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TAISA VENÄLÄINEN

PLASMA FATTY ACID COMPOSITION, DIETARY COMPONENTS AND CARDIOMETABOLIC RISK FACTORS IN CHILDREN

Plasma Fatty Acid Composition, Dietary Components And Cardiometabolic Risk Factors In Children

- Cross-Sectional Associations And Effect of a Lifestyle Intervention

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Author's address: Physiology/Institute of Biomedicine/School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Supervisors: Professor Ursula Schwab, Ph.D.
Clinical Nutrition/Institute of Public Health and Clinical Nutrtion/School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Docent Jyrki Ågren, Ph.D.
Physiology/Institute of Biomedicine/School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Docent Vanessa de Mello Laaksonen, Ph.D.
Clinical Nutrition/Institute of Public Health and Clinical Nutrtion/School of Medicine
University of Eastern Finland
KUOPIO
FINLAND

Reviewers: Professor Bryndís Eva Birgisdóttir, Ph.D.
Faculty of Food Science and Nutrition
University of Iceland
REYKJAVIK
ICELAND

Docent Riitta Freese, Ph.D
Department of Food and Environmental Sciences
University of Helsinki
HELSINKI
FINLAND

Opponent: Docent Kirsi Laitinen, Ph.D.
Institute of Biomedicine
University of Turku
TURKU
FINLAND

Venäläinen, Taisa
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ABSTRACT

Evidence on association of plasma fatty acid composition with cardiometabolic risk and the effects of lifestyle interventions on plasma fatty acid composition in children is limited. Plasma fatty acid composition is known to be an indicator of dietary fat quality, but the associations of other dietary factors with plasma fatty acids remain unknown in children. The principal objective of this thesis was to investigate the effects of a 2-year dietary and physical activity intervention on plasma fatty acid composition in a population based sample of Finnish children participating in the Physical Activity and Nutrition in Children (PANIC) study. The aims of this doctoral thesis were also to investigate the associations of food consumption with plasma fatty acid composition, including estimated desaturase and elongase activities, as well as their relationships with cardiometabolic risk factors.

We conducted a 2-year controlled dietary and physical activity intervention based on Finnish nutrition and physical activity recommendations in a population sample of 506 children aged 6-8 years. Food consumption was assessed by food records and plasma fatty acid composition by gas chromatography. Desaturase and elongase activities were estimated as product-to-precursor fatty acid ratios. Cardiometabolic risk was assessed using a continuous cardiometabolic risk score variable.

A higher consumption of vegetable oil-based margarines (fat 60-80%) was related to a lower proportion of saturated and monounsaturated fatty acids and higher proportions of polyunsaturated fatty acids in plasma cholesteryl esters, phospholipids and triacylglycerols. A higher consumption of high-fiber grain products and a lower consumption of candy associated with lower proportions of monounsaturated fatty acids in plasma. The proportions of several saturated fatty acids and that of palmitoleic acid were directly associated with cardiometabolic risk score whereas the proportions of many polyunsaturated fatty acids were inversely associated with it. The proportions of polyunsaturated fatty acids tended to increase in the intervention group but decreased in the control group due to the lifestyle intervention.

To conclude, it is possible to affect plasma fatty acid composition by a 2-year individualized and family-based lifestyle intervention aiming at enhancing overall diet quality, increasing physical activity and decreasing sedentary behavior. Of note, plasma fatty composition is not only a biomarker for dietary fat quality but also reflects the consumption of high-fiber grain products and foods high in sugar, such as candy. These findings also reinforce the evidence that fatty acid metabolism is closely associated with cardiometabolic risk, already in childhood.

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TIIVISTELMÄ

Lasten veren rasvahappojen yhteys aineenvaihdunta- ja verenkiertoelimistön sairauksien vaaraan sekä elintapaohjauksen vaikutus veren rasvahappoihin on epäselvä. Verestä erotetun plasman rasvahappokoostumus heijastelee ruokavalion rasvan laatua, mutta yhteydet muihin ruoka-aineisiin kaipaavat vielä lisäselvitystä. Tämän väitöskirjatyön päätavoitteena oli selvittää 2-vuotisen elintapaohjauksen vaikutusta plasman rasvahappokoostumukseen suomalaislasten väestöotoksessa osana Lasten liikunta ja ravitsemus -tutkimusta. Lisäksi tavoitteena oli selvittää lasten ruokavalion yhteyksiä plasman rasvahappoihin ruoka-ainetasolla sekä tutkia rasvahappojen yhteyttä aineenvaihdunta- ja verenkiertoelimistön sairauksien vaaraan lapsilla.

Perhekeskeinen, 2-vuotinen liikunta- ja ravitsemusinterventio toteutettiin 506 8-6-vuotiaan lapsen otoksessa Suomalaisten ravitsemussuositusten ja Varhaiskasvatuksen liikuntasuosituksen mukaisesti. Ruoankäyttö määritettiin neljän päivän ruokapäiväkirjalla. Veren rasvahapot mitattiin kaasukromatografialla. Desaturaasi- ja elongaasiaktiivisuudet laskettiin tuote/lähtöaine-rasvahapposuhteella. Aineenvaihdunta- ja verenkiertoelimistön sairauksien vaaraa arvioitiin summamuuttujalla, johon pisteytettiin eri vaaratekijät.

Runsaampi runsasrasvaisten kasvirasvalevitteiden käyttö oli yhteydessä matalampaan tyydyttyneiden ja kertatyydyttymättömien sekä suurempaan monityydyttymättömien rasvahappojen osuuteen plasman kolesteryyliestereissä, fosfolipideissä ja triasyyloglyseroleissa. Runsaampi täysjyväviljan ja vähäisempi makeisten käyttö oli yhteydessä matalampaan kertatyydyttymättömien rasvahappojen osuuteen plasmassa. Useiden tyydyttyneiden rasvahappojen sekä palmitoleiinihapon osuuksien ja aineenvaihdunta- ja verenkiertoelimistön sairauksien vaaran välillä oli suora yhteys. Elintapainterventio vaikutti monityydyttymättömien rasvahappojen osuuden suurenemiseen interventioryhmässä, kun taas kontrolliryhmässä monityydyttymättömien rasvahappojen osuus plasmassa pieneni kahden vuoden aikana.

Tämä väitöskirjatyön johtopäätöksenä voidaan todeta, että lasten plasman rasvahappokoostumukseen on mahdollista vaikuttaa elintapainterventiolla, jonka tavoitteena on lasten ruokavalion laadun parantaminen, liikunnan lisääminen ja fyysisesti passiivisen elämäntavan vähentäminen. Huomion arvoista on myös se, että plasman rasvahappokoostumus ei heijastele ainoastaan ruokavalion rasvan laatua vaan myös hiilihydraattien laatua. Väitöskirjatyön tulokset vahvistavat myös aiempia tuloksia siitä, että rasvahappoaineenvaihdunta on tiiviisti yhteydessä aineenvaihdunta- ja verenkiertoelimistön sairauksien vaaratekijöiden kasaantumiseen jo lapsilla.

Luokitus: QT 235, QT 256, QU 85, QU 90, QU 93, WK 820, WS 130

Yleinen suomalainen asiasanasto: rasvahapot; triglyseridit; lipidit; entsyymit; aineenvaihduntahäiriöt; metabolinen oireyhtymä; riskitekijät; ruokavaliot; ruoka-aineet; ravitsemus; elintavat; fyysinen aktiivisuus; liikunta; interventio; lapset; Suomi

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Kuopio, February 2017

Taisa Venäläinen

List of the original publications

This dissertation is based on the following original publications:

I Venäläinen T, Schwab U, Ågren J, de Mello V, Lindi V, Eloranta AM, Kiiskinen S, Laaksonen D, Lakka T. Cross-sectional associations of food consumption with plasma fatty acid composition and estimated desaturase activities in Finnish children. *Lipids 49:467-79, 2014.*

II Venäläinen T, Ågren J, Schwab U, de Mello V, Lindi V, Eloranta AE, Laaksonen D, Lakka T. Cross-sectional associations of plasma fatty acid composition and estimated desaturase and elongase activities with cardiometabolic risk in Finnish children – The PANIC Study. *Journal of Clinical Lipidology 10(1):82-91, 2016.*

III Venäläinen T, Viitasalo A, Schwab U, Eloranta AM, Haapala E, Jalkanen H, de Mello V, Laaksonen D, Lindi V, Ågren J and Lakka T. The effect of a 2-y dietary and physical activity intervention on plasma fatty acid composition and estimated desaturase and elongase activities in children: the PANIC Study. *American Journal of Clinical Nutrition 104(4):964-972, 2016.*

Contents

Abbreviations

BMI-SDS	BMI-standard deviation score
CE	Cholesteryl ester
D5D	Δ5 desaturase
D6D	Δ6 desaturase
DPA	Docosapentaenoic acid (22:5n-3)
DHA	Docosahexaenoic acid (22:6n-3)
EPA	Eicosapentaenoic acid (20:5n-3)
HDL	High density lipoprotein
LDL	Low density lipoprotein
MUFA	Monounsaturated fatty acid
PANIC	Physical activity and nutrition in children
PL	Phospholipid
PUFA	Polyunsaturated fatty acid
SCD	Stearoyl-CoA desaturase
SFA	Saturated fatty acid
TG	Triacylglycerol
VLDL	Very low density lipoprotein

1 Introduction

Metabolic syndrome is a prevalent condition among adults but rising alarmingly also among children (1–3). It is a cluster of several metabolic disorders such as abdominal adiposity, insulin resistance, dyslipidemia and high blood pressure that can be affected by both genetic and environmental factors, such as diet and physical activity. Dietary factors are of utmost importance among the environmental factors. In particular, the quality of dietary fat has an essential role in human health and in the pathogenesis of metabolic syndrome (4).

Plasma fatty acid composition is known to reflect the quality of dietary fat in children and adults. Especially dietary intakes of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA) are mirrored to the plasma composition of those fatty acids (5,6). Plasma monounsaturated fatty acids (MUFA) are more likely to reflect the intake of SFA than MUFA (7). However, most of the interventions that have investigated the effect of diet on plasma fatty acid composition have modified the quality of fat in the subject's diet rather than other components of diet. Of note, other lifestyle factors, such as physical activity, have not been considered modifiers in the intervention studies regarding plasma fatty acids in children.

Plasma fatty acid composition is usually analyzed by measuring several components of the circulating system, such as cholesteryl esters (CE), phospholipids (PL), triacylglycerols (TG), total plasma lipids and free fatty acids. This doctoral thesis concentrates in three fractions, CE, PL and TG, since it has previously been established that the plasma fatty acid composition of CE and PL reflect the dietary intake of preceding weeks and months and TG reflects the dietary intake of last few meals (8,9). Concurrently, it has been reported that plasma fatty acid composition can be altered by changing the quality of dietary fat and therefore the dietary fat intake of preceding weeks or months can be assessed quite reliably from plasma CE and PL. Fasting plasma TG, however, are suggested to describe the endogenous metabolism of fatty acids rather than the fat quality of last meal. Therefore, the plasma fatty acid composition of TG seems to be excellent choice when investigating the associations of plasma fatty acids with cardiometabolic risk factors in children. This doctoral thesis investigated this topic in children.

The aims of this doctoral thesis were to investigate the effect of a 2-year dietary and physical activity intervention on plasma fatty acid composition in school- aged children and to investigate the associations of food consumption with plasma fatty acid composition as well as the associations of plasma fatty acid composition with the cardiometabolic risk factors in childhood.

2 Review of the Literature

2.1 FATTY ACIDS

2.1.1 Structure and nomenclature

The chemical structure of fatty acids in plasma and tissue lipids consist of carbon, hydrogen and oxygen (10). These components form a carbon chain with carboxyl and methyl tail. Carbon chains with single or double bonds vary in length. SFA have only single bonds whereas MUFA have one double bond and PUFA two or more double bonds in the carbon chain. Unsaturated fatty acids are categorized into n-series (omega series). By the location of the first double bond from the methyl end, unsaturated fatty acids belong either in *n-9* (omega 9), *n-7* (omega 7), *n-6* (omega 6) or *n-3* (omega 3) series.

Fatty acids are basic elements of some lipids, such as plasma CE, PL and TG that are of the essence for this thesis. In general, lipids are complex molecular structures that consist of wide series of molecular species with different chemical structures and functions (11) and they can be found in several tissues in human body, such as blood adipose tissue. Lipids can be classified in many ways according to their chemical structure or behavior (12). One simple way is to classify plasma lipids into CE, PL, TG and non-esterified fatty acids (free fatty acids) that are carried by in lipoproteins. Very low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) consist of different relative amounts of CE, PL and TG (see Chapter 2.2). CE are formed from fatty acid and cholesterol by an ester bond between the carboxylate group of a fatty acid and the hydroxyl group of cholesterol (Figure 1). Most PL consist of a hydrophilic phosphate head and two hydrophobic fatty acid tails that are linked together with a glycerol molecule (Figure 2). TG contains and three fatty acids that are esterified to a glycerol molecule (Figure 3). TG is the main form of fat in the diet.

Fatty acids are named by the number of carbon atoms and the number and position of double bonds. Fatty acids are also systematically named by the standards of International Union of Pure and Applied Chemistry (IUPAC) (10). For example, a fatty acid with 16 carbon atoms in its chain and one double bond in *cis* configuration between carbon 9 and carbon 10 from the carboxy end of the molecule is named as 9-*cis*-hexadecenoic acid, (9Z)-hexadec-9-enoic acid or *cis*-Δ^9 hexadecenoic acid. However, it has also a trivial name palmitoleic acid and a shorthand notation 16:1n-7 where n-7 indicates the position of double bond counted from the methyl end of carbon chain. Trivial names are commonly used and they are based on the natural source or some other feature of the fatty acid. However, some fatty acids are best known by their systematic name or abbreviation, such as eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The shorthand notation of EPA is 20:5n-3 so it has 20 carbon atoms in the chain with five double bonds starting from the third carbon from the methyl end. DPA is 22:5n-3 and thus has otherwise the same structure than EPA but has two more carbons in the chain. DHA has same amount of carbon atoms in the chain than DPA but has six double bonds starting from the third carbon from methyl end and thus the shorthand notation is 22:6n-3.

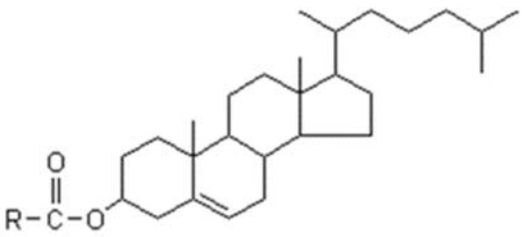

Figure 1. The structure of a cholesteryl ester (R = fatty acid hydrocarbon tail).

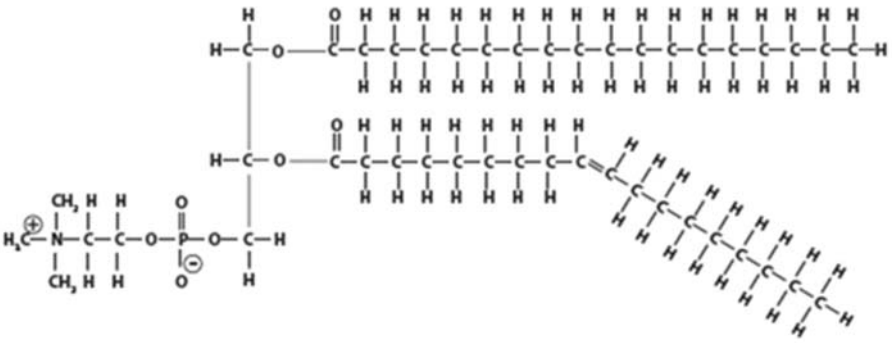

Figure 2. The structure of a phospholipid (1-stearoyl-2-oleoyl-sn-glysero-3-phosphatidylcholine).

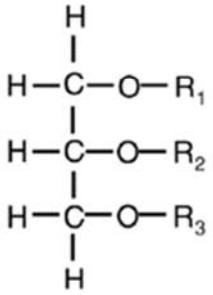

Figure 3. The structure of a triacylglycerol (R1, R2, R3 = fatty acid).

2.1.2 Fatty acids in the body

Fatty acids can exist either as free fatty acids in the body or they can combine with other molecules and constitute lipids eg. CE, PL and TG. Together with proteins, lipids form important components of a cell membrane, mitochondria and parts of cytoplasm. Fat is an excellent source of energy, but fatty acids also affects the properties of a cell membrane and cell energy expenditure and are involved in the gene expression and metabolic signaling in the human body (11). Therefore the fatty acid composition of membrane lipids has an effect many physiological and pathophysiological processes in the body (13).

Several fatty acids have the capability to regulate the expression and the activity of factors that are included in transcription. Therefore, those fatty acids have a role in regulating gene expression and protein production in cells. Certain PUFA also serve as precursors to eicosanoids, which are a wide group of bioactive molecules produced by enzymes like cyclooxygenases and lipoxygenases (14). Eicosanoids, such as prostaglandins, prostacyclins, thromboxanes, leukotrienes and epoxyeicosatrienoic acids have roles in inflammation, regulation of blood pressure, blood clotting, modification of immune system, regulation of reproductive processes and tissue growth and regulation of the sleep and wake cycle (15).

2.1.2 Metabolism of fatty acids

Besides using fat as an energy source, a human body also synthesizes, desaturates and lengthens fatty acids endogenously (Nelson, Chow). SFA are synthesized from acetyl-CoA in *de novo* lipogenesis in the cell cytosol (16). This synthesis of fatty acids has been found to be low if the dietary intake of fat is moderate or high, whereas high dietary intake of carbohydrates stimulates *de novo* lipogenesis (17).

Desaturation and elongation are steps of a metabolic pathway in which dietary and endogenous SFAs are lengthened and converted to MUFA and higly PUFA are synthetized from dietary *n-3* fatty acids (e.g. α-linolenic acid) and *n-6* fatty acids (e.g. linoleic acid) in the liver and adipose tissue (Figure 4.) (18). Desaturases and elongases are enzymes that activate this metabolic pathway. Desaturases add a double bond to the fatty acid and elongases lengthen the fatty acid by adding two carbon molecules to the carbon chain. Beta-oxidation is a process where two carbon atoms are removed from the chain. This step is needed for the metabolic pathways of *n-3* and *n-6* fatty acids.

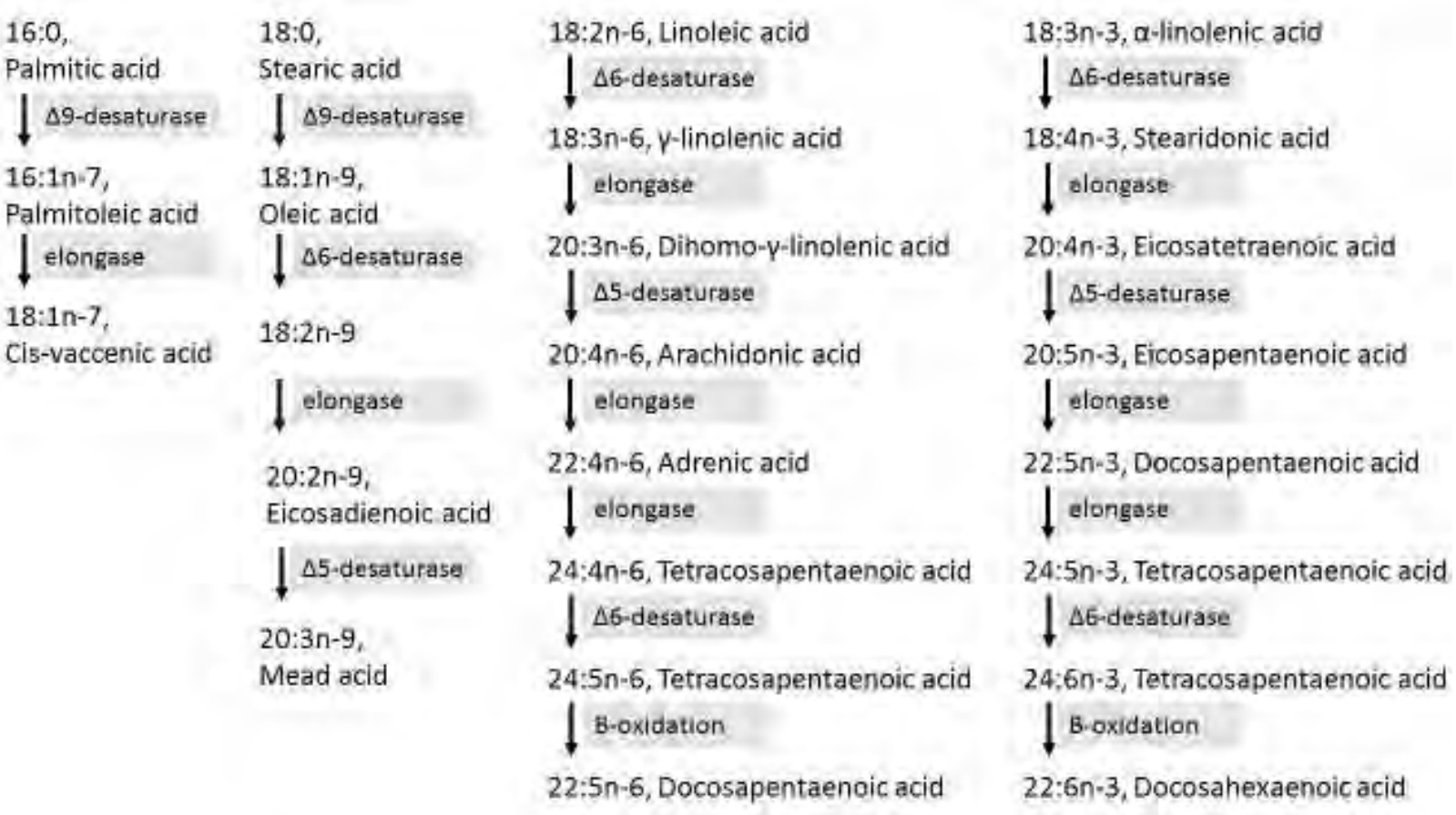

Figure 4. An outline of the main desaturation and elongation steps in metabolism of fatty acids in the human body. The endogenous pathway on the left starts from palmitic acid that is the end product of *de novo* syhthesis. On the right the metabolism pathways of *n-6* and *n-3* fatty acids.

Desaturases and elongases have roles in the endogenous metabolism of *n-7*, *n-9*, *n- 6* and *n-3* fatty acid families. Stearoyl-CoA desaturase (SCD), Δ9 desaturase, is an enzyme that converts of SFA (eg. palmitic acid and stearic acid) to MUFA (eg. palmitoleic acid and oleic acid) in the metabolic pathway of *n-7* and *n-9* fatty acids.

Δ6 desaturase (D6D) has an important role in the first step of the conversion of linoleic acid and α-linolenic acid to longer-chain more unsaturated fatty acids (eg. arachidonic acid and DHA) (Figure 4). Moreover, D6D is needed for the production of 24:5n-6 and 24:6n-3.

Δ5 desaturase (D5D) is needed for one step in the metabolism of *n-6* and *n-3* PUFA as it converts dihomo-γ-linolenic acid (20:3n-6) to arachidonic acid (20:4n-6) (Figure 4) and docosatetraenoic acid (20:4n-3) to EPA (20:5n-3) (Figure 4). Also MUFA can be converted by Δ6 and Δ5 desaturase (e.g. oleic acid to 22:3n-9) but in humans this happens significantly only in the deficiency of linoleic and α-linolenic acids.

Linoleic and α-linolenic acid are the starting points of the metabolic pathways of *n-6* and *n-3* fatty acids (Figure 4). Only plants have the ability to synthesize these essential fatty acids by the help of Δ12 and Δ15 desaturases (19). Humans can not convert oleic acid into linoleic acid neither linoleic acid into α-linolenic acid because of the lack of Δ12 and Δ15 desaturases. This is why linoleic and α-linolenic acids are considered as essential fatty acids and are needed from the diet.

2.2 PLASMA FATTY ACID COMPOSITION

Fatty acids can be assayed from various human tissues, including cells of the immune system, buccal cells, adipose tissue, erythrocytes and blood (20–24). However the most reported in the literature are fatty acid compositions of blood plasma or serum, erythrocytes and adipose tissue. In addition to whole plasma, the fatty acid composition is usually described in CE, PL and TG, which are three major plasma lipid fractions and have their own unique composition (see Chapters 2.2.1, 2.2.2 and 2.2.3). Plasma also contains albumin-bound non-esterified fatty acid fraction but its composition has been less frequently analyzed due to its small amount and analytical difficulties. The fatty acid composition of these fractions is usually expressed as a percentage (mol% or mass%) of total amount of fatty acids (25). In this thesis, all the proportions of fatty acids are expresses as mol%.

In fasting state most of plasma lipoproteins carrying CE, PL and TG originate from the liver and smaller part from the intestine. The relative amounts of CE, PL and TG differ greatly between individual lipoprotein particles. VLDL are rich in TG and deliver fatty acids to tissues. After removal of most TG, VLDL particles finally form LDL particles with high cholesterol content. The proportion of TG is low in HDL particles, which contain mostly cholesterol and PL. All lipoproteins contain both cholesterol and CE but only part of them originate from VLDL and HDL secreted by liver. Lecithin-cholesterol acyltransferase, which is present in HDL, catalyzes the esterification of free cholesterol. This enzyme produces most of the plasma CE esterification and is necessary for the reverse transport of cholesterol from other tissues to liver (26).

The plasma PL fraction consists of several PL species. Phosphatidylcholine is the most abundant (about 70-80 %), sphingomyelin form the second largest fraction (about 15-20%) and the rest consists mostly of phosphatidylethanolamine, phosphatidylinositol and lysophosphatidylcholine (27). The structure of sphingomyelin differs from other major plasma and membrane PL because it has one fatty acid attached to sphingosine backbone. The fatty acid composition of sphingomyelin is also specific as it contains very long chain SFA and MUFA like lignoceric acid (24:0) and nervonic acid (24:1n-9)

2.2.1 Cholesteryl esters

Previous studies have established a specific fatty acid composition of plasma CE for different age groups of children and adolescents (23,28–30). It has been reported that most abundant SFA in CE is palmitic acid with a proportion of 10-12% among 6-8-years old boys and girls. The proportion of major MUFA, oleic acid, is approximately 20% in CE and that of linoleic acid is around 50%. Thus, linoleic acid is the most abundant fatty acid in plasma CE among children aged 6-8 years according to previous studies.

In adults, similar fatty acid composition of CE has been established (7,31,8). Linoleic acid covers around 50% of CE, followed by oleic acid that have the proportion of 20% of CE. The proportion of palmitic acid in CE is around 13% among adults. The proportion of arachidonic acid is around 5-6% of CE both in children and in adults (29).

2.2.2 Phospholipids

There are few studies among children that demonstrate that palmitic acid and linoleic acid cover 25-28% and 20-23% of plasma PL (23,29). The proportion of stearic acid in PL is 14-15% and that of oleic acid 13-14%. The second abundant PUFA in PL is arachidonic acid with the proportion of 9%. Other PUFA such as α-linolenic acid, dihomo-γ-linolenic acid, EPA and DHA covers 0.6%, 3%, 1.2% and 4%, respectively, out of the PL.

In adults the most abundant fatty acid in PL is palmitic acid that have the proportion of 24-29% accordin to the previous studies (29,32,33). In the same studies the proportion of linoleic acid in plasma PL varies between 18-23%. PL are also abundant of stearic acid, 13-18%, oleic acid, 10-15%, and arachidonic acid, 9-11%.

2.2.3 Triacylglycerols

The fatty acid composition of plasma TG among 5-10-year old children is abundant with oleic acid with the proportion of 40% (23,29). Palmitic acid and linoleic acid have been found in proportions of 25-28% and 14%. The proportion of arachidonic acid is 1.1-1.3% of TG. The PUFA such as α-linolenic acid, dihomo-γ-linolenic acid, γ-linolenic acid, EPA and DHA have all been found to have proportions under 1% of TG.

In adults the previous studies have shown that the most abundant fatty acid in plasma TG is oleic acid with a proportion in the range of 37-42% (8,34–37). TG are also abundant with palmitic acid with the proportion that varies between 27 and 30%. In the same studies the proportion of linoleic acid had quite wide range of 11-18.5%.

2.2.4 Calculation of estimated desaturase and elongase activities

To study endogenous desaturase and elongase activities a liver biopsy is needed. It is therefore unethical to study actual desaturase and elongase activities in large population samples of humans (38). Therefore, the ratios of the proportions of individual fatty acids in plasma, indicating desaturation and elongation steps that produce longer and more unsaturated fatty acids (13), have been widely used as surrogate measures of actual desaturase and elongase activities (25,39). These ratios have been shown to be good estimates on the actual activity of the desaturases and elongases (40–42).

SCD activates the desaturation of palmitic acid into palmitoleic acid or the desaturation of stearic acid into oleic acid. Therefore, estimation of SCD is calculated from the following ratios: 16:1n-7/16:0 or 18:1n-9/18:0.

D6D converts linoleic acid to γ-linolenic acid, which is subsequently elongated to dihomo-γ-linolenic acid. Thus the ratios for calculation of estimated desaturase activities are 18:3n-6/18:2n-6 and 20:3n-6/18:2n-6. The latter is often used to calculate the estimated D6D activity in PL because of more reliable quantification of dihomo-γ-linolenic acid than γ-linolenic acid in this fraction (43).

D5D has a role in the conversion of dihomo-γ-linolenic acid to arachidonic acid. Therefore, the ratio for estimation of D5D is 20:4n-6/20:3n-6.

The estimation of elongase activity is calculated by dividing *cis*-vaccenic acid by palmitoleic acid since the precursor fatty acid, 16:1n-7, is elongated to the product fatty acid, 18:1n-7. By calculating this estimate from the ratio of *cis*-vaccenic acid to palmitoleic acid, it may represent the activity of elongase 5 or 6 or both (42).

2.3 FACTORS AFFECTING PLASMA FATTY ACID COMPOSITION

Plasma fatty acid composition has been found to be influenced by several factors including diet, age, gender, physical activity, obesity, genetic factors and endogenous metabolism of fatty acids (13,30,37,44–47). The factors of essence for this doctoral thesis are reviewed below.

2.3.1 Diet

Plasma fatty acid composition is a reliable indicator of the quality of dietary fat in adults (8,45,48,49,5) and children (30). The fatty acid composition of plasma CE and PL reflects the dietary fat quality of the last weeks or months (8,50–52), whereas the fatty acid composition of plasma TG represents the dietary intake from preceding days (9).

Previous cross-sectional studies have reported positive correlations between dietary fatty acid intakes and plasma fatty acids. Plasma total SFA and PUFA, especially have been reported to reflect the dietary intakes of those in adults (8,45,5) but also in children (23,30,53,6). However, the results of the association of dietary intake of MUFA and that of plasma are in inconsistance and even lacking (7,45,54). Quite the contrary, the proportion of MUFA in plasma seems not reflect dietary MUFA intake but rather SFA intake (5).

Diet rich in SFA is reported to increase the proportion of palmitic acid and decrease the proportion of linoleic acid in plasma CE (55). Furthermore, the proportion of myristic acid in CE correlates well with the intake of SFA (56). The proportion of pentadecaenoic acid (15:0) has been found to be a good biomarker for dairy fat intake (57–59). It has been reported previously that diet rich in PUFA and linoleic acid correlates positively with the proportion of linoleic acid in plasma CE (56). The proportions of EPA and DHA in CE and PL have also been found to reflect fish intake in both children (6) and adults (60).

Few cross-sectional studies on adults have looked into the relationship of dietary fat with estimated desaturase activities. Lower intake of total fat, MUFA, and PUFA and higher intake of SFA seem to be associated with higher estimated SCD activity (61).

The human body can utilize dietary carbohydrate only to a certain extend and excess consumption of carbohydrate is converted to fat in *de novo* lipogenesis (62). Diet high in carbohydrate has been reported to decrease the proportion of oleic acid in plasma CE (55) and increase the estimated SCD activity (Flowers and Ntambi 2009). There is no evidence on the association of the quality of dietary carbohydrate with the estimated desaturase activities. However, higher intake of fiber is associated with higher activity of estimated SCD (61).

The assessment of dietary data is problematic as one should take into account the time frame in which dietary fatty acids metabolize and move along to tissues, such as plasma. Various methods for dietary assessment exist such as food records for several days, a 24-hour dietary recalls or a food frequency questionnaires. Food records are considered to be a superior method for collecting dietary data (63). The dietary data is collected from the records that are subjectively filled in by the respondents during predefined days, usually 4-7 days, overlapping both weekdays and weekends. Food records contain information on all food and liquid consumed, including portion sizes, place and time of eating. The respondent fills out the food records in real time and therefore this method does not rely on memory. This method is really time consuming for the respondent and can be burdensome. Reporting foods and drinks for four days keeps the burden modest. However, there have to be enough days recorded in the food diary so that the actual, long-term, habitual consumption of food and dietary intakes of nutrients are accurate enough since the daily variation in the consumption of foods may be quite large. One consideration in using food records is careful instructions

beforehand and reviewing the food record upon return by a clinical nutritionist. When applying this method to the children, the instructions are given to the parents as they fill in the food record on behalf of their child. Also, it is recommended to do the reviewing of the record with the parents as soon as possible after keeping the record.

2.3.2 Age and gender

There seem to be no gender differences in plasma fatty acid composition before the age of 12 in children (30,64). Some gender differences begin to show at the age of 15, showing boys having higher proportion of palmitic acid, stearic acid and oleic acid and a lower proportion of linoleic acid in plasma compared with girls in this age group (30).

There are earlier reports that gender has an effect on the plasma fatty acid composition in adults (8,65). The greatest gender differences seem to be in CE, followed by PL and TG (8,66). There is a difference in the proportion of stearic acid, palmitoleic acid, oleic acid, linoleic acid, α-linolenic acid, dihomo-γ-linolenic acid and DHA in CE between middle-aged Finnish men and women. The difference can be found in palmitic acid, stearic acid and oleic acid in PL whereas in α-linolenic acid, dihomo-γ-linolenic acid and DHA in TG. In a population based survey among New Zealand adolescents and adults, women had lower proportions of EPA and DPA and higher proportion of DHA in PL when compared with men (66). It has been speculated that hormonal differences between genders may have effect on the synthesis of DHA (27).

2.3.3 Other factors

There are inconsistent findings about physical activity affecting plasma fatty acid composition (67). Some changes have been seen in composition of plasma CE but no effects on the PL were observed (68). However, some studies have found that higher physical activity may have an effect to fatty acid composition of muscle PL by increasing the proportions of *cis*-vaccenic acid, oleic acid, DHA and total MUFA and PUFA (68–70).

The effect of genetic factors on the plasma fatty acid composition have been investigated in twin studies (71–73). Kang et al. (1976) did not find any effect of genetic variation on the plasma fatty acid composition whereas the studies of Kunesova et al (2002a, 2002b) suggest that there is a genetic influence on dietary habits or endogenous fatty acid metabolism, and that the selection of fatty acids to phosphatidylcholine is under strong genetic control (71–73).

The encoding of D6D and D5D occur by the FADS2 and FADS1 genes that are located in the chromosome 11 (74). Polymorphisms in these genes are associated with plasma fatty acid composition (74,75). The polymorphism in FADS gene has an especially strong association with the elevated plasma proportion of arachidonic acid that is known to be a precursor for inflammation in the body (75). There are fewer studies on the SCD gene that encodes SCD and the results are still unclear (76–78).

2.4 PLASMA FATTY ACID COMPOSITION AND CARDIOMETABOLIC RISK FACTORS

Metabolic syndrome is a cluster of cardiometabolic risk factors, such as insulin resistance, increased waist circumference, dyslipidemia and elevated blood pressure, and the condition is found in children as well (1,2,79). Metabolic syndrome, type 2 diabetes and cardiovascular diseases have been observed to affect fatty acid composition in plasma and adipose tissue (80–84). Children with metabolic syndrome seem to have plasma fatty acid composition characterized by a higher proportion of palmitoleic acid and a lower proportion of arachidonic acid (80).

In adults some fatty acids in plasma have been associated with cardiovascular disease mortality, stroke, transient ischemic attack (TIA), ischemic heart disease and atrial fibrillation

(85–90). Most of these studies have analyzed the fatty acid composition of plasma CE for their study purpose. To review the adult studies briefly, higher proportions of PUFA were associated with lower cardiovascular disease mortality and lower risk of stroke and TIA. Moreover, some plasma SFA, myristic and palmitic acids, and some MUFA, palmitoleic acid and oleic acid, were directly associated with cardiosvascular disease mortality (91).

Certain plasma fatty acid composition in childhood may also predict the risk of developing metabolic syndrome, type 2 diabetes and cardiovascular diseases in adulthood (3). Moreover, different proportions of fatty acids in plasma have been related to single features of metabolic syndrome, such as insulin resistance, dyslipidemia and elevated blood pressure (3,45,92–94). Having these cardiometabolic risk factors in childhood have been associated with an increased risk of type 2 diabetes, cardiovascular diseases and premature mortality in adulthood (95–97).

2.4.1 Continuous risk scores for cardiometabolic risk in children

The definition of metabolic syndrome in children and adolescents is under debate and the definition for diagnostic criteria has not yet been established (98,99). Since there is no universal definition of the metabolic syndrome in children or adolescence, previous studies have commonly used continuous risk scores to assess the acccumulation of cardiometabolic risk factors in children (100–103). These continuous risk scores have usually been calculated as a sum of the risk factors of metabolic syndrome such as fasting glucose and insulin concentration, lipid concentrations, blood pressure, and adiposity. Ekelund and partners (2007) calculated the continuous metabolic risk score by summing the Z-scores of hypertension ([systolic BP+diastolic BP]/2); hyperglycaemia (fasting plasma glucose); insulin resistance (fasting insulin); fasting HDL-cholesterol × –1; and fasting TG (103).

The risk scores are a more sensitive and less controversial way to describe cardiometabolic risk in children than dichotomous definitions for metabolic syndrome (102,104,105). Furthermore, the use of continuous risk scores increases the statistical power in the analyses of the studies that investigate the associations of exposures, such as diet or physical activity, with the dichotomous outcome, such as metabolic syndrome (106).

2.4.2 Insulin

The results of a previous study showed that higher proportions of palmitoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid and EPA and a lower proportion of linoleic acid in plasma CE are related to higher concentration of fasting insulin among adolescents (107). The same study also found that higher proportions of myristic acid, stearic acid, γ-linolenic acid, dihomo-γ-linolenic acid and EPA in PL are associated to higher fasting insulin concentration. Folsom and partners (1996) found that the fasting concentration of insulin is directly associated with the proportion of SFA and the proportion of palmitoleic acid and inversely associated with the proportion of oleic acid in plasma PL among adults (108). Measuring the fasting insulin concentration is a robust way of diagnosing insulin resistance. The results of previous studies in adults have determined a specific plasma fatty acid pattern that is related to insulin resistance. The pattern includes higher proportions of palmitic and palmitoleic and dihomo-γ-linolenic acids and a lower proportion of linoleic acid (13,109–111).

Increased SCD and D6D activities and decreased D5D activity in plasma and erythrocyte membrane have been found to be associated with a worsened insulin sensitivity in adults (20,38,112,113). Similarly, the results of two earlier studies among children and adolescents suggested that increased estimated D6D and decreased estimated D5D activity in plasma are associated with increased plasma concentration of insulin (107,114).

A better quality of dietary fat has been associated with insulin sensitivity in several studies focusing on adults. It has been reported that substituting dietary saturated fat to unsaturated fat seem to improve insulin sensitivity (109,111,115,116,4). Improvements in dietary fat quality affects the plasma fatty acid composition which may influence insulin

action through several mechanisms, such as affecting membrane lipid composition, glucose metabolism and signal-transduction pathways (45).

2.4.2 Glucose

A previous study in adolescents found no associations of plasma fatty acid composition of CE and PL with fasting glucose concentration (107). The results of previous study in adults suggested that there is an association between plasma fatty acid composition and hyperglycaemia (92). A lower proportion of linoleic acid in plasma is associated with higher risk of developing hyperglycaemia.

There is some evidence that the plasma fatty acid composition is different in subjects with impaired glucose tolerance and type 2 diabetes mellitus compared with healthy subjects (117,118). The findings suggest that subjects with impaired glucose tolerance or type 2 diabetes have higher proportions of palmitic, palmitoleic and arachidonic acids and a lower proportion of linoleic acid in plasma (117). The mechanisms behind these associations is suggested to be a lower insulin sensitivity (111,112,117).

Increased estimated SCD and D6D activities and decreased D5D activity in plasma and erythrocyte membrane have been found to be associated with an increased risk of type 2 diabetes in adults (20,38,112,113).

2.4.3 Plasma triacylglyceride and HDL cholesterol concentrations

There are some previous studies that have investigated the associations of plasma fatty acids and plasma lipid concentrations in children and adolescents (107,114,119). Shortly, in CE, the proportions of myristic acid, palmitic acid, palmitoleic acid, α-linolenic acid, γ-linolenic acid, dihomo-γ-linolenic and EPA are reported to be directly associated with the concentration of TG whereas linoleic acid showed an inverse association. The proportions of myristic acid, stearic acid, palmitoleic acid, γ-linolenic acid and dihomo-γ-linolenic acid in plasma PL seem to associate directly with the concentration in TG. Heptadecanoic acid and linoleic acid in PL have inverse associations with the concentration of TG. The proportions of palmitic acid, stearic acid and dihomo-γ-linolenic acid in CE showed inverse associations with the concentration of HDL cholesterol. A higher proportion of α-linolenic acid and a lower proportion of dihomo-γ-linolenic acid in PL are associated with a higher HDL cholesterol concentration. Moreover, the proportion of EPA in whole blood is directly related to HDL cholesterol concentration.

The results of two earlier studies among children and adolescents suggested that increased estimated D6D and decreased estimated D5D activity are associated with increased plasma concentration of TG and decreased concentration of HDL cholesterol (107,114).

2.4.4 Blood pressure

High blood pressure is a risk factor for cardiometabolic diseases such as metabolic syndrome and type 2 diabetes (120). In adults, high blood pressure is inversely associated with the proportion of plasma linoleic acid but directly associated with the proportion of total SFA in plasma (94). Moreover, higher proportions of EPA and DHA in plasma have been reported to be associated with lower blood pressure in adults (121). There is some evidence that higher proportions of SFA, MUFA and omega-3 PUFA in plasma CE among children may be associated with higher blood pressure in adulthood (122). Two previous studies in a same sample of 8-11 years old children found that the proportion of DHA in whole blood was directly associated with systolic and diastolic blood pressure in boys (118,122).

2.4.5 Waist circumference

The results of two earlier studies regarding the associations of plasma fatty acid composition with waist circumference suggest that the proportions of stearic acid, palmitoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid are directly and those of oleic acid and linoleic acid inversely associated with waist circumference (107,124). There is also a

previous observation of a direct relation between estimated D6D activity and waist circumference. One study has investigated the relation of estimated desaturases activities to waist to height-ratio and results show that estimated SCD and D5D activities have inverse association with the waist to height-ratio in children whereas the estimated D6D activity has direct association with it (114).

2.5 LIFESTYLE INTERVENTIONS AND PLASMA FATTY ACID COMPOSITION

Enhancing diet and increasing physical activity from childhood on are key factors in the prevention of type 2 diabetes and cardiovascular disease (125,126). One of the reasons for the beneficial effects of these lifestyle changes could be their influence on plasma fatty acid composition. It has been reported that a one year lifestyle intervention has no effect on the plasma fatty acid composition in adults (113). However, the study found an association between changes in plasma CE fatty acids and change in insulin resistance, assessed by HOMA-IR. Based on the results, the changes in the proportions of myristic acid, palmitoleic acid, γ-linolenic acid and dihomo-γ-linolenic acid are directly associated with the changes in HOMA-IR, whereas the proportions of oleic acid and arachidonic acid are inversely associated with it. Furthermore, increased estimated SCD and D6D activities and decreased estimated D5D activity seem to be associated with worsened insulin resistance.

Most of the interventions that have studied the effect of lifestyle changes on the plasma fatty acid composition are purely dietary interventions and the topic has usually been the adherence of the subjects to the suggested changes in fat intake (5,50,52,127). Moreover, most of these studies include adults only. In a previous study, a decrease in the proportion of stearic acid in plasma CE was reported in adults during a 6-week Healthy Nordic Diet intervention (128). Previous short-term dietary intervention studies focusing on the effect of diet to the plasma fatty acid composition have resulted in a decrease in the estimated SCD activity and an increase in estimated D5D activity (43,128) and also a decrease in estimated D6D activity (43).

A dietary intervention beginning in infancy aiming to decrease dietary SFA and cholesterol intake (129) resulted in lower proportions of SFA and higher proportions of PUFA in serum TG fraction after five years of follow-up among five year old children (64). The study found no changes in the fatty acid compositions of serum CE and PL during the intervention. Other studies on the effects of dietary interventions on plasma fatty acid composition in children are scarce. There are no changes reported in plasma fatty acid composition in CE or PL during lifestyle intervention among children before this thesis. Moreover, evidence on the effects of physical activity or combined dietary and physical activity interventions on plasma fatty acid composition among children is limited.

3 Aims of the study

The general goals of the doctoral thesis were to investigate how a 2-year dietary and physical activity intervention affects plasma fatty acid composition in school- aged children and to investigate the associations of food consumption with plasma fatty acid composition as well as the associations of plasma fatty acid composition with the cardiometabolic risk factors. The aims of the study are outlined in the Figure 5.

1. To examine the associations of the consumption of variety of food items with fatty acid composition and estimated desaturase and elongase activities in plasma CE and PL in a population sample of children 6-8 years of age. We hypothesized that the consumption of foods, that are main sources of dietary SFA, MUFA and PUFA, but also fiber and sugar, are related to plasma fatty acid composition and estimated desaturase and elongase activities in plasma CE and PL. *Study I*.

2. To investigate the associations of plasma fatty acid composition, as well as estimated desaturase and elongase activities, in TG and PL with cardiometabolic risk score and single cardiometabolic risk factors in a population sample of 6-8-year old children. We hypothesized that higher proportions of SFA and lower proportions of PUFA in plasma TG and PL are associated with higher cardiometabolic risk score in children. *Study II*.

3. To study the effects of a 2-year individualized and family-based dietary and physical activity intervention on plasma fatty acid composition of CE and PL as well as estimated desaturase and elongase activities in a population sample of school-aged children. We hypothesized that a lifestyle increases the proportion of PUFA and decreases the proportion of MUFA and SFA in plasma CE and PL among children. *Study III*.

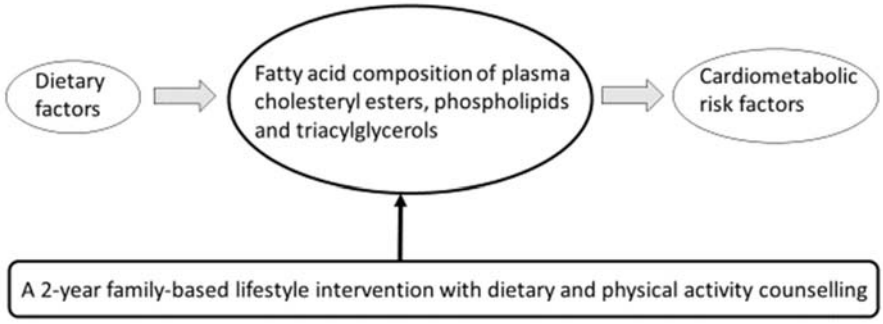

Figure 5. Outlines of the study.

4 Subjects and methods

4.1 STUDY POPULATION AND DESIGN

4.1.1 Study population

The analyses of the present doctoral thesis are based on the baseline and 2-year follow-up data of the Physical Activity and Nutrition in Children (PANIC) study, which is a controlled dietary and physical activity intervention study in a population sample of children from the city of Kuopio, Finland (Clinical trial number NCT01803776). The study has primarily been carried out at the Institute of Biomedicine, University of Eastern Finland, Kuopio campus, Finland. Altogether 736 children 6–8 years of age who were registered for the first grade in 16 primary schools of Kuopio were invited to participate in the baseline study in 2007–2009. Invitation letters were sent by mail to the principal custodian of the children who were asked to contact the research secretary for participation. Of the 736 invited children, 512 (70%) participated in the baseline study that was conducted in 2007-2009 (Figure 6). Based on the comprehensive school health examination data, the participants did not differ in age, sex distribution, or BMI-standard deviation score (BMI-SDS) from all children who started the first grade in Kuopio during the years 2007–2009 (data not shown).

Six children were excluded from the intervention study because of severe physical disability or withdrawal during baseline examinations. The 506 eligible children were then allocated to the intervention group (306 children, 60%) or the control group (200 children, 40%) by matching them according to the location (urban compared with rural) and size (large compared with small) of the schools to minimize differences in baseline characteristics between the groups. Dividing the children in the intervention or control groups according to schools made it possible to organize after-school exercise clubs conducted at schools only for the intervention group and to avoid non-intentional intervention in the control group. More children were included in the intervention group than in the control group because of a larger number of dropouts expected in the intervention group and to have sufficient statistical power for comparison between the groups. Therefore we ended up with 9 intervention schools with only intervention subjects and 7 control schools with only control subjects.

Of the 506 children who participated in the baseline study, 440 (87%) attended the 2-year follow-up study (Figure 6). The median (interquartile range) of the intervention time period was 2.1 (2.1-2.2) years in both groups. Altogether 45 (15%) children in the intervention group and 21 (11%) children in the control group dropped out during the 2-year follow-up. There were no differences in baseline characteristics between the drop-outs in the intervention and control group (data not shown). The children and their parents gave their written informed consent. The PANIC Study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo.

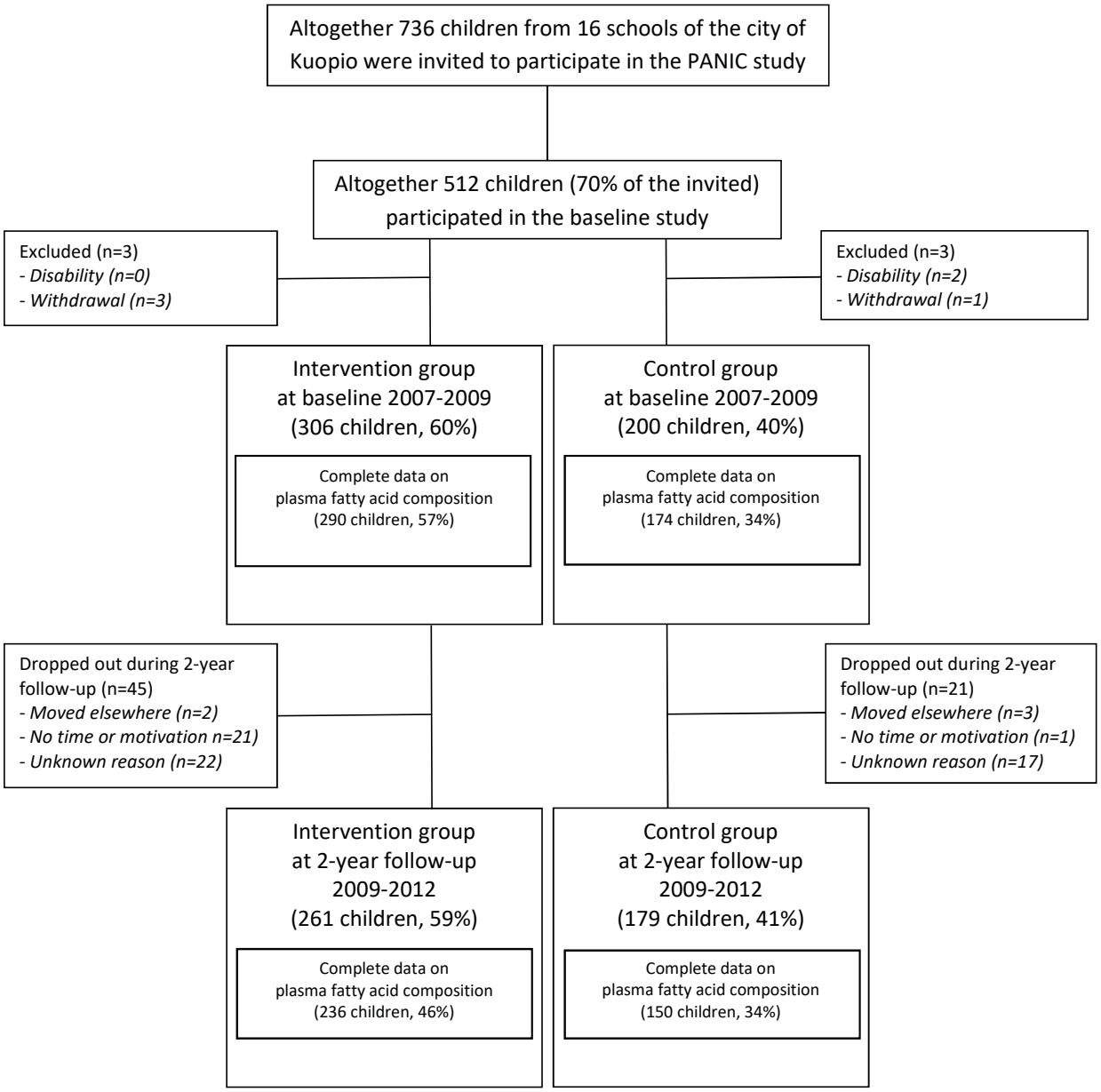

Figure 6. Flowchart of the PANIC Study and the number of children with complete data on plasma fatty acid composition (Study III). PANIC, Physical Activity and Nutrition in Children.

Study 1 used the baseline data of the PANIC study. The final study population was 423 children (208 girls and 215 boys) with complete data on diet, plasma fatty acid composition and other variables needed in the statistical analyses.

Study 2 used the baseline data of the PANIC study. Data on plasma fatty acid composition and cardiometabolic risk factors at baseline were available for 384 children (188 girls, 196 boys).

Study 3 used both the baseline data and the 2-year follow-up data of the PANIC study. Data on plasma fatty acid composition was available for 464 children (290 children in the intervention group, 174 children in the control group) at baseline and for 386 children (236 children in the intervention group, 150 children in the control group) at 2-year follow-up (Figure 6).

4.1.2 Physical activity and dietary intervention

Children and their parents allocated to the intervention group had six dietary counseling sessions of 30-45 minutes and six physical activity counseling sessions of 30-45 minutes during the 2-year intervention period. The dietary and physical activity counseling sessions occurred at 0.5, 1.5, 3, 6, 12 and 18 months after baseline. Authorized clinical nutritionists and specialists in exercise medicine of the PANIC study gave detailed and individualized advice on how to enhance diet quality, increase physical activity and decrease sedentary behavior. The children and their families also received fact sheets on diet quality, physical activity and sedentary behavior, verbal and written information on opportunities to exercise in Kuopio and some financial support for physical activity, such as exercise equipment and admission for indoor sports. The children were also encouraged to participate in after school exercise clubs organized by the PANIC study and supervised by trained exercise instructors. In the exercise clubs, the children had the opportunity to engage in different kinds of physical activities.

The goals of the intervention were 1) to decrease the consumption of foods high in saturated fat and particularly high-fat dairy and meat products, 2) to increase the consumption of foods high in unsaturated fat and particularly high-fat vegetable-oil-based margarines, vegetable oils, and fish, 3) to increase the consumption of vegetables, fruit, and berries, 4) to increase the consumption of whole grain products and other foods high in dietary fiber, 5) to decrease the consumption of foods high in sugar, particularly sugar-sweetened beverages, sugar-sweetened dairy products, and candies, 6) to decrease the consumption of foods high in salt and the use of salt in cooking, and 7) to avoid excessive energy intake, 8) to increase total physical activity by emphasizing its diversity, 9) to decrease total and particularly screen-based sedentary behavior. These goals were based on the Finnish Nutrition Recommendations (130) and the Finnish Recommendations for Physical Activity of School-aged Children (131).

The children and their parents in the control group received verbal and written advice on health improving diet and physical activity according to the Finnish recommendations at baseline but no active intervention.

4.2 METHODS

4.2.1 Assessment of plasma fatty acids composition

Trained research personnel draw the venous blood samples into EDTA vacuum tubes after a 12-hour overnight fast at baseline and at the 2-year follow-up. The samples were centrifuged for ten minutes (3500rpm) by CompactStar CS4 device (VWR®, Radnor, Pennsylvania, USA) and stored at -80°C for approximately three and a half years. Plasma fatty acids were assessed from the blood samples of the baseline and the 2-year follow-up so that the samples of the two timepoints from the same child were analyzed at the same time. Plasma fatty acids were analyzed by a rapid method with a single aminopropyl solid phase

glass column (132). The plasma samples were extracted with chloroform-methanol (2:1) and the lipid fractions were separated by solid phase extraction with an aminopropyl column. Fatty acids in plasma CE, PL and TG were transmethylated with 14% boron trifluoride in methanol and were analyzed by 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE, USA) equipped with a 25-meter FFAP column (Agilent Technologies, Inc., Wilmington, DE, USA). Cholesteryl nonadecanoate (Nu Chek Prep, Inc., Elysian, MA, USA), trinonadecanoin and phosphatidylcholine dinonadecanoyl (Larodan Fine Chemicals, Malmö, SWE) served as internal standards. The relative amount of fatty acid was expressed as a percentage of the total amount fatty acids reported (mol%) (133).

Study I and *Study III* focused on the CE and PL fractions because those fractions reflect the dietary fat intake from preceeding weeks and monts that is longer time of period than that TG reflects. The fraction of TG reflects the dietary fat intake of preceding few days. *Study 2* used the fractions of PL and TG in the analyses because diet was not of interest but cardiometabolic risk was.

4.2.2 Calculation of estimated desaturase and elongase activities

The desaturase and elongase activities in plasma CE, PL and TG were estimated as the ratio of the single product fatty acid divided by the single precursor fatty acid (20,43). In terms of fatty acids in CE and TG, the formulas were set as follows: SCD = 16:1n-7/16:0, D6D = 18:3n-6/18:2n-6, D5D = 20:4n-6/20:3n-6. In terms of PL, the same ratios were used except for the D6D that equaled as 20:3n-6/18:2n-6. The ratio for estimation of the elongase activity was 18:1n-7/16:1n-7 in all fractions.

4.2.3 Assessment of food consumption at baseline

Food and drink consumption and total energy intake were assessed by food records administered by the parents within 2 weeks from the blood sampling at baseline. Complete data from food records of four predefined consecutive days were available for 393 (93 %) children and from food records of three consecutive days for 30 (7 %) children at baseline *in Study I*. Data from the food records of 2 weekdays and 2 weekend days, 3 weekdays and 1 weekend day and 2 weekdays and 1 weekend day were included in the analyses. The parents were instructed to record all food and drink consumption of their child at home and outside home. Clinical nutritionists checked the food records and filled in the missing information together with the children and their parents. The food records were analyzed using the Micro-Nutrica® dietary analysis software, Version 2.5 (The Social Insurance Institution of Finland). Data on food consumption obtained is based on laboratory analyses on the nutrient compositions of different food items used in Finland and on international analyses of the nutrient compositions of food items (134). In *Study I*, the focus was on the associations of the main sources of dietary fat and carbohydrate with plasma fatty acid composition. A clinical nutritionist updated the software by adding new food items and products with their precise nutritional contents received from the producers and schools.

4.2.4 Assessment of cardiometabolic risk factors

Body height, body weight, waist circumference and blood pressure were assessed by trained study nurses. Body height was measured in a Frankfurt position using a wall-mounted stadiometer and body weight was measured using the InBody 720® bioimpedance device (Biospace, Seoul, Korea) that measures body weight as well as body composition. Body mass index (BMI) was calculated as body weight (kg) divided by body height squared (m^2). BMI-SDS was assessed by Finnish growth references (135). Waist circumference was measured after expiration at mid-distance between the bottom of the rib cage and the top of the iliac crest with an unstretchable measuring tape to accuracy of 0.1 cm. Body height, body weight and waist circumference were measured three times and the mean of the nearest two values was used for the analyses. Blood pressure was measured manually by a calibrated aneroid sphygmomanometer (HEINE 130 GAMMA G7, Herrsching, Germany) after a rest of five

minutes. Three measurements in the sitting position at 2-minute intervals were performed. The mean of all three values was used both for the systolic and diastolic blood pressure.

Biochemical analyses were performed by trained research personnel and were done using Cobas 6000 analysers (Hitachi High Technology Co, Tokyo, Japan) in the Eastern Finland Laboratory Centre Joint Authority Enterprise. Plasma glucose concentration was analyzed by a hexokinase method (Roche Diagnostics Co, Mannheim, Germany). An electrochemiluminescence immunoassay with the sandwich principle (Roche Diagnostics Co, Mannheim, Germany) was used to analyze serum insulin concentration. Plasma concentration of TG was analyzed by a colorimetric enzymatic assay (Roche Diagnostics Co, Mannheim, Germany). To analyze plasma HDL cholesterol concentration, a homogeneous enzymatic colorimetric assay was used (Roche Diagnostics Co, Mannheim, Germany).

We calculated a continuous cardiometabolic risk score similarly to the previously published scores (101,103) using continuous Z-score variables by the following sum: glucose + insulin - HDL cholesterol + TG + waist circumference + the mean of systolic and diastolic blood pressures. The Z-score of HDL cholesterol was multiplied by -1, because it is inversely associated with cardiometabolic risk. A higher cardiometabolic risk score indicates a higher cardiometabolic risk. The score was calculated for the same sample of children aged 6–8 years in participating the PANIC study.

4.2.5 Other assessments

Current height as a percentage of predicted adult height was used as a measure of maturity and was calculated for a sample of children aged 6–8 years participating in the PANIC study. The boy's predicted adult height (cm) was calculated as follows: the mean of the height of Finnish men (178.6 cm) + [the SD of the height of Finnish men (6.0 cm) x the SD of the child's predicted adult height from the average of the predicted adult height of Finnish children]. The girl's predicted adult height was calculated as follows: the mean of the height of Finnish women (165.3 cm) + [the SD of the height of Finnish women (5.4 cm) x the SD of the child's predicted adult height from the average of the predicted adult height of Finnish children]. The SD of the child's predicted adult height from the average of the predicted adult height of Finnish children was calculated according to the national guidelines as follows: (the arithmetic mean of the father's and mother's height - 171) / 10. Body fat percentage was measured by a dual energy X-ray absorptiometry (DXA) method with the Lunar DXA device (Lunar Prodigy Advance; GE Medical Systems, Madison, WI, USA).

Habitual physical activity during a usual week was assessed by the PANIC Physical Activity Questionnaire administered by the parents at home. The types of physical activity included organized sports, structured exercise, unstructured physical activity, commuting to and from school and physical activity during recess. The frequency of each type of physical activity, expressed in sessions per week, and the duration of a single session of each type of physical activity, expressed in hours per session to the accuracy of half an hour, were asked. The amount of each physical activity type was calculated by multiplying frequency with duration and was expressed in minutes per day. The amount of total physical activity was calculated by adding the amounts of each physical activity type and was expressed in minutes per day. All children in the first grade had 90 minutes of physical education per week that was included in total physical activity. The PANIC Physical Activity Questionnaire was validated by using the Actiheart monitor combining heart rate and accelerometry measurements (Actiheart, CamNtech, Cambridge, UK) in a subsample of 38 children examined at baseline of the PANIC Study (136). Total physical activity measured by the questionnaire correlated positively with total physical activity measured by the Actiheart monitor ($r = 0.37$, $p = 0.033$).

Habitual sedentary behavior during usual five weekdays and two weekend days was also assessed by the PANIC Physical Activity Questionnaire administered by the parents at home. The types of sedentary behavior included watching TV and videos, using a computer and playing video games, using a mobile phone and playing mobile games, listening to music,

playing a musical instrument, reading, writing, drawing, doing arts and crafts, playing board games and resting. Electronic media time (EMT) was calculated by adding watching TV and videos, using a computer and playing video games and using a mobile phone and playing mobile games. The amount of total sedentary behavior was calculated by adding the times spent in each sedentary behavior and was expressed in minutes per day weighted by the number of weekdays and weekend days.

The annual household income was asked from both parents and coded into three categories (≤ 30,000, 30,001–60,000 and > 60,000 €/year). If the parents reported different categories, the higher category was used in the analyses.

4.2.6 Sample size calculations

The original power calculations were performed according to the primary outcomes of the PANIC Study, which were BMI-SDS, waist circumference, fasting serum insulin, fasting plasma glucose, HOMA-IR, fasting plasma TG, HDL cholesterol, LDL cholesterol, systolic and diastolic blood pressure, and the cardiometabolic risk score. The plasma fatty compositions of CE and PL were secondary outcomes of the PANIC study. We determined the number of children required at 2-year follow-up to detect a ≥ 0.30-SD difference in the primary outcomes between the intervention group (60% of children) and the control group (40% of children) with a power of 80% and a two-sided *P* value for the difference between the groups of 0.05, in spite of a 20% loss to follow-up or missing data. According to these power calculations, we would need a sample size of ≥ 275 children in the intervention group and ≥ 183 children in the control group at baseline and ≥ 220 children in the intervention group and ≥ 147 children in the control group at 2-year follow up for the whole PANIC study. In the *Study III* which was a secondary outcome study, the fatty acid data was available for 236 children in the intervention group and 150 children in the control group at 2-year follow-up, which is in the range of our original power calculation.

4.2.7 Statistical methods

The IBM SPSS statistics software, version 19.0 and 21.0 (IBM Corp.), was used for statistical analyses. The normalities of the distributions of the variables were verified by the Kolmogorov-Smirnov test and visual assessment. In *Studies I* and *II*, the associations of food consumption with plasma fatty acid composition and the associations of plasma fatty acid composition with cardiometabolic risk factors, respectively, were analyzed by linear regression models. Before statistical analyses, logarithmic transformations were performed for the proportions of palmitoleic acid, α-linolenic acid and EPA in CE and for the proportions of myristic acid, behenic acid, lignoceric acid, palmitoleic acid, nervonic acid, α-linolenic acid, dihomo-γ-linolenic acid, EPA and osbond acid in PL in *Study I* and for plasma concentration of TG and waist circumference in S*tudy II* because of the skewed distributions of those variables. To assess differences in basic characteristics between girls and boys, the *t* test for independent samples was used in *Studies I* and *II*. Since there were no sex differences in the plasma fatty acid composition or cardiometabolic risk score, data from both genders were pooled for linear regression analyses for *Studies I* and *II*. In S*tudy I*, data were adjusted for age, sex, physical activity and total energy intake. Further adjustment for BMI-SDS was made but it did not alter the results. In *Study II*, the linear regression models were adjusted for age, sex, body fat percentage and current height as a percentage of predicted adult height. We also made further adjustments for physical activity, electronic media time, total energy intake and household income by adding them one by one to the original model. These further adjustments did not alter the results. In *Study I* Benjamini–Hochberg false discovery rate was used to adjust results for multiple comparisons (137). Associations with a false discovery rate-adjusted *P* value of < 0.05 were considered statistically significant for all tests in *Study I*. In S*tudy II*, the Bonferroni correction for multiple comparisons was used. Therefore, associations in TG and PL with $P < 0.003$ and $P < 0.002$, respectively, were considered

statistically significant. In addition, associations of estimated desaturase and elongase activities with $P < 0.004$ were considered statistically significant in S*tudy II*.

In *Study III*, differences in basic characteristics between the intervention group and the control group at baseline were tested by the *t* test for independent samples except for the difference in sex distribution that was tested by the X^2 test. The effects of the 2-year intervention on plasma fatty acid composition were analyzed by using the linear mixed models. Study group (intervention group, control group), time (baseline, 2-year follow-up), and group*time interaction were included as fixed factors in the model. Participant and school were included as random effects in the model to account for intraparticipant and intraschool correlations between the repeated measures for each participant. The interaction between study group and time was analyzed after adjustment for age and sex. Further adjustments for BMI-SDS were also made. Therefore, the linear mixed models were as follows: (study group, time, age, sex, group*time interaction) and (study group, time, age, sex, BMI-SDS, group*time interaction). Differences and interactions with 2-sided *P* values < 0.05 were considered statistically significant.

5 Results

5.1 CHARASTERISTICS OF THE CHILDREN

The girls were shorter but had attained a higher percentage of their predicted adult height than boys at baseline (Table 1). Girls also had lower waist circumference, higher concentration of serum insulin, and lower concentrations of plasma fasting glucose and HDL cholesterol than boys. The boys were more physically active and had a higher energy intake than the girls at baseline. The girls consumed less vegetable oil, low-fat vegetable oil-based margarine (fat<60%), skimmed milk (fat<1%), cheese (fat>17%), red meat, chocolate and hot chocolate powder, low-fiber grain products, candy and sugar-sweetened beverages than the boys (Table 2).

Table 1. Characteristics of the children at baseline.

Characteristics	**All Children (n=423)**		**Girls (n= 208)**		**Boys (n=215)**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P value***
Age (years)	7.6	0.4	7.6	0.4	7.7	0.4	0.29
Body weight (kg)	26.9	4.9	26.6	5.1	27.2	4.7	0.18
Body height (cm)	128.8	5.6	127.9	5.7	129.7	5.5	**0.001**
BMI (kg/m²)	16.1	2.1	16.1	2.2	16.1	2.0	0.80
BMI-standard deviation score[a]	-0.18	1.07	-0.16	1.06	-0.19	1.08	0.72
Current height as a percentage of predicted adult height[b]	74.6	3.6	76.9	3.0	72.5	2.6	**<0.001**
Waist circumference, cm	56.7	5.7	56.2	5.9	57.3	5.3	**0.033**
Fasting serum insulin, mU/l	4.54	2.30	4.89	2.21	4.20	2.34	**0.002**
Fasting plasma glucose, mmol/l	4.81	0.36	4.77	0.37	4.86	0.33	**0.009**
Fasting plasma triacylglycerols, mmol/l	0.60	0.25	0.62	0.25	0.58	0.24	0.066
Fasting plasma high-density lipoprotein cholesterol, mmol/l	1.60	0.31	1.57	0.31	1.64	0.31	**0.013**
Systolic blood pressure, mmHg	100.33	7.41	99.86	7.56	100.73	7.25	0.225
Diastolic blood pressure, mmHg	61.15	7.19	60.93	7.40	61.29	7.01	0.608
Cardiometabolic risk score	-0.03	3.47	0.05	3.44	-0.15	3.47	0.544
Physical activity (min/d)	110.8	42.0	102.2	39.1	119.1	43.1	**<0.001**
Total energy intake (kcal/d)	1632	317	1542	288	1719	320	**<0.001**

Mean values with standard deviations; *P* values are from *t* tests for independent samples; Differences with $P<0.05$ are considered statistically significant.

a) Calculated using Finnish references (Saari et al. 2011).

b) All children n=384; Girls n=188; Boys n=196.

Table 2. Food consumption of children at baseline.

Food consumption (g/d)	**All children (n= 423)**		**Girls (n= 208)**		**Boys (n= 215)**		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	***P* value**
Butter and butter-oil mixture	6.0	7.4	5.5	6.4	6.4	8.2	0.18
Vegetable oil	2.9	3.0	2.6	2.6	3.2	3.3	**0.03**
Vegetable oil-based margarine (fat 60-80%)	7.1	7.9	7.3	7.9	6.9	8.0	0.56
Low-fat vegetable oil-based margarine (fat<60%)	3.8	6.8	3.2	5.5	4.5	7.8	**0.04**
Milk (fat≥1%)	192.0	227.3	194.2	222.1	190.5	233.1	0.87
Skimmed milk (fat<1%)	376.0	291.4	338.1	270.6	414.4	305.9	**0.01**
Sour milk products (fat≥1%)[a]	84.0	79.6	79.3	68.5	88.4	89.0	0.24
Low-fat sour milk products (fat<1%)[a]	18.8	53.3	18.6	51.4	19.0	55.2	0.93
Cheese (fat>17%)	7.7	10.5	6.4	7.9	8.9	12.4	**0.01**
Low-fat cheese (fat≤17%)	7.3	11.6	8.0	11.7	6.7	11.5	0.24
Ice cream and pudding	26.2	31.4	25.9	30.9	26.5	32.1	0.86
Red meat[b]	79.0	40.1	70.4	33.9	87.0	43.7	**<0.001**
Poultry	17.1	21.7	17.0	22.6	17.3	20.9	0.89
Fish	11.7	17.6	10.4	16.0	13.0	19.1	0.13
Egg	13.4	11.2	13.2	11.3	13.7	11.2	0.59
Chocolate and hot chocolate powder	9.5	12.0	7.9	10.6	11.0	13.1	**0.01**
High-fiber grain products (fiber≥5%)[c]	63.3	40.3	62.1	36.8	64.5	43.6	0.54
Low-fiber grain products (fiber < 5%)[c]	112.1	52.3	107.9	44.6	116.1	58.6	**0.09**
Sugar and honey	9.5	8.0	9.5	7.8	9.4	8.2	0.92
Candy[d]	21.0	25.3	18.9	24.2	23.1	26.2	**0.09**
Sugar-sweetened beverages[e]	139.3	132.3	128.0	120.9	150.2	142.2	**0.08**
Artificially sweetened beverages	43.1	81.2	39.5	72.8	46.9	88.6	0.35

P values are from *t* tests for independent samples. Differences with $P<0.05$ are considered statistically significant.

[a]Sour milk products include yoghurt, curdled milk, sour milk and quark.

[b]Red meat includes all meat other than poultry.

[c]Grain products includes bread, cereal, porridge, flour, pasta and rice.

[d]Candy includes all candy other than chocolate.

[e]Sugar-sweetened beverages include lemonades and all juices other than fresh juices.

5.2 FATTY ACID COMPOSITION IN PLASMA CHOLESTERYL ESTERS, PHOSPHOLIPIDS AND TRIACYLGLYCEROLS (STUDY I)

The characteristic features of fatty acid compositions in different plasma lipid fractions were found as expected (Table 3). There were, however, also some common features. Palmitic acid was the most abundant SFA and oleic acid most abundant MUFA in all fractions. Linoleic acid constituted over half of all fatty acids in CE and it was also the major PUFA in PL and TG.

Table 3. Plasma fatty acid composition (mol%) of cholesteryl esters, phospholipids and triacylglycerols among children 6-8 years old.

Fatty acid		**Cholesteryl esters (n=423)**		**Phospholipids (n=423)**		**Triacylglycerols (n=423)**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
SFA							
Myristic acid	14:0	0.82	0.26	0.45	0.12	2.27	1.1
Pentadecanoic acid	15:0			0.19	0.05		
Palmitic acid	16:0	11.36	0.68	29.16	1.00	26.02	2.86
Margaric acid	17:0			0.34	0.05		
Stearic acid	18:0	0.62	0.17	13.42	0.82	2.95	0.77
Arachidic acid	20:0			0.43	0.07		
Behenic acid	22:0			0.79	0.15		
Lignoceric acid	24:0			0.65	0.14		
MUFA							
Palmitoleic acid	16:1n-7	2.52	0.78	0.57	0.17	3.49	0.99
Oleic acid	18:1n-9	20.25	1.81	10.49	1.21	40.84	2.93
Cis-vaccenic acid	18:1n-7	1.08	0.14	1.38	0.16	2.29	0.29
Eicosenoic acid	20:1n-9+11			0.28	0.04	0.58	0.10
Nervonic acid	24:1n-9			1.50	0.33		
PUFA							
Linoleic acid	18:2n-6	52.72	3.24	20.98	2.06	14.55	2.88
γ-linolenic acid	18:3n-6	0.93	0.44			0.45	0.22
α-linolenic acid	18:3n-3	1.05	0.22	0.41	0.11	1.75	0.58
Dihomo-γ-linolenic acid	20:3n-6	0.70	0.12	2.83	0.49	0.34	0.09
Arachidonic acid	20:4n-6	5.88	1.05	8.37	1.20	1.64	0.69
Eicosapentaenoic acid	20:5n-3	1.26	0.54	1.29	0.54	0.65	0.68
Adrenic acid	22:4n-6			0.32	0.06		
Osbond acid	22:5n-6			0.22	0.05		
Docosapentaenoic acid	22:5n-3			1.28	0.20	0.66	0.27
Docosahexaenoic acid	22:6n-3	0.80	0.21	4.66	1.03	1.51	1.06
SCD (16:1n-7/16:0)		0.22	0.07	0.02	0.01	0.13	0.03
D6D (18:3n-6/18:2n-6)		0.02	0.01			0.03	0.02
D6D (20:3n-6/18:2n-6)				0.13	0.03		
D5D (20:4n-6/20:3n-6)		8.48	1.98	3.06	0.76	4.98	1.98
Elongase (18:1n-7/16:1n-7)		0.46	0.14	2.63	0.73	0.70	0.18

SCD, stearoyl-CoA desaturase; D6D, Δ6 desaturase; D5D, Δ5 desaturase.

5.3 PLASMA SATURATED FATTY ACIDS (STUDIES I, II AND III)

5.3.1 Food consumption and plasma saturated fatty acids

A higher consumption of butter and butter-oil mixture and milk (fat≥1.0%) were associated with a higher proportion of myristic acid in CE (Table 4). A higher consumption of vegetable oil-based margarines (fat 60-80%) was related to a lower proportion of palmitic acid and stearic acid in plasma CE.

The consumptions of butter and butter-oil mixture and milk (fat≥1.0%) were directly associated with the proportion of pentadecanoic acid in plasma PL (Table 4). A higher consumption of candy was associated with a lower proportion of margaric acid in plasma PL.

5.3.2 Plasma saturated fatty acids and cardiometabolic risk factors

The proportions of myristic and stearic acid in plasma PL were directly associated with cardiometabolic risk score and the plasma concentration of TG (Table 5). Moreover, the proportion of stearic acid in PL was directly related to the concentration of insulin. The proportions of myristic acid and palmitic acid in plasma TG were directly associated with cardiometabolic risk score and with the concentrations of serum insulin and plasma TG. The proportion of myristic acid was also directly associated with the fasting concentration of glucose. A higher proportion of stearic acid was related to a higher concentration of insulin.

5.3.3 The effect of the lifestyle intervention on plasma saturated fatty acids

The proportion of stearic acid in CE decreased in the intervention group but not in the control group after adjustment for age and sex ($p=0.001$ for group*time interaction, Table 6). There were no interactions of group and time between the intervention group and control group in PL.

Table 4. The associations of the food consumption with saturated fatty acids in plasma cholesteryl esters, phospholipids and triacylglycerols (n=423).

	SFA in Cholesteryl Esters			*SFA in Phospholipids*							
Food group	***14:0***	***16:0***	***18:0***	***14:0****	***15:0***	***16:0***	***17:0***	***18:0***	***20:0***	***22:0****	***24:0****
Butter and butter-oil mixture	**0.150**[b,d]	0.076	0.064	0.079	**0.266**[c,f]	-0.064	**0.154**[b]	-0.061	-0.077	-0.039	-0.009
Vegetable oil	-0.080	**-0.107**[a]	0.042	-0.045	-0.021	-0.103 [a]	0.061	0.034	0.069	0.078	0.043
Vegetable oil-based margarine (fat 60-80 %)	**-0.124**[a]	**-0.173**[c,d]	**-0.182**[c,d]	-0.061	**-0.097**[a]	-0.026	-0.089	-0.089	0.077	0.051	0.074
Low-fat vegetable oil-based margarine (fat<60%)	-0.122[a]	0.007	-0.038	-0.040	-0.050	-0.006	-0.058	0.073	-0.0005	0.011	0.002
Milk (fat≥1%)	**0.146**[b,d]	0.082	0.038	0.077	**0.218**[c,e]	0.032	**0.125**[a]	-0.032	**-0.104**[a]	-0.055	-0.065
Skimmed milk (fat<1%)	-0.005	**0.105**[a]	-0.090	0.030	-0.080	0.054	-0.032	-0.005	**0.193**[c,e]	**0.108**[a]	**0.115**[a]
Sour milk products (fat≥1%)	0.002	-0.092	0.045	0.025	0.005	-0.012	0.008	-0.056	**0.129**[a]	**0.114**[a]	**0.132**[b]
Low-fat sour milk products (fat<1%)	-0.018	-0.012	0.048	0.046	0.015	-0.019	**0.116**[a]	-0.002	0.026	0.005	**-0.096**[a]
Cheese (fat>17%)	0.026	0.030	0.049	-0.011	0.096	-0.070	**0.120**[a]	0.053	0.003	-0.004	0.000
Low-fat cheese (fat≤17%)	0.094	0.096	-0.067	0.051	0.056	0.095	0.082	-0.093	-0.071	-0.085	-0.011
Ice cream and pudding	**0.125**[a]	0.040	**0.112**[a]	**0.136**[b]	0.037	**0.127**[a]	-0.031	-0.020	0.008	-0.010	-0.043
Red meat	-0.050	-0.026	0.059	-0.091	-0.078	**-0.104**[a]	0.021	**0.104**[a]	-0.087	-0.058	-0.034
Poultry	0.049	0.027	0.024	0.062	0.064	0.035	-0.018	-0.055	-0.007	0.008	0.011
Fish	-0.007	-0.003	-0.055	-0.064	-0.091	-0.030	-0.068	0.013	-0.036	-0.023	-0.034
Egg	-0.016	0.017	0.048	-0.003	0.038	-0.079	0.068	-0.042	0.033	0.055	0.063
Chocolate and hot chocolate powder	0.020	0.027	0.089	-0.038	-0.047	0.047	**-0.125**[a]	**0.099**[a]	0.007	0.019	0.004
High-fiber grain products (fiber≥5%)	-0.025	0.061	**-0.110**[a]	0.016	0.016	-0.002	**0.114**[a]	-0.092	-0.030	-0.057	-0.012
Low-fiber grain products (fiber < 5%)	**-0.110**[a]	-0.074	0.004	-0.026	0.008	-0.035	0.017	-0.026	0.004	0.047	0.019
Sugar and honey	-0.016	0.096	-0.028	-0.057	-0.071	0.081	-0.076	-0.099	0.087	0.060	0.051
Candy	-0.009	-0.086	0.039	-0.030	**-0.151**[b]	0.020	**-0.200**[c,e]	0.078	-0.015	0.005	-0.018
Sugar-sweetened beverages	-0.047	**-0.122**[a]	0.020	-0.051	-0.050	-0.061	-0.049	0.064	-0.032	0.006	0.011
Artificially sweetened beverages	-0.022	0.025	0.042	0.046	-0.030	0.002	**-0.107**[a]	0.025	-0.004	0.013	0.032

Values are standardized regression coefficients from linear regression models adjusted for age, sex, physical activity and total energy intake.
*Logarithmic transformation was carried out, because of skewed distributions. The *P* values of <0.05 are bolded. SFA; saturated fatty acid.

[a]*P*<0.05.
[b]*P*<0.01.
[c]*P*<0.001.
[d]*P*<0.05, after FDR-adjustment for multiple comparisons.
[e]*P*<0.01, after FDR-adjustment for multiple comparisons.
[f]*P*<0.001, after FDR-adjustment for multiple comparisons.

Table 5. Associations of saturated fatty acids in plasma phospholipids and triacylglycerols with cardiometabolic risk factors (n=384).

Fatty acid	Cardiometabolic risk score	Insulin	Glucose	Triacylglycerols[a]	HDL cholesterol	Systolic BP	Diastolic BP	Waist circumference[a]
Phospholipids								
14:0	**0.159**[b]	0.137	0.097	**0.192**[b]	-0.002	0.110	0.070	0.018
15:0	-0.078	-0.067	-0.064	-0.023	0.086	-0.030	0.071	-0.061
16:0	-0.026	-0.053	-0.109	0.083	-0.065	-0.018	-0.096	0.010
17:0	-0.091	-0.108	-0.096	-0.051	0.055	-0.086	0.082	-0.036
18:0	**0.251**[b]	**0.202**[b]	0.150	**0.235**[b]	-0.051	0.135	0.079	0.082
20:0	-0.095	-0.091	-0.039	**-0.238**[b]	-0.040	-0.094	-0.018	0.077
22:0	-0.058	-0.042	0.050	**-0.199**[b]	0.001	-0.087	0.005	0.061
24:0	-0.108	-0.072	0.003	**-0.225**[b]	0.072	-0.068	0.066	0.010
Triacylglycerols								
14:0	**0.353**[b]	**0.313**[b]	**0.241**[b]	**0.462**[b]	-0.060	0.096	0.131	-0.012
16:0	**0.291**[b]	**0.217**[b]	0.042	**0.428**[b]	-0.098	0.149	0.046	0.087
18:0	0.132	**0.167**[b]	0.079	0.043	-0.019	0.086	0.005	0.089

Values are standardized regression coefficients (β) from linear regression models adjusted for age, sex and the current height as a percentage of predicted adult height. HDL; High-density lipoprotein. BP; Blood pressure.

a)Logarithmic transformation was carried out because of a skewed distribution.

b)Statistical significance after Bonferroni correction for multiple comparisons: The *P* values with <0.002 for phospholipids and *P*<0.003 for triacylglycerols are bolded.

Table 6. Saturated fatty acids (mol%) in plasma cholesteryl esters and phospholipids in the intervention group and in the control group at baseline and after 2-year follow-up.

	Intervention group				**Control group**				
	Baseline *n*=290	**2-year follow-up *n*=236**	**2-year change in mean**	**P for 2-year change**	**Baseline *n*=174**	**2-year follow-up *n*=150**	**2-year change in mean**	***P* for 2-year change**	***P* for group*time interaction**
Cholesteryl esters									
Total SFA	12.8 (12.6, 13.0)	12.7 (12.5, 12.8)	-0.1	**0.027**	12.9 (12.7, 13.1)	12.8 (12.6, 13.1)	-0.1	0.809	0.233
14:0	0.82 (0.79, 0.86)	0.82 (0.78, 0.86)	-0.004	0.845	0.84 (0.80, 0.89)	0.85 (0.80, 0.90)	0.01	0.864	0.789
16:0	11.4 (11.2, 11.5)	11.2 (11.1, 11.4)	-0.2	**0.024**	11.4 (11.3, 11.6)	11.4 (11.2, 11.5)	-0.04	0.463	0.401
18:0	0.65 (0.61, 0.70)	0.62 (0.57, 0.66)	-0.03	**0.001**	0.61 (0.56, 0.67)	0.63 (0.58, 0.69)	0.02	0.129	**0.001**
Phospholipids									
Total SFA	45.4 (45.2, 45.6)	45.5 (45.3, 45.7)	0.1	0.231	45.4 (45.2, 45.7)	45.6 (45.4, 45.9)	0.2	**0.027**	0.320
14:0	0.46 (0.43, 0.49)	0.48 (0.45, 0.51)	0.02	0.111	0.45 (0.41, 0.49)	0.46 (0.42, 0.50)	0.01	0.467	0.673
15:0	0.19 (0.18, 0.20)	0.19 (0.18, 0.20)	-0.003	0.493	0.19 (0.18, 0.21)	0.20 (0.19, 0.22)	0.01	0.087	0.077
16:0	29.1 (28.9, 29.4)	29.0 (28.7, 29.2)	-0.1	**0.014**	29.3 (29.0, 29.6)	29.1 (28.8, 29.4)	-0.2	0.058	0.964
17:0	0.34 (0.32, 0.35)	0.33 (0.32, 0.34)	-0.01	**0.024**	0.34 (0.33, 0.36)	0.34 (0.33, 0.36)	-0.001	0.739	0.252
18:0	13.4 (13.3, 13.6)	13.7 (13.5, 13.8)	0.3	**<0.001**	13.4 (13.2, 13.5)	13.6 (13.5, 13.8)	0.2	**<0.001**	0.563
20:0	0.44 (0.42, 0.45)	0.43 (0.41, 0.45)	-0.01	0.106	0.42 (0.40, 0.44)	0.42 (0.41, 0.45)	0.001	0.227	0.051
22:0	0.80 (0.77, 0.84)	0.81 (0.77, 0.84)	0.01	0.503	0.77 (0.73, 0.82)	0.80 (0.76, 0.84)	0.03	**0.038**	0.227
24:0	0.65 (0.62, 0.68)	0.66 (0.63, 0.69)	0.01	0.365	0.65 (0.61, 0.69)	0.66 (0.63, 0.70)	0.01	0.132	0.537

Values are means (95% confidence intervals) of proportion of saturated fatty acids in plasma cholesteryl esters and phospholipids at baseline and after 2-year follow-up, the 2-year changes in the means and p-values for the differences between the means at baseline and 2-year follow-up from the linear mixed models adjusted for age and sex. Study group, time and their interactions were included as fixed factors in the model and participant and school were included as random effects in the model. The *P* values of <0.05 are bolded. SFA; saturated fatty acid.

5.4 PLASMA MONOUNSATURATED FATTY ACIDS (STUDIES I, II AND III)

5.4.1 Food consumption and plasma monounsaturated fatty acids

A higher consumption of vegetable oil-based margarines (fat 60-80%) was associated with lower proportions of palmitoleic and oleic acids in CE (Table 7). A higher consumption of sour milk products (fat≥1%) was related to a higher proportion of oleic acid in plasma CE, whereas higher consumption of low-fat cheese (fat≤17%) was associated with a lower proportion of oleic acid in plasma CE (Table xx). The consumption of high-fiber grain products was inversely associated with the proportion of oleic acid in plasma CE. The consumption of candy was directly related to the proportions of palmitoleic acid and oleic acid in plasma CE.

The consumption of vegetable oil-based margarine (fat 60-80%) was inversely related to the proportion of palmitoleic acid in plasma PL. A higher consumption of high-fiber grain products was associated with a lower proportion of oleic acid in plasma PL. A higher consumption of chocolate and hot chocolate powder was related to a lower proportion of eicosanoic acid in PL. The consumption of skimmed milk was directly associated with the proportion of nervonic acid in PL.

5.4.2 Plasma monounsaturated fatty acids and cardiometabolic risk factors

A higher proportion of palmitoleic acid in PL was associated with a higher cardiometabolic risk score and a higher plasma concentration of TG (Table 8). A higher proportion of oleic acid in plasma PL associated with a higher fasting concentration of TG. The proportion of *cis*-vaccenic acid in PL was inversely related to cardiometabolic risk score and the concentrations of insulin, glucose and TG. The proportion of palmitoleic acid in TG was directly associated with cardiometabolic risk score and the concentrations of serum insulin and plasma TG.

5.4.3 The effect of the lifestyle intervention on plasma monounsaturated fatty acids

The proportion of total MUFA in CE did not change significantly in the intervention group but increased in the control group ($p=0.007$ for group*time interaction, Table 9). The proportion of oleic acid in CE tended to decrease in the intervention group but increased in the control group ($p=0.002$ for group*time interaction). The proportion of total MUFA in PL tended to decreased in the intervention group but did not change significantly in the control group after adjustment for age and sex ($p=0.021$ for group*time interaction. The proportion of oleic acid in PL did not change significantly in the intervention group but tended to increase in the control group ($p=0.023$ for group*time interaction).

Table 7. The associations of the food consumption with monounsaturated fatty acids in plasma cholesteryl esters and phospholipids (n=423).

	MUFA in Cholesteryl Esters			*MUFA in Phospholipids*				
Food group	***16:1n-7****	***18:1n-9***	***18:1n-7***	***16:1n-7****	***18:1n-9***	***18:1n-7***	***20:1n-9+11***	***24:1n-9****
Butter and butter-oil mixture	-0.001	0.015	-0.002	-0.045	0.016	0.044	0.002	-0.068
Vegetable oil	**-0.128**[a]	-0.035	-0.041	-0.079	-0.024	-0.026	0.092	0.066
Vegetable oil-based margarine (fat 60-80 %)	**-0.220**[c,e]	**-0.163**[b,d]	-0.046	**-0.185**[c,d]	**-0.105**[a]	0.053	**0.103**[a]	0.096
Low-fat vegetable oil-based margarine (fat<60%)	0.010	-0.096	0.049	0.035	-0.090	-0.025	-0.019	0.021
Milk (fat≥1%)	0.019	0.001	-0.039	-0.048	0.024	-0.051	-0.072	**-0.122**[a]
Skimmed milk (fat<1%)	0.070	-0.001	**0.129**[b]	**0.103**[a]	-0.015	0.038	0.026	**0.192**[c,e]
Sour milk products (fat≥1%)	0.092	**0.164**[b,d]	0.066	**0.105**[a]	**0.108**[a]	0.033	0.028	0.074
Low-fat sour milk products (fat<1%)	-0.030	0.015	0.036	0.051	0.091	0.046	0.057	0.032
Cheese (fat>17%)	-0.021	-0.057	-0.026	-0.005	-0.016	-0.006	0.038	0.029
Low-fat cheese (fat≤17%)	-0.011	**-0.147**[b,d]	0.005	0.026	**-0.107**[a]	0.030	-0.045	0.015
Ice cream and pudding	**0.109**[a]	**0.142**[b]	-0.066	**0.121**[a]	**0.146**[b]	-0.034	0.025	-0.072
Red meat	-0.016	0.014	-0.038	-0.025	0.022	-0.053	-0.007	-0.059
Poultry	0.023	-0.012	0.007	-0.002	0.009	-0.045	0.025	0.009
Fish	-0.040	-0.021	-0.068	-0.069	-0.021	-0.076	-0.019	-0.006
Egg	-0.033	-0.043	-0.033	-0.034	**-0.106**[a]	-0.038	**-0.105**[a]	0.058
Chocolate and hot chocolate powder	0.063	**0.101**[a]	-0.026	0.051	0.030	-0.095	**-0.157**[b,d]	**-0.116**[a]
High-fiber grain products (fiber≥5%)	-0.094	**-0.205**[c,e]	0.089	-0.054	**-0.169**[b,d]	**0.125**[a]	**0.104**[a]	**0.098**[a]
Low-fiber grain products (fiber < 5%)	-0.074	-0.066	0.012	-0.007	-0.013	0.053	0.018	0.029
Sugar and honey	0.031	-0.035	0.052	-0.025	**-0.122**[a]	0.003	-0.063	0.091
Candy	**0.166**[b,d]	**0.189**[c,d]	-0.100	0.097	**0.149**[b]	**-0.120**[a]	-0.097	-0.088
Sugar-sweetened beverages	0.018	0.109[a]	-0.044	-0.022	-0.007	-0.026	-0.047	-0.010
Artificially sweetened beverages	0.021	0.003	0.060	0.072	0.027	0.017	0.027	0.016

Values are standardized regression coefficients from linear regression models adjusted for age, sex, physical activity and total energy intake.
*Logarithmic transformation was carried out, because of skewed distributions. The *P* values of <0.05 are bolded. MUFA; monounsaturated fatty acid.
[a]*P*<0.05. [d]*P*<0.05, after FDR-adjustment for multiple comparisons.
[b]*P*<0.01. [e]*P*<0.01, after FDR-adjustment for multiple comparisons.
[c]*P*<0.001. [f]*P*<0.001, after FDR-adjustment for multiple comparisons.

Table 8. Associations of monounsaturated fatty acids in plasma phospholipids and triacylglycerols with cardiometabolic risk factors (n=384).

Fatty acid	Cardiometabolic risk score	Insulin	Glucose	Triacylglycerols[a]	HDL cholesterol	Systolic BP	Diastolic BP	Waist circumference[a]
Phospholipids								
16:1n-7	**0.159**[b]	0.065	-0.048	**0.322**[b]	-0.086	0.091	0.012	0.073
18:1n-9	0.141	0.048	0.047	**0.305**[b]	-0.089	0.117	0.003	-0.081
18:1n-7	**-0.217**[b]	**-0.239**[b]	**-0.230**[b]	**-0.225**[b]	-0.052	-0.109	0.004	-0.054
20:1n-9+11	-0.104	-0.142	-0.144	-0.136	-0.159	-0.077	0.005	-0.070
24:1n-9	-0.110	-0.090	-0.073	**-0.262**[b]	-0.052	-0.099	-0.039	0.080
Triacylglycerols								
16:1n-7	**0.289**[b]	**0.158**[b]	0.084	**0.512**[b]	-0.087	0.035	0.082	0.072
18:1n-9	-0.100	-0.127	-0.032	-0.144	-0.066	-0.044	-0.051	-0.046
18:1n-7	-0.020	-0.080	-0.058	-0.061	-0.025	-0.071	0.037	0.116
20:1n-9+11	**-0.171**[b]	-0.135	-0.080	**-0.246**[b]	0.031	-0.108	0.011	-0.050

Values are standardized regression coefficients (β) from linear regression models adjusted for age, sex and the current height as a percentage of predicted adult height. HDL; High-density lipoprotein. BP; Blood pressure.

a)Logarithmic transformation was carried out because of a skewed distribution.

b)Statistical significance after Bonferroni correction for multiple comparisons: The *P* values with <0.002 for phospholipids and <0.003 for triacylglycerols are bolded.

Table 9. Monounsaturated fatty acids (mol%) in plasma cholesteryl esters and phospholipids in the intervention group and in the control group at baseline and after 2-year follow-up.

	Intervention group				**Control group**				
	Baseline *n*=290	**2-year follow-up *n*=236**	**2-year change in mean**	**P for 2-year change**	**Baseline *n*=174**	**2-year follow-up *n*=150**	**2-year change in mean**	***P* for 2-year change**	***P* for group*time interaction**
Cholesteryl esters									
Total MUFA	24.1 (23.7, 24.4)	23.9 (23.5, 24.3)	-0.2	0.260	23.4 (23.0, 23.9)	24.0 (23.5, 24.4)	0.6	**0.010**	**0.007**
16:1n-7	2.59 (2.49, 2.69)	2.64 (2.54, 2.74)	0.05	0.360	2.42 (2.29, 2.55)	2.56 (2.43, 2.69)	0.14	**0.030**	0.259
18:1n-9	20.5 (20.1, 20.7)	20.2 (19.9, 20.5)	-0.3	0.086	20.0 (19.6, 20.3)	20.4 (20.0, 20.7)	0.4	**0.012**	**0.002**
18:1n-7	1.08 (1.05, 1.10)	1.06 (1.04, 1.09)	-0.02	0.099	1.06 (1.03, 1.09)	1.04 (1.01, 1.07)	-0.02	0.062	0.662
Phospholipids									
Total MUFA	14.3 (14.1, 14.6)	14.1 (13.9, 14.4)	-0.2	0.052	14.0 (13.8, 14.3)	14.2 (13.9, 14.5)	0.2	0.158	**0.021**
16:1n-7	0.58 (0.55, 0.61)	0.58 (0.56, 0.61)	0.001	0.937	0.55 (0.51, 0.58)	0.56 (0.53, 0.59)	0.01	0.393	0.536
18:1n-9	10.5 (10.3, 10.7)	10.4 (10.2, 10.6)	-0.1	0.160	10.4 (10.1, 10.6)	10.6 (10.3, 10.8)	0.2	0.074	**0.023**
18:1n-7	1.39 (1.36, 1.42)	1.38 (1.35, 1.41)	-0.01	0.512	1.38 (1.34, 1.41)	1.34 (1.30, 1.37)	-0.04	**0.010**	0.106
20:1n-9+11	0.28 (0.28, 0.29)	0.28 (0.27, 0.29)	-0.004	0.205	0.29 (0.28, 0.30)	0.28 (0.27, 0.29)	-0.01	**0.003**	0.119
24:1n-9	1.53 (1.44, 1.63)	1.49 (1.40, 1.59)	-0.04	**0.046**	1.46 (1.34, 1.57)	1.45 (1.33, 1.56)	-0.01	0.784	0.303

Values are means (95% confidence intervals) of proportion of monounsaturated fatty acids in plasma cholesteryl esters and phospholipids at baseline and after 2-year follow-up, the 2-year changes in the means and p-values for the differences between the means at baseline and 2-year follow-up from the linear mixed models adjusted for age and sex. Study group, time and their interactions were included as fixed factors in the model and participant and school were included as random effects in the model. The P values of <0.05 are bolded. MUFA; monounsaturated fatty acid.

5.5 PLASMA POLYUNSATURATED FATTY ACIDS (STUDIES I, II AND III)

5.5.1 Food consumption and plasma polyunsaturated fatty acids

The consumption of vegetable oil-based margarine (fat 60-80%) was directly related to the proportions of linoleic acid and α-linolenic acid in plasma CE (Table 10). The consumption of low-fat cheese (fat≤17%) was directly associated with the proportion of arachidonic acid in plasma CE. The consumption of skimmed milk was directly associated with the proportion of EPA in plasma CE. The consumptions of low-fat cheese (fat≤17%) and fish were directly associated with the proportion of DHA in plasma CE, whereas candy consumption was inversely associated with it.

Vegetable oil-based margarine (fat 60-80%) consumption was directly associated with the proportions of linoleic acid and α-linolenic acid in plasma PL (Table 11). A higher consumption of skimmed milk associated with a lower proportion of linoleic acid in plasma PL. The consumption of ice cream and pudding was inversely associated with the proportion of DHA in plasma PL. A lower consumption of low-fiber grain products was related to a higher proportion of EPA in plasma PL.

Table 10. The associations of the food consumption with polyunsaturated fatty acids in plasma cholesteryl esters (n=423).

Food group	***18:2n-6***	***18:3n-6****	***18:3n-3***	***20:3n-6***	***20:4n-6***	***20:5n-3****	***22:6n-3***
Butter and butter-oil mixture	-0.012	-0.078	-0.075	**-0.105**[a]	0.005	0.003	-0.043
Vegetable oil	0.072	-0.050	-0.016	-0.066	0.018	0.022	0.048
Vegetable oil-based margarine (fat 60-80 %)	**0.216**[c,e]	-0.080	**0.222**[c,e]	-0.025	-0.070	0.030	-0.013
Low-fat vegetable oil-based margarine (fat<60%)	0.014	0.003	0.019	0.013	**0.106**[a]	0.012	0.092
Milk (fat≥1%)	-0.005	-0.010	-0.008	-0.044	-0.021	-0.051	-0.088
Skimmed milk (fat<1%)	**-0.108**[a]	0.070	0.021	**0.142**[b]	0.078	**0.151**[b,d]	**0.139**[b]
Sour milk products (fat≥1%)	-0.052	-0.017	0.009	**0.116**[a]	**-0.126**[a]	-0.023	-0.012
Low-fat sour milk products (fat<1%)	0.003	-0.072	0.048	-0.060	-0.028	0.025	0.050
Cheese (fat>17%)	0.041	-0.023	-0.047	-0.038	0.001	-0.054	-0.069
Low-fat cheese (fat≤17%)	-0.019	0.000	0.016	0.045	**0.156**[b,d]	**0.120**[a]	**0.163**[b,d]
Ice cream and pudding	-0.095	0.054	0.030	0.021	-0.041	**-0.107**[a]	**-0.142**[b]
Red meat	-0.011	0.031	-0.064	-0.045	0.068	-0.045	-0.026
Poultry	-0.017	-0.072	0.057	-0.091	-0.002	0.052	0.092
Fish	0.004	0.001	0.057	-0.051	-0.046	**0.115**[a]	**0.169**[c,d]
Egg	0.007	-0.036	-0.073	**-0.107**[a]	0.079	0.024	0.095
Chocolate and hot chocolate powder	-0.081	0.058	-0.068	-0.012	0.016	-0.028	0.045
High-fiber grain products (fiber≥5%)	0.071	**-0.110**[a]	**0.097**[a]	0.016	**0.115**[a]	**0.132**[b]	**0.137**[b]
Low-fiber grain products (fiber < 5%)	**0.125**[a]	-0.087	-0.031	-0.011	-0.048	**-0.135**[b]	-0.037
Sugar and honey	-0.061	-0.018	-0.034	0.011	0.082	**0.150**[b]	0.089
Candy	-0.084	**0.124**[a]	-0.048	0.003	-0.087	-0.096	**-0.153**[b,d]
Sugar-sweetened beverages	-0.008	0.084	0.024	0.015	-0.056	-0.095	**-0.110**[a]
Artificially sweetened beverages	-0.030	-0.024	-0.079	0.001	0.071	-0.021	0.042

Values are standardized regression coefficients from linear regression models adjusted for age, sex, physical activity and total energy intake.
*Logarithmic transformation was carried out, because of skewed distributions. The *P* values of <0.05 are bolded.

[a]*P*<0.05. [d]*P*<0.05, after FDR-adjustment for multiple comparisons.
[b]*P*<0.01. [e]*P*<0.01, after FDR-adjustment for multiple comparisons.
[c]*P*<0.001. [f]*P*<0.001, after FDR-adjustment for multiple comparisons.

Table 11. The associations of the food consumption with polyunsaturated fatty acids in plasma phospholipids (n=423).

Food group	***18:2n-6***	***18:3n-3****	***20:3n-6****	***20:4n-6***	***20:5n-3****	***22:4n-6***	***22:5n-6****	***22:5n-3***	***22:6n-3***
Butter and butter-oil mixture	0.044	-0.002	**-0.111**[a]	0.045	0.020	0.022	-0.003	0.004	-0.004
Vegetable oil	0.000	-0.069	-0.096	0.047	0.031	-0.013	-0.006	0.063	0.043
Vegetable oil-based margarine (fat 60-80 %)	**0.159**[b,d]	**0.158**[b,d]	-0.045	-0.040	0.002	**-0.142**[b]	-0.091	0.034	-0.041
Low-fat vegetable oil-based margarine (fat<60%)	-0.058	0.040	0.015	0.071	0.016	-0.021	-0.026	-0.067	0.087
Milk (fat≥1%)	0.086	0.058	-0.042	-0.005	-0.051	0.045	0.048	-0.039	-0.093
Skimmed milk (fat<1%)	**-0.213**[c,e]	-0.009	0.097	0.010	**0.147**[b]	-0.026	-0.062	**0.148**[b]	**0.109**[a]
Sour milk products (fat≥1%)	-0.038	0.012	**0.128**[a]	**-0.113**[a]	-0.003	0.022	0.026	0.030	-0.025
Low-fat sour milk products (fat<1%)	-0.034	0.042	-0.078	-0.046	-0.003	-0.046	-0.059	0.048	0.038
Cheese (fat>17%)	0.011	-0.062	-0.013	0.035	-0.039	**0.106**[a]	0.089	0.042	-0.021
Low-fat cheese (fat≤17%)	-0.069	-0.027	0.017	**0.119**[a]	0.064	-0.061	0.016	-0.052	0.088
Ice cream and pudding	-0.051	0.008	0.066	-0.019	-0.085	0.094	**0.162**[b,d]	-0.009	**-0.167**[b,d]
Red meat	-0.036	-0.034	-0.010	**0.130**[a]	-0.031	**0.105**[a]	0.099	-0.082	0.008
Poultry	-0.021	-0.006	**-0.113**[a]	-0.030	0.042	-0.084	**-0.124**[a]	-0.038	**0.107**[b]
Fish	0.028	0.032	-0.066	-0.091	0.095	**-0.189**[c,e]	**-0.155**[b,d]	-0.057	**0.149**[b]
Egg	0.001	-0.061	**-0.113**[a]	**0.139**[a]	0.017	-0.008	-0.015	-0.039	**0.106**[a]
Chocolate and hot chocolate powder	-0.023	-0.032	0.017	-0.019	-0.036	0.042	0.029	**-0.106**[a]	-0.004
High-fiber grain products (fiber≥5%)	-0.012	0.021	-0.014	0.085	**0.101**[a]	**-0.120**[b]	-0.089	-0.007	**0.134**[b]
Low-fiber grain products (fiber < 5%)	**0.111**[a]	-0.001	-0.050	-0.007	**-0.175**[b,d]	0.037	0.034	0.003	-0.074
Sugar and honey	-0.054	-0.084	-0.050	0.058	**0.113**[a]	-0.048	-0.062	**0.130**[a]	0.101
Candy	0.060	0.008	0.028	-0.092	-0.097	0.046	-0.000	-0.087	**-0.153**[b]
Sugar-sweetened beverages	0.023	0.031	0.078	0.008	-0.053	**0.118**[a]	0.087	0.028	-0.049
Artificially sweetened beverages	-0.041	**-0.130**[b]	-0.024	0.051	-0.051	0.047	0.026	-0.030	-0.013

Values are standardized regression coefficients from linear regression models adjusted for age, sex, physical activity and total energy intake.
*Logarithmic transformation was carried out, because of skewed distributions. The *P* values of <0.05 are bolded.

[a]P<0.05. [d]P<0.05, after FDR-adjustment for multiple comparisons.
[b]P<0.01. [e]P<0.01, after FDR-adjustment for multiple comparisons.
[c]P<0.001. [f]P<0.001, after FDR-adjustment for multiple comparisons.

5.5.2 Plasma polyunsaturated fatty acids and cardiometabolic risk factors

The proportion of arachidonic acid in PL was inversely related to the plasma concentration of TG (Table 12). A lower proportion of linoleic acid in TG was associated with a higher cardiometabolic risk score and higher concentrations of serum insulin and plasma TG. The proportions of arachidonic acid and EPA in TG were inversely associated with cardiometabolic risk score and plasma concentration of TG whereas both of these fatty acids were directly associated with HDL cholesterol concentration. A lower proportion of DHA in TG was associated with a higher cardiometabolic risk score and a higher plasma concentration of TG.

5.5.3 The effect of the lifestyle intervention on plasma polyunsaturated fatty acids

The proportion of total PUFA in CE tended to increase in the intervention group but decreased in the control group ($p=0.007$ for group*time interaction, Table 13). The proportion of linoleic acid in CE did not change significantly in the intervention group but decreased in the control group ($p=0.038$ for group*time interaction). The proportion of α-linolenic acid in CE increased in the intervention group but decreased in the control group ($p<0.001$ for group*time interaction).

The proportion of total PUFA in PL did not change significantly in the intervention group but decreased in the control group ($p=0.019$ for group*time interaction). The proportion of α-linolenic acid in PL tended to increase in the intervention group but tended to decrease in the control group ($p=0.015$ for group*time interaction).

Table 12. Associations of polyunsaturated fatty acids in plasma phospholipids and triacylglycerols with cardiometabolic risk factors (n=384).

Fatty acid	Cardiometabolic risk score	Insulin	Glucose	Triacylglycerols[a]	HDL cholesterol	Systolic BP	Diastolic BP	Waist circumference[a]
Phospholipids								
18:2n-6	-0.059	0.055	0.140	-0.080	0.163	-0.071	-0.068	-0.083
18:3n-3	0.075	0.094	-0.003	0.051	-0.077	0.052	0.014	0.004
20:3n-6	**0.222**[b]	0.050	-0.006	**0.306**[b]	**-0.171**[b]	0.090	0.075	0.122
20:4n-6	-0.117	-0.082	-0.126	**-0.224**[b]	0.031	-0.003	0.071	0.048
20:5n-3	-0.109	-0.071	-0.079	-0.131	0.108	-0.030	0.028	0.023
22:4n-6	0.067	-0.043	-0.050	0.099	-0.084	0.085	0.109	0.012
22:5n-6	0.025	-0.062	-0.082	0.099	-0.139	0.044	0.060	-0.083
22:5n-3	-0.109	**-0.175**[b]	-0.140	-0.121	-0.071	0.021	-0.031	0.003
22:6n-3	-0.057	-0.055	-0.052	-0.140	-0.056	-0.064	0.011	0.022
Triacylglycerols								
18:2n-6	**-0.239**[b]	**-0.173**[b]	-0.073	**-0.323**[b]	0.083	-0.100	-0.061	-0.067
18:3n-3	-0.014	0.014	0.014	-0.110	-0.054	-0.041	0.015	-0.004
18:3n-6	0.063	0.094	**0.158**[b]	0.140	0.104	-0.001	0.056	-0.079
20:3n-6	-0.145	-0.126	-0.060	-0.131	0.089	-0.049	0.029	-0.077
20:4n-6	**-0.268**[b]	-0.127	-0.078	**-0.396**[b]	**0.222**[b]	-0.057	-0.064	0.002
20:5n-3	**-0.216**[b]	-0.113	-0.070	**-0.278**[b]	**0.171**[b]	-0.067	-0.062	-0.023
22:5n-3	**-0.376**[b]	**-0.313**[b]	**-0.229**[b]	**-0.432**[b]	**0.189**[b]	-0.066	0.004	-0.079
22:6n-3	**-0.220**[b]	-0.145	-0.102	**-0.326**[b]	0.127	-0.065	0.006	-0.022

Values are standardized regression coefficients (β) from linear regression models adjusted for age, sex and the current height as a percentage of predicted adult height. HDL; High-density lipoprotein. BP; Blood pressure.

a)Logarithmic transformation was carried out because of a skewed distribution.

b)Statistical significance after Bonferroni correction for multiple comparisons: The *P* values of <0.002 are bolded.

Table 13. Polyunsaturated fatty acids (mol%) in plasma cholesteryl esters and phospholipids in the intervention group and in the control group at baseline and after 2-year follow-up.

	Intervention group				**Control group**				
	Baseline *n*=290	**2-year follow-up *n*=236**	**2-year change in mean**	***P* for 2- year change**	**Baseline *n*=174**	**2-year follow-up *n*=150**	**2-year change in mean**	***P* for 2-year change**	***P* for group*time interaction**
Cholesteryl esters									
Total PUFA	63.1 (62.7, 63.5)	63.5 (63.1, 63.9)	0.4	0.086	63.7 (63.2, 64.2)	63.2 (62.7, 63.7)	-0.5	**0.034**	**0.007**
18:2n-6	52.4 (51.9, 52.9)	52.6 (52.0, 53.1)	0.2	0.586	53.2 (52.6, 53.7)	52.6 (51.9, 53.2)	-0.6	**0.027**	**0.038**
18:3n-3	1.05 (1.02, 1.09)	1.08 (1.05, 1.12)	0.03	**0.044**	1.07 (1.03, 1.12)	1.01 (0.96, 1.05)	-0.06	**0.001**	**<0.001**
18:3n-6	0.96 (0.91, 1.01)	0.99 (0.94, 1.05)	0.03	0.351	0.90 (0.83, 0.97)	0.97 (0.90, 1.04)	0.07	0.086	0.445
20:3n-6	0.71 (0.69, 0.73)	0.73 (0.71, 0.74)	0.02	**0.015**	0.68 (0.66, 0.70)	0.70 (0.68, 0.72)	0.02	**0.025**	0.816
20:4n-6	5.91 (5.72, 6.09)	6.00 (5.82, 6.19)	0.09	0.074	5.85 (5.63, 6.06)	5.92 (5.70, 6.14)	0.07	0.286	0.780
20:5n-3	1.26 (1.17, 1.34)	1.29 (1.20, 1.39)	0.03	0.436	1.27 (1.16, 1.39)	1.29 (1.17, 1.40)	0.02	0.795	0.778
22:6n-3	0.82 (0.78, 0.86)	0.81 (0.77, 0.85)	-0.01	0.618	0.79 (0.75, 0.84)	0.78 (0.73, 0.83)	-0.01	0.432	0.761
Phospholipids									
Total PUFA	40.2 (40.0, 40.5)	40.3 (40.1, 40.6)	0.1	0.399	40.5 (40.2, 40.9)	40.2 (39.8, 40.5)	-0.3	**0.020**	**0.019**
18:2n-6	20.9 (20.6, 21.2)	21.1 (20.8, 21.4)	0.2	0.066	21.2 (20.8, 21.6)	21.1 (20.7, 21.5)	-0.1	0.554	0.108
18:3n-3	0.41 (0.39, 0.43)	0.42 (0.40, 0.44)	0.01	0.068	0.41 (0.38, 0.43)	0.39 (0.37, 0.41)	-0.02	0.094	**0.015**
20:3n-6	2.86 (2.79, 2.93)	2.89 (2.82, 2.96)	0.03	0.357	2.78 (2.70, 2.86)	2.82 (2.74, 2.91)	0.04	0.308	0.822
20:4n-6	8.35 (8.11, 8.58)	8.30 (8.06, 8.54)	-0.05	0.522	8.40 (8.12, 8.68)	8.27 (7.99, 8.55)	-0.13	0.159	0.481
20:5n-3	1.27 (1.19, 1.36)	1.29 (1.20, 1.38)	0.02	0.695	1.30 (1.20, 1.41)	1.36 (1.25, 1.46)	0.06	0.289	0.558
22:4n-6	0.33 (0.32, 0.34)	0.32 (0.31, 0.32)	-0.01	**0.002**	0.31 (0.30, 0.32)	0.31 (0.29, 0.32)	-0.001	0.138	0.461
22:5n-6	0.22 (0.21, 0.23)	0.22 (0.21, 0.22)	-0.005	0.073	0.21 (0.20, 0.22)	0.21 (0.20, 0.22)	-0.004	0.230	0.858
22:5n-3	1.28 (1.23, 1.33)	1.29 (1.24, 1.34)	0.01	0.805	1.26 (1.20, 1.32)	1.20 (1.14, 1.26)	-0.06	**<0.001**	**0.003**
22:6n-3	4.68 (4.48, 4.88)	4.50 (4.30, 4.70)	-0.18	**0.008**	4.69 (4.45, 4.93)	4.56 (4.32, 4.80)	-0.13	0.106	0.692

Values are means (95% confidence intervals) of proportion of polyunsaturated fatty acids in plasma cholesteryl esters and phospholipids at baseline and after 2-year follow-up, the 2-year changes in the means and p-values for the differences between the means at baseline and 2-year follow-up from the linear mixed models adjusted for age and sex. Study group, time and their interactions were included as fixed factors in the model and participant and school were included as random effects in the model. The *P* values of <0.05 are bolded. PUFA; polyunsaturated fatty acid.

5.6 ESTIMATED DESATURASE AND ELONGASE ACTIVITIES (STUDIES I, II AND III)

5.6.1 Food consumption and estimated desaturase and elongase activities

The consumption of vegetable oil-based margarine (fat 60-80%) was inversely associated and candy consumption was directly related to estimated SCD activity in plasma CE (Table 14). The consumption of vegetable oil-based margarine (fat 60-80%) was directly associated and candy consumption was inversely related to estimated elongase activity in plasma CE.

The consumption of vegetable oil-based margarine (fat 60-80%) was inversely associated with estimated SCD activity in plasma PL (Table 14). The consumption of skimmed milk was directly related to estimated D6D activity in plasma PL. The consumption of sour milk products (fat≥1%) was inversely associated and egg consumption was directly related to estimated D5D activity in plasma CE and PL. The consumption of vegetable oil-based margarine (fat 60-80%) was directly associated whereas the consumptions of sour milk products (fat≥1%) and ice cream and pudding were inversely associated with estimated elongase activity in plasma PL.

5.6.2 Estimated desaturase and elongase activities and cardiometabolic risk factors

Estimated SCD activity in plasma TG was directly associated with both cardiometabolic risk score and plasma concentration of TG (Table 15). Estimated D6D activity was directly related to cardiometabolic risk score. Estimated D5D activity was inversely associated with the plasma concentration of TG. A lower estimated elongase activity in plasma TG was associated with a higher cardiometabolic risk score and higher concentration of serum insulin.

Higher estimated SCD and D6D activities in plasma PL were associated with a higher cardiometabolic risk score and a higher plasma concentration of TG (Table 15). A lower estimated D5D activity was associated with a higher cardiometabolic risk score and a higher plasma concentration of TG. Estimated elongase activity in plasma PL was inversely associated with cardiometabolic risk score and with the plasma concentration of TG.

5.6.3 The effect of the lifestyle intervention on estimated desaturase and elongase activities

Estimated elongase activity in CE did not change significantly in the intervention group but decreased in the control group ($p=0.050$ for group*time interaction, Table 16). The intervention had no effects on estimated desaturase activities in plasma CE. The intervention had no effects on estimated desaturase or elongase activities in plasma PL.

Table 14. The associations of the food consumption with estimated desaturase and elongase activities in plasma cholesteryl esters and phospholipids (n=423).

	Cholesteryl esters				*Phospholipids*			
Food group	***SCD****	***D6D****	***D5D****	***Elongase***	***SCD****	***D6D****	***D5D****	***Elongase***
Butter and butter-oil mixture	-0.017	-0.070	0.084	-0.023	-0.039	**-0.110**[a]	**0.114**[a]	0.050
Vegetable oil	**-0.109**[a]	-0.055	0.074	**0.100**[a]	-0.069	-0.077	**0.099**[a]	0.071
Vegetable oil-based margarine (fat 60-80%)	**-0.192**[c,e]	**-0.102**[a]	-0.038	**0.194**[c,f]	**-0.190**[c,e]	**-0.107**[a]	0.006	**0.202**[c,f]
Low-fat vegetable oil-based margarine (fat<60%)	0.010	0.001	0.071	0.012	0.037	0.040	0.028	-0.040
Milk (fat≥1%)	0.001	-0.009	0.034	-0.043	-0.054	-0.074	0.038	0.031
Skimmed milk (fat<1%)	0.049	0.078	-0.050	-0.016	**0.100**[a]	**0.172**[c,d]	-0.070	-0.094
Sour milk products (fat≥1%)	**0.115**[a]	-0.008	**-0.201**[c,e]	-0.072	**0.111**[a]	0.120[a]	**-0.165**[b,d]	**-0.103**[a]
Low-fat sour milk products (fat<1%)	-0.028	-0.067	0.022	0.053	0.055	-0.047	0.027	-0.026
Cheese (fat>17%)	-0.028	-0.026	0.030	0.010	0.004	-0.015	0.036	0.013
Low-fat cheese (fat≤17%)	-0.032	0.003	0.093	0.004	0.015	0.043	0.061	-0.022
Ice cream and pudding	**0.104**[a]	0.062	-0.051	**-0.125**[a]	**0.109**[a]	0.070	-0.058	**-0.134**[b,e]
Red meat	-0.011	0.030	0.082	-0.001	-0.013	0.006	0.085	0.012
Poultry	0.018	-0.065	0.076	-0.020	-0.007	-0.081	0.066	-0.020
Fish	-0.040	0.001	0.013	0.037	-0.068	-0.064	-0.002	0.041
Egg	-0.038	-0.035	**0.160**[b,d]	0.003	-0.025	-0.093	**0.172**[b,d]	0.004
Chocolate and hot chocolate powder	0.060	0.064	0.006	-0.079	0.047	0.022	-0.028	-0.098
High-fiber grain products (fiber≥5%)	**-0.110**[a]	**-0.111**[a]	0.064	**0.120**[a]	-0.056	-0.004	0.059	**0.106**[a]
Low-fiber grain products (fiber < 5%)	-0.060	-0.096	-0.013	0.067	-0.002	-0.089	0.031	0.025
Sugar and honey	0.012	-0.008	0.049	-0.014	-0.037	-0.013	0.068	0.018
Candy	**0.190**[c,e]	**0.125**[a]	-0.071	**-0.178**[c,f]	0.099	-0.005	-0.076	**-0.131**[a]
Sugar-sweetened beverages	0.045	0.078	-0.058	-0.029	-0.015	0.051	-0.051	0.014
Artificially sweetened beverages	0.016	-0.018	0.055	-0.004	0.075	-0.001	0.048	-0.064

Values are standardized regression coefficients from linear regression models adjusted for age, sex, physical activity and total energy intake. The *P* values of <0.05 are bolded. SCD, stearoyl-CoA desaturase *(16:1n-7/16:0)*; D6D, Δ6 desaturase (18:3n-6/18:2n-6 in cholesteryl esters, 20:3n-6/18:2n-6 in phospholipids); D5D, Δ5 desaturase (20:4n-6/20:3n-6).*Logarithmic transformation was carried out, because of skewed distributions.

[a]*P*<0.05. [d]*P*<0.05, after FDR-adjustment for multiple comparisons.

[b]*P*<0.01. [e]*P*<0.01, after FDR-adjustment for multiple comparisons.

[c]*P*<0.001. [f]*P*<0.001, after FDR-adjustment for multiple comparisons.

[c]*P*<0.001. [f]*P*<0.001, after FDR-adjustment for multiple comparisons.

Table 15. Associations of estimated desaturase and elongase activities in plasma and phospholipids and triacylglycerols with cardiometabolic risk factors (n=384).

	Cardiometabolic risk score	**Insulin**	**Glucose**	**Triacylglycerols[a]**	**HDL cholesterol**	**Systolic BP**	**Diastolic BP**	**Waist circumference[a]**
Phospholipids								
SCD (16:1n-7/16:0)	**0.168[b]**	0.072	-0.040	**0.323[b]**	-0.082	0.098	0.024	0.075
D6D (20:3n-6/18:2n-6)	**0.208[b]**	0.012	-0.073	**0.286[b]**	**-0.221[b]**	0.096	0.089	**0.146[b]**
D5D (20:4n-6/20:3n-6)	**-0.205[b]**	-0.069	-0.069	**-0.338[b]**	0.132	-0.045	-0.0004	-0.041
Elongase (18:1n-7/16:1n-7)	**-0.209[b]**	-0.121	-0.023	**-0.338[b]**	0.059	-0.138	0.007	-0.113
Triacylglycerols								
SCD (16:1n-7/16:0)	**0.192[b]**	0.077	0.069	**0.376[b]**	-0.061	-0.024	0.070	0.044
D6D (18:3n-6/18:2n-6)	**0.175[b]**	**0.167[b]**	**0.149[b]**	**0.286[b]**	0.028	0.047	0.084	-0.033
D5D (20:4n-6/20:3n-6)	-0.122	-0.011	-0.020	**-0.211[b]**	0.116	-0.012	0.028	-0.047
Elongase (18:1n-7/16:1n-7)	**-0.291[b]**	**-0.177[b]**	-0.103	**-0.519[b]**	0.088	-0.085	-0.051	-0.026

Values are standardized regression coefficients (β) from linear regression models adjusted for age, sex and the current height as a percentage of predicted adult height. HDL; High-density lipoprotein. BP; Blood pressure. SCD; stearoyl CoA-desaturase. D6D; Δ6 desaturase. D5D; Δ5 desaturase.
a) Logarithmic transformation was carried out because of a skewed distribution.
b) Statistical significance after Bonferroni correction for multiple comparisons: The *P* of <0.004 are bolded.

Table 16. Estimated desaturase and elongase activities in plasma cholesteryl esters and phospholipids in the intervention group and in the control group at baseline and after 2-year follow-up.

	Intervention group				**Control group**				
	Baseline *n*=290	**2-year follow-up *n*=236**	**2-year change in mean**	***P* for 2-year change**	**Baseline *n*=174**	**2-year follow-up *n*=150**	**2-year change in mean**	***P* for 2-year change**	***P* for group*time interaction**
Cholesteryl esters									
SCD (16:1n-7/16:0)	0.23 (0.22, 0.24)	0.24 (0.23, 0.25)	0.01	0.173	0.21 (0.20, 0.22)	0.23 (0.21, 0.24)	0.02	**0.017**	0.304
D6D (18:3n-6/18:2n-6)	0.019 (0.017, 0.020)	0.019 (0.018, 0.020)	0.001	0.361	0.017 (0.016, 0.019)	0.019 (0.017, 0.020)	0.002	0.068	0.390
D5D (20:4n-6/20:3n-6)	8.50 (8.15, 8.84)	8.45 (8.10, 8.80)	-0.05	0.659	8.87 (8.46, 9.27)	8.73 (8.32, 9.15)	-0.14	0.336	0.633
Elongase (18:1n-7/16:1n-7)	0.45 (0.44, 0.47)	0.45 (0.43, 0.46)	-0.01	0.318	0.48 (0.46, 0.50)	0.44 (0.42, 0.46)	-0.04	**0.001**	0.050
Phospholipids									
SCD (16:1n-7/16:0)	0.020 (0.019, 0.021)	0.020 (0.019, 0.021)	0.0002	0.725	0.019 (0.018, 0.020)	0.019 (0.018, 0.020)	0.001	0.277	0.526
D6D (20:3n-6/18:2n-6)	0.139 (0.135, 0.143)	0.139 (0.135, 0.143)	0.00004	0.984	0.133 (0.128, 0.138)	0.136 (0.131, 0.141)	0.003	0.315	0.438
D5D (20:4n-6/20:3n-6)	3.01 (2.88, 3.13)	2.97 (2.84, 3.09)	-0.04	0.385	3.13 (2.98, 3.28)	3.03 (2.88, 3.18)	-0.1	0.086	0.421
Elongase (18:1n-7/16:1n-7)	2.60 (2.49, 2.71)	2.59 (2.48, 2.70)	-0.01	0.796	2.69 (2.55, 2.82)	2.55 (2.41, 2.68)	-0.14	**0.043**	0.153

Values are means (95% confidence intervals) of estimated desaturase and elongase activities in plasma cholesteryl esters and phospholipids at baseline and after 2-year follow-up, the 2-year changes in the means and p-values for the differences between the means at baseline and 2-year follow-up from the linear mixed models adjusted for age and sex. Study group, time and their interactions were included as fixed factors in the model and participant and school were included as random effects in the model. The *P* values of <0.05 are bolded. SCD; stearoyl CoA-desaturase. D6D; Δ6 desaturase. D5D; Δ5 desaturase.

6 Discussion

6.1 PRINCIPAL FINDINGS

A new finding of this doctoral thesis is that a higher consumption of high-fiber grain products is associated with a lower proportion of oleic acid in plasma CE and PL and that a higher consumption of candy is related to higher proportion of palmitoleic acid and oleic acid in plasma CE among children. The results also showed that a higher consumption of vegetable oil-based margarine (fat 60-80%) is related to higher plasma proportions of PUFA such as linoleic acid and α-linolenic acid, lower in SFA such as myristic acid, palmitic acid and stearic acid and lower in MUFA such as palmitoleic acid and oleic acid.

Another novel finding in children is that a lower estimated elongase activity in plasma TG and PL was associated with a higher cardiometabolic risk score. Moreover, estimated SCD and D6D activities in plasma TG and PL were directly associated with cardiometabolic risk score. The study also showed that higher proportions of myristic and palmitoleic acid in plasma PL and TG and a lower proportion of linoleic acid in plasma TG were associated with higher cardiometabolic risk score and plasma concentration of TG.

The results demonstrated that a 2-year individualized and family-based dietary and physical activity intervention in school-aged children attenuated the decrease in the proportion of total PUFA and the increase in the proportion of total MUFA in plasma CE and PL relative to the control group. The proportion of α-linolenic acid increased and the proportion of oleic acid decreased in the intervention group compared with the control group. Moreover, the proportion of stearic acid in plasma CE decreased in the intervention group relative to the control group.

6.2 STRENGHTS AND LIMITATIONS

6.2.1 Study population and study design

A major strength of the PANIC study is a relatively large population sample of healthy children who have no diseases and have not been exposed to smoking, alcohol or medication. The strengths of this doctoral thesis also include detailed assessment of dietary factors by food records in the *Study I*, the use of a continuous cardiometabolic risk score in the *Study II*, a carefully conducted, individualized and family-based dietary and physical activity intervention in the *Study III* and a detailed fatty acid analysis in *Studies I, II and III.* As the sensitivity of individual fatty acid to dietary or metabolic changes may be different in plasma CE, PL and TG, separate analysis can reveal changes not seen in all fractions or whole plasma. Furthermore, the number of measurements of possible confounding factors, including physical activity, electronic media time, total energy intake, body fat percentage and household income, that were controlled for in the analyses is a strength.

The PANIC study had a representative population sample of 6-8-year old children from the city of Kuopio since all the children from the age group were invited to the study by letters to their parents. Of all the children invited, 70% participated to the baseline examinations of the PANIC study. This rate of participating is quite high which means that the results of this doctoral thesis can apply to the general population of the Finnish children in this age group. Based on the comprehensive school health examination data, the participants did not differ in age, sex distribution or body mass index - standard deviation score (BMI-SDS) from all children who started the first grade in Kuopio during years 2007–2009. These features were the only ones available for both participants and non-participants.

Therefore, the selection bias should be taken into account such in a way that there might be a possibility that the families with higher awareness of health relating lifestyle participated in the study.

A weakness of the *Study III* is that we did not randomly allocate the children in the intervention and control groups. However, we divided the children into groups by matching them according to the location and size of the schools. This allowed us to organize after school exercise clubs that minimized the non-intentional intervention in the control group. This is why we controlled for the possible random effect of the schools in the mixed model analyses.

We excluded six children from the intervention study during baseline examinations. These six children either had severe physical disability or withdrawed from the study. Of the 506 children who participated in the baseline study, 440 (87%) attended the 2-year follow-up study. Altogether 45 (15%) children in the intervention group and 21 (11%) children in the control group dropped out during the 2-year follow-up. The drop-out rate of 13% during the intervention was relatively low, though the burden of the intervention was quite heavy for the families. Although no differences in age, sex distribution, or BMI-SDS between the drop-outs in the intervention and control group were found, these issues could have affected the results of this doctoral thesis by diminishing the statistical power of the analyses. However, the power calculation of the PANIC study showed enough power in the analyses despite the drop-outs.

One weakness is that the cross-sectional study design in *Studies I and II* does not allow us to draw conclusions about the time order or causality either of the relationships of dietary factors with plasma fatty acid composition nor the relationships of plasma fatty composition with cardiometabolic risk factors. However, in *Study III* we were able to utilize the longitudinal study design. The strengths of the intervention (*Study III*) were a relatively large population sample of children and a carefully conducted, individualized and family-based dietary and physical activity intervention with a long follow-up. The median (interquartile range) of the intervention period was 2.1 (2.1-2.2) years in both groups which is an adequate time to have an effect on plasma fatty acid composition.

6.2.2 Assessment of food consumption

We made the dietary analysis based on food consumption rather than nutrient intake since lack of these type of studies in children. Moreover, the results of this thesis are easier to adopt to the practise when we dealed with the consumption of food items rather than nutrient intake. In the *Study I*, the food consumption was assessed by food records of four consecutive predefined days which is a strength. We also included 30 food records of three consecutive days in the analyses since those records were completed with the most care and were considered inclusive by a clinical nutritionist. The number of four day food records was substantially higher (393 records). While having some limitations to it, a food record is considered to be a superior method in assessing dietary factors (63). A food record is based on the actual food consumption of the respondent that fills in all the food and drinks in real time or as soon as possible after consumption. Therefore, the biases resulting from bad memory are absent. Keeping a food record for four days is less burden to the respondent than a seven day food record. Moreover, a four day food record is a valid method for assessing the intake of energy nutrients and has been suggested to adequately represent the diet of 6-year-old children at a group level in a previous Finnish study (138). However, reporting food and drinks for four days may not be a valid assessment for intakes of some nutrients, such as MUFA, PUFA and iron that may need longer period of time to be assessed accurately. These nutrients have wide day-to-day variation and therefore the food record may have to be kept longer than four days. In this doctoral thesis the food consumption of the children at the level of foodstuff was of interest. We did not study intakes of nutrients. Of note, however, that total energy intake was considered as a confounding factor in the

analysis of *Study I.* The parental assistance in filling out the food record has been shown to diminish underreporting of energy intake (139) and therefore in the PANIC study the parents took the responsibility of filling the food diaries on behalf their 6-8-year old children at the baseline.

The parents were instructed by a clinical nutritionist to record all food and drink consumption of their child at home and outside home. The schools provided us the information about A clinical nutritionist also reviewed the food records at their return and filled in the missing information together with the children and their parents. Carefulness in instructing and reviewing the food record has minimized the errors in this self-reporting, open-ended dietary assessment method. However, the motivation of the respondent, ability to assess and fill in their food consumption and misreporting are challenges of this method. Misreporting is often due to the changes in eating for the sake of easing of the writing or underreporting the foods and drinks that are being considered unhealthy (63,140).

Data on food consumption obtained is based on laboratory analyses on the nutrient compositions of different food items used in Finland and on international analyses of the nutrient compositions of food items (134). We used Micro-Nutrica® dietary analysis software to analyze the consumption of certain food groups. A clinical nutritionist updated the software by adding new food items and products with their precise nutritional contents received from the producers. The main sources of dietary fat and carbohydrate were the food groups of interest in the *Study I*. The consumption of certain foods may have an effect on the whole dietary pattern or may replace other foods in the diet. However, we had an opportunity to take other dietary factors into account in the analyses of the *Study I* that reduced the possibility of residual confounding due to other dietary factors.

6.2.3 Assessment of cardiometabolic risk

Another strength is that we used a continuous cardiometabolic risk score and assessed several individual features of the metabolic syndrome in the *Study II*. A continuous cardiometabolic risk score is a more sensitive and less controversial way to describe cardiometabolic risk in children than dichotomous definitions for metabolic syndrome (102,104,105). In fact, the approved definition of paediatric metabolic syndrome is still lacking. By continuous risk scores the early signs of metabolic dysfunctions in the body were detected more sensitive way among the study population of children who were healthy. Moreover, the continuous risk scores are increasingly used in the studies among children over the world (102). Previous studies have calculated the continuous risk score among population samples of children and adolescents aged 6-17 years and these studies have been conducted both in Europe and United States as well as in India (105,141,142). Moreover, the validity on the use of continuous risk scores in pediatric research has been concluded in an earlier study and therefore it is valid to create risk scores that are specific to the study population (105).

6.2.4 Assessment of plasma fatty acid composition

The blood samples at baseline and 2-year follow-up for the analysis of fatty acid composition were taken into EDTA vacuum tubes after 12-h fasting. The plasma was separated by centrifugation and stored at -80°C for approximately three and a half years. A previous study has shown that plasma fatty acid composition remains the same in the storage of -80°C for at least ten years (143). In this doctoral thesis, the storage was conducted at the same temperature but for the shorter period of time. The analysis of plasma fatty acid composition was made after both the baseline and the 2-year plasma samples were ready for the study subject. The samples of the two timepoints from the same children were analyzed at the same time. This dimished the error that could have been caused by the gas chromatography device.

The analysis of plasma fatty acid composition was made using a method by Ågren et al (1992). The lipids were extracted from plasma samples with chloroform-methanol (2:1) that is a solvent with a feature of good recovery of lipids in plasma. Extracted lipids were separated into plasma lipid fractions with a single aminopropyl solid phase glass column. Afterwards fatty acids were esterified and analyzed with a gas chromatography. This method is widely used and offers high recovery and purity more rapidly than thin layer chromatography (132).

Caution should be taken when interpreting the results regarding different plasma fatty acid fractions since the day to day variation of the fatty acid composition of plasma CE, PL and TG varies. The plasma fatty acid composition of CE and PL are more solid whereas the fatty acid composition of TG is more sensitive to change during the day (144). For this reason and for the results of previous studies (8,50–52,9) it has been determined that the fatty acid compositions of plasma CE and PL reflect the dietary fat intake of the last weeks or months and that of plasma TG reflects the dietary fat quality of few last meals consumed. However, even after fatty test meal the fatty acid composition of VLDL TG is very similar with its fasting composition whereas the fatty acid composition of chylomicron TG resembles closely that of test meal (145). This suggests that especially in the fasting state the endogenous fatty acid status and metabolism are important determinants of plasma TG composition.

The findings of this doctoral thesis also suggest that fatty composition of TG better reflect the endogenous metabolism of fatty acids in the liver than fatty composition of CE or PL. Fatty acid composition of TG also strongly reflects recent dietary SFA and PUFA compositions, especially with respect to myristic acid, linoleic acid and long-chain *n–3* fatty acids (146). However, the time interval between the drawing of blood samples and the completion of the dietary records varied, which decreased our ability to assess the association of diet with the fatty acid composition of TG in *Study I* (52). Moreover, the fractions have their distinct compositions of fatty acid with different proportions, and selective mechanisms favoring incorporation of certain fatty acid may dilute the effects of both dietary and metabolic changes. An association being present in only one or two fractions does not necessarily mean that the association does not represent a real dietary or metabolic effect. Although interpreting these results may be problematic, this doctoral thesis showed consistent results of plasma fatty acid composition having associations with food consumption and cardiometabolic risk factors.

A weakness in *Studies I, II and III* is that we were not able to assess the true enzyme activities, because liver biopsy would be needed for that purpose (38). We used ratios calculated from the proportions of individual fatty acids for the estimation of desaturase and elongase activities in plasma lipid fractions. This approach has been widely used (39,133). However, changes in estimated desaturase and elongase activities may also be due to other factors than actual changes in these activities, such as a high dietary intake of certain fatty acid or changes in the preceding or subsequent desaturation or elongation steps.

Another weakness of this doctoral thesis is that we could not investigate the metabolic pathways of fatty acid synthesis and fatty acid transformation in adipose tissue, liver and skeletal muscle, all of which may have an effect on plasma fatty acid composition (45). In addition, we could not control for genetic factors that could have modified the association between food consumption and plasma fatty acid composition (71,72). Especially the polymorphism in FADS 1 and FADS 2 could have been of interest of controlling for, since they have been reported to affect plasma fatty acid composition (74).

6.3 PLASMA SATURATED FATTY ACIDS

6.3.1 The associations of food consumption with plasma saturated fatty acids

We found that a higher consumption of butter and butter-oil mixture as well as a higher consumption of milk (fat≥1%) were associated with a higher proportion of myristic acid in

plasma CE. The explanation for this is that dairy fat has an abundance of myristic acid. Higher consumptions of these foods rich in milk fat were also related to higher proportions of pentadecaenoic acid and margaric acid in plasma PL. These odd chain fatty acids have been reported to be biomarkers for milk fat intake (57–59,147). The explanation for the stronger associations of consumption of foods containing lots of milk fat with pentadecanoic acid than with margaric acid may be that margaric acid reflects also fish consumption (148,149). However, fish consumption was not related to margaric acid in our study, opposite to the results of the European Prospective Investigation into Cancer and Nutrition (EPIC) Study (148). The reason for this observation may be that only few children in our study population ate fish.

A higher consumption of vegetable-oil-based margarine (fat 60-80%) was also related to a lower proportion of two SFA, palmitic and stearic acid, in plasma CE. These observations suggest that children who consume more vegetable-oil-based margarine (fat 60-80%) eat less foods rich in SFA than other children. However, this is only speculation that needs further investigation by the PANIC study group.

Skimmed milk was the only food that was directly associated with the proportion of arachidic and nervonic acid in PL. Arachidic and nervonic acid originate practically exclusively from sphingomyelin suggesting that skimmed milk increases the proportion of sphingomyelin in plasma PL or changes the composition of sphingomyelin.

6.3.2 The associations of plasma saturated fatty acids with cardiometabolic risk factors

In plasma TG, the proportions of myristic and palmitic acids were directly associated with cardiometabolic risk score and plasma concentrations of insulin and TG. This indicates that the composition of plasma TG changes to a more saturated direction with its increasing concentration. One explanation for these findings may be the increased *de novo* fatty acid synthesis in liver (150) that can be activated by a high intake of carbohydrates and positive energy balance (62). Also the activity of SCD is induced by glucose and the increased activity seems to be protective towards hepatic fat accumulation (146). High SFA intake may also upregulate SCD activity (151). Our other finding of direct association of estimated SCD activity in plasma CE with the consumption of candy but not with the consumption of major SFA sources in children suggests, however, that the intake of carbohydrates and positive energy balance may have had greater effect on plasma fatty acid composition than SFA intake per se. The predominant role of *de novo* synthesis is also supported by the lack of association between the proportions of odd-chain fatty acids (15:0 and 17:0) in PL with cardiometabolic risk score or plasma TG concentration linked to our findings of a direct association between these fatty acids in plasma PL with the consumption of SFA food sources and an inverse association with the consumption of candy.

We found inverse associations between the proportions of arachidonic, behenic and lignoceric acids in plasma PL with the plasma concentration of TG. These fatty acids originate almost exclusively from sphingomyelin indicating that the relative amount of sphingomyelin in plasma PL decreases or that the fatty acid composition of sphingomyelin changes towards shorter chain fatty acids with the increasing plasma TG. Changes in the amount or composition of plasma sphingomyelin may be of great importance since sphingomyelin is an inhibitor of several lipolytic enzymes, eg. lecithin-cholesterol acyltransferase, endothelial lipase and hepatic lipase, and it has been proposed to have both protective and pro-atherogenic effect (152–154).

6.3.3 The effect of lifestyle intervention on plasma saturated fatty acids

We found that the proportion of stearic acid in CE decreased in the intervention group but not in the control group among children. In a previous study with adults, a decrease in the proportion of stearic acid in plasma CE was reported during a 6-week Healthy Nordic Diet intervention (128). The PANIC study group have previously shown that the intervention decreased slightly the consumption of butter-based spreads (155), which may contribute to

this finding. However, the intake of butter and butter-oil mixture was not associated with stearic acid in CE at baseline in the *Study I*. In addition, a dietary intervention to decrease dietary SFA and cholesterol intake resulted in a lower proportion of palmitic acid in serum TG but in a higher proportion of stearic acid in serum CE among children 5 years of age (64). Therefore, the more probable explanation for the decreased proportion of stearic acid in CE is the increased consumption of vegetable oil-based margarine in the intervention group. In line with this, a decrease in the proportion of stearic acid in CE but not in PL has been found after a rapeseed oil diet (43).

6.4 PLASMA MONOUNSATURATED FATTY ACIDS

6.4.1 The associations of food consumption with plasma monounsaturated fatty acids

A higher consumption of vegetable-oil-based margarine (fat 60-80%) was also related to lower proportions of palmitoleic and oleic acids in plasma CE and to a lower proportion of palmitoleic acid in plasma PL. These findings suggest that the high PUFA intake decreases SCD activity and thereby the endogenous production of MUFA (see Chapter 6.6.1).

Consistent with the results of previous study (43) a higher consumption sour milk products (fat≥1%), and thus a lot of SFA, were associated with a larger proportion of oleic acid in plasma CE. As stated before, the proportion of MUFAs in plasma does not reflect dietary MUFA intake but rather SFA intake (5). There is also some previous evidence that a higher intake of SFA in the Western diet is related to a higher intake of MUFA (156).

We observed that a higher consumption of candy was associated with higher proportions of palmitoleic and oleic acids in plasma CE. The reason for these findings may be that children who eat more candy also eat more foods rich in SFA. However, these associations remained similar even after taking milk, sour milk products and ice cream and pudding into account, which suggests that these associations are related more likely to high intake of carbohydrates than to intake of SFA. It has been reported earlier that an increase in intake of carbohydrates, rather than an intake of SFA, is associated with increased plasma SFA proportions and that way with increased proportion of palmitoleic acid in plasma (157). Another explanation for these observations may be that candy includes fudge that contains a lot of SFA. However, only few children in our study population consumed fudge and we could not analyse the relationships of the consumption of fudge with the proportions of different SFA in plasma CE or PL.

Interestingly, we observed that a higher consumption of high-fiber grain products was associated with a lower proportion of oleic acid in plasma CE and PL. One explanation for this could be that fat in whole grains is mainly polyunsaturated fat and the total fat content of whole grains is low. In contrast, whole grains have abundance of fermentable carbohydrates that can be fermented to short-chain fatty acid in the colon (158). Increased short-chain fatty acid production has been reported to relate to decreased serum lipid levels (159), which may also modify the fatty acid composition of different plasma lipid fractions. In addition, dietary fiber may also affect lipid metabolism by other mechanisms, such as increasing fecal fat excretion and decreasing intestinal uptake of cholesterol and fatty acids (160). Finally, children who consumed less high-fiber grain products may have had a higher consumption of foods rich in SFA which are known to correlate positively with MUFA intake in Western people not favouring olive oil (156).

6.4.2 The associations of plasma monounsaturated fatty acids with cardiometabolic risk factors

In plasma TG and PL, the proportion of palmitoleic acid was directly associated with cardiometabolic risk score and plasma concentrations of insulin and TG. A previous study has reported that the proportion on palmitoleic acid in plasma increases due to the increased activation of SCD in the hepatic *de novo* fatty acid synthesis (13). Since the results of this

doctoral thesis also showed direct associations of estimated SCD activity in plasma TG and PL with plasma concentration of TG, this view is supported. The activity of SCD is induced by glucose and the increased activity seem to protect from hepatic fat accumulation (146) and fatty acids are released into the blood stream. Thus, high intake of carbohydrates and furthermore, the positive energy balance activates the *de novo* fatty acid synthesis in liver (62) which may be one explanation for the results.

We found an inverse association of the proportion of nervonic acid in plasma PL and the plasma concentration of TG. This fatty acid originates from sphingomyelin. Thus this finding only confirms the earlier speculation of the idea that the relative amount of sphingomyelin in plasma PL or the proportions of very long chain fatty acids in sphingomyelin decrease with the increasing concentration of plasma TG.

6.4.3 The effect of lifestyle intervention on plasma monounsaturated fatty acids

We found a decrease in the proportion of oleic acid in CE in the intervention group compared with the control group. However, since the margarines in Finland are rich in rapeseed oil, the intake of MUFA increased also in the intervention group (155), and yet the proportion of MUFA and oleic acid was found to decrease in CE and PL compared with the control group. This could be a consequence of more efficient incorporation of linoleic acid and α-linolenic acid into plasma lipid fractions replacing MUFA. The pattern with increased proportion of oleic acid and decreased proportions of linoleic acid and alpha-linoleic acid seen in the control group may suggest increased *de novo* lipogenesis and SCD activity whereas this development may have been prevented by the better diet quality in the intervention group. This view is also supported, despite the lack of the significant interaction between group and time, by the increased proportion of palmitoleic acid and increased estimated SCD activity in CE in the control group.

6.5 PLASMA POLYUNSATURATED FATTY ACIDS

6.5.1 The associations of food consumption with plasma polyunsaturated fatty acids

A higher consumption of vegetable oil-based margarine (fat 60-80%) was related to higher proportions of linoleic and α-linolenic acid in plasma CE and PL. These findings provide further evidence for the results of other Finnish studies in adults and children (56,161). The reason for the direct associations of vegetable oil-based margarines (fat 60-80%) with plasma linoleic and α-linolenic acid is that margarines in Finland mainly contain rapeseed oil that is rich in these PUFA.

The consumption of ice cream and pudding was directly associated with the proportion of osbond acid and inversely related to the proportion of DHA in plasma PL. This could be due to a low intake of DHA in children with the high consumption of ice cream or pudding. A low intake of *n-3* fatty acid was associated with decreased DHA and increased levels of *n-6* fatty acid, especially osbond acid, among animals (162). However, a positive correlation between the concentration of DHA and osbond acid in erythrocyte PL was reported in Canadian preschool children (163). In the present study the proportion of DHA in plasma PL correlated negatively with two *n-6* fatty acids, adrenic acid ($r=-0.413$, $p>0.001$) and osbond acids ($r=-0.238$, $p>0.001$). One possible explanation for this discrepancy could be the low dietary intake of DHA and hence a low proportion of DHA in erythrocyte PL among children in the Canadian study (163). As DHA and osbond acid are produced by the same pathway, it could be suspected that their concentrations change in the same direction when the activities of desaturases or other participating enzymes change considering that those fatty acids are not competing from the same enzymes. However, already a small increase in the dietary intake of DHA can cause a significant response in its proportion in plasma and membrane lipids (164). It is possible that the replacement of *n-6* fatty acid by dietary DHA in PL overwhelms the impact of the possible differences in desaturase activities.

The consumption of skimmed milk was directly related to the proportion of EPA in plasma CE. One explanation could lie behind the speculation that perhaps the children who drink skimmed milk, also eat foods rich in EPA or the foods that are rich in the fatty acids that have a role in desaturation and elongation process of EPA. As this association was not explained by fish consumption, this finding could at the same time be due to changes in elongase or desaturase activities (165).

We observed that a higher consumption of candy was associated with a lower proportion of DHA in plasma CE and the same tendency was seen in plasma PL. The reason for this finding may be that children who eat more candy, eat less fish. However, these associations remained similar even after taken fish intake into account. We could adjust for certain foods that could have an effect on the whole dietary pattern but these speculations need further investigation by the PANIC study group.

6.5.2 The associations of plasma polyunsaturated fatty acids with cardiometabolic risk factors

In plasma TG, the proportions of most PUFA were inversely associated with cardiometabolic risk score and plasma concentration of TG. These findings are in line with the results of a previous study (166). In the present study, lower proportions of linoleic acid, arachidonic acid, EPA, DPA and DHA in plasma TG was associated with higher cardiometabolic risk score and plasma TG concentration. The associations with concentration of TG most likely explain the results of the cardiometabolic risk score. The fatty acid proportions of TG in circulating blood are affected by endogenous fatty acid metabolism rather than diet because in this doctoral thesis the time between last meal and blood sampling was approximately 12 hours. These findings suggest that, in addition to possibly decreased *de novo* synthesis of SFA and MUFA, increased desaturation and elongation pathway of *n-3* and *n-6* fatty acids is associated with lower plasma concentration of TG. Conversely, the proportions of the same PUFA were directly associated with the concentration of HDL cholesterol. These metabolic pathways of *n-3* and *n-6* fatty acids naturally need the substrate fatty acid to be accessible for the body to use. These substrates, linoleic and α-linolenic acids are needed from the diet because the human body cannot produce them itself. It has been established before that higher dietary intake of PUFA is associated with lower mortality of cardiovascular diseases (167,168).

Interestingly, the proportion of DPA but not those of EPA or DHA in plasma TG and PL was inversely associated with serum insulin concentration. Fish products contain all these *n-3* fatty acids, but the concentration of DPA is usually lower than those of EPA and DHA and the effect of increased fish or fish oil intake on its concentration in plasma is modest compared to EPA and DHA (164). Therefore, it could be speculated that the proportion of DPA reflects more accurately the efficiency of desaturation and elongation pathway than EPA and DHA.

6.5.3 The effect of lifestyle intervention on plasma polyunsaturated fatty acids

We found an increase in the proportion of α-linolenic acid in CE in the intervention group compared with the control group. In addition, the proportion of linoleic acid decreased in the control group. These findings may be caused by the substantial increase in the consumption of vegetable oil-based margarine (fat 60-80 %) and increase in the intake of PUFA in the intervention group, as previously reported by the PANIC study group (155). In addition, the results of the *Study I* showed that the consumption of vegetable oil-based margarine is directly associated with the proportion of linoleic and α-linolenic acid in CE at baseline. These findings together may result to the higher intake of linoleic acid and α-linolenic acid in the intervention group and may explain the differences in their proportions in CE. However, it is also possible that higher intake of PUFA has had beneficial effect on liver fatty acid metabolism as PUFA has been shown to reduce liver fat content, *de novo* lipogenesis and SCD activity (169,170). The pattern with increased proportion of oleic acid

and decreased proportions of linoleic acid and alpha-linoleic acid seen in the control group may suggest increased *de novo* lipogenesis and SCD activity whereas this development may have been prevented by the better diet quality in the intervention group.

6.6 ESTIMATED DESATURASE AND ELONGASE ACTIVITIES

6.6.1 Consumption of foods and estimated desaturase and elongase activities

We found an inverse association between the consumption of vegetable oil-based margarine (fat 60-80%) with estimated SCD activity in plasma CE and PL. The explanation for this observation may be that a higher PUFA intake inhibits SCD and D6D activities (151). We also found that the consumption of candy was directly associated with estimated SCD and D6D activities in plasma CE and PL. One reason for this could be that a high carbohydrate intake increases *de novo* lipogenesis and SCD activity (171). Changes in estimated desaturase activities may also be due to other factors than actual changes in these activities, such as a higher dietary intake of certain fatty acid or changes in the preceding or subsequent desaturation or elongation steps.

We observed the association of a higher consumption of sour milk products (fat≥1%) with a lower estimated D5D activity in plasma CE and PL. The lower D5D activity may contribute to the higher estimated D6D activity in plasma PL due to the accumulation of dihomo-γ-linolenic acid that is used in the calculation of D6D activity in PL. Moreover, a higher consumption of egg was associated with a higher estimated D5D activity in plasma CE and PL. This is probably explained by a high content of arachidonic acid in egg and that arachidonic acid is used to calculate the estimated D5D activity in both CE and PL. However, the consumption of egg had a weak inverse association with the proportion of dihomo-γ-linolenic acid in plasma CE and PL, suggesting that arachidonic acid affects the elongation or desaturation steps between linoleic and arachidonic acid.

The consumption of vegetable oil-based margarine (fat 60-80%) was directly associated with estimated elongase activity in plasma CE and PL, whereas the consumption of candy was inversely related to estimated elongase activity in CE. These associations are opposite to those found between the consumption of these foods and SCD activity, suggesting that possible inhibition of SCD activity by PUFA is associated with increased elongase activity and induction of SCD activity by carbohydrates with decreased elongase activity. However, as the end product in the calculation of SCD activity estimate is the same as the precursor fatty acid in the calculation of elongase activity estimate, there is a possibility that the change in one enzyme activity contributes to the estimate of the other enzyme.

6.6.2 Estimated desaturase and elongase activities and cardiometabolic risk

There are no previous studies on the association of the estimated elongase activity in plasma lipid fractions with cardiometabolic risk in children. Recently, higher estimated elongase activity calculated from the fatty acid composition of erythrocyte membranes has been related to improved glucose tolerance among adults (20). Consistent with these results, we found that higher estimated elongase activity in TG was associated with a lower cardiometabolic risk score and fasting serum insulin concentration among children. This estimated elongase activity calculated from the ratio of *cis*-vaccenic acid to palmitoleic acid may describe the activity of elongase 6 or elongase 5, which may also be involved in the production of dihomo-γ-linolenic acid and DPA (165). The proportion of this precursor fatty acid, palmitoleic acid, was directly associated with cardiometabolic risk score suggesting that elongase 5 activity is decreased in children with a higher cardiometabolic risk score. In addition, the proportion of DPA in plasma TG was inversely associated with cardiometabolic risk score and insulin, glucose and triacylglycerol concentrations. By contrast, the proportion of dihomo-γ-linolenic acid in plasma PL associated directly with cardiometabolic risk score and plasma TG concentration. However, it could be speculated that this finding is not related

to increased elongase activity but to accumulation of dihomo-γ-linolenic acid due to increased D6D and decreased D5D activity. In any case, it cannot be definitely concluded based on this elongase estimate whether the endogenous activity of elongase is actually changed or is the estimate affected by the changes in desaturase activities. On the other hand, changes in elongase activities may also affect SCD, D5D and D6D estimates.

We also found that higher estimated D6D activity in TG and PL and lower estimated D5D activity in PL were associated with a higher cardiometabolic risk score. Previous studies have reported higher D6D and lower D5D activity in plasma lipid fractions among obese children with metabolic syndrome than other children (80,81,172). The studies in adults have reported similar relations of D6D and D5D activities to type 2 diabetes (38). D6D activity is enhanced by increased insulin secretion (173), which could explain these associations. Together these findings support the view that desaturase activities may have an impact on cardiometabolic risk via glucose metabolism, starting in childhood. On the other hand, genetic variations in genes encoding D6D and D5D activities have shown to be related to the development of type 2 diabetes (38). This indicates that changes in desaturase activities may predispose to impaired glucose tolerance.

6.6.3 The effect of lifestyle intervention on estimated desaturase and elongase activities

The results of the present study showed no effect of lifestyle intervention on the estimated desaturase activities in children. This is in accordance with an exercise and dietary counseling study among adults showing no changes in desaturase estimates after 1-year (113). Previous short-term dietary intervention studies have resulted in a decrease of the estimated SCD activity and an increase of estimated D5D activity (43,128) and also in decrease of estimated D6D activity (43). This difference might be explained by the greater modification of dietary fatty acid composition in these studies. However, it is also possible that rapid substantial changes in the intake of fatty acids used in the calculation of estimates, e.g. palmitoleic acid and linoleic acid, may affect their amounts in plasma and thus desaturase estimates without or in addition to actual change in enzyme activity.

We found decreased estimated elongase activity in CE in the control group. As this estimate was calculated from the ratio of *cis*-vaccenic acid to palmitoleic acid, it may represent the activity of elongase 5 or 6 or both (42). The proportion of DPA acid in PL decreased in the control group, which may also indicate decreased elongase 5 activity. A high proportion of palmitoleic acid and a low elongase activity estimate calculated from fatty acids in erythrocyte membranes have been associated with worsening of hyperglycemia (20) and also with higher plasma concentration of C-reactive protein (174), which indicates that low elongase activity and accumulation of palmitoleic acid may be biomarkers for unfavorable metabolic changes.

6.7 PLASMA FATTY ACIDS AND PHYSICAL ACTIVITY

There are no previous intervention studies about the effects of physical activity on plasma fatty acid composition in children and there are only a few studies in adults (175,176). Aerobic training has been reported to increase the proportions of oleic acid, vaccenic acid and DHA and to decrease the proportion of arachidonic acid in muscle PL and the proportion of dihomo-γ-linolenic acid in serum CE but not in muscle TG or other fatty acids in serum PL or CE compared with the control group (68,69,177). Nevertheless, enhancing dietary fat quality is a more important way of improving plasma fatty acid composition than physical activity.

7 Conclusions and Future Implications

It is known that plasma fatty acid composition reflects the dietary intake of fatty acids among adults and children. The dietary studies in children have been made in the 1980s and 1990s and since then the food culture and the consumption of foods has drastically changed. This doctoral thesis brings updated information about the associations of the quality of dietary fat with plasma fatty acids but also novel information about the associations of other food consumption with plasma fatty acids in children. As expected, we found that higher consumptions of foods rich in vegetable fat are related to higher plasma proportions of PUFA and lower proportions of SFA and MUFA. A new finding of the thesis is that a higher consumption of high-fiber grain products and a lower proportion of candy is associated with a lower proportion of MUFA in plasma. This observation gives new insight to how quality of dietary carbohydrate associates with plasma fatty acids.

This thesis also offers knowledge about which fatty acids in plasma may have an association with cardiometabolic risk already in childhood. However, it is comforting to conclude that it is possible to affect plasma fatty acid composition by a 2-year individualized and family-based lifestyle intervention that aimed at enhancing overall diet quality, increasing physical activity and decreasing sedentary behavior. The principal finding of this doctoral thesis is that a lifestyle intervention prevented the decrease in the proportion of total PUFA and linoleic acid as well as increased the proportion of α-linolenic acid relative to the control group. It is important to affect the plasma fatty acid composition through dietary modification since this doctoral thesis also concludes that greater proportions of myristic and palmitoleic acid and a smaller proportion of linoleic acid, as well as higher estimated SCD and D6D activities and a lower estimated elongase activity, in plasma fatty acid fractions are associated with cardiometabolic risk factors among children. The most favorable dietary effect on plasma fatty acid composition in children may be enhancing the quality of dietary fat and the quality of dietary carbohydrates, since the results of the present doctoral thesis in a population sample of primary school children suggests that plasma fatty composition is not only a biomarker for dietary fat quality, but also reflects the consumption of high-fiber grain products and foods high in sugar. However, dietary intervention studies are needed to clarify the effect of changes in the quality of dietary carbohydrates on plasma fatty composition.

These findings on the beneficial effects of dietary and physical activity intervention on plasma fatty acid composition in children may be useful in developing lifestyle counselling strategies to prevent metabolic syndrome, type 2 diabetes and cardiovascular diseases starting in childhood. However, further investigation is needed for finding out the separate effects of dietary counseling and physical activity counseling on the plasma fatty acid composition.

These findings also reinforce the evidence that fatty acid metabolism is closely associated with cardiometabolic risk, starting already from childhood. Further studies are needed to establish the mechanisms behind these associations. Also, it would be important to determine a set of specific fatty acids, "a fatty acid score", that would be associated with cardiometabolic risk starting from childhood.

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TAISA VENÄLÄINEN

Plasma fatty acids are known to reflect dietary fat in children and in adults. They are also associated with cardiometabolic risk factors, such as dyslipidemia and elevated blood pressure among adults. In this thesis, the associations of food consumption, also other than dietary sources of fat, with plasma fatty acid composition in children, were examined. Also, the associations of plasma fatty acids with cardiometabolic risk factors were of interest. The effect of a lifestyle intervention on the plasma fatty acid composition was the main goal of this thesis. Novel associations of the quality of dietary carbohydrates with plasma fatty acids, and plasma fatty acids with cardiometabolic risk factors were found. Moreover, it was concluded that it is possible to affect the plasma fatty acid composition by a lifestyle intervention among children.

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