Milk Fat Intake and Conjugated Linoleic Acid (CLA) Supplementation

Dietary Markers and Associations to Clinical and Biochemical Characteristics

ANNIKA SMEDMAN
Dissertation presented at Uppsala University to be publicly examined in Auditorium Minus, Gustavianum, Uppsala, Thursday, April 14, 2005 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in Swedish.

Abstract

In the present thesis dietary markers for intake of milk fat, associations between intake of milk fat and risk factors for coronary heart disease (CHD), and the effects of supplementation with conjugated linoleic acid (CLA) to healthy humans are investigated.

The dietary fat quality is one of the main lifestyle factors affecting risk for CHD. When studying the associations between diet and health it is important to have accurate dietary information. Objective dietary markers increase the possibilities to interpret dietary surveys.

In a study of 62 men we demonstrated that the milk fatty acid pentadecanoic acid (15:0) measured in serum lipids can be used as marker for intake of fat from milk products. In the same study we observed inverse correlations between intake of milk fat and certain risk factors for CHD, especially anthropometric variables.

To further investigate these findings we supplemented humans with CLA, naturally present in milk. CLA has in animals and in vitro been ascribed positive effects on adiposity and glucose and lipid metabolism. When supplementing humans with CLA we observed a slight decrease in body fat, but no effects on other anthropometric variables or serum lipids. However, markers of lipid peroxidation and inflammation increased. From a second supplementation study we concluded that CLA trans 10, cis 12 induced lipid peroxidation more than did a mixture of isomers.

We conclude that the inverse associations between milk fat intake and CHD risk factors, and the effects of CLA, are interesting and need further investigation. However, according to current knowledge, the general population is still advised to have a limited intake of total and saturated fat and to instead choose unsaturated fats. In addition, there is to date no medical reasons for humans to take CLA as supplements.

Keywords: Pentadecanoic acid (15:0), milk products, milk fat, dietary marker, coronary heart disease, anthropometric variables, conjugated linoleic acid (CLA), lipid peroxidation, inflammation, F2 isoprostane, prostaglandin F2, human

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ISSN 1651-6206
ISBN 91-554-6170-0
urn:nbn:se:uu:diva-4820 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-4820)
Till Sten
List of Papers

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

I Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors
A Smedman, I-B Gustafsson, L Berglund and B Vessby
American Journal of Clinical Nutrition 1999;69(1):22-29 1

II Conjugated linoleic acid supplementation in humans—metabolic effects
A Smedman and B Vessby
Lipids 2001;36(8):773-781 2

III Conjugated linoleic acid induces lipid peroxidation in humans
S Basu, A Smedman and B Vessby

IV Conjugated linoleic acid increased C-reactive protein (CRP) in humans
A Smedman, S Basu and B Vessby
British Journal of Nutrition. In press 4

V Isomer-specific effects of conjugated linoleic acid on lipid peroxidation in humans: regulation by alpha-tocopherol and cyclo-oxygenase-2 inhibitor
A Smedman, B Vessby and S Basu
Clinical Science 2004;106(1):67-73 5

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## Abbreviations

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<th>Abbreviation</th>
<th>Full Form</th>
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<tr>
<td>15:0</td>
<td>pentadecanoic acid</td>
</tr>
<tr>
<td>17:0</td>
<td>heptadecanoic acid</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CLA</td>
<td>conjugated linoleic acid</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
</tr>
<tr>
<td>DBP</td>
<td>diastolic blood pressure</td>
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<tr>
<td>HDL</td>
<td>high density lipoprotein</td>
</tr>
<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
</tr>
<tr>
<td>M/I</td>
<td>insulin sensitivity index</td>
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<tr>
<td>MUFA</td>
<td>monounsaturated fatty acid(s)</td>
</tr>
<tr>
<td>NEFA</td>
<td>non-esterified fatty acid(s)</td>
</tr>
<tr>
<td>P</td>
<td>probability</td>
</tr>
<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor-1</td>
</tr>
<tr>
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<td>prostaglandin F&lt;sub&gt;2α&lt;/sub&gt;</td>
</tr>
<tr>
<td>PPAR</td>
<td>peroxisome proliferator-activated receptor</td>
</tr>
<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid(s)</td>
</tr>
<tr>
<td>RIA</td>
<td>radio-immuno assay</td>
</tr>
<tr>
<td>SAD</td>
<td>sagittal abdominal diameter</td>
</tr>
<tr>
<td>SBP</td>
<td>systolic blood pressure</td>
</tr>
<tr>
<td>SFA</td>
<td>saturated fatty acid(s)</td>
</tr>
<tr>
<td>TAG</td>
<td>triacylglycerols</td>
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<tr>
<td>TNF-α</td>
<td>tumour necrosis factor-α</td>
</tr>
<tr>
<td>TNFR1 and 2</td>
<td>tumour necrosis factor receptor 1 and 2</td>
</tr>
<tr>
<td>ULSAM</td>
<td>Uppsala Longitudinal Study of Adult Men</td>
</tr>
<tr>
<td>VLDL</td>
<td>very low density lipoprotein</td>
</tr>
<tr>
<td>WHR</td>
<td>waist hip-ratio</td>
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</table>
Introduction

The prevalence of coronary heart disease (CHD) and diabetes type 2 is increasing worldwide. Increasing are also the metabolic diseases abdominal obesity, insulin resistance, dyslipidaemia and hypertension, which are part of the so called metabolic syndrome. Contributing to the development of CHD are both genetic and lifestyle factors. Lifestyle factors important with regard to CHD are physical activity, smoking and dietary habits.

Dietary fat quality and coronary heart disease

The quality of the dietary fat is of importance for the development of CHD (1-3). Intake of saturated fat in different populations is directly related to serum cholesterol concentrations and mortality in CHD (4, 5). It has, however, been difficult to confirm these associations within populations and in prospective studies (6, 7). Two main reasons have been proposed to explain why it is difficult to confirm these associations within groups. Firstly, the hypothesis could be false. Secondly, it could be due to the nature of the dietary data, either in that the reported intake of a food item or nutrient studied could have a day-to-day variation within persons that is greater than the variation between persons. It could also partly be due to weaknesses of dietary assessment methods.

Dietary fat quality and insulin resistance

Insulin resistance is a central part of the metabolic syndrome. Individuals with the metabolic syndrome are of a higher risk for developing type 2 diabetes, cardiovascular disease and death from all causes. Evidence based on human studies show that dietary fat quality influences several of the metabolic abnormalities clustering in the metabolic syndrome (8). Epidemiological studies have related insulin resistance to higher intake of saturated fat and lower intake of unsaturated fat (9, 10). The weaker relations between polyunsaturated fatty acids (PUFA) in the diet and insulin sensitivity in epidemiological studies are possibly due to difficulties in assessing the intake of PUFA. However, fatty acid compositions of serum or muscle used as dietary markers have repeatedly shown positive relations between PUFA and improved insulin sensitivity (11, 12).
Few intervention studies of adequate size and length have been performed in order to study the relationship between dietary fatty acid quality and insulin sensitivity (8). However, the KANWU study, included 162 subjects who participated for three months. The results indicate that the insulin sensitivity is reduced by the high saturated fat diet and unaffected by a diet rich in monounsaturated fatty acid (MUFA) (13). Similar results have been reported by Summers et al. where a PUFA rich diet increased insulin sensitivity compared to a diet rich in saturated fatty acids (SFA), and by Pérez-Jiménez where substitution of carbohydrates and MUFA for SFA improved insulin sensitivity (14, 15).

Dietary fat quality and serum lipoproteins
Epidemiological studies have shown that increased levels of total and low density lipoprotein (LDL) cholesterol levels and decreased HDL cholesterol levels strongly predict cardiovascular morbidity and mortality (16-18).

Findings from randomised clinical trials have rendered a general opinion regarding the effects of dietary fatty acids on serum lipids. It is experienced that decreased amounts of saturated fatty acids result in lower LDL cholesterol levels. In addition, replacement of saturated fat with mono- or polyunsaturated fat lowers HDL cholesterol slightly, but does not affect triacylglycerides. Trans fatty acids from hydrogenated vegetable oils increase LDL and may decrease HDL cholesterol (16). However, different saturated fatty acids have different effects on lipoprotein levels. Myristic acid (14:0) and palmitic acid (16:0) increase LDL cholesterol more than does lauric acid (12:0). Stearic acid (18:0) is almost neutral in this aspect (19).

Dietary fat quality and risk for coronary heart disease
It is clear that, within certain limits, the quality of the dietary fat is of greater importance for cardiovascular disease protection than is the absolute amount ingested (2, 8). The evidence from both intervention and epidemiological studies agree, and they provide compelling grounds for dietary treatment (2, 16). With regard to the dietary fat, the advice to prevent and treat the metabolic syndrome is to consume a limited amount of saturated fat, and instead choose foods containing unsaturated fats, according to the best studies available today.

Milk products and risk for coronary heart disease
Fat from milk and milk products is one of the main dietary sources of saturated fats. The SFAs lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0), that are present in milk fat, have repeatedly been found to be hypercholesterolaemic in controlled interventions studies (20). Thus, milk products have been concluded to contribute to an increased risk for CHD (21),
which suggests that intake of from milk products should be restricted. Contrarily, intake of milk products and fat from milk products has also been found to be inversely associated to risk of CHD (22-30). Different factors in milk or milk fat have been proposed as possible candidates to explain the inverse relations observed between intake of milk fat and coronary heart disease risk (31). Components of milk products that have been mentioned are calcium, bioactive peptides, folic acid and vitamins B6 and B12 (31). Conjugated linoleic acid (CLA) is another component of milk fat that has been suggested to be a factor that could possibly contribute to the inverse associations between milk fat intake and risk for CHD.

Dietary markers

In studies of diet and health the quality of the information about dietary habits is of great importance. However, accurate assessments of dietary intake in free living subjects are difficult to obtain. Forgetfulness and reluctance to report the true intake may result in misreporting of the intake of, for example, energy and dietary fat, and relative overestimations of nutrients such as dietary fibre, vitamins, and minerals (32-34). To enhance the reliability of dietary assessments a combination of dietary surveys and biomarkers of ingested nutrients, groups of food items or energy, have been suggested.

A dietary marker should be specific for a nutrient or group of food items, and the concentration of the marker should be possible to analyse in for example body tissues, urine or blood, and should correlate to the dietary intake (35). Dietary markers that have been validated and applied in nutritional studies are for example urinary nitrogen excretion for estimation of protein intake, urinary 3-methylhistidine excretion for intake of meat, urinary concentration of electrolytes as sodium and potassium, concentration of for example ascorbic acid in the blood for intake of vitamin C, and fatty acid composition of serum lipids, erythrocytes and adipose tissue for the fat quality of the diet (35).

The fatty acid composition of the serum lipids, thrombocytes and the adipose tissue are known to partially reflect the relative fatty acid composition of the diet. The strongest correlations between intake and proportions in body compartments have been found for, for example, the essential fatty acids linoleic acid (18:2 n-6) and α-linolenic acid (18:3 n-3) and the long-chain PUFA eicosapentaenoic acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3) (36-43). The endogenously synthesised unsaturated and saturated fatty acids (SFA) are weaker.

Markers of dairy fat intake

In the rumen of ruminants the bacterial flora can synthesise fatty acids relatively specific for ruminants, pentadecanoic (15:0) and heptadecanoic acid
(17:0) (44). Since these fatty acids consist of an uneven number of carbon atoms, they cannot be synthesized in the human body. In a study Wolk et al. observed that the proportions of 15:0 and 17:0 in adipose tissue reflected milk fat consumption in women (45), and could thus possibly be used as markers for intake of milk fat. There are 15:0 and 17:0 also in fish. However, compared to the amounts from milk fat, that is of minor quantity.

Conjugated linoleic acid, CLA

The last ten years CLA has received increasing attention due to the potential metabolic and chemoprotective properties demonstrated in animal and \textit{in vitro} models. Currently, CLA is marketed as a dietary supplement that is claimed to have anti-obesity effects.

![Figure 1. Structures of CLA trans 10, cis 12, CLA cis 9, trans 11 and linoleic acid.](image)

The term CLA refers to a number of positional and geometric isomers of octadecadienoic acid (18:2) with two conjugated double bonds (46, 47). In the linoleic acid molecule the double bonds are separated by two single bonds, while the double bonds in the CLA molecule are separated by one single bond, i.e. the double bonds in CLA are conjugated (Figure 1). A double bond in CLA can be either in a \textit{cis} or a \textit{trans} configuration, and the two double bonds occur in all combinations of the two configurations: \textit{cis-cis}, \textit{cis-trans}, \textit{trans-cis}, or \textit{trans-trans}. The double bonds can be situated anywhere between the 7th and 14th carbon atom in the CLA molecule (48). The
two naturally most abundant isomers of CLA are \textit{cis} 9, \textit{trans} 11 and \textit{trans} 10, \textit{cis} 12.

CLA \textit{cis} 9, \textit{trans} 11 is synthesised from linoleic acid by bacteria (\textit{Butyrovibrio fibrisolvens}) in the rumen of ruminants (49). Smaller amounts of the isomer \textit{cis} 9, \textit{trans} 11 can also be synthesised from vaccenic acid (18:1 \textit{trans} 11) by the action of delta-9 desaturase in cows and in humans (50, 51).

The major source of CLA in the human diet is fat from ruminants, thus mainly fat from milk products, but also fat from beef and mutton products (47). Thus, CLA \textit{cis} 9, \textit{trans} 11 is the major CLA isomer in the diet. Pasteurised Swedish milk contain on average 5.9 mg CLA/g fat (52). A corresponding figure from Germany is 9.8 mg/g fat (53). CLA in cheese vary with the type of cheese from 4.0 to 17.0 mg/g fat (53), and ruminant meat products have been reported to contain 2.7 mg/g veal and 5.6 mg/g mutton (47). The amount of CLA, predominantly \textit{cis} 9, \textit{trans} 11, in the diet has been estimated to be 160 mg/day in Swedish women and men (54), 350 mg/day and 430 mg/day in German women and men, respectively (53), and 127 mg/day in American women (55).

Effects of CLA in animal and \textit{in vitro} models

CLA has in \textit{in vitro} and animal models been observed to have a variety of biological effects. These effects include improved serum lipid profile, decreased aortic lipid deposition and inhibited tumorigenesis (56, 57) (reviews). Moreover, CLA reduces body fat accumulation in animal models (58, 59) and has been ascribed effects on glucose metabolism (60, 61). The isomer \textit{trans} 10, \textit{cis} 12 has been suggested to have effects on body fat and glucose metabolism (62, 63). Furthermore, CLA has been suggested to have antioxidative properties (64-66) and to be anti-inflammatory and / or immune ameliorating \textit{in vitro} (67).

Effects of CLA in humans

At the initiation of the first CLA study of the present thesis there had been very few studies of effects of CLA in humans. At present a number of studies of the effects of CLA, with mixed or single isomer preparations, have been performed (56, 57, 68, 69) (reviews). The focus has mainly been on effects on body composition, lipid and glucose metabolism, and immune function. However, the results of studies are partly conflicting, and the effects of CLA in humans are as yet not well characterised.
Lipid peroxidation

When lipids are oxidised without release of energy the reaction is called lipid peroxidation. Polyunsaturated fatty acids are more prone to lipid peroxidation than saturated and monounsaturated fatty acids. The lipid peroxidation reactions occur rapidly either through catalysis by free radicals (non-enzymatic lipid peroxidation) or by enzymes (enzymatic lipid peroxidation) (70). A schematic diagram of non-enzymatic and enzymatic conversion of arachidonic acid is shown in Figure 2.

![Figure 2. Non-enzymatically and enzymatically induced lipid peroxidation of arachidonic acid to formation of 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$, respectively.](image)

Non-enzymatic lipid peroxidation

Oxidative stress is a situation with an excess of pro-oxidants (free radicals and other reactive species) in relation to antioxidants in the body. This state may have arisen by either an increase in formation of prooxidants or a decrease in antioxidants. Free radicals can react with lipids, DNA and proteins, which can eventually be followed by tissue injuries or several states of disease.

The non-enzymatic lipid peroxidation in vivo is initiated when a double bond in e.g. a polyunsaturated fatty acid reacts with an oxidant inducer to form a free radical intermediate. Further, the reaction leads to a chain reaction were peroxyl radicals react with other polyunsaturated fatty acids until a termination reaction is initiated by for example chain-breaking antioxidants (71). Products of non-enzymatic lipid peroxidation not only take part in pathophysiology, but are also essential parts in a range of normal physiological regulations, including vascular control, defence against microorganisms, cell signalling and generation and degradation of cells (71). In a controlled situation in the body there is a balance between the produced oxidants and the antioxidant defence system.
F₂-isoprostanes are formed in vivo by peroxidation of arachidonic acid (Figure 2), and are rapidly metabolised and excreted in the urine (72, 73). 8-Iso-PGF₂ₐ is a major F₂-isoprostane formed in vivo, and is thus an endogenous indicator of oxidative stress in vivo. 8-Iso-PGF₂ₐ can be measured in both plasma and urine with a specific and sensitive radioimmunoassay (RIA) (74) or by GC/MC (75). Elevated concentrations of 8-iso-PGF₂ₐ in body fluids have been observed in humans in conditions proposed to be associated with an increased free radical production, such as smoking (76-78), diabetes mellitus (79, 80), vascular reperfusion (81-83), hypercholesterolaemia (84), atherosclerotic lesion (85) and rheumatic disease (86).

Enzymatic lipid peroxidation

Lipid peroxidation may also occur via enzymatically catalysed pathways. Prostaglandins are derived from arachidonic acid through the cyclooxygenase (COX) pathway (Figure 2) (87). Prostaglandin F₂ₐ (PGF₂ₐ) is one of the major primary prostaglandins formed in vivo among other prostaglandins and thromboxanes. PGF₂ₐ is vasoconstrictive and is involved in several inflammatory processes (71). PGF₂ₐ has a very short half-life in vivo and may be formed as an artefact during blood sampling (88, 89), and is thus difficult to quantify in vivo. 15-Keto-dihydro-PGF₂ₐ, a stable metabolite of PGF₂ₐ, can however be quantified in plasma and urine with RIA (90). Measurements of 15-keto-dihydro-PGF₂ₐ is thus regarded as an indicator of PGF₂ₐ formation both in physiology (91) and pathophysiology (92, 93). Enhanced PGF₂ₐ formation (15-keto-dihydro-PGF₂ₐ) has been shown in patients with rheumatic diseases, diabetes type 2 and smoking (78, 80) and in experimental animals with septic shock (92, 94).

Inflammatory biomarkers

Atherosclerosis is nowadays generally accepted as an inflammatory disease, and population studies show strong associations between indices of inflammation and abnormal lipid and carbohydrate metabolism, obesity and atherosclerosis (95-99). It is, however, unclear whether different biomarkers for inflammation are merely indicators, or if they contribute to progression and development of atherosclerotic disease (98).

Acute phase proteins

C-Reactive protein

C-Reactive protein (CRP) is an acute phase protein and a circulatory indicator for inflammation that has been suggested to be a component of the metabolic syndrome (95, 98, 100). Mildly increased concentrations of CRP, within the clinical normal range, may predict future cardiovascular events
Visceral fat has been found to be a promoter of low-grade inflammation, which can explain a part of the association between CRP and features of the metabolic syndrome (102).

*Tumour necrosis factor-α*

Tumour necrosis factor-α (TNF-α) is a cytokine secreted partly by the adipose tissue. In addition to its established role in the immune system, TNF-α affects metabolism of adipose tissue and glucose homeostasis. Human obesity is positively related to TNF-α expression in adipose and muscle tissue (103, 104), and plasma levels of TNF-α are positively associated to body mass index (BMI), fasting glucose and serum triglycerides, and inversely related to high density lipoprotein cholesterol (104). Thus, high TNF-α levels are associated to abnormalities characteristic of the metabolic syndrome, and have further been suggested to be involved in the pathogenesis of non-insulin-dependent diabetes mellitus and insulin resistance (104).

*Prostaglandin-F₂α*

Prostaglandins are well known as indicators of the inflammatory process (105) and 15-keto-dihydro-PGF₂α is a potent indicator of cyclooxygenase mediated acute and chronic inflammation (86, 90, 92-94).
Aims

The overall aims of the present thesis were to investigate the associations between intake of milk products and fat from milk products and risk factors for coronary heart disease, and to study the effects of supplementation with conjugated linoleic acid, CLA, to healthy humans.

And the specific aims were to:

- Investigate whether pentadecanoic acid (15:0) and heptadecanoic acid (17:0) can be used as dietary markers for milk products (Paper I).
- Study the associations between intake of milk products and fat from milk products, on the one hand, and risk factors for cardiovascular disease on the other (Paper I).
- Investigate the effects on body composition, body weight, abdominal adiposity, blood pressure, serum lipid concentrations and fatty acid composition of the serum lipids after CLA supplementation to humans (Paper II).
- Study the effects of CLA supplementation on indicators of non-enzymatic lipid peroxidation (8-iso-PGF$_{2\alpha}$), enzymatic lipid peroxidation (15-keto-dihydro-PGF$_{2\alpha}$), and serum concentrations of tocopherols. (Paper III).
- Investigate the effects of CLA supplementation on markers of inflammation (CRP, TNF-$\alpha$, TNFR1, TNFR2 and VCAM-1) (Paper IV).
- Study possible mechanisms behind the CLA induced lipid peroxidation by supplementing humans with CLA in combination with $\alpha$-tocopherol or cyclooxygenase-2 inhibitor (Paper V).
- Examine the abilities of different isomer preparations of CLA to induce lipid peroxidation (Paper V).
In the study of Paper I we investigated the possibility to use proportions of 15:0 and 17:0 as markers for intake of milk fat. We also correlated milk fat intake to risk factors for CHD and found inverse associations.

To explore whether the inverse findings of Paper I could be explained by CLA we supplemented women and men with CLA and analysed effects on metabolic variables (Paper II) and lipid peroxidation (Paper III).

In the study of Paper IV we investigated the effects of CLA on markers of inflammation.

To search for the possible mechanisms behind the CLA induced lipid peroxidation we performed a second intervention study with different CLA isomers, vitamin E and COX-2 inhibitor (Paper V).
Subjects, study designs and methods

Subjects

Paper I

The participants of Paper I took part in the 70-year reinvestigation of the population based cohort “Uppsala Longitudinal Study of Adult Men” (ULSAM). The ULSAM study has previously been described in detail (106, 107). At the 70-year survey 1221 men were reinvestigated. Additional physiological analyses, including fatty acid analyses of the serum lipids, were performed on 66 men in consecutive order. These 66 subjects were included in the present study. Of these 66 men 62 had completed a 7-day dietary record (Table 1).

Papers II, III and IV

Healthy subjects were recruited by advertisements in the local newspaper and among university employees. Fifty-three persons, 27 men and 26 women, between 23 and 63 years of age were included and randomly assigned to either a CLA supplemented group or a control group before entering the study. In Table 1 some baseline characteristics of the participants are shown. At baseline there were no statistical differences between the groups with regard to the variables in Table 1.

Two groups of subjects including 25 persons each was calculated to give a statistical power of 80% to detect a difference in serum or LDL cholesterol of 9% if $P < 0.05$. 

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Table 1. Clinical characteristics of the subjects in papers I, II, III, IV and V (mean (SD)).

<table>
<thead>
<tr>
<th>Clinical variable</th>
<th>Paper I (n=62)</th>
<th>Paper II, III and IV (n=53)</th>
<th>Paper V (n=60)</th>
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<td>Sex (men/women)</td>
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<td>25/35</td>
<td></td>
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<tr>
<td>Age (yr.)</td>
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<td>45.4 (11.7)</td>
<td>38.4 (14.6)</td>
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<tr>
<td>Weight (kg)</td>
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<td>75.7 (15.3)</td>
<td>75.9 (17.2)</td>
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<td>BMI (kg/m$^2$)</td>
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<td>25.1 (4.1)</td>
<td>25.3 (4.6)</td>
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<td>Waist (cm)</td>
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<td>84 (13)</td>
<td></td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>99.0 (6.1)</td>
<td>101 (7)</td>
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</tr>
<tr>
<td>WHR</td>
<td>0.94 (0.05)</td>
<td>0.8 (0.1)</td>
<td></td>
</tr>
<tr>
<td>SAD (cm)</td>
<td>22 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>29.3 (6.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
<td>24%</td>
<td></td>
<td>8% and 5% snuff users</td>
</tr>
<tr>
<td>Serum triacylglycerides (mmol/L)</td>
<td>1.28 (0.59)</td>
<td>1.4 (0.8)</td>
<td>1.2 (0.6)</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/L)</td>
<td>5.93 (1.01)</td>
<td>5.6 (1.1)</td>
<td>5.2 (1.2)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.34 (0.26)</td>
<td>1.3 (0.3)</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>HDL triacylglycerides (mmol/L)</td>
<td>0.10 (0.07)</td>
<td>0.12 (0.07)</td>
<td>0.09 (0.05)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>4.01 (0.94)</td>
<td>3.8 (1.0)</td>
<td></td>
</tr>
<tr>
<td>LDL triacylglycerides (mmol/L)</td>
<td>0.39 (0.12)</td>
<td>3.21 (1.03)</td>
<td></td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>3.11 (0.96)</td>
<td>3.2 (1.1)</td>
<td>2.4 (1.0)</td>
</tr>
<tr>
<td>VLDL cholesterol (mmol/L)</td>
<td>0.35 (0.26)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VLDL triacylglycerides (mmol/L)</td>
<td>0.80 (0.69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-I (g/L)</td>
<td>1.37 (0.24)</td>
<td>1.36 (0.20)</td>
<td></td>
</tr>
<tr>
<td>Apo B (g/L)</td>
<td>1.02 (0.25)</td>
<td>0.94 (0.27)</td>
<td></td>
</tr>
<tr>
<td>Apo(a) (U/L)</td>
<td>217 (262)</td>
<td>297 (311)</td>
<td></td>
</tr>
<tr>
<td>NEFA (mmol/L)</td>
<td>0.50 (0.17)</td>
<td>0.41 (0.19)</td>
<td></td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>5.80 (1.77)</td>
<td>4.5 (0.7)</td>
<td></td>
</tr>
<tr>
<td>Fasting insulin (mU/L)</td>
<td>8.6 (11.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral glucose tolerance test (AUC)</td>
<td>189 (114)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M (100 mg (kg b.w.)$^{-1}$ (min.$^{-1}$)</td>
<td>5.93 (2.08)</td>
<td>6.08 (2.78)</td>
<td></td>
</tr>
<tr>
<td>M/I (100 mg (kg b.w.)$^{-1}$ (min.$^{-1}$) (mU/L)$^{-1}$</td>
<td>6.08 (2.78)</td>
<td>148 (14)</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>148 (14)</td>
<td>122 (13)</td>
<td></td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>84 (9)</td>
<td>72 (8)</td>
<td></td>
</tr>
</tbody>
</table>

Paper V

Healthy subjects were recruited among university students and previous participants. Sixty persons, 35 women and 25 men, were included and all completed the study. The characteristics of participants are shown in Table 1. At inclusion the participants were randomly divided into six groups. The participants reported all kinds of medication used during the study. Three persons reported that they had used oestrogen replacement therapy during
the study, 17 used oral contraceptives, three persons had taken paracetamol less than two days prior to an investigation, and three subjects reported medication coded as “other” at some occasion during the study.

Inclusion criteria (Papers II, III, IV and V)
The participants of the interventions studies were not to have any medication, any known disease, or abnormal results on analyses of concentrations of CRP, thyroid stimulating hormone, blood glucose or serum lipids. The participants had to be weight stable at inclusion, and were not to follow any dietary restrictions or to have any intentions to change weight, food habits or physical activity during the studies.

Ethical considerations
All study protocols were approved by the Research Ethics Committee of the Medical Faculty at Uppsala University, and all participants gave their informed consent prior to the studies.

Study designs and compounds
Papers II, III and IV
**Study design**
The intervention was randomised, double blind and placebo controlled. The participants were randomly assigned to participate in either the control group or the CLA group (Figure 4). During two initial weeks all participants were given the control capsules, containing olive oil (4.2 g per day). During the following 12 weeks the participants were given either control capsules or capsules containing 4.2 g of CLA. At three occasions, before start, and during the fifth and ninth week of the study, the participants performed 3-days weighed dietary records.

![Figure 4. Design of study II, III and IV.](image)
Compounds
The CLA capsules were composed of 75.9% CLA with equal amounts of the CLA isomers $cis$ 9, $trans$ 11 and $trans$ 10, $cis$ 12, respectively. In addition to CLA the capsules contained oleic acid (18:1n-9) (14.0%), palmitic acid (16:0) (4.4%), stearic acid (18:0) (1.5%), linoleic acid (18:2n-6) (0.4%) and minor amounts of other fatty acids. All fatty acids were non-esterified. The control and CLA capsules were identical in appearance. All capsules were provided by Natural Ltd A/S (Oslo, Norway). The participants were asked not to change their habits regarding diet and physical activity and to abstain from any dietary supplementation with vitamins, minerals or fatty acids prior to and during the study.

Paper V
Study design
The study was randomised with control groups (Figure 5). The CLA supplementation was single blinded. The participants were randomly assigned to one of six groups. During the first two weeks groups 2 and 5 received $\alpha$-tocopherol, and groups 3 and 6 received a cyclooxygenase-2 (COX-2) inhibitor. Groups 1 and 4 did not receive any supplementation during these initial weeks. During the third to sixth week all groups were supplemented with CLA. Groups 1, 2 and 3 were assigned “CLA mix” capsules, and groups 4, 5 and 6 were assigned “CLA 1012” capsules. Thus, group 1 acted as control for groups 2 and 3, while group 4 was control for groups 5 and 6. Groups 1 and 4 received identical treatments except for the diverging isomer compositions of the CLA preparations. Correspondingly, groups 2 and 5 were parallel, and so were groups 3 and 6.

![Figure 5. Design of study V.](image)
Compounds

Two different preparations of CLA were used. The first CLA preparation, further termed "CLA mix", consisted of 38.0% of CLA trans 10, cis 12; 36.5% of the two CLA isomers cis 9, trans 11 and CLA trans 8, cis 10; 12.2% of oleic acid (18:1 cis 9); 4.5% of palmitic acid (16:0); 2.4% of stearic acid (18:0); 1.1% of linoleic acid (18:2 cis 9, cis 12); and less than 1.1% of other fatty acids and CLA isomers. The CLA mix capsule contained 742 mg oil, out of which 578 mg was CLA (77.9%) and the participants were asked to take six capsules per day, in total containing 3.5 g CLA. The second CLA preparation, referred to as "CLA 1012", consisted mainly (85.1%) of the CLA isomer trans 10, cis 12, but also of 9.1% of CLA cis 9, trans 11 and 1.5% of oleic acid (18:1 cis 9). The CLA 1012 capsule contained 752 mg oil, out of which 709 mg was CLA (94.2%) and the participants were asked to take six capsules per day, corresponding to 4.0 g of CLA. The CLA capsules were provided by Natural Ltd A/S (Oslo, Norway), who also performed the analyses of the capsule contents. Vitamin E was given as 200 mg per day of d-α-tocopherol acetate (IDO-E®, Pharmacia Upjohn, Stockholm, Sweden). As a COX-2 inhibitor rofecoxib (12.5 mg / day) was used (Vioxx®, Merck Sharp & Dohme, Haarlem, Netherlands).

Methods

Examinations and samplings (Papers II, III, IV and V)

All examinations and blood sampling were made in the morning after an over night fast. The participants were asked not to perform any heavy physical activity the day before, or in the morning prior to an examination. The participants were not allowed to take any dietary supplements two weeks before or during the interventions. Furthermore, the participants were asked not to change their habits with regard to diet and physical activity, and not to have the ambition to change their body weight during the studies.

Morning urine samples were collected in the morning in the subjects’ homes, prior to an examination. Twenty-four hour urinary samples were collected in cans the day before an examination. The participants were asked to store the cans in a refrigerator until the examination.

Blood samples were immediately placed on ice and all serum and plasma samples were stored at -70°C within 1 hour after collection. Urinary samples were stored at -70°C within 1 hour from the subjects’ arrival at the clinic, until analysis.
Dietary assessments

Paper I
An optically readable precoded food record was used to assess the dietary intake. The food record used is a modification of a pre-coded record book used by the Swedish National Food administration and Statistics Sweden used in a nation wide household and dietary survey in 1989 (108). This record book has been validated in young adults (108) and in middle-aged persons (109). The intake of foods and nutrients were calculated using Food Database of the Swedish National Food Administration. The amounts of milk, cream, cheese, ice cream, butter, and the amounts of fat from these groups of foods were calculated, and the amount of fat contributed by these foods was summarised.

Papers II, III and IV
At three occasions, before start, and during the fifth and ninth week of the study, the participants performed 3-days weighed dietary records, including two week days and one week end day. Dietary data from the records were computerised using a commercial diet analysis software (MATs 4.03e, Rudans Lättdata, Västerås, Sweden). Calculations were made using the PC version 2.97 of the Food Database of the Swedish National Food Administration (Swedish National Food Administration, Uppsala, Sweden). The fat provided by CLA and control capsules, respectively, was not included in the calculations of intakes.

Anthropometric measurements
Body weight was determined to the nearest kilogram and height to the nearest centimetre, wearing light indoor clothing and no shoes. BMI was calculated as the body weight in kilograms divided by the square of the height in meters. The waist circumference was measured midway between the lowest rib and the iliac crest, and the hip circumference at the widest part of the hip, and the waist to hip ratio was calculated. The sagittal abdominal diameter was measured as the height of the stomach when lying on the back on a firm bed with the knees bent. The percentage body fat was calculated from the three compartment model based on measurements of skin fold thickness, using Harpenden skin fold callipers (John Bull, British Indicators Ltd., St Albans, Great Britain) and body water volume, estimated by a multi frequency bioelectric impedance analyser Hydra 4200 (Xitron Technologies Inc., San Diego, CA, USA) as described by Forslund et al. (110).
Fatty acid composition

Serum lipids

**Paper I**

The fatty acid composition of the serum lipids was analyzed by gas-liquid chromatography (GLC), as described in detail by Boberg *et al.* (111); in short, plasma lipids were extracted with chloroform. Butylated hydroxy toluene and NaH$_2$PO$_4$ was added prior to evaporation under nitrogen. Phospholipids, triacylglycerides and cholesterol esters were separated by thin layer chromatography. The lipids esters were transmethylated with methanol and H$_2$SO$_4$. The Hewlett Packard GLC system used for the analyses consisted of a GC 5890, an automatic sampler 7671A, an integrator 3392A, and a 25 m Nordion Fused Silica capillary column NS-351, with helium as the carrying gas. The temperature was programmed to 100-210°C. Standards from Nu Chek (Nu Chek Prep, Inc., Elysian, MN, USA) were used for identification of the individual fatty acids and as a control of the GLC system. The amounts of fatty acids are given as the relative percentage of the sum of the fatty acids analyzed.

**Paper II**

The extraction, separation and methylation of the plasma lipids were performed as described in detail by Boberg *et al.* (111). The fatty acid methyl esters were separated with gas chromatography using a Hewlett Packard GLC system consisting of a HP 5890 Series II GC apparatus, HP 7673 automatic sampler, HP 3365A Series II Chemstation integrator software and a 50m x 0.25mm CP-Sil 88 Chrompack capillary column, with helium as carrying gas. Standards from Nu Check were used for identification of the individual fatty acids and as a control of the GC system. The technique used is not optimal for resolving different CLA isomers. Thus, the peaks identified as CLA when using a reference standard (Sigma Chemical, St Louis, MO, USA) were added and the sum of total CLA is presented here as “CLA”. The proportions of fatty acids are given as the relative percentage of the sum of the fatty acids analyzed.

**Thrombocytes (Paper II)**

Blood was drawn using minimal venous pressure. A 16 G butterfly cannula was used to collect blood drop by drop. Thrombocyte clot was received through gentle centrifugation 120xG for 20 minutes, washed in saline and dissolved with a Polytron mixer. From the dissolved clot the total platelet lipids were extracted with chloroform-methanol including butylated hydroxy toluene. The fatty acid composition was analyzed as in the serum lipids.
Apparent desaturase activities (Paper II)

The desaturase activities were assessed by calculating product to precursor ratios as follows: 20:4n-6/20:3n-6 for Δ-5 desaturase, 18:3 n-6/18:2 n-6 for Δ-6 desaturase in triglycerides and cholesterol esters, 20:3 n-6/18:2 n-6 for Δ-6 desaturase in phospholipids and thrombocytes and 18:1 n-9/18:0 and 16:1 n-7/16:0 for Δ-9 desaturase activities.

Lipoprotein and apolipoprotein analyses (Papers I and II)

Serum very low density lipoprotein (VLDL), low density lipoprotein (LDL, Paper II), and high density lipoprotein (HDL) were isolated by a combination of preparative ultra-centrifugation (112) and precipitation with a sodium phosphotungstate and magnesium chloride solution (113). Triacylglyceride (TAG) and cholesterol concentrations in serum and in the isolated lipoprotein fractions were measured by enzymatic methods (Instrumentation Laboratories, Lexington, MA, USA) in a Monarch 2000 centrifugal analyzer. The coefficients of variation of the analyses of serum cholesterol and serum triacylglycerides were 2% and 4%, respectively. The concentrations of apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) were measured by immunoturbidimetry in a Monarch apparatus. Apolipoprotein(a) (apo(a)) was in Paper I determined by a Pharmacia Apo(a) RIA method (Pharmacia (a) RIA, Pharmacia Diagnostics AB, Uppsala, Sweden 1985) The coefficient of variation of the analysis of apolipoprotein(a) is approximately 5% according to the manufacturer. In Paper II Apo(a) was analysed using a Coda Automated EIA automatic ELISA analyzer (Bio-Rad Laboratories, Hercules, CA, USA) using Mercodia apo(a) reagens (Mercodia AB, Uppsala, Sweden). Serum free fatty acids were measured by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany).

Tocopherol concentration (Papers II, III and IV)

Plasma α- and γ-tocopherol concentrations were assayed by using HPLC with fluorescence detection (114). In brief, 500 µl plasma was extracted with 500 µl ethanol containing 0.005 % butylated hydroxytoluene and 2 ml hexane. A volume of 20 µl of the supernatant was injected to a HPLC column (LiChrospher 100 NH2 250x4 mm). The fluorescence detector had an excitation wave length of 295 nm and an emission wave length of 327 nm. Plasma tocopherol concentrations were adjusted for serum lipid concentrations by dividing the tocopherol concentration with the sum of serum concentrations of cholesterol and TAG, as suggested by Thurnham et al. (115).
Glucose and insulin (Paper II)
Blood glucose was measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany). Plasma insulin was analyzed using an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim) in an ES300 automatic analyzer (Boehringer Mannheim, Germany).

Insulin sensitivity and glucose tolerance tests (Paper I)
The insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp procedure according to DeFronzo et al. (116), as described in detail by Pollare et al. (117). In short, the insulin sensitivity index (M/I) was calculated by dividing the glucose disposal during the last hour of the clamp (mg/kg/min) (M) by the actual insulin concentration during the test (mU/L) multiplied by 100 (I). The oral glucose tolerance test was performed by giving 75 g of glucose in 300 ml of water. Plasma glucose was measured before and 30, 60, 90, and 120 minutes after the ingestion of glucose. The result of the oral glucose tolerance test was expressed as the area under the glucose curve (AUC).

Blood pressure (Paper I)
Supine systolic and diastolic blood pressures were measured with a mercury manometer after a 10 minutes’ rest. The value was recorded twice to the nearest even figure. The mean of these two figures was used.

Plasminogen activator inhibitor-1 activity (Paper II)
The amount of active plasminogen activator inhibitor-1 (PAI-1) was analyzed in citrate plasma using a commercially available bioimmunoassay (Chromolize PAI-1 kits, Biopool AB, Umeå, Sweden).

8-Iso-PGF$_{2\alpha}$, non-enzymatic lipid peroxidation (Papers III and V)
The urinary samples from this study were analysed for free 8-iso-PGF$_{2\alpha}$ without any extraction by a radioimmunoassay, as described in (74). In brief, an antibody was raised in rabbits by immunization with 8-iso-PGF$_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by 1,1’-carbonyldimidazole method. The cross-reactivity of the antibody with 8-iso-15-keto-13,14-dihydro-PGF$_{2\alpha}$, 8-iso-PGF$_{3\beta}$, PGF$_{2\alpha}$, 15-keto-PGF$_{2\alpha}$, 15-keto-13,14-dihydro-PGF$_{2\alpha}$, TXB$_2$, 11β-PGF$_{2\alpha}$, 9β-PGF$_{2\alpha}$ and 8-iso-PGF$_{3\alpha}$ was 1.7, 9.8, 1.1, 0.01, 0.01, 0.1, 0.03, 1.8 and 0.6%, respectively. The detection limit of the assay was 23 pmol/l. The urinary concentrations of 8-iso-
PGF$_{2\alpha}$ were adjusted for creatinine values. Urinary creatinine was measured by a commercial kit (IL™ Test by Monarch Instrument, Amherst, NH, USA).

15-Keto-dihydro-PGF$_{2\alpha}$, enzymatic lipid peroxidation (Papers III, IV and V)

15-Keto-dihydro-PGF$_{2\alpha}$ was analysed without any extraction by a radioimmunoassay, as described in (90). In brief, an antibody was raised in rabbits by immunization with 15-keto-dihydro-PGF$_{2\alpha}$ coupled to bovine serum albumin at the carboxylic acid by 1,1´-carbonyldiimidazole method. The cross-reactivity of the antibody with PGF$_{2\alpha}$, 15-keto-PGF$_{2\alpha}$, PGE$_2$, 15-keto-13,14-dihydro-PGE$_2$, 8-iso-15-keto-13,14-dihydro-PGF$_{2\alpha}$, 11β-PGF$_{2\alpha}$, 9β-PGF$_{2\alpha}$, TXB$_2$ and 8-iso-PGF$_{3\alpha}$ was 0.02, 0.43, <0.001, 0.5, 1.7, <0.001, <0.001, <0.001, and 0.01%, respectively. The detection limit of the assay was 45 pmol/l. The urinary concentrations of 15-keto-dihydro-PGF$_{2\alpha}$ were adjusted for urinary creatinine concentrations.

C-reactive protein (Paper IV)

Plasma CRP was measured with the use of a rabbit anti-human CRP (Dako A/S, Glostrup, Denmark) as capture antibody, rabbit anti-human CRP (Peroxidase conjugated, Dako P0227) for detection and human CRP high control (Dako X0926) as standard. For all reactions, if not indicated otherwise, TMB one substrate (Dako S1600) was used as substrate. The detection limit was 0.1 µg/L and the inter assay CV 8%.

Tumour necrosis factor-α (Paper IV)

Plasma TNF-α was measured with the use of mouse anti-human TNF-α (R&D Systems Europe, Abingdon, Oxon, United Kingdom) as capture antibody, rabbit anti-human TNF-α (Biotin conjugated, R&D BAF210) and streptavidin conjugated with ALP for detection and AMPAK (Dako K6200) as substrate. The detection limit was 0.5 pg/mL and the inter assay CV 18%.

Tumour necrosis factor receptors (Paper IV)

sTNFR1 mouse anti-human TNFR1 (MAB625, R&D Systems Europe, Oxon, UK) was used for coating and biotinylated goat anti-human TNFR1 (BAF225, R&D) and avidin conjugated with HRP (Dako A/S, Glostrup, Denmark) was used for detection. The detection limit was 6.25 pg/mL and the inter assay CV 12%.
sTNFR2 was measured in a similar way with rabbit anti-human TNFR2 (HP9003, Hbt, Uden, The Netherlands) used as coating antibody, biotinylated mouse anti-human TNFR2 (HM2008, Hbt) and avidin conjugated with HRP (Dako A/S, Glostrup, Denmark) for detection. The detection limit was 15.6 pg/mL and the inter assay CV 12%.

Vascular cell adhesion molecule-1 (Paper IV)
sVCAM-1 was analysed with mouse anti-human VCAM-1 (MCA907, Serotec Ltd, Oxford, UK) as coating antibody and biotinylated mouse anti-human VCAM-1 (MCA1479B, Serotec) and avidin conjugated with HRP (Dako A/S, Glostrup, Denmark) for detection. The detection limit was 3.75 ng/mL and the inter assay CV 7%.

Statistical analyses
The statistical analyses were performed using SAS (SAS Institute Inc., Cary, NC, USA) and STATA (Stata Corporation, TX, USA) statistical software. *P* values less or equal to 0.05 were considered as statistically significant. Variables with a skewed distribution (*W*<0.95 in Shapiro-Wilk’s *W* test for normality) were logarithmically transformed (log 10) prior to analysis.

Correlation analyses of Pearson correlations were performed on continuous variables and Spearman’s correlations on skewed or ordinal variables. For a post hoc regression analysis of the association between the changes of two variables, an analysis of the standard residuals of the regression was performed (Paper IV).

Group comparisons were performed using Student’s *t*-tests, un-paired tests for analyses within groups and paired tests for differences between groups. If variables were not normally distributed after logarithmic transformation analyses of differences within a group were analysed using Wilcoxon matched pairs signed-rank tests were used, and differences between groups using Wilcoxon rank-sum tests (Paper IV).

When statistically analysing the effect of a supplementation, the difference in a supplemented group was compared to the difference in the control group. That is, a change *within* a group was not regarded as an effect of a treatment, unless it was different from the change in the control group.

Calculation of percental changes was performed as: ((mean value after – mean value before) / mean value before) x 100.

In Paper II the main statistical analyses were performed “according to protocol”, i.e. excluding participants with a low compliance, who had taken less than 80% of the prescribed number of capsules. Additional analyses were also made according to “intention to treat”, i.e. all subjects were included irrespective their degree of compliance. Results from the intention to
treat analyses are presented when different from the according to protocol analyses.

In Paper V calculations adjusting the analyses for one factor at the time showed that use of oral contraceptives affected the results of the interventions. All analyses shown, except paired t-tests, are thus adjusted for use of oral contraceptives using linear multiple regression analyses.
Results

Paper I

Figure 6. Schematic diagram showing how the relations between dietary intake, clinical characteristics and fatty acid composition of serum lipids were studied.

15:0 as a marker for intake of milk fat

The proportion of 15:0 in both serum cholesterol esters and phospholipids was positively correlated with intake of fat from milk and cream, with intake of ice cream and fat from ice cream, with intake of butter and fat from butter, and with the total amount of fat provided by milk products (Table 2). There were no significant associations between the proportion of 15:0 and intake of cheese or fat from cheese.

Table 2. The proportions of 15:0 in serum cholesterol esters and phospholipids correlated to energy-adjusted intake of milk products and fat from milk products using Spearman’s rank correlation (n = 62 for CE and n = 61 for PL).

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>15:0 in cholesterol esters</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fat from milk and cream</td>
<td>0.26</td>
<td>0.039</td>
</tr>
<tr>
<td>ice-cream</td>
<td>0.27</td>
<td>0.036</td>
</tr>
<tr>
<td>fat from ice-cream</td>
<td>0.27</td>
<td>0.036</td>
</tr>
<tr>
<td>butter</td>
<td>0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>fat from butter</td>
<td>0.36</td>
<td>0.004</td>
</tr>
<tr>
<td>fat from milk products</td>
<td>0.46</td>
<td>&lt;0.0001</td>
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<tr>
<td><strong>15:0 in phospholipids</strong></td>
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<td></td>
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<tr>
<td>fat from milk and cream</td>
<td>0.34</td>
<td>0.008</td>
</tr>
<tr>
<td>ice-cream</td>
<td>0.26</td>
<td>0.041</td>
</tr>
<tr>
<td>fat from ice-cream</td>
<td>0.26</td>
<td>0.041</td>
</tr>
<tr>
<td>butter</td>
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<td>0.051</td>
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<tr>
<td>fat from butter</td>
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<td>0.051</td>
</tr>
<tr>
<td>fat from milk products</td>
<td>0.34</td>
<td>0.008</td>
</tr>
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</table>

*r* = correlation coefficient
15:0 in relation to intake of nutrients and fatty acids

Relations between the proportion of 15:0 in serum cholesterol esters and phospholipids, respectively, and the estimated dietary intake of 4:0–10:0 and 14:0 were positive. Negative associations were found between the proportion of 15:0 in cholesterol esters and the percentage of energy intake as PUFA.

15:0 in relation to other fatty acids in serum

The proportion of 15:0 in cholesterol esters was positively related to 15:0 and 17:0 in phospholipids and to 16:0 in cholesterol esters, whereas 15:0 in phospholipids was positively related to 17:0 in phospholipids. There were also positive relations between 15:0 in cholesterol esters and phospholipids and the long-chain n-3 fatty acids.

Associations between milk products and clinical characteristics

Inverse associations were observed between intake of milk products and fat from milk products, on one hand, and several risk factors for coronary heart disease, on the other (Table 3). The associations remained largely unchanged after statistical adjustments for life style factors as physical activity, but weakened somewhat after adjustments for intake of meat, beer, vegetables and root-crops and potatoes (Table 3).

Table 3. Association between energy adjusted intake of fat from milk products and clinical variables

<table>
<thead>
<tr>
<th>Crude correlations</th>
<th>Adjustments:</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Physical activity</td>
<td>Intake of meat, beer, vegetables, root crops &amp; potatoes</td>
</tr>
<tr>
<td>Fat from milk &amp;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cream</td>
<td></td>
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</tr>
<tr>
<td>Weight</td>
<td>↓</td>
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</tr>
<tr>
<td>Waist</td>
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<tr>
<td>Hip</td>
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<td>↓</td>
</tr>
<tr>
<td>BMI</td>
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<td>↓</td>
</tr>
<tr>
<td>WHR</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>SBP</td>
<td>(↑)</td>
<td>(↑)</td>
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<tr>
<td>TG</td>
<td>(↑)</td>
<td></td>
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<tr>
<td>HDL-TG</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Apo A1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>↓</td>
<td></td>
</tr>
<tr>
<td>OGTT</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Fat from cheese</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Waist</td>
<td>(↑)</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>(↑)</td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>(↑)</td>
<td></td>
</tr>
<tr>
<td>DBP</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chol</td>
<td>Apo(a)</td>
</tr>
<tr>
<td>----------------</td>
<td>------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Chol</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

↑ or ↓ = positive or negative association with a P value ≤ 0.05. (↑) or (↓) = positive or negative association with a P value > 0.05 and ≤ 0.10.

**15:0 in relation to clinical variables**

The proportion of 15:0 in cholesterol esters correlated negatively to body weight, waist and hip circumferences and BMI, and 15:0 in phospholipids correlated negatively to hip circumference (data not shown). After adjustment for intake of meat, beer, potatoes, root crops and vegetables the inverse associations between 15:0 in cholesterol esters and body weight and BMI remained (data not shown).
Paper II

Compliance and tolerance

All participants completed the trial. The compliance, counted as percentage of eaten capsules out of those prescribed, was equal to or more than 91% in 46 of the participants, between 81% and 90% in three, and 80% or less of the capsules in three of the participants. Participants with a compliance of 80% or less were regarded as low compliers, and were excluded from the main statistical analyses. The capsules were well tolerated, and only a few subjects reported mild diarrhoea at some occasions. There were no effects on serum concentrations of the liver enzymes aspartate amino transferase (ASAT) or alanine amino transferase (ALAT). Fifty out of the 53 subjects completed the 3x3 days weighed dietary registrations. The participants did not change their dietary intake during the study with regard to energy, fat, carbohydrates or protein, respectively (data not shown).

Anthropometric variables

The proportion of body fat was reduced more in the CLA group than in the control group (Table 4), while there were no differences between changes in body weight, BMI, waist to hip ratio and sagittal abdominal diameter (Table 4). When analysing the data according to intention to treat (including all 53 participants) reduction of body fat in the CLA group was 3.7%, \( P < 0.001 \) (\( P = 0.07 \) for difference between the groups). There was no relation between change in body fat and the proportion of body fat at start (data not shown).

Table 4. Change in anthropometric variables after CLA supplementation

<table>
<thead>
<tr>
<th></th>
<th>Absolute change (range)</th>
<th>Percental change</th>
<th>( P ) for difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.21 (-2 – 3)</td>
<td>0.28</td>
<td>0.664</td>
</tr>
<tr>
<td>CLA</td>
<td>0.4 (-6 – 4)</td>
<td>0.55</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.06 (-0.7 – 1.1)</td>
<td>0.25</td>
<td>0.655</td>
</tr>
<tr>
<td>CLA</td>
<td>0.14 (-1.9 – 1.5)</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>WHR n=48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.0 (-0.1 – 0.1)</td>
<td>1.04</td>
<td>0.560</td>
</tr>
<tr>
<td>CLA</td>
<td>0.0 (-0.1 – 0.1)</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>SAD (cm) n=48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.1 (-2.5 – 2.0)</td>
<td>0.57</td>
<td>0.423</td>
</tr>
<tr>
<td>CLA</td>
<td>0.0 (-2.0 – 2.5)</td>
<td>-0.19</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.4 (-3.8 – 1.9)</td>
<td>-1.23</td>
<td>0.050</td>
</tr>
<tr>
<td>CLA</td>
<td>-1.1 (-3.9 – 1.7)</td>
<td>-3.84 *</td>
<td></td>
</tr>
</tbody>
</table>

* \( P = 0.0006 \) for difference within the group.
Serum lipids and apolipoproteins

The serum concentration of apolipoprotein B increased in the CLA group compared to the control group, whereas LDL triacylglycerides decreased. When including all 53 participants in the statistical analyses there were no differences between the changes in serum lipid, apolipoprotein or nonesterified fatty acid concentrations in the two groups.

Table 5. Change in serum lipids, glucose, insulin and PAI-1 after CLA supplementation (n = 50)

<table>
<thead>
<tr>
<th></th>
<th>Absolute change</th>
<th>Percental change</th>
<th>P for difference between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.17</td>
<td>2.82</td>
<td>0.432</td>
</tr>
<tr>
<td>CLA</td>
<td>0.29**</td>
<td>5.35</td>
<td></td>
</tr>
<tr>
<td>HDL chol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.16***</td>
<td>12.1</td>
<td>0.204</td>
</tr>
<tr>
<td>CLA</td>
<td>0.09**</td>
<td>7.35</td>
<td></td>
</tr>
<tr>
<td>LDL chol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.09</td>
<td>2.23</td>
<td>4.15</td>
</tr>
<tr>
<td>CLA</td>
<td>0.20*</td>
<td>5.46</td>
<td></td>
</tr>
<tr>
<td>LDL/HDL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.30*</td>
<td>-9.25</td>
<td>0.168</td>
</tr>
<tr>
<td>CLA</td>
<td>-0.10</td>
<td>-3.14</td>
<td></td>
</tr>
<tr>
<td>TAG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.23**</td>
<td>-17.8</td>
<td>0.445</td>
</tr>
<tr>
<td>CLA</td>
<td>-0.07</td>
<td>-4.82</td>
<td></td>
</tr>
<tr>
<td>HDL TAG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.02</td>
<td>-26.2</td>
<td>0.869</td>
</tr>
<tr>
<td>CLA</td>
<td>-0.01</td>
<td>-7.69</td>
<td></td>
</tr>
<tr>
<td>LDL TAG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.04*</td>
<td>-8.84</td>
<td>0.039</td>
</tr>
<tr>
<td>CLA</td>
<td>0.01</td>
<td>2.06</td>
<td></td>
</tr>
<tr>
<td>VLDL chol (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.01***</td>
<td>1.19</td>
<td>0.577</td>
</tr>
<tr>
<td>CLA</td>
<td>0.11***</td>
<td>13.0</td>
<td></td>
</tr>
<tr>
<td>VLDL TAG (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.13</td>
<td>-20.0</td>
<td>0.785</td>
</tr>
<tr>
<td>CLA</td>
<td>-0.01</td>
<td>-0.45</td>
<td></td>
</tr>
<tr>
<td>NEFA (mmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>0.02</td>
<td>3.40</td>
<td>0.941</td>
</tr>
<tr>
<td>CLA</td>
<td>0.01</td>
<td>3.02</td>
<td></td>
</tr>
<tr>
<td>Apo A1 (g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-0.62</td>
<td>-0.45</td>
<td>0.472</td>
</tr>
<tr>
<td>CLA</td>
<td>-2.88</td>
<td>-2.16</td>
<td></td>
</tr>
<tr>
<td>Apo B (g/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>-1.0</td>
<td>-1.06</td>
<td>0.04</td>
</tr>
<tr>
<td>CLA</td>
<td>5.77**</td>
<td>6.24</td>
<td></td>
</tr>
<tr>
<td>Apo(a) (U/l)^n=48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>16.9</td>
<td>4.20</td>
<td>0.482</td>
</tr>
<tr>
<td>CLA</td>
<td>12.8</td>
<td>5.47</td>
<td></td>
</tr>
</tbody>
</table>
Blood glucose (mmol/l) *n=49*

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>CLA</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-0.06</td>
<td>0.11</td>
<td>0.053</td>
</tr>
<tr>
<td>Plasma insulin (mU/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-0.64</td>
<td>2.45</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-2.30</td>
<td>-1.16</td>
<td>0.600</td>
</tr>
</tbody>
</table>

PAI-1 (U/ml) | control | CLA     |       |
|            | 3.69    | 5.08    | 0.575 |
|            | 43.2    | 32.4    |       |

* P < 0.05, ** P < 0.01, *** P < 0.0001 for differences within groups.

Glucose metabolism and fibrinolysis

There was a borderline significant difference between the changes of fasting blood glucose in the groups, with a decrease in the control group and an increase in the CLA group, as shown in Table 5. When including all participants there were no differences between the two groups with regard to changes in fasting glucose, insulin or PAI-1.

Fatty acid composition

**Serum lipids**

In the serum phospholipids the proportion of stearic acid (18:0), docosatetraenoic acid (22:4n-6) and docosapentaenoic acid (22:5n-3) increased, and palmitic acid (16:0), oleic acid (18:1n-9), γ-linolenic acid (18:3n-6) (*P* = 0.065) and dihomo-γ-linolenic acid (20:3n-6) decreased in the CLA group, as compared to the control group. The changes of fatty acid composition of serum cholesterol esters and triglycerides were similar to the changes of the serum phospholipids (data not shown).

**Thrombocytes**

The fatty acid composition in the thrombocytes changed with an increase of docosapentaenoic acid (22:5n-3) and a decrease of oleic acid (18:1n-9) and dihomo-γ-linolenic acid (20:3n-6).

**Estimated desaturase activities**

The changed proportions of the fatty acid composition in the serum lipids correspond to an increase of the estimated Δ 5 desaturase activity (20:4n-6/20:3n-6) in the CLA group as compared to the control group, whereas the estimated activities of Δ 6 desaturase (18:3 n-6/18:2 n-6 in triglycerides and cholesterol esters and 20:3 n-6/18:2 n-6 in phospholipids) and Δ 9 desaturase (18:1 n-9/18:0) decreased as compared to the control group (Table 6). Also in the thrombocytes there was an increase in Δ 5 (20:4 n-6/20:3 n-6) and decrease in Δ 6 (20:3 n-6/18:2 n-6) estimated activities in the CLA group as
compared to the control group while Δ9 (18:1 n-9/18:0) was unchanged (Table 6). No changes were seen in the activity of Δ9 desaturase calculated as the ratio between 16:1 n-7 and 16:0 in any of the compartments analyzed.

**Table 6. Changes in estimated desaturase activities after CLA supplementation**

<table>
<thead>
<tr>
<th>Desaturase</th>
<th>Fatty acid ratio</th>
<th>CE</th>
<th>PL</th>
<th>TG</th>
<th>TR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ-9</td>
<td>16:1 n-7/16:0</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Δ-9</td>
<td>18:1/18:0</td>
<td>↓</td>
<td>↓</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>Δ-9</td>
<td>18:3 n-6/18:2 n-6</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Δ-5</td>
<td>20:4 n-6/20:3 n-6</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

CE = cholesterol esters, PL = phospholipids, TG = triacylglycerols, TR = thrombocytes. ↓ or ↑ = change statistically significant compared to the change in the control group, n.s. = not significantly different compared to the control group.

The effects of CLA and control supplementation on any of the parameters analyzed were not significantly different in men and women, and were not changed when adjusting for reported mean fat (data not shown).

**Paper III**

Non-enzymatic lipid peroxidation, 8-iso-PGF2α

Supplementation with CLA for three months resulted in 333% increase in concentrations of urinary 8-iso-PGF2α (Table 7), with a clear difference from the change in the control group.

**Table 7. 8-iso-PGF2α, 15-keto-dihydro-PGF2α and tocopherols in control and CLA supplemented groups given as means (SEM) before and after the study**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>CLA (n = 28)</th>
<th>P for diff. between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>8-iso-PGF2α (nmol/mmol crea)</td>
<td>0.34 (0.03)</td>
<td>0.36 (0.04)</td>
<td>0.50 (0.07)</td>
</tr>
<tr>
<td>15-keto-dihydro-PGF2α (nmol/mmol crea)</td>
<td>0.51 (0.07)</td>
<td>0.54 (0.07)</td>
<td>0.70 (0.09)</td>
</tr>
<tr>
<td>s-α-tocopherol (mg/mmol)</td>
<td>1.60 (0.04)</td>
<td>1.60 (0.04)</td>
<td>1.59 (0.05)</td>
</tr>
<tr>
<td>s-γ-tocopherol (mg/mmol)</td>
<td>0.08 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.10 (0.01)</td>
</tr>
</tbody>
</table>

crea = creatinine, m = morning urine, n.s. = not statistically significant, s = serum, u = 24 h urine. Symbols for differences within the groups: * P = 0.0024, ** P < 0.0001.
Enzymatic lipid peroxidation, 15-keto-dihydro-PGF$_{2\alpha}$

The urinary concentration of 15-keto-dihydro-PGF$_{2\alpha}$ increased with 129% in the CLA supplemented group as compared to the control group (Table 7). The individual changes in the concentrations of 15-keto-dihydro-PGF$_{2\alpha}$ in the urine were positively correlated to those of urinary 8-iso-PGF$_{2\alpha}$ ($r = 0.46$, $P = 0.0006$).

Paper IV
Effects of CLA supplementation

CRP increased after CLA supplementation, compared to the control group Table 8. In contrast, CLA supplementation did not have any statistically significant effect on the differences between the groups with regard to plasma levels of TNF-α, sTNFR1, sTNFR2 or sVCAM-1 (Table 8). There was no effect of gender or BMI (data not shown).

Table 8. Concentrations of indicators of inflammation before and after intervention with CLA or control capsules, given as medians and ranges. Differences within the groups are based on Wilcoxon matched pairs signed-rank tests, and differences between the groups are based on Wilcoxon rank-sum tests.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 25)</th>
<th>CLA (n = 28)</th>
<th>$P$ for diff. between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/l)</td>
<td>1.76 (0.07-31.5)</td>
<td>1.24 (0.08-46.4)</td>
<td>3.27 (0.02-20.6)</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>4.23 (1.18-14.1)</td>
<td>4.00 (1.21-7.41)</td>
<td>4.81 (0.17-20.6)</td>
</tr>
<tr>
<td>sTNFR1 (ng/ml)</td>
<td>631 (291-4590)</td>
<td>676 (257-1890)</td>
<td>528 (168-1790)</td>
</tr>
<tr>
<td>sTNFR2 (ng/ml)</td>
<td>9.8 (0.8-750)</td>
<td>12.6 (0.02-640)</td>
<td>9.5 (1.6-500)</td>
</tr>
<tr>
<td>sVCAM1 (ng/ml)</td>
<td>930 (190-13210)</td>
<td>1010 (50-25030)</td>
<td>680 (180-7500)</td>
</tr>
</tbody>
</table>

$a$ $n = 24$. Symbols for differences within the groups: * $P<0.05$, ** $P<0.01$.

Correlations between changes

In a post hoc regression analysis of the whole population (n = 53), the change in CRP was correlated to the change in 15-keto-dihydro-PGF$_{2\alpha}$ ($r = 0.315$, $P = 0.024$) (data not shown). There was no correlation between change in TNF-α and change in 15-keto-dihydro-PGF$_{2\alpha}$.
Paper V

Compliance and tolerance

The compliance, measured as proportion of capsules or tablets eaten out of the prescribed number, was 96%. The treatments were generally well tolerated. However, one subject in group number 6 reported symptoms of gastritis, probably due to the consumption of the COX-2 inhibitor. Half of the subjects reported intestinal symptoms as diarrhoea or flatulence, probably due to the CLA capsules. No negative effects on liver enzymes (aspartate aminotransferase and alanine aminotransferase in serum) were observed.

Three of the subjects were light smokers with a reported smoking rate of one to seven cigarettes per day. Another five persons reported that they were tobacco snuff users, however the amount used is difficult to estimate. No tobacco use was allowed in the morning prior to the examinations. Adjustments for use of tobacco did not alter the results appreciably.

By random the participants in groups 3 and 6 had lower urine concentrations of 15-keto-dihydro-PGF$_{2a}$ at baseline than the participants in the other groups. However, this did not affect the results.

Effects of intervention with \( \alpha \)-tocopherol or COX-2 inhibitor

Supplementation with \( \alpha \)-tocopherol only (groups 2 and 5) during the first and second week of the study did not affect the basal concentrations of urinary 8-iso-PGF$_{2a}$ or 15-keto-dihydro-PGF$_{2a}$ as compared to the control groups (groups 1 and 4) (data not shown). Neither did addition of the COX-2 inhibitor (groups 3 and 6) affect the urinary concentrations of 8-iso-PGF$_{2a}$ or 15-keto-dihydro-PGF$_{2a}$ during weeks one and two, as compared to the control groups (data not shown).

Table 9. Change in urinary concentration of 8-iso-PGF$_{2a}$ and 15-keto-dihydro-PGF$_{2a}$ after supplementation with CLA mix (group 1) and CLA 1012 (group 4), given as means (SD)

<table>
<thead>
<tr>
<th></th>
<th>Week 2</th>
<th>Week 6</th>
<th>Change (%)</th>
<th>P for diff. within the group</th>
<th>P for diff. between groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLA mix (group 1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-iso-PGF$_{2a}$ (nmol/mmol crea)</td>
<td>0.24 (0.09)</td>
<td>0.65 (0.31)</td>
<td>171</td>
<td>&lt;0.0001</td>
<td>0.035</td>
</tr>
<tr>
<td>15-keto-dihydro-PGF$_{2a}$ (nmol/mmol crea)</td>
<td>0.46 (0.14)</td>
<td>0.79 (0.38)</td>
<td>72</td>
<td>0.024</td>
<td>0.028</td>
</tr>
<tr>
<td>CLA 1012 (group 4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-iso-PGF$_{2a}$ (nmol/mmol crea)</td>
<td>0.19 (0.02)</td>
<td>1.07 (0.74)</td>
<td>463</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>15-keto-dihydro-PGF$_{2a}$ (nmol/mmol crea)</td>
<td>0.43 (0.12)</td>
<td>1.10 (0.40)</td>
<td>156</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>
Lipid peroxidation induced by different CLA isomer preparations

The urinary concentrations of both 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ increased in the CLA mix group (group 1) as well as in the CLA 1012 group (group 4) (Table 9). However, the supplementation with CLA 1012 gave a larger increase in 8-iso-PGF$_{2\alpha}$ than did CLA mix. Similarly, the increase in 15-keto-dihydro-PGF$_{2\alpha}$ was greater after CLA 1012 than after the CLA mix supplementation (Table 9).

![Graph A](image1.png) 8-iso-PGF$_{2\alpha}$

![Graph B](image2.png) 15-keto-dihydro-PGF$_{2\alpha}$

![Graph C](image3.png) 8-iso-PGF$_{2\alpha}$

![Graph D](image4.png) 15-keto-dihydro-PGF$_{2\alpha}$

*Figure 7.* Change in urinary concentration of 8-iso-PGF$_{2\alpha}$ (A and C) and 15-keto-dihydro-PGF$_{2\alpha}$ (B and D) calculated as differences at 2 and 6 weeks of supplementation, given as mean and SEM.

CLA supplementation with addition of α-tocopherol or COX-2 inhibitor

Addition of α-tocopherol did not affect the formation of urinary 8-iso-PGF$_{2\alpha}$ (Figure 7 A and C) or 15-keto-dihydro-PGF$_{2\alpha}$ (Figure 7 B and D) induced by supplementation with CLA mix (group 2) or CLA 1012 (group 5).

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Subjects given CLA 1012 in combination with COX-2 inhibitor (group 6) had a smaller increase in urinary 15-keto-dihydro-PGF$_{2\alpha}$ as compared to the increase in the control group, group 4, that only got the CLA 1012 ($P = 0.007$) (Figure 7 D). However, the COX-2 inhibitor did not affect the formation of urinary 8-iso-PGF$_{2\alpha}$ or 15-keto-dihydro-PGF$_{2\alpha}$ induced by the CLA mix (group 3) (Figure 7 A and B). Neither did the COX-2 inhibitor affect the CLA 1012 induced concentrations of 8-iso-PGF$_{2\alpha}$ (group 6) (Figure 7 C). There were no differences in the changes of plasma concentrations of 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ after CLA mix or CLA 1012 supplementation in combination when combined with the COX-2 inhibitor (groups 3 and 6) (data not shown).

Serum concentrations of tocopherols
Supplementation with 200 mg α-tocopherol during the first two weeks (groups 2 and 5) increased the serum concentrations of α-tocopherol (from 1.55 to 2.46 mg/mmol) as compared to control groups 1 and 4 (from 1.53 to 1.56 mg/mmol, $P < 0.0001$ for a difference between supplemented and controls). The serum concentrations of γ-tocopherol decreased (from 0.10 to 0.04 mg/mmol) in the α-tocopherol supplemented subjects as compared to controls (from 0.09 to 0.11 mg/mmol) ($P < 0.0001$ for difference between supplemented and controls) during the first two weeks.
Discussion

15:0 och 17:0 as dietary markers

Our results indicate that 15:0 in serum lipids can be used as a marker for milk fat intake (Paper I). In the present material the proportions of 17:0 in serum cholesterol esters were too low be used as dietary marker. The possibility to use 15:0 and 17:0 as markers for intake of dairy products has also been concluded by others using the fatty acid composition of adipose tissue (45, 118-120) and serum (118, 120).

After intervention with diets rich in milk fat proportions of 15:0 and 17:0 have increased, which confirm their function as biomarkers (13, 121).

Both 15:0 and 17:0 are also present in fish (122-124). Analyses of Baltic sea herring show that concentrations of 15:0 are lower than concentrations of 17:0 (122) However, the absolute amounts of 15:0 and 17:0 from fish are probably small in comparison to the contribution from milk fat. The correlations between 15:0 and acid eicosapentaenio acid (20:5 n-3) and docosahexaenoic acid (22:6 n-3), respectively, could indicate that the 15:0 in serum in the present study is of marine origin. However, it is more likely that this association mirrors an enzymatic competition for desaturases and elongases in the synthesis of long chain PUFA. Another explanation could be that persons who have a higher intake of milk products also tend to have a high intake of fish.

Milk fat and clinical characteristics

We found inverse associations between intake of milk products and fat from milk products and certain risk factors for CHD in elderly men in Paper I. These results are supported by the negative correlations between 15:0 in serum and weight, waist and hip circumferences and BMI, also found in this population. It appears as if the associations between intake of cheese and fat from cheese on the one hand, and clinical characteristics on the other, differs from associations for intake of other milk products and fat from milk products studied in the this study. The explanations of these observations are not yet known.
It is well documented that the SFAs lauric acid (12:0), myristic acid (14:0) and palmitic acid (16:0) are hypercholesterolaemic in controlled interventions studies (20), and milk fat is one of the main dietary sources of saturated fats. Thus, milk products have been proposed to contribute to an increased risk for CHD (21).

One possible explanation for the inverse associations between milk fat intake and risk factors for CHD could be that intake of milk products could be a part of a life style pattern characterised by for example a healthy diet. When adjusting for physical activity the associations remained essentially unchanged, while adjustments for possible confounding factors of the diet such as intake of meat, vegetables, root crops and beer weakened the associations somewhat, although the main findings remained (Table 3). This suggests that the inverse correlations to some extent, but not completely, are explained by other life style factors. Similar observations have been made by Rosell et al., who found that an observed inverse association between dairy fat intake and sagittal abdominal diameter was not notably affected by physical activity and alcohol intake (120).

Similar associations have also been found between intake or markers for intake of milk and milk fat on one hand, and for example reduced risk of acute myocardial infarction (125), lower serum concentrations of cholesterol and Apo B in 15-year-olds (29), the insulin resistance syndrome in overweight (25), stroke and hypertension (30), LDL particle size in men (126) and BMI (127), on the other hand.

Several factors in milk or milk fat have been proposed as possible candidates to explain the inverse relations observed between intake of milk fat and certain risk factors for CHD. Components of milk products that have been mentioned are calcium, potassium, whey protein, bioactive peptides, folic acid and vitamins B6 and B12 (30, 31, 128, 129). However, adjustments for calcium intake did not change the correlations in the present study (Table 3). Another component of milk fat that has been proposed as a possible explanation of the inverse findings is CLA. Possibly, there is/are factor(s) in milk products or fat from milk products that, when eaten habitually for long periods, could affect risk factors for CHD through mechanisms yet unknown.

Even though there have been a number of reports indicating an inverse association between intake of milk fat and risk factors for CHD, the current dietary recommendations of a limited amount of total and saturated fat, and thus fat from milk products, still remains.
Effects of CLA supplementation

Anthropometric variables

After supplementation of CLA to healthy women and men we observed a slight decrease in body fat (Paper II), while there were no changes in BMI, body weight, waist-to-hip ratio or sagittal diameter. Several studies on the effects of CLA on body anthropometric variables have now been published. Two studies, apart from ours, have shown effects on body fat content (130, 131), and one have shown a decreased abdominal obesity (132). However, no studies on humans have shown any effects on body weight (56).

In animal models CLA, and especially trans 10, cis 12, has been found to decrease body fat accumulation. However, the effects seem to differ between species, where body fat content of mice is affected to a higher degree than for example rat, rabbits or pigs (56).

The effects of CLA on adipose tissue in animals have been suggested to be due to an increased energy expenditure / thermo genesis. The corresponding situation could, however, not be confirmed in humans by Zambell et al. (133).

From studies in vitro and in animals CLA has been concluded to affect enzymes involved in fat metabolism. In mice hepatic fatty acid synthase activity (134) and mRNA (135) have been observed to increase, leading to an accumulation of fat in the liver, while fatty acid synthase mRNA in adipose tissue have been reported to decrease (135, 136). Moreover, in mice activities of carnitine palmityltransferase-1, involved in fatty acid oxidation, were increased in muscle and adipose tissue, but not affected in liver (58). Thus, in mice CLA appears to decrease synthesis and increase oxidation of fatty acids in non-hepatic tissues, while hepatic fatty acid synthesis is increased relatively more than the oxidation (134). This gives a net effect of decreased lipid accumulation in adipose tissue, and liver steatosis and increased liver weight (134).

The main part of studies of CLA and body composition in animal models are made in growing animals, while most studies in humans are made in grown up individuals. There is thus a possibility that a part of the differences in effects on body composition are due to diverging responsiveness in growing and mature individuals. An interesting question is whether CLA would have a different effect in humans after for example a weight-loss intervention, or after smoking cessation, to prevent a weight gain that is common in such situations.

Whether there are any antiadipogenic effects by CLA in humans, and how they could be mediated is yet to be found out.
Lipoproteins and apolipoproteins

In Paper II we found an increase in Apo B in the CLA group as compared to the control group, but there were no other effects of CLA supplementation as compared to the control group. We observed a difference between the groups in effects on LDL triacylglycerols. This effect was however explained by a decrease in the control group, and was presumably not related to the CLA intervention.

A few studies on CLA effects in animal models, but not all, have suggested positive effects by CLA on blood lipid concentrations and development of atherosclerosis (56, 137, 138). In humans there are, however, no convincing studies showing positive effects by CLA on blood lipid concentrations. On the contrary, there are data suggesting that especially CLA trans 10, cis 12 may have adverse effects on the serum lipoprotein concentrations. Most published studies have hitherto not shown any significant effects of the mixed isomer CLA preparations on plasma lipid concentrations, although there are some suggestions of potentially negative effects with reductions of the HDL cholesterol concentrations (130, 139). Similar to our study (Paper II), increased concentrations of apo B in very low density lipoproteins (VLDL) was seen in a recent study of diabetic subjects (140). In a one-year supplementation study of overweight subjects the only difference between the CLA supplemented and the control group was an increase of Lp(a) in the group given a free fatty acid CLA preparation (141).

When a CLA preparation containing mainly the trans 10, cis 12 isomer was given to abdominally obese men (139) HDL cholesterol concentrations decreased as compared to the control group, with a similar but less pronounced reduction in the CLA mixture preparation group. Simultaneously there was an increase of VLDL triglycerides \( (P = 0.06) \) while the LDL cholesterol concentrations were un-affected. In a recent study (142) the trans 10, cis 12 isomer impaired the ratio between total cholesterol and HDL as compared to cis 9, trans 11. One study has reported a certain reduction of VLDL cholesterol by the cis 9, trans 11 (143), whereas others did not observe any effects of the cis 9, trans 11 on serum lipid concentrations (144).

Glucose and insulin

In Paper III we observed no effect on insulin concentration, but a tendency to increased glucose concentrations after CLA supplementation compared to the control group \( (P = 0.053) \).

Medina et al. has found increased insulin levels after CLA supplementation (145). However, generally CLA preparations with mixed isomers appear to have limited effects on glucose and insulin metabolism in healthy subjects. There was no significant effect on insulin sensitivity, as reflected by the HOMA index, in healthy men when supplemented for four weeks (132).
In contrast, significantly impaired insulin sensitivity, as measured by HOMA, and higher blood glucose concentrations both in fasting and post-prandially were recently reported when well-controlled diet-treated type-2-diabetic patients were supplemented with a CLA mixture (140).

It is believed that possible negative effects of CLA on glucose homeostasis are mainly related to CLA \( \text{trans} \ 10, \text{cis} \ 12 \). This has been suggested by Risérus et al., who found that insulin sensitivity, measured by the euglycemic, hyperinsulinemic clamp technique, was reduced in obese men after supplementation with \( \text{trans} \ 10, \text{cis} \ 12 \) (139). Similar effects, but less pronounced, were found after supplementation with a preparation containing mainly \( \text{cis} \ 9, \text{trans} \ 11 \), but also smaller amounts of \( \text{trans} \ 10, \text{cis} \ 12 \) in a group of abdominally obese men (146), but not when supplemented with a mixed CLA preparation (139). In a study by Tricon et al. there was no difference in insulin sensitivity, estimated with HOMA, when comparing supplementations with \( \text{trans} \ 10, \text{cis} \ 12 \) and \( \text{cis} \ 9, \text{trans} \ 11 \) (142).

Serum fatty acid composition and estimated desaturase activities

The changes in fatty acid composition of serum phospholipids, cholesterol esters and triacylglycerides induced by the CLA intervention correspond to a decreased \( \Delta-9 \) activity (18:1 n-9/18:0), which has been observed previously in experimental animals (147, 148). A decrease in the activity of \( \Delta-9 \) desaturase has been suggested to be due to an inhibitory effect of CLA on the mRNA expression of stearoyl-CoA desaturase, an enzyme catalyzing the \( \Delta-9 \) desaturation (147, 148). This effect has been ascribed \( \text{trans} \ 10, \text{cis} \ 12 \) (147, 149).

The estimated \( \Delta-5 \) desaturase activity increased in the CLA group compared to the control group. The \( \Delta-5 \) desaturase activity in humans has been suggested to be inversely related to proportion of body fat (150), insulin levels (12) and risk for myocardial infarction (151). The \( \Delta-6 \) desaturase activity (18:3n-6/18:2n-6) decreased after CLA treatment, as observed earlier in \textit{in vitro} rat liver microsomes (148). The increased proportion of docosapentaenoic acid (22:5n-3) and the unchanged docosahexaenoic acid (22:6n-3) supports the indications of a decreased \( \Delta-6 \) desaturase activity.

The altered fatty acid profile of the serum lipids and thrombocytes, especially the decreased proportions of dihomo-\( \gamma \)-linolenic acid (20:3n-6) and increased activity of \( \Delta-5 \) desaturase may lead to an altered eicosanoid metabolism. However, the proportions of arachidonic acid (20:4 n-6) and eicosapentaenoic acid (20:5 n-3) were unchanged.
Lipid peroxidation induced by CLA

To our knowledge, our study was the first to demonstrate that high doses of CLA induce lipid peroxidation (Paper III). The CLA induced lipid peroxidation was indicated as increases urine concentrations of 8-iso-PGF$_2$α, a marker for free radical-induced lipid peroxidation, and 15-keto-dihydro-PGF$_2$α, a marker for enzymatic lipid peroxidation. These findings have later on been confirmed in Paper V and by Risérus et al. (152). The latter also observed a normalisation of both 8-iso-PGF$_2$α and 15-keto-dihydro-PGF$_2$α concentrations within two weeks after cessation of CLA supplementation (152). This normalisation suggests that supplementation with CLA can initiate eicosanoid formation as long as the intake continues. The normalised eicosanoid concentrations four weeks after termination of CLA supplementation indicate that no accumulation of eicosanoids has been obtained.

In both the study in Paper V and in studies by Risérus et al. (146, 153) the CLA induced increase in both 8-iso-PGF$_2$α and 15-keto-dihydro-PGF$_2$α were greater from CLA trans 10, cis 12 than from the mixed CLA preparations, or capsules containing mainly cis 9, trans 11. The increases seen after trans 10, cis 12 supplementations were approximately twice as high as after intervention with preparation containing approximately half as much of trans 10, cis 12.

Previous observations have suggested that CLA preparation containing mainly either cis 9, trans 11 or trans 10, cis 12 both have decreased insulin sensitivity in obese men (139, 146). Interestingly, the increases in 8-iso-PGF$_2$α observed in these studies were positively correlated to the aggravated insulin resistance, suggesting a possible link between lipid peroxidation and insulin resistance.

The observations of increased free radical-induced lipid peroxidation, estimated with 8-iso-PGF$_2$α, are in contrast to the suggestion that CLA in vitro has antioxidative properties (64-66). Moreover, our findings of larger inductions of lipid peroxidation after CLA trans 10, cis 12 stand in contrast to the suggestion that trans 10, cis 12 is a stronger antioxidant in vitro than cis 9, trans 11 or α-tocopherol (154). These differences are probably due to the differences in models used, and should be investigated further.

When investigating possible mechanisms for the increase in 15-keto-dihydro-PGF$_2$α by giving CLA trans 10, cis 12 in combination with a COX-2 inhibitor we observed a less increase in 15-keto-dihydro-PGF$_2$α (Paper V). This suggests that the CLA induced increase in 15-keto-dihydro-PGF$_2$α partly is mediated via COX-2. We did not observe any effect on eicosanoid formation when combining CLA mix preparation with COX-2 inhibitor. This is probably due to the lower increase in 15-keto-dihydro-PGF$_2$α seen after CLA mix than after CLA trans 10, cis 12 supplementation.

α-Tocopherol is a chain breaking antioxidant preventing lipid peroxidation in membranes by scavenging free radicals (155). We wanted to investi-
gate whether the CLA induced lipid peroxidation could be decreased by \( \alpha \)-tocopherol. However, we did not observe any difference in increase of 8-iso-PGF\(_{2\alpha} \) or 15-keto-dihydro-PGF\(_{2\alpha} \) when giving CLA in combination with \( \alpha \)-tocopherol (Paper V). From these results we cannot draw any conclusions regarding the ability of \( \alpha \)-tocopherol to affect CLA induced lipid peroxidation, possibly partly due to a low statistical power. In an experimental design, \( \alpha \)-tocopherol in extremely high doses has previously been observed to reduce both 8-iso-PGF\(_{2\alpha} \) and 15-keto-dihydro-PGF\(_{2\alpha} \) during hepatic oxidative injury in rats (156).

During the initial two weeks of the intervention study in Paper V, when giving the subjects \( \alpha \)-tocopherol but no CLA, we did not see any effects on markers of lipid peroxidation, which is in line with previous reports (157, 158). However, a decreased concentration of 8-iso-PGF\(_{2\alpha} \) was seen after supplementation with 400 IU/d of \( \alpha \)-tocopheryl acetate to non-smokers (159).

In Paper III we observed an increase in \( \gamma \)-tocopherol after CLA supplementation, compared to the control group. This could possibly be due to a compensatory increase in the antioxidative defence in the situation of increased lipid peroxidation. Another possible explanation could be that the decrease in body fat could lead to a concomitant release of \( \gamma \)-tocopherol from the adipose tissue (A. Kamal-Eldin, personal communication November 2004).

### Effect of CLA on markers of inflammation

15-Keto-dihydro-PGF\(_{2\alpha} \) has been shown to be elevated in various acute and chronic diseases and in experimental studies of inflammation (70, 86, 160). Following the observations that CLA induces profound increases in 15-keto-dihydro-PGF\(_{2\alpha} \) we wanted to further study the effects on other markers of inflammation. We analysed serum concentrations of CRP, TNF-\( \alpha \), TNF receptors 1 and 2, and VCAM-1 before and after the 12-week supplementation with the mixed CLA preparation. The CRP concentrations increased, but there were no changes in TNF-\( \alpha \), TNF receptors or VCAM-1 in the CLA group compared to the control group. Interestingly, the change in CRP was correlated to the change in 15-keto-dihydro-PGF\(_{2\alpha} \).

Results from previous studies of the effects of CLA on parameters of inflammation have been diverging. In animal and \textit{in vitro} models CLA have been suggested to have anti-inflammatory and / or immune ameliorating effects (67). In addition to the study in Paper IV, only a limited number of studies performed in humans have shown effects on inflammation. Risérus \textit{et al.} found increases in CRP in obese men after supplementation with CLA \textit{trans} 10, \textit{cis} 12 (153). However, Moloney and co-workers did not find any effects on CRP or IL-6 in diabetic subjects (140). Neither did Tricon \textit{et al.}
observe any effect on CRP or \textit{ex vivo} cytokine production (161). Kelley \textit{et al.} did not find any change in indices of immune status in healthy women of a mixture of CLA isomers (162). Albers \textit{et al.} observed beneficial effects on initiation of specific response to hepatitis B vaccination of healthy women after CLA supplementation (163). However, the same group did not observe any effects TNF-\(\alpha\) response in \textit{ex vivo} mononuclear cells (163).

So far, effects of CLA on markers of inflammation are inconclusive and must be investigated further.

\section*{Concluding remarks}

This thesis adds knowledge about the associations between intake of milk fat and risk factors for coronary heart disease, and about the effects of supplementation with CLA to healthy humans.

We can conclude that the saturated fatty acid pentadecanoic acid (15:0) in serum lipids can be used as a marker for intake of fat from milk products. Moreover, the intake of fat from milk products is inversely related to certain risk factors for coronary heart disease, especially to anthropometric variables, in a group of elderly men. What explains inverse associations between risk factors for CHD and intake of milk products and fat from milk products is not yet known. It could possibly partly be a result of other coherent lifestyle factors, or it could be due to a component of milk or milk fat that, if habitually eaten during long periods of life, could have favourable effects of the human body. However, until these questions are answered it must be emphasised that current knowledge advise the general population to have a limited intake of total and saturated fat and instead choose unsaturated fats, and to increase the intake of fruits and vegetables.

From the CLA supplementation studies we learnt that the CLA supplementation decreased the proportion body fat marginally, while there essentially were no other effects on anthropometric variables or serum lipids. We observed remarkable increases in lipid peroxidation, which was higher after a preparation containing mainly CLA \textit{trans} 10, \textit{cis} 12 than after the preparation with a mixture of isomers. Further, we observed that CLA supplementation increased C-reactive protein concentrations, a marker for acute phase inflammation.

We could not determine whether CLA is the explaining factor behind the inverse associations between intake of milk products and fat from milk products. Intervention studies with high CLA doses rather indicate opposite effects, with increased markers of inflammation, increased lipid peroxidation and decreased insulin sensitivity. However, we do not know the effects of CLA as a component of the diet, or in lower doses, during longer periods.

Continued controlled intervention studies in humans are needed to further explore the effects of CLA and the isomers. When comparing results from
studies of the effects of CLA, the results seem to be largely depending on the model used, and therefore the inference of results must be made with caution. There is, however, to date not medical reasons for humans to take CLA as supplements.
Conclusions

From the present studies we conclude that:

- The saturated fatty acid pentadecanoic acid (15:0) can be used as a marker for intake of milk products and fat from milk products (Paper I).

- Intake of milk products and milk fat was inversely related to anthropometry and some risk factors for cardiovascular disease in 70-year-old men (Paper I).

- CLA slightly reduced the relative amount of body fat in healthy women and men, but did not affect other anthropometric measurements or serum lipid concentrations (Paper II).

- CLA increased the urinary and plasma concentrations of 8-iso-PGF$_{2\alpha}$, a marker of free radical-induced lipid peroxidation, and urinary concentrations of 15-keto-dihydro-PGF$_{2\alpha}$, an indicator for enzymatically mediated lipid peroxidation (Paper III).

- The CRP concentration increased after CLA supplementation, but there was no effect on TNF-α, TNF-α receptors 1 and 2 or VCAM-1. The change in CRP was positively correlated to the change in 15-keto-dihydro-PGF$_{2\alpha}$ (Paper IV).

- The magnitude of the increase in 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ seen after CLA supplementation seems to differ between CLA isomers. Capsules containing mainly trans 10, cis 12 induced a larger increase in 8-iso-PGF$_{2\alpha}$ and 15-keto-dihydro-PGF$_{2\alpha}$ than did supplementation with a mixture of CLA isomers (Paper V).

- The CLA induced increase in 15-keto-dihydro-PGF$_{2\alpha}$ is suggested to partly be mediated via cyclooxygenase-2 (Paper V).
Sammanfattning på svenska (Summary in Swedish)

Förekomsten av hjärt-kärlsjukdom och diabetes typ 2 (åldersdiabetes) ökar nu världen över. Ökar gör även bukfetma, insulinresistens, felaktiga blodfettsvärden och högt blodtryck, sjukdomar som ingår i det som kallas metabola syndromet. Utvecklingen av de nämnda sjukdomarna påverkas både av ärtlighet och livsstilsfaktorer. Bland livsstilsfaktorer som påverkar risken för hjärt-kärlsjukdom kan nämnas motion, rökning och matvanor.

Det är väl känt att både mängden fett i kosten och fettets sammansättning är av betydelse för utvecklandet av hjärt-kärlsjukdom. Mättat fett har ofta relaterats till en sämre insulinaktivitet och till förhöjda nivåer av både total mängd kolesterol i blodet och till LDL-kolesterol, det som ibland kallas ”det onda kolesterolet”. I studier där man bytt ut mättat fett mot enkel- eller fleromättat fett har man däremot sett att både insulinaktivitet och blodfetten förbättras. Det anses idag, inom vissa gränser, att sammansättningen av fettet i kosten är viktigare än den totala mängden, när det gäller förebyggande av hjärt-kärlsjukdom. De allra flesta av oss bör alltså konsumera en begränsad mängd mättat fett och istället välja matvaror som innehåller omättat fett.

Vid studier av kost och hälsa är korrekt information om matvanor av stor betydelse, men någon helt rättvisande kostundersökningsmetod finns inte idag. Objektiva dietära markörer kan däremot öka möjligheterna att tolka information från kostundersökningar. Fettsyrasammansättningen i t ex blod och fettväv har visat sig reflektera fettsyrasammansättningen i kosten. I mjölkfett finns två mättade fettsyror som är relativt specifika för fett från idisslande djur, pentadekansyra (15:0) och heptadekansyra (17:0). De består av ett udda antal kolatomer och kan därför inte syntetiseras i människokroppen. Vi har i en tvärsnittsstudie av svenska 70-åriga män kunnat bekräfta att koncentrationerna av 15:0 och 17:0 i serum kan användas som dietära markörer för intag av fett från mjölkprodukter (artikel I).

En kost rik på mättat fett har, som nämnts ovan sedan länge förknippats med en förhöjd risk för hjärt-kärlsjukdom. Mjölkprodukter är en av de stora källorna av mättat fett i den västerländska kosten. Man har därför föreslagit att det finns ett samband mellan intag av mjölkprodukter och eb ökad risk för hjärt-kärlsjukdom. Vi har i en tvärsnittsstudie av en grupp 70-åriga svenska män undersökt hur intag av mjölkprodukter samt fett från mjölkprodukter

De oväntade sambanden påminde om effekter som observerats när man givit djur en fettsyra som finns bland annat i mjölkfett, konjugerad linolsyra (CLA). Detta ledde oss vidare till att studera effekterna av supplementering med CLA till människor. CLA är en fettsyra med samma kemiska sammansättning som linolsyra (18:2), som är en av de vanligare omättade fettsyrorna i kosten. CLA skiljer sig från linolsyra genom att de två dubbelbindningarna i CLA sitter närmare samman, dvs. är konjugerade. CLA lanseras idag som ett fetmareducerande ”kosttillskott”. De påstådda effekterna är huvudsakligen baserade på studier av djur och celler (in vitro-studier), och effekterna på människor är ännu inte fullt kartlagda.

I en dubbel blind, placebo kontrollerad interventionsstudie gav vi 4,2 g/dag av CLA eller kontrollolja till friska kvinnor och män under 12 veckor. Denna interventionsstudie ligger till grund för resultaten i artiklarna II, III och IV.

I artikel II undersökte vi effekterna av CLA på variabler som kroppsvikt, BMI, bukfetma, andel kroppsfett, blodfetter, fettsyrasammansättning i blodet, nivåer av socker och insulin i blodet och antropometriska mått (kroppsmått). Vi såg en marginell minskning av kroppsfettandel, men ingen effekt på kroppsvikt, BMI, mått på bukfetma, blodfetter, blodsocker eller insulin i CLA gruppen jämfört med kontrollgruppen.


I artikel IV ville vi undersöka hur CLA-supplementering påverkar andra markörer för inflammation, utöver 15-keto-dihydro-PGF2α. Markörerna vi använde var C-reaktivt protein (CRP), tumör nekros faktor-α (TNF-α), receptorerna för TNF-α (TNFR1 och TNFR2) och vaskulär celladhesions molekyl-1 (VCAM-1). CLA ökade nivåerna av CRP, men påverkade inte TNF-
α, TNFR1, TNFR2 eller VCAM-1. Detta tyder på att CLA-supplementering kan öka CRP, som är en markör för inflammation.

I den femte artikeln (V) ville vi undersöka bakgrunden till höjningen av 8-iso-PGF$_{2\alpha}$ och 15-keto-dihydro-PGF$_{2\alpha}$ vi sett i artikel III. Vi genomförde en ny randomiserad, kontrollerad interventionsstudie med tre parallella grupper. Grupp ett supplementerades med CLA, och var kontrollgrupp. Grupp två fick CLA och α-tokoferol (vitamin E), en antioxidant som skulle kunna hämma den friradikalmedierade lipidperoxidationen. Grupp tre fick CLA och en hämmer av enzymet cyklooxygenas-2, för att möjliggöra den enzymatiskt medierade lipidperoxidationen. Vi ville i denna studie även undersöka om olika isomerer (varianter) av CLA har olika effekt på lipidperoxidation, och använde två olika beredningar av CLA-kapslar. Från den här studien sammanfattade vi att den CLA-inducerade ökningen av 15-keto-dihydro-PGF$_{2\alpha}$ till viss del troligen är medierad via cyklooxygenas-2, och att CLA-isomeren trans 10, cis 12 ger en större lipidperoxidation än en blandning av CLA-isomerer.

Sammanfattning


Våra studier av effekterna av CLA-supplementering tyder inte på att det finns några goda effekter av CLA i de ganska stora mängder vi givit våra deltagare. Andra studier i vår grupp har visat att CLA till och med sänker insulininkänsligheten i människor med bukfetma. Vad de ökade nivåerna av markörer för lipidperoxidation och inflammation betyder vet vi ännu inte. Fortsatta studier av effekterna av CLA på människor behövs för att utreda dessa effekter vidare. Enligt den samlade kunskapen idag finns det ingen medicinsk anledning att åta CLA som tillskott.
Thank you!

I wish to express my sincere gratitude and appreciation to everyone who has contributed to this thesis. Especially I would like to thank:

*Bengt Vessby*, my supervisor, for encouraging me, for letting me share your deep knowledge on clinical nutrition research and scientific thinking, and for introducing me to the fascinating world of fatty acids.

*Samar Basu*, my supervisor, for personal engagement and for sharing your endless knowledge and ideas. Thank you also for being such an excellent travel company, for all our long discussions about fishing and small island in the archipelago, and for letting me get acquainted with your nice family.

*Marianne Carlsson*, head of the Department for Public Health and Caring Sciences, for being an excellent boss.

*Hans Lithell and Lars Lannfelt*, former and current heads of the Unit for Geriatrics for giving me the opportunity to carry out my studies at the department and for creating a productive atmosphere.

All the *subjects* for your enthusiasm and interest, and for eating lots of large, brown capsules.

*Siv Tengblad, Eva Sejby and Barbro Simu* for all laboratory analyses, for your nice company, and for contributing to a nice atmosphere at the department.

*The staff at former metabolic ward*, for good collaboration and for help with blood samplings and examinations.

*Rawya Mohsen* for excellent help with data management, my computer and very nice company during coffee breaks.

*Brita Karlström Ledins* for your warmth and kindness and for being a skilful nutritionist to look up to.

*Lars Berglund* for statistical advice and fun efterfester.

*Inga-Britt Gustafsson* for your encouragement in the initial stages of my work, and for valuable advice on dietary assessments.

*Johanna Helmersson, Agneta Andersson, Cecilia Nälssén, Achraf Daryani, Eva Södergren, Anette Järv, Ulf Risérus and Anders Sjödin*, my friends and current or former colleagues at the Unit for Clinical Nutrition Research, for our interesting discussions during nutrition seminars, for creating a nice atmosphere in our research group, and for all our nice coffee breaks.
Liisa Byberg for many wise words regarding research, parenthood, knitting and other essential things, but most of all for being my friend.

My dear friend Erika Olsson, who made me stay in Uppsala and made me enjoy my stay.

Margareta Öhrvall and Merike Boberg your support and discussions about science and life.

Roger Olsson for nice collaborations and help with the bio-impedance analyser.

Karin Modin and Inger Stenström for current and former help with administrative matters.

Friends and current or former colleagues at the Unit for Geriatrics: Ann-Cristin Åberg (especially for the semla!), Per-Erik Andersson, Kristina Björklund, Kristina Dunder, Klara Edlund Halvarsson, Anu Hedman, Arvo Hänni, Lena Kilander, Richard Reneland, Claes Risinger, Kristina Ström-Möller, Johan Sundström, Bernice Wiberg, Martin Wohlin, Björn Zethelius and Johan Årnlöv for fruitful discussions and nice company during coffee breaks.

The dieticians Susanne Fredén, Marie Lemcke, Agneta Nilsson, Britt Stålnacke, Marie von Post Skagegård and Helena Peterson and the cook Marina Spoverud Älvebratt for nice and inspiring discussions about dietetics, nutrition and lot of other things.

Sköna Fröjden for enjoyable and productive meetings.

Bertil, Annika, Paola, Gustav, Oscar och Bobo för ert intresse för om det är Bregott eller Lätta som gäller.

Nina, Min Allra Käraste Syster, för allt. Nu kommer jag och målar!

Gustaf, min bror, för omtanke och bl a datorhjälp.

Mamma Karin för ditt genuina intresse för mitt ämne och för barnvakten bittida och sent.

Pappa Kristers, som alltid trodde att jag kunde och nog skulle ha tyckt att det här var kul.

Anna-Karin och Einar, kära svärföräldrar, för ert intresse för mina studier och för att ni hållit med både barnvakta och skrivarstuga.

Ebba för att du visat mig vad som egentligen är viktigt, och bidragit genom att dra mitt intresse ifrån avhandlingen.

Min älskade Sten för ditt stora stöd, din uppmuntran och ditt intresse för mitt ämne. – Även om du påstod att 17:0 heter stearinsyra!
Grants

This work was financially supported by the Ernfors Foundation, Mary, Åke and Hans Ländell’s Foundation, the Swedish Dairy Association, the Swedish Diabetes Foundation, Swedish Fund for Industrial and Technical Development, the Swedish Medical Association, and the Swedish Medical Research Foundation. We also thank Natural Lipids Ltd AS, Norway, for supplying the CLA preparations.
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