxidation after n-6 PUFA, despite a higher intake than recommended by the authorities (14% energy instead of the recommended maximum 10% energy\textsuperscript{8}). An important conclusion from this thesis is that the results do not support a pro-inflammatory effect of linoleic acid, in line with observations discussed by others\textsuperscript{141,155-157}. The beneficial effects by linoleic acid observed with regard to conditions such as cardiovascular disease\textsuperscript{66,79,80,158} may thus not be counterbalanced by an increased inflammation. Also in other studies
linoleic acid do not increase lipid peroxidation\textsuperscript{145,159}. In fact, plasma lipid peroxidation reduces after diets high in MUFA or n-6 PUFA compared with a relatively high SFA intake\textsuperscript{159}. In contrast to our results, one study has observed an increase in urinary 8-iso-PGF\textsubscript{2α} after a diet high in linoleic acid (12% energy). This was however only valid within the group and not when compared with the control group\textsuperscript{122}. In line with our results, increased production of pro-inflammatory prostaglandins after a high intake of linoleic acid is not observed\textsuperscript{160}. Supplementation with 8 g safflower oil (rich in linoleic acid) for 16 weeks decreased CRP concentrations but the levels at baseline were very high (55-80 mg/l), indicating that acute infections may have affected the study outcome\textsuperscript{161}. A high intake of linoleic acid reduces circulating levels of adhesion molecules\textsuperscript{146,162}. Supplementation with the longer-chain n-6 PUFA, γ-linolenic acid (18:3 n-6) and arachidonic acid (20:4 n-6), do not promote an inflammatory response\textsuperscript{163}. As previously reviewed, a moderately increased intake of arachidonic acid does not appear to have any major inflammatory effects\textsuperscript{164}. Moreover, the conversion of linoleic acid to arachidonic acid is tightly regulated\textsuperscript{160,165}. Only about 0.2% of dietary linoleic acid is converted to arachidonic acid\textsuperscript{165}. This is also supported by results from Paper IV where arachidonic acid did not increase in serum cholesterol esters in spite of a high linoleic acid intake.

In the HEPFAT study reported in Paper IV, plasma concentrations of IL-1Ra and sTNF-R2 were decreased during the n-6 PUFA-diet compared with the SFA-diet indicating certain anti-inflammatory properties of linoleic acid and/or pro-inflammatory effects of SFA. Other inflammatory markers were not affected by the diet in the HEPFAT study. One explanation may be that IL-1Ra and sTNF-R2 have a lower coefficient of variation, thus they are more sensitive to detect small changes. In accordance, serum IL-1Ra but not IL-1β is related to plasma n-3 and n-6 PUFA\textsuperscript{108}, indicating IL-1Ra to be a more sensitive inflammatory marker. One may find it strange that a reduction in IL-1Ra and sTNF-R2 is interpreted as beneficial since these markers have anti-inflammatory properties\textsuperscript{20,45,166}. Administration of IL-1Ra improves glycaemia in patients with type 2 diabetes\textsuperscript{45} and neutralisation of soluble TNF-α by TNF-receptors increases insulin sensitivity in diabetic animals\textsuperscript{27}. However, even if they do not act pro-inflammatory themselves, their plasma concentrations reflect the activity of the pro-inflammatory cytokines IL-1 and TNF-α\textsuperscript{28,166-168}. Notably, in observational studies, they predict development of type 2 diabetes\textsuperscript{40,42,169} and IL-1Ra is elevated in plasma several years before diabetes onset\textsuperscript{169}. Linoleic acid has also been shown to reduce plasma concentrations of IL-6, TNF-α and sTNF-receptors postprandially, whereas a meal high in SFA increased postprandial IL-6 levels\textsuperscript{168}.

Whereas dietary SFA, compared with MUFA, increased the expression of pro-inflammatory genes in adipose tissue\textsuperscript{170} we observed no such effects in
Paper IV. This study also observed decreased gene expression of PPAR-γ after SFA and down-regulating effects of MUFA on genes involved in lipogenesis such as SREBP-1. The correction for multiple testing in this study has however been questioned. SCD-1 gene expression in adipose tissue was not affected by a modified fat quality even though a change in the serum index was observed (Paper IV). This may be due to that the effects occurred in other tissues (such as the liver) rather than in adipose tissue. In line with this, serum cholesterol ester SCD-1 index has been suggested to reflect hepatic rather than adipose tissue desaturase activity. Another reason for the current negative findings on gene expression might be that the selected target genes did not fully capture a potential effect on adipose tissue inflammation during PUFA and SFA treatment.

The ratio between n-6 and n-3 PUFA

A balanced intake, and thus also membrane composition, of n-6 and n-3 PUFA has been suggested as an important factor in the regulation of the inflammation system. Plasma n-6/n-3 ratio has also been positively related to inflammation markers. However, it may be inappropriate to sum all n-6 fatty acids since individual fatty acids may have diverse metabolic effects. Also, when fatty acids are grouped together according to their saturation, measurement reliability is lower than for the individual fatty acids. In a non-controlled study, a reduction in the ratio between linoleic acid and α-linolenic acid from 32 to 2 led to a decline in plasma TNF-α levels. However, the change in the ratio was mainly due to an increased intake of α-linolenic acid (from 0.4 g/joule to 2.6 g/joule) rather than decreased linoleic acid intake (from 9.8 g/joule to 8.9 g/joule). Further, the n-6/n-3 ratio was raised to 14:1 in study IV without any signs of increased inflammation, lipid peroxidation or oxidative stress. Plasma TNF-α did not differ between groups supplemented with α-linolenic acid or linoleic acid for 12 weeks. Thus, the absolute amounts of n-3 and n-6 fatty acids may be more important than their ratio, as well as an adequate intake of long-chain n-3 fatty acids for the balanced regulation of inflammation.

Interventional studies versus observational studies

In evidence-based medicine, studies are evaluated according to their scientific value. In these grading systems, randomised controlled trials are considered the gold standard. Interventional studies can show causality whereas observational studies only establish associations. Moreover, the randomisation process limits the risk for confounders affecting the results. However, randomised controlled trials demand considerable resources and are not al-
ways the best study design within the nutritional field. Observational studies make it possible to study the impact of a factor on an outcome during a longer time period\textsuperscript{178}. In drug trials, impact of one active component affecting mainly one body function is generally investigated. In contrast, food contains many active components (both macronutrients and micronutrients) able to modify several body functions (e.g. body weight, peripheral insulin resistance, hepatic glucose production) which may partly explain diverse results in dietary interventions. A major drawback with observational studies within the nutritional field is that it is difficult to study specific nutrients without impact of other factors, especially since other lifestyle factors often are related. A specific fatty acid pattern may for example reflect a healthy lifestyle rather than the fatty acids per se. It is often difficult (if not impossible) to avoid residual confounding in observational dietary studies, resulting in limited interpretation of the observed associations that in turn will lead to weaker conclusions.

Dietary fat quality may only have a minor impact on inflammation, which is why a longer treatment period than a couple of weeks/months may be needed to be able to assess the small changes. These minor effects can be investigated in observational studies but with the risk of possible confounding factors and risk of reporting bias. In dietary interventions carried out during a long treatment period, good compliance may however be difficult to achieve throughout the study. Therefore, both interventional and observational studies (of high quality) should be included when investigating health impact of dietary components. Even though effects of dietary fat quality on risk markers may be moderate or small, the clinical impact may be of relevance in the long-term.

**SCD-1 and Inflammation**

The positive correlations between MUFA (i.e. palmitoleic and oleic acids) and CRP seen in the observational studies (Paper I and II) may first seem surprising since MUFA generally is associated with beneficial effects on metabolic risk factors\textsuperscript{179}. It should however be noted that palmitoleic acid only constitutes a small part of the fat in the Swedish diet\textsuperscript{5}. The palmitoleic acid content in serum cholesterol esters may rather reflect the endogenous conversion from palmitic acid by SCD-1. Indeed, both palmitoleic acid and SCD-1 index increase in response to SFA intake\textsuperscript{180}, results also supported by animal data suggesting that dietary SFA induce SCD-1 activity and hence increase endogenous fatty acid synthesis of MUFA\textsuperscript{181}. Moreover, in the Western societies, especially in elderly populations and decades ago, oleic acid is mainly provided by animal derived fat, and not from olive oil, which is the major source for MUFA in for example the Mediterranean countries.
Thus, MUFA in cholesterol esters reflects dietary saturated fat intake rather than dietary MUFA intake\textsuperscript{14}, suggesting that the present correlation between MUFA, SCD-1 index and CRP probably reflects high meat and dairy fat intake. SCD-1 index can also be influenced by a high carbohydrate intake (especially from sugars) via \textit{de novo} lipogenesis\textsuperscript{182-184}. This endogenous synthesis of fat is however believed to be small in Western populations due to the relatively high fat intake\textsuperscript{3,4,184,185}. Whereas SCD-1 index shows a strong positive relation to insulin resistance in Amerindians living under Western conditions, this association is absent in Amerindians with traditional hunter/gatherer lifestyle despite a higher SCD-1 index. Thus, if the diet is very low in fat and high in non-refined carbohydrate the plasma SCD-1 index might reflect enzyme activity in adipose tissue rather than in the liver, whereas activity in the latter probably is the case in Western diets with a higher fat content\textsuperscript{186}. SFA was not related to inflammation, perhaps because inflammation is promoted by a metabolic disordered state associated with elevated SCD-1 activity rather than the SFA intake per se.

In mice, SCD-1 deficiency attenuated adipocyte inflammation\textsuperscript{187}. In contrast, other studies show, in spite of beneficial effects on metabolic syndrome features, an elevated pro-inflammatory state after SCD-1 inhibition\textsuperscript{188,189} A decreased conversion of SFA into MUFA and thus augmentation of pro-inflammatory SFA has been suggested to cause this inflammatory effect\textsuperscript{188}. This effect was attenuated by supplementation of very long-chain n-3 PUFA\textsuperscript{190}.

Desaturase indices as estimates of their activities

In this thesis, desaturase activities were estimated by the ratio between the end product and the substrate, e.g. 16:1/16:0. Ideal would be to measure mRNA or protein expression of these enzymes. This is however difficult to achieve in larger epidemiological studies. Further, the most relevant organ to study is probably the liver. This requires liver biopsies, which is an invasive method associated with ethical concerns. Since indices were estimated by fasting serum cholesterol esters composition, they probably mainly reflect hepatic desaturation. The SCD-1 index is used as a marker of SCD-1 activity in several other studies\textsuperscript{16,182,191}. There are animal studies and \textit{in vitro} data supporting that the ratios reflect SCD-1 activity\textsuperscript{192-196}. In humans, both SCD-1 mRNA expression in adipose tissue and plasma SCD-1 ratio increased after rosiglitazone treatment\textsuperscript{197}. Moreover, the SCD ratios in adipose tissue reflect adipose tissue gene expression of SCD whereas no correlations were observed for $\Delta 5$- and $\Delta 6$-desaturases\textsuperscript{198}. SCD-1 ratio in plasma VLDL is also well correlated with hepatic SCD-1 mRNA expression\textsuperscript{199}. Associations between gene expression of $\Delta 5$- and $\Delta 6$-desaturases and these fatty acid ratios
Polymorphism in FADS1 and FADS2 (the genes encoding Δ5-desaturase and Δ6-desaturase, respectively) is associated with the ratios of 20:4 n-6/18:2 n-6 and 20:5 n-3/18:3 n-3 in red blood cell membranes. Strong association between arachidonic acid and also other PUFA in serum phospholipids and FADS polymorphism has also been reported.

Potential mechanisms

As discussed earlier, linoleic acid is sometimes claimed to promote inflammation via the conversion of linoleic acid to arachidonic acid and thereby an increased production of pro-inflammatory eicosanoids. However, in accordance with the studies presented here, potential pro-inflammatory effects of linoleic acid should be questioned. Further studies are needed to determine whether it is neutral or, in fact, may have anti-inflammatory properties. Linoleic acid derivates have been proposed to act anti-inflammatory by suppressing pro-inflammatory cytokine secretion from macrophages. Arachidonic acid may, in addition to being a substrate for pro-inflammatory eicosanoid production, also serve as a precursor for potent anti-inflammatory mediators. Incorporation of long-chain n-3 PUFA into the cell membrane reduces the production of eicosanoids derived from arachidonic acid in a dose-dependent manner. Eicosanoids derived from n-3 PUFA are often less biologically active compared with those derived from arachidonic acid. Long-chain n-3 PUFA also exert anti-inflammatory actions by influencing phagocytosis, inflammatory cytokine production and T cell reactivity. Whereas raised intake of long-chain n-3 PUFA increases incorporation of these fatty acids in cell membranes at the expense of arachidonic acid, high linoleic acid intake only increases arachidonic acid incorporation to a minor extent.

A possible role of SFA in inflammation has been demonstrated in vitro. When stimulating human cells with palmitic acid, the gene expression and protein production of IL-6 increased. In contrast, linoleic acid prevented this palmitic acid-induced up-regulation of IL-6 and thus seemed to act anti-inflammatory. In another in vitro study, SFA did not inhibit cytokine-induced endothelial activation whereas an increased inhibitory effect was observed for fatty acids with increasing number of double bonds (MUFA < n-6 PUFA < n-3 PUFA). The exact mechanisms behind pro- or anti-inflammatory effects of fatty acids are unknown but signalling via TLR, mainly TLR2 and TLR4, is proposed as central. SFA-stimulated TLR activation induces NF-κB activation. As a result inflammatory genes such as cytokines and COX are expressed. In contrast, unsaturated fatty acids can suppress this activation induced by TLR agonists. However, some in vitro studies have observed pro-inflammatory effects of li-
noleic acid, via activation of NF-kB\textsuperscript{212}. The ability of some SFA (palmitic and stearic acids) to induce inflammation \textit{in vitro} should however not be generalised to all SFA such as the short-chain fatty acids myristic and lauric acid\textsuperscript{207,213}. SFA is also proposed to induce inflammatory gene expression through endoplasmatic reticulum stress, generation of reactive oxygen species and ceramides (potent pro-inflammatory factors), as well as protein kinase C signalling\textsuperscript{214}. SFA also reduces the anti-inflammatory properties of HDL particles\textsuperscript{215}.

**Assessment of inflammatory markers and oxidative stress**

In this thesis, inflammatory status was measured by circulating levels of inflammatory markers. Thus, it cannot be excluded that dietary effects on inflammation could have been observed if assessed directly in target tissues such as endothelium or adipose tissue. However, no changes in mRNA expression of inflammatory genes were observed in adipose tissue in study IV. On the other hand, samples were collected in subcutaneous abdominal adipose tissue, leaving dietary consequences in the visceral compartment or other tissues unknown. Also, gene expression from isolated adipocytes and adipose tissue may differ\textsuperscript{216}. Therefore, one may speculate that the washing of adipose tissue biopsies could have affected the amount of non-adipocytes in the samples and therefore also the amount of inflammatory gene expression. Even though the inflammatory expression is lower in samples assessed by needle biopsies with less stroma cells compared with surgical biopsies\textsuperscript{217}, inflammation has been observed in samples from needle biopsies\textsuperscript{170}.

Another alternative when investigating inflammatory status is to extract cells and investigate their response to stimuli. For example, excretion of cytokines from lipopolysaccharide-stimulated peripheral blood mononuclear cells has been assessed\textsuperscript{134,155}. Also, postprandial effects may be studied as a complement to systemic inflammation\textsuperscript{118,119,124}.

CRP occurs in circulation as pentamers in its natural form but may disseminate into monomeric subunits. Monomeric CRP is proposed to have more potent inflammatory properties than its pentameric form\textsuperscript{218}, and it may be relevant to measure these isoforms separately. However, because the dissemination probably occurs localized, monomeric CRP can not be detected in peripheral circulation\textsuperscript{218}. Moreover, CRP was only measured once at each investigation. Several measurements during a time period would have given a more robust estimate of the inflammatory status. This could have been difficult to achieve in the two interventional studies since they were per-
formed during a relatively short period. However, in the observational studies this would have given more valid results. Even though we excluded those with CRP levels >10 mg/l, other conditions than low-grade inflammation (such as genetic, demographic and behavioural factors) could have contributed to the concentrations\textsuperscript{219}.

Urinary 8-iso-PGF\textsubscript{2α} was used as a marker of oxidative stress. When assessed in urine, the concentration reflects whole-body free radical lipid peroxidation rather than specific peroxidation in different tissues\textsuperscript{25}. This is also valid for urinary levels of 15-keto-dihydro-PGF\textsubscript{2α}. Even though analyses of both 8-iso-PGF\textsubscript{2α} and 15-keto-dihydro-PGF\textsubscript{2α} were performed in duplicate, several measurements over time would have been optimal. This was however difficult due to the short study designs. It has also been discussed that the metabolic rate of isoprostanes may be affected whereas overall lipid peroxidation rate is unchanged. To detect such effects, the metabolites (i.e. 2,3-dinor-8-iso-PGF\textsubscript{2α} and 2,3-dinor-5,6-dihydro-8-iso-PGF\textsubscript{2α}) have to be measured as well\textsuperscript{220}. It cannot be excluded that other oxidative stress markers (such as oxidative damage to DNA) could have shown an effect. However, due to the suggested increased lipid peroxidation upon PUFA consumption, 8-iso-PGF\textsubscript{2α} was found to be the most relevant oxidative stress marker.

Possible clinical implications of increased linoleic acid intake

About one third of the American population has CRP concentrations above 3 mg/l\textsuperscript{219}. When compared with CRP <1 mg/l, individuals with CRP levels >3 mg/l have an almost two-fold increased risk for all-cause mortality (hazard ratio: 1.9)\textsuperscript{221}. One standard deviation higher log\textsubscript{e} CRP concentration is associated with 37% increased risk of coronary heart disease and 55% higher risk of vascular mortality after adjustments for conventional risk factors\textsuperscript{49}. Change in log\textsubscript{e} CRP concentrations by one standard deviation is also associated with a 30% increased risk of developing type 2 diabetes\textsuperscript{41}.

In Paper I and II, one can only speculate about possible clinical impact. The participants in the highest quartile of serum linoleic acid had 0.89 mg/l and 1.06 mg/l lower CRP concentrations compared with those in the lowest quartiles, respectively. This corresponds to a decrease of 0.5 standard deviation of log\textsubscript{e} CRP. Individuals in the highest quartiles of serum SCD-1 index had 0.83 mg/l and 0.89 mg/l higher CRP levels than those in the lowest quartiles, corresponding to 0.4 and 0.5 standard deviations of log\textsubscript{e} CRP. This could be compared with effects achieved by weight loss and statins in intervention studies. For each kg of weight loss, CRP is estimated to decline by 0.13
mg/l\textsuperscript{222}. In the JUPITER trial, rosuvastatin treatment for 12 months reduced CRP levels by 2.0 mg/l (from 4.2 mg/l at baseline). At the end of the trial (also LDL-cholesterol was lowered), major cardiovascular events and all-cause mortality were reduced\textsuperscript{52}.

In Paper IV, a high intake of n-6 PUFA decreased sTNF-R2 (-108 ng/l) and IL-1Ra (-7 ng/l) and a high SFA diet increased sTNF-R2 (94 ng/l) and IL-1Ra (23 ng/l). It would have been interesting to know if a longer duration of these interventions would have caused larger effects or if the treatment period was long enough to result in a steady-state. Notably, an annual increase in IL-1Ra by 11 ng/l have been observed 6 years before development of type 2 diabetes, after adjustment for obesity\textsuperscript{169}. Higher plasma levels of IL-1Ra\textsuperscript{42} and sTNF-R2\textsuperscript{40} are associated with approximately 60-80\% increased risk for diabetes.

**Strengths and limitations**

One strength of this thesis is the inclusion of both observational studies and randomised controlled dietary interventions, thus, providing scientific data from different angels and the study of different Swedish populations. Also, the thesis was able to investigate a hypothesis generated in the first two observational studies, i.e. a possible anti-inflammatory effect of linoleic acid, in a randomised controlled trial. This is highly motivated since there still are only few clinical trials conducted in humans investigating the impact of n-6 PUFA on inflammatory status.

Since the studies reported in Paper I and II are of observational nature, no conclusions regarding causality can be drawn. In Paper I, CRP was not measured at the age of 50. Instead adjustments were made for erythrocyte sedimentation rate to address the possibility that low-grade inflammation at baseline explained the longitudinal correlations. Interestingly the relationships remained, supporting the possibility that an altered fatty acid composition may precede inflammation. Due to the time difference of 20 years between the measurements, there is a possibility that the subjects could have changed their diet when CRP levels were assessed. No food records were assessed at baseline to support fatty acid composition data. Serum fatty acid composition is however, compared with dietary registration, a more objective method that limits reporting bias\textsuperscript{1,11,12,78}. But, it is only a marker of dietary fat quality and not a quantitative measurement; thus, if one fatty acid increases, the proportions of the other decrease. In particular, those fatty acids occurring in low proportions in serum are uncertain markers of their dietary intake\textsuperscript{103}. The lack of food records did not allow us to adjust for other dietary components that might influence these associations, e.g. carbohy-
urate intake (especially sugar intake). This may be relevant when investigating associations with certain SFA and MUFA, e.g. palmitic and palmitoleic acids.\textsuperscript{223} Another strength is that both the ULSAM- and PIVUS-cohorts were population-based, although it should be noted that the correlations in Paper I were performed in a healthy sub-sample free from diseases that may contribute to inflammation (i.e. cardiovascular disease, diabetes and cancer). Thus, the associations between fatty acid composition at baseline (age 50) and CRP at age 70 was probably not biased by already existing disease-caused inflammation at baseline. Fatty acid composition was measured in serum cholesterol esters and not total plasma or serum as in several other studies.\textsuperscript{105,106,108,110} The results are therefore less sensitive to confounding by blood lipid changes such as hypertriglyceridemia.\textsuperscript{11,224} Strengths of Paper I include the longitudinal design, the large sample with complete fatty acid composition data and adjustments of several relevant covariates including directly measured insulin resistance at follow-up. In Paper II, the assessment of a large number of inflammatory and endothelial function molecules is a strength. Other studies have only measurements of a few markers of inflammation without any markers of endothelial function. Unfortunately, only men were included in Paper I, although women were included in Paper II which increases the generalisability of that study. However, the applicability of observed results to other ages is unknown but links between fatty acids and inflammation have also been indicated in younger subjects.\textsuperscript{105,106,109} Although the study population in Paper II may seem small it appears to be representative for the whole cohort concerning several parameters. Our results in Paper I are also in accordance with previous findings from the ULSAM-cohort where a similar fatty acid pattern was related to the metabolic syndrome,\textsuperscript{191} insulin resistance,\textsuperscript{87} and cardiovascular disease.\textsuperscript{96,225} The multicentre design of the LIPGENE study increased the generalisability by including several countries and thereby also diverse genetic set-ups, dietary habits, living conditions and environmental factors. This may however also have contributed to the lack of dietary effects in that study. The HEPFAT study was smaller and only conducted at one centre. Even though the inclusion criteria of the HEPFAT study were limited to individuals with abdominal obesity, the study included participants with and without non-insulin dependent type 2 diabetes, treatment with antihypertensive agents or lipid-lowering drugs; thus having a broad distribution in the general population. Inclusion criteria in the interventional studies reported in Paper III and IV were the metabolic syndrome and abdominal obesity, respectively. Therefore the participants were likely to have an ongoing low-grade inflammation. The participants in the LIPGENE study had average CRP levels above 3 mg/l whereas the participants in the HEPFAT study only had CRP concen-
trations of 1.7 mg/l at baseline. Due to low CRP concentrations, an interventional effect may be more difficult to detect. Inflammation markers such as CRP could have been measured at screening to ensure a basal inflammatory state, but the primary endpoints in the LIPGENE and the HEPFAT studies were, rather than inflammation, insulin sensitivity and liver fat content, respectively. Apart from the use of a randomised controlled study design (similar to that of Paper III), strengths in the HEPFAT study included low drop-out rate, assessment of serum cholesterol ester fatty acid composition as a compliance marker and that body weight and n-3 PUFA intake were kept unchanged during the intervention period.

Future perspective

In this thesis, a relationship between serum fatty acids and systemic inflammation was reported. However, in contrast to the findings in the observational studies, the impact of dietary fat quality in the interventional studies was none or only minor. To further shed light in this area it would be interesting to perform a clinical trial with the primary aim to investigate effects of different types of fat on inflammation and lipid peroxidation. In such a trial individuals with a low-grade inflammation could be selected in advance by only including subjects with CRP concentrations above 3 mg/l. Also, study of postprandial inflammatory effects would be relevant and interesting to examine.

It is suggested that different types of SFA (i.e. short-, medium- and long-chain SFA) possess diverse biological functions. Research exploring the impact of altered dietary intake of different SFA would therefore be of great interest.

To investigate if the genotype affects the response to different diets could also contribute to more knowledge within this area. Important genes could for example be SCD-1 or TNF-α. The dietary impact on gene expression would also be of interest to study further. Another approach than used in Paper IV could be microarray techniques. Such approach could provide information about global gene expression changes and thus increasing the chance of identifying significant genes as well as more novel genes.
Conclusions

- High proportions of MUFA in serum cholesterol esters and a high SCD-1 index are related to increased CRP concentrations, independently of obesity, lifestyle factors and insulin resistance. In these Swedish populations, these associations may partly be a reflection of a long-term high SFA intake. It may also be a reflection of a link between lipogenic activity and inflammation.

- A low proportion of linoleic acid (18:2 n-6, the major dietary n-6 PUFA) in serum cholesterol esters, indicating a low vegetable fat intake, is associated with higher CRP levels.

- Supplementation with a low dose of n-3 PUFA for 12 weeks did not affect systemic inflammation and lipid peroxidation in individuals with the metabolic syndrome.

- Neither the macronutrient proportions (i.e. fat versus carbohydrates) nor a diet high in MUFA from e.g. olive oil or a diet high in SFA did influence markers of inflammation or oxidative stress.

- A high intake of dietary n-6 PUFA (linoleic acid) during 10 weeks does not increase systemic inflammation and lipid peroxidation. Instead it may reduce plasma levels of some inflammatory markers compared with a diet rich in SFA.

- Taken together, this thesis suggests that systemic low grade-inflammation is to some extent associated with certain dietary fatty acids. In addition, indices of endogenous fatty acid desaturation (i.e. increased SCD-1 activity index) were also shown to be associated with higher systemic inflammation.
Svensk sammanfattning

Typerna av fett i kosten påverkar risken för att drabbas av typ 2-diabetes och hjärt-kärlsjukdom. Vid fetma ses ofta en låggradig inflammation som tros spela en roll i utvecklingen av dessa sjukdomar. Inflammatoriska molekyler har bland annat visats kunna hämma insulinsignalering och därmed bidra till insulinresistens. Dessutom påverkar inflammationsaktiviteten stabiliteten hos åderförkalkningsplack.

Fettsyror utgör byggstenarna i fetter. Den huvudsakliga fettkällan i kosten är triglycerider som består av tre fettsyror bundet till glycerol. Fettsyror kan vara mättade, enkelomättade eller fleromättade beroende på om de innehåller dubbelbindningar, samt antalet sådana. Ju fler dubbelbindningar en fettsyra har desto mer oregelbunden är dess form och desto mjukare blir fettet. De fleromättade fetterna delas in i n-3 och n-6 beroende på var deras dubbelbindningar är placerade i fettsyrakedjan, d.v.s. om den första dubbelbindningen sitter vid 3:e eller 6:e kolatomen räknat från fettsyrans methylende. Linolsyra (18:2 n-6) och α-linolensyra (18:3 n-3) är s.k. essentiella fettsyror och måste tillföras via maten för att upprätthålla en normal tillväxt och funktion hos vävnader. Övriga fettsyror kan även bildas i kroppen genom enzymer som kallas desaturaser (inför dubbelbindningar i kolskelettet) och elongaser (tillför nya kolatomer till kolskelettet). Desaturasernas aktivitet kan uppskattas genom beräkning av fettsyrakvoter, d.v.s. kvoten mellan metabolit och substrat. Generellt kan man säga att mättade fettsyror framför allt förekommer i animaliska fetter medan omättade fettsyror finns i stor mängd i vegetabiliskt fett. De långkedjiga n-3 fettsyrorna finns framför allt i fet fisk.

De långkedjiga n-3 fettsyrorna har beträffats som antiinflammatoriska medan n-6 fettsyror har tillskrivits proinflammatoriska egenskaper. Som grund för detta ligger framför allt att längre n-6 fettsyror agerar som substrat för inflammatoriska molekyler. Huruvida n-6 fettsyror från växtfetter har några proinflammatoriska effekter hos människa är dock ännu oklart och behöver testas ytterligare i kliniska kontrollerade studier. Mättade fettsyror har visats kunna öka inflammationssignaler i cellstudier. I denna avhandling studerades vilka effekter olika typer av fettsyror har på en låggradig inflammation associerad med fetma. Två observationsstudier (studie I och II) och två interventionsstudier (studie III och IV) ingår i avhandlingen.

Studie III och IV baserades på två randomiserade kontrollerade studier; LIPGENE- och HEPFAT-studien. I studie III (LIPGENE-studien) jämfördes två högfettskostor, rika på antingen mättat eller enkelomättat fett, och två lågfettskostor med eller utan berikning av långkedjiga n-3 fettsyror. Efter 12 veckor sågs inga effekter på inflammation eller oxidativ stress vid någon av kosterna. I studie IV (HEPFAT-studien) undersöktes effekter av en kost rik på antingen linolsyra (n-6) eller mättat fett. Efter 10 veckors behandling med högt intag av linolsyra sågs inga tecken på ökad inflammation eller oxidativ stress. Istället sågs en sänkning av två inflammationsmarkörer (interleukin-1 receptor antagonist [IL-1Ra] och tumor necrosis faktor receptor-2 [TNF-R2]) jämfört med den mättade fettkosten, vilket indikerar en viss antiinflammatorisk effekt av linolsyra och/eller en proinflammatorisk verkan av mättat fett.

Den gamla uppfattningen att n-6 fettsyror i kosten ökar inflammationen i kroppen medan n-3 fettsyror minskar den, tycks i enlighet med nyare studier vara en alltför förenklad och potentiellt felaktig uppfattning. I denna avhandling, som inkluderar både epidemiologiska och kliniska kontrollerade studier, sågs inga antiinflammatoriska effekter av n-3 fettsyror. Detta skulle kunna bero på att dosen n-3 var relativt låg. Däremot fanns tecken på att n-6 fettsyror i kosten skulle kunna sänka inflammationsstatus, något som är av betydelse eftersom linolsyra utgör majoriteten av det fleromättade fett vi äter. Det är rimligt att anta att en kost som sänker kroppens inflammationsaktivitet på lång sikt minskar risken för att insjukna i sjukdomar där den låggradiga inflammationen är förhöjd, t.ex. diabetes och hjärt-kärlsjukdom.
I would like to express my sincere gratitude to all that have contributed to this thesis, and to all that have supported and encouraged me during these years. I wish to express especial thanks to:

My supervisor Ulf Risérus for sharing your knowledge and your enormous interest in nutritional research. Your never ending enthusiasm for science is very encouraging and your exceptional memory for references is impressing.

Co-supervisor and head of the section for Clinical Nutrition and Metabolism, Tommy Cederholm for providing valuable comments, support and for creating a friendly, stimulating working atmosphere at the unit.

Bengt Vessby, professor emeritus, for initiating fat research and its impact on diseases at the department and for continuing to share your enormous knowledge.

Lars Lannfelt, professor of Geriatrics and heat of the ICTUS group, for continuing the ULSAM cohort and for valuable support.

Samar Basu, co-author and head of the section for Oxidative Stress and Inflammation, for sharing your massive knowledge about isoprostanes and prostaglandins.

Lars Lind, co-author and professor at the Department of Medical Sciences, for initiating the PIVUS cohort and for giving valuable advices.

Co-authors for your invaluable contributions and comments improving the articles.

All former and present colleagues and friends at the department for fruitful discussions, both of scientific and non-scientific (but yet as important) nature. You have made working a joy.

All personnel involved in data collection in the ULSAM, PIVUS, LIPGENE and HEPFAT studies for their massive contribution.
The participants in the ULSAM, PIVUS, LIPGENE and HEPFAT studies. Without your effort and interest none of this would have been possible.

All friends for good times and joy over the years. Special thanks to my bio-medicine friends for going through the same experiences and following ups and downs but mostly for all the fun we have together.

My mom and dad for their endless love, total support and being a safety in my life. My sister and brother with families for being there and giving so much joy. All my relatives and pseudo-relatives for the knowledge of always having people supporting me.

Daniel, by sharing the same education, you always give me good advices and thoughts when needed. But most of all you support me, love me and brighten my life.
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