UNIVERSITY OF TURKU

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The Cardiovascular Risk in Young Finns Study

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To My Family

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ABSTRACT

Background: The links between fatty acids (FAs) and cardiometabolic outcomes are topics of debate. There is a lack of data, from childhood to adulthood, regarding the links between dietary or serum FA composition and cardiometabolic health. The intake of omega-6 (n-6) polyunsaturated FAs (PUFAs) has been linked to both inflammation and oxidative reactions, which are potentially adverse states with regards to cardiometabolic health.

Aims: To study at a population level: 1) how FA intake (% from the total FAs) is associated with the corresponding serum FA proportions (% from the total FAs); whether serum FA proportions or FA intake in childhood are associated with 2) blood pressure or 3) carotid artery intima media thickness (cIMT) in adulthood; 4) how the adulthood FA composition affects certain cardiometabolic outcomes, such as obesity, insulin resistance (HOMA-IR), blood pressure or non-alcoholic fatty liver in adulthood; and 5) whether adulthood serum FA proportions are associated with inflammation and/or LDL oxidation.

Methods: Baseline of the Young Finns Study was conducted in 1980 (3–18 years old children, n=3596). Adulthood follow-ups for clinical data and outcomes were conducted in 2001 (n=2284), 2007 (n=2204) and/or 2011 (n=2063). Serum cholesteryl ester (CE) FA proportions were measured in 1980 and serum total FA proportions in 2001. The intake of FAs was characterized by a 48-h recall.

Results: In childhood (1980), dietary intake and serum CE proportions of FAs correlated well with each other (r=0.30 for saturated FAs (SFAs), r= -0.19 for monounsaturated FAs (MUFAs) and r=0.57 for PUFAs). Childhood CEFAs were associated with adult blood pressure in both sexes and with cIMT in females 27 years later in adulthood (1980->2007). Serum SFA, MUFA and omega-3 (n-3) PUFA proportions showed direct and omega-6s (linoleic acid, 18:2n-6, in particular) inverse associations. Dietary data, i.e. the P/S ratio (PUFAs/SFAs) and the intake of SFAs, exhibited links which were in line with these serum-based findings.

In adulthood (2001), dietary intake and serum total proportions of FAs correlated only weakly with each other, omega-3 PUFAs being an exception (r=0.40). Serum SFA and MUFA proportions showed direct, and omega-6s (+omega-3s borderline significantly) inverse links with prevalent obesity, high HOMA-IR and/or blood pressure in multivariable models. With regard to individual FAs, γ-linolenic (18:3n-6), dihomo-γ-linolenic (20:3n-6) and eicosatetraenoic acids (20:4n-3) displayed direct associations (especially with obesity), whereas the links with linoleic acid were inverse. Adulthood FA intake data did not support these outcome findings. An association profile for the corresponding incident outcomes including fatty liver (2001->2011) was weaker and in many cases statistically non-significant, but its trends were very similar as compared to the cross-sectional data. In addition, serum SFA and MUFA proportions were directly and PUFAs, including omega-6s, inversely associated with C-reactive protein levels and oxidized LDL lipids or proteins in adults (2001 data).

Conclusions: These data suggest that childhood and adulthood serum FA proportions are associated with adulthood cardiometabolic outcomes. Serum SFAs are associated with a higher outcome risk, whereas PUFAs, omega-6s and particularly linoleic acid are associated with a lower risk. The role of omega-3 PUFAs and MUFAs remains unclear. These data support dietary recommendations to replace SFAs partly with unsaturated FAs rich in omega-6 PUFAs already in childhood to improve an individual's cardiometabolic health.

KEYWORDS: Cardiometabolic diseases, desaturation degree, diet, longitudinal study, serum fatty acids, metabolism, risk factors

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TIIVISTELMÄ

Tausta: Rasvahappojen (RH) terveysvaikutukset ovat jatkuvan keskustelun aihe. Tällä hetkellä ei ole olemassa lapsuudesta aikuisuuteen ulottuvaa tietoa RH:jen kardiometabolisista terveysvaikutuksista. Omega-6 (n-6) monityydyttymättömät RH:t (PUFA) on liitetty tulehdusreaktioihin ja hapetusstressiin, jotka ovat kardiometabolisten sairauksien riskitekijöitä.

Tavoite: tutkia väestöaineistossa: 1) Miten RH:jen saanti ravinnosta (% RH:jen kokonaissaannista) heijastuu seerumin RH%-koostumukseen; onko lapsuuden seerumi-näytteiden RH-koostumus tai RH:jen saanti ravinnosta yhteydessä 2) verenpaineeseen tai 3) kaulavaltimon intima-median paksuuteen (cIMT) aikuisiässä 4) liittyykö aikuisuuden RH-koostumus kardiometabolisiin riskitekijöihin/sairauksiin kuten lihavuus, insuliini-resistenssi (HOMA-indeksi), verenpaine ja ei-alkoholiperäinen rasvamaksa 5) selittääkö seerumin RH-koostumus aikuisuudessa elimistön tulehdus- ja/tai LDL:n hapettumis-reaktioita.

Menetelmät: Lasten Sepelvaltimotaudin Riskitekijät-tutkimus (LASERI) alkoi vuonna 1980 (tutkittavat 3–18-vuotiaita, n=3596). Aikuisiän kliinisen terveydentilan tutkimukset suoritettiin vuosina 2001 (n=2284), 2007 (n=2204) ja/tai 2011 (n=2063). Seerumin kolesteroliesteri (KE)-fraktion RH-koostumus määritettiin vuonna 1980 ja seerumin kokonais-RH-koostumus vuonna 2001 otetuista näytteistä. RH-saanti ravinnosta perustui edeltävän 48-tunnin ruuankäyttöön.

Tulokset: Lapsuuden (1980) RH-saanti ravinnosta oli yhteydessä seerumin KE-fraktion RH-koostumuksen kanssa (r=0.30 tyydyttyneille RH:ille (SFA), r= -0.19 kertatyydyttymättömille RH:ille (MUFA) ja r=0.57 PUFA:lle). KE-fraktion RH-osuudet olivat yhteydessä verenpaineeseen (pojat+tytöt) sekä kaulavaltimon intima-median paksuuteen (tytöillä) aikuisiässä 27 vuotta myöhemmin siten, että MUFA, SFA ja omega-3 (n-3)-osuudet olivat yhteydessä lisääntyneeseen ja omega-6 (linolihappo, 18:2n-6) alentuneeseen verenpaine- ja cIMT-riskiin. Ravitsemusdata, P/S-suhde (PUFA/SFA) ja SFA-saanti, tukivat edellä mainittuja seerumilöydöksiä.

Aikuisiässä (2001), RH-saanti ravinnosta oli ainoastaan heikosti yhteydessä seerumin kokonais-RH-koostumuksen kanssa, omega-3 RH:illa oli kuitenkin vahva yhteys (r=0.40). Seerumin SFA- ja MUFA-osuudet osoittivat suoria ja omega-6 (+omega-3 lähes tilastollisesti merkitsevästi joissakin malleissa) käänteisiä yhteyksiä lihavuuteen, HOMA-indeksiin, ja/tai verenpaineeseen monimuuttuja-malleissa. Yksittäisistä RH:ista γ-linoleeni (18:3n-6)-, dihomo-γ-linoleeni (20:3n-6)- ja eikosatetraeenihappo (20:4n-3) osoittivat suoria ja linolihapppo käänteisiä yhteyksiä, erityisesti lihavuuden kanssa. RH:jen dieetti-data ei tukenut näitä seerumipohjaisia havaintoja. Vastaavat insidenssi-yhteydet, mukaan lukien rasvamaksa-yhteydet (2001 vs. 2011), olivat ennustavuus-trendiltään samansuuntaisia, mutta useimmista yhteyksistä puuttui tilastollinen merkitsevyys. Lisäksi, seerumin SFA- ja MUFA-osuudet olivat yhteydessä lisääntyneeseen C-reaktiivisen proteiinin ja LDL:n hapettuneiden lipidien ja proteiinien määrään, mutta vastaavat PUFA- ja omega-6 PUFA-yhteydet olivat käänteisiä (2001 data).

Johtopäätökset: Tutkimuksen havainnot tukevat sitä, että lapsuuden ja aikuisuuden seerumin RH-koostumus on yhteydessä aikuisiän kardiometabolisiin riskitekijöihin, SFA lisääntyneeseen ja PUFA, omega-6-RH:t ja erityisesti linolihappo alentuneeseen riskiin. Omega-3- ja MUFA-rasvahappojen vaikutukset jäävät epäselviksi. Tulokset tukevat suosituksia tyydyttyneen rasvan osittaiseksi korvaamiseksi runsaasti omega-6-rasvoja sisältävällä tyydyttymättömällä rasvalla jo lapsuusiässä kardiometabolisen terveyden parantamiseksi.

AVAINSANAT: kardiometaboliset sairaudet, metabolia, riskitekijät, seurantatutkimus, ruokavalio, seerumin rasvahapot, tyydyttymättömyysaste

Table of Contents

Abbreviations

BMI	Body mass index
BP	Blood pressure
CE	Cholesteryl ester
CEFA	Cholesteryl ester fatty acid
CHD	Coronary heart disease
cIMT	Common carotid artery intima media thickness
CVD	Cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DBP	Diastolic BP
DISC	Dietary Intervention Study in Children
E%	% of energy intake
FA	Fatty acid
HDL	High-density lipoprotein
HOMA-IR	Homeostatic model-based insulin resistance
KANWU	Kuopio, Aarhus, Naples, Wollongong and Uppsala Study
LDL	Low-density lipoprotein
MUFA	Monounsaturated FA
n-3 (n-3) PUFA	Omega-3 polyunsaturated FA
n-6 (n-6) PUFA	Omega-6 polyunsaturated FA
PL	Phospholipid
P/S ratio	Dietary PUFA/SFA ratio
PUFA	Polyunsaturated FA
RCT	Randomized clinical trial
SBP	Systolic BP
SD	Standard deviation
SFA	Saturated FA
STRIP	Special Turku coronary Risk factor Intervention Project
TGs	Triglycerides
T2D	Type 2 diabetes
UFA	Unsaturated fatty acid
YFS	the Cardiovascular Risk in Young Finns Study

FAs

14:0	Myristic acid
15:0	Pentadecanoic acid
16:0	Palmitic acid
16:1n-7	Palmitoleic acid
17:1n-7	Heptadecaenoic acid
18:0	Stearic acid
18:1n-9	Oleic acid
18:1n-7	Octadecenoic acid
18:2n-6	Linoleic acid (LA)
18:3n-6	γ-Linolenic acid
18:3n-3	α-Linolenic acid (ALA)
20:1n-9	Eicosenoic acid
20:2n-6	Eicosadienoic acid
20:3n-6	Dihomo-γ-linolenic acid
20:4n-6	Arachidonic acid (AA)
20:4n-3	Eicosatetraenoic acid
20:5n-3	Eicosapentaenoic acid (EPA)
22:1n-9	Docosenoic acid
22:4n-6	Docosatetraenoic acid
22:5n-3	Docosapentaenoic acid (DPA)
22:6n-3	Docosahexaenoic acid (DHA)

Definitions

Cardiometabolic outcome = obesity, higher homeostatic model-based insulin resistance, hypertension, elevated systolic or diastolic BP, non-alcoholic fatty liver or higher cIMT

C-reactive protein (CRP) = an acute-phase protein of hepatic origin whose concentration increases in response to acute or chronic inflammation.

Essential FAs = PUFAs that are necessary for growth and normal physiological functions, but cannot be synthesized in the body (linoleic acid and α-linolenic acid).

Non-essential FAs = the body is capable of synthesizing non-essential FAs.

oxLDLlipids = a measure of the concentration of conjugated dienes in LDL lipids

oxLDLprot = a measure of the level of oxidized apolipoprotein B-100 in LDL particles

List of Original Publications

This thesis is based on the following original publications, which are referred to in the text by Roman numerals. Previously unpublished data are additionally presented.

I **Kaikkonen JE**, Jula A, Mikkilä V, Viikari JS, Moilanen T, Nikkari T, Kähönen M, Lehtimäki T, Raitakari OT. Childhood serum cholesterol ester fatty acids are associated with blood pressure 27 y later in the Cardiovascular Risk in Young Finns Study. Am J Clin Nutr 2012; 95:1422–31.

II **Kaikkonen JE**, Jula A, Mikkilä V, Juonala M, Viikari JS, Moilanen T, Nikkari T, Kähönen M, Lehtimäki T, Raitakari OT. Childhood serum fatty acid quality is associated with adult carotid artery intima media thickness in women but not in men. J Nutr 2013;143:682–9.

III **Kaikkonen JE**, Jula A, Viikari JSA, Juonala M, Hutri-Kähönen N, Kähönen M, Lehtimäki T, Raitakari O. Dietary and serum fatty acid proportions: associations with obesity, insulin resistance, blood pressure and fatty liver: The Cardiovascular Risk in Young Finns Study. Manuscript.

IV **Kaikkonen JE**, Kresanov P, Ahotupa M, Jula A, Mikkilä V, Viikari JS, Kähönen M, Lehtimäki T, Raitakari OT. High serum n-6 fatty acid proportion is associated with lowered LDL oxidation and inflammation: the Cardiovascular Risk in Young Finns Study. Free Radic Res 2014;48:420–6.

1 Introduction

According to a report of the American Heart Association, a suboptimal diet quality is the leading risk factor for death and poor health in the United States (2016 update) (Mozaffarian et al. 2016). Even childhood nutrition may play a role in the development of adult health (Jääskeläinen et al. 2012). It has been shown that dietary patterns from childhood to adulthood are associated with adulthood cardiovascular risk factors, such as the concentrations of serum total and low-density lipoprotein (LDL) cholesterol, apolipoprotein B, blood pressure (BP), inflammatory markers (C-reactive protein) and carotid artery intima-media thickness (cIMT, a marker of pre-clinical atherosclerosis) (Mikkilä et al. 2007; Mikkilä et al. 2009). A life-long tracking of adverse dietary habits, leading to long-term exposure to unhealthy dietary patterns, is a potential effect mediating mechanism (Kaikkonen, Mikkilä, and Raitakari 2014). One of the macronutrients determining the food quality of a particular dietary pattern is fat, and in particular, the composition of dietary fatty acids (FAs). FAs are needed in the body to synthesize phospholipids (PLs) for membranes, for energy storage and production, and metabolic and/or regulatory purposes (Berg, Tymoczko, and Stryer 2012). The circulating total FA pool is a combination of esterified FAs in triglycerides (TGs, 44-59% of the circulating FAs), cholesteryl esters (CEs, 14-21%) and PLs (24-31%), and non-esterified FAs (free FA pool, 3-6%) (Hodson, Skeaff, and Fielding 2008). Circulating FA concentrations are strongly linked to lipoprotein concentrations. For this reason, it is reasonable to use the proportions of different circulating FAs (% of the total concentration or mass) when investigating their health effects. Both diet and endogenous FA metabolism are affecting the concentrations of circulating FAs. Thus, serum FA proportions can be used to some extent to reflect dietary intake of FAs (Hodson, Skeaff, and Fielding 2008).

A high LDL cholesterol concentration is an independent risk factor for cardiovascular disease (CVD) (Briel et al. 2009). The Diet-Heart Hypothesis, developed by Dr. Ancel Keys in the Seven Countries Study in the 1950's, stated that dietary FAs determine CVD risk since they influence the LDL cholesterol concentration. In that study, a high serum LDL cholesterol concentration was associated with the intake of saturated (SFAs) FAs and the low concentration with

the intake of polyunsaturated FAs (PUFAs) (Keys 1957). During the last 60-70 years, population-level data has been collected to support the replacement of SFAs partly with PUFAs in the diet, to lower the levels of cardiometabolic risk factors and the risk of CVD. Firstly, several long-term intervention trials with a long enough duration (>2 years) and confirmed compliance (Morris JN and the committee 1968; Dayton et al. 1969; Leren 1970; Turpeinen et al. 1979), and meta-analyses/reviews (Sacks et al. 2017; Hooper et al. 2015; Schwab, U., Lauritzen, Tholstrup, Haldorssoni, Riserus, Uusitupa, and Becker 2014a; Mozaffarian, Micha, and Wallace 2010; Skeaff and Miller 2009) based on these core findings, provide the strongest evidence to support the benefits of replacing SFA with unsaturated fatty acids (UFAs). With regard to these interventions, a decrease in serum cholesterol by 15% to 20% has reduced cardiovascular events on average by 30% which is similar to the reduction observed after initiating statin treatment (Sacks et al. 2017). Secondly, the data from prospective observational cohort studies and related meta-analyses (Jakobsen et al. 2009; Farvid et al. 2014; Li et al. 2015) have complemented the data obtained from the intervention studies. Since the 1970s, due to national counselling and dietary recommendations, Finnish people have modified their dietary fat consumption behavior according to recommendations, and this has been reflected in decreased rates of CVD morbidity and mortality (Puska 2009).

However, the national FINDIET 2007, 2012 and 2017 surveys (Paturi et al. 2008; Helldán et al. 2013; Valsta et al. 2018) have shown that fat and SFA intakes, proportioned to the total energy, have once again started to increase in the diet of Finnish people. On the other hand, there is at least one recent meta-analysis of randomized controlled trials (RCTs) with a duration of 4 to 28 weeks, which has not found serum lipid-profile (LDL cholesterol) related benefits for the replacement of SFA with UFAs (Hannon et al. 2017). In addition, Hamley and co-workers (Hamley et al. 2017) have re-analyzed the old RCTs by omitting 'inadequately' controlled trials and concluded that FA intake does not affect the risk of coronary heart disease (CHD). Recent meta-analyses have also suggested that highly elevated omega-6 FA (n-6 PUFA) intake could, without simultaneous long-chain omega-3 FA (n-3 PUFA) supplementation, increase rather than lower the risk of CHD morbidity and mortality (Ramsden et al. 2010; Ramsden et al. 2013; Calder 2013). These deviating observations are one reason why we started to study the health effects of SFAs and PUFAs in this thesis. Furthermore, the health effects of dietary and circulating monounsaturated FAs (MUFAs), and those of the ratio of n-6/n-3 PUFAs are also to some extent unclear (see details below).

Virgin olive oil, which is rich in MUFAs, has been linked to a reduced risk of cardiometabolic outcomes (Buckland and Gonzalez 2015). However, there is increasing evidence that the health effects of olive oil may be dependent on its polyphenol content, i.e. not the MUFA itself (Covas et al. 2006). In addition, recent

large-scale population studies have found that serum MUFA proportions are associated with an elevated risk for cardiometabolic outcomes (Mahendran et al. 2013; Würtz et al. 2015; Kaikkonen et al. 2017). Δ9-Desaturase (stearoyl-CoA desaturase-1) activity is responsible for the conversion of body SFAs to MUFAs (Murakami et al. 2008; Berg, Tymoczko, and Stryer 2012) and has been linked to cardiometabolic risk factors (Warensjö, Riserus, and Vessby 2005; Murakami et al. 2008). In order to further study whether MUFAs have beneficial or adverse health effects, in this thesis we examined the links between cardiometabolic outcomes, including obesity, insulin resistance, elevated BP or hypertension and fatty liver, with dietary MUFAs, serum MUFAs and indicators of desaturase activity.

The optimal n-6/n-3 PUFA ratio in the diet has been the topic of ongoing debate. N-3 PUFAs have been considered as anti-inflammatory FAs and n-6 PUFAs as inflammatory FAs. Particularly, arachidonic acid (20:4n-6) has been linked to harmful inflammatory reactions (Schmitz and Ecker 2008). In addition, one major problem with PUFAs is that they are easily oxidized, and oxidative reactions (such as LDL oxidation) have been shown to be linked/play a role in the etiology of cardiometabolic outcomes, such as obesity, type 2 diabetes (T2D), dyslipidemia and atherosclerosis (Halliwell and Gutteridge 1989). In the light of these data, it seemed of interest to examine the health effects of PUFAs, in particular n-6 PUFAs. This thesis will examine how both serum n-6 PUFA proportions and n-3 PUFAs are associated with the markers of inflammation and LDL oxidation.

Even though different cardiometabolic outcomes are closely linked with each other, FAs may have direct and independent effects on individual metabolic outcomes (Ulven and Christiansen 2015). In addition, the health effects of individual FAs, such as linoleic acid (18:2n-6) or stearic acid (18:0), require further investigation. In this thesis, multivariable models have been used to examine, obesity and lipoprotein lipid-independent links between individual circulating FAs and different cardiometabolic outcomes, such as insulin resistance, elevated BP or hypertension and fatty liver.

There is a lack of long-term prospective data, from childhood to adulthood, regarding the health effects of FAs (Kaikkonen, Mikkilä et al. 2013). Growing children may be more sensitive than adults to the effects of FAs. On the other hand, metabolic outcomes, such as fatty liver, may affect circulating levels of FAs, for example, by increasing the secretion of very-low-density lipoproteins (Kaikkonen et al. 2017). For this reason, long-term prospective studies, such as this thesis, are needed to examine associations (and to approximate causality) between FAs and cardiometabolic outcomes.

Prospective cohorts, such as the Cardiovascular Risk in Young Finns Study (YFS), have several benefits. Mostly they include large populations with relatively long follow-ups. The extensive collection of data makes it possible to control study

findings over a variety of confounders. Since the participants choose their own diet, adherence to the diet is not a problem. However, in population studies, inaccurate ascertainment of nutrient intake data may hamper the detection of biologically important associations (Sacks et al. 2017). For this reason, circulating FA proportions have been used as indicators of dietary FA intakes. However, serum FAs are being continuously metabolized, which may weaken their associations with dietary intake (Hodson, Skeaff, and Fielding 2008). This thesis has examined links between dietary intake of FAs and their serum proportions. Both dietary intake of FAs and their proportions in serum have been used as explanatory variables in different outcome models.

The YFS study is a population-based cohort in which children, aged 3-18 years at baseline in 1980 (Raitakari et al. 2008), have now been followed with extensive health data collection for 40 years. Data for dietary and serum FA proportions used in this thesis were collected at baseline in childhood (some validation data also in 1983 and 1986) and in 2001 (some background data also in 2007 and 2011) in early adulthood of the study participants. Cardiometabolic outcomes were assessed in adulthood and in 2001, 2007 and/or 2011. The main purpose of this thesis study was to examine at a population level how 1) Serum FA proportions reflect dietary intake of FAs; 2) Serum cholesteryl ester fatty acid (CEFA) proportions in childhood are associated with BP in adulthood; 3) Serum CEFA proportions in childhood are associated with sub-clinical atherosclerosis, i.e., cIMT in adulthood; 4) Serum total (free+esterified) FA proportion profile in early adulthood is associated with different cardiometabolic outcomes, i.e., obesity, high homeostatic model-based insulin resistance (HOMA-IR), elevated BP or non-alcoholic fatty liver in adulthood; 5) Serum total FA proportions in early adulthood are associated with the markers of elevated inflammation and lipoprotein oxidation, i.e. the factors known to be linked with an increased risk of cardiometabolic outcomes.

2 Review of the Literature

2.1 Structure of Fatty Acids

FAs can be categorized bin several ways, such as by their 1) carbon-chain length, i.e., short (≤4 carbon atoms), medium (6-12 carbon atoms), long (14-21 carbon atoms) and very long-chain (≥22 carbon atoms) FAs or 2) even or odd number of carbon atoms in their chains 3) number and location of double bonds 4) cis–trans isomerism of double bonds (**Figure 1**). SFAs have no double-bonds in their carbon chains. MUFAs have one double-bond, and PUFAs have at least two double-bonds. A high number of double bonds means a high desaturation degree (Gunstone 1996; Berg, Tymoczko, and Stryer 2012).

With regard to the systematic chemical names, carbon atoms included in the carbon chains of the FAs are numbered starting from their functional group, i.e., carboxyl group. For linoleic acid (trivial name), the abbreviation is LA, the International Union of Pure and Applied Chemistry chemical name is octadeca-9,12-dienoic acid and in shorthand 18:2 (18 carbon atoms: 2 double bonds). With regard to FAs, the common way is to give numbers for carbon atoms starting from the terminal methyl end and categorize FAs by the location of the first double bond in their methyl end. In this way, UFAs can be labeled as omega-3 (n-3), omega-6 (n-6), omega-7 (n-7) or omega-9 (n-9) FAs (for example, linoleic acid, 18:2n-6). These FA families cannot be interconverted. Trans-FAs are PUFAs with at least one double-bond in the trans-position (hydrogen atoms are at the opposite sides of the double-bond) (Gunstone 1996).

Serum/plasma esterified FAs are located in TGs (45%), in CEs (15%) or in PLs (35%), lipoprotein particles being their main carrier. There is also a small albumin-bound free fraction (<5%) (Jula et al. 2005), rich in SFAs, originating from the adipose tissue as a result of lipolysis (Hodson, Skeaff, and Fielding 2008; Berg, Tymoczko, and Stryer 2012).

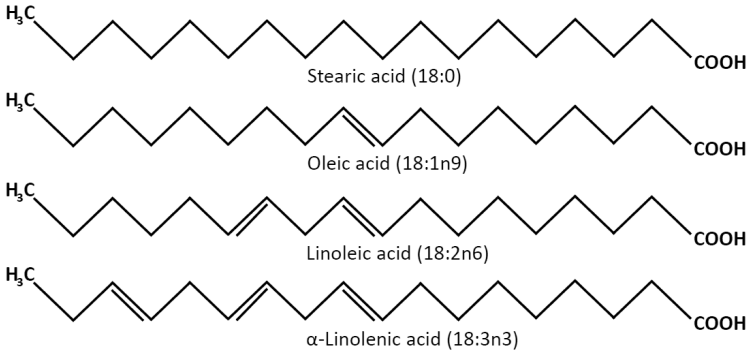


Figure 1. Structure of trans-FAs containing 18 carbon atoms with their increasing desaturation degree**.** As a difference to trans-FAs, hydrogen atoms of cis-FAs are at the same side of the double bonds resulting in the fact that there are turn/turns in their carbon chains. For this reason, cis-FAs have a lower melting point compared to trans-FAs. Figure data is modified from the work of Gunstone (Gunstone 1996).

2.2 Fatty Acid Metabolism

2.2.1 Fat Intake and Transfer in the Body

Dietary fat is mainly ingested as TGs (95%) and to a lesser extent as PLs and CEs (5%). To enable absorption, these esterified FAs have to be hydrolyzed in the small intestine by several lipases (pancreatic lipase for TGs, phospholipase A2 for PLs and pancreatic CE hydrolase for CEs). Following hydrolysis and absorption, the fat digestion end-products (free FAs and monoglycerides, cholesterol, lysophospholipids), are re-esterified in the intestinal epithelium resulting in TGs, CEs and PLs. These are transferred into the bloodstream as a part of chylomicrons (consisting of TGs, CEs, PLs, free cholesterol and apolipoproteins). Most of the mass of chylomicrons consists of TG, which are hydrolyzed by lipoprotein lipase at the capillary endothelium. The free FAs formed in this way are mainly transferred either to adipose tissue for storage as TGs or to muscle cells for energy producing beta-oxidation. The remaining chylomicron remnants, which are rich in cholesterol, are delivered to the liver for the purposes of hepatic cholesterol metabolism (Murray et al. 1990; Berg, Tymoczko, and Stryer 2012).

2.2.2 Body Metabolism and Fatty Acids

FA-related metabolism includes catabolic processes that produce energy and anabolic processes that create biologically important molecules (PLs, TGs, CEs, second messengers, lipid mediators and ketone bodies) (Berg, Tymoczko, and Stryer 2012; Murray et al. 1990), see **Figure 2** for the main pathways. There are several

transcription factors, such as sterol regulatory element binding protein-1 (SREBP1) and peroxisome proliferator activated receptors (PPARs) regulating the metabolism of FAs related to adipogenesis and lipogenesis. UFA consumption, n-3 PUFAs in particular, has been found to induce the activation of several genes involved in lipid oxidation and thermogenesis, resulting in an increased FA beta-oxidation rate and a reduced risk of adipogenesis. In addition, UFA consumption has been found to suppress the expression of many lipogenic genes. On the other hand, consumption of long-chain SFAs (≥14 carbon atoms) may lead to increased lipogenesis and adipogenesis (Hammad and Jones 2017). As one possible mechanism, it has been proposed that membrane ceramides, such as specific 16:0 ceramide, formed from 16:0 SFA, are associated with lowered mitochondrial beta-oxidation and an increased prevalence of obesity and obesity-related cardiometabolic outcomes (Fucho et al. 2017). These ceramide-mediated mechanisms of SFAs require further investigation.

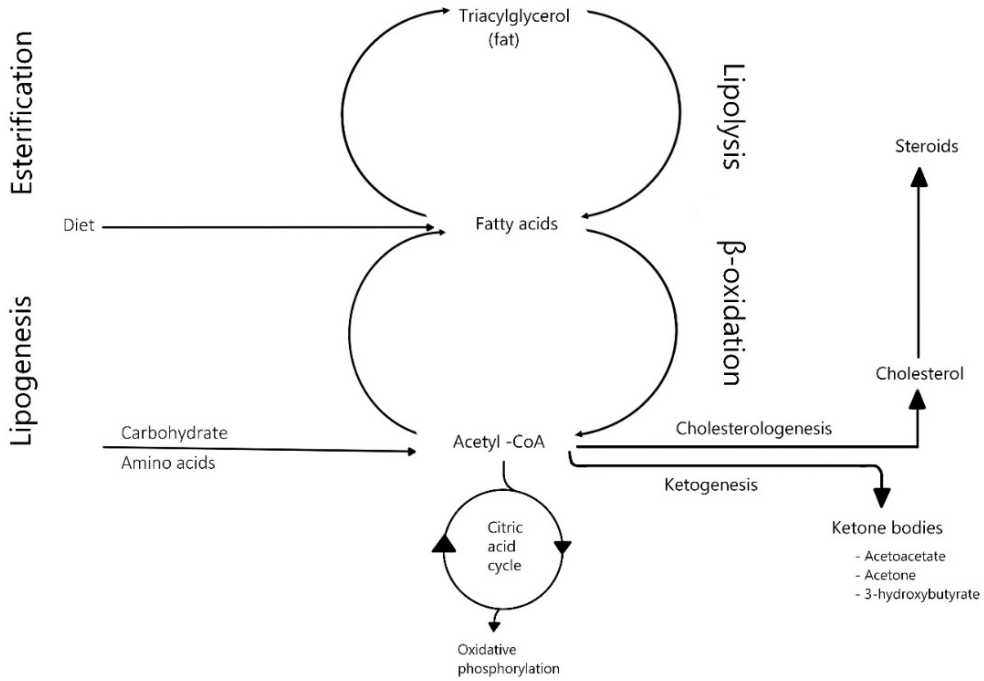


Figure 2. General view of the FA-related body metabolism. Figure data has been modified from Harper's Biochemistry (Murray et al. 1990).

FAs have numerous effects that can influence cell-signaling and pathways linked to cardiometabolic outcomes. Firstly, membrane FAs modify membrane fluidity and lipid layer properties thus regulating signaling pathways and the function of proteins (e.g. influencing insulin receptor binding and/or its affinity). Incorporation of FAs

into membranes can also alter the function of Na^+ channels and L-type Ca^{2+} channels, which might underlie the capacity of n-3 PUFAs to reduce cardiomyocyte excitability and, therefore, account for the antiarrhythmic potential of n-3 PUFAs. Secondly, FAs can directly interact with membrane proteins and ion channels. Activation of the G protein-coupled membrane receptor 40 (GPR40) and GPR120 by long-chain UFAs can reduce inflammation, improve glucose homeostasis or regulate body weight. Thirdly, an important effect of dietary FAs is mediated through direct regulation of the expression of genes related to metabolic pathways. For example, UFAs are natural ligands for nuclear receptors, such as peroxisome proliferator-activated receptor-α and retinoid X receptors. FAs can also regulate other transcription factors, such as nuclear factor- κB, which might not require their direct binding. Finally, dietary FAs are metabolized into diverse sets of highly bioactive metabolites. For example, after their release from PLs (mediated by cytosolic phospholipase A2), both n-3 PUFAs and n-6 PUFAs are converted through enzymatic and non-enzymatic processes into eicosanoids, such as leukotrienes, isoprostanes and specialized pro-resolving mediators (SPMs) such as resolvins. These highly bioactive metabolites participate in numerous pathways involved in cardiometabolic diseases. In addition, cytochrome P450 can convert arachidonic acid into epoxyeicosatrienoic acid, which has shown robust and diverse metabolic effects in animal models, including anti-inflammatory, antifibrotic and pro-vasodilatory properties (Wu, Micha, and Mozaffarian 2019; Ulven and Christiansen 2015; Riserus, Willett, and Hu 2009; Weijers 2012).

SFAs are mainly synthetized in the liver, and to a lesser extent in other tissues, such as adipose tissue. Starvation and the related low concentrations of plasma glucose are associated with decreased synthesis of FAs; over-nutrition exerts an opposite effect. In the first phase, acetyl coenzyme A is changed to malonyl coenzyme A in the cytosol. This is followed by chain elongation steps catalyzed by multi-enzyme complex named FA synthase, palmitic acid (16:0) being the main end product of this cascade. The chain elongation process can continue with the addition of two carbons to form stearic acid (18:0). The synthesis of longer chain FAs and the increase in their desaturation degree (by membrane-bound desaturases adding double-bonds to carbon locations Δ9, Δ6 or Δ5) occurs via several two-carbon elongation and desaturation steps (**Table 1**) (Nakamura and Nara 2004). With regard to n-6 and n-3 PUFAs, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are essential dietary-based FAs which are further processed by the FA metabolism. Only plants have the ability to synthesize these essential FAs since they possess Δ12- and Δ15-desaturases (Nakamura and Nara 2004; Lee et al. 2016). The affinity of Δ6-desaturase is highest for α-linolenic acid (18:3n-3) following linoleic (18:2n-6) and oleic acids (18:1n-9) (Brenner and Peluffo 1966). Diet can also regulate specific enzymes, such as Δ6-desaturase activity. This has been studied in rats *in vivo* by using different dietary

intakes, and in cell culture experiments by using varying concentrations of nutrients in the cell culture media. For example, a low amount of proteins, and a high amount of linoleate, arachidonate, glycerol, fructose or glucose have been recognized as inhibitors of Δ6-desaturase activity, whereas a lack of essential FAs, high protein levels and high insulin activity seem to accelerate the activity (Brenner 1981). FA metabolism decreases the proportions of certain circulating FAs (precursors) while increasing the proportions of product FAs (Nakamura and Nara 2004). This may influence the health effects of dietary FAs, i.e., some of them may lose their effects, and for some others, the health role may be over-estimated. In humans, smoking may increase the circulating levels of MUFAs (Hodson, Skeaff, and Fielding 2008), and statins elevate the levels of serum long-chain PUFAs via increased elongase, Δ5-desaturase and Δ6-desaturase activities (Jula et al. 2005). In addition, genetic variation in the enzymes participating in FA metabolism affects the circulating PUFA levels (Dorajoo et al. 2015). For example, the intake of linoleic acid seems to have FA desaturase 1 genotype specific effects on the levels of circulating esterified arachidonate as well as on the inflammatory response (Lankinen et al. 2019).

Table 1. Metabolism of n-9, n-7, n-6 and n-3 series of FAs.

Elongation/ desaturation	N7 series	N9 series	N6 series	N3 series
	Diet/synthesis	Diet/synthesis	Diet	Diet
	16:0 ➡ (elongase)	18:0		
Δ9-Desaturase	⬇	⬇		
	16:1n-7	18:1n-9	18:2n-6	18:3n-3
Δ6-Desaturase	⬇	⬇	⬇	⬇
	16:2n-7	18:2n-9	18:3n-6	18:4n-3
Elongation	⬇	⬇	⬇	⬇
	18:2n-7	20:2n-9	20:3n-6	20:4n-3
Δ5-Desaturase	⬇	⬇	⬇	⬇
	18:3n-7	20:3n-9	20:4n-6	20:5n-3
Elongation		⬇	⬇	⬇
		22:3n-9	22:4n-6	22:5n-3
Elongation			⬇	⬇
			24:4n-6	24:5n-3
Δ6-Desaturase			⬇	⬇
			24:5n-6	24:6n-3
β-Oxidation			⬇	⬇
			22:5n-6	22:6n-3

Table data has been modified from the works of (Nakamura and Nara 2004; Jula et al. 2005).

2.3 Dietary Recommendations for Fatty Acids

Different organizations, such as the American Heart Association, the United States Department of Agriculture, the World Health Organization, the Nordic Council of Ministers and the National Nutrition Council in Finland have set recommendations for a healthy diet. The recommendations about the FA intake are very similar in their content from one organization to another (**Table 2**). In the latest recommendations, restricting the intake of SFAs, replacement of SFAs with UFAs, limiting the intake of trans-FAs, with the priority being to balance the total energy intake with the energy consumption. Nonetheless, as argued by Astrup and co-workers, in the future, there may be valid reasons to take different fat-containing food matrixes into consideration when setting guidelines for dietary FAs (Astrup et al. 2019).

Table 2. Dietary recommendations of FAs for adults set by different organizations.

	AHA 2006–2015	**USDA** 2015–2020	**WHO** 2010–2018	**Nordic and Finnish** 2012–2014[8-9]
Total fat	25–35E%[1]	20-35E%[4]	<30E%[6] 20-35E%[7]	25-40E%
SFAs	5–6E%[2]	<10E%[5]	<10E%[6]	<10E%
Trans-FAs	Limited as low as possible[2]	As low as possible[5]	<1E%[6]	As low as possible
MUFAs	Majority of fats as UFAs[2]	Replace SFAs with UFAs[5]	Positive effects on lipids when SFAs or carbohydrates are replaced by MUFAs[7]	10–20E% (2/3 of fats as UFAs)
PUFAs	Majority of fats as UFAs[2] n-6 PUFAs, 5–10E%[3]	Replace SFAs with UFAs[5]	6–11 E%, including n-3 PUFAs 0.5–2 E%[7]	PUFAs 5–10 E%, including n-3 PUFAs ≥1 E%, (2/3 of fats as UFAs)

For each point, the newest recommendation found has been presented. AHA=American Heart Association; USDA=United States Department of Agriculture; WHO=World Health Organization. 1=(Lichtenstein et al. 2006); 2=(AHA online recommendations 2015); 3=(Harris et al. 2009); 4=(Recommendations of USDA and US Department of Health and Human Services 2010); 5=(USDA dietary guidelines for Americans 2015-2020); 6=(WHO. Healthy diet 2018); 7= (WHO, FAO. Fats and Fatty Acids in Human Nutrition 2010); 8=(Nordic nutrition recommendations 2012); 9=(Suomalaiset ravitsemussuositukset = Finnish dietary recommendations 2014).

2.4 Dietary Sources for Fatty Acids

In Finland in 2017, the percentage contribution of fat to the total energy intake was 38.7% in men and 37.7 in women (Valsta et al. 2018). The main sources of SFAs were 1) milk products (particularly in women) 2) meat and egg foods (particularly in men) 3) fat blend spreads and oils (particularly in men). For MUFAs, the sources were 1) meat and egg-based foods (particularly in men) 2) fat blend spreads and oils

(particularly in men) 3) cereal products. For n-6 PUFAs, the sources were 1) fat blend spreads and oils (particularly in men) 2) meat and egg-based foods (particularly in men) 3) cereal products, and for n-3 PUFAs 1) fat blend spreads and oils (particularly in men) 2) fish foods 3) meat and egg-based foods (particularly in men) (Valsta et al. 2018).

According to the FINDIET Surveys, the previously observed improved trend in the dietary habits of the Finnish adult population concerning the quality of fat has ceased and possibly even reversed. Between 1992 and 2007, SFA intakes steadily decreased from 15E% to 12E%. However, there was an increase in SFA intake from 12.6E% to 15.1E% in men and 11.9E% to 14.4E% in women between the years 2007 and 2017 (Paturi et al. 2008; Valsta et al. 2018; Laatikainen et al. 2019). Total fat intake has increased not only because of increased intake of SFAs but also through increased intake of UFAs, i.e., vegetable oils (Paturi et al. 2008; Helldán et al. 2013; Valsta et al. 2018). See **Table 3** for the intake of FAs in 2007, 2012 and 2017.

Following long-term beneficial trend started from the early eighties, concentrations of total cholesterol and LDL cholesterol started to elevate between the years 2007 and 2012, following a modest decrease between the years 2012 and 2017. High-density lipoprotein cholesterol has remained at a constant level (Laatikainen et al. 2019).

Table 3. FA intakes, E% (mean±SD or mean) in Finland.

	Men n=959 2007	**n=795 2012**	**n=780 2017**	**Women n=1080 2007**	**n=913 2012**	**n=875 2017**
SFAs*	12.6 ± 4.2	13.7 ± 4.1	15.1	11.9 ± 3.9	13.5 ± 4.2	14.4
MUFAs	11.8 ± 3.5	12.9 ± 3.6	14.6	10.8 ± 3.2	12.4 ± 3.6	14.3
PUFAs	5.8 ± 2.2	6.2 ± 2.3	6.8	5.6 ± 2.1	6.3 ± 2.5	6.9
n-6s PUFAs	4.5 ± 1.8	4.5 ± 1.8	5.1	4.4 ± 1.8	4.5 ± 2.0	5.1
18:2n-6		4.4 ± 1.8	4.7		4.5 ± 2.0	4.7
n-3s PUFAs	1.3 ± 0.6	1.4 ± 0.7	1.6	1.2 ± 0.6	1.5 ± 0.7	1.7
18:3n-3		1.2 ± 0.5	1.2		1.2 ± 0.6	1.3
Trans FAs	0.4 ± 0.1	0.4 ± 0.2	0.4	0.4 ± 0.1	0.4 ± 0.2	0.4

Subjects were aged 18-74 years. Data were collected by a 48-hour dietary recall as a part of the National FINDIET Study (Paturi et al. 2008; Helldán et al. 2013; Valsta et al. 2018). *=intake is above recommendations. In 2007, mean daily intake of fat was 79g in men and 56g in women. In 2017, the values were 97g (38.7E%) and 75g (37.7E%), respectively.

The FA composition of selected oils is presented in **Figure 2**. There are clear differences in the sources of FAs belonging even to the same group of saturation degree. For example, soybean oil, corn oil and soft margarine are common sources

for linoleic acid (18:2n-6), whereas meat, poultry and eggs are major sources for arachidonic acid (20:4n-6). Fatty fish, such as salmon, mackerel, herring and trout are important sources for n-3 PUFAs, however, α-linolenic acid (18:3n-3) can be obtained from flax, chia, walnuts and rapeseed oil (canola oil). With regard to MUFAs, olive oil, rapeseed oil, beef tallow, lard and avocado are important sources for oleic acid (18:1n-9), whereas macadamia nuts and blue-green algae are high in palmitoleic acid (16:1n-7). Thus, rapeseed oil is a source for both MUFAs and n-3 PUFAs, but also for linoleic acid (18:2n-6). With respect to the SFAs, meat and fully-hydrogenated vegetable oils are important sources for stearic acid (18:0), palm oil and most fats and oils, such as butter and milk fat for palmitic acid (16:0), and beef tallow, cacoa butter and butter and milk fat for myristic acid (14:0) (Vannice G. 2014). It is also important to notice that other macronutrients may affect the levels of non-essential FAs, i.e. the FAs that body is able to synthetize for itself. For example, a high carbohydrate intake may lead to an increase in circulating TGs and related FAs, SFAs in particular (Parks 2001).

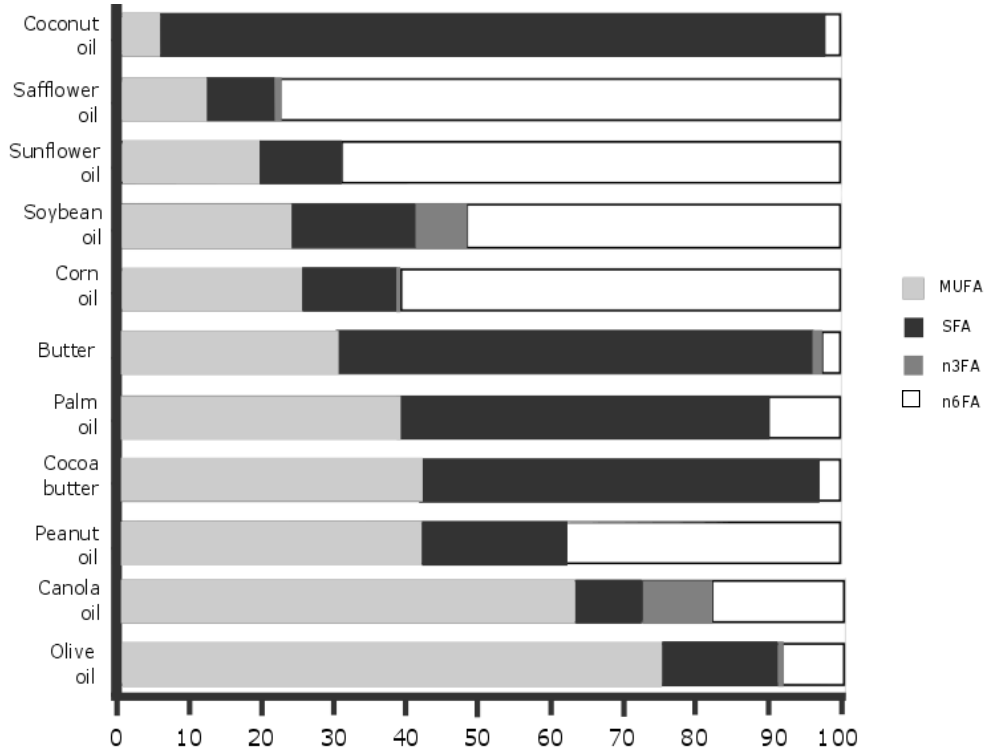


Figure 2. Average FA compositions of selected fats and oils. Canola oil=rapeseed oil. Figure data is modified from the work of Degirolamo and co-workers (Degirolamo C., Rudel L. L 2010).

2.5 Intake in Europe versus WHO Recommendations

Recently, SFA and PUFA intakes have been investigated in 24 countries, focusing on Europe. Reported mean intakes ranged from 28.5 to 46.2% of total energy (E%) for total fat, from 8.9 to 15.5 E% for SFA and from 3.9 to 11.3 E% for PUFA. The mean intakes met the recommendation for total fat (20–35 E%) in 15 countries; and for SFA (<10 E%) in two countries, i.e., Israel and Portugal; and for PUFA (6–11 E%) in 15 countries (Eilander, Harika, and Zock 2015). Intake of SFA and PUFA, and the P/S ratio (dietary PUFA/SFA ratio) are presented for selected countries in **Figure 3**. The highest P/S ratio was observed in Russia whereas Finland displayed an average value.

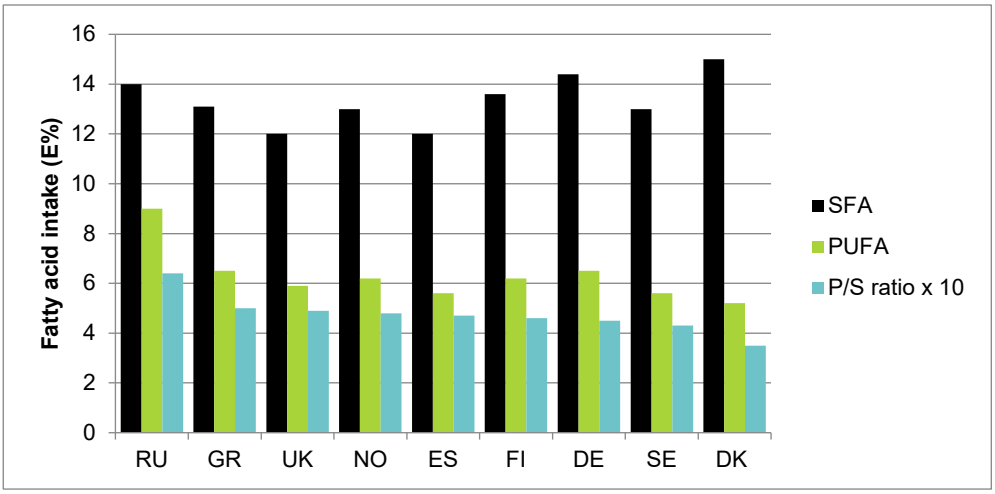


Figure 3. Dietary intake of SFA E% and PUFA E%, and the P/S ratio (x10) in selected European countries arranged according to the lowering P/S ratio. DE=Germany, DK=Denmark, ES=Spain, FI=Finland, GR=Greece, NO=Norway, SE=Sweden, RU=Russia, UK=United Kingdom. Figure data is modified from the work of Eilander and co-workers (Eilander, Harika, and Zock 2015).

2.6 Blood Fractions as an Indicator of Dietary Fatty Acid Intake

On the basis of dietary assessment methods (recalls, food frequency questionnaires, food intake diaries), it is difficult to reliably characterize the long-term dietary intake of FAs. For this reason, many studies have measured FA levels from different blood fractions, such as red blood cell membrane PLs or serum TG, cholesterol or PL fractions, as indicators of dietary intake (Moore et al. 1977; Vessby et al. 1980; Riboli, Ronnholm, and Saracci 1987; Zock et al. 1997; Takkunen et al. 2013). When

one is interested in metabolism, then serum TG esters should reflect the fat quality from the preceding days (Moore et al. 1977), and both CE and PL fractions from the preceding weeks to several months (Vessby et al. 1980; Riboli, Ronnholm, and Saracci 1987; Zock et al. 1997). The long-time indicators are possibly the most reliable measures of the dietary intake, since there will be variations in the intake of FAs on daily/weekly/monthly basis. However, FA metabolism does change the serum FAs towards levels and composition regulated by the body metabolism. On the other hand, the amount and composition of ingested FAs affect the FA metabolism (Brenner 1981; Holman 1986; Wang, Y. et al. 2005). For these reasons, there is no fraction which can precisely characterize the long-term intake. Adipose tissue FAs have been considered as the most reliable marker of long-term FA intake, reflecting the intakes over the last 0.5 to 2 years (Hodson, Skeaff, and Fielding 2008). The requirement for an invasive fat biopsy obviously limits the use of this fraction in large scale studies. During the fasting state, the serum free fraction (mainly albumin–bound non-esterified FAs) is released from the adipose tissue, thus reflecting the FA content of the adipose tissue (Hodson, Skeaff, and Fielding 2008). On the other hand, following a dietary intake of fats, the levels of circulating free FAs reflect the recent intake of FAs (Hodson, Skeaff, and Fielding 2008).

Fasting plasma total FAs vs. intake of FAs have had correlation coefficients of r=0.11-0.23 for SFA, r=0.04-0.31 for MUFA, r=0.17-0.38 for n-6 PUFAs and 0.23-0.47 for n-3 PUFAs (a summary data of adults from 3-4 studies, **Table 3**) (Hodson, Skeaff, and Fielding 2008). With regard to serum CE fraction in children, it has been shown earlier that linoleate (18:2n-6) correlates particularly well with the dietary P/S ratio, i.e., r=0.57 (Moilanen, Räsänen et al. 1985). The correlations between dietary FAs and plasma CEFAs or PL FAs are presented for SFAs, MUFAs and PUFAs in Table 3 and for selected individual FAs in **Table 4**. Long-chain n-3 PUFAs, 22:6n-3 in particular, seemed to exhibit the highest associations between dietary intakes and their plasma concentrations.

Table 3. Correlation coefficients between dietary intake of FAs and plasma proportions of FAs[a] in different circulating fractions.

Author	Adult subjects	Fraction	Dietary Assessment	SFAs	MUFAs	n-6 PUFAs	n-3 PUFAs	PUFAs
Andersen et al.[b]	125 M	Total	FFQ	**0.23**[c]	0.08			**0.20**
Kuriki et al.	79 F	Total	7 DDR	0.13	**0.31**	0.17	**0.47**	
Sun et al.	306 F	Total	FFQ	**0.16**	0.04	**0.21**	**0.30**	**0.23**
Baylin et al.	196 M+F	Total	FFQ	0.11	0.14	**0.38**	**0.23**	
Ma et al.	3570 M+F	CE	FFQ	**0.23**	**-0.09**			**0.31**
Asciutti-Moura et al.	53 M+F	CE	7 DDR	0.19				
Asciutti-Moura et al.	53 M+F	PL	7 DDR	0.17				
Hodge et al.	4439 M+F	PL	FFQ	0.16[d]	0.46[d]	0.38[d]	0.57[d]	0.39[d]
Asciutti-Moura et al.	53 M+F	FFA	7 DDR	0.11				

[a]Dietary intake was expressed as percentage of the total fat intake and circulating FAs were expressed as a percentage of the total FA weight or concentration.
[b]References can be found from the original work.
[c]Correlation coefficients in bold indicate significance of $P<0.05$ as reported by the original papers.
[d]Statistical significance was not reported. DDR=day diet record, F=female, FFQ=food frequency questionnaire, M=male. Table data has been modified from the work of Hodson and co-workers (Hodson, Skeaff, and Fielding 2008).

Table 4. Correlation coefficients between dietary intake and plasma proportions of selected individual FAs in different circulating fractions.

Author	Adult subjects	Fraction	Dietary Assessment	16:0	18:0	18:1 n-9	18:2 n-6	18:3 n-3	22:6 n-3
Andersen et al.[c]	125 M	Total	FFQ[a]	0.11		0.09	0.16	**0.28**[d]	**0.52**
Kuriki et al.	79 F	Total	7 DDR[a]	0.10	0.14	**0.30**	0.16	**0.24**	**0.59**
Sun et al.	306 F	Total	FFQ[a]	**0.12**	0.06	**0.12**	**0.25**	**0.23**	**0.48**
Baylin et al.	196 M+F	Total	FFQ[a]	0.14	0.01	**0.21**	**0.41**	**0.39**	**0.31**
Astorg et al.	276 M	Total	15x24h[b] recall				**0.22**	0.06	**0.25**
Sarkkinen et al.	160 M+F	CE	5x3 DDR[a]	**0.34**			**0.49**		
Ma et al.	5370 M+F	CE	FFQ[a]	**0.19**			**0.28**	**0.21**	**0.42**
Asciutti-Moura et al.	53 M+F	CE	7 DDR[a]			0.21	0.23		
Asciutti-Moura et al.	53 M+F	PL	7 DDR[a]			0.13	**0.29**		
Hodge et al.	4439 M+F	PL	FFQ[a]	0.17[e]		0.45[e]	0.58[e]	0.24[e]	0.78e[e]
Asciutti-Moura et al.	53 M+F	FFA	7 DDR[a]			0.09	0.18		

Dietary intake was expressed as percentage of total fat intake[a] or as E%[b] and circulating FAs were expressed as a percentage of the total FA weight or concentration.
[c]References can be found from the original work.
[d]Correlation coefficients in bold indicate significance of $P<0.05$ as reported by original papers.
[e]Statistical significance was not reported. DDR=day diet record, F=female, FFQ=food frequency questionnaire, M=male. Table data has been modified from the work of Hodson and co-workers (Hodson, Skeaff, and Fielding 2008).

2.7 Fatty Acid Composition of Different Plasma Fractions and Adipose Tissue

There are clear differences in the FA composition between different fractions. Linoleic acid (18:2n-6) is the predominant FA in the CE fraction, whereas palmitic acid (16:0) dominates the PL fraction. In other fractions, such as TGs, plasma free fraction and adipose tissue, oleic acid (18:1n-9) has the highest concentration relative to the other FAs. See **Figure 4** for FA composition in different fractions (a mean from seven separate studies). Nikkari and co-workers (Nikkari et al. 1983) have earlier studied the FA composition of different circulating fractions in boys in the YFS Study. Their observations were very similar with the findings presented in Figure 4. For example, linoleic acid (18:2n-6) showed following proportions: 11.5±2.8% for free FAs, 13.5±4.4% for TGs, 22.7±2.7% for PLs and 53.1±4.7% for CEs. For oleic acid (18:1n-9), the proportions were 35.7±3.1, 40.1±3.0, 12.7±1.3 and

19.7±2.6 and for palmitic acid (16:0), the proportions were 24.3±1.9, 24.7±2.4, 25.2±1.0 and 10.5±0.6%, respectively. Regardless of these fraction-based differences, most FAs, such as PUFAs% in CEs or PLs, have put individuals into the same order with regard to their circulating FA% levels. However, some caution is needed when comparing palmitic acid (16:0) or stearic acid (18:0) in these lipid fractions since their levels may differ greatly (Marklund et al. 2017). In earlier examinations of the YFS, in particular the plasma CEFA composition has reflected the daily intake of margarine, oils and butter in children (Moilanen et al. 1983). For this reason, CEFA fractions and their outcome associations have been examined in this thesis.

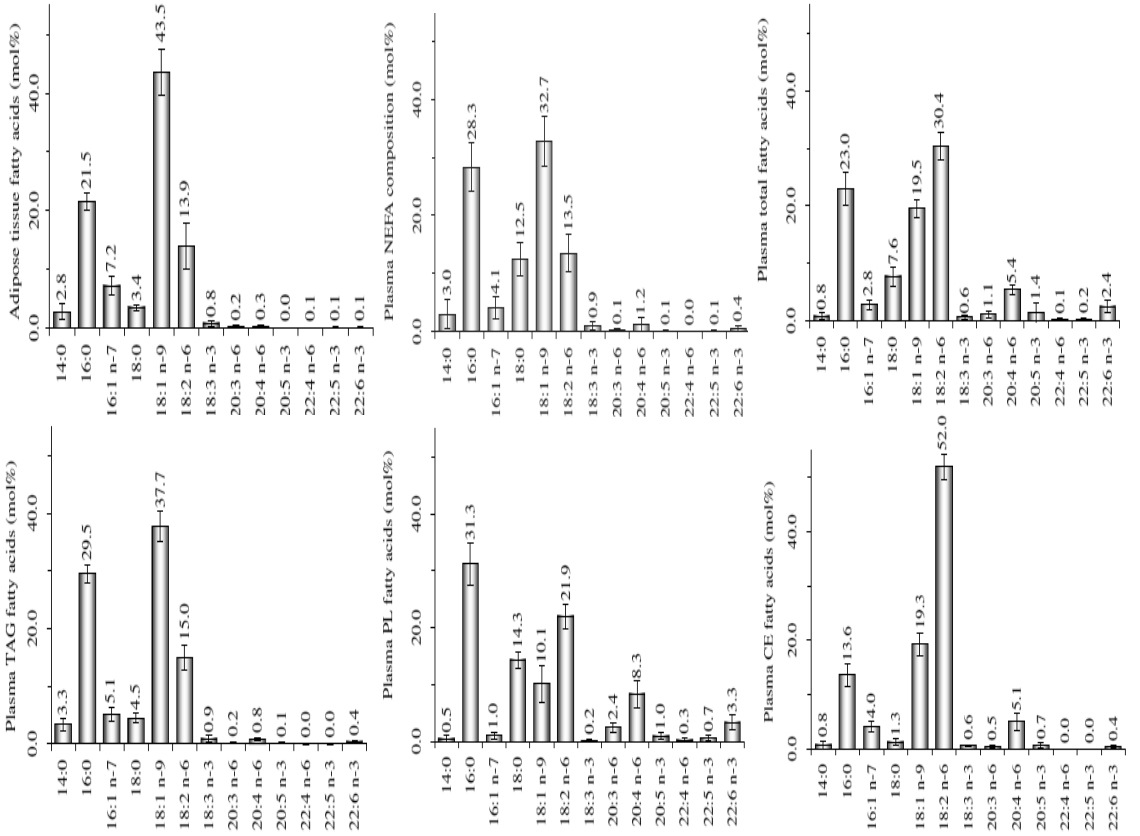


Figure 4. FA proportions (% from the total concentration) of adipose tissue and different blood fractions. Data are expressed as mean values from seven separate studies. Error bars represent standard deviation. NEFA=non-esterified fatty acid. Figure data has been modified from the work of Hodson and co-workers (Hodson, Skeaff, and Fielding 2008).

2.8 Measurement Methods for Fatty Acids

Traditionally, circulating FAs have been measured by gas chromatographic separation, followed by either flame ionization (Moilanen and Nikkari 1981) or mass spectrometric detection (Kaikkonen, Vilppo et al. 2013). Prior to chromatographic analysis, different lipid fractions can be separated by solvent extraction and, for example, by thin layer chromatography (Moilanen and Nikkari 1981; Cao et al. 2006). Recently, nuclear magnetic resonance-based analyses have been developed for the determination of the total FA composition (Soininen et al. 2015). The advantage of nuclear magnetic resonance is the minimal sample handling needed and fast analysis, on the other hand, its sensitivity is lower than that available with mass spectrometric techniques. Total free FAs can be measured by enzymatic colorimetric methods (Steffen et al. 2015). Since the concentrations of individual FAs are strongly intercorrelated, reflecting the levels of circulating lipid fractions, they have usually been expressed as proportions (concentration% or weight%) of the total amount of FAs.

2.9 Introduction to Fatty Acids and Cardiometabolic Outcomes

2.9.1 Dietary Fatty Acids

Abdominal obesity, impaired glucose or lipid metabolism, T2D, high BP or hypertension and increased inflammation are closely linked cardiometabolic outcomes (Grundy et al. 2005). International dietary recommendations advise individuals to keep their consumption of total fat between 25 (20) – 35 (40)% of total energy and to reduce consumption of SFAs to <10 E% by replacing the SFAs partly with PUFAs and MUFAs to lower the risk for metabolic outcomes and related CVD (Mach et al. 2020). These recommendations are based on intervention studies conducted in adults (with a duration ≥2 years, proven adherence and standard outcome ascertainment) showing approximately a 30% reduction in CVD events when SFA was partly replaced with PUFA (Morris JN and the committee 1968; Dayton et al. 1969; Leren 1970; Turpeinen et al. 1979) and the related meta-analyses/reviews (Sacks et al. 2017; Hooper et al. 2015; Mozaffarian, Micha, and Wallace 2010; Skeaff and Miller 2009). This is supported by the evidence accumulated from epidemiological population studies and related meta-analyses and reviews (Jakobsen et al. 2009; Farvid et al. 2014). For example, Nurses' Health Study and Health Professionals Follow-up Study, carried out between 1980 to 2010 involving 84,628 women and 42,908 men participants showed in their statistical models that replacement of 5% of energy intake from SFAs with an equal intake from PUFAs or MUFAs could lower the CHD risk by 25% or 15%, respectively (Li

et al. 2015). There is a large consensus that a higher intake of PUFAs is related with a reduced CHD risk (Mozaffarian, Micha, and Wallace 2010; Astrup et al. 2011). However, there are individual studies which have questioned the diet-heart hypothesis (Schwab, U. and Uusitupa 2015; Ramsden et al. 2016). For example, one recent meta-analysis questioned those earlier findings because of inadequate randomization and controlling in some of the RCTs included in the meta-analyses (Hamley 2017). Recently, the impact of fat quality has also been questioned due to observations showing that high carbohydrate diets are directly associated with the risk of incident metabolic syndrome irrespective of their impact on the FA composition (the KNHANES 2007–2014 study including 38, 766 adult Korean females) (Park et al. 2017). A too low fat intake may also lead to metabolic outcomes. In this same cohort, the incidence of metabolic syndrome was significantly higher in the ≤15E% fat intake group accompanied by lower daily energy intake, compared to the reference group (≥25E% fat intake) (Park, Ahn, and Lee 2016). It is important to notice that in cohort studies, it may not be possible to take all the necessary confounders, such as quality of carbohydrates or amount of fiber into account in statistical analyses, leading to inconsistent findings.

The strongest health benefits have been found for n-6 PUFAs which are dominating the dietary intake of PUFAs. However, a recent meta-analysis indicated that higher intakes of n-3 PUFAs, but not n-6 PUFAs, are associated with a lower risk of the metabolic syndrome (Jang H 2020). In line with this supposition, it was recently reported in an US cohort of young adults that the intake of non-fried fish and n-3 PUFAs could prevent incident metabolic syndrome 25 years later in their middle-age (Kim et al. 2016). It has also been claimed that highly elevated n-6 PUFA intake could, without simultaneous n-3 PUFA supplementation, increase rather than attenuate the risk of CHD morbidity and mortality (Ramsden et al. 2010; Ramsden et al. 2013; Calder 2013).

2.9.2 Circulating Fatty Acids

In several studies, circulating PUFAs and in particular linoleic acid (18:2n-6) have been associated with a lower risk and SFAs with a higher risk of cardiometabolic outcomes, such as T2D (Kroger et al. 2011; Wang, L. et al. 2003; Hodge et al. 2007) or cardiovascular events and/or mortality (Laaksonen et al. 2005; Warensjö et al. 2008; Clarke et al. 2009). However, specific odd-chain PL FAs, 15:0 and 17:0 have displayed inverse associations with T2D (Kroger et al. 2011; Hodge et al. 2007; Krachler et al. 2008). N-3 PUFAs have exhibited inverse associations particularly with blood pressure (Geleijnse et al. 2002). A recent review concluded that the association between blood docosahexaenoic acid, 22:6n-3, and the metabolic syndrome is unclear in obese children (Lassandro et al. 2015). In addition, the role

of MUFA is uncertain (Joris and Mensink 2016) since its serum proportions have been linked with increased cardiometabolic and CHD risk (Mahendran et al. 2013; Würtz et al. 2015; Kaikkonen et al. 2017).

In addition to FA proportions, also indicators of desaturase and elongase activity, i.e., specific FA ratios, seem to be markers or play a role in the etiology of cardiometabolic outcomes. For example in a Swedish Uppsala cohort of middle-aged and older men, high activity of estimated Δ9- and Δ6-desaturases, as well as low estimated Δ5-desaturase activity predicted the development of the metabolic syndrome (Warensjö, Riserus, and Vessby 2005). See Tables 6-9 for additional studies.

2.9.3 Long-Term Intervention Studies Started in Childhood

There are only a few long-term intervention studies which have started in childhood and continued to adulthood. With regard to ABC-intervention (Campbell et al. 2014), children born in the US between 1972 and 1977 were divided to intervention (n=57) and reference (n=54) groups. Healthy nutrition (details remained unclear), consisting of 2 meals and snacks, advised by a nutritionist and approved by a local health department, was one of the intervention components in the childcare center. Children randomly assigned to the treatment group when they were aged 0 to 5 years had a significantly lower prevalence of common cardiometabolic risk factors (blood pressure, metabolic syndrome) in their mid-30s. However, it is difficult to distinguish the effect of diet from the other components of the intervention, i.e., better pediatric care, improved cognitive and non-cognitive skills.

With regard to dietary interventions, the major long-term study that has continued from childhood to adulthood is the Finnish STRIP study (Special Turku coronary Risk factor Intervention Project). It started in the early 1990s and was designed to examine the influences of dietary modification counselling (low SFA, low cholesterol diet) on dietary intakes, serum lipid and lipoprotein concentrations and growth and development of infants and children (Simell et al. 2009). This intervention has proven to be effective in lowering SFA intake and serum LDL cholesterol concentration from infancy until the age of 19 years (Niinikoski et al. 2012). In particular in boys, significant intervention effects were evident in various lipoprotein measures, indicating a more favorable dietary lipid profile (Niinikoski et al. 2012). The STRIP intervention was also associated with improved insulin sensitivity in healthy adolescents aged 15-20 years (Oranta et al. 2013). These beneficial effects remained even 6 years after the end of the intervention (at the age of 26 years), since a higher proportion of intervention group participants met the ideal total cholesterol concentration and optimal LDL cholesterol concentration. In addition, those who had received the intervention had also lower glucose levels and HOMA-IR than the participants in the control group (Pahkala et al. 2020).

Similarly, the DISC (Dietary Intervention Study in Children), (carried out in 1988-1997) was a randomized controlled clinical trial of a reduced-fat dietary intervention to decrease serum LDL cholesterol (increased fiber intake was also encouraged) in children with elevated LDL cholesterol concentrations in the United States (Kwiterovich et al. 1997). Follow-up findings were based on the data collected 9 years after the end of the intervention. Female participants in the intervention group had lower systolic BP, lower plasma glucose and large very-low-density lipoprotein particle concentrations, indicating benefits for later glycemic control and BP (Dorgan et al. 2011). There is also one more recently started intervention study in children, the Eastern Finnish PANIC study (the Physical Activity and Nutrition in Children) (Venäläinen et al. 2016). On the basis of cross-sectional baseline data (collected in 2007-2009) of this study, greater proportions of plasma myristic and palmitoleic acids (16:1n-7) and a smaller proportion of linoleic acid (18:2n-6), as well as higher estimated Δ9- and Δ6-desaturase activity, and a lower estimated elongase activity, were associated with cardiometabolic risk factors among children (n=512, aged 6-8 years) (Venäläinen et al. 2016).

The following chapters review the literature in more detail, focusing on the last 20 years and associations found between FAs and different cardiometabolic outcomes.

2.10 Fatty Acids and Body Fat Mass

Approximately 2.5 million adult Finns are overweight, and of these, every fourth is obese (Koponen et al. 2018). Overweight (body mass index 25.0-29.9 kg/m^2) and obesity (body mass index ≥30 kg/m^2) develop when energy intake is higher than energy expenditure for an extended period of time, with this being regulated by the brain-based "lipostatic regulation system" (Speakman 2004). In addition to the body mass index, another widely used measure for obesity is waist circumference: 100 cm for men and 90 cm for women being clinical cut-off values for meaningful central obesity (Lihavuus, lapset, nuoret, aikuiset, käypä hoito-suositus 2020). Obesity, and central obesity in particular, play a crucial role in the metabolic syndrome with its cardiometabolic outcomes, such as T2D, (hypertension) and dyslipidemia (Grundy et al. 2005). It has been proposed that obesity-based elevated circulating levels of (non-esterified) FAs could result in insulin resistance (Qatanani and Lazar 2007). Usually, weight loss requires changes in the diet and exercise habits. Even a weight loss of 5-10% of body weight may be extremely important in reducing the risk of pre-diabetes/diabetes (Magkos et al. 2016), and a weight loss of around 15 kg is often a priority to achieve a remission of T2D in overweight or obese individuals (McCombie et al. 2017). Weight loss was the most important predictor of T2D remission also in the Diabetes Remission Clinical Trial (DiRECT) (Lean et al. 2019;

Thom et al. 2020). Modification of dietary and/or the circulating composition of FAs could be a potential tool promoting a modest weight regulation (Schwab et al. 2014; Liu et al. 2018). When compared to other macronutrient classes (carbohydrates and protein), FAs yield the most adenosine triphosphate on an energy per gram basis, when they are completely oxidized to CO_2 and water by β-oxidation and the citric acid cycle. All of the FAs contain the same amount of energy (37 KJ per a gram of fat) (Berg, Tymoczko, and Stryer 2012).

In obesity, the presence of excess fat impairs normal cellular functions. In an attempt to maintain normality, this excess fat is collected into lipid droplets, and simultaneously, there is also a tendency towards an increased beta-oxidation of FAs (Aon, Bhatt, and Cortassa 2014). However, in this respect, the quality of fat seems to matter. In animal models, an increased consumption of long-chain n-3 PUFAs has been suggested to exert anti-obesity effects (Buckley and Howe 2009). FAs regulate the expression of the genes involved in lipid and energy metabolism. For example, one possible mechanism to explain this phenomenon is that PUFAs, but not SFAs or MUFAs, suppress the induction of lipogenic genes by inhibiting the expression and processing of transcription factor sterol regulatory element binding protein-1c. This evokes suppressed fat deposition and increased fat oxidation (Nakamura et al. 2004; Buckley and Howe 2009). Based on human experiments with labelled FAs, the long-chain SFAs, such as 18:0 compared to 12:0, may lead to weight gain since long-chain FAs are poorly oxidized, as compared to other fats (DeLany et al. 2000).

Some reviews, meta-analyses and individual clinical trials or population studies investigating the associations between dietary intake of FAs or circulating FAs and body fat mass are presented in **Table 6**. The role of n-3 PUFA (Du et al. 2015; Zhang, Y. Y. et al. 2017) and MUFA (Hammad and Jones 2017) intakes on fat mass remains unclear. Dietary source may affect the associations between MUFA intake and body fat mass. An increased intake of MUFA from animal sources by 1% has been associated with a weight gain of 0.29 kg (95% CI: 0.25, 0.33 kg), whereas MUFA from plant sources has not been associated with weight gain (Liu et al. 2018). The study reports have pointed to the health benefits of higher dietary and/or circulating PUFAs (linoleic acid, 18:2n-6, in particular) and estimated Δ5-desaturase activity to be associated with normal weight, and increased dietary and/or circulating SFAs, trans-fat and estimated Δ9- and Δ6-desaturase activity to be linked with obesity. However, the findings are not totally consistent. With regard to circulating individual PUFA proportions, dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3) have shown study-specific, either direct (in adults) or inverse (in children and adolescents) associations with body fat (Warensjö, Ohrvall, and Vessby 2006; Tang et al. 2019). As well as being due to age-difference, this inconsistency may also be due to genetic variation in FA metabolism between individuals and/or populations (Glaser et al. 2011). Prostaglandin-metabolism may

also affect FA metabolism since 20:4n-6 is a precursor for prostaglandins, thromboxanes (series 2), and leukotrienes (series 4). In addition, 20:3n-6 is a precursor for prostaglandins (series 1) and 20:5n-3 a precursor for prostaglandins, thromboxanes (series 3) and leukotrienes (series 5) (Das 2006).

In general, the intakes of SFAs have been associated with higher body weights and linoleic acid (18:2n-6) with lower body weight, whereas the role of MUFAs and n-3 PUFAs is much less clear.

Table 6. FAs and parameters of body fat mass.

Study	Observation	Population
INTAKE OF FAs:		
(Parker et al. 2019)	n-3 PUFAs did not appear to be an effective agent for reducing waist circumference or body fat% (or the liver fat) in overweight men.	Intervention/RCT: the effect of 12-week supplementation with n-3 PUFAs from fish oil vs. placebo on body composition and the liver fat in a double-blind randomized controlled trial in 50 healthy overweight men (BMI 25-29.9 kg/m^2). Daily fish oil dose: 1728 mg marine TGs, of which 588 mg of eicosapentaenoic acid (20:5n-3) and 412 mg of docosahexaenoic acid (22:6n-3), combined with 200 mg of coenzyme Q10, or placebo (olive oil capsules).
(Liu et al. 2018)	Compared with equivalent changes in carbohydrate intake, a 5% increase in energy from SFAs and a 1% increase in energy from trans-fat were associated with 0.61 kg and 0.69 kg greater weight gain per 4-y period, respectively. A 5% increase in energy from PUFAs was associated with less weight gain (-0.55 kg). Increased intake of MUFAs from animal sources by 1% was associated with weight gain of 0.29 kg, whereas MUFAs from plant sources was not associated with weight gain.	Three prospective US cohorts (baseline 1976-89), the Health Professionals Follow-Up Study (HPFS), the Nurses' Health Study (NHS), and the NHS II with their 121,335 men and women, free of diabetes, CVD, cancer, or obesity over a 20- to 24-y follow-up (every 4 year). Cohort-specific results were pooled with the use of a random-effect meta-analysis.
(Zhang, Y. Y. et al. 2017)	Based on the meta-analysis of nine studies, a statistically non-significant difference was revealed in weight loss between n-3 PUFA and placebo.	A total of 11 RCTs (up to 5/2015) involving 617 participants were included in this meta-analysis. Treatment duration from three to 24 weeks with a daily dosage of 2-6 grams of n-3 PUFAs.
(Hammad and Jones 2017)	Dietary composition of FAs affects obesity (i.e., long-chain SFAs elevating and PUFAs lowering the risk, the role of MUFAs remained unclear) and, FAs may interact with polymorphisms in several genes, such as PPARγ, ADRB2, FTO, LEP, LEPR, ADIPOQ, HSL, LPL, GLP1R, CLOCK, CEBPB, BDNF, REV-ERB-ALPHA circadian, STAT3, IL6, TNFα, TACE, APOA5, APOA2, APOB and LRP1, modifying the link between FAs and obesity (see detailed gene names from the original article).	Review of over 40 trials or population studies (n=60-11091) including one meta-analysis, up to the year 2017.
(Du et al. 2015)	Fish oil had no effect in reducing body weight. These findings do not support an anti-obesity role of n-3 PUFAs in overweight/obese subjects.	A meta-analysis of 21 RCTs with 1652 individuals to investigate the influence of fish oil on some parameters of body composition in overweight/obese adults. The median

		n-3 PUFA dose was 1.92 g/day (range: 0.54–11.3 g/day), and the ratio of EPA to DHA varied from 0.23 to 1.55. Mean duration was 12 weeks.
(Krishnan and Cooper 2014)	Dietary SFAs are likely more obesogenic than MUFAs, and PUFAs. The UFAs appear to be more metabolically beneficial, specifically MUFAs≥PUFAs >SFAs, as evidenced by the higher thermogenesis and fat oxidation following high-fat meals or diets.	Review of the literature up to the year 2013 with studies that compared MUFAs, PUFAs, and SFAs related to diet-induced thermogenesis, energy expenditure, or fat oxidation in response to a high-fat meal challenge, or long-term dietary intervention comparing these FAs.
(Smith et al. 2013)	A high intake of SFA was associated with an increased BMI, and higher waist and hip circumferences (no waist association among African Americans). The strongest associations were observed in white subjects with TT allele carriers of rs2306692 (low-density lipoprotein-related receptor protein 1, LRP1).	Meta-analyses using data from 14 studies of US and European whites and 4 of African Americans (n=584-9189).
(Bjermo et al. 2012)	Compared to SFA intake, n-6 PUFA intake reduced liver fat without weight loss. PUFAs had beneficial effect on plasma lipid concentrations (compared to parallel SFA intake).	Intervention: 67 abdominally obese Swedish subjects, aged 50-64 years, 66% women, with 10-wk isocaloric diet high in/baked on 18:2n-6 (margarines, oils, seeds) or high in/baked on SFAs (butter).
(Phillips et al. 2012)	Gene-SFA interaction: FTO rs9939609 was associated with obesity measures, especially in those subjects with the metabolic syndrome, which was further exacerbated by high dietary SFA intake at baseline and 7.5 years later.	Prospective European case-control study, the LIPGENE study (n = 1754), mean age of 51 years at baseline, 40% females.
CIRCULATING FAs:		
(Tang et al. 2019)	Five erythrocyte PL SFAs (14:0, 16:0, 17:0, 18:0 and 20:0) were significantly higher, and 9 PUFAs (18:3n-3,20:3n-3, 20:5n-3, 22:5n-3, 22:6n-3, 18:2n-6, 20:2n-6, 20:3n-6, and 20:4n-6) were significantly lower in cases than in controls. Pooled results from the comparative meta-analysis were consistent for 22:5n-3, 22:6n-3 and 18:2n-6, although high heterogeneity was found between studies. In the logistic regression model, erythrocyte PL 16:0 and 18:0 were directly and 20:4n-6 and 22:6n-3 inversely associated with obesity in children and adolescents.	A case-control study including 1442 obese and 1442 normal-weight Chinese children and adolescents, aged 6 to 17 years, 26% girls. Circulating FA composition between cases and controls were compared both in this study (MUFAs were not analyzed) and literature-based meta-analysis.
(Rosqvist et al. 2017)	Serum PL/CE linoleic acid% (18:2n-6) was inversely related to body fat storage including visceral adipose tissue and trunk fat whereas palmitic acid% (16:0) was less consistently but directly associated.	A population-based cross-sectional sample of 287 elderly subjects, aged 70 years, 48% women, in the PIVUS cohort in Sweden.

(Aglago et al. 2017)	Serum PL Δ9-desaturase activity, palmitoleic acid % (16:1n-7), and dihomo-γ-linolenic acid % (20:3n-6) were directly associated with BMI. Total n-6 PUFAs % and the n-6/n-3 PUFA ratio were positively associated with WHR, while odd-chain FAs %, pentadecanoic (15:0) and heptadecanoic acid (17:0) showed inverse associations with the adiposity indicators. There are some variation in the findings between different outcomes of fat mass.	A cross-sectional study in 372 healthy Mexican women with a mean age of 50 years.
(Simopoulos 2016)	The high dietary n-6/n-3 PUFA ratio (as well as red blood cells membrane PL n-6/n-3 PUFA ratio) is likely a risk factor for obesity, whereas n-3s decrease the risk. As potential mechanisms, n-3 and n-6 PUFAs may elicit divergent effects on fat gain, adipogenesis, lipid homeostasis, brain-gut – adipose tissue axis and systemic inflammation.	Review of literature, human+animal studies (selection criteria were not presented in detail for the literature collected).
(Fekete et al. 2015)	The dihomo-γ-linolenic acid (20:3n-6) proportion (in plasma total lipids, PLs and CEs) was higher in overweight or obese subjects, compared to normal-weight controls. In addition, the plasma PL dihomo-γ-linolenic acid (20:3n-6)/linoleic acid (18:2n-6) ratio was higher and estimated Δ5-desaturase activity lower in overweight/obese subjects.	Review including 1, 575 participants in 21 case-control studies.
(Vinknes et al. 2013)	Plasma markers of Δ9-desaturase activity were associated with increased adiposity.	Population-based, cross-sectional study of 2021 elderly, 71-74 years old, 54% women, from the Hordaland Health Study in Western Norway.
(Warensjö, Ohrvall, and Vessby 2006)	The proportions of serum CE palmitic acid (16:0), palmitoleic acid (16:1n-7), stearic acid (18:0), γ-linolenic acid (18:3n-6), dihomo-γ-linolenic acid (20:3n-6), arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3) and indicators of Δ9- and Δ6-desaturase activity (specific FA ratios) had direct associations, and an indicator of Δ5-desaturase activity and linoleic acid (18:2n-6) inverse associations with the markers of obesity.	A cross-sectional setting of the population consisting of men (n=554) and women (n=295) with a mean age of 41 years who took part in a health survey concerning CHD in Sweden.

BMI=body mass index; CHD=coronary heart disease; CVD=cardiovascular disease; FA=fatty acid; MUFA=monounsaturated fatty acid; n-3=omega-3; n-6=omega-6;

PUFA=polyunsaturated fatty acid; RCT=randomized controlled trial; SFA=saturated fatty acid; UFA=unsaturated fatty acid.

Estimation of desaturase activity (examples): Δ9-desaturase activity: the 16:1n-7)/16:0 and/or the 18:1n-9/18:0 ratios; Δ6-desaturase activity: the 18:3n-6/18:2n-6 ratio; Δ5-desaturase activity: the 20:4n-6/20:3n-6 and/or the 20:5n-3/20:4n-3 ratios.

2.11 Fatty Acids and Insulin Resistance or Type 2 Diabetes

Over half a million Finnish adults have pre-diabetes or T2D (Koponen et al. 2018). These are long-term cardiometabolic outcomes characterized by high blood sugar levels, insulin resistance and in T2D later also by a (relative) lack of insulin. Usually, pre-diabetes leads to T2D. The diagnostic criteria for T2D are fasting plasma glucose≥7 mmol/L (for pre-diabetes 6.1-6.9 mmol/L) or the 2-hour glucose value >11 mmol/L (for pre-diabetes 7.8-11 mmol/L) in an oral glucose tolerance test or HbA1c≥48 mmol/mol, ≥6.5%) (World Health Organisation 1999; Diabetes. Käypä hoito-suositus, 2018). In addition, the HOMA-IR has been used in large population studies as a validated measure of insulin resistance.

Abdominal obesity is a strong risk factor for insulin resistance. The FA composition may exert obesity-dependent or independent effects on insulin resistance and T2D. It has been suggested that there is a connection between (free) SFAs or trans-FAs, and lipotoxicity, inflammation and insulin resistance. The FA composition has also been speculated to affect the function of cell membranes. Especially a high content of long-chain PUFAs in cell membranes may accelerate both insulin independent and dependent (i.e. by influencing on insulin receptor binding and/or insulin's affinity for its receptor) glucose intake into the cells. PUFA-rich membranes may also be more flexible than membranes rich in SFA-based PLs, for example, it has been postulated that this may attenuate diabetes-related tissue hypoxia (Malcom et al. 1989; Qatanani and Lazar 2007; Riserus, Willett, and Hu 2009; Weijers 2012; Estadella et al. 2013).

There are several meta-analyses, individual RCTs and population studies linking dietary intake of FAs or circulating FAs to the risk of pre-diabetes and T2D (**Table 7**). Due to the heterogeneity in the meta-analyses reported (regarding designs/targets of individual studies), the strongest evidence of the beneficial health effects of dietary UFAs has emerged from individual RCTs that have been planned to study the association between dietary fats and insulin resistance. In the KANWU Study (Kuopio, Aarhus, Naples, Wollongong and Uppsala Study, n=162), the substitution of dietary SFAs for MUFAs improved insulin sensitivity in healthy men and women (Vessby et al. 2001). In the Finnish study of hypercholesterolemic men (n=120) (Jula et al. 2002), the reduction of energy intake from SFAs and trans-UFAs to ≤10 E% and replacing them partly with MUFAs and PUFAs rich in n-3 PUFAs (+increased intake of fruits, vegetables and dietary fiber), decreased the levels of serum insulin by 14%. In addition, the Finnish STRIP study showed that life-time dietary modification counselling starting in infancy and continued until early adulthood (low SFAs, low cholesterol) could lower the SFA intake and improve insulin sensitivity in adolescence and early adulthood (Simell et al. 2009; Pahkala et al. 2020).

With regard to circulating FA proportions, the literature supports the beneficial effects of total n-6 PUFAs and linoleic acid (18:2n-6), in particular, in reducing the risk of T2D (Wu et al. 2017). An inverse association has been reported between T2D and the circulating plant-origin phospholipid n-3 PUFAs (α-linolenic acid, 18:3n-3) whereas no convincing associations have been detected between T2D and marine-derived n-3 PUFAs (Forouhi et al. 2016). With respect to SFAs, specific SFA proportions, odd-numbered chain 15:0 and 17:0 SFAs (Santaren et al. 2014; Huang et al. 2019), or long or very long-chain 20:0, 22:0 and 24.0 SFAs have been inversely associated with incident T2D (Lemaitre et al. 2015). In several studies, palmitoleic acid (16:1n-7), γ-linolenic acid (18:3n-6) and/or dihomo-γ-linolenic acid (20:3n-6) have consistently shown direct links with T2D, impaired glucose and/or insulin metabolism (Vessby et al. 1994; Wang, L. et al. 2003; Kurotani et al. 2012; Forouhi et al. 2016). Similarly, the estimated high Δ9-desaturase and Δ6-desaturase activity, and low Δ5-desaturase and elongase activity have been suggested to display direct links with impaired glucose metabolism and T2D (Kurotani et al. 2012; Lankinen et al. 2015).

Schwab and co-workers (2014) reviewed the literature (607 quality graded papers) some years ago related to forming of Nordic dietary recommendations (Schwab et al. 2014). Their main findings/conclusions were: ”the evidence for a direct association between total fat intake and risk of T2D was found inconclusive, whereas there was limited-suggestive evidence from biomarker studies that circulating linoleic acid (18:2n-6) is inversely associated with the risk of T2D. Beneficial effect of dietary MUFAs both on insulin sensitivity and fasting plasma/serum insulin concentration was considered as probable in comparisons of MUFAs and carbohydrates vs. SFAs, whereas no effect was found on fasting glucose concentration in these comparisons. However, there was limited-suggestive evidence in biomarker studies that odd-chain SFA found in milk fat and fish may be inversely related to T2D, but these associations have not been supported by controlled studies. The evidence for an association between dietary n-3 PUFAs and T2D was found to be inconclusive.”

Table 7. FAs and insulin resistance, pre-diabetes and/or type 2 diabetes.

Study	Observation	Population
INTAKE META-ANALYSES:		
(Brown et al. 2019)	Evidence suggests that increasing n-3s, n-6s, or total PUFAs has little or no effect on prevention and treatment of T2D.	Eighty-three RCTs, up to 12/2018. Duration of at least 24 months. Participants were adults with or without pre-diabetes/diabetes. Eligible interventions could be dietary advice, supplementation (taken orally as oil, foods, or capsules), or diet provided. Primary analyses assessed effects of total PUFAs n-6s, long-chain n-3s, and α-linolenic acid (18:3n-3) separately using random effects meta-analysis.
(Wanders et al. 2019)	Plant-derived PUFAs did not significantly affect fasting glucose, but lowered fasting insulin and HOMA-IR. In dose-response analyses, a 5% increase in energy (E%) from PUFAs significantly reduced serum insulin by 5.8 pmol/L.	A meta-analysis of 13 RCTs published up to 1/2018 investigating the effects of a diet high in plant-derived PUFAs as compared with SFAs or carbohydrates, and measured markers of glucose metabolism and insulin resistance as outcomes.
(Qian et al. 2016)	This analysis provided evidence that consuming diets high in MUFAs (vs. high in carbohydrates) could improve all the key metabolic syndrome risk factors among patients with T2D. A diet high in MUFAs vs. PUFAs showed improvements in glucose values only.	Review and meta-analysis of 24 intervention studies high in MUFAs vs. high in carbohydrates with 1460 participants. Four studies high in MUFAs vs. high in PUFAs with 44 participants.
(Imamura et al. 2016)	Replacing 5% energy from carbohydrate with MUFAs lowered HbA1c (-0.09%), 2h post-challenge insulin (-20.3 pmol/L), and HOMA-IR (-2.4%). Replacing carbohydrate with PUFAs significantly lowered HbA1c (-0.11%) and fasting insulin (-1.6 pmol/L). Replacing SFAs with PUFAs significantly lowered glucose, HbA1c, C-peptide, and HOMA-IR. Based on gold-standard acute insulin response in ten trials, PUFAs significantly improved insulin secretion capacity (+0.5 pmol/L/min) whether replacing carbohydrate, SFAs, or even MUFAs. Conclusion: most consistent favorable effects were seen with PUFAs, which was linked to improved glycaemia, insulin resistance, and insulin secretion capacity.	A meta-analysis of RCT trials up to 11/2015 (30% of these published before the year 2001) that tested effects of macronutrient intake on blood glucose, insulin, HbA1c, insulin sensitivity, and insulin secretion in adults (102 trials, with 4220 adults, 55% females, across 239 dietary arms). A multiple-treatment meta-regression was used to estimate dose-response effects of isocaloric replacements between SFAs, MUFAs, PUFAs, and carbohydrate, adjusted for protein, trans fat, and dietary fiber.

(Zhang, M., Picard-Deland, and Marette 2013)	No associations were found between the intake of fish/seafood or marine n-3 PUFAs and the incidence of T2D. However, intake of oily fish lowered the risk of T2D. Conclusion: different nutrients other than n-3 resulted in the observed risk reduction of oily fish.	A meta-analysis of 10 prospective studies (549 955 participants) to examine the relationship between fish/seafood intake and risk of T2D. Six prospective studies (346 710 participants) to study association between marine long-chain n-3 PUFA intake and risk of T2D.
(Zhou, Tian, and Jia 2012)	N-3 PUFA intake did not associate with the risk of T2D.	A meta-analysis of ten prospective cohort studies with 506 665 subjects with a follow-up from 4.1 to 15 years. Highest vs. lowest intake.
INTERVENTION STUDIES:		
(Oranta et al. 2013)	After follow-up of 15 to 20 years, the (PUFA+MUFA)/SFA ratio and the dietary fiber intake were higher in the intervention group compared with the control group. HOMA-IR was 7.5% lower in the intervention group. The intervention effect was equal in females and males.	In the STRIP Study: follow-up parameters including HOMA-IR were determined annually between the participants' age of 15 to 20 years in 245 intervention (a diet lower in SFAs and cholesterol) and 273 control adolescents, 47% females.
(Kaitosaari et al. 2006)	Intervention children consumed less total fat and SFAs than the control children. The HOMA-IR was lower in intervention children than in control children. There was a significant association between SFA intake and HOMA-IR. However, this association was diluted into statistical non-significance when adjusted for the known determinants of HOMA-IR.	In the STRIP Study (baseline in 1990), 1062 healthy 7-month-old infants were randomized to the biannual individualized intervention (*n*=540) and control (*n*=522) groups. Follow-up parameters including HOMA-IR were determined in a random subgroup of 78 intervention children and 89 control children at the age of 9 years, 51% girls.
(Jula et al. 2002)	Dietary treatment decreased activity of serum insulin by 14%, having a beneficial effect also on serum lipids. In addition, the intervention potentiated the cholesterol lowering effect of simvastatin and counteracted the fasting insulin-elevating effect of simvastatin.	A controlled cross-over trial (1997-1998) in 120 previously untreated hypercholesterolemic Finnish (Turku area) men, aged 35-64 years, randomly allocated either to a habitual diet or dietary treatment groups (+randomization of the both groups for simvastatin or placebo). Dietary intervention (n=60): to reduce energy intake from SFAs+ trans-UFAs to no more than 10 E% by replacing them partly with MUFAs and PUFAs rich in n-3 PUFAs and to increase intake of fruits, vegetables, and dietary fiber.
(Vessby et al. 2001)	A change of the proportions of dietary FAs, decreasing SFAs and increasing MUFAs, improved insulin sensitivity but had no effect on insulin secretion. A beneficial impact of the fat quality on insulin sensitivity was not seen in individuals with a high fat intake (> 37	The KANWU study with a total of 162 healthy subjects from Kuopio, Aarhus, Naples, Wollongong and Uppsala, aged 30-65 years, 47% females, chosen at random to receive a controlled, isoenergetic diet for 3 months containing a median of 37 E% of fat with either a high proportion of SFAs

	E%). The addition of n-3 PUFAs to the diet did not affect insulin sensitivity.	(17 E%) or MUFAs (23 E%). Within each group there was a second assignment at random to supplements with fish oil (3.6 g n-3 PUFAs/d) or placebo.
CIRCULATING FAs:		
(Huang et al. 2019)	This meta-analysis showed that the per standard deviation increase in odd-chain SFAs pentadecanoic acid (15:0) and heptadecanoic acid (17:0) was associated with a reduced risk of incident T2D, while the increase in one even-chain SFAs, myristic acid (14:0), was associated with an increased risk of incident T2D.	A total of 19 studies were included for systematic review, and 10 prospective studies for meta-analyses.
(Wu et al. 2017)	Higher linoleic acid (18:2n-6) proportion was associated with a lower risk of T2D, association being similar in different lipid compartments, including PL, plasma, CEs, and adipose tissue. Proportions of arachidonic acid (20:4n-6) were not significantly associated with T2D risk.	Pooled meta-analyses for the biomarker linoleic acid (18:2n-6) and its metabolite arachidonic acid (20:4n-6) and incident T2D in 20 prospective cohorts (Iceland, the Netherlands, the USA, Taiwan, the UK, Germany, Finland, Australia, Sweden, and France), sampled between 1970 and 2010 (39 740 adults including 4347 T2D cases).
(Imamura et al. 2017)	A combination of individual FAs (principal component analysis of 27 individual plasma phospholipid FAs), characterized by high concentrations of linoleic acid (18:2n-6), odd-chain FAs, and very long-chain FAs, was associated with lower incidence of T2D.	Eight European countries in EPIC-InterAct, a prospective nested-case-cohort study with 27 296 adults including 12 132 incident T2D cases (baseline between 1991-1998, and the end of the follow-up in 2007).
(Guo et al. 2017)	Circulating n-3 PUFAs, i.e., docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3), but not their dietary intake, were associated with a lower metabolic syndrome risk (impaired glucose metabolism being one component).	A meta-analysis of 27 case-control or cross-sectional studies with the characterization of circulating FAs and/or FA intake data.
(Cabout et al. 2017)	Serum linoleic acid (18:2n-6) was inversely associated with fasting and post-load glucose in cross-sectional, but not in prospective analyses.	This prospective study included 667 participants (mean age 69 years, 51% females) followed for 8 years (2000-2008) in the population-based Hoorn study in the Netherlands.
(Forouhi et al. 2016)	Inverse association of circulating plant-origin phospholipid n-3 PUFA (α-linolenic acid, 18:3n-3), but no convincing association of marine-derived n-3 PUFAs (eicosapentaenoic acid, 20:5n-3 and docosahexaenoic acid, 22:6n-3) with T2D. The most abundant n-6 PUFAs (linoleic acid, 18:2n-6) and eicosadienoic acid (20:2n-6) were inversely associated with T2D whereas γ-linolenic acid (18:3n-6), dihomo- γ-linolenic acid (20:3n-6), docosatetraenoic acid (22:4n-6) and docosapentaenoic acid (22:5n-3) showed direct links.	A prospective case-control study of 12 132 incident T2D cases vs. 15 919 sub-cohort participants (the EPIC-InterAct Case-Cohort Study).

(Johnston et al. 2016)	Higher PL stearic acid (18:0) and γ-linolenic acid (18:3n-6) and lower octadecenoic acid (18:1n-7) predicted consistently lower insulin sensitivity and β-cell function.	A population of 447 individuals (with a mean baseline age of 50 years and 73% of them females) with the elevated risk for T2D followed for 6 years in the PROMISE cohort located in London and Toronto areas (baseline between 2004-2006).
(Yu et al. 2016)	Docosapentaenoate (22:5n-3), as a part of plasma metabolomics profile, showed a direct association with incident T2D.	Prospective Chinese study, based on the Shanghai Women's/Men's Health Study, with 976 men and women (aged 40–74 years, baseline 1997-2006), of whom 73 suffered from incident T2D.
(Bigornia et al. 2016)	"De Novo Lipogenesis Pattern", associated with a diet high in sugar and low in n-6 PUFAs, may adversely affect HOMA-IR.	Participants of the Boston Puerto Rican Health Study (n=922, aged 45–75 y, >70% females, baseline 2004-2009) with a 2-year follow-up. On the basis of principal component analysis, 5 FA patterns were derived: *1*) relatively high de novo lipogenesis FAs and low n-6 PUFAs, *2*) high very-long-chain SFAs, *3*) high n-3 PUFAs, *4*) high linoleic acid (18:2n-6) and low arachidonic acid (20:4n-6), and *5*) high *trans*-FAs.
(Lankinen et al. 2015)	Plasma PL, CE and TG total SFAs, palmitoleic acid (16:1n-7), dihomo-γ-linolenic acid (20:3n-6) and estimated Δ9- and Δ6-desaturase activity significantly predicted the worsening of glycaemia, whereas total PUFAs, linoleic acid (18:2n-6) and elongase activity (18:1n-7/16:1n-7) predicted a decrease in the glucose AUC (area under curve). Estimated Δ6-desaturase activity and dihomo-γ-linolenic acid (20:3n-6) were associated with an increased risk of incident T2D. Findings were consistent across the 3 different lipid fractions.	The Metabolic Syndrome in Men (METSIM) study sub-cohort of 1302 Finnish men, examined between 2005-2010, aged 45-68 years, followed for 5.9 years. Seventy-one participants developed incident T2D.
(Lemaitre et al. 2015)	Plasma PL very long-chain SFAs, major components of ceramides and sphingomyelins, i.e., arachidic acid (20:0), behenic acid (22:0), and lignoceric acid (24:0) were inversely associated with incident T2D. These associations may be mediated by lower TGs and palmitic acid (16:0).	A total of 3179 older US adults, 61% females, from the Cardiovascular Health Study, with a mean age of 75 years at study baseline (1992-1993), were followed through 2011 resulting in 284 cases with incident T2D.
(Santaren et al. 2014)	Serum pentadecanoic acid (15:0), a short-term marker of dairy food intake, was inversely associated with incident T2D.	The Insulin Resistance Atherosclerosis Study (IRAS) with its 659 US adults, mean age 55 years, 55% females, followed for 5 years (between years 1992-1999). Diabetes status was assessed by using oral-glucose-tolerance tests.

(Virtanen et al. 2014)	Serum n-3 PUFAs, i.e., eicosapentaenoic acid (20:5n-3) + docosapentaenoic acid (22:5n-3) + docosahexaenoic acid (22:6n-3), biomarkers for fish intake, were associated with long-term lower risk of T2D. Serum α-linolenic acid, (18:3n-3) or dietary intake of n-3 PUFAs did not show any significant association.	A total of 2212 Finnish men from the prospective, population-based Kuopio Ischemic Heart Disease Risk Factor study (KIHD), aged 42-60 years, followed for a mean of 19.3 years with 422 incident T2D cases.
(Mahendran et al. 2013)	Cross-sectional baseline: FFAs, total MUFAs, SFAs, and n-7 and n-9 MUFAs were increased in fasting and 2-h hyperglycemia, whereas n-3 and n-6 PUFAs were decreased. Follow-up study showed that FFAs, total MUFAs, and SFAs and n-7 and n-9 MUFAs, predicted a worsening of hyperglycemia and development of incident T2D. N-6 PUFAs, mainly linoleic acid (18:2n-6), were associated with reduced risk for the worsening of hyperglycemia and conversion to T2D.	Cross-sectional and longitudinal analyses of the population-based the Metabolic Syndrome in Men (**METSIM)** study (examined between 2005-2010). There were 9398 baseline men (mean age of 57 years). For 4335 men, there were a follow-up of 4.5 years (with 276 incident T2D cases).
(Kurotani et al. 2012)	High levels of serum CE and/or PL stearic (18:0), palmitoleic (16:1n-7), or dihomo-γ-linolenic acids (20:3n-6); estimated Δ9- and Δ6-desaturase activity; and low levels of serum linoleic acid (18:2n-6) or estimated Δ5-desaturase activity might be associated with higher insulin resistance estimated by C-peptide concentration.	A cross-sectional study of 437 Japanese office employees, 41% women, aged 21-67 years, examined in 2006.
(Djousse et al. 2011)	Plasma PL n-3 PUFAs, eicosapentaenoic (20:5n-3)+docosahexaenoic acid (22:6n-3) had borderline significant and α-linolenic acid (18:3n-3) statistically significant inverse association with the risk of incident T2D.	Prospective data analysis in 3088 older US men and women, 61% females, mean age of 75 years, with a median follow-up of 10.6 years with 204 cases of incident T2D, from the Cardiovascular Health Study (1992–2007).
(Hodge et al. 2007)	In plasma PLs, direct associations with T2D were seen for stearic acid (18:0) and total SFAs, whereas an inverse association was seen for linoleic acid (18:2n-6). Dietary 18:2n-6, palmitic acid (16:0) and stearic acid (18:0) were directly associated with incident T2D (before adjustment for body size).	A prospective case-cohort study (baseline in 1990-94), The Melbourne Collaborative Cohort Study (MCCS), of 3737 adults aged 36–72 y with a follow-up of 4 years (self-reported T2D incidence with 346 cases).
(Wang, L. et al. 2003)	Total SFAs in plasma CE and PL were directly associated with incident T2D. In CEs, the proportions of palmitic (16:0), palmitoleic (16:1n-7), and dihomo-γ-linolenic (20:3n-6) acids were directly, and the proportion of linoleic acid (18:2n-6) inversely associated with the incident T2D. In PL, the proportions of palmitic (16:0) and stearic acid (18:0) were directly associated with incident T2D.	Among 2909 US adults of the prospective the Atherosclerosis Risk in Communities (ARIC) Study (baseline 1987-89), aged 45–64 y, >50% females, 252 incident diabetes cases were observed within a follow-up of 9 years.

(Laaksonen et al. 2002)	Serum esterified and non-esterified SFAs were directly associated and PUFAs inversely associated with incident impaired fasting glucose (n=56) or T2D (n=34).	A prospective Kuopio Ischemic Heart Disease Risk Factor study (KIHD) cohort of 895 normoglycemic Finnish men, mean age of 52 years (baseline 1984-1989), followed for 4 years.
(Vessby et al. 1994)	Higher serum CE proportions of SFAs and palmitoleic acid (16:1n-7), and a low proportion of linoleic acid (18:2n-6), and a relatively high content of γ-linolenic (18:3n-6) and dihomo-γ-linolenic (20:3n-6) acids were associated with the elevated risk of T2D.	Normoglycemic Swedish (Uppsala) men (n=1,753) and those who later developed diabetes (*n*=75), aged 50 years at baseline in 1970-73 with a follow-up of 10 years (re-examination in 1980-84).

CE=cholesteryl ester; FA=fatty acid; HOMA-IR= homeostatic model-based insulin resistance; MUFA=monounsaturated fatty acid; n-3=omega-3; n-6=omega-6; PL=phospholipid; PUFA=polyunsaturated fatty acid; RCT=randomized controlled trial; SFA=saturated fatty acid; TG=triglycerides; T2D=type 2 diabetes; UFA=unsaturated fatty acid.

Estimation of desaturase activity (examples): Δ9-desaturase activity: the 16:1n-7)/16:0 and/or the 18:1n-9/18:0 ratios; Δ6-desaturase activity: the 18:3n-6/18:2n-6 ratio; Δ5-desaturase activity: the 20:4n-6/20:3n-6 and/or the 20:5n-3/20:4n-3 ratios.

2.12 Fatty Acids and Elevated Blood Pressure or Hypertension

Approximately two million adult Finns have an elevated BP. It has been recommended that systolic BP 140 mmHg or more and/or diastolic BP 90 mmHg or more (during the clinical examination) need lifestyle changes or lifestyle changes+medical treatment. Approximately one million Finns have been prescribed medication for BP, but only 40% of them have reached target values for BP (Kohonnut verenpaine. Käypä hoito-suositus, 2020). With regard to a dietary approach to lower BP, a well-known clinical trial, Dietary Approaches to Stop Hypertension (DASH) showed that a diet rich in fruits, vegetables, and low-fat dairy foods and with reduced saturated and total fat could substantially lower BP (Appel et al. 1997). This DASH diet is still a reference diet as a way to lower BP.

In rats, dietary fats seem to influence BP levels, as summarized and discussed by Wang and co-workers (Wang, L. et al. 2010): Experimental studies have found that feeding rats with SFAs results in an impaired endothelial function (enhanced noradrenaline sensitivity and blunted endothelium-dependent relaxation), enhanced sympathetic nervous system activity and elevated BP. In contrast, consumption of long-chain n-3 PUFAs seemed to modulate plasma PL composition and cell membrane fluidity of rats, increasing the production of vasodilators, and reducing cardiac adrenergic activity, which lowered the animals' BP. The incorporation of n-6 PUFAs into the cell membrane appeared to change the balance between vasoconstrictors and vasodilators, however, the subsequent net effects on BP have varied in different animal models. Similarly, MUFAs also may be able modify membrane PL composition and vascular reactivity in rats, but their effects on BP are unclear (Wang, L. et al. 2010).

Large studies from the US using semi-quantitative food frequency questionnaires, the Nurses' Health Study (58 218 women) and the Health Professionals' Follow-up Study (30 681 men), have not found any association between SFA, MUFA or PUFA intakes and BP (Witteman et al. 1989; Ascherio et al. 1992). Data from randomized clinical trials suggest that a diet low in SFAs and high in PUFAs may not decrease BP (Aro et al. 1998), except when the baseline the P/S ratio is low (Puska et al. 1983). In the STRIP Study, a diet with low content in SFAs and cholesterol, and a higher content in PUFAs, resulted in a 1 mmHg smaller BP increase as children grew up from 7 months to 15 years of age (Niinikoski et al. 2009).

The OmniHeart Feeding Study, carried out in the US, reported evidence for a BP-lowering effect of replacing SFAs with MUFAs as a part of a healthy lifestyle diet (Appel et al. 2005). However, based on a meta-analysis of different intervention trials, it was unclear whether the replacement of SFAs with MUFAs diet alone could lower the BP (Shah, Adams-Huet, and Garg 2007). Overall, the inconsistent findings

for MUFAs seem to be at least partly dependent on the dietary source of MUFAs (olive oil in the Mediterranean area vs. meat, dairy products and butter in the USA and the Northern Europe) (Alonso, Ruiz-Gutierrez, and Martinez-Gonzalez 2006) and the amount of total fat intake, i.e., the high total fat intake may prevent the beneficial effects (Rasmussen et al. 2006).

The strongest evidence for beneficial effects of FAs has emerged from interventions examining the effects of n-3 PUFAs on BP. A meta-regression analysis of 36 trials (1966-2001) concluded that a high intake of fish oil with n-3 PUFAs (median fish oil dose of 3.7 g/day) could reduce BP in adults (Geleijnse et al. 2002). There are also other reviews and/or meta-analyses with very similar findings in which n-3 PUFAs (Morris, Sacks, and Rosner 1993; Campbell et al. 2013) have lowered BP. Particularly, long-chain docosahexaenoic acid (22:6n-3) may exert beneficial influences on BP (Mori et al. 1999).

Some additional studies have been presented in **Table 8**. Similarly, the strongest evidence regarding the BP lowering capabilities of FAs emerges from RCT meta-analyses where the effects of n-3 PUFAs have been investigated (Miller, Van Elswyk, and Alexander 2014). For other FAs, both the intake and biomarker data are inconsistent. On the basis of findings of Murakami and co-workers (Murakami et al. 2008), estimated Δ9- and Δ6-desaturase activity was positively associated with diastolic or systolic BP, whereas estimated Δ5-desaturase and elongase activity showed inverse associations. These findings related to FA metabolism are similar with those observed in connection with elevated fat mass and glucose metabolism or T2D (Tables 6 and 7).

Table 8. FAs and blood pressure or hypertension.

Study	Observation	Population
INTAKE OF FAs:		
(Jovanovski et al. 2019)	In the context of low SFAs, high-MUFA diets in isocaloric substitution for high-carbohydrate diets did not affect BP in individuals with and without hypertension.	Fourteen RCTs (>3 weeks duration), 980 participants. The optimal macronutrient profile, in the context of a low-SFA diet, is controversial. The purpose was to assess the effect of high-MUFA diets in isocaloric substitution for high-carbohydrate diets on systolic BP and diastolic BP.
(Colussi et al. 2017)	n-3 PUFA intervention was associated with significant improvement in vascular function and lowering of BP in hypertensive participants, in particular.	Review article/summary of meta-analyses consisting of 111 n-3 PUFA interventions in 7233 normotensive participants, and 70 interventions in 3258 hypertensive participants. Meta-analyses were carried out between 1993-2014.
(Vafeiadou et al. 2015)	Replacement of SFAs with MUFAs or n-6 PUFAs lowered BP. There was no influence on the percentage of flow-mediated dilatation or other measures of vascular function.	Intervention of the Dietary Intervention and Vascular Function (DIVAS) study: a randomized, controlled, single-blind, parallel-group dietary SFA, MUFA or n-6 PUFA rich intervention for 16 weeks. A total of 195 participants, aged 21-60 years, 56% females from the United Kingdom (2009-2012).
(Miller, Van Elswyk, and Alexander 2014)	Compared with placebo, eicosapentaenoic acid (20:5n-3)+docosahexaenoic acid (22:6n-3) provision reduced systolic BP and diastolic BP in the meta-analyses of all studies combined. The strongest effects of eicosapentaenoic acid (20:5n-3)+docosahexaenoic acid (22:6n-3) were observed among untreated hypertensive subjects.	Seventy RCTs: to examine the effect of eicosapentaenoic acid (20:5n-3)+docosahexaenoic acid (22:6n-3), without upper dose limits and including food sources (mainly fish oils), on BP in RCTs representing the adult general populations. Treatment duration >3 weeks, no medical treatment for hypertension.
(Miura et al. 2008)	Linear regression analyses, adjusted for confounders, showed a non-significant inverse relationship of linoleic acid (18:2n-6) intake to systolic and diastolic BP. When analyzed for 2238 “non-intervened” individuals (not on a special diet, not consuming nutritional supplements, no diagnosed CVD or T2D, and not taking medication for high BP, CVD, or T2D), the relationships of 18:2n-6 with BP was stronger and statistically significant.	The cross-sectional International Study of Macro-Micronutrients and Blood Pressure (INTERMAP) study from 17 population samples in China, Japan, United Kingdom, and US (4680 men and women, aged 40-59 years, 1996-1999).
(Psaltopoulou et al. 2004)	Olive oil intake and the MUFAs/SFAs intake ratio had an inverse association with BP.	The cross-sectional European Prospective Investigation into Cancer and Nutrition (EPIC-Greece) study with 20 343 normotensive volunteers, 57% females, aged 20-86 years, recruited in 1994-1999.

(Hajjar and Kotchen 2003)	There were higher consumptions of MUFAs and PUFAs (and cholesterol) in regions with the highest mean BP (=the south). Analyses were adjusted for age, gender, ethnicity, BMI and total energy consumption.	NHANES III, Third National Health and Nutritional Examination Survey with 17 752 adult participants, 53% females, mean age of 48 years, recruited in 1988-94. Observational study to compare four US regions, northeast, midwest, south and west.
CIIRCULATING FAs:		
(Wolters et al. 2016)	Low baseline whole blood arachidonic acid (20:4n-6) levels in the whole sample and high n-3 PUFA levels in thin/normal weight children were associated with lower subsequent BP. Linoleic acid (18:2n-6) did not show any statistically significant associations. Association data was not shown for SFAs and MUFAs.	A subsample of 1267 children, 49% girls, aged 2–9 years at baseline (2007-8) of the European IDEFICS (Identification and prevention of dietary- and lifestyle-induced health effects in children and infants) cohort. Systolic and diastolic BP were measured at baseline and after two and six years.
(Virtanen et al. 2012)	Higher serum long-chain n-3 PUFA concentration, i.e., eicosapentaenoic acid (20:5n-3) + docosapentaenoic acid (22:5n-3) + docosahexaenoic acid (22:6n-3) had a modest inverse association with systolic BP in older men and women.	A cross-sectional data of 396 men and 372 women, aged 53-73 years, from the Kuopio Ischemic Heart Disease Risk Factor study (KIHD) cohort (data collected in 1998-01). Participants with ischemic heart disease, stroke, diabetes or hypertension treatment were excluded.
(Murakami et al. 2008)	Estimated Δ9-desaturase activity was positively associated with diastolic BP, Δ6-desaturase activity showed positive associations with systolic BP. Δ5-desaturase activity showed independent negative associations with systolic BP. Elongase activity was associated negatively with systolic and diastolic BP.	A cross-sectional set of 640 female Japanese dietetic students aged 18 to 22 years. Data was collected in 2007.
(Zheng et al. 1999)	Lowered levels of plasma cholesteryl ester linoleic acid (18:2n-6) and the PUFA/SFA ratio, and elevated levels of palmitic (16:0) and arachidonic acid (20:4n-6) were associated with a higher risk of incident hypertension.	Prospective the Atherosclerosis Risk in Communities (ARIC) Study with 1975 normotensive and 413 hypertensive US participants, >50% females, followed for six years. Baseline was in 1987-95.
(Grimsgaard et al. 1999)	Direct associations were found between plasma phospholipid total FA or SFA levels and BP, whereas the linoleic acid (18:2n-6) concentration was inversely associated with BP.	A cross-sectional Norwegian study with 4033 men, aged 40-42 years, recruited in 1988-89.

BMI=body mass index; BP=blood pressure; CVD=cardiovascular disease; FA=fatty acid; MUFA=monounsaturated fatty acid; n-3=omega-3; n-6=omega-6; PUFA=polyunsaturated fatty acid; RCT=randomized controlled trial; SFA=saturated fatty acid; T2D=type 2 diabetes.

Estimation of desaturase activity (examples): Δ9-desaturase activity: the 16:1n-7)/16:0 and/or the 18:1n-9/18:0 ratios; Δ6-desaturase activity: the 18:3n-6/18:2n-6 ratio; Δ5-desaturase activity: the 20:4n-6/20:3n-6 and/or the 20:5n-3/20:4n-3 ratios.

2.13 Fatty Acids and Cardiovascular Outcomes with Atherosclerosis Etiology

The quality of dietary fat has been linked to dyslipidemia (elevated total and LDL cholesterol concentrations), atherosclerosis and increased risk of CHD. In intervention trials, replacement of SFA intake partly with PUFA intake has consistently let to a decreased risk of CHD (Sacks et al. 2017; Mozaffarian, Micha, and Wallace 2010; Astrup et al. 2011; Schwab et al. 2014). In more detail, Schwab and co-workers concluded (Schwab et al. 2014): "there was convincing evidence that partial replacement of SFAs with PUFAs decreases the risk of CVD, especially in men. This finding was supported by an association with biomarkers of PUFA intake; the evidence of a beneficial effect of dietary total PUFAs, n-6 PUFAs, and linoleic acid (18:2n-6) on CVD mortality was limited suggestive." See **Table 9** for some additional and more recent trials and cohort studies. Although a high intake of PUFAs has generally been related with a reduced CHD risk (Mozaffarian, Micha, and Wallace 2010; Astrup et al. 2011), some meta-analyses have suggested that there was no association in the trials which had been carried out (Hamley 2017) or that an elevated n-6 PUFA intake could, without simultaneous adequate n-3 PUFA intake, increase rather than attenuate the risk of CHD morbidity and mortality (Ramsden et al. 2010; Ramsden et al. 2013; Calder 2013). On the other hand, the cardiovascular benefits of pure n-3 PUFA supply are also not fully consistent (ASCEND Study Collaborative Group et al. 2018). Nowadays, one reason for these varying findings may be the high-class medical care, which is reducing the relative benefits of the healthy nutrition. Circulating SFAs, and FAs associated metabolically with SFAs, such as circulating MUFAs, have had direct links with the risk for cardiovascular events (Warensjö et al. 2008; Würtz et al. 2015). Estimates of desaturase activity (Δ9-, Δ6- and Δ5-desaturase) and elongase activity seemed to have similar CV event associations than those observed for the fat mass (BMI), BP and T2D and/or impaired glucose metabolism (see above).

Increased wall thickness of the carotid artery, including the combined thicknesses of the intima and media layers (cIMT) measured with ultrasound has been used as a marker for sub-clinical atherosclerosis. A large body of evidence has revealed the relationship between increased cIMT and a future risk of adverse cardiovascular events. In a systematic review and meta-analysis of 14 studies with 45, 828 asymptomatic individuals who underwent a single cIMT measurement and were followed-up for 11 years, cIMT was associated with the risk of first myocardial infarction or stroke (Den Ruijter et al. 2012). It has been earlier demonstrated in the YFS that when the determination of serum FA profile, i.e., serum FAs, was added to the prediction models together with conventional risk factors, this improved the prediction of incident high cIMT in young adults over a 6-year follow-up (Würtz et al. 2012). In particular, a higher serum docosahexaenoic acid (22:6n-3) concentration

was associated with a decreased risk for incident high cIMT (Würtz et al. 2012). In a cross-sectional study with 108 elderly women (aged 70±4 years, mean±SD), PUFA and linoleic acid (18:2n-6) intake in particular, have been inversely associated with cIMT. Similarly, a PUFA intake of >9 g/day was found to be associate with a low atherosclerotic plaque prevalence (Mazza et al. 2018). Thus, there is some evidence that PUFAs could lower CVD/CHD risk by reducing the development of the early stages of arterial atherosclerosis.

Table 9. FAs and the risk of cardiovascular events.

Study	Observation	Population
REPLACEMENT STUDIES OR RCTs:		
(Lenighan, McNulty, and Roche 2019)	Conclusion: replacement of SFAs with PUFAs has the potential to reduce risk of CVD and T2D. Benefits of eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) have been well-characterized, less is known of the role of α-linolenic acid (18:3n-3). The current dietary guideline for α-linolenic acid (18:3n-3) is 0.5E%; however, evidence suggests that benefit is observed at levels greater than 2 g/d (0.6–1 E%). Thus, additional RCTs are needed to find optimal α-linolenic acid (18:3n-3) dose.	Review of the literature: replacement of SFAs with PUFAs, and with α-linolenic acid (18:3n-3) in particular. Metabolic changes related to replacement, such as alterations in cholesterol homeostasis and adipose tissue inflammation, were discussed.
(ASCEND Study Collaborative Group et al. 2018)	There was no significant difference in the risk of serious incident vascular events between those who were assigned to receive n-3 PUFA supplementation and those who were assigned to receive placebo.	The ASCEND Study intervention: 15,480 British patients with diabetes but without evidence of atherosclerotic CVD were randomly assigned to receive 1-g capsules containing either n-3 PUFAs (FA group) or matching placebo (olive oil) daily. Baseline 2005-2011, a mean follow-up of 7.4 years.
(Sacks et al. 2017)	In summary, randomized controlled trials that lowered intake of dietary SFAs and replaced it with PUFA-rich vegetable oil reduced CVD by ≈30%, similar to the reduction achieved by statin treatment. Replacement of SFAs with UFAs lowered low-density-lipoprotein cholesterol. This recommended shift from SFAs to UFAs should occur simultaneously in an overall healthful dietary pattern, such as DASH or the Mediterranean diet.	The American Heart Association advisory. Several long-term intervention trials with long enough duration (>2 years) and confirmed compliance (Morris JN and the committee 1968; Dayton et al. 1969; Leren 1970; Turpeinen et al. 1979), and meta-analyses based on these core findings, provide the strongest evidence to support benefits to replace SFAs with UFAs.
(Hamley 2017)	When pooling results from only the adequately controlled trials, FA intake did not have an effect on CHD events. In contrast, the pooled results from all trials, including the inadequately controlled trials, suggested that replacing SFAs with mostly n-6 PUFAs would significantly reduce the risk of total CHD.	A meta-analysis of eleven RCTs, focusing on trials that most accurately tested the effect of replacing SFAs with mostly n-6 PUFAs. Clinical trials were identified from earlier meta-analyses. Relevant trials were categorized as 'adequately controlled, 5 pcs' or 'inadequately controlled 5-6 pcs' depending on differences between interventions that were not related to SFA or PUFA intake. Lower intake of trans-FAs in the intervention group being one possible confounding factor.

(Hooper et al. 2015)	Reducing dietary SFAs lowered the risk of cardiovascular events by 17%. Subgrouping suggested that the reduction in cardiovascular events was seen in studies that primarily replaced SFA calories with PUFAs.	Fifteen RCTs (published 1965-2006) with 53 300 participants of whom 8% had a cardiovascular event.
(Ramsden et al. 2013)	The intervention group (n=221) had higher rates of CVD and CHD deaths than controls (n=237) (CVD and CHD deaths have not been reported earlier). In addition, an updated meta-analysis of linoleic acid (18:2n-6) intervention trials showed no evidence of cardiovascular benefit.	Evaluation of recovered data from the Sydney Diet Heart Study (RCT, carried out in 1966-73), 458 men aged 30-59 years with a recent coronary event. INTERVENTION: replacement of dietary SFAs with n-6 PUFAs in secondary prevention.
(Mozaffarian, Micha, and Wallace 2010)	In RCTs, the replacement of 5% of energy from SFAs with PUFAs reduced CHD risk 10%.	Eight RCT trials (searched up to 6/2009) with a follow up of 1-8 years, 1042 events among a total of 13 614 participants. Intervention: PUFA consumption 14.9 E%. Controls: PUFA consumption 5.0E%.
(Astrup et al. 2011)	In populations with a Western diet, the replacement of 1% of energy from SFAs with PUFAs lowered LDL cholesterol and was likely to produce a reduction in CHD incidence of ≥2-3%. Insufficient evidence existed to judge the effect on CHD risk of replacing SFAs with MUFAs.	Expert panel conclusion. The meeting was held in Copenhagen, Denmark, in May, 2010.
(Ramsden et al. 2010)	Mixed n-3+n-6 PUFA intervention (to replace SFAs and trans-FAs), reduced the risk of myocardial infarction events+CHD deaths by 22%, whereas the pure n-6 PUFA intervention tended to increase these risks (death from all causes being statistically significant).	Ten RCTs (661 non-fatal MI events + CHD deaths among 11275 subjects with a follow-up of 2-8 years) with the increase in n-6 PUFA intake or both n-3 and n-6 PUFA intakes were characterized. In the final meta-analysis, eight RCTs were included (n=8239). Two studies were omitted due to incomplete data or lack of randomization.
(Skeaff and Miller 2009)	Convincing RCT evidence that the replacement of SFAs with PUFAs reduced CHD events and CHD deaths significantly. (Cohort studies, and effects of MUFAs and n-3 PUFAs were also handled in this paper).	Meta-analysis: four trials for CHD deaths (with a follow up of 3.3-5 years, 61 events among 2102 participants) and 6 trials for CHD events (with a follow up of 2-5 years, 288 events among 3002 participants).
(de Lorgeril et al. 1994)	Use of a Mediterranean α-linolenic acid (18:3n-3) rich diet for 27 months to partially replace SFAs with UFAs. The experimental group consumed significantly less lipids, SFAs, cholesterol, and linoleic acid (18:2n-6) but more oleic and α-linolenic acids. A 73% reduction of the risk of acute myocardial infarction recurrence was observed.	RCT: a French prospective, randomized single-blinded secondary prevention trial in which myocardial infarction patients were divided to experimental (n = 302) or control groups (n = 303) to compare the effect of a Mediterranean alpha-linolenic acid-rich diet on of acute myocardial infarction recurrence.

INTAKE IN COHORT STUDIES:		
(Innes and Calder 2020)	Long-term prospective cohort studies demonstrate an association between higher intakes of fish, fatty fish and marine n-3 PUFAs, i.e., eicosapentaenoic acid (20:5n-3)+docosahexaenoic acid (22:6n-3) or their higher levels in the body and lower risk of developing CV events in the general population. With regard to possible mechanisms, 20:5n-3 and 22:6n-3 may beneficially modify a number of known risk factors for CVD, such as blood lipids, BP, heart rate and heart rate variability, platelet aggregation, endothelial function and inflammation. RCTs: evidence for primary prevention is weak, but secondary prevention with eicosapentaenoic acid (20:5n-3) + docosahexaenoic acid (22:6n-3), or 20:5n-3 alone, may help high risk patients.	Literature review up to the year 2020 (cohort studies, case-control studies and RCT trials), focusing on dietary intake of eicosapentaenoic acid (20:5n-3)+docosahexaenoic acid (22:6n-3) or their circulating levels and the risk of cardiovascular events.
(Zhu, Bo, and Liu 2019)	This meta-analysis of cohort studies suggested that total fat, SFA, MUFA or PUFA intakes were not associated with the risk of CVD. A higher trans-FA intake was associated with a greater risk of CVDs in a dose-response fashion. Sub-group analysis suggested inverse associations between PUFAs intake and CVDs risk among studies followed up for more than 10 years.	Meta-analysis of prospective cohort studies (56 studies for SFAs (1989-), 43 for MUFAs (1990-) and 45 for PUFAs (1990-), published up to 7/2018), reporting associations between dietary FA intake and the risk of CVD (the highest vs. the lowest categories of the intake of FAs).
(Jiao et al. 2019)	In patients with T2D, a higher intake of PUFAs, in comparison with carbohydrates or SFAs, was associated with lower total mortality and CVD mortality when comparing the highest with the lowest quarter. In models that examined the theoretical effects of substituting PUFAs for other fats, isocalorically replacing 2% of energy from SFAs with total PUFAs or linoleic acid (18:2n-6) was associated with 13% lower CVD mortality, respectively.	Two prospective cohorts with a total of 11 264 participants with T2D in the Nurses' Health Study (1980-2014) and Health Professionals Follow-Up Study (1986-2014). The aim was to study the association of dietary FAs with CVD and total mortality in patients with T2D. Dietary fat intake was assessed using validated food frequency questionnaires, updated every two to four years.
(Zong et al. 2018)	Significantly lower CHD risk was observed when intake of SFAs, *trans* fats, or refined carbohydrates were replaced by plant-based MUFAs, but not animal-based MUFAs. These findings support a beneficial role of MUFAs in long-term CHD prevention, when plant-based foods such as vegetable oils, nuts, and related products are the primary sources.	The prospective US Nurses' Health Study (1990-2012) with its 63,442 women, aged 45-69 years and, the Health Professionals Follow-Up Study (1990-2012) with its 29,942 men, aged 44-79 years at baseline in 1990.

(Harcombe, Baker, and Davies 2017)	Total fat or SFA intake was not associated with CHD mortality. Conclusion: epidemiological evidence does not support the present dietary fat guidelines.	A systematic review and meta-analysis of 7 prospective cohort studies (published 1985-2012) with 89 801 participants (aged 30-79 years at baseline, 94% male) and with a mean follow-up of 11.9 years. All studies excluded participants with previous heart disease. In addition, a literature review was carried out.
(Blekkenhorst et al. 2015)	High SFA intake was associated with the risk of atherosclerotic vascular disease mortality (the highest SFA quartile showing 16% vs. the lowest 5% mortality risk. Although there was a strong positive association between SFA intake and LDL cholesterol, LDL cholesterol was not associated with atherosclerotic vascular disease mortality.	A prospective cohort of 1469 women living in Western Australia, with a mean age of 75 years at baseline in 1998 followed over 10 years. There were 134 (9.1%) atherosclerotic vascular disease deaths.
(Schwingshackl and Hoffmann 2014)	These results indicated an overall risk reduction of all-cause mortality (11%), cardiovascular mortality (12%), cardiovascular events (9%), and stroke (17%) when comparing the top versus bottom third of the intake of MUFA, olive oil, oleic acid (18:1n-9), and MUFA:SFA ratio. In the subgroup analyses, this significant correlation could only be observed between higher intakes of olive oil and reduced risk of all-cause mortality, cardiovascular events, and stroke, respectively.	A meta-analysis of thirty-two prospective cohort studies, 42 reports, published 1991-2014, including 841,211 adult males and females. Sample size varied between 161 and 161,808 with a follow-up time ranging from 3.7 to 30 years.
(Jakobsen et al. 2009)	Each 5% of energy consumed as PUFAs, as a replacement for SFAs, was associated with a 13% lower risk of coronary events, and 26% lower risk for coronary deaths. MUFA and carbohydrate did not show any beneficial effects. The associations suggest that replacing SFAs with PUFAs rather than MUFAs or carbohydrates prevents CHD over a wide range of intakes.	A prospective study with eleven pooled American and European cohorts with 5249 incident CHD events among 344 696 subjects, 4-10 years of follow-up.
(Xu et al. 2006)	Intakes of total fat, SFAs, and MUFAs were strong predictors of CHD in American Indians aged 47-59 years with 185 CHD vs. 1474 no CHD individuals. In older Indians, this association was not found.	A prospective (the Strong Heart Study) cohort of middle-aged and older (baseline in 1993-95, aged 47-79 years) American Indians (n=2,938) followed for a mean of 7.2 years with 436 incident CHD cases.
CIRCULATING FAs IN COHORT STUDIES:		
(Del Gobbo et al. 2016)	In continuous (per 1-SD increase) multivariable-adjusted analyses, seafood and plant-derived circulating n-3 PUFA biomarkers, α-linolenic acid (18:3n-3), docosapentaenoic acid	A meta-analysis of nineteen studies (blood sampling 1970-2007) published up to 11/2014, comprising 16 countries, 45 637 individuals of whom 7973 experienced

	(22:5n-3) and docosahexaenoic acid (22:6n-3), were modestly but statistically significantly associated with a lower incidence of fatal CHD. Docosapentaenoic acid (22:5n-3) was also associated with a lower risk of total CHD (fatal+non-fatal).	an incident CHD, aged 18-97 years, 37% females. There were 17 prospective cohort or prospective nested-case-control studies and two retrospective studies. N-3 PUFA measures were carried out either from total plasma, phospholipids, cholesteryl esters or adipose tissue.
(Würtz et al. 2015)	High serum MUFA proportion was associated with increased cardiovascular event risk, while high n-6 PUFA and docosahexaenoic acid (22:6n-3) levels were associated with a lower risk.	A prospective study of 3 population-based cohorts, n=13 441, including 1741 incident cardiovascular events within a follow-up of 11 to 23 years. (the National Finnish FINRISK study, the Southall and Brent Revisited (SABRE) study and British Women's Health and Heart Study).
(Warensjö et al. 2008)	The proportion of serum CE linoleic acid (18:2n-6) was inversely related, whereas serum FAs associated with SFA intake, palmitic acid (16:0), palmitoleic (16:1n-7)) and dihomo-γ-linolenic acid (20:3n-6) were directly related to total and cardiovascular mortality. Δ5-desaturase activity was inversely and Δ9- and Δ6-desaturase activity directly associated with CV mortality. The role of desaturase activity on CV events was discussed.	Swedish (Uppsala) prospective cohort: community-based sample of 2009 men, recruited in 1970-4) in which 50-y-old men were followed for a maximum of 33.7 years with 1012 total and 461 CV deaths.

CHD=coronary heart disease; CV=cardiovascular; CVD=cardiovascular disease; FA=fatty acid; LDL=low-density-lipoprotein; MUFA=monounsaturated fatty acid; n-3=omega-3; n-6=omega-6; PUFA=polyunsaturated fatty acid; RCT=randomized controlled trial; SFA=saturated fatty acid; T2D=type 2 diabetes; UFA=unsaturated fatty acid.

Estimation of desaturase activity (examples): Δ9-desaturase activity: the 16:1n-7)/16:0 and/or the 18:1n-9/18:0 ratios; Δ6-desaturase activity: the 18:3n-6/18:2n-6 ratio; Δ5-desaturase activity: the 20:4n-6/20:3n-6 and/or the 20:5n-3/20:4n-3 ratios.

2.14 Fatty Acids and Non-Alcoholic Fatty Liver

Central obesity and insulin resistance are important risk factors for the development of non-alcoholic fatty liver. For this reason, the risk factors for these cardiometabolic outcomes are very similar. Small-scale intervention trials support the view that SFAs are metabolically more harmful for the human liver than UFAs or simple sugars (Luukkonen et al. 2018). In a Swedish intervention, 67 abdominally obese participants, aged 50-64 years, 66% women, received 10-week isocaloric diet high in/baked in 18:2n-6 (margarines, oils, seeds) or high in/baked in SFAs (butter). Compared to the SFA intake, the n-6 PUFA intake reduced liver fat without causing a weight loss (Bjermo et al. 2012). Similarly, in their RCT, Rosqvist and coworkers showed that palm oil SFAs markedly induced liver fat and serum ceramides, whereas sunflower oil PUFAs prevented these events during excess energy intake and weight gain in overweight individuals (Rosqvist et al. 2019). In the YFS population (Kaikkonen et al. 2017), serum total SFA and MUFA proportions were directly and n-6 PUFA proportion inversely associated both with prevalent and incident fatty liver. These associations were independent of age, sex, BMI, physical activity, alcohol consumption and smoking. Similarly, in their cross-sectional study of 546 Swedish elderly men, Petersson and co-workers found that the serum CEFA composition and particularly low linoleic acid (18:2n-6), but not n-3 PUFAs, were associated with the markers of liver fat (alanine aminotransferase activity) independently of lifestyle, obesity and insulin resistance (Petersson et al. 2010). Similarly, desaturation products including palmitoleic, oleic, γ-linolenic and dihomo-γ-linolenic acids (20:3n-6), and Δ6- and Δ9-desaturase activity indices were directly related to alanine aminotransferase activity (all $P<0.05$). One Finnish study has linked altered FA metabolism, i.e., estimated higher Δ6- and Δ9-desaturase and lower Δ5-desaturase activity, with prevalent fatty liver (Walle et al. 2016).

The current therapies of fatty liver focus on the treatment of co-morbidities, such as obesity, hyperglycemia and dyslipidemia and hypertension since there are no specific pharmaceutical treatments for this disease (Ei-alkoholiperäinen rasvamaksatauti, (NAFLD). Käypä hoito-suositus, 2020). Reductions of alcohol intake and an increase of n-3 PUFA intake have been suggested as therapies (Jump et al. 2018). However, a 12-week supplementation of n-3 PUFA from fish with a daily dose of 588 mg of eicosapentaenoic acid (20:5n-3) and 412 mg of docosahexaenoic acid (22:6n-3), was not able to decrease the liver fat content (Parker et al. 2019).

2.15 Fatty Acids and Inflammation or Lipid Peroxidation

2.15.1 Inflammation

Obesity-related inflammation is mediated by the release of adipocytokines by the adipocytes. It has been claimed in review articles that dietary components, such as FAs, could play a role in inflammation. In particular trans-FAs and SFAs have been postulated to be pro-inflammatory (Calder et al. 2011). Recent evidence with hormone and inflammation marker data suggests that all of the individual SFAs do not influence on metabolism and inflammation similarly. Serum PL SFAs with an even number of carbon atoms in their carbon chains (14:0, 16:0 and 18:0) were observed to associate directly with resistin and inversely with adiponectin levels, whereas odd chain SFAs, such as 15:0 and 17:0 showed inverse associations with leptin and plasminogen activator inhibitor-1 (Kurotani et al. 2017). With regard to PUFAs, the common view has been that the eicosanoids that had originated from n-6 PUFAs are potent inflammatory factors. In contrast, those originating from n-3 PUFAs are considered to be anti-inflammatory (Schmitz and Ecker 2008; Calder et al. 2011). This is due to well-characterized metabolism of arachidonic acid (20:4n-6). This FA can become oxidized mainly enzymatically forming isoprostanoids and prostaglandins, which have been linked to lipid peroxidation, inflammatory reactions, arterial vasoconstriction, platelet aggregation and an elevated risk of CVD (Davies and Roberts 2011). However, a large cross-sectional population study from the US (n=17,689 males and females with a mean age of 46 years) demonstrated recently that low age, sex and race-adjusted intake of total PUFAs and linoleic acid (18:2n-6) would be associated with lowered C-reactive protein levels (Mazidi et al. 2017). Linoleic acid is the precursor FA (a source molecule) for arachidonic acid (Table 5). This finding, even although from a cross-sectional trial, suggests that also n-6 PUFAs might tend to decrease rather than increasing the level of inflammation. Finally, the effects of n-6 PUFAs seem to be genetically determined since linoleic acid may have FA desaturase 1 genotype-specific effects on the levels of circulating esterified arachidonate and on the inflammatory response (Lankinen et al. 2019).

On the basis of animal and cell culture experiments, free FAs may regulate body functions, such as inflammatory reactions, via free FA receptors (G protein-coupled receptors). To date, several receptors have been identified and characterized that are activated by the free FAs of various chain lengths. In particular, free FA receptor 1 (GPR40), expressed in pancreatic beta cells, and free FA receptor 4 (GPR120), expressed in the intestine, adipocytes, and pro-inflammatory macrophages, are activated by long-chain SFAs and UFAs, while free FA receptor 3 (GPR41) and free FA receptor 2 (GPR43) are activated by short-chain FAs (Del Guerra et al. 2010)(Cox

et al. 2016)(Kimura et al. 2020). With regard to n-3 PUFAs, one of their suggested anti-inflammatory effects is the down-regulation of pro-inflammatory cytokines in adipose tissue, such as tumor necrosis factor-α, interleukin-6 and monochemoattractant protein-1. SFAs are the acyl component of lipopolysaccharides, which are ligands of toll-like receptor-4 (Teng et al. 2014). While SFAs were found to stimulate toll-like receptor-4 signaling pathway, eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3) were found to inhibit this pathway (Teng et al. 2014). On the basis of recent meta-analysis in humans (Jiang et al. 2016), n-3 PUFA supplementation was claimed to reduce the levels of inflammatory eicosanoids, such as thromboxane B2 in subjects with high CVD risk.

The recent discovery of electrophilic nitro-fatty acids (NO2-FAs) provides one mechanism for UFAs to modulate cell signaling. These compounds are endogenously formed by redox reactions of nitric oxide- and nitrite- derived nitrogen dioxide with UFAs (detected in human plasma and urine). NO_2-FAs are potent anti-inflammatory and antioxidant cell signaling mediators and they have been demonstrated to exert protective effects in numerous pre-clinical animal models of diseases including cardiovascular, pulmonary and renal fibrosis (Khoo and Schopfer 2019).

2.15.2 Lipid Peroxidation

PUFAs have a susceptibility to become easily oxidized by free radicals or by non-radical oxygen species, since PUFAs are the only FAs to contain at least two double-bonds interspersed by methylene groups with unstable hydrogen-carbon bonds (Bochkov et al. 2010). Lipid peroxidation and its prevention by antioxidants have been studied for decades (Stocks et al. 1974). In *in vitro* studies with human samples exposed to highly oxidative conditions, n-6 PUFA supplementation (linoleic acid, 18:2n-6) has been associated with an elevated oxidation susceptibility of LDL (Abbey et al. 1993; Reaven et al. 1993). However, there is evidence that low serum n-6 PUFA or 18:2n-6 levels might be associated with more inflammation, i.e., as assessed by C-reactive protein levels (Petersson et al. 2008; Petersson et al. 2009; Kaska et al. 2014) or with higher lipid peroxidation *in vivo* (Kaikkonen, Vilppo et al. 2013). Hypercholesterolemia and a high serum LDL cholesterol concentration have been linked with elevated lipid peroxidation (Davies and Roberts 2011). In contrast to hypercholesterolemia and its treatment with statin drugs, partial replacement of SFAs with UFAs has not reduced serum ubiquinol-10 or beta-carotene concentrations, which are needed, in addition to α-tocopherol, in the reduction or prevention of oxidative reactions (Jula et al. 2002). There is also some evidence that SFA intake impairs the antioxidant potential of high-density lipoprotein (HDL) and increases serum levels of oxidized lipoproteins, whereas the antioxidant potential of HDL is enhanced after mainly n-6 PUFA consumption (Cedo et al. 2015).

3 Aims

The Cardiovascular Risk in Young Finns Study (YFS) is an ongoing epidemiological multicenter study to evaluate risk and preventive factors of metabolic outcomes and CVD from childhood to adulthood. The present study is based on the FA proportions determined in the childhood of the study participants in 1980 or in their early adulthood in 2001, and cardiometabolic outcome data collected between the years 2001-2011 in adulthood.

The major aims of the present study were to study whether (**Figure 6**):

1) Serum FA proportions reflect dietary intake of FAs (I, II, III)
2) Serum CEFA proportions in childhood are associated with elevated BP or hypertension in adulthood (I).
3) Serum CEFA proportions in childhood are associated with subclinical atherosclerosis, i.e., common carotid artery intima-media thickness (cIMT) in adulthood (II).
4) Serum total (free+esterified) FA proportion profile in early adulthood is associated with different cardiometabolic outcomes, such as obesity, high homeostatic model-based insulin resistance (HOMA-IR), elevated BP, or non-alcoholic fatty liver in adulthood (III).
5) Serum total (free+esterified) FA proportions in early adulthood are associated with the markers of inflammation and low-density lipoprotein oxidation (IV).

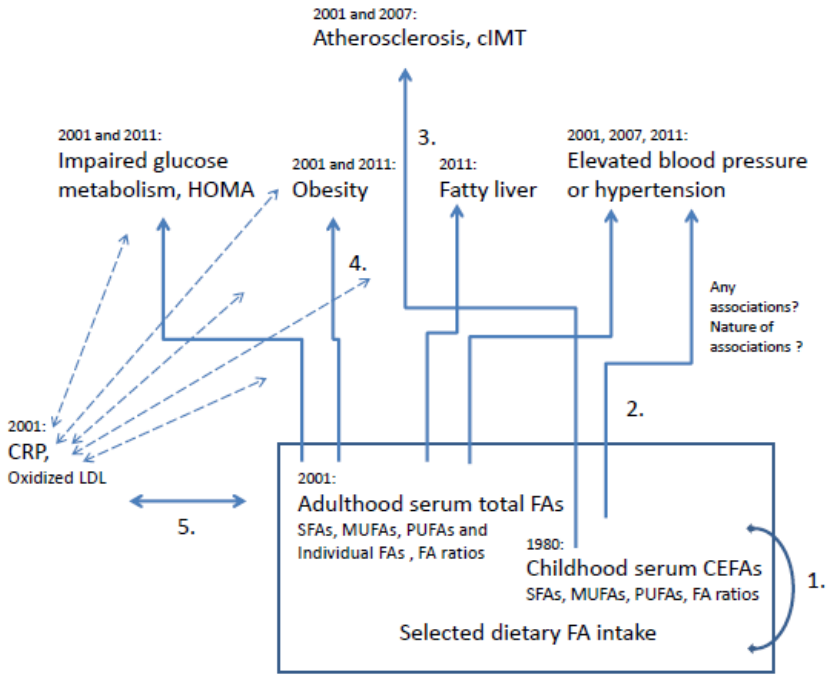


Figure 6. Summary of the associations investigated in this thesis (solid arrows). C-reactive protein (CRP) and oxidized LDL are to some extent associated with the outcomes (not investigated in this thesis, dashed arrows). Bidirectional arrow=cross-sectional study setting.

4 Materials and Methods

4.1 Description of the Cardiovascular Risk in Young Finns Study

The participants in the YFS study lived in 5 university cities and 12 adjacent rural communities. In 1980, 4320 children and adolescents aged 3, 6, 9, 12, 15, and 18 years were chosen by university city-specific randomization from the Finnish national population register to obtain a sample that would represent Finnish children living either in western or eastern Finland (Raitakari et al. 2008). A total of 3596 individuals (83.1%) of the invited population actually participated in the baseline examination. In half of the population, quantitative information on their food consumption was collected by using a 48-h dietary recall. The follow-up examinations with clinical data were carried out on the years 2001, 2007 and 2011 for this thesis. In 2007 and 2011, food consumption data was gathered with food frequency questionnaires from nearly all of the participants (including FA intake data presented in this thesis).

The availability of FA and cardiometabolic outcome data formed the basis for the selection of subjects in different sub-studies (n=803 to 2200). Detailed exclusion and inclusion criteria have been presented in connection with the original publications. The main variables are presented in **Table 10**. Childhood CEFA proportions (the year 1980 data) were linked with BP and hypertension (I) and subclinical atherosclerosis, i.e., cIMT (II), data collected 27 y later in adulthood. The adulthood cross-sectional (the year 2001 data) and prospective (2001 ->2011) cardiometabolic outcome analyses concerned serum total FA proportions which were linked either with obesity, high HOMA-IR, elevated BP, or non-alcoholic fatty liver (III). In addition, associations between serum total FAs and the biomarkers of inflammation (C-reactive protein) and lipoprotein oxidation (conjugated dienes and oxidized proteins in LDL) were studied cross-sectionally (2001 data) (IV). See also Figure 6 for the associations investigated.

Table 10. Cohort description of the YFS and the FA and the outcome variables used in the present study.

n	Study year	Age cohorts																FA proportions	Outcomes
3596	**1980**	**3**	**6**	**9**	**12**	**15**	**18**											Childhood CEFAs, n=803-823 and dietary intake of FAs, n=724-795 (I, II)	
	1983		6	9	12	15	18	21										(CEFAs) (II)	
	1986			9	12	15	18	21	24									(CEFAs) (II)	
2284	**2001**								**24**	**27**	**30**	**33**	**36**	**39**				Serum total FAs in adulthood, n=975-2200 and dietary intake of FAs, n=991-996 (III, IV)	(cIMT), obesity, high HOMA-IR, high BP or hypertension, conjugated dienes or oxidized proteins in LDL and C-reactive protein levels
2204	**2007**										**30**	**33**	**36**	**39**	**42**	**45**		Serum total FAs by NMR, this thesis only	cIMT, high BP or hypertension
2063	**2011**											**34**	**37**	**40**	**43**	**46**	**49**	Serum total FAs by NMR, this thesis only	Obesity, high HOMA-IR, high BP or hypertension, fatty liver

Variable names in parenthesis mean that they have been used for confirmatory purposes (supplemental data).

4.2 Determination of Circulating Fatty Acids

4.2.1 Serum Cholesteryl Ester Fatty Acids (CEFAs)

Serum CEFAs were determined so that the participants presented, as well as possible, boys and girls and all age groups and all domestic regions in the YFS (Räsänen et al. 1985; Moilanen, Nikkari et al. 1985). Serum sample handling involved several steps: chloroform-methanol extraction for serum lipids, fractioning of CEs from other lipids with thin-layer chromatography, saponification of CEs with potassium hydroxide–ethanol and esterification of FAs with hydrogen chloride–methanol. The formed FA methyl esters were analyzed by a gas chromatograph with flame ionization detector (Moilanen and Nikkari 1981). Individual FAs were presented as percentages of the total FA concentration.

Individual CEFAs were subdivided into *1*) SFAs: myristic acid, 14:0; palmitic acid, 16:0 and stearic acid, 18:0 *2*) MUFAs: palmitoleic acid, 16:1n-7; *cis*-10

heptadecaenoic acid, 17:1 and oleic acid, 18:1n-9 or *3*) PUFAs: linoleic acid, 18:2n-6; γ-linolenic acid 18:3n-6; dihomo-γ-linolenic acid 20:3n-6; arachidonic acid, 20:4n-6; α-linolenic acid, 18:3n-3; eicosapentaenoic acid, 20:5n-3 and docosahexaenoic acid, 22:6n-3.

4.2.2 Serum Total Fatty Acid Proportions

Similarly, serum proportions of total FAs (free FAs + esterified FAs in CE, PL and TG fractions) were analyzed by a gas chromatograph with flame ionization detector (Jula et al. 2002). Individual FAs were subdivided into 1) SFAs: myristic acid, 14:0; pentadecanoic acid, 15:0; palmitic acid, 16:0 and stearic acid, 18:0 2) MUFAs: palmitoleic acid, 16:1n-7; octadecenoic acid, 18:1n-7; oleic acid, 18:1n-9; eicosenoic acid, 20:1n-9 and docosenoic acid, 22:1n-9 3) PUFAs: linoleic acid, 18:2n-6; γ-linolenic acid, 18:3n-6; eicosadienoic acid, 20:2n-6; dihomo- γ-linolenic acid, 20:3n-6; arachidonic acid, 20:4n-6; docosatetraenoic acid, 22:4n-6, α-linolenic acid, 18:3n-3; eicosatetraenoic acid, 20:4n-3; eicosapentaenoic acid, 20:5n-3 and docosapentaenoic acid, 22:5n-3, docosahexaenoic acid, 22:6n-3, and 20:2n-9. The subject-specific mean number of double bonds per FA was also calculated from the FA proportions.

Nuclear magnetic resonance-based FA analyses (Soininen et al. 2015) were carried out for the serum collected in 2007 and 2011 (for additional FA data included in this thesis only). Gas-chromatographic and nuclear magnetic resonance-based analysis methods were found very similar in their analytical result of both levels and distribution. With regard to between-method comparisons, Pearson correlation coefficients were $r \geq 0.92$ for all the main proportions of total FAs, i.e., SFAs, MUFAs, n-6 PUFAs and n-3 PUFAs (n=2196, the year 2001 YFS data). Würst and co-workers (Würtz et al. 2015) have published a comparison between these two analytical methods (as supplemental material).

4.2.3 Indicators of Fatty Acid Metabolism

With regard to FA metabolism, the ratio of serum stearic acid (18:0) to palmitic acid (16:0) was calculated as an elongase activity index. The palmitoleic acid (16:1n-7)/palmitic acid (16:0) and the oleic acid (18:1n-9)/stearic acid (18:0) ratios were calculated as an index of Δ9-desaturase activity. Furthermore, the γ-linolenic acid (18:3n-6)/linoleic acid (18:2n-6) ratio was calculated to reflect Δ6-desaturase activity and the arachidonic acid (20:4n-6)/dihomo-γ-linolenic acid (20:3n-6) and the eicosapentaenoic acid (20:5n-3)/eicosatetraenoic acid (20:4n-3) ratios to refer to Δ5-desaturase activity.

4.3 Collection of Food-Composition Data Including Intake of FAs (1980-2007)

4.3.1 48-h Dietary Recall (1980 and 2001)

Participants (aged 15-18 years) provided quantitative information on their food consumption by means of a 48-h dietary recall in which a trained interviewer gathered detailed information on all foods and beverages consumed by subjects during the 2 days before the interview (5 interview-days per week). Children aged 3 to 12 years were interviewed together with their mothers, fathers, or some other accompanying adult. Food-composition data used in the 1980s were computed based on the Finnish food-composition tables maintained by the Department of Nutrition, University of Helsinki, and on analytical data obtained from the local food-processing industry (Räsänen et al. 1985; Räsänen et al. 1991). The intake of FAs was proportioned to the total energy intake, i.e., intake per 1000 kcal or E% or to the total FA intake. Dietary intake variables included also vitamin E, red meat and monthly portions for fruits and vegetables.

4.3.2 Food frequency Questionnaire (2007 and 2011)

For the intake-data, a validated 131-item food frequency questionnaire (FFQ) and the Fineli database were used (Fineli. Finnish Food Composition Database. Release 7. Helsinki, Finland, the National Public Health Institute, Nutrition Unit, 2007; http://www.fineli.fi). Consumptions of alcohol and sodium were estimated as grams per day and milligrams per day, respectively. Because of the skewed distribution, alcohol consumption was categorized into 4 groups.

4.4 Outcomes in Adulthood

4.4.1 Obesity and HOMA Index (2001 and 2011)

An individual was defined as obese if her/his BMI was higher than 30 kg/m^2. The rest of the study population formed a non-obese group. Serum glucose and insulin levels were used to calculate HOMA-IR index (homeostatic model-based insulin resistance (glucose, mmol/L x insulin, mU/L)/22.5). Since there was a wide age distribution among the subjects (from 24 to 39 years in 2001), the HOMA-IR was categorized for logistic regression by forming age and sex -specific percentiles, 80% being used as a cutoff point: ≥80%=1 vs. <80%=0. In addition, a continuous log-transformed HOMA-IR variable was used. Glucose and insulin levels were also used as log-transformed continuous variables.

4.4.2 Blood Pressure and Hypertension (2001, 2007 and 2011)

BP was measured by using a random-zero sphygmomanometer with the subject in the sitting position after 5 min of rest. Korotkoff's fifth phase was used as the sign of diastolic BP, and the first phase was used as the sign of systolic BP. Readings were performed ≥3 times on each subject with at least one minute intervals; the average of these measurements was used in the statistical analysis.

Participants were defined to have elevated BP in adulthood if they received medication for hypertension or their systolic BP or diastolic BP belonged to the highest age and sex-specific 20% percentile. In parallel analyses, a subject was defined as being clinically hypertensive when he or she had medication for hypertension, or his or her systolic BP was ≥140 mm Hg or diastolic BP was ≥90 mm Hg. The parents' history of hypertension was gathered in the childhood of the participants by a questionnaire.

4.4.3 cIMT (2001 and 2007)

Sub-clinical atherosclerosis was estimated by measuring cIMT using ultrasonography. It was measured on the posterior wall of the left common carotid artery, approximately 10 mm proximal to the carotid bifurcation. The examination was performed using Acuson Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA), equipped with a 13.0 MHz linear array transducer (Raitakari et al. 2003).

4.4.4 Estimation of Liver Fat (2011)

The amount of liver fat was measured by using ultrasonography. The examinations were performed with Acuson Sequoia 512 ultrasound mainframes using 4.0 MHz adult abdominal transducers. A trained sonographer graded the liver fat status from the ultrasonographic images using five widely accepted criteria for fatty liver: 1) the liver-to-kidney contrast, 2) parenchymal brightness, 3) deep beam attenuation, 4) bright vessel walls, and 5) visibility of the neck of the gallbladder (Kaikkonen et al. 2017).

Ultrasonography was used to assess fatty liver only in 2011. With regard to incident fatty liver (2001-2011), individuals with Bedogni's fatty liver index >30 in 2001 were excluded from the analyses. The fatty liver index was calculated based on BMI, waist circumference, serum TG concentration and γ-glutamyl-transferase activity. An index value < 30 (negative likelihood ratio = 0.2) rules out and value ≥ 60 (positive likelihood ratio = 4.3) rules in the fatty liver (Bedogni et al. 2006).

4.4.5 Oxidized LDL and High-Sensitive C-Reactive Protein (2001)

The status of LDL oxidation was estimated by two separate methods, i.e. by assessing LDL conjugated diene concentration (oxLDLlipids) and the levels of oxidized LDL proteins (oxLDLprot) *in vivo*. In the assay of oxLDLlipids, the serum LDL fraction was precipitated with buffered heparin (Ahotupa et al. 1998). Lipids were extracted from the precipitated lipoproteins by chloroform-methanol (2:1), dried under nitrogen and re-dissolved in cyclohexane. The concentration of peroxidized lipids was assessed spectrophotometrically at 234 nm (Ahotupa et al. 2010). In the present statistical analyses, the diene concentration was divided by the concentration of serum apolipoprotein B. Validation studies for the assay have ruled out interference by non-specific substances or the precipitate sensitive on the procedure (Ahotupa et al. 1998) and shown that diene conjugation is a measure of the oxidative LDL lipid modifications found in all LDL lipid classes. When serum samples were kept at -80°C, the levels of oxidized lipoprotein lipids did not change during even prolonged (up to 8 years) follow-up. The coefficient of variation was 4.4% for within-assay precision and 4.5% for the between-assay precision. In addition, we measured the numbers of oxidized apolipoprotein B-containing LDL lipoprotein particles in serum (oxLDLprot) by a monoclonal antibody-based enzyme-linked immunosorbent assay (Oxidized LDL ELISA kit, Mercodia, Sweden). The method was based on binding the mouse monoclonal antibody 4E6 to a conformational epitope in oxidized apolipoprotein B-100 (Holvoet et al. 2006). High-sensitive C-reactive protein was analyzed by standard methods (Juonala et al. 2006).

4.5 Covariates

Covariate-data used was mainly adulthood-data (both childhood and adulthood covariates were used in Work II). Use of oral contraceptives (estrogen and/or progesterone, no vs. yes), pregnancy (no vs. yes), medication (no vs. yes), family history of hypertension (no vs. yes), use of table salt, i.e., sodium or potassium (added never vs. added following tasting vs. added always, 1 to 3), smoking (never or more seldom than daily vs. daily), alcohol consumption (doses per day), the number of years of education and leisure-time physical activity index (varied between 8 to 23 in children aged 3–6y, and between 5 to 15 in older subjects) were characterized by using questionnaires and/or an interview (Telama et al. 2005; Raitakari et al. 2008; Juonala et al. 2010). The physical activity index was determined by combining the information from questions concerning the frequency and intensity of physical activity, frequency and hours spent on vigorous physical activity, the average duration of a physical activity session and participation in

organized physical activity (Telama et al. 1985). Socioeconomic position was characterized by educational status (1=comprehensive school; 2=secondary education, not academic; 3=academic). Height, weight and waist circumference were measured during the study visit, following calculation of body mass index (kg/m^2). All laboratory analyses were carried out on overnight fasting samples. Serum glucose, serum apolipoprotein B, serum activity of γ-glutamyl transferase and alanine aminotransferase, LDL cholesterol, HDL cholesterol, and TG concentrations were measured by using standard methods (Juonala et al. 2004; Juonala et al. 2008; Raitakari et al. 2008; Juonala et al. 2010). The TG/HDL cholesterol ratio was used in some analyses to adjust for lipid status. Serum insulin concentrations were measured by using a microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot).

4.6 Statistical Analyses

Normal distributions of study variables were assessed by using visual assessment and/or the Kolmogorov-Smirnov test. For variables with a distribution skewed to the right, such as BMI, glucose, insulin and TG levels, a log transformation was carried out prior to statistical analyses. Alcohol consumption was categorized or its crude value was used as a continuous variable. A t-test for independent samples was used to assess differences between men or women, or between cases and controls, in the characteristics of FA proportions, and in the factors known to associate with the outcomes investigated. Nonparametric Spearman's correlations or Pearson or Partial correlations adjusted for age and sex were calculated to obtain correlation coefficients between FA proportions and the continuous outcome variables. With regard to regression analyses with missing covariate values, a mean substitution was used to form a complete data set. A 2-tailed P value < 0.05 was considered statistically significant (works I, II and IV). For work III, following Bonferroni-correction with 41 dietary and serum variables, a P-value <0.001 was defined as statistically significant and a P-value between 0.001 and 0.05 as borderline significant. IBM SPSS Statistics software (versions 19-22; SPSS Inc) was used to carry out statistical analyses.

4.6.1 Work I

The regression models were constructed in several steps by gradually increasing the number of covariates that were allowed to enter the models. Linear and logistic regression models were conducted for different CEFA proportions or their ratios (1980) and age as fixed covariates after inclusion of factors known to modulate adulthood BP (2007), i.e., BMI, alcohol use, sodium intake, smoking, physical

activity, family history of hypertension (forward method: *P*-entry < 0.05 and *P*-removal > 0.10). In the last step, serum lipids (i.e., LDL cholesterol, HDL cholesterol, and TGs) and serum insulin were further examined in the model (forward method: *P*-entry < 0.05 and *P*-removal > 0.10). With regard to logistic regression models, CEFA dummies were formed and ORs for the highest one-third vs. the lowest one-third were reported. CEFA proportions or their ratios were individually entered into the multivariate models because they correlated strongly with each other.

4.6.2 Work II

The relationships between FA variables (1980) and cIMT (2007) were examined with linear regression models. The models were constructed in several steps by gradually increasing the number of covariates that were allowed to enter the models to examine the potential confounding of non-lipid and lipid risk variables. First, the CEFA of interest alone (step 1a), or the CEFA of interest and age (step 1b), was modeled as the independent variables. Second, the childhood (step 2a) or childhood and adulthood risk variables (step 2b), other than serum lipids, were allowed to enter the models using a stepwise technique ($P<0.05$ for entry and $P>0.1$ for removal). These risk variables (covariates) included childhood and/or adulthood BMI, physical activity, serum insulin, systolic BP, alcohol use, smoking, the number of years of education, serum glucose, and T2D. Finally, serum lipid variables were allowed to enter the models: childhood LDL cholesterol, HDL cholesterol, and TGs in step 3a and childhood and adulthood lipids as well as drug use for dyslipidemia in step 3b. The regression analyses were first conducted after stratification by sex and then stratified by sex and different age groups.

Confirmatory analyses were performed using data collected during several study visits. We calculated the mean of the standardized (Z-scored) FA values collected in 1980, 1983, and/or 1986 and the mean of the standardized adult cIMT values collected in 2001 and/or 2007. This increased the number of individuals included in the models from 823 to 1025. Identical statistical methods and the same youth and adulthood covariate set were used both for primary analyses and confirmatory analyses.

4.6.3 Work III

In work III, besides outcome associations of total PUFAs, MUFAs and SFAs (2001), also individual FAs were studied equally. Prior to t-testing or logistic regression for variable differences between cases and controls, the variables were Z-scored (mean=0, SD=1). Z-scoring made it possible to compare the magnitudes of the

associations between different variables, and thus, to find the strongest FA links with the endpoints (2001, 2011). We also formed two summary variables: 1) the total FA status was defined as (SFAs+MUFAs)/PUFAs and 2) the number of cardiometabolic outcomes (including obesity, high insulin resistance and elevated BP, values ranging from 0 to 3). Univariate general linear model was used to form a figure regarding age and sex-adjusted FA status vs. number of outcomes.

The two-step logistic regression models included: First, the associations of standardized FA variables with each cardiometabolic outcome were examined with models including age and sex as covariates. Then, additional covariates were specifically selected for each outcome to construct fully adjusted models. For prevalent and incident obesity, further adjustments were made for physical activity, educational socioeconomic status, smoking and the monthly portions for fruits and vegetables. For prevalent and incident HOMA-IR, further covariates included BMI, leisure-time physical activity, alanine aminotransferase, the triglyceride/high-density lipoprotein (HDL) cholesterol ratio and smoking. For elevated BP, further adjustments were made for BMI, leisure-time physical activity, HOMA-IR levels, the triglyceride/HDL cholesterol ratio, smoking and salt use. For incident fatty liver, same set of covariates was used as for BP, except that the salt intake and the triglyceride/HDL cholesterol ratio were replaced with alcohol use. Ultrasound examination was not performed in 2001. Therefore, to exclude possible cases with prevalent fatty liver in 2001, we removed participants with the Bedogni's fatty liver index>30 (Bedogni et al. 2006) and those with the known risk level alcohol use, i.e., over 20 g/day in women and 30 g per day in men in 2001, from the incident fatty liver models.

As tested with obesity (main risk factor for other outcomes), there were no consistent sex interactions in the FA associations characterized which was tested by the logistic regression models supplemented by a sex*FA-variable interaction term (data not shown).

4.6.4 Work IV

Age and contraceptive use-adjusted partial correlation coefficients were calculated separately for men and women to test the associations between the markers of LDL oxidation or inflammation and their FA determinants (2001 cross-sectional data). However, there were no sex-interactions in the FA associations characterized when being tested by the general linear models constructed by sex, FA variable and the sex*FA variable interaction term. For this reason, men and women were pooled in the regression analyses in which the FA variable, sex and age were modeled as explanatory variables (Step 1). In Step 2, Step 1 variables+physical activity, use of

contraceptives, vitamin E intake, smoking, BMI, alcohol intake, glucose, insulin, systolic BP and LDL cholesterol were included in the models.

4.7 Ethics

This study was carried out in accordance with the recommendations of the Declaration of Helsinki. The YFS was approved by the local ethics committee. All participants signed written informed consent forms during the follow-up studies and their parents gave their consent at baseline in 1980.

5 Results

5.1 Study Population in 1980, 2001, 2007 and 2011

The clinical characteristics of the study population are presented in **Table 11**. There was a decreasing trend in the LDL cholesterol concentration between the years 1980 and 2007 (not tested statistically). After that, an opposite trend was evident. In 2011, the LDL cholesterol concentration was at the same level as observed than in 2001. As evaluated below (**Figure 7**), there was a simultaneous increasing trend in the intake of SFAs between the years 2007 and 2011.

Table 11. Clinical characteristics of the same study participants in 1980, 2001, 2007 and 2011 (n=1418-1686). Female subjects who were pregnant in 2001, 2007 or/and 2011 have been omitted.

		1980		2001		2007		2011	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age range (years)		3-18		24-39		30-45		34-49	
Sex (male%)		47.0							
Body mass index (kg/m^2)		18.0	3.1	25.0	4.4	26.0	4.7	26.5	5.0
Waist circumference (cm)				84.0	12.2	88.7	13.4	91.8	14.0
Serum LDL cholesterol (mmol/L)		**3.41**	**0.82**	**3.28**	**0.84**	**3.11**	**0.78**	**3.27**	**0.83**
Serum HDL cholesterol (mmol/L)		1.56	0.31	1.28	0.31	1.33	0.32	1.33	0.33
Systolic BP (mmHg)		113	12	116	13	121	14	119	14
Diastolic BP (mmHg)		69	9	71	11	76	11	75	10
Serum triglycerides (mmol/L)		0.66	0.31	1.31	0.87	1.38	0.92	1.34	1.29
Serum C-reactive protein (U/L)		1.1	3.2	1.7	3.7	1.8	3.8	1.6	2.7
Serum Insulin (mU/L)		9.9	5.9	7.6	5.4	9.3	17.9	9.9	13.6
Serum glucose (mmol/L)				5.06	0.82	5.34	0.88	5.39	0.96
HOMA-based insulin resistance index				1.75	1.54	2.32	4.08	2.66	7.08
Serum total SFAs, %				37.8	2.2	37.4	2.0	37.3	2.1
Serum total MUFAs, %				25.9	3.1	27.0	2.8	26.6	2.9
Serum total n-6 PUFAs, %				31.9	3.2	31.2	3.0	31.5	3.2
Serum total n-3 PUFAs, %				4.5	1.0	4.4	1.1	4.6	1.0
Serum CE SFAs, %	n=629	12.4	0.9						
Serum CE MUFAs, %		26.0	3.6						
Serum CE n-6 PUFAs, %		58.7	4.6						
Serum CE n-3 PUFAs, %		3.0	0.5						

CE=cholesteryl ester; MUFAs=monounsaturated FAs; n-3=omega-3; n-6=omega-6; PUFAs= polyunsaturated FAs; SFAs=saturated FAs. Fatty acid intake data is presented in Figures 6 and 7.

5.2 Dietary Intake of Fatty Acids versus Serum Fatty Acid Proportions

5.2.1 Trends in Fatty Acid Intakes (1980-2011) and Serum Total Fatty Acid Proportions (2001-2011) at a Population Level

This thesis determined intakes and serum proportions of FAs both in childhood and in adulthood. For this reason, it was deemed interesting to examine how the FA composition of the diet and in serum has changed during the follow-up of 31 years (not tested statistically).

Since the 1980s (1980-2011), the greatest reduction trend (39.9%) was observed in the mean intake of SFAs **(Figure 7).** In practice, there was no change in the intake of MUFAs, whereas both n-6 PUFA (15.3%) and n-3 PUFA intakes (76.5%) increased during the follow-up of 31 years. Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) were used as their indicators since there were no possibility to characterize total n-3 and n-6 PUFA intakes in 1980. However, the reduction in mean SFA intake seemed to cease and change into a modest (11.6%) elevation between the years 2007 and 2011. This trend was observed also in the P/S ratio with its modestly lowered levels. When collecting dietary data, a 48h recall was used in 1980 and 2001, whereas a food frequency questionnaire was utilized in 2007 and 2011.

Changes in dietary intake did not affect the corresponding proportions of serum total FAs (**Figure 8**). In practice, serum FA proportions were at a constant level during the years 2001-2011. The PUFAs/SFAs and the n-3s/n-6s FA ratios also stayed at a constant level (none of these level changes were tested statistically).

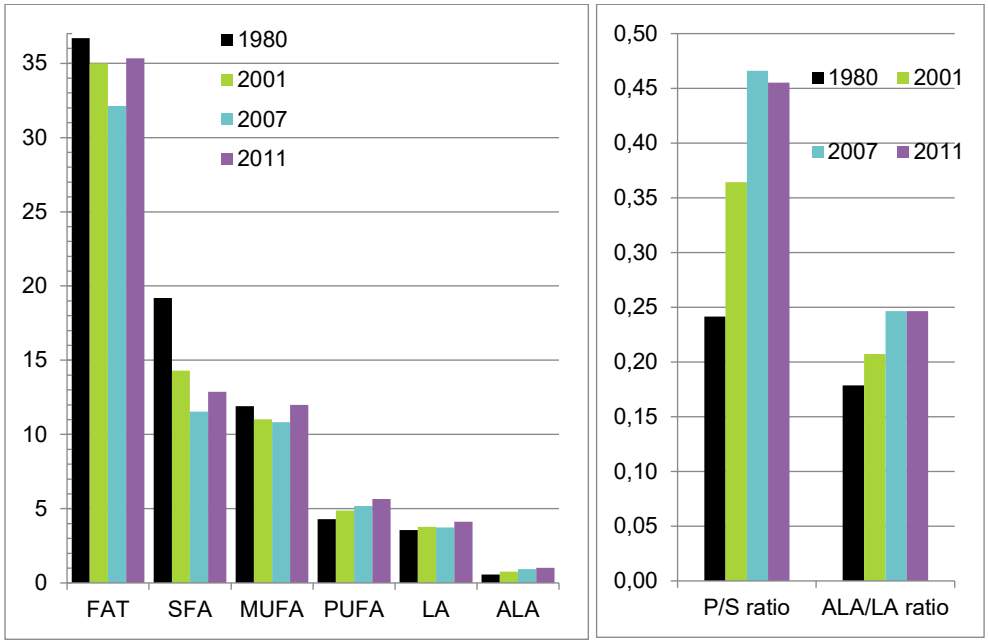


Figure 7. A) Mean **dietary** intakes of fats (E%), and B) the P/S and ALA/LA ratios in the YFS population (n=1037-1996) over the time of 31 years. FAT=total fat; SFA=saturated FAs; MUFA=monounsaturated FAs; PUFA=polyunsaturated FAs; ALA= α-linolenic acid, 18:3n-3; LA=linoleic acid, 18:2n-6; P/S=dietary PUFA/SFA ratio.

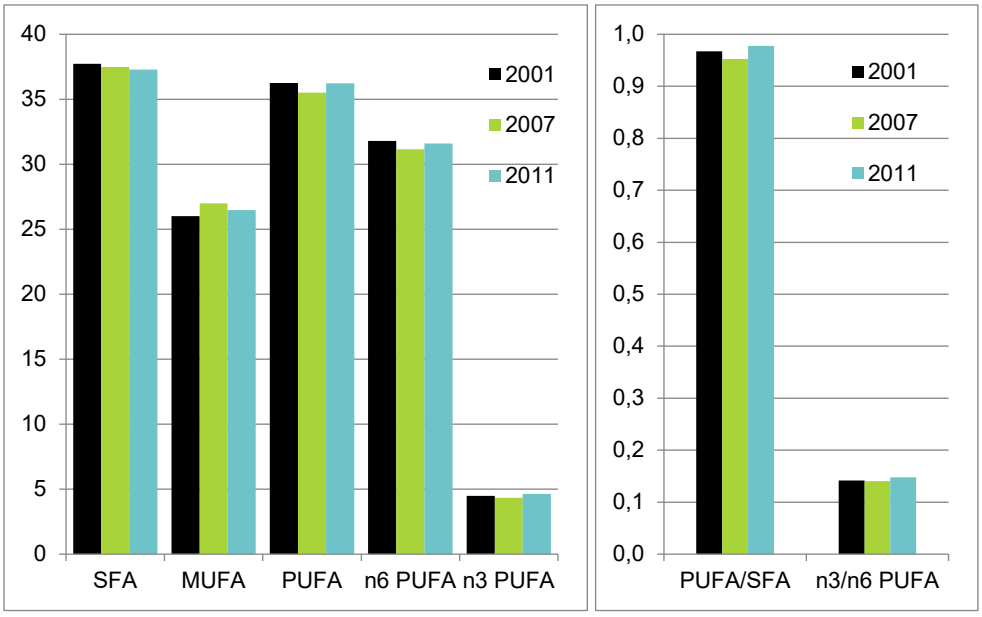


Figure 8. A) Proportions of **serum** FAs in the YFS population (n=1014-1925) over the time of 10 years in adulthood. The same NMR-based analysis method was used in all the time points. SFA=saturated FAs; MUFA=monounsaturated FAs; PUFA=polyunsaturated FAs.

5.2.2 Correlation Between Dietary Fatty Acids and Serum CEFAs (I)

Dietary FAs (proportions of the total FA intake) and serum CEFA proportions correlated strongly with each other in 1980 in childhood (n=795). The strongest correlation was observed for 18:2n-6, $r = 0.61$ ($P < 0.001$). The correlation coefficient was $r = 0.30$ ($P < 0.001$) for SFAs and $r = 0.57$ ($P < 0.001$) for PUFAs. Dietary MUFA intake and the proportion of MUFAs in CE fraction correlated inversely, $r = -0.19$ ($P < 0.001$).

5.2.3 Correlation between Dietary Fatty Acids and Serum Total Fatty Acids (III)

The associations between adulthood dietary intake of FAs (proportions of the total FA intake) and serum total FA proportions were weaker (according to the year 2001 cross-sectional data, n=991). The strongest association r=0.40 ($P < 0.001$) was observed between dietary n-3 PUFAs and serum total n-3 PUFA proportions. For SFAs, the correlation coefficient was r=0.14 ($P<0.001$); for MUFAs, the coefficient was r = 0.05 (ns) and for n-6 PUFAs r=0.04 (ns).

5.2.4 Comparisons between Dietary and Serum Proportions of Fatty Acids (III)

There seemed to be a clear difference in the dietary intake of FAs (proportions of the total FA intake) vs. serum total FA proportions (not tested statistically, **Figure 9**). In serum, SFA and MUFA proportions were weaker and n-3 PUFA and n-6PUFA proportions higher compared to intake data in 2001.

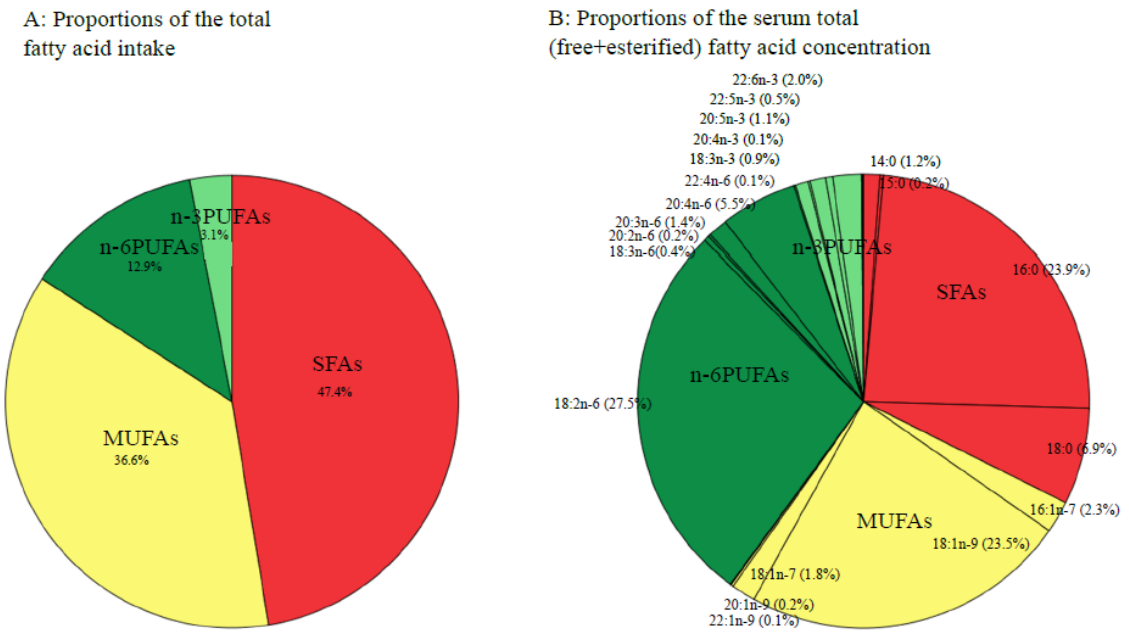


Figure 9. Fatty acid (FA) proportions related to daily FA intake and serum total FA concentration in the year 2001 data (n=991). MUFAs=monounsaturated FAs; PUFAs=polyunsaturated FAs; SFAs=saturated FAs. n-3=omega-3; n-6=omega-6. This figure has been presented in the original work III.

5.3 Fatty Acids and Cardiometabolic Outcomes

5.3.1 Childhood CEFAs versus Blood Pressure or Hypertension in Adulthood (I)

In 803 boys and girls, age-adjusted correlations showed that several childhood markers of desaturase and elongase activity (1980) were associated with adulthood BP (2007). In particular, the CE palmitoleic acid (16:1n-7)/palmitic acid (16:0) ratio, a marker of Δ9-desaturase activity, seemed to be associated with systolic BP and diastolic BP both in men and women. The CE stearic acid (18:0)/palmitic acid (16:0) ratio, a marker of elongase activity, was also associated with BP in men. See details in the **Original Work I.**

With respect to the fully-adjusted linear regression models in males (**Table 12**), serum CE SFAs (B=2.97, P<0.001 for systolic BP; B=1.48, P=0.015 for diastolic BP), and proportions of CE MUFAs (B=0.61, P=0.001 for systolic BP; B=0.27, P=0.078 for diastolic BP), as well as those of CE n-3 PUFAs (B=5.50, P<0.001 for systolic BP; B=2.47, P=0.015 for diastolic BP), were directly associated with BP, whereas the CE n-6 PUFA proportion, was inversely associated with BP (B=−0.56,

P<0.001 for systolic BP; *B*=−0.27, *P*<0.018 for diastolic BP). Serum CE SFA and PUFA associations were supported by dietary intake data. Associations were stronger for systolic BP than for diastolic BP. In females, the associations between CEFA proportions and BP were weaker than in males. For example, the childhood CE SFA proportion and the CE PUFAs/SFAs ratio did not correlate with adult systolic BP (**Table 12**). The strongest associations were found in 3 to 6 years old girls and in 9-12 years old boys. Overall, CE n-6 PUFAs which are derived mainly from vegetable oils and margarines, showed inverse BP associations, whereas FAs derived mainly from animal fats in this population (n-3 PUFAs, SFAs, MUFAs) exhibited direct BP associations.

The above-mentioned findings were confirmed with a dichotomous hypertension variable (age and sex specific the highest 20% for systolic BP or diastolic BP or medication for hypertension vs. the lowest 80%) by using CEFA dummies. Clinically determined hypertension was also used as an outcome with similar findings (data not presented). See details in **Original work I.**

Table 12. Linear regression for the childhood CEFA determinants of adulthood systolic BP and diastolic BP.

	Women (n = 396)				Men (n = 343)			
	SBP		DBP		SBP		DBP	
	B	*P*	*B*	*P*	*B*	*P*	*B*	*P*
Model 1 (SFA)								
Step 1	0.403	0.599	0.147	0.804	2.949	<0.001	1.458	0.019
Step 2	0.358	0.638	−0.012	0.984	2.974	<0.001	1.477	0.015
Model 2 (MUFA)								
Step 1	0.437	0.021	0.353	0.015	0.577	0.002	0.244	0.111
Step 2	0.440	0.019	0.345	0.016	0.612	0.001	0.266	0.078
Model 3 (PUFA)								
Step 1	−0.349	0.027	−0.276	0.023	−0.535	<0.001	−0.259	0.038
Step 2	−0.362	0.021	−0.246	0.039	−0.564	<0.001	−0.274	0.025
Model 4 (n−6)								
Step 1	−0.322	0.030	−0.264	0.020	−0.531	<0.001	−0.256	0.027
Step 2	−0.334	0.023	−0.237	0.034	−0.560	<0.001	−0.269	0.018
Model 5 (18:2n−6)								
Step 1	−0.250	0.072	−0.185	0.083	−0.479	<0.001	−0.213	0.058
Step 2	−0.242	0.079	−0.157	0.134	−0.511	<0.001	−0.234	0.034
Model 6 (n−3)								
Step 1	1.210	0.368	1.780	0.085	5.243	<0.001	2.449	0.018
Step 2	1.179	0.376	1.705	0.092	5.500	<0.001	2.472	0.015
Model 7 (PUFA:SFA)								
Step 1	−1.427	0.153	−1.001	0.192	−3.851	<0.001	−1.917	0.019
Step 2	−1.381	0.163	−0.799	0.288	−4.070	<0.001	−2.005	0.012
Model 8 [PUFA:(MUFA + SFA)]								
Step 1	−4.719	0.032	−3.670	0.030	−7.316	0.001	−3.212	0.071
Step 2	−4.703	0.031	−3.354	0.043	−7.892	<0.001	−3.560	0.041
Model 9 (n−6:n−3)								
Step 1	−0.208	0.140	−0.223	0.040	−0.563	<0.001	−0.226	0.040
Step 2	−0.199	0.155	−0.208	0.051	−0.602	<0.001	−0.244	0.024
Model 10 (dietary P:S ratio)								
Step 1	−4.308	0.419	−4.468	0.275	−15.637	0.002	−5.200	0.214
Step 2	−3.823	0.469	−4.712	0.239	−15.817	0.002	−5.396	0.189

Step 1 included a fixed linear regression model for CEFA and age plus a forward model (*P*-entry = 0.05 and *P*-removal = 0.10) for the factors that modulate adult BP such as BMI, alcohol use, sodium intake, smoking, physical activity, and family history of hypertension. Step 2 included step 1 + a forward model (*P*-entry=0.05 and *P*-removal=0.10) for serum LDL cholesterol, serum HDL cholesterol, serum triglycerides, and serum insulin. Subjects who took medication for hypertension (n = 59) or had missing drug information (n=5) have been omitted. DBP, diastolic blood pressure; P:S, PUFA:SFA; SBP, systolic blood pressure. This table has been presented in original work I.

5.3.2 Childhood Serum CEFAs and Adulthood cIMT (II)

In 374 males and 449 females, links between childhood FA quality (serum CEFAs and dietary intake in 1980) and adult sub-clinical atherosclerosis (estimated by the cIMT in 2007) were investigated (**Table 13**).

In females, after adjustment for age and childhood non-lipid risk markers, the childhood CE SFA (B = 11.3; P = 0.011), CE MUFA (B = 2.5; P = 0.025), and CE n-3 PUFA (B = 16.2; P = 0.035) percentages were directly associated with adult

cIMT. In contrast, the CE n-6 PUFA percentage was inversely associated with cIMT ($B = -2.3$; $P = 0.008$). These associations were only marginally diluted following further adjustments for childhood lipid risk variables. Following adjustment for both childhood and adulthood risk factors, many of the associations remained statistically significant or borderline significant. Overall, the strongest associations were seen in the oldest age groups. In males, these associations were generally weak and non-significant ($P > 0.05$) after controlling for confounders. There were some differences in the FA variables between sexes. In particular, the arachidonic acid (20:4n-6)/dihomo-γ-linolenic acid (20:3n-6) ratio, an estimate of Δ5-desaturase activity, was higher in female subjects (see details for variables in the **Original work II, Table 1**).

Statistically significant relationships were also observed between childhood dietary intake data and adult cIMT. In particular, the intake of SFAs (E%), was directly associated with adulthood cIMT in females.

The above findings were confirmed by forming a mean value from the CEFA data characterized in 1980, 1983 and/or 1986. With regard to cIMT, a mean value of the cIMT data characterized in 2001 and/or 2007, was used. With regard to females, this data provided very similar statistically significant or borderline significant findings compared to primary data presented above. See details in the supplemental material of original **work II**. In males in the non-adjusted models, dietary intake of SFAs (E%) and serum n-3 PUFA proportion were directly associated with the cIMT values. Following adjustment for covariates, these relationships became statistically non-significant.

Table 13. Linear regression models examining childhood serum cholesteryl ester fatty acid and dietary FA intake as predictors of adulthood common carotid artery intima media thickness in 2007.

		cIMT of females in 2007, μm								cIMT of males in 2007, μm							
CEFA and dietary FA intake models with		All females[3]		3–6 y old females		9–12 y old females		15–18 y old females		All males[4]		3–6 y old males		9–12 y old males		15–18 y old males	
1980 data	Step[2]	B	*P*	B	*P*	B	*P*	B	*P*	B	*P*	B	*P*	B	*P*	B	*P*
Adjusted for childhood factors																	
CEFA variables, *n*		449		137		141		171		374		115		112		147	
SFAs, %	1a	6.0	0.19	2.8	0.68	20.4	0.007	8.4	0.32	−1.1	0.87	2.1	0.84	11.4	0.31	−11.2	0.26
	2a	11.3	0.011	2.7	0.70	20.1	0.006	8.4	0.31	−3.8	0.52	2.7	0.78	10.2	0.35	−10.1	0.31
	3a	9.6	0.032	2.7	0.70	20.1	0.006	5.8	0.48	−3.8	0.52	6.3	0.53	10.2	0.35	−10.1	0.31
MUFAs, %	1a	1.6	0.16	0.0	0.98	1.3	0.50	5.8	0.011	0.6	0.72	−0.6	0.76	2.7	0.33	1.7	0.54
	2a	2.5	0.025	−0.0	0.99	1.7	0.36	5.2	0.024	0.6	0.70	0.2	0.93	2.0	0.46	1.6	0.58
	3a	2.0	0.07	−0.0	0.99	0.6	0.74	5.3	0.018	0.6	0.70	0.9	0.67	2.0	0.46	1.6	0.58
PUFAs, %	1a	−1.5	0.12	−0.0	0.98	−2.1	0.18	−4.5	0.015	−0.2	0.88	0.6	0.73	−2.3	0.31	−0.3	0.90
	2a	−2.4	0.009	0.0	0.99	−2.4	0.12	−4.1	0.028	−0.1	0.95	−0.0	1.00	−1.6	0.46	−0.3	0.91
	3a	−2.0	0.032	0.0	0.99	−2.4	0.12	−4.0	0.028	−0.1	0.95	−0.6	0.73	−1.6	0.46	−0.3	0.91
18:2n6, %	1a	−1.6	0.06	−0.4	0.76	−2.2	0.13	−4.0	0.013	−0.4	0.74	1.0	0.54	−2.5	0.21	−0.7	0.73
	2a	−2.3	0.005	−0.3	0.78	−2.3	0.10	−3.6	0.023	−0.2	0.83	0.6	0.72	−2.0	0.31	−0.7	0.74
	3a	−1.9	0.019	−0.3	0.78	−2.3	0.10	−3.6	0.025	−0.2	0.83	0.1	0.97	−2.0	0.31	−0.7	0.74
n6 PUFAs, %	1a	−1.6	0.08	−0.1	0.92	−2.1	0.15	−4.4	0.013	−0.3	0.81	0.6	0.74	−2.2	0.30	−0.4	0.86
	2a	−2.3	0.008	−0.1	0.95	−2.3	0.11	−4.0	0.023	−0.2	0.89	−0.0	0.99	−1.6	0.43	−0.3	0.88
	3a	−1.9	0.028	−0.1	0.95	−2.3	0.11	−3.9	0.023	−0.2	0.89	−0.5	0.75	−1.6	0.43	−0.3	0.88
n3 PUFAs, %	1a	20.3	0.013	6.8	0.52	23.6	0.09	26.1	0.09	9.8	0.37	−1.2	0.94	15.4	0.41	9.1	0.64
	2a	16.2	0.035	6.7	0.53	20.4	0.14	26.0	0.08	6.9	0.50	2.2	0.88	16.0	0.37	6.7	0.73
	3a	13.2	0.09	6.7	0.53	20.4	0.14	26.9	0.07	6.9	0.50	0.1	0.99	16.0	0.37	6.7	0.73
Dietary variables, *n*		443		133		139		171		372		115		111		146	
Dietary P:S ratio	1a	−27.9	0.40	19.0	0.71	−107	0.046	−44.1	0.43	7.0	0.88	−32.2	0.68	54.5	0.52	−32.1	0.65
	2a	−54.9	0.08	19.4	0.71	−121	0.020	−46.9	0.40	3.7	0.93	−33.2	0.65	56.9	0.49	−34.0	0.64
	3a	−41.9	0.18	19.4	0.71	−121	0.020	−47.0	0.39	3.7	0.93	−30.7	0.67	56.9	0.49	−34.0	0.64
Dietary SFAs, *E%*	1a	1.9	0.08	−1.7	0.30	3.3	0.06	3.7	0.06	2.5	0.10	1.5	0.51	0.4	0.87	0.5	0.86
	2a	2.4	0.022	−1.7	0.30	3.7	0.026	3.9	0.049	0.7	0.65	1.8	0.41	0.6	0.80	0.6	0.81
	3a	2.2	0.034	−1.7	0.30	3.7	0.026	3.9	0.048	0.7	0.65	1.8	0.40	0.6	0.80	0.6	0.81
Adjusted for childhood and adulthood factors																	
CEFA variables, *n*		449		137		141		171		374		115		112		147	
SFAs, %	1b	11.3	0.011	2.7	0.70	20.1	0.008	8.4	0.31	−1.7	0.78	0.8	0.94	11.2	0.33	−10.1	0.31
	2b	10.4	0.014	1.6	0.82	19.1	0.008	10.1	0.19	−7.6	0.18	−1.5	0.87	7.3	0.45	−13.2	0.17
	3b	7.6	0.08	1.6	0.82	19.3	0.007	7.7	0.32	−7.7	0.18	1.9	0.84	7.3	0.45	−13.4	0.15
MUFAs, %	1b	2.5	0.027	−0.0	0.99	1.4	0.48	5.2	0.024	1.2	0.42	0.4	0.86	2.7	0.34	1.6	0.58
	2b	1.5	0.17	−0.1	0.92	1.0	0.57	3.8	0.08	−0.4	0.76	−0.1	0.96	0.6	0.82	1.3	0.64
	3b	0.9	0.42	−0.1	0.92	1.0	0.57	4.0	0.06	−0.7	0.63	0.6	0.76	0.6	0.82	0.4	0.89
PUFAs, %	1b	−2.4	0.011	0.0	0.99	−2.1	0.17	−4.1	0.028	−0.6	0.61	−0.1	0.97	−2.2	0.33	−0.3	0.91
	2b	−1.6	0.07	0.1	0.95	−1.9	0.22	−3.1	0.07	0.8	0.49	0.3	0.83	−0.5	0.80	0.5	0.82
	3b	−1.1	0.24	0.1	0.95	−1.9	0.22	−3.1	0.07	1.0	0.40	−0.2	0.88	−0.5	0.80	1.1	0.60
18:2n6, %	1b	−2.3	0.005	−0.3	0.78	−2.1	0.13	−3.6	0.023	−0.7	0.50	0.5	0.75	−2.5	0.22	−0.7	0.74
	2b	−1.7	0.034	−0.2	0.87	−1.8	0.18	−2.8	0.07	0.5	0.62	1.0	0.53	−1.2	0.50	−0.5	0.79
	3b	−1.2	0.14	−0.2	0.87	−1.8	0.18	−2.7	0.07	0.6	0.54	0.5	0.76	−1.2	0.50	−0.0	0.99
n6 PUFAs, %	1b	−2.3	0.008	−0.1	0.95	−2.1	0.15	−4.0	0.023	−0.6	0.57	−0.0	0.98	−2.1	0.31	−0.3	0.88
	2b	−1.6	0.06	0.0	0.97	−1.9	0.19	−3.0	0.07	0.7	0.51	0.4	0.81	−0.5	0.77	0.5	0.81
	3b	−1.1	0.21	0.0	0.97	−1.9	0.19	−3.0	0.07	0.9	0.42	−0.1	0.95	−0.5	0.77	1.1	0.60
n3 PUFAs, %	1b	18.6	0.017	6.7	0.53	21.4	0.13	26.0	0.08	8.3	0.42	−0.3	0.99	15.5	0.41	6.7	0.73
	2b	12.4	0.10	2.3	0.83	18.8	0.16	16.9	0.23	−2.3	0.82	−6.2	0.67	9.3	0.56	2.3	0.91
	3b	9.5	0.21	2.3	0.83	20.1	0.13	17.9	0.20	−1.6	0.87	−8.8	0.53	9.3	0.56	0.6	0.97
Dietary variables, *n*		443		133		139		171		372		115		111		146	
Dietary P:S ratio	1b	−51.4	0.10	19.4	0.71	−107	0.046	−46.9	0.40	−6.3	0.89	−42.2	0.58	55.5	0.51	−34.0	0.64
	2b	−43.0	0.16	21.5	0.67	−110	0.030	−41.2	0.43	56.7	0.18	−14.8	0.83	126	0.08	15.4	0.83

	3b	−24.3	0.43	21.5	0.67	−110	0.030	−41.2	0.42	56.1	0.18	−12.6	0.86	126	0.08	11.5	0.87
Dietary SFAs, *E%*	1b	2.3	0.028	−1.7	0.30	3.5	0.045	3.9	0.049	0.8	0.57	2.0	0.38	0.4	0.88	0.6	0.81
	2b	1.7	0.10	−2.2	0.18	3.5	0.036	3.4	0.07	−1.1	0.45	0.6	0.80	−1.2	0.60	−0.9	0.72
	3b	1.1	0.26	−2.2	0.18	3.5	0.036	3.3	0.07	−1.0	0.47	0.5	0.81	−1.2	0.60	−0.9	0.72

Values are regression coefficients indicating the change in cIMT in μm in response to 1 unit of change in the explanatory factor (serum CEFA or dietary variable). Models were adjusted for the childhood, or childhood and adulthood lipid and nonlipid risk variables (primary analyses). CEFA, cholesteryl ester FA; cIMT, carotid artery mean intima media thickness; P:S ratio, PUFA:SFA ratio; SBP, systolic BP.

[2]Step 1a: the CEFA or dietary variable of interest was included in the model. Step 2a: step 1a variable and age were included in the model + stepwise regression (P-entry = 0.05 and P-removal = 0.10) for childhood BMI, physical activity, serum insulin, and systolic BP. Step 3a: step 1a variable + step 2a variables + stepwise regression (P-entry = 0.05 and P-removal = 0.10) for childhood serum LDL cholesterol, HDL cholesterol, and TGs. Step 1b: the CEFA or dietary variable of interest and age were included in the model. Step 2b: step 1b variables + stepwise regression (P-entry = 0.05 and P-removal = 0.10) for childhood BMI, physical activity, serum insulin and systolic BP, and for adulthood BMI, alcohol use, smoking, physical activity, the number of education years, serum insulin, plasma glucose, type 2 diabetes, and systolic BP. Step 3b: step 1b variables + step 2b variables + stepwise regression (P-entry = 0.05 and P-removal = 0.10) for childhood serum LDL cholesterol, HDL cholesterol, and TGs, and for adulthood serum LDL cholesterol, HDL cholesterol, TGs, and drug for dyslipidemia.

[3]In women (all of the women included), CEFAs and dietary variables accounted for 0.2–1.4% of the variation in cIMT. The final models with childhood factors accounted for 11.9–14.3%, and the final models with childhood and adulthood factors accounted for 17.9–20.7% of the variation in cIMT.

[4]In men (all of the men included), CEFAs and dietary variables accounted for 0.0–0.7% of the variation in cIMT. The final models with childhood factors accounted for 14.1–14.2%, and the final models with childhood and adulthood factors accounted for 22.9–24.4% of the variation in cIMT.

This table has been presented in original work II.

5.3.3 Adulthood Serum Total Fatty Acid Profile versus Cardiometabolic Outcomes, Including Obesity, High HOMA-IR, Elevated Blood Pressure and Fatty Liver in Adulthood (III)

Cross-Sectional Associations (2001 data)

With regard to the age and sex-adjusted associations, SFAs and MUFAs were directly and total n-6 PUFA proportion inversely associated with obesity, high HOMA-IR and elevated BP (**Table 14**). Total n-3 PUFA also had inverse borderline significant associations with obesity and BP. Many of these associations were also statistically significant or borderline significant in the fully-adjusted models meaning that they displayed independency of the other cardio-metabolic outcomes. The high PUFA/SFA ratio had the strongest inverse fully-adjusted association with the outcomes.

Dietary intake data did not support these serum-based findings (**see work III, Table 1 for details**), since the links observed with the serum data were absent.

Specific FAs associated also similarly with all the cardiometabolic outcomes. However, several of them showed deviations in their associations compared to their own group of desaturation-degree. With respect to SFAs, stearic acid (18:0) did not show any age and sex-adjusted links. Myristic (14:0) and palmitic acids (16:0), with an even number of carbons in their chains, consistently showed direct outcome associations, whereas pentadecanoic acid (15:0) had an inverse link with BP (fully-adjusted models).

With regard to specific MUFAs, only palmitoleic acid (16:1n-7) and oleic acid (18:1n-9) showed consistent direct age and sex-adjusted associations with the outcomes. With regard to the specific MUFAs, the increasing carbon chain length was systematically linked to a decreasing risk for cardiometabolic outcomes. With respect to specific PUFAs, linoleic acid (18:2n-6) showed consistent inverse associations with all of the outcomes. Gamma-linolenic acid (18:3n-6), dihomo-gamma-linolenic acid (20:3n-6) and eicosatetraenoic acid (20:4n-3) showed, in contrast to n-6 or n-3 PUFAs as groups, strong direct associations with obesity and high HOMA-IR, in particular. Docosatetraenoic acid (22:4n-6) exhibited a strong direct association with BP. With respect to long-chain n-3 PUFAs, docosapentaenoic acid (22:5n-3) and docosahexaenoic acid (22:6n-3) showed inverse links (some associations were borderline significant) with the outcomes. Several of the above-mentioned links were also statistically significant in the fully-adjusted models.

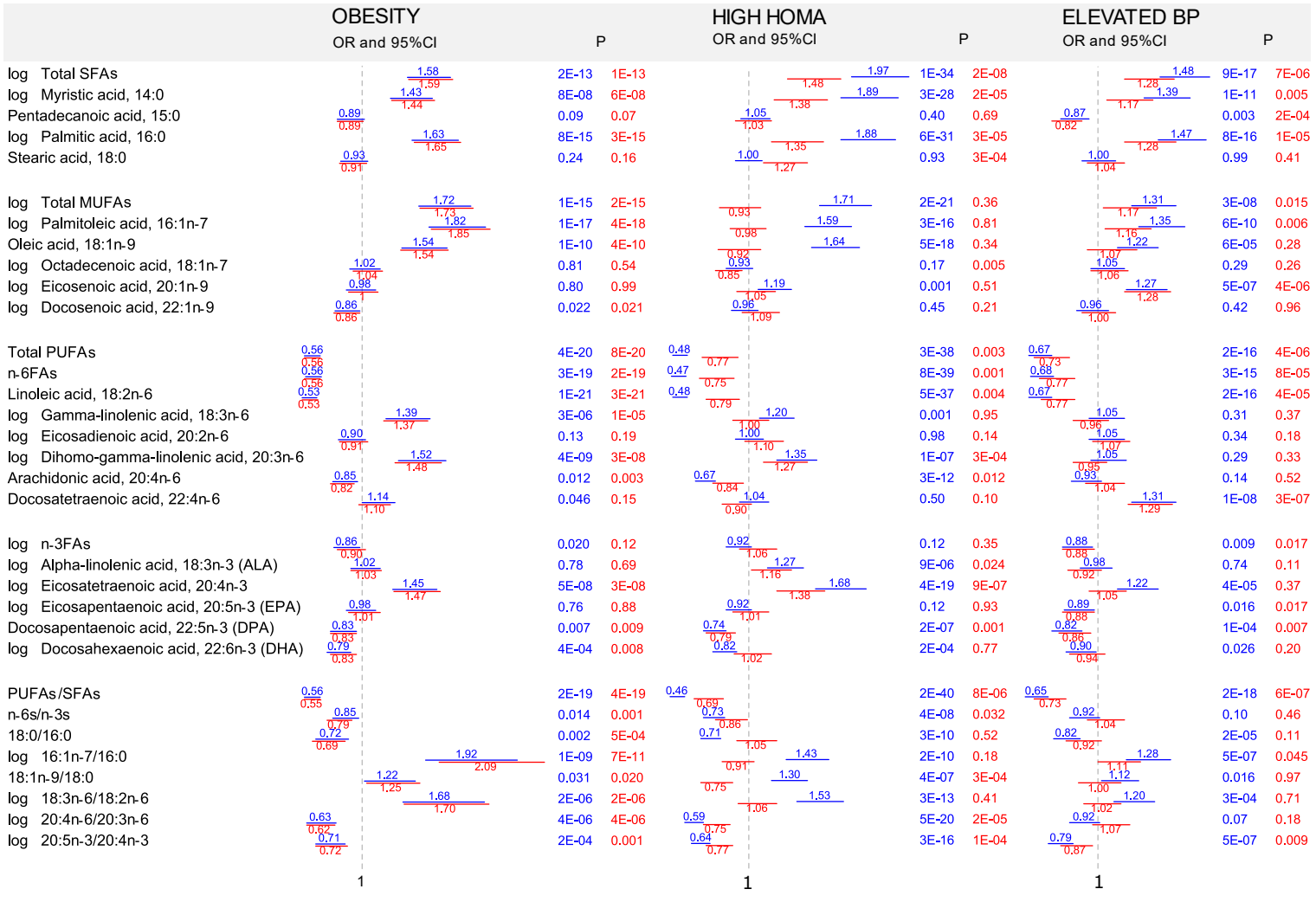
With regard to the FA ratios and estimates of FA metabolism, the 16:1n-7/16:0 and the 18:1n-9/18:0 (estimates of Δ9-desaturase activity) and the 18:3n-6/18:2n-6 (Δ6-desaturase activity) ratios had direct age and sex-adjusted, significant or borderline significant, associations with the outcomes. However, the ratios 18:0/16:0 (elongase activity), 20:4n-6/20:3n-6 and 20:5n-3/20:4n-3 (Δ5-desaturase activity) were inversely associated (some associations being statistically non-significant) with the outcomes. Several of these links remained statistically significant following full adjustment.

Prospective Associations (2001-2011 data)

With respect to the 10-year incident obesity, HOMA-IR, fatty liver and elevated BP, the association profiles of FAs were weaker (many of them were only borderline significant or non-significant) but very similar in terms of their profile with the year 2001 cross-sectional data (**Table 15**). Once again, low PUFA proportions and high SFA and MUFA proportions were associated (or tended to be linked) with the cardiometabolic outcomes. Many similar (but weaker) associations were seen between FAs and incident fatty liver, as were seen between FAs and obesity. Specific FAs and FA ratios also showed similar (but weaker) association profiles than had

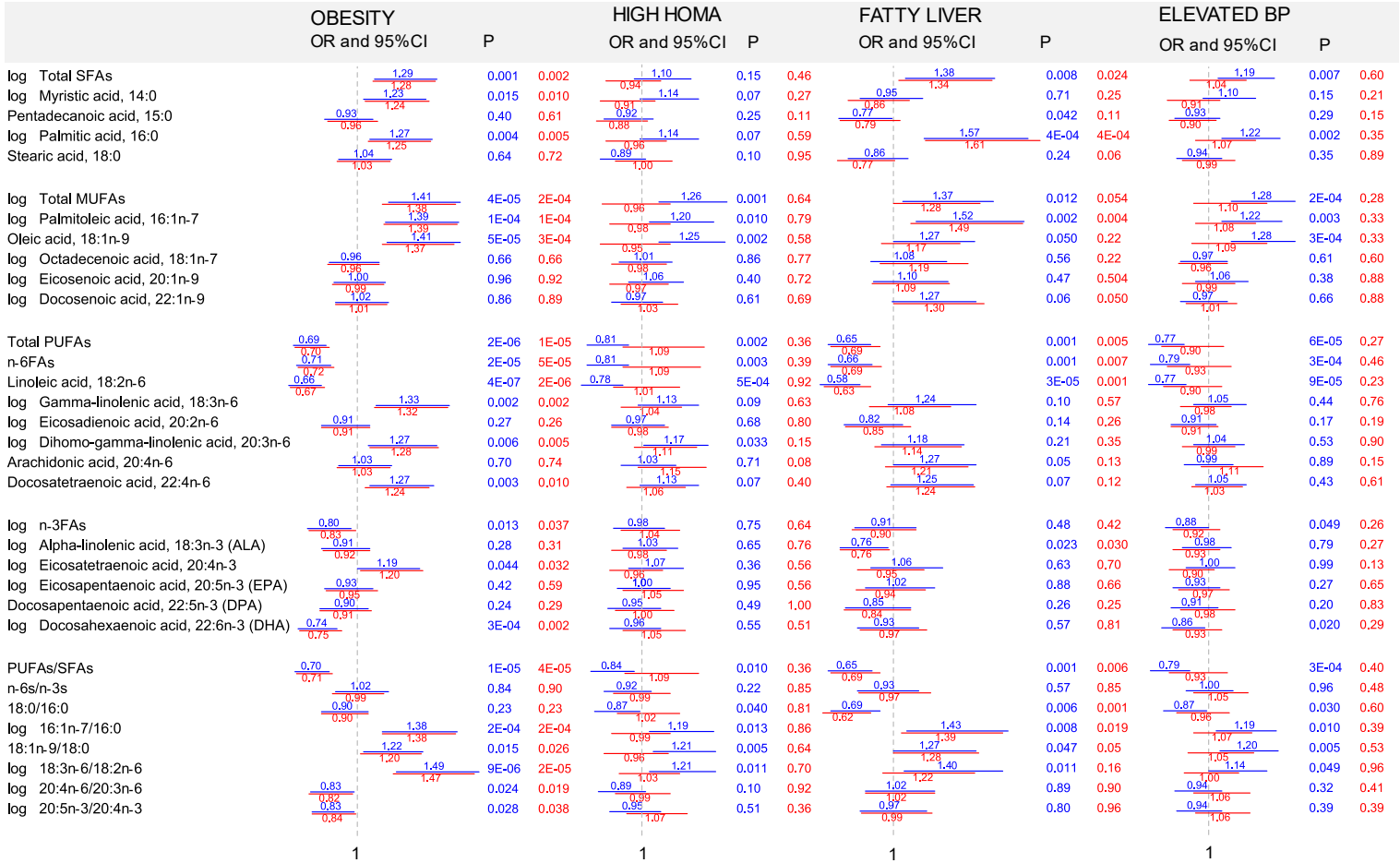
been observed in the cross-sectional data. Age and sex-adjusted linoleic acid (18:2n-6) was inversely associated with all the outcomes, but only the obesity association remained following full adjustment. Palmitic acid (16:0) showed a direct fatty liver association in the fully adjusted model. Fully-adjusted models for incident HOMA-IR or elevated BP did not show any statistically significant associations.

Table 14. Cross-sectional associations of FA proportions in 2001 with different outcomes in 2001. Odds ratios for prevalent obesity (BMI>30 kg/m^2 vs. ≤30 kg/m^2, n=271 obese out of 2200 participants, high HOMA-IR (age and sex specific percentiles ≥80% vs. <80%, n=444 high HOMA-IR out of 2199 participants) and elevated BP (age and sex specific diastolic or systolic BP percentiles≥80% or medication for hypertension vs. <80% without medication, n=647 elevated BP out of 2187 participants). Values are odd ratios and their 95% confidence intervals per 1-SD increment in the FA measures. Each FA measure was tested separately in logistic regression models adjusted for sex and age (blue bars). Additionally, obesity associations were adjusted for leisure-time physical activity, education-based socioeconomic status, smoking and the number of monthly portions for fruits and vegetables (red bars); HOMA-IR associations for body mass index, leisure-time physical activity, alanine aminotransferase activity, smoking and the triglycerides/HDL cholesterol ratio (red bars); and BP associations for body mass index, leisure-time physical activity, smoking, HOMA-IR values, salt intake and the triglyceride/HDL cholesterol ratio (red bars). Pregnant subjects were excluded. MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SFA, saturated FA. This table has been presented in original work III.



	OBESITY P		HIGH HOMA P		ELEVATED BP P	
log Total SFAs	2E-13	1E-13	1E-34	2E-08	9E-17	7E-06
log Myristic acid, 14:0	8E-08	6E-08	3E-28	2E-05	1E-11	0.005
Pentadecanoic acid, 15:0	0.09	0.07	0.40	0.69	0.003	2E-04
log Palmitic acid, 16:0	8E-15	3E-15	6E-31	3E-05	8E-16	1E-05
Stearic acid, 18:0	0.24	0.16	0.93	3E-04	0.99	0.41
log Total MUFAs	1E-15	2E-15	2E-21	0.36	3E-08	0.015
log Palmitoleic acid, 16:1n-7	1E-17	4E-18	3E-16	0.81	6E-10	0.006
Oleic acid, 18:1n-9	1E-10	4E-10	5E-18	0.34	6E-05	0.28
log Octadecenoic acid, 18:1n-7	0.81	0.54	0.17	0.005	0.29	0.26
log Eicosenoic acid, 20:1n-9	0.80	0.99	0.001	0.51	5E-07	4E-06
log Docosenoic acid, 22:1n-9	0.022	0.021	0.45	0.21	0.42	0.96
Total PUFAs	4E-20	8E-20	3E-38	0.003	2E-16	4E-06
n-6FAs	3E-19	2E-19	8E-39	0.001	3E-15	8E-05
Linoleic acid, 18:2n-6	1E-21	3E-21	5E-37	0.004	2E-16	4E-05
log Gamma-linolenic acid, 18:3n-6	3E-06	1E-05	0.001	0.95	0.31	0.37
log Eicosadienoic acid, 20:2n-6	0.13	0.19	0.98	0.14	0.34	0.18
log Dihomo-gamma-linolenic acid, 20:3n-6	4E-09	3E-08	1E-07	3E-04	0.29	0.33
Arachidonic acid, 20:4n-6	0.012	0.003	3E-12	0.012	0.14	0.52
Docosatetraenoic acid, 22:4n-6	0.046	0.15	0.50	0.10	1E-08	3E-07
log n-3FAs	0.020	0.12	0.12	0.35	0.009	0.017
log Alpha-linolenic acid, 18:3n-3 (ALA)	0.78	0.69	9E-06	0.024	0.74	0.11
log Eicosatetraenoic acid, 20:4n-3	5E-08	3E-08	4E-19	9E-07	4E-05	0.37
log Eicosapentaenoic acid, 20:5n-3 (EPA)	0.76	0.88	0.12	0.93	0.016	0.017
Docosapentaenoic acid, 22:5n-3 (DPA)	0.007	0.009	2E-07	0.001	1E-04	0.007
log Docosahexaenoic acid, 22:6n-3 (DHA)	4E-04	0.008	2E-04	0.77	0.026	0.20
PUFAs/SFAs	2E-19	4E-19	2E-40	8E-06	2E-18	6E-07
n-6s/n-3s	0.014	0.001	4E-08	0.032	0.10	0.46
18:0/16:0	0.002	5E-04	3E-10	0.52	2E-05	0.11
log 16:1n-7/16:0	1E-09	7E-11	2E-10	0.18	5E-07	0.045
18:1n-9/18:0	0.031	0.020	4E-07	3E-04	0.016	0.97
log 18:3n-6/18:2n-6	2E-06	2E-06	3E-13	0.41	3E-04	0.71
log 20:4n-6/20:3n-6	4E-06	4E-06	5E-20	2E-05	0.07	0.18
log 20:5n-3/20:4n-3	2E-04	0.001	3E-16	1E-04	5E-07	0.009

Table 15. Prospective associations of FA proportions in 2001 with different outcomes in 2011. ORs for incident obesity (BMI>30 kg/m^2 vs. ≤30 kg/m^2, 163 obese out of 1414 participants), high HOMA-IR (age and sex specific percentiles≥80% vs. <80%, n=255 high HOMA-IR out of 1289 participants), fatty liver (n=70 fatty liver out of 975 individuals) and elevated BP (age and sex specific diastolic or systolic BP percentiles≥80% or medication for hypertension vs. <80% without medication, n=342 elevated BP out of 1088 participants). For obesity incidence, obese individuals, BMI>30 kg/m^2, in 2001 were excluded. For high HOMA-IR incidence, subjects with HOMA-IR percentile≥80% in 2001 were excluded. For fatty liver, individuals with Bedogni's fatty liver index>30 and the risk level use of alcohol in 2001 were excluded. For elevated BP, individuals with elevated BP in 2001 were excluded (see the criteria above). Values are odd ratios and their 95% confidence intervals per 1-SD increment in the FA measures. Each FA measure was tested separately in logistic regression models adjusted for sex and age (blue bars). Further adjustments (red bars) were carried out as described in Table 2. Fatty liver was adjusted for body mass index, leisure-time physical activity, smoking, HOMA-IR values and alcohol intake (red bars). Pregnant subjects, either in 2001 or 2011, were excluded. MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SFA, saturated FA. This table has been presented in original work III.


OBESITY
OR and 95%CI
P
HIGH HOMA
OR and 95%CI
P
FATTY LIVER
OR and 95%CI
P
ELEVATED BP
OR and 95%CI
P
log Total SFAs
log Myristic acid, 14:0
Pentadecanoic acid, 15:0
log Palmitic acid, 16:0
Stearic acid, 18:0
log Total MUFAs
log Palmitoleic acid, 16:1n-7
Oleic acid, 18:1n-9
log Octadecenoic acid, 18:1n-7
log Eicosenoic acid, 20:1n-9
log Docosenoic acid, 22:1n-9
Total PUFAs
n-6FAs
Linoleic acid, 18:2n-6
log Gamma-linolenic acid, 18:3n-6
log Eicosadienoic acid, 20:2n-6
log Dihomo-gamma-linolenic acid, 20:3n-6
Arachidonic acid, 20:4n-6
Docosatetraenoic acid, 22:4n-6
log n-3FAs
log Alpha-linolenic acid, 18:3n-3 (ALA)
log Eicosatetraenoic acid, 20:4n-3
log Eicosapentaenoic acid, 20:5n-3 (EPA)
Docosapentaenoic acid, 22:5n-3 (DPA)
log Docosahexaenoic acid, 22:6n-3 (DHA)
PUFAs/SFAs
n-6s/n-3s
18:0/16:0
log 16:1n-7/16:0
18:1n-9/18:0
log 18:3n-6/18:2n-6
log 20:4n-6/20:3n-6
log 20:5n-3/20:4n-3
1
1
1
1

In summary, serum FA proportions had several outcome associations which were independent from common cardiometabolic risk factors. For example, adjustment for BMI did not dilute the associations of high HOMA-IR or BP into statistical non-significance. This concerned mainly cross-sectional associations, but also included those for incident obesity and fatty liver. Finally, we formed a summary variable reflecting the composition of serum FA proportions, i.e. (SFAs%+MUFAs%)/PUFAs%. This summary variable was strongly linked with an increasing number of prevalent cardiometabolic outcomes (0 to 3) which the subject was suffering (**Figure 10**). Thus, a non-optimal serum FA composition was strongly associated with the severity of the cardiometabolic state.

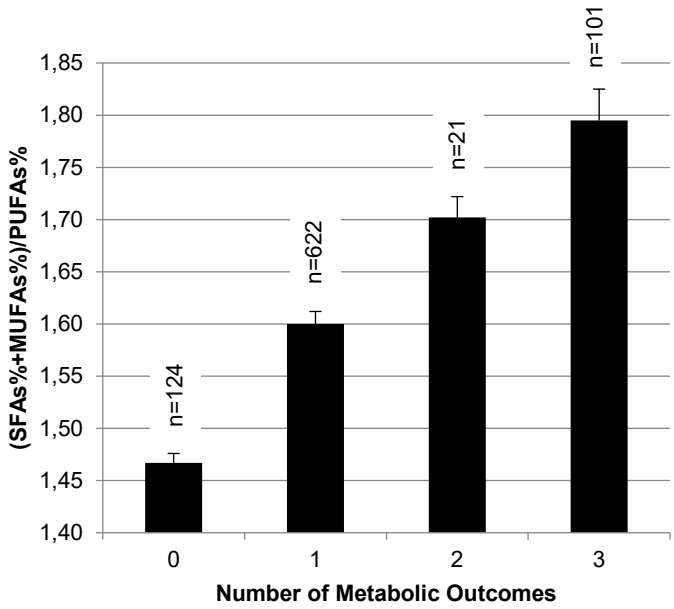


Figure 10. Serum FA composition, i.e., (SFAs%+MUFAs%)/PUFAs%, vs. the number of cardiometabolic outcomes (the year 2001 cross-sectional data, *beta*=0.12, *P*<0.001 for the trend, n=2200). Bars denote means and their standard errors. Values presented were adjusted for age and sex. With regard to the cardiometabolic outcome score, obesity (0 vs. 1), high HOMA-IR (0 vs. 1) and high BP (0 vs. 1) were summarized. Criteria for different outcomes have been described in the methods section. Liver fat was not included in the outcome score since it was not estimated in 2001. MUFA, monounsaturated FA; PUFA, polyunsaturated FA; SFA, saturated FA. The number of subjects per group is given next to the error bars. This figure has been presented in original work III.

5.3.4 Adulthood Serum Total Fatty Acids versus Lipoprotein Oxidation Markers and C-Reactive Protein (IV)

In this cross-sectional sub-study (2001 data), we examined associations between serum total FA proportions and 1) the status of LDL oxidation *in vivo* (conjugated dienes=oxLDLlipids and antibody-based oxidized proteins=oxLDLprot) or 2) the C-

reactive protein levels (inflammatory marker) in 2196 YFS participants. Simple age and contraceptive use-adjusted correlations of serum total FA proportions with the markers of in vivo LDL oxidation and inflammation have been presented in **Original work IV, Table 2**.

With regard to fully-adjusted linear regression models, the main findings were as follows: low SFA and MUFA proportions, and high n-6 PUFAs, the PUFA/SFA and the n-6/n-3 ratios were all associated with reduced levels of oxLDLlipids, oxLDLprot and C-reactive protein (**Table 16**). N-3 PUFAs showed conflicting findings since they were directly associated with oxLDLprot but inversely with oxLDLlipids and C-reactive protein. The variable indicating the mean number of double bonds per FA, showed constant inverse associations with all of the explained variables investigated. These observations suggest that PUFAs are inversely and SFAs and MUFAs directly related with LDL oxidation and inflammation.

Table 16. Linear regression models between FA variables and the markers of in *vivo* LDL oxidation or inflammation (2001).

Serum FA%s		**oxLDLprot***		**oxLDLlipids: LDL dienes/ApoB***		**Serum C-reactive protein***	
	Step	*B*	*P*	*B*	*P*	*B*	*P*
SFAs	1	5.8	<0.001	12.7	<0.001	51.1	<0.001
	2	5.3	<0.001	11.5	<0.001	17.3	<0.001
MUFAs	1	7.8	<0.001	12.1	<0.001	39.2	<0.001
	2	7.8	<0.001	12.2	<0.001	18.2	<0.001
PUFAs	1	-5.4	<0.001	-9.4	<0.001	-33.3	<0.001
	2	-5.8	<0.001	-9.9	<0.001	-14.8	<0.001
n-6s	1	-6.4	<0.001	-9.6	<0.001	-33.2	<0.001
	2	-6.1	<0.001	-10.1	<0.001	-13.4	<0.001
n-3s	1	6.1	0.007	-6.9	<0.001	-35.7	<0.001
	2	0.5	0.79	-4.5	0.014	-23.8	0.004
n-6s/n-3s	1	-10.0	<0.001	-6.3	<0.001	-13.2	0.020
	2	-5.4	<0.001	-6.2	<0.001	2.4	0.63
PUFAs/SFAs	1	-102	<0.001	-194	<0.001	-715	<0.001
	2	-106	<0.001	-201	<0.001	-303	<0.001
Double bonds per	1	-143	<0.001	-389	<0.001	-1159	<0.001
FA	2	-200	<0.001	-376	<0.001	-486	<0.001

Data are unstandardized regression coefficients multiplied by 10^3. *The variable was log-transformed. Linear regression models contained two steps: Step 1: FA variable, sex and age were included in the model. Step 2: step 1+physical activity, use of contraceptives, vitamin E intake*, smoking, body mass index*, alcohol intake, glucose*, insulin,* systolic BP and LDL cholesterol were included in the model. apoB=apolipoprotein B; FA=fatty acid; MUFA=monounsaturated FA; PUFA=polyunsaturated FA; SFA=saturated FA. This table has been presented in the original work IV.

5.3.5 Summary of the Findings Presented in This Thesis

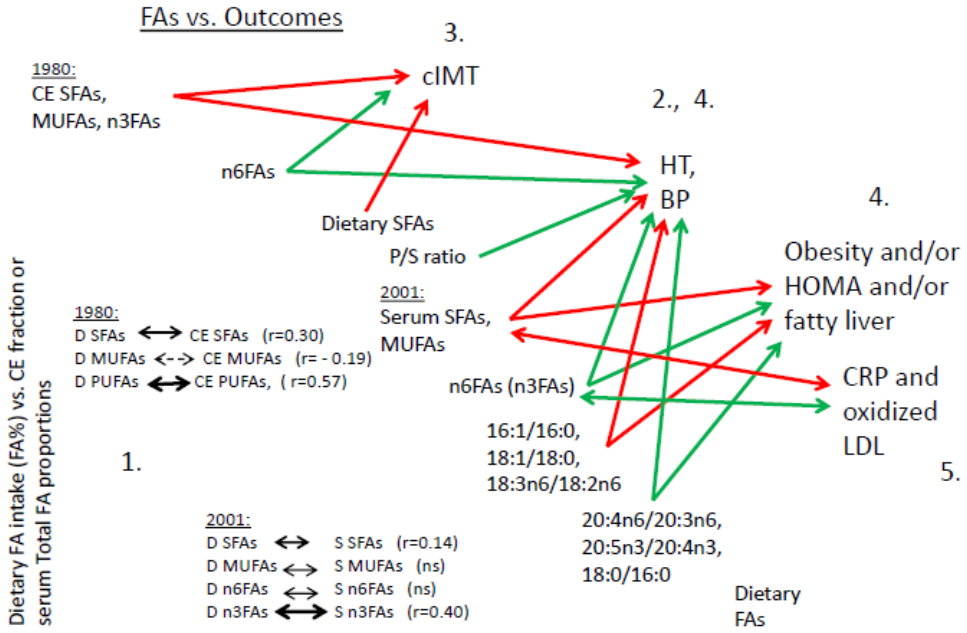


Figure 11. Summary of the main findings or trends emerging from this study. With regard to outcome associations, risk-increasing links have been presented in red and risk-lowering links in green color. With respect to the links between dietary and serum FA proportions, the weight of the arrows illustrates the strength of the association with that of a dashed arrow representing an inverse association. Bidirectional arrow=only cross-sectional data available. BP=blood pressure; CE=cholesteryl ester; cIMT=common carotid artery intima media thickness; CRP=C-reactive protein; D=dietary; HOMA=homeostatic model-based insulin resistance; HT=hypertension; ns=non-significant, $P \geq 0.05$; r=coefficient of variation, S=serum total.

6 Discussion

This thesis has examined whether childhood or adulthood dietary intake of FAs and serum FA proportions are associated with the prevalent or incident cardiometabolic outcomes in adulthood (Figure 6). Childhood-adulthood sub-studies had a follow-up of almost 30 years in which subjects who were children or young adolescents at baseline have been followed over the critical years in their development. Replacing the consumption of SFAs partly with PUFAs and MUFAs is one central pillar in the international dietary recommendations to prevent cardiometabolic outcomes (Mach et al. 2020). This thesis provides further evidence for the importance of adhering to dietary recommendations, in particular this concerns efforts to increase the dietary (and serum) n-6 PUFAs/SFAs ratio already in childhood.

6.1 Childhood CEFAs and Adulthood Cardiometabolic Outcomes (I, II)

Childhood serum CEFA proportions were used as indicators of dietary intake of FAs. CE SFAs, MUFAs and n-3 PUFAs, shown to derive from animal fat (Moilanen, Räsänen et al. 1985), were directly associated, and n-6 PUFAs, which originated from vegetable oils and margarine (Moilanen, Räsänen et al. 1985), inversely associated with the elevated BP or hypertension and cIMT in adulthood (see summary of the findings in **Figure 11**). Statistical models were adjusted for the common cardiometabolic lipid and non-lipid risk factors. Associations with BP were stronger in men, whereas cIMT associations were only seen in women. Men had higher BP values, but this confounder was taken into account in the statistical analyses since in a part of the models, the highest 20% percentile in BP indicated elevated BP, and it was formed separately for men and women. The childhood dietary intake data supported these findings. Overall, these findings are in line with the beneficial cardiometabolic effects found in connection with long-term FA intervention studies started only in childhood stimulating early childhood environment in general (Campbell et al. 2014), or leading to healthier dietary habits, i.e. the STRIP and DISC studies (Kwiterovich et al. 1997; Simell et al. 2009; Dorgan et al. 2011; Niinikoski et al. 2012; Oranta et al. 2013; Pahkala et al. 2020). The data from the YFS has also shown that an unhealthy childhood-lifestyle is associated with

sub-clinical atherosclerosis and its progression in adulthood (Juonala, Viikari, and Raitakari 2013). Cardiometabolic outcomes, such as obesity, T2D, dyslipidemia, non-alcoholic fatty liver or hypertension are increasingly prevalent also in the younger age-groups (Alisi, Carpino, and Nobili 2013; Poyrazoglu, Bas, and Darendeliler 2014). Imbalanced nutrition and nutrition behavior (Nicklas et al. 2001; Osei-Assibey et al. 2012) have been suggested to play an important role in their development. Overall, these findings emphasize the role of childhood nutrition as an important determinant for adulthood cardiometabolic health. These findings are also in line with the DASH study, which showed that a diet rich in fruits, vegetables, and low-fat dairy foods and with reduced amounts of saturated and total fat can substantially lower BP (Appel et al. 1997).

Since women were premenopausal, we expected that stronger associations between CEFAs and cIMT would have been observed in men (II). However, the CEFA link with cIMT, independent of the traditional risk factors of CVD, was found in women only. This finding is at odds with the findings related to CEFAs vs. BP (I). However, BP and cIMT may only partially have the same disease etiology. CIMT is an estimate of generalized atherosclerosis with arterial thickening (Bots et al. 1996) and later plaque formation. In addition to atherosclerosis, also arteriosclerosis in general with its increased collagen and decreased elastin content of the arterial media layers, has been linked with elevated BP (Braunwald et al. 1987; Cecelja and Chowienczyk 2009). We did not test the possible differences in arterial stiffness (pulse wavelength velocity) between the sexes, or associations of FAs with the estimates of stiffness. Finally, we do not have any firm explanations for the observed sex-based differences. In the YFS, the relationships between childhood risk factors and adulthood cardiometabolic outcomes have often been stronger in men than in women (Kaikkonen, Mikkilä et al. 2013).

6.2 Adulthood Serum Total Fatty Acids and Cardiometabolic Outcomes (III, IV)

We suggest that serum total FA proportions are strongly regulated by the body metabolism since their correlations with dietary intake were weak. There are also challenges in the assessment of dietary intake. The dietary intake of FAs did not show any consistent links with the cardiometabolic outcomes. One reason for this may be that since the 1980's, dietary habits have become more similar between individuals. Nowadays, pure animal fat users are rare.

The findings related to adulthood serum total FA proportions vs. adulthood outcomes were very similar with the above childhood findings. As groups, serum SFAs and MUFAs were directly and total n-6 PUFAs (+n-3 PUFAs borderline significantly in some models) inversely associated with all of the prevalent outcomes

(cross-sectional data, Table 14). These findings were consistent since also the summary variable reflecting FA composition, i.e., the (SFAs%+MUFAs%)/ PUFAs% ratio, was linearly associated with the number of cardiometabolic outcomes. This variable regarding serum FA status was formed based on our findings, i.e., SFAs and MUFAs having direct and PUFAs inverse associations with the outcomes. In addition, SFA and MUFAs are metabolically linked together via delta-9 desaturase activity, i.e. there is an active conversion of SFAs to MUFAs. For this reason, it is reasonable to combine these two FA classes and compare their sum with the PUFAs. Several serum FA proportions were also prospectively associated (significantly or borderline significantly) with the future outcomes, obesity and fatty liver in particular (Table 15), supporting the proposal that unfavorable FA composition might participate in the etiology of cardiometabolic outcomes. The cross-sectional data regarding serum total FA proportions and the markers of LDL oxidation or inflammation was very similar with the above outcome data (Table 16). For example, these outcome findings support recent meta-analyses and reviews which have concluded that the intakes of long-chain SFAs should be reduced and partly substituted with PUFAs to decrease body weight (Liu et al. 2018; Hammad and Jones 2017) or that an increased intake of MUFAs from animal sources (the main source of MUFAs in northern Europe) is associated with a weight gain (Liu et al. 2018). Overall, it was the FA metabolism rather than the dietary intake which seemed to play a role in the adulthood FA associations since the estimates of FA metabolism (and specific FA proportions related to this metabolism) showed strong associations with the outcomes, obesity in particular.

6.3 Fatty Acid Classes or Individual FAs versus the Cardiometabolic Outcomes

6.3.1 PUFAs and SFAs

In Finland, the North-Karelia project demonstrated that a change in dietary habits, resulting in higher PUFA/SFA intake and the serum HDL/LDL cholesterol ratios, was associated with lowered rates of cardiovascular morbidity and mortality (Puska 2009). Similarly, a meta-analysis (Mozaffarian, Micha, and Wallace 2010) consisting of eight intervention trials with 13 614 participants and 1 042 CHD events in their statistical models detected a 10% reduction in the CHD risk for each 5% energy of increased PUFA. In addition, Astrup and co-workers (Astrup et al. 2011) concluded that in populations who consume a Western diet, the replacement of 1% of energy from SFAs with PUFAs would lower LDL cholesterol and be likely to produce a reduction in CHD incidence by ≥2-3%. In support of this theory, in elderly

women, linoleic acid (18:2n-6) intake had an inverse association with prevalent cIMT (Mazza et al. 2018).

Our findings support these earlier observations since low SFA and high PUFA and linoleic acid (18:2n-6) proportions in particular were inversely associated with all of the investigated cardiometabolic outcomes (I-IV). They are also in line with the observations linking plasma PL and CE fraction and erythrocyte PL 18:2n-6 to a lowered risk of incident T2D, in some models even following adjustment for body size (Wang, L. et al. 2003; Hodge et al. 2007; Kroger et al. 2011). In addition, lowered levels of skeletal muscle PL PUFAs have been associated with decreased insulin sensitivity (Borkman et al. 1993). The present findings are also in line with the performed interventions, supporting the view that partial replacement of dietary SFAs with UFAs can improve insulin sensitivity (Vessby et al. 2001; Jula et al. 2002; Kaitosaari et al. 2006; Oranta et al. 2013). The age and sex-adjusted linoleic acid (18:2n-6) proportion was also inversely associated with the incident fatty liver. These data support our earlier observations with the metabolomics data conducted with nuclear magnetic resonance-based serum FA analyses in which the serum total SFA proportion was directly and the n-6 PUFA proportion inversely associated with the incident non-alcoholic fatty liver (Kaikkonen et al. 2017). Our findings are also consistent with some small-scaled RCTs. In a Swedish intervention with 67 abdominally obese participants, the n-6 PUFA intake reduced liver fat as compared to SFA intake (Bjermo et al. 2012). Similarly, in their RCT of overweight individuals, Rosqvist and coworkers showed if the SFA intake was as palm oil, this could markedly induce liver fat, whereas sunflower oil PUFA prevented its formation during excess energy intake and weight gain (Rosqvist et al. 2019). In a Finnish intervention with 38 middle-aged overweight subjects, extra SFA (1000 kcal/day) for 3 weeks increased lipolysis, whereas an equal amount of UFAs exerted an opposite effect. SFAs had the greatest effect on the increase of intrahepatic triglycerides, insulin resistance and harmful ceramides (Luukkonen et al. 2018).

A part of the n-6 PUFAs showed direct outcome associations. These n-6 PUFA findings are in line with a recent study (Yary et al. 2016) in which high γ-linolenic acid (18:3n-6) and dihomo-γ-linolenic acid (20:3n-6) proportions were associated with the incident T2D. Our direct outcome associations related to circulating 18:3n-6 and 20:3n-6 are also very similar to the results of a Swedish study examining obese participants (Warensjö, Ohrvall, and Vessby 2006). This thesis has confirmed these adverse trends of n-6 PUFAs to relate to prevalent and incident obesity, in particular.

6.3.2 MUFAs

Dietary intake of MUFAs had no associations with the cardiometabolic outcomes (III), whereas serum MUFA proportions in childhood and in adulthood were

systematically and directly associated with the outcomes (I-IV). Previous findings from large cohorts support this finding (Mahendran et al. 2013; Würtz et al. 2015; Kaikkonen et al. 2017). However, the mechanisms behind this association are not clear. The dietary intake of MUFAs was either inversely or not at all associated with the circulating MUFA proportions. Earlier, similar inverse associations were found in another Finnish study with middle-aged men and women (Nikkari et al. 1995).

In the body, MUFA concentrations are under continuous dynamic metabolism. In particular, Δ9-desaturase activity can convert SFAs into MUFAs, which may result in a MUFA concentration that does not reflect its dietary intake (Murakami et al. 2008). In the present data, the ratios of 16:1/16:0 and/or 18:1/18:0, estimates of Δ9-desaturase activity, were directly associated with the outcomes (I, III). Thus, this enzyme activity might at least partly explain the direct association between CE MUFA proportion and the outcomes. Earlier, increased estimates of Δ9- and Δ6-desaturase activity have been associated with elevated BP (Murakami et al. 2008).

The dietary source of MUFAs may also determine whether the MUFA intake or serum MUFA levels are associated either directly or inversely with the outcomes. In northern Europe and the US, the main dietary source of MUFAs has been animal fat (meat, dairy products, butter), whereas in the Mediterranean diet, the main source has been olive oil (Alonso, Ruiz-Gutierrez, and Martinez-Gonzalez 2006). Nowadays in Finland, vegetable oils (such as canola oil), spreads, cereal products, and vegetable and potato foods are also important MUFA sources (approximately 50% of the total intake) (Valsta et al. 2018). This might link MUFA intake partially with the PUFA intake. However, there was no evidence of this link in our outcome associations. MUFAs and PUFAs had consistently opposite associations with the outcomes.

In the Strong Heart study (n=2938), a high intake of MUFAs (and SFAs) has predicted increased CHD mortality in middle-aged and older (aged 47-59 years) subjects followed for a mean of 7.2 years (Xu et al. 2006). Schwingshackl and co-workers conducted a meta-analysis of 12 intervention trials where high intake of MUFAs (energy percentage from MUFA >12%) was compared to low intake (energy percentage from MUFA ≤ 12%) (Schwingshackl, Strasser, and Hoffmann 2011). Following adjustment for the common CV risk factors, a high intake of MUFAs was found to have beneficial effects on fat mass and on diastolic and systolic BP (Schwingshackl, Strasser, and Hoffmann 2011). Nonetheless, when MUFAs have been used to replace the dietary SFAs, the benefits of such a substitution have remained unclear (Jakobsen et al. 2009; Astrup et al. 2011).

6.3.3 N-3 PUFAs

One unexpected finding was that the childhood CE n-3 PUFA proportion was directly associated with the adulthood BP and cIMT (I, II), whereas in adulthood, serum total n-3 PUFAs tended to show inverse outcome associations (III, IV).

In childhood samples, serum n-3 PUFAs may simply reflect the animal fats present in the diet. It has been previously shown that the proportion of CE n-3 PUFAs correlates with fish intake, but also with butter, which is of animal origin (Moilanen, Räsänen et al. 1985). This may be due to the generally used method of frying fish in butter in Finland, or to the consumption of butter and rapeseed oil (rich in SFAs and partially hydrogenated n-3 PUFAs) for bread spreads and cooking in Finland (Puska 2009). Highly salted fish, rich in n-3 PUFAs, may also elevate BP. In adulthood, the role of canola oil as a source for n-3 PUFAs is probably more important, linking n-6 PUFA intake to n-3 PUFA intake. In addition, there is a competition between n-3 and n-6 PUFAs with respect to their metabolism, since they share the same enzymatic pathways. It has been observed that the accumulation of long-chain n-3 PUFAs in tissues is more effective when competing amounts of n-6 analogs do not greatly exceed the amounts of n-3s (Cleland et al. 1992). In the YFS subjects, the mean dietary n-6/n-3 ratio has decreased by approximately 30% during the past 30 years (the LA/ALA ratio as an estimate, Figure 7). This change may result in the phenomenon that the inverse n-3 PUFA links in childhood have changed to direct trends when they reached adulthood. The dietary LA/ALA ratio is lower in Finland, as compared with many other countries, such as the USA (Harika et al. 2013). Previously, it has been shown that the beneficial effects of n-3 PUFAs on BP are observed only when the intake of fish oil or n-3 PUFAs has been high (median 3.7g per day) (Geleijnse et al. 2002).

Overall, the literature is quite consistent about the beneficial role of n-3 PUFAs to promote CV health, particularly to lower CHD mortality and sudden cardiac deaths (Mozaffarian and Wu 2011). There is an increasing body of evidence that n-3 PUFAs could reduce cardiomyocyte excitability and, therefore, exert antiarrhythmic effects (Wu, Micha, and Mozaffarian 2019). The literature emphasize the role of n-3 PUFAs in secondary prevention of CHD (de Lorgeril et al. 1994; Innes and Calder 2020). Recent large-scale meta-analyses of RCTs support the view that n-3 PUFA intake could also lower BP in hypertensive individuals (Miller, Van Elswyk, and Alexander 2014; Colussi et al. 2017). It was not possible to investigate secondary prevention in our young cohort. However, also some doubts regarding its benefits have been presented (Risk and Prevention Study Collaborative Group et al. 2013). For example, a daily supply of 226 mg of eicosapentaenoic acid (20:5n-3) combined with 150 mg of docosahexaenoic acid (22:6n-3), 1.9 g of α-linolenic acid (18:3n-3), or both for 40 months did not significantly reduce the rate of major CV events in a trial mostly involving male patients who had had a myocardial infarction

and who were receiving drug therapy (Kromhout et al. 2010). The authors emphasized that the dose used in this trial was low.

6.3.4 Arachidonic Acid

The role of 20:4n-6 on health has been unclear. There is at least one previous study (Zheng et al. 1999) in which plasma levels of CE 20:4n-6 have been directly associated with BP. Arachidonic acid is a metabolite of the predominant linoleic acid (18:2n-6), and its high concentration can be a marker of a high consumption of 18:2n-6 containing vegetable oils such as sunflower oil. On the other hand, it is a precursor for isoprostanoids, prostaglandins and leukotrienes, which have been linked to lipid peroxidation, inflammatory reactions, arterial wall vasoconstriction, platelet aggregation and the elevated risk of CHD (Fontana et al. 2001; Davies and Roberts 2011). Our adulthood findings (IV), with two parallel *in vivo* LDL oxidation markers and C-reactive protein, confirm that PUFAs, and in particular n-6 PUFAs, are associated with reduced LDL oxidation and inflammation rather than their increased levels. It was found here that n-6 PUFAs or arachidonic acid had no adverse outcome links with the metabolic diseases (I-III). In the literature, there is evidence supporting the proposal that the metabolism of 20:4n-6 can lead to the formation of anti-inflammatory cytochrome P450 epoxygenase-derived eicosanoids (Node et al. 1999). In addition, earlier findings suggest that there is no association between circulating 20:4n-6 and its major metabolite, i.e., F2-isoprostanes. High plasma free PUFA/SFA and n-6/n-3 PUFA ratios have also associated inversely with the levels of $F_{2\alpha}$-isoprostanes (Kaikkonen, Vilppo et al. 2013).

6.3.5 Other Individual Fatty Acids and Indicators of Fatty Acid Metabolism

On the basis of earlier findings, we hypothesized that the effects of FAs on health may vary according to the carbon chain length (DeLany et al. 2000), desaturation within FA classes, and between an even or odd number of carbon atoms (Huang et al. 2019), therefore, the FAs should also be examined individually.

FA metabolism has a tendency to increase the chain-length of FAs by elongase activity and the degree of desaturation by desaturase activity (Guillou et al. 2010). In the present study, the following three FA ratios, 18:0/16:0 (reflecting elongase activity), the 20:4n-6/20:3n-6 and the 20:5n-3/20:4n-3 ratios (reflecting Δ5-desaturase activity) had inverse links, whereas also the three ratios, 16:1n-7/16:0, the 18:1n-9/18:0 (reflecting Δ9-desaturase activity) and the 18:3n-6/18:2n-6 ratios (reflecting Δ6-desaturase activity) had all direct links with the cardiometabolic outcomes, and obesity in particular (Table 14). Thus, this present study suggests that

it is apparently FA metabolism which is associated with the cardiometabolic outcomes. These findings related to indicators of FA metabolism are very similar with the Swedish study (Warensjö, Ohrvall, and Vessby 2006).

With respect to SFAs, palmitic acid (16:0) showed direct outcome associations in fully adjusted models (for example with incident fatty liver). Stearic acid (18:0) was a neutral FA showing non-significant associations. In comparison to other SFAs, it is known that stearate is a poor substrate for TG and CE synthesis, but is easily changed by the Δ9-desaturase activity to oleate (18:1) (Sampath and Ntambi 2005). In contrast to the risk-increasing myristic (14:0) and palmitic acids (16:0), pentadecanoic acid (15:0) with an odd number of carbon atoms was mainly inert (but displaying an inverse association with BP).

The increasing chain length of MUFAs was systematically linked to a decreasing risk of the cardiometabolic outcomes, obesity in particular. Elongase activity also had or tended to have inverse associations with the prevalent outcomes or with the incident fatty liver. Thus, this trend of MUFAs may be due to elongase activity. Palmitoleic acid (16:1n-7) simply showed the strongest cross-sectional obesity association. High Δ9-desaturase activity (high 16:1/16:0 ratio), i.e., effective conversion of 16:0 to 16:1 may partially explain this strong MUFA association. Oleic acid (18:1n-9) was also associated with the obesity. This is in line with the animal studies where high Δ9-desaturase activity has been linked to the development of obesity in animal models (Ntambi et al. 2002).

6.3.6 Dietary Intake and Serum Total Fatty Acid Proportions

We found only weak correlations between dietary FA intake and the corresponding serum total FA proportions **(**III**).** This can be due to several reasons; first, serum FA proportions are under continuous metabolism, such as lipogenesis, lipolysis, beta-oxidation and the related transfer of FAs between the liver and other tissues (Rui 2014). In particular, the FA metabolism with its carbon chain transformations (Guillou et al. 2010), will affect circulating levels of FAs. Serum total FA proportions are a sum of esterified TG, CE and PL fractions, including also a minor free fraction of FAs. Non-fasting free FAs and fasting/non-fasting TGs best reflect the recent fat intake (from hours to days), whereas CE and PL esters are representative of fat intake over some weeks or months (Moore et al. 1977; Vessby et al. 1980; Riboli, Ronnholm, and Saracci 1987; Zock et al. 1997; Takkunen et al. 2013). Secondly, it is challenging to estimate precisely the dietary intake. Typically, consumption of unhealthy food items or energy intake is under-estimated (Bingham 1991; Poppitt et al. 1998). In addition, the programs used to calculate the intake of FAs (such as national Fineli-database for food composition, fineli.fi) are using information which is based on average diets. In the present study, dietary recall

reflected the intake of the preceding 48 hours. A longer time is required to evaluate the FA composition of the individuals' diet in detail. Nowadays, 7-day recalls have been used as a reference method (Yuan et al. 2018).

6.3.7 Fatty Acids and Inflammation

Inflammation plays a role in the etiology of cardiometabolic outcomes (Haffner 2006). However, some genetic studies (Nordestgaard and Zacho 2009) have suggested that inflammation might be just an innocent bystander in a cascade leading to CVD. The findings of this thesis (IV, Figure 11), adjusted for the common CVD risk factors, suggest that SFAs and MUFAs rather than PUFAs are linked with inflammation (and cardiometabolic outcomes). The earlier literature also describes some mechanisms to account for the harmful effects of SFAs. In peripheral tissues, it has been speculated that inflammatory signaling in obesity can be activated by SFAs which bind to toll-like receptors that phosphorylate the inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) (Arkan et al. 2005; Cai et al. 2005; Lumeng, Bodzin, and Saltiel 2007), activating the inflammatory signaling cascade that then induces the phosphorylation of the serine residue in insulin receptor substrate 1 (IRS-1) (Aguirre et al. 2002; Gao et al. 2002; Carvalheira et al. 2005), which in turn, triggers the insulin resistance.

With regard to PUFAs, the common view has been that eicosanoids originating from n-6 PUFAs are more potent inflammatory factors than those derived from n-3 PUFAs (Schmitz and Ecker 2008). However, genome-wide interaction studies have shown that the genetic background modulates the associations between FAs and inflammatory markers (Veenstra et al. 2017). On the basis of *in vitro* experiments, specific epoxygenase-derived eicosanoids, even though those originated from n-6 PUFAs, have shown anti-inflammatory and vasodilatory effects (Node et al. 1999). Thus, this earlier finding, together with our observations, do seem to provide one potential mechanism behind the observations found in this thesis in which serum n-6 PUFAs rather than n-3 PUFAs were associated with a lowered prevalence and incidence of cardiometabolic outcomes (I, II, III).

6.3.8 Fatty Acids and LDL Oxidation *in vivo*

Oxidation of biomolecules may play an important role in the etiology of cardiometabolic outcomes. For example, it has been shown recently that oxidized LDL (both elevated LDL conjugated diene concentration and oxidized LDL apolipoprotein B as its markers), is associated with an elevated risk for fatty liver (Kaikkonen et al. 2016).

According to the oxidation theory of atherosclerosis, LDL oxidation, such as peroxidation of the LDL n-6 PUFAs, might be one potential mechanism leading to atherosclerosis (Steinberg et al. 1989; Abbey et al. 1993; Reaven et al. 1993; Davies and Roberts 2011). *In vitro* models have also shown that dietary cholesterol increases the susceptibility of LDL to oxidative modification (Schwab, U. S. et al. 2000). PUFAs have a susceptibility to become oxidized by either free radicals or by non-radical oxygen species, since these are the only FAs to contain at least two double-bonds interspersed by methylene groups and these are susceptible to oxidative attacks due to their unstable hydrogen-carbon bonds (Bochkov et al. 2010). In *in vitro* studies with human samples, linoleic acid (18:2n-6) supplementation has been associated with the elevated LDL oxidation susceptibility (Abbey et al. 1993; Reaven et al. 1993). Our *in vivo* findings at the population level do not support this hypothesis (IV). However, it is important to note that in those previous studies the oxidation susceptibility has been measured under highly oxidative conditions, not necessarily reflecting the situation *in vivo*. In contrast in the present study, we measured levels of non-induced LDL dienes and oxidized proteins, i.e. their baseline or *in vivo* levels.

Due to their chemical structure, SFAs cannot themselves become oxidized. However, the plasma free SFA concentration has been shown to directly associate with plasma lipid peroxidation *in vivo* (Kaikkonen, Vilppo et al. 2013). One important dietary source for SFAs is red meat, rich in heme iron which can catalyze oxidative reactions (White and Collinson 2013). For example, in a Fenton reaction, Fe^{2+} reacts with hydrogen peroxide, producing highly reactive hydroxyl radicals which can react with different biomolecules (Halliwell and Gutteridge 1989). According to some studies, red meat consumption may associate with increased LDL oxidation (Cocate et al. 2015).

6.4 Strengths

The main strength of the YFS is the longitudinal study design with its high participation rate and extensive data collection, which makes it possible to explore environmental factors, socioeconomic background, life style factors, anthropometrics, and morbidity in the same participants first as children and then when they grew up to be adults. This longitudinal study design has included regular follow-ups and a collection of a diverse set of carefully measured phenotypes, expression data and background information, including data about the genetic background. With regard to the vascular measurements, the study has the potential to provide important insights of the mechanisms of cardiometabolic outcomes and atherosclerosis, as it will be possible to test and integrate at the population level concepts that have emerged from experimental studies. The cohort provides a unique

opportunity to study the development of lifestyle and risk factors from childhood to adulthood, i.e., to examine the lifetime burden. It is possible to study the childhood determinants of adult health in a way that has not been previously possible. The cumulative data allow testing the hypothesis that common cardiometabolic outcomes and outcomes have their origin in early life. In the near future, the study also allows examinations over three generations to characterize epigenetic mechanisms linked to cardiometabolic outcomes.

The personnel of all the clinical centers have been trained similarly and with care for the data collections. All of the centers have had identical procedures and tools for the data and sample collection. Standardized analysis methods have been used over the years. Possible changes in the equipment and in the analysis methods have been handled with care. For several variables, such as cholesterol, there has been a need for level-corrected values.

6.5 Limitations

One shortcoming was that observational studies like the YFS cannot establish causality. Due to the relatively young YFS study population, it is not yet possible to estimate the associations between childhood risk factors with "hard" disease end points, such as CVD. In addition, the generalizability of the findings is limited to white European subjects, and the loss of some subjects is an inevitable problem in such very long-term studies. Furthermore, the original enrolment of the subjects occurred over a wide age range (3–18 years) resulting in a lack of early childhood data for a large number of the study subjects (Raitakari et al. 2008). In addition, baseline subjects were at different phases of development, i.e., either children at pre-puberty or adolescents/young adults. Due to the wide age-range, most of the participants with insulin resistance or hypertension were present in the oldest age groups. For this reason, we carried out statistical analyses with age and sex-specific categorized HOMA-IR and BP values (80% percentile as a cutoff point).

FAs were expressed as proportions (%) from the total amount since the circulating concentrations of FAs correlate strongly with each other and with lipoprotein levels. Even though this is a generally used approach to present the quality of the circulating FAs, it does not take into account their actual concentrations: a high proportion does not necessarily mean a high concentration if the total amount of FAs is low. It might be reasonable to adjust statistical models for the total fat intake since there is some evidence that a too high total fat intake may hide beneficial effects of the fat quality (Vessby et al. 2001; Rasmussen et al. 2006), or a too low total FA intake can have adverse cardiometabolic effects (Park, Ahn, and Lee 2016). This adjustment was not done in the present study. In addition, FAs were determined from CE fraction in childhood and from total serum in adulthood,

making it impossible to directly compare FA proportions between these two fractions. It was also not possible to adjust childhood CEFA associations for adulthood CEFAs. Thus, it is unclear whether childhood CEFA associations (with cIMT and BP) are independent from adulthood CEFA levels. However, it is known that dietary habits are tracking over the years, as shown with the dietary patterns in this same cohort (Mikkilä et al. 2005). In addition, serum levels of CEFAs track over six years at a minimum, as examined earlier in the YFS (Moilanen et al. 1992). Adulthood total FA proportions were also very constant over a time of 10 years as shown in this thesis (Figure 7).

Full dietary data was available for less than half of the study subjects. It is challenging for the study participants to remember exactly what they have eaten within the last 48 hours. Typically, the intake of unhealthy food items are under-estimated and healthy ones over-estimated. However, measurement of serum FA levels provides information which is free from this kind of data collection bias. Serum total FA levels are dependent on FA metabolism, however, CE fraction SFAs and PUFAs reflect quite well the corresponding dietary intake. For several reasons, the present childhood-adulthood findings cannot be easily compared with the results of other studies. We have measured FAs from the CE fraction and most of the population studies have used dietary intake data or other fractions. In addition, there has been much variability in food intake in different consumers in the 1980s in Finland, i.e. there were fruit and vegetable users, as well as those who continued to consume a traditional diet (Mikkilä et al. 2005). Nowadays, the changes in fruit and vegetable use are smaller. With respect to population studies, nutrients contain energy, an increased intake of one nutrient will lead to a decreased intake of another. Thus, it is difficult to investigate the effects of individual nutrients on health. Similarly, there are also problems with clinical randomized trials investigating changes in fat intake since there is no possibility to arrange a real placebo group. One shortcoming of the present study was that the quality or amount of carbohydrates, i.e., the source molecules for endogenous FAs, was not controlled in statistical analyses. Similarly, control over the intake of fiber may be crucial since dietary fiber can participate in the elevation of odd-chain FA levels (15:0 and 17:0) since gut-derived propionate is used for their hepatic synthesis in humans (Weitkunat et al. 2017).

With respect to endpoints, there was no possibility to characterize cross-sectional associations between FAs and fatty liver since the liver fat status was not assessed in 2001. We did not test the well-characterized associations between FA proportions and serum lipids, however, fatty liver is strongly linked to dyslipidemia, i.e., high TG and low HDL cholesterol concentrations in particular (Kaikkonen et al. 2017). Lipids were included in the statistical models.

With regard to methods, mainly standard clinical laboratory methods were used. However, it is possible that the immunological method quantifying oxidized LDL proteins (in apolipoprotein B) also recognizes other lipoprotein particles with a similar epitope structure (Ahotupa et al. 1998). With respect to statistical methods, it may be that some fully adjusted regression models were over-adjusted. This concerns work III in particular. However, age and sex-adjusted associations were also evaluated in that study. Furthermore, ultrasound is a generally used method for assessing fatty liver, but it has a limited performance, compared to magnetic resonance imaging when the steatosis is <30% on liver biopsy (Bohte et al. 2011).

6.6 Future Aspects

In the literature, varying health effects of FAs may be due to several reasons. Among the SFAs or UFAs, individual FAs seem to have even opposite health effects. In addition, there are differences in the total FA intake, follow-up times, duration of interventions and/or the replacement procedures between the studies. Furthermore, the interactions of dietary and circulating FAs with individual-specific genetic, nutritional and other environmental factors, including dietary source of FAs, or even effects spanning over generations, i.e. the epigenetic regulation of energy balance and body metabolism, may also affect the findings.

There is evidence that dietary FA composition can modulate metabolic outcomes by interacting with obesity-related genes (as shortly reviewed in Table 6) (Hammad and Jones 2017). Furthermore, at least palmitic acid (16:0) can post-translationally modify proteins and their functions in a process called palmitoylation in which palmitic acid is covalently linked to the proteins through a thioester bond (Fatima et al. 2019). In the future, it might be reasonable to examine the metabolic regulation caused by FAs in more detail in the YFS. Genome-wide association/interaction studies and expression data available are offering some tools for this purpose. For example, leptin and adiponectin levels seem to regulate together with FAs, the metabolic homeostasis (Stern, Rutkowski, and Scherer 2016). G protein –coupled receptor 120 (GPR120) is a receptor for long-chain free FAs, suggested to regulate several functions, such as gut hormone secretion, islet function, food preference, osteoclastogenesis, anti-inflammation, adipogenesis and appetite control (Mo et al. 2013). The combination of common genetic variations in the GPR120 gene and dietary fat intake is a possible determinant of body mass index and T2D (Mo et al. 2013; Waguri et al. 2013). There is also some evidence that dietary linoleic acid (18:2n-6) interacts with FA desaturase 1 genetic variability to modulate obesity and HDL-cholesterol-related traits (Dumont et al. 2018) or that dietary PUFAs and polymorphisms in peroxisome proliferator activated receptor gamma (PPARG) interact regulating serum lipid concentrations (Pihlajamäki et al. 2015). In the near

future in the YFS, the effects of genes on energy balance and body metabolism (such as that FAs) can be studied over three generations, focusing on epigenetic regulation.

Childhood FA – adulthood outcome associations should be investigated at the level of individual FAs and also by taking the source of FAs into account in order to study whether the association profile is similar with the adulthood findings (III). Principal component analysis (PCA) might help to categorize the individual FAs to cardiometabolic risk factors or protective factors over their desaturation degrees. The interactions between FA metabolism and dietary/non-dietary environmental exposures should also be investigated in more detail. For example, smoking may affect FA metabolism and related FA composition by increasing circulating MUFA levels (Hodson, Skeaff, and Fielding 2008). The cotinine data of the YFS offer tools to assess even the possible effects of passive smoking on FA levels. Overall, these kinds of interactions of FAs with environmental factors may partly explain the present MUFA findings. With regard to the regulation of the synthesis, it is known that an excess of dietary PUFAs suppresses elongase activity, i.e., Elovl5 and Elovl6 gene expression (Wang, Y. et al. 2005). In addition, for example, Δ6-desaturase activity is inhibited by fasting, linoleate, arachidonate, low protein levels, glucose, aging, thyroxine and glucocorticoids, and accelerated by insulin, the lack of essential dietary FAs and proteins (Brenner 1981). It might be interesting in the future to study to determine the factors associated with the circulating FA concentrations or proportions. Furthermore, it might be worthwhile analyzing FA levels from red blood cells (reflecting intake and metabolism of several months) or even from adipose tissue, which reflects intake and metabolism of 0.5 to 2 years (Katan et al. 1997; Hodson, Skeaff, and Fielding 2008). The determination of FA levels/proportions from several different fractions and body locations might help to better understand the link between FAs and cardiometabolic outcomes. However, a 12-week trial with fish and camelina sativa oil, rich in n-3 PUFAs, has affected similarly the n-3 PUFA composition in erythrocyte membranes as determined in plasma PLs, CEs and TGs (Manninen et al. 2019). Alterations in FA profiles may be one factor distinguishing metabolically healthy and unhealthy (with respect to the inflammatory state) obese subjects from each other (Perreault et al. 2014). Free FAs should also be one of the interests in any future interaction and disease-mechanism studies, since it is known that increased fasting levels of non-esterified FAs in the circulation are markers for metabolic outcomes, such as obesity and diabetes (Steffen et al. 2015). Fasting levels of non-esterified FAs reflect levels and composition of FAs in adipose tissues. Elevated serum free FA levels have been shown to promote inflammation and insulin resistance in the liver and skeletal muscle (Steffen et al. 2015).

N-6 (n-3) FAs originate from the diet only, in contrast, SFAs and MUFAs are dietary and metabolic in their origin and their levels are under regulation by the body.

Regarding health, the correct intake of PUFAs might thus be more important than that of SFAs or MUFAs. The relative importance of FAs should be investigated, for example, in suitable study settings and/or statistical analyses. Since body FAs are a combination of both dietary intake and metabolism, it might be interesting to try to form a sum variable from the intakes and serum fractions and to use this information to achieve better FA-based forecasting for different outcome models.

Sample handling and analysis methods have been developed since the 1980s. For this reason, in the future it might be interesting to exploit these novel rapid technologies to separate FAs from different lipid fractions and to analyze them by mass spectrometric or NMR-based methods. The analysis of circulating nitro-fatty acids (NO_2-FAs) might provide tools to clarify cell signaling mechanisms (Khoo and Schopfer 2019).

7 Conclusions

The present data suggest that

1) In childhood, dietary intake of FAs and serum CEFA proportions correlate quite well with each other (MUFAs had inverse links), whereas in adulthood, dietary intake and serum total FA proportions correlate only weakly. Statistically significant links were found for serum total SFAs, and n-3 PUFAs in particular). These findings support the view that serum CEFA proportions can be used as an indicator for FA intake (reflecting the FA intake of the last 2-3 weeks), but serum total FA proportions reflect mainly FA metabolism.

2&3) Childhood foods containing animal fats (SFAs, MUFAs and n-3 PUFAs) were associated with the increased cardiometabolic risk, and foods containing vegetable oils (n-6 PUFAs) with the reduced risk in adulthood. This was characterized by the links between serum CEFA proportions with the BP and cIMT.

4) In adulthood, serum total FA proportions, i.e., high SFAs or MUFAs and low n-6 PUFAs, or n-3 PUFAs borderline significantly in some models, reflecting mainly FA metabolism, were linked with several prevalent and/or incident cardiometabolic outcomes, such as obesity, insulin resistance, high BP and/or non-alcoholic fatty liver. Dietary sources are very similar for plant-based α-linolenic and linoleic acids, whereas the long-chain n-3 PUFAs are origin from animal sources. The links of individual outcomes were independent from the common cardiometabolic risk factors. A summary variable of the serum FA composition, i.e., the (SFAs%+MUFAs%)/PUFAs% ratio was also linearly associated with the severity of cardiometabolic state (number of outcomes). Some individual FAs in adulthood, such as stearic acid (18:0) as an inert FA, or γ-linolenic (18:3n-6), dihomo-γ-linolenic (20:3n-6) and eicosatetraenoic acid (20:4n-3) showed associations which were in conflict with the links of their own group of desaturation degree. This reflects the importance of utilizing specific FAs as markers for cardiometabolic outcomes. Total n-6 PUFAs

or arachidonic acid (20:4n-6) did not show any harmful outcome associations. FA ratios reflecting Δ9- and Δ6-desaturase activity were directly and FA ratios reflecting Δ5-desaturase and elongase activity inversely associated with cardiometabolic outcomes, prevalent and incident obesity in particular. This reflects the importance of considering FA metabolism when examining the associations between serum total FAs and cardiometabolic outcomes.

5) Serum n-6 PUFAs (and n-3 PUFAs partially) had an inverse, whereas SFAs and MUFAs exhibited direct associations with oxidative reactions and inflammation. These findings are in line with the observed beneficial effects of n-6 PUFAs and might also provide one mechanism for the health effects of PUFAs towards cardiometabolic outcomes.

In Finland, the mean intake of SFAs has increased in the 2010s, and it is higher than currently recommended. This thesis provides further evidence for the importance of adhering to dietary recommendations and in particular, this concerns efforts to increase the dietary (and serum) PUFA/SFA ratio. As a novel finding, even childhood PUFA intake rich in n-6 PUFAs seems to lower the cardiometabolic risk 30 years later in adulthood. The role of MUFAs and n-3 PUFAs remains unclear. This may be due to confounding by their varying dietary sources or matrixes (mainly animals in origin in Finland), FA metabolism (for example, linking MUFAs to SFAs), interactions between FAs and the environment, and/or the genetic background. With regard to the links between FAs and cardiometabolic outcomes, there is a need to examine the role of these confounding factors in more detail in the near future.

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