ASSOCIATION OF QUALITY AND QUANTITY OF FAT INTAKE WITH LIPOPROTEIN SUBCLASSES

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Several studies have shown significant associations between intake of various fatty acids and lipoproteins. However, little is known about the associations between the consumption of different dietary fatty acids and lipoprotein subclasses. The aim of this Master's thesis was to investigate the cross-sectional association of dietary fat intake with subclasses of lipoproteins in elderly women.

Altogether 554 women (aged≥ 65 years), who were part of population based OSTPRE cohort study filled out three days' food record and had blood sampling. The food records were used to calculate intake of dietary fat including polyunsaturated fatty acids, monounsaturated fatty acids, saturated fatty acids (SFA), total fat, palmitic acid, stearic acid, linoleic acid, linolenic acid, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. Lipoprotein subclasses were determined by nuclear magnetic resonance spectroscopy. Participants answered to the questions about lifestyle and health information such as intake of dietary supplements and medications, physical activity, disease status, menopause time, smoking and alcohol intake through self-administrated postal questionnaire at baseline. Data have been collected between February 2003 and May 2004 from women who were born in 1932-1941.

Data were analyzed by IBM SPSS statistics software (version 21). Pearson and Spearman correlation coefficients were used to investigate the correlations between dietary fat and lipoprotein subclasses. The ANCOVA test was conducted to find the relation between tertiles of SFA (E%) and lipoprotein subclasses with adjustment for physical activity, BMI, age, smoking and intake of lipid lowering drugs. The pairwise differences were checked by Bonferroni test.

Most of the significant correlations were observed between SFA and lipoprotein subclasses. For example, SFA positively associated with higher concentrations small, medium and large LDL particles. After adjustment for covariates, the intake of SFA significantly associated with lower size of LDL particles ($p=0.04$) and lower amount of TG in small VLDL ($p= 0.046$). Furthermore, higher intake of SFA tended to be in significant relation with higher amount of phospholipid in small LDL ($p=0.06$).

Higher dietary intake of SFA associated with lower size of LDL which might increase the risk of heart diseases. Further studies of SFA intake and lipoprotein subclasses are needed to confirm the result and to clarify the clinical meanings of these associations.

CONTENTS:

1. INTRODUCTION

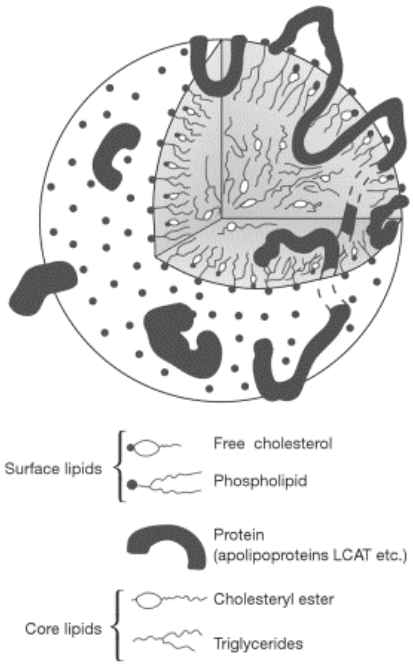
Lipid profile of plasma contains the broad spectrum of lipid molecules that can be divided into six categories, including glycerolipids, fatty acyls, glycerophospholipids, sterols, sphingolipids and prenols (Quehenberger et al. 2010). Plasma lipid profile is an important factor that exerts a powerful effect on various diseases, especially cardiovascular diseases (CVD). This profile depends on the synthesis of lipid in the body and intake of fat from diet (Kwiterovich 2009). Therefore, the quality and quantity of fat intake are very important. In addition, since lipid cannot circulate throughout the body individually and needs lipoproteins as carriers, the concentration of lipoproteins in plasma is a momentous factor. There are five different types of lipoproteins with various functions, densities, sizes and compositions and each can be separated into diverse subclasses using methods like nuclear magnetic resonance spectroscopy (Ala-Korpela 2008, Ross et al. 2012). Considering the fact that, the concentration of lipoproteins can be improved by the help of diet as an environmental factor, many researches have provided valuable knowledge about the effect of diet on the quantity of lipoproteins in circulation. However, further studies have revealed that the distributions of different subclasses of lipoproteins are extremely important in predicting the future diseases (Krauss 2001, Felder et al. 2008, Gerber & Bernis 2012, Kim et al. 2012, Filippatos et al. 2013, Pirillo et al. 2013). In this research, the aim is to examine the association between quality and quantity of fat intake and serum lipoprotein subclasses.

2. LITERATURE REVIEW

2.1 Lipoprotein

Triglycerides (TG), phospholipids, cholesterols and other lipids need special molecules to circulate in the blood. The chemical structure of lipoproteins allows them to transport the lipids throughout the blood stream and body as a whole. These particles are made from lipids and proteins (Brody 1999). As shown in Picture 1, lipoprotein is a spherical molecule with polar lipids (phospholipids and cholesterol) in the surface and hydrophobic lipids (cholesterol esters and TG) in the core. Furthermore, the protein parts of this molecule named apolipoproteins or apoproteins are attached to the surface of particles. Lipoproteins are more soluble and detectable for enzyme and receptors by the help of their proteins (Warrell et al. 2003, Ross et al. 2012).

There are five types of lipoproteins, including chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL). These particles, which are made in the small intestine and liver, vary based on functions, densities, sizes and compositions. In addition, they are affected by diet and have different effect on health (Brody 1999, Moredich et al. 2005, Ross et al. 2012).



Picture 1: lipoprotein, source: (Warrell et al. 2003)

2.2 Lipoprotein Subclasses

Earlier researchers were exploring the relation between plasma concentration of lipoproteins and diet, these days new analysis methods that define various subclasses of lipoproteins provide the opportunity to study more thoroughly lipoprotein metabolism. Subclasses of lipoproteins can be differentiated by their density and size, and they are measured by diverse methods such as nuclear magnetic resonance spectroscopy (NMR), vertical rotor ultracentrifugation, and gradient gel electrophoresis (Moredich et al. 2005, Schaefer et al. 2009, Gerber & Berneis 2012).

2.2.1 Lipoprotein Subclasses Defined By NMR Method

NMR is a method which is based on the principle that each subclass of lipoproteins has a unique signal in a magnetic field (Felder et al. 2008), and it provides the opportunity of measuring broad spectrum of metabolites (more than 100 metabolites by a single analytical method) (Kettunen et al. 2012). Although multi- metabolic characteristics of serum lead to creation of overlapping signals, the content of metabolites and concentration of them can be measured by empirical setting and advanced computational methods (Inouye et al. 2010).

In overall, numerous subclasses of lipoproteins can be determined by NMR method (Ala-Korpela 2008). For example, in a study involving prediabetic participants, more than 30 variables related to lipoprotein subclasses were defined using NMR. Lipoprotein particles were classified into nine subclasses, including three subclasses of VLDL, IDL, two subclasses of LDL and three subclasses of HDL (Festa et al. 2005).

2.2.2 Lipoprotein Subclasses Defined By Other Methods than NMR

Besides the NMR method, other methods can be used to classify the lipoproteins. Two subclasses of LDL can be determined by non-denaturing gradient gel electrophoresis and analytic ultracentrifugation. These two methods show that the most abundant form of LDL subclass is denser and smaller one called LDL type B. On the contrary, there is LDL type A which is bigger, less dense and less pathogenic than LDL type B (Krauss 2001). In addition, several subclasses of HDL can be defined by mentioned methods, for example; HDL is classified into two categories using ultracentrifugation method providing HDL2 and HDL3. HDL3 is smaller and denser than HDL2. These subclasses of HDL can have further division based on their size (HDL2b, HDL2a, HDL3a, HDL3b and HDL3c) by non-denaturing gradient gel electrophoresis method (Pirillo et al. 2013).

2.3 Factors Affecting Subclasses of Lipoproteins

The concentration of lipoprotein subclasses in our body is affected by genetic factors (Krauss 2001, Shah 2006, Kettunen et al. 2012). It is estimated that 50- 70% of the variation in the concentration of different lipoprotein particles is heritable (Kettunen et al. 2012). On the other hand, non-genetic factors such as diet, age, menopausal state and obesity are related to the concentration of lipoprotein subpopulation in plasma. Moreover, numerous diseases significantly correlate with the distribution of lipoprotein subclasses in the blood stream (Williams et al. 1993, Krauss 2001, Freedman et al. 2004, Mäntyselkä et al. 2012).

2.3.1 Demographic Characteristics

Concentrations of small LDL and small HDL were higher in adult men as compared to the younger boys using nondenaturing gradient gel electrophoresis (Williams et al. 1993, Krauss 2001). Using NMR method, it was shown that the concentration of large HDL had a negative

correlation with age only in women, but not in men (Freedman et al. 2004). In addition, the concentrations of small and medium HDL increased with age in women. That led to lower average size of HDL in older women than in younger women. Furthermore, LDL size had weak negative correlation with age in both genders (Freedman et al. 2004).

Regarding gender, men usually have more atherogenic lipoprotein subclasses' profile than women (Freedman et al. 2004). They had smaller size of LDL and smaller size of HDL. In addition, men had higher concentration of VLDL and larger mean size of VLDL than women. The larger mean size of VLDL in men was due to having higher concentrations of large and medium VLDL as compared to women (Freedman et al. 2004). Furthermore, postmenopausal women had higher concentrations of small LDL and small HDL than premenopausal women (Williams et al. 1993, Krauss 2001).

2.3.2 Plasma Concentration of Lipids, Lipoproteins and Apolipoproteins

The lipoproteins and lipid profile of plasma associate with each other. High percentage of LDL type B was usually seen in patients with TG level higher than 1.58-1.81 mmol/l. Patients who had a TG level lower than 1.13-1.24 mmol/l often did not have high prevalence of small LDL (Krauss 2001, Gerber & Berneis 2012). In addition, the size of LDL negatively correlated with the concentration of HDL in plasma (Shah 2006). Concentrations of LDL, TG, and total cholesterol had negative correlations with HDL particle size, as well. Moreover, Tian and Fu (2010) reported that even though the impact of diverse hyperlipidemia on the distribution of HDL subclasses was mildly different, high concentration of small HDL and low concentration of large HDL were usually seen in hyperlipidemic subjects.

2.3.3 Adiponectin

Adiponectin is a protein that is secreted from adipocytes into the blood stream and have strong correlation with insulin sensitivity (Shin & Kim 2011, Vanhala et al. 2011). It was found that adiponectin concentration had positive correlations with LDL particle size and concentration of HDL2 (Shin & Kim 2011) reflecting the more favorable profile of lipoprotein particles (Vanhala et al. 2011). In addition, it had negative correlation with the concentration of HDL3 in plasma. It

should be mentioned that the relation of LDL particle size and adiponectin was not significant after adjusting for TG levels (Shin & Kim 2011).

2.3.4 Obesity

Obesity is another factor, which is related to lipid profile. It has been reported that the concentrations of HDL and HDL2 increased by weight loss (Moriyama et al. 2014). A cohort study with 6.5 years follow up showed that even mild weight loss shifted the lipoprotein profile to a more favorable one (Mäntyselkä et al. 2012). The higher proportion of larger HDL was seen in participants who lost weight. In addition, the concentrations of small, medium and large LDL besides the mean size of VLDL decreased in this group (Mäntyselkä et al. 2012).

2.3.5 Lipid Lowering Drugs

Drugs modulating cholesterol and fatty acids have impacts on the composition of lipoprotein subclasses. Statins most often are used to decrease the concentration of cholesterol in circulation (Rizzo & Berneis 2006).

The effect of atorvastatin on subclasses of lipoproteins defined by the NMR method was analyzed in two studies (Soedamah et al. 2003, Ikewaki et al. 2009). While Ikewaki and his colleagues reported that atorvastatin decreased the concentrations of all LDL subclasses with higher reduction in small LDL, Soedamah and his coworkers found that atorvastatin only decreased the concentrations of large and medium LDL. In addition, both groups demonstrated that atorvastatin increased the size of HDL particles (Soedamah et al. 2003, Ikewaki et al. 2009). Positive correlations between atorvastatin therapy and size of LDL and VLDL were found, as well (Ikewaki et al. 2009). Furthermore, atorvastatin decreased the concentrations of medium and small VLDL (Soedamah et al. 2003).

Simvastatin therapy has a favorable impact on HDL subclasses. Increased concentration of large HDL and declined concentration of intermediate HDL were seen after intake of simvastatin in comparison to ezetimibe (Berthold et al. 2014).

An interventional study showed that treatment with pravastatin decreased the concentration of IDL and increased the size of VLDL and LDL (Otvos et al. 2002). In addition, in this study, pravastatin differently affected lipoprotein subclasses of participants who had small LDL at baseline and participants who had large LDL at baseline. In other words, pravastatin increased the average size of LDL and decreased the concentration of LDL particles in subjects with small LDL more than in subjects with large LDL at baseline. In addition, while it did not have an effect on the size of HDL in subjects with large LDL at baseline, it increased the average size of HDL in participants who had small LDL at baseline (Otvos et al. 2002).

Various statins are suggested to have different effects on concentration and size of lipoprotein subclasses in circulation (Rizzo & Berneis 2006). A systematic review study reported that statins had none to moderate effect on LDL subclasses. While limited changes were seen in LDL size after treatment with pravastatin and simvastatin, fluvastatin and atorvastatin had more favorable effects on LDL subclasses (Rizzo & Berneis 2006). In addition, a cohort study showed that the intake of niacin plus lovastatin decreased the concentration of smaller and denser LDL more than either simvastatin or atorvastatin. To be more specific, the amount of participants who shift from pattern B of LDL to pattern A of LDL was three or more times higher in niacin plus lovastatin group in comparison to atorvastatin and simvastatin groups. In addition, the higher increase in the proportion of larger HDL (HDL2b) was seen in subjects' plasma after intake of niacin plus lovastatin as compared to simvastatin or atorvastatin (Bays & McGovern 2003).

2.3.6 Diseases Related to Lipoprotein Profile

Lipoprotein profile of plasma significantly correlates with numerous diseases most importantly, with CVD and type 2 diabetes. Therefore, improving the lipoprotein profile might reduce the prevalence or severity of these two chronic diseases.

Cardiovascular diseases

CVDs are the main reason of death not just in Europe, but also throughout the world (WHO 2014). Several studies have revealed that the predominance of LDL type B is related to two or three times increased risk of CVD (Krauss 2001, Gerber & Berneis 2012). One study, considered it even more pathogenic by reporting sevenfold increase in the risk of CVD in subjects with

small LDL higher than 2.59 mmol/l (Kim et al. 2012). This correlation can be explained by four mechanisms. First, LDL receptors bind small LDL with less readiness. Second, the small LDL infiltrates easier into the wall of arteries (40-50% faster than large LDL). Third, the small LDL binds more tightly with the proteoglycan of arteries and fourth the small LDL oxidizes more quickly in comparison to large LDL (Moredich et al. 2005, Shah 2006, Kim et al. 2012).

Moreover, it is proved that the HDL has protective effects on heart diseases, but further studies on different subclasses of HDL showed that the larger HDL is more protective than the smaller HDL (Felder et al. 2008). The concentration of HDL2 has had a negative association with progression and severity of coronary lesions. Furthermore, it was discovered that HDL2 is significantly lower in patients who had myocardial infarction (Pirillo et al. 2013).

Type 2 Diabetes

Diabetes or insulin resistance exerts impacts on the lipoprotein subclasses. Higher small LDL concentration was seen in participants with type 2 diabetes or prediabetes (Krauss 2001, Gerber & Berneis 2012, Magge et al. 2012, Filippatos et al. 2013). Moreover, the unfavorable profile of HDL (lower HDL2 and higher small HDL) have been measured in subjects with type 2 diabetes, poor glycemic control and prediabetes (Medina-Bravo et al. 2013, Pirillo et al. 2013, Filippatos et al. 2013). In addition, intensive management of glucose in patients with type 2 diabetes was accompanied by higher concentrations of larger VLDL, LDL and medium HDL subclasses besides lower concentration of small HDL (Azar et al. 2013).

2.4 Diet and Lipoprotein Subclasses

In several studies significant associations between subclasses of lipoproteins and intake of various nutrients or foods e.g. carbohydrates, fructose, fatty acids and alcohol have been found (Mukamal et al. 2007, Schaefer et al. 2009, Annuzzi et al. 2012). In this part, the impact of different dietary fatty acids on lipoprotein subclasses will be explained. Since the limited number of studies used NMR method for determining the lipoprotein subclasses, the effect of quality and quantity of fat intake on lipoprotein subclasses is explained in two parts. First, the effect of fat intake on lipoprotein subclasses determined by NMR methods. Second, the effect of fat intake on lipoprotein subclasses determined by methods other than NMR. In addition, tables 1, 2, 3, 4 and

5 show the summaries of included studies about the association between lipoprotein subclasses and intake of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), saturated fatty acids (SFA), trans fatty acids and quantity of fat, respectively.

2.4.1 Polyunsaturated Fatty Acids

NMR Studies

Fish and long chain n-3 fatty acids

Fish contains long chain n-3 PUFA (eicosapentaenoic acid and docosahexaenoic acid) which can affect lipoprotein subclasses. Although it is agreed that the high intake of fish and diet rich in fatty fish cannot change the size of LDL and IDL (Li et al. 2004, Erkkila et al. 2014), there are conflicting evidence about the impact of fish intake on other subclasses of lipoproteins. In an interventional study on coronary heart disease patients, the high intake of fatty fish (4 times per week) increased the size of HDL (Erkkila et al. 2014). In addition, another interventional study in subjects with impaired glucose metabolism aimed to find the effect of diet rich in bilberries (300 g/day), whole grain and fatty fish (3 times per week) on the metabolic profile of serum and activities of lipid transfer protein. In this study, the higher intake of fish increased the concentration of large HDL and mean size of HDL (Lankinen et al. 2014). On the contrary, one intervention study showed that high fish diet (8 times per week) did not affect HDL particles defined by NMR (Li et al. 2004). In addition, Li and his colleagues concluded that the concentration of large HDL and the size of HDL particles decreased after consumption of low fish diet (2 times per week). High fish diet reduced the concentrations of medium and small VLDL significantly, as well (Li et al. 2004). Moreover, components of large HDL changed after intake of a diet rich in fatty fish. Erkkila and her colleagues demonstrated that fatty fish diet increased the concentrations of total lipids, cholesterol and cholesterol esters in large HDL (Erkkila et al. 2014). The result of another study also showed that the higher intake of fish associated with increased concentrations of lipid components of large HDL (Lankinen et al. 2014).

In an observational study, the correlation between dietary intake of n-3 fatty acids and lipoprotein subclasses was investigated (Annuzzi et al. 2012). The dietary intake was measured by food frequency questionnaire. In addition, it should be kept in mind that EPA and DHA were

the two main n-3 fatty acids in this study, and they were derived from consumption of salmon and seal. The results of the study demonstrated that the dietary intake of n- 3 fatty acids associated with decreased concentration of large VLDL and smaller mean size of VLDL. Moreover, it associated with increased concentration of large HDL, concentration of large LDL and average size of HDL. In addition, the intake of n-3 fatty acids had negative correlations with the concentrations of small and medium HDL only in women, and it had positive correlations with average size of LDL and the concentration of large LDL in men. Furthermore, considering the fact that the intakes of carbohydrate and sugar affect lipoprotein subclasses, the impact of n-3 fatty acids on concentration of large VLDL, concentration of large HDL and the size of VLDL and HDL particles remained significant even after adjustment for intake of carbohydrate and sugar (Annuzzi et al. 2012).

The effects of daily n-3 fatty acids intake on lipoprotein subclasses were analyzed in an interventional study on type 2 diabetes patients (Mostad et al, 2008). In this study, intervention group consumed fish oil enriched with n-3 fatty acids (5.9 g/day n-3 fatty acids) and control group had the same amount of corn oil (8.5 g/day n-6 fatty acids). Based on the result of this study, n-3 fatty acids intake reduced the size of VLDL and concentrations of large VLDL and small HDL particles. In addition, n-3 fatty acids intake did not have any effect on the subclasses of LDL (Mostad et al, 2008).

Furthermore, Burdge and his colleagues conducted an interventional study involving healthy men to find the effect of acute fish oil consumption (7.3 fold more DHA and EPA than usual UK diet) on lipoprotein subclasses (Burdge et al. 2009). They found that fish oil consumption changed significantly the size and concentration of VLDL particles. Consumption of a single meal rich in fish oil increased the concentration of VLDL particles and decreased the size of them. However, acute intake of fish oil did not have a significant effect on the size and concentration of LDL and HDL particles (Burdge et al. 2009).

Moreover, in another study the effects of n-3 fatty acids intake on lipoprotein profile were analyzed by comparing the effect of n-3 fatty acids (DHA and EPA, 4g/day) plus atorvastatin and placebo plus atorvastatin (Maki et al. 2011). Although following changes were seen in both

groups, the changes were significantly greater in n-3 fatty acids group as compared to the placebo group. Intake of n-3 fatty acids plus atorvastatin decreased the mean size of VLDL, concentrations of medium, large and total VLDL, concentrations of IDL and small LDL. In addition, it increased the average size of LDL, concentrations of small HDL and large HDL. On the contrary, concentrations of small HDL and total HDL had the larger increase in the placebo group in comparison to n-3 fatty acid group. Furthermore, the size of HDL particles did not change in either group. Finally, more participants shifted from pattern B of LDL to pattern A in n-3 fatty acids plus atorvastatin group as compared to the atorvastatin group (Maki et al. 2011).

Usually, the intakes of EPA and DHA are analyzed together by supplementing with fish oils, while a randomized controlled trial aimed to find the effect of DHA on lipoprotein subclasses individually using 5 mg/day algal DHA oil containing 2 g DHA (Neff et al. 2011). It was found that DHA intake declined the average size of VLDL particles, and increased the average size of LDL and HDL particles. In addition, algal DHA decreased the concentrations of small LDL, large VLDL, medium VLDL and medium HDL particles. Higher concentrations of large LDL and large HDL were identified in plasma after intake of algal DHA oil, as well (Neff et al. 2011).

Walnut

Walnuts contain both n-3 and n-6 PUFA. Effects of walnut consumption on lipoprotein subclasses were analyzed in an intervention study by adhering study participants to four diets sequentially, including habitual diet (HD), habitual diet and walnuts (HD+W), low fat diet (LD), and lastly low fat diet and walnuts (LD+W) (Almario 2001). Dose of 48 grams walnuts per day was provided for the subjects who were in HD+W or LD+W diets. Eating walnuts did not change the size of VLDL, IDL and HDL particles, but the LD+W decreased the concentrations of LDL and IDL in comparison to LD. Moreover, HD+W declined the cholesterol content of small LDL (Almario 2001).

Olive, sunflower and rapeseed oils

An interventional study used mixture of olive, sunflower and rapeseed oils were used as sources of PUFA in healthy subjects. The diet of control group included 7% of energy from LA and 0.4% of energy from ALA and the dietary fat was a mixture of olive oil, sunflower oil, rapeseed

oil and 25% hard stock (fully hydrogenated palm kernel and palm oil). Dietary fat of the low LA group (3% of energy from LA, 0.4 % of energy from ALA) consisted of 63% of olive oil, 9% of rapeseed oil and 28% of hard stock. Moreover, the high ALA group (7% of energy from LA, 1.1% of energy from ALA) consumed 20% sunflower oil, 5% olive oil, 52.5% rapeseed oil and 22.5% hard stock (Goyens & Mensink 2005). They found that the concentration of LDL cholesterol decreased in the high ALA group as compared to control and low LA groups. In addition, concentrations of VLDL subclasses decreased in both low LA and high ALA groups that mainly were due to decline in medium and small VLDL subclasses in low LA and high ALA groups, respectively. Furthermore, they did not find any changes in HDL, LDL and VLDL particle size. Finally, there was not any significant correlation between lipoprotein profile and the ratio of ALA to LA (Goyens & Mensink 2005).

Other Methods

In an observational study on healthy sedentary men the associations between diet and lipoproteins subclasses were analyzed (Williams et al. 1986). It was found that PUFA intake had negative correlations with concentrations of VLDL and LDL cholesterol. In addition, higher intake of PUFA significantly associated with lower concentrations of small LDL, small IDL and small VLDL particles. All of the mentioned correlations except for small VLDL were significant after adjustment for age, percentage of body fat and cigarette use. On the contrary, a cross-sectional study showed that PUFA consumption was positively associated with the size of LDL particles only in patients with type 2 diabetes or impaired glucose metabolism and not in a person with a normal glucose metabolism (Bos et al. 2007).

On the basis of a cross-sectional study, Bogl and her colleagues concluded that the twins who consumed higher amounts of n-3 fatty acids had higher concentration of large HDL and lower concentration of small HDL in comparison to their co-twins with lower consumption of n-3 fatty acids (Bogl et al. 2011). In addition, the substitution of one energy percentage from n-3 fatty acids for other kinds of fat had significant correlation with larger mean size of HDL. They did not find any significant correlation between intake of n-3 fatty acids and LDL particle size. Moreover, in some interventional studies the effects of DHA and EPA supplementations on lipoprotein subclasses were analyzed. Intake of n-3 fatty acid supplements (4g/day from 60%

EPA, 40% DHA) increased the concentration of HDL2, but it did not affect the LDL subpopulations in active males (Thomas et al. 2004). In a crossover study, participants consumed 3 different kinds of capsules, including capsules containing control oil, capsules containing 1.8 g EPA+DHA/ day or capsules containing 0.7 g EPA+ DHA/ day in a randomized order. The results of this study showed that even moderate intake of EPA and DHA from fish oil increased the concentrations of HDL2, total HDL and total LDL (Caslake et al. 2008). In addition, the lower VLDL cholesterol concentration was seen in plasma after moderate intake of DHA and EPA (Caslake et al. 2008). Furthermore, the effect of DHA was analyzed individually. Based on a result of a randomized control trial study on hyperlipidemic children, DHA consumption (1.2g/day) increased the concentrations of large LDL by 91% and large HDL by 14%. DHA intake decreased the concentration of small LDL by 48% in these children, as well (Engler et al. 2005).

Table 1. Summary of included studies about the association between intake of PUFA and lipoprotein subclasses.

Author	Study design	Participants/ Sample size	Interventions	Method of determining lipoprotein subclasses	Main findings
1. Interventions					
Almario 2001	Interventional study	18 subjects (5 men and 13 postmenopausal women; age 60± 8 y)	1) habitual diet for 4 weeks, 2) habitual diet plus walnuts for 6 weeks, 3) low fat diet for 6 weeks and 4) low fat diet plus walnuts for 6 weeks.	NMR	Habitual diet plus walnuts ↓ the cholesterol content of small LDL in comparison to habitual diet.
Burdge et al. 2009	Interventional study	11 healthy men (age 58± 5 y)	1) Reference meal or usual UK diet and 2) meal enriched with fish oil in a random order. There were at least 14 day washout periods.	NMR	Meal rich in fish oil ↓ the mean size of VLDL particles during postprandial period. It did not affect the LDL and HDL particles.
Caslake et al. 2008	Randomized, Double- blind, placebo-controlled crossover study	312 adult subjects (age 20-70 y)	1) Capsules contained EPA+ DHA (0.7/day), 2) capsules contained EPA+DHA (1.8 g/day) and 3) capsules contained control oil in a random order and for 8 weeks. There was 12 week washout period.	nondenaturing gradient gel electrophoresis	Fish oil ↑ the concentrations HDL2.
Engler et al. 2005	Randomized, double-blind, placebo-controlled, crossover study	20 children with hyperlipidemia. (age 9-19 y)	1) 1.2 g/day of DHA or 2) 1.2g/day of placebo for 6 weeks.	Vertical Auto Profile II	DHA significantly ↑ the concentrations of large LDL by 91% and ↓ the concentration of small LDL by 48%. It ↑ the concentration of large HDL by 14%.
Erkkila et al. 2014	Randomized, controlled, Parallel study	33 patients with coronary heart disease (age 61.0 ± 5.8 y)	1) Fatty fish diet (from fish likes salmon, rainbow and trout), 2) lean fish diet (from fish like pike, perch and cod) or 3) control diet (from lean beef, pork and chicken instead of fish) for 8 weeks.	NMR	Concentrations of total lipid, cholesterolester and, cholesterol in very large HDL ↑ after intake of fatty fish diet and ↓ after consumption of control and lean fish diets. Fatty fish diet ↑ mean size of HDL. Fish diets did not affect VLDL and IDL particles.
Goyens & Mensink 2005	Randomized, double-blind, controlled, parallel study	54 subjects including 21 men (age 52.6± 13.7 y) and 33 women (age 47.7± 11.1 y)	1) Control diet, 2) low LA diet or 3) high ALA diet for 6 weeks.	NMR	Concentration of VLDL particles ↓ in low LA and high ALA groups. This reduction was mainly due to ↓ of medium VLDL in the low LA group and small VLDL in the high ALA group.

Author	Study design	Participants/ Sample size	Interventions	Method of determining lipoprotein subclasses	Main findings
Lankinen et al. 2014	Randomized, controlled, Parallel study	131 subjects with impaired glucose metabolism and features of metabolic syndrome (age 40-70 y)	1) Healthy diet (fatty fish, bilberries, whole grain and grain product with low postprandial insulin response), 2) WGED diet (whole grain and low postprandial insulin response grain product) or 3) control diet (refined wheat bread) for 12 weeks.	NMR	Higher intake of fish associated with larger size of HDL particles, increased concentrations of lipid components of large HDL and higher concentration of large HDL.
Li et al. 2004	Interventional study	22 men and women (age> 40 y)	1) High fish diet or 2) low fish diet for 24 weeks.	NMR	High fish diet ↓ the concentration of medium and small VLDL. Low fish diet ↓ the large HDL and the size of HDL particles.
Maki et al. 2011	Randomized, double-blind, placebo-controlled, parallel study	245 subjects with mixed dyslipidemia (18-79 y)	1) POM3 (prescription of omega-3 acid) plus atorvastatin (10 mg/day) or placebo plus atorvastatin (10mg/day) for 8 weeks. 2) Elevated dosage of atorvastatin (20mg/day) for 4 weeks. 3) Elevated dosage of atorvastatin (40 mg/day) for 4 weeks.	NMR	The lower concentration of small LDL particles, higher concentration of larger LDL particles and larger mean size of LDL were seen after intake of POM3 diet as compared with placebo diet. More participants shifted from pattern B of LDL to pattern A in POM3 group as compared to the placebo group.
Mostad et al, 2008	Randomized, placebo controlled, parallel study	26 subjects with normal TG level and type 2 diabetes without insulin treatment (age 40-75 y)	1) Fish oil diet or 2) corn oil diet for 9 weeks.	NMR	n-3 fatty acid ↓ the mean size of VLDL, concentrations of large VLDL and small HDL (without correlation with reduction of insulin sensitivity). It did not affect LDL particles.
Neff et al. 2011	Randomized, Double blind, controlled, parallel study	36 healthy, obese or overweight subjects (18-65 y)	1) Algal DHA oil or 2) placebo (an oil mixture of corn and soybean) for 4.5 months.	NMR	Smaller mean size of VLDL, larger mean size of HDL and LDL were seen in participants who consumed DHA diet in comparison to the placebo group. Intake of DHA also ↑ the concentrations of large LDL and large HDL, and ↓ the concentrations of small LDL and medium HDL.
Thomas et al. 2004	Interventional study	10 active males (age 25± 1.5 y)	1) n-3 fatty acids for 4 weeks. 2) 60 minutes treadmill exercise before and after 4 weeks supplementation.	Modified heparin-MnCl2-dextran sulfate and Lipoprint methods	N-3 fatty acid supplementation ↑ the concentrations of HDL2. The size of LDL particles and concentrations of LDL subclasses did not change by intake of n-3 fatty acids. Intake of n-3 fatty acids and exercise ↑ the concentration of HDL3 and LDL1 in comparison to baseline measurements.
2. Observational Studies					

Author	Study design	Participants/ Sample size	Interventions	Method of determining lipoprotein subclasses	Main findings
Annuzzi et al. 2012	Cross-sectional study	977 subjects (age women 40.7± 15.2, men 41.2± 14.8 y)	-	NMR	After adjustment for potential confounders (age, BMI, total energy intake and percentage of energy from fat), intake of n-3 significantly correlated with lower concentration of large VLDL and smaller size of VLDL particles, higher concentration of large HDL and larger size of HDL particles. The correlations remained significant after adjustment for carbohydrate and sugar.
Bogl et al. 2011	Cross-sectional study	24 healthy twin pairs (age 23-33 y)	-	Non-denaturing gradient gel electrophoresis	The consumption of one percent of energy from n-3 fatty acid instead of other kinds of fats was correlated with larger mean size of HDL in both genders. This relation remained significant after controlling the effect of genetic and shared environment by within- pair comparison in twin pairs.
Bos et al. 2007	Cross-sectional study	758 subjects with normal, impaired glucose metabolism and type II diabetes	-	Gel-filtration chromatography	PUFA intake negatively correlated with size of LDL in participants with type 2 diabetes and in subjects with impaired glucose metabolism.
Williams et al. 1986	Cross-sectional study	77 healthy, sedentary men (age 30-55 y)	-	Analytical ultracentrifugation	Higher intake of PUFA negatively correlated with concentrations of smaller LDL, IDL and VLDL particles.

PUFA= Polyunsaturated fatty acids, DHA= Docosahexaenoic acid, EPA= Eicosapentaenoic acid, LA= Linoleic acid, ALA= Alpha linolenic acid.

2.4.2 Monounsaturated Fatty Acids

NMR method

Perez-Martinez and his coworkers demonstrated that subjects who ate a meal rich in olive oil (36% of energy from MUFA) had more favorable lipoprotein profile in comparison to participants who took a meal rich in butter (35% of energy from SFA) and walnuts (16% of energy from PUFA). It was found that a meal rich in MUFA increased the size of TG rich lipoproteins more than a meal rich in PUFA. In addition, lower number of TG rich lipoproteins were seen after consumption of MUFA as compared to SFA and PUFA (Perez-Martinez et al. 2011). On the contrary, results of an interventional study showed that the intake of these diverse fatty acids did not have significantly different effects on the lipoprotein subclasses (Thijssen & Mensink 2008). In this study, participants used 3 different diets in a random order for 5 weeks. The compositions of diets were similar except for 7% of energy that came from oleic acid (MUFA), stearic acid (SFA) or linoleic acid (PUFA) (Thijssen & Mensink 2008).

Other Methods

A cross-sectional study on relations of lifestyle factors and lipoprotein subclasses revealed that the ratio of cholesterol in small LDL to cholesterol in large buoyant LDL had negative correlation with intake of MUFA (Parlesak et al. 2014). It was in agreement with an interventional study, which was done by Zambon and his colleagues. In that study, participants randomly consumed high carbohydrate hypocaloric diet (60% of calories from complex carbohydrate, and 25% of calories from fat, which contained 10% from SFA, 7% from MUFA, 8% from PUFA) or olive oil enriched hypocaloric diet (40% of calorie from complex carbohydrate, 45% of calories from fat which contained 10% from SFA, 27% from MUFA and 8% from PUFA) for 6 months. Lower concentration of small and dense LDL and lower ratio of LDL to HDL cholesterol were seen in plasma after 6 months' consumption of high MUFA diet. In addition, high MUFA diet increased the cholesterol content of HDL2 particles while it did not have any effect on cholesterol content of HDL3. Moreover, both diets decreased the concentration of LDL cholesterol and weight of the participants (Zambon et al. 1999). Furthermore, in an interventional study in moderately hypercholesterolemic subjects the impact of MUFA as a replacement for SFA on lipoprotein subclasses' profile was assessed. In this study, participants consumed low MUFA diet (7.8% of energy from MUFA and 14.7% of energy

from SFA), moderate MUFA diet (10.3% of energy from MUFA and 11.2% of energy from SFA) and high MUFA diet (13.7% of energy from MUFA and 7.3% of energy from SFA) for 6 weeks in random order. The higher consumption of MUFA decreased the concentrations of total LDL, small LDL and large LDL. Higher intake of MUFA did not have a significant effect on the distribution of LDL particles and lower concentration of small and large LDL were due to lower concentration of total LDL cholesterol after higher intake of MUFA. Since the higher intake of MUFA in this study was accompanied with the lower intake of SFA, it was not clear if this reduction happened due to reduction of SFA in diet, or an increase of MUFA in diet or even a combination of both of them (Gill et al. 2003).

Table 2. Summary of included studies about the association between intake of MUFA and lipoprotein subclasses.

Author	Study design	Participants/ Sample size	Intervention	Method of determining lipoprotein subclasses	Main findings
1. Interventions					
Gill et al. 2003	Interventional study	35 subjects with moderate hypercholesterolmia (17 men and 18 women, age 55± 5.6 y)	1) Low MUFA diet, 2) moderate MUFA diet and 3) high MUFA diet for 6 weeks and in a random order. There were at least 8 week washout period.	Dextran sulfate-magnesium chloride dual-precipitation, gradient ultracentrifugation, discontinuous density gradient centrifugation	The distribution of LDL subclasses did not change by consumption of various amounts of MUFA. However, since LDL cholesterol ↓ with higher intake of MUFA in a dose response trend, the higher intake of MUFA ↓ the concentration of LDL2 and LDL3 by 21.5 and 24.5% respectively.
Perez-Martinez et al. 2011	Randomized crossover study	20 men (age 22± 1.8 y)	1) Butter meal, 2) olive oil meal and 3) walnut meal. Mentioned meal had 1g fat and 7 mg cholesterol per kg body weight.	NMR	Olive oil meal ↓ the number of TRL particles in comparison to other meals. Olive oil meal also ↑ the mean size of TRL particles as compared to walnut meal.
Thijssen & Mensink 2008	Randomized, crossover study	45 participants (27 women, 18 men, age 28–66 y)	7% of energy in various diets was obtained from 1) stearic acid, 2) oleic acid or 3) linoleic acid. The duration of each diet was 5 weeks	NMR	No significant changes were found in the concentration and mean size of lipoprotein subclasses among various diets.
Zambon et al. 1999	Interventional study	34 normolipidemic, premenopausal women (age 22-39 y)	1) High carbohydrate hypocaloric diet or 2) olive oil enriched hypocaloric diet for 6 months.	Density gradient ultracentrifugation	High MUFA diet ↑ cholesterol in HDL2. The concentration of small LDL ↓ significantly after intake of high MUFA diet. The concentration of HDL3 did not change after consumption of both diets.
Observational Studies					
Parlesak et al. 2014	Cross-sectional study	265 healthy men (20-67 y)	-	Ultracentrifugation	Higher intake of MUFA associated with lower ratio of cholesterol in small dense LDL to cholesterol in large buoyant LDL.

MUFA= monounsaturated fatty acids and TRL= Triacylglycerols-rich lipoproteins

2.4.3 Saturated Fatty Acids

There was not any article published about the effect of SFA intake on lipoprotein subclasses determined by NMR method, except as control group in previously mentioned studies.

In an interventional study the effects of low fat (24% of energy from fat, 6% SFA, 12%MUFA and 4% PUFA) versus high fat diet (46% of energy from fat, 18% SFA, 13% MUFA, 12% PUFA) on lipoprotein subclasses were explored. It was found that the higher consumption of SFA (myristic and palmitic acids) correlated with higher concentration of large LDL and larger mean size of LDL particles. Intake of SFA also had significant, negative correlation with concentration of small LDL (Dreon et al. 1998). In addition, in a cross-sectional study involving healthy men, Sjogren and his colleagues indicated that pentadecanoic acid, 15:0 and heptadecanoic acid, 17:0 derived from milk products had negative correlation with concentration of small LDL particles (Sjogren et al 2004).

Table 3. Summary of included studies about the association between intake of SFA and lipoprotein subclasses.

Author	Study design	Participants/ Sample size	Intervention	Method of determining lipoprotein subclasses	Main findings
1. Interventions					
Dreon et al. 1998	Randomized Crossover study	103 healthy men (age>20 y)	1) High fat diet or 2) low fat diet for 6 weeks.	Analytic ultracentrifugation and Nondenaturing polyacrylamide gradient gel electrophoresis	Higher intake of SFA correlated with a lower concentration of small LDL, higher concentration of large LDL and larger mean size of LDL particles.
2. Observational Studies					
Sjogren et al 2004	Cross-sectional study	291 healthy men (age 63± 0.6 y)	-	Polyacrylamide gradient gel electrophoresis, protein-staining	Higher intake of pentadecanoic acid and heptadecanoic acid correlated with a lower concentration of small LDL.

SFA= saturated fatty acids

2.4.4 Trans Fatty Acids

NMR Studies

In an interventional study the effects of industrially produced trans fatty acids intake and natural trans fatty acids intake on lipoprotein subclasses were compared (Chardigny et al 2008). It was found that the consumption of industrially produced trans fatty acids decreased the number of HDL particles, especially by reduction of large HDL particles in women. In addition, the women consuming trans fatty acids from natural sources had higher LDL cholesterol as compared to women who ate industrially produced trans fatty acids. This increase was due to increase in the concentration of large LDL (Chardigny et al. 2008).

Other Methods

There is a debate over the effect of trans fatty acid on lipoprotein subclasses defined by methods other than NMR. In an interventional study 5 different diets (all of them contained 30% of energy from fat) were used in a random order for 35 days. In each diet two thirds of fat were supplied by different fat in which the amount of trans fatty acids ranged from 0.6 g trans fatty acids/100 g fat to 26.1 g trans fatty acids/100 g fat. The result of this study showed that the higher dietary intake of trans fatty acids decreased the size of LDL particles in a dose dependent fashion. Furthermore, higher intake of trans fatty acids increased the cholesterol content of small and medium LDL (Mauger et al. 2003). Contradicting this result, in a cross-sectional study, Kim and Campos reported that trans fatty acids intake positively correlated with size of LDL particles (Kim & Campos 2003). It should be mentioned that the first interventional study can explain the causal relation better than the second observational study. In other words, since the researchers have control over the exposure in an interventional study, the isolated effect of exposure on outcome can be analyzed (DiPietro 2010).

Table 4. Summary of included studies about the association between intake of trans fatty acids and lipoprotein subclasses.

Author	Study design	Participants/ Sample size	Intervention	Method of determining lipoprotein subclasses	Main findings
1. Interventions					
Chardigny et al 2008	Randomized, double-blind, controlled, crossover study	46 healthy participants, including 22 men and 24 women (age 27.6± 7.1)	1) Industrially produced trans fatty acids and 2) natural trans fatty acid for 3 weeks. There was a week washout period.	NMR	Natural sources of trans fatty acid ↑ the concentration of large HDL cholesterol and ↓ the concentrations of large LDL cholesterol in women in comparison to industrially produced trans fatty acids.
Mauger et al. 2003	Interventional study	36 subjects, 18 men and 18 postmenopausal women (age 63± 6 y)	5 different diets in random order for 35 days. Two third of fat in various diets were provided by products with different level of trans fatty acids, including 1) semiliquid margarine, 2) soft margarine, 3) shortening, 4) stick margarine and 5) butter.	Nondenaturing, 2–16% polyacrylamide gradient gel electrophoresis	Higher intake of trans fatty acids ↓ the mean size of LDL particles in dose response manner. The concentrations of cholesterol in large and medium LDL ↑ with higher intake of trans fatty acids.
2. Observational studies					
Kim & Campos 2003	Cross-sectional study	414 subjects	-	Gradient gel electrophoresis on 2% to 16% no denaturing polyacrylamide gels	The mean size of LDL particles had positive correlation with intake of trans fatty acids. This correlation remained significant even after adjustment for potential confounders including age, gender, energy intake, BMI, LDL, HDL and TG.

TG= Triglycerides

2.4.5 Quantity of Fat

NMR Studies

An interventional study aimed to estimate the effects of reduced carbohydrate diet and reduced fat diet on lipoprotein subclasses in overweigh and obese healthy subjects (LeCheminant et al. 2010). In the first 3 months of the study all of the participants consumed a very low energy diet to lose weight. Then, in next 9 months, participants consumed either reduced carbohydrate diet (around 20% of energy from carbohydrate) or reduced fat diet (around 30% of energy from fat) to maintain weight. LeCheminant and his colleagues found that the reduced fat diet significantly increased the concentrations of total, large and small HDL. It increased the concentrations of total, large, medium and small VLDL, as well. In addition, the amount of weight loss was not different between reduced fat diet and reduced carbohydrate diet groups (LeCheminant et al. 2010). In another study, overweight subjects were randomized into either low carbohydrate, ketogenic diet (lower than 20 g carbohydrate /day) or low fat diet (lower than 30% of energy from fat and lower than 300 mg of cholesterol per day) (Westman 2006). According to this study, low fat diet decreased the concentrations of large VLDL, small LDL and LDL particles. Furthermore, low fat diet increased the concentration of large HDL and average size of LDL and HDL particles. In addition, it should be mentioned that the weight of the participants reduced by consumption of both diets (Westman 2006). Moreover, in an interventional study that involved hyperlipidemic patients for 8 weeks, the concentration of small LDL increased by 36% after intake of low fat diet (30% of energy from fat with intake of trans fatty acid and SFA lower than 10%) (Stoernell et al. 2008).

The acute effect of a high fat meal on lipoprotein subclasses was assessed in an interventional study (Wojczynski et al 2011). It was found that while the concentration of total LDL particles and small LDL decreased due to consumption of a high fat meal (83% of energy from fat, 14% of energy from carbohydrate and 3% of energy from protein), this meal increased the concentration of large LDL in plasma. In addition, high fat meal decreased the concentrations of total VLDL, medium and small VLDL particles. High fat meal increased the concentrations of total HDL and medium HDL in men, but it did not affect large HDL. Furthermore, the lower concentration of small HDL particles and higher concentration of medium HDL particles were seen in women's plasma in response to a high fat meal (Wojczynski et al 2011).

Other Methods

An interventional study involving men on low fat (24% of energy from fat, 60% of energy from carbohydrate) and high fat diets (46% of energy from fat, 38% of energy from carbohydrate) showed that lower intake of fat decreased HDL3a, HDL2a and HDL2b concentrations (William et al. 1995). In addition, it decreased the peak diameter and mean diameter of HDL. It should be mentioned that changes in the mean diameter of HDL and concentration of HDL2b were greater in men with LDL pattern A than those with LDL pattern B (William et al. 1995).

In an interventional study on healthy, non-obese males with normal lipid profile effects of short term, high fat versus low fat diet consumption on lipoprotein subclasses were analyzed (Guay et al. 2012). Participants consumed two different diets, including high fat diet (37% of energy from fat and 50% of energy from carbohydrate) and low fat diet (25% of energy from fat and 62% of energy from carbohydrate) for three days in random order. High fat diet increased the size of LDL particles, concentration of medium LDL and cholesterol content of large and medium LDL particles as compared to low fat diet. In addition, the concentration of small LDL particles decreased after intake of high fat diet in comparison to low fat diet (Guay et al. 2012).

Table 5. Summary of included studies about the association quantity of fat intake and lipoprotein subclasses.

Author	Study design	Participants/ Sample size	Intervention	Method of determining lipoprotein subclasses	Main findings
1. Interventions					
Guay et al. 2012	Randomized, double-blind, crossover study	12 healthy and non-obese men with normal plasma lipid profile (age 27.1± 3.9 y)	1) High fat diet (37% of energy from fat and 50% of energy from carbohydrate) and 2) low fat diet (25% of energy from fat and 62% of energy from carbohydrate) for 3 days. There were 2 week washout period.	Polyacrylamide gradient gel electrophoresis	High fat diet ↑ the mean size of LDL particles in comparison to low fat diet. High fat diet ↓ the concentration of small LDL particles.
LeChemi nant et al. 2010	Quasi-experimental study	35 healthy, obese or overweight, middle age subjects	1) Reduced carbohydrate diet (around 20% of energy from carbohydrate) or 2) reduced fat diet (around 30% of energy from fat) for 9 months.	NMR	Both diets ↑ the concentrations of large and small HDL and large VLDL. Restricted fat diet ↑ the concentration of medium and small VLDL, as well. The amount of weight loss was not significantly different between and within groups.
Stoernell et al. 2008	Randomized, parallel study	28 hypertriglyceridemic participants	1) Low carbohydrate (15% of energy from carbohydrate and 55-65% of energy from fat) or 2) low fat diets (50-60% of energy from carbohydrate and 30% of energy from fat, lower than 10% of energy from SFA and trans fatty acids) for 8 weeks.	NMR	Low fat diet ↑ the concentration of small LDL by 36%.
Westman 2006	Randomized, parallel study	119 healthy, hyperlipidemic, overweight or obese subjects (age: 18-65 y)	1) Low carbohydrate, ketogenic diet (lower than 20g charbohydrate/ day) or 2) low fat, low calorie diet (lower than 30% of energy from fat, lower than 300 mg cholesterol/ day) for 6 months.	NMR	Low fat diet ↓ the concentrations of large VLDL, small LDL and LDL particles. It ↑ the concentration of large HDL and the mean size of LDL and HDL particles in comparison to baseline measurements.
William et al. 1995	Randomized Crossover study	105 men (age 48.9 ±11. 1 y)	1) Low fat diet (24% of energy from fat and 60% of energy from carbohydrate) and 2) high fat diet (46% of energy from fat and 38% of energy from carbohydrate) for 6 weeks.	Gradient-gel electrophoresis	Low fat diet ↓ the concentrations of HDL3a, HDL2a and HDL2b, mean size of HDL and peak diameter of HDL particles in comparison to high fat diet. The higher amount of reduction in concentration of HDL2b and mean diameter of HDL was seen in men with LDL pattern A than those with LDL pattern B.
Wojczynski et al 2011	Interventional study	1048 subjects (age: 18-87 y)	high fat meal (83% of energy from fat, 14% of energy form carbohydrate and 3% of energy from protein)	NMR	High fat meal ↓ the number of total LDL particles after 6 hours and the highest amount of ↓ was seen for the number of small LDL. High fat meal also ↓ the total number of VLDL particles owing to reduced concentration of medium

					and small VLDL. It ↑ the concentration of large VLDL and chylomicron among men with normal TG level.

SFA= Saturated fatty acids

3. OBJECTIVES

The main aim was to study the association between the quality and quantity of dietary fat intake and lipoprotein subclasses in postmenopausal women older than 65 years.

The specific aim was to study the association of SFA, MUFA, n-3 and n-6 PUFA intake with lipoprotein subclasses in plasma.

4. METHODS

This study was based on the baseline data of Osteoporosis Risk Factor and Prevention- Fracture Prevention Study (OSTPRE-FPS) (Kärkkäinen et al. 2010). It was an intervention study that began in 2003 and lasted for 3 years. OSTPRE-FPS was planned to analyze the impact of calcium and vitamin D supplementation on falls and fractures in postmenopausal women older than 65 years. OSTPRE-FPS was accepted by a research ethics committee of Kuopio University Hospital in October 2001. Moreover, participants completed paper based informed consent form at baseline (Kärkkäinen et al. 2010).

4.1 Participants

The participants of this study were part of the population based OSTPRE cohort of women who were born in 1932-1941. The interest of participation in the study was asked by postal enquiries from August to December of 2002. Minimum age of 65 years at the end of November 2002 and residing in Kuopio during trial were inclusion criteria of the study. In this study 3432 volunteering women with 63.5% response rate were participated (Kärkkäinen et al. 2010). From this population, 750 participants were asked to have laboratory tests and fill 3 days food records at baseline. Dietary food records of 554 participants were valid for analysis (Järvinen et al. 2012).

4.2 Baseline Measurements

Baseline measurements started in February 2003 and finished in May 2004. Anthropometric measurements were done at baseline. Weight was measured by a digital calibrated scale (Philips, type HF 351/00) and height was determined by calibrated wall meter. In addition, BMI was calculated dividing weight (in kg) by the square of height (in meters) (Kärkkäinen et al. 2010). Furthermore, lifestyle and health information such as intake of dietary supplements and medications, physical activity, disease status, menopause time, smoking and alcohol intake were asked through self-administrated postal questionnaire at baseline (Jarvinen et al. 2012. Participants were asked if they had a diagnosed diabetes treated with insulin, oral diabetic medications or diet.

4.3 Lipoprotein Subclasses

Blood samples of participants who were fasted for at least 10 hours were collected at baseline, and the lipoprotein subclasses were assessed by the NMR spectroscopy method. Bruker AVANCE 3 spectrometer, which work at 500.36 MHz was used for measuring the NMR information. Serum of blood sample was separated by centrifugation. 300 μl of each sample was mixed with 300 μl of sodium phosphate buffer. Next, the automatic sample preparation was done by the Gilson Liquid Handler. In this procedure at first, 300 μl of buffer was added to the NMR tubes, then 300 μl of serum was transformed to the tube. After that, aspirating mixed it completely.

Serum metabolome was detected via three different molecular windows (LIPO, LMWM and LIPID). The LIPO and LMWM were run from the native serum sample in a same experiment and the LIPID data from lipid extracts afterwards. LIPO windows illustrate the wide range of overlapped resonances that are mostly derived from various lipid molecules in different lipoprotein particles. These data were collected by 80 k data points after four dummy scans that used eight transients. LMWM shows signals from low molecular weight metabolites and LIPID window provides detailed molecular information on serum lipids (Inouye et al. 2010).

In this study, we used data only from LIPO window, which includes concentrations of lipoprotein particles, including chylomicrons, extremely large VLDL, very large VLDL, large VLDL, medium VLDL, small VLDL, very small VLDL, IDL, large LDL, medium LDL, small LDL, very large HDL, large HDL, medium HDL and small HDL. Moreover, amounts of total lipid, phospholipid, total cholesterol, cholesterol ester, free cholesterol and triglyceride in mentioned lipoprotein subclasses were measured. Mean diameters of VLDL, LDL and HDL particles were measured, as well.

4.4 Dietary Habits

Participants were asked to fill out 3 days' food record at baseline. The instruction of filling records and food record were sent to the women, and the subjects were asked to bring the complete form on the research visit. It was demanded to fill the records in 3 successive days (2 weekdays and 1 weekend). Regards to fat intake, questions were asked about the quality of fat

consumed in bread, cooking, and baking. Furthermore, participants were asked to recall frequency of fish consumption and nutritionist asked about the unclear parts of the diaries through phone calls. Five hundred and fifty four participants returned the dietary records. Finally, the intakes of nutrients were calculated by Nutrica program version 2.5 (Finnish social insurance institute, Turku. Finland) (Jarvinen et al. 2012).

4.5 Statistical Analysis

Data were analyzed by IBM SPSS statistics software (version 21). Mean ± SD, and valid percentage are reported. Normality of variables was checked by the Kolmogorov-Smirnov test, and the skewed variables were transformed by log 10 transformation. Transformed variables were used for the analysis, but back transformed means are reported. The Pearson correlation coefficient was used to analyze the correlations between normally distributed dietary factors and lipoprotein subclasses. The Spearman correlation coefficient was used to evaluate the correlations between non-normally distributed dietary factors and lipoprotein subclasses. Frequency of fish intake was classified into three categories, including no use or once per month, 0.5-1 times per week and two or more times per week. Kruskall-Wallis test was used to test the differences in distribution of VLDL and HDL subclasses among the categories of fish intake. The relation between tertiles of SFA (E%) and lipoprotein subclasses was analyzed by ANCOVA test with adjustment for physical activity, BMI, age, smoking and intake of lipid lowering drugs. However, unadjusted mean is reported. The Bonferroni test was conducted to test the pairwise differences. It should be mentioned that since the participants were elderly women who did not have a lot of physical activity, the physical activity was categorized into two categories, including two times or less per week and more than three times per week. In addition, smoking status was categorized into current smoker and not current smoker. The level of significance was set to p value less than 0.05 for all of the tests, and 2 tailed p values were reported.

5. RESULT

5.1 Basic Characteristics

Clinical and biochemical characteristics of participants are shown in Table 6. Altogether, 555 mostly nonsmoker women participated in the study. Mean BMI of participants was in the overweight range. In addition, there were not a lot of variations in the age of participants. Almost half of the participants had hypertension. Various kinds of diabetes were seen in 9.3 percent of participants. CHD was the most predominant heart problem in the sample group. In addition, around 25 percent of the population used the lipid lowering drug.

Table 6. Clinical and biochemical characteristics of participants[1].

Women (n=555)	
Age (y)[2]	67.85± 1.87
BMI (kg/m2)[2]	28.77± 4.73
Current smoker (%)[3]	5.4
Hypertension (%)	41.1
Coronary heart diseases (%)	16.6
Stroke (%)	1.1
Transient ischemic attack (%)	6.8
Other heart disease (%)	8.8
High serum cholesterol (%)	31.9
Diabetes treated with insulin (%)	1.8
Diabetes treated with oral medication (%)	4.1
Diabetes treated with diet (%)	3.4
Intake of lipid lowering drugs (%)	24.7
Physical activity 2 times or less per week (%)	40.4
Physical activity more than 3 times per week (%)	59.6

[1]Mean ± SD is reported for continuous variable and valid percentage for categorical variables.

[2]n= 554

[3]n= 503

Table 7 shows the distribution of lipoprotein subclasses in the population. According to the table, the concentration of 14 lipoprotein subclasses were measured. The concentration of very small VLDL was more than other subclasses of VLDL. Large LDL had the highest concentration, and

medium LDL had the lowest concentration between subclasses of LDL. Furthermore, although the highest proportions of total lipid, total cholesterol, cholesterol ester and free cholesterol were seen in large LDL, the greatest amount of phospholipid and triglycerides were seen in small HDL and medium HDL, respectively. In addition, extremely large VLDL had the lowest proportion of total lipid, phospholipid, total cholesterol, cholesterol ester and free cholesterol. The lowest amount of triglycerides was seen in very large HDL, as well.

Table 7. Lipoprotein subclasses of participants.

	women (n=547)
VLDL	
Concentration of chylomicrons and extremely large VLDL particles (nmol/l)	0.13± 0.11
Total lipids in chylomicrons and extremely large VLDL (mmol/l)	0.028± 0.025
Phospholipids in chylomicrons and extremely large VLDL (mmol/l)	0.003± 0.003
Total cholesterol in chylomicrons and extremely large VLDL (mmol/l)	0.005± 0.005
Cholesterol esters in chylomicrons and extremely large VLDL (mmol/l)	0.003± 0.003
Free cholesterol in chylomicrons and extremely large VLDL (mmol/l)	0.002± 0.002
Triglycerides in chylomicrons and extremely large VLDL (mmol/l)	0.020± 0.017
Concentration of very large VLDL particles (nmol/l)	0.65± 0.65
Total lipids in very large VLDL (mmol/l)	0.064± 0.0635
Phospholipids in very large VLDL (mmol/l)	0.010± 0.011
Total cholesterol in very large VLDL (mmol/l)	0.014± 0.013
Cholesterol esters in very large VLDL (mmol/l)	0.007± 0.007
Free cholesterol in very large VLDL (mmol/l)	0.007± 0.006
Triglycerides in very large VLDL (mmol/l)	0.040± 0.040
Concentration of large VLDL particles (nmol/l)	4.27± 3.47
Total lipids in large VLDL (mmol/l)	0.247± 0.202
Phospholipids in large VLDL (mmol/l)	0.045± 0.036
Total cholesterol in large VLDL (mmol/l)	0.058± 0.048
Cholesterol esters in large VLDL (mmol/l)	0.031± 0.024
Free cholesterol in large VLDL (mmol/l)	0.027± 0.024
Triglycerides in large VLDL (mmol/l)	0.145± 0.119
Concentration of medium VLDL particles (nmol/l)	16.05± 8.73
Total lipids in medium VLDL (mmol/l)	0.539± 0.290
Phospholipids in medium VLDL (mmol/l)	0.110± 0.056

Total cholesterol in medium VLDL (mmol/l)	0.156± 0.077
Cholesterol esters in medium VLDL (mmol/l)	0.093± 0.042
Free cholesterol in medium VLDL (mmol/l)	0.064± 0.036
Triglycerides in medium VLDL (mmol/l)	0.274± 0.162
Concentration of small VLDL particles (nmol/l)	33.00± 10.27
Total lipids in small VLDL (mmol/l)	0.658± 0.199
Phospholipids in small VLDL (mmol/l)	0.151± 0.042
Total cholesterol in small VLDL (mmol/l)	0.268± 0.074
Cholesterol esters in small VLDL (mmol/l)	0.173± 0.049
Free cholesterol in small VLDL (mmol/l)	0.095± 0.028
Triglycerides in small VLDL (mmol/l)	0.239± 0.094
Concentration of very small VLDL particles (nmol/l)	47.42± 10.17
Total lipids in very small VLDL (mmol/l)	0.606± 0.129
Phospholipids in very small VLDL (mmol/l)	0.169± 0.041
Total cholesterol in very small VLDL (mmol/l)	0.315± 0.065
Cholesterol esters in very small VLDL (mmol/l)	0.217± 0.043
Free cholesterol in very small VLDL (mmol/l)	0.097± 0.024
Triglycerides in very small VLDL (mmol/l)	0.122± 0.037
IDL	
Concentration of IDL particles (nmol/l)	122.26± 29.33
Total lipids in IDL (mmol/l)	1.235± 0.303
Phospholipids in IDL (mmol/l)	0.324± 0.078
Total cholesterol in IDL (mmol/l)	0.777± 0.206
Cholesterol esters in IDL (mmol/l)	0.557± 0.146
Free cholesterol in IDL (mmol/l)	0.220± 0.062
LDL	
Concentration of large LDL particles (nmol/l)	192.03± 50.69
Total lipids in large LDL (mmol/l)	1.364± 0.365
Phospholipids in large LDL (mmol/l)	0.337± 0.077
Total cholesterol in large LDL (mmol/l)	0.907± 0.270
Cholesterol esters in large LDL (mmol/l)	0.647± 0.203
Free cholesterol in large LDL (mmol/l)	0.260± 0.069
Triglycerides in large LDL (mmol/l)	0.120± 0.033
Concentration of medium LDL particles (nmol/l)	149.94± 43.17
Total lipids in medium LDL (mmol/l)	0.762± 0.220

Phospholipids in medium LDL (mmol/l)	0.204± 0.044
Total cholesterol in medium LDL (mmol/l)	0.502± 0.168
Cholesterol esters in medium LDL (mmol/l)	0.357± 0.135
Free cholesterol in medium LDL (mmol/l)	0.145± 0.033
Triglycerides in medium LDL (mmol/l)	0.055± 0.018
Concentration of small LDL particles (nmol/l)	173.68± 47.33
Total lipids in small LDL (mmol/l)	0.488± 0.134
Phospholipids in small LDL (mmol/l)	0.144± 0.028
Total cholesterol in small LDL (mmol/l)	0.309± 0.101
Cholesterol esters in small LDL (mmol/l)	0.221± 0.082
Free cholesterol in small LDL (mmol/l)	0.088± 0.020
Triglycerides in small LDL (mmol/l)	0.034± 0.011
HDL	
Concentration of very large HDL particles (nmol/l)	519.47± 228.67
Total lipids in very large HDL (mmol/l)	0.530± 0.234
Phospholipids in very large HDL (mmol/l)	0.240± 0.125
Total cholesterol in very large HDL (mmol/l)	0.272± 0.110
Cholesterol esters in very large HDL (mmol/l)	0.199± 0.077
Free cholesterol in very large HDL (mmol/l)	0.072± 0.033
Triglycerides in very large HDL (mmol/l)	0.019± 0.008
Concentration of large HDL particles (nmol/l)	1172.70± 566.13
Total lipids in large HDL (mmol/l)	0.734± 0.362
Phospholipids in large HDL (mmol/l)	0.360± 0.158
Total cholesterol in large HDL (mmol/l)	0.340± 0.193
Cholesterol esters in large HDL (mmol/l)	0.266± 0.146
Free cholesterol in large HDL (mmol/l)	0.074± 0.047
Triglycerides in large HDL (mmol/l)	0.035± 0.020
Concentration of medium HDL particles (nmol/l)	1912.49± 412.82
Total lipids in medium HDL (mmol/l)	0.806± 0.179
Phospholipids in medium HDL (mmol/l)	0.392± 0.078
Total cholesterol in medium HDL (mmol/l)	0.369± 0.100
Cholesterol esters in medium HDL (mmol/l)	0.295± 0.079
Free cholesterol in medium HDL (mmol/l)	0.073± 0.022
Triglycerides in medium HDL (mmol/l)	0.046± 0.015
Concentration of small HDL particles (nmol/l)	4846.44± 566.78

Total lipids in small HDL (mmol/l)	1.074± 0.127
Phospholipids in small HDL (mmol/l)	0.585± 0.078
Total cholesterol in small HDL (mmol/l)	0.434± 0.072
Cholesterol esters in small HDL (mmol/l)	0.321± 0.064
Free cholesterol in small HDL (mmol/l)	0.112± 0.017
Triglycerides in small HDL (mmol/l)	0.055± 0.015
Mean diameter of VLDL, LDL and HDL	
Mean diameter for VLDL particles (nm)	36.14± 1.23
Mean diameter for LDL particles (nm)	23.67± 0.14
Mean diameter for HDL particles (nm)	9.99± 0.26

Mean ± SD is reported for all of variables.

Table 8 shows the dietary intakes of nutrients among participants. According to the table, around 18% of energy was derived from protein, 50% of energy was supplied by carbohydrate, and 31% percent of energy was derived from total fat. Participants approximately obtained 12% of energy from SFA, 9% of energy from MUFA and 5% of energy from PUFA. In average, they consumed 0.12 g/day EPA and 0.28 g/day DHA, as well.

Table 8. Dietary intake of participants.

Dietary intake of participants	
n= 554	
	Mean ± SD
Energy (kcal/d)	1568± 372
Protein (E%)	17.6± 3.1
Carbohydrates (E%)	49.1± 5.8
Fat (E%)	31.1± 5.6
Saturated fatty acids (E%)	12.2± 3.1
Monounsaturated fatty acids (E%)	9.8± 2.4
Polyunsaturated fatty acids (E%)	5.1± 1.4
Palmitic acid C16 (g/d)	9.91± 3.95
Stearic acid C18 (g/d)	3.85± 1.63
Linoleic acid C18:2 (g/d)	5.88± 2.34
Linolenic acid C18:3 (g/d)	1.45± 0.78
Arachidonic acid C20:4 (g/d)	0.11± 0.08
Eicosapentaenoic acid C20:5 (g/d)	0.12± 0.14
Docosahexaenoic acid C22:6 (g/d)	0.28± 0.33

5.2 Association between Dietary Fat and Lipoprotein Subclasses

The results of correlation analyses are reported in Table 9. Besides to the fatty acids reported in the table, arachidonic acid, palmitic acid, linoleic acid and linolenic acid were entered in the correlation analysis, but they did not have significant correlation with lipoprotein subclasses. Based on the results of these analyses, intake of PUFA had significant positive correlations with triglycerides in medium and small VLDL, concentration of small VLDL and mean diameter of VLDL. In addition, smaller mean diameter of LDL significantly related to higher intake of MUFA, PUFA and total fat. Furthermore, total fat intake had positive correlations with free cholesterol in medium and small LDL, concentration of small LDL, total lipid and phospholipid in small LDL. Higher intake of EPA significantly correlated with higher total cholesterol, cholesterol ester and free cholesterol in very small VLDL while, intake of DHA had positive correlation only with total cholesterol and cholesterol ester in very small VLDL. In addition, EPA and DHA negatively correlated with triglycerides in chylomicron and extremely large VLDL. EPA had negative correlation with the mean diameter of VLDL, as well.

SFA (E%) had positive correlations with total cholesterol in very small VLDL, medium and small HDL, large, medium and small LDL and IDL. In addition, it positively associated with higher concentrations of IDL, small, medium and large LDL particles. Higher intake of SFA also significantly related with higher phospholipid in IDL, large LDL, medium LDL and small LDL. It had significant positive correlations with cholesterol ester in IDL, large, medium and small LDL, very small VLDL and small HDL. Moreover, total lipid and free cholesterol in IDL, large, medium and small LDL positively correlated with intake of SFA.

Furthermore, intake of protein had negative correlations with lots of the lipoprotein subclasses, including total lipid and total cholesterol in IDL, total cholesterol and cholesterol ester in large LDL, phospholipid, total cholesterol and cholesterol ester in medium LDL, total cholesterol and cholesterol ester in small LDL, free cholesterol in very large HDL and total cholesterol in medium HDL. In addition, further correlation analysis revealed that the intake of protein had negative correlations with intake of SFA and total fat.

Moreover, the intake of EPA plus DHA had positive correlations with cholesterol ester and total cholesterol in very small VLDL ($p = 0.011$ and 0.020, respectively). Triglycerides in

chylomicron and extremely large VLDL had negative correlation with intake of EPA plus DHA as well (p= 0.041). In addition, further analysis revealed that there was not any significant difference in distribution of VLDL and HDL subclasses among the three categories of fish intake (no use or once per month, 0.5-.1 times per week and two or more times per week).

Table 9. Correlation coefficient from Pearson and Spearman correlation analysis.

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
VLDL									
Concentration of chylomicron and extremely large VLDL (mol/L)	0.02	-0.028	0.043	0.058	0.002	0.036	0.048	-0.078	-0.081
Total lipid in chylomicron and extremely large VLDL (mmol/L)	0.022	-0.028	0.043	0.057	0.003	0.034	0.048	-0.076	-0.08
Phospholipid in chylomicron and extremely large VLDL (mmol/L)	0.023	-0.014	0.036	0.066	0.002	0.051	0.029	-0.067	-0.07
Total cholesterol in chylomicron and extremely large VLDL (mmol/L)	0.049	-0.034	0.044	0.044	-0.001	0.018	0.06	-0.047	-0.057
Cholestrolester in chylomicron and extremely large VLDL (mmol/L)	0.065	-0.051	0.05	0.037	0.000	0.011	0.071	-0.038	-0.048
Free cholesterol in chylomicron and extremely large VLDL (mmol/L)	0.025	-0.01	0.032	0.05	0.002	0.028	0.037	-0.068	-0.076
Triglycerides in chylomicron and extremely large VLDL (mmol/L)	0.015	-0.029	0.045	0.061	0.001	0.037	0.048	**-.087***	**-.089***
Concentration of very large VLDL (mol/L)	0.017	0.002	0.027	0.056	0.003	0.047	0.021	-0.075	-0.076
Total lipid in very large VLDL (mmol/L)	0.019	-0.001	0.029	0.056	0.002	0.046	0.025	-0.074	-0.076
Phospholipid in very large VLDL (mmol/L)	0.025	-0.011	0.038	0.057	0	0.04	0.037	-0.075	-0.08
Total cholesterol in very large VLDL (mmol/L)	0.041	-0.023	0.044	0.051	-0.006	0.028	0.053	-0.054	-0.062
Cholesterolester in very large VLDL (mmol/L)	0.042	-0.023	0.038	0.047	-0.003	0.029	0.047	-0.049	-0.055
Free cholesterol in very large VLDL (mmol/L)	0.036	-0.025	0.05	0.054	-0.008	0.029	0.056	-0.059	-0.068
Triglycerides in very large VLDL (mmol/L)	0.009	0.009	0.02	0.056	0.005	0.051	0.011	-0.08	-0.078
Concentration of large VLDL (mol/L)	0.01	0.015	0.016	0.064	0.003	0.077	-0.004	-0.063	-0.059
Total lipid in large VLDL (mmol/L)	0.011	0.015	0.016	0.063	0.003	0.075	-0.003	-0.062	-0.059

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
Phospholipid in large VLDL (mmol/L)	0.013	0.009	0.017	0.062	0.007	0.071	0.001	-0.063	-0.062
Total cholesterol in large VLDL (mmol/L)	0.029	0.004	0.015	0.054	0.007	0.061	0.006	-0.051	-0.051
Cholesterolester in large VLDL (mmol/L)	0.038	-0.01	0.015	0.047	0.008	0.06	0.008	-0.04	-0.04
Free cholesterol in large VLDL (mmol/L)	0.02	0.009	0.017	0.058	0.007	0.061	0.004	-0.063	-0.064
Triglycerides in large VLDL (mmol/L)	0.004	0.018	0.015	0.066	0.003	0.081	-0.008	-0.067	-0.061
Concentration of medium VLDL (mol/L)	-0.02	0.004	-0.001	0.062	0.023	0.08	-0.007	-0.047	-0.046
Total lipid in medium VLDL (mmol/L)	-0.017	0.002	-0.001	0.06	0.023	0.078	-0.006	-0.047	-0.046
Phospholipid in medium VLDL (mmol/L)	-0.013	-0.002	0.001	0.059	0.022	0.075	-0.003	-0.047	-0.048
Total cholesterol in medium VLDL (mmol/L)	0.016	-0.017	0.01	0.047	0.017	0.059	0.011	-0.035	-0.041
Cholesterolester in medium VLDL (mmol/L)	0.059	-0.023	0.018	0.031	0.014	0.036	0.029	-0.027	-0.037
Free cholesterol in medium VLDL (mmol/L)	0.019	0.014	0.01	0.061	0.009	0.08	-0.012	-0.049	-0.05
Triglycerides in medium VLDL (mmol/L)	-0.033	0.012	-0.005	0.067	0.025	**.086***	-0.017	-0.052	-0.047
Concentration of small VLDL (mol/L)	-0.005	-0.006	0.001	0.06	0.026	**.086***	-0.012	-0.005	-0.011
Total lipid in small VLDL (mmol/L)	0	-0.011	0.004	0.059	0.024	0.083	-0.007	0	-0.007
Phospholipid in small VLDL (mmol/L)	0.007	-0.005	-0.004	0.058	0.024	0.083	-0.023	0.01	0.005
Total cholesterol in small VLDL (mmol/L)	0.032	-0.05	0.032	0.046	0.012	0.054	0.045	0.036	0.022
Cholesterolester in small VLDL (mmol/L)	0.046	-0.069	0.047	0.038	0.005	0.037	0.075	0.05	0.034
Free cholesterol in small VLDL (mmol/L)	0.006	-0.014	0	0.053	0.027	0.078	-0.009	0.011	0.004

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
Triglyceride in small VLDL (mmol/L)	-0.027	0.015	-0.018	0.059	0.038	**.094***	-0.042	-0.029	-0.03
Concentration of very small VLDL (mol/L)	0.038	-0.03	0.029	0.034	-0.008	0.033	0.035	0.056	0.047
Total lipid in very small VLDL (mmol/L)	0.043	-0.034	0.034	0.032	-0.012	0.028	0.045	0.063	0.053
Phospholipid in very small VLDL (mmol/L)	0.035	-0.054	0.033	0.024	-0.005	0.02	0.052	0.05	0.038
Total cholesterol in very small VLDL (mmol/L)	0.072	-0.045	0.055	0.023	-0.033	0.007	**.085***	**.107***	**.095***
Cholesterolester very small VLDL (mmol/L)	0.066	-0.029	0.058	0.02	-0.062	-0.034	**.095***	**.117****	**.103***
Free cholesterol in very small VLDL (mmol/L)	0.045	-0.031	0.04	0.026	-0.017	0.029	0.058	**.088***	0.077
Triglycerides in very small VLDL (mmol/L)	-0.014	0.014	-0.033	0.033	0.043	0.067	-0.057	0.004	-0.002
IDL									
Concentration of IDL (mol/L)	0.039	**-.101***	0.057	0.013	-0.006	-0.024	**.104***	0.013	-0.003
Total lipid in IDL (mmol/L)	0.024	**-.089***	0.053	0.009	-0.007	-0.069	**.109***	0.017	0
Phospholipid in IDL (mmol/L)	0.039	**-.111****	0.061	0.011	-0.005	-0.029	**.113****	0.008	-0.009
Total cholesterol in IDL (mmol/L)	0.029	**-.094***	0.068	0.012	-0.021	-0.067	**.124****	0.026	0.009
Cholesterolester in IDL (mmol/L)	0.051	**-.110****	0.061	0.008	-0.009	-0.033	**.127****	0.023	0.007
Free cholesterol in IDL (mmol/L)	0.016	-0.078	0.055	0.007	-0.014	-0.072	**.113****	0.027	0.011
Triglycerides in IDL (mmol/L)	0.004	-0.001	-0.044	0.005	0.041	-0.023	-0.026	0.021	0.014
LDL									
Concentration of large LDL (mol/L)	0.028	**-.113****	0.072	0.029	-0.012	-0.013	**.115****	0	-0.018

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
Total lipid in large LDL (mmol/L)	0.03	**-.114****	0.073	0.028	-0.013	-0.014	**.117****	0.002	-0.016
Phospholipid in large LDL (mmol/L)	0.036	**-.113****	0.075	0.03	-0.019	-0.014	**.124****	0.002	-0.014
Total cholesterol in large LDL (mmol/L)	0.024	**-.102***	0.073	0.024	-0.015	-0.053	**.124****	0.007	-0.011
Cholesterolester in large LDL (mmol/L)	0.025	**-.103***	0.074	0.028	-0.015	-0.045	**.123****	0.005	-0.012
Free cholesterol in large LDL (mmol/L)	0.034	**-.115****	0.072	0.015	-0.011	-0.024	**.129****	0.008	-0.009
Triglycerides in large LDL (mmol/L)	-0.008	-0.058	0.017	0.016	0.017	-0.008	0.011	-0.001	-0.012
Concentration of medium LDL (mol/L)	0.016	**-.116****	0.08	0.045	-0.016	0.002	**.111****	-0.006	-0.025
Total lipid in medium LDL (mmol/L)	0.018	**-.116****	0.081	0.044	-0.017	0.002	**.114****	-0.005	-0.023
Phospholipid in medium LDL (mmol/L)	0.034	**-.112****	**.092***	0.049	-0.026	-0.034	**.130****	-0.005	-0.018
Total cholesterol in medium LDL (mmol/L)	0.014	**-.101***	0.071	0.032	-0.011	-0.039	**.115****	0.001	-0.018
Cholesterolester in medium LDL (mmol/L)	0.011	**-.099***	0.068	0.034	-0.009	-0.033	**.110****	0	-0.019
Free cholesterol in medium LDL (mmol/L)	0.029	**-.118****	**.097***	0.041	-0.032	-0.008	**.136****	0.004	-0.012
Triglycerides in medium LDL (mmol/L)	-0.012	-0.055	-0.009	0.012	0.044	-0.045	0.017	-0.026	-0.037
Concentration of small LDL (mol/L)	0.013	**-.119****	**.087***	0.058	-0.023	0.012	**.111****	-0.002	-0.017
Total lipid in small LDL (mmol/L)	0.015	**-.119****	**.089***	0.058	-0.025	0.011	**.115****	0	-0.015
Phospholipid in small LDL (mmol/L)	0.021	**-.117****	**.104***	0.073	-0.046	0.017	**.126****	-0.005	-0.011
Total cholesterol in small LDL (mmol/L)	0.008	**-.103***	0.075	0.037	-0.014	-0.033	**.116****	0.004	-0.012
Cholesterolester in small LDL (mmol/L)	0.004	**-.102***	0.07	0.036	-0.01	-0.03	**.111****	0.002	-0.015

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
Free cholesterol in small LDL (mmol/L)	0.029	**-.117****	**.108***	0.059	-0.044	0.009	**.131****	0.009	-0.003
Triglycerides in small LDL (mmol/L)	-0.023	-0.074	0.01	0.04	0.037	0.019	0.005	-0.046	-0.06
HDL									
Concentration of very large HDL (mol/L)	-0.014	-0.06	0.023	-0.021	0.009	-0.033	0.028	0.032	0.044
Total lipid in very large HDL (mmol/L)	-0.013	-0.063	0.026	-0.019	0.007	-0.033	0.032	0.033	0.045
Phospholipid in very large HDL (mmol/L)	-0.017	-0.046	0.006	-0.028	0.017	-0.03	0.009	0.03	0.044
Total cholesterol in very large HDL (mmol/L)	-0.01	-0.08	0.055	-0.002	-0.014	-0.031	0.062	0.036	0.047
Cholesterolester in very large HDL (mmol/L)	-0.013	-0.079	0.055	0.001	-0.015	-0.027	0.06	0.036	0.047
Free cholesterol in very large HDL (mmol/L)	-0.005	**-.085***	0.06	-0.003	-0.017	-0.042	0.07	0.032	0.047
Triglycerides in very large HDL (mmol/L)	0.01	-0.056	0.009	-0.027	0.04	-0.059	0.036	-0.057	-0.076
Concentration of large HDL (mol/L)	-0.02	-0.01	-0.012	-0.036	0.015	-0.029	-0.007	0.024	0.032
Total lipid in large HDL (mmol/L)	0.021	-0.008	-0.001	-0.03	-0.014	-0.03	-0.006	0.026	0.033
Phospholipid in large HDL (mmol/L)	0.026	-0.016	-0.001	-0.026	-0.015	-0.031	-0.004	0.025	0.032
Total cholesterol in large HDL (mmol/L)	0.021	-0.007	0.004	-0.026	-0.017	-0.025	-0.009	0.031	0.04
Cholesterolester in large HDL (mmol/L)	0.022	-0.006	0.004	-0.026	-0.018	-0.026	-0.008	0.03	0.039
Free cholesterol in large HDL (mmol/L)	-0.019	-0.01	-0.011	-0.033	0.013	-0.023	-0.009	0.036	0.045
Triglycerides in large HDL (mmol/L)	0.012	0.001	-0.039	-0.07	0.048	-0.056	0.001	-0.009	-0.017
Concentration of medium HDL (mol/L)	0.029	0.001	0.012	0.041	-0.028	0.026	0.002	0.007	0.024

	Energy (kcal)	Protein (E%)	Fat (E%)	MUFA (E%)	Carbohydrates (E%)	PUFA (E%)	SFA (E%)	Eicosapentaenoic acid (g/d)	Docosahexaenoic acid (g/d)
Total lipid in medium HDL (mmol/L)	0.028	0.001	0.013	0.041	-0.03	0.026	0.002	0.012	0.029
Phospholipid in medium HDL (mmol/L)	0.027	-0.016	0.013	0.04	-0.023	0.017	0.004	0.007	0.023
Total cholesterol in medium HDL (mmol/L)	0.014	**-.101***	0.071	0.032	-0.011	-0.039	**.115****	0.001	-0.018
Free cholesterol in medium HDL (mmol/L)	0.024	0	0.004	0.029	-0.026	0.017	-0.003	0.033	0.048
Cholesterolester in medium HDL (mmol/L)	0.021	0.022	0.015	0.039	-0.044	0.033	-0.004	0.024	0.042
Triglycerides in medium HDL (mmol/L)	0.054	0.003	-0.039	-0.005	0.039	0.004	-0.025	-0.041	-0.05
Concentration of small HDL (mol/L)	0.019	-0.019	0.042	0.068	-0.035	0.024	0.037	-0.013	-0.005
Total lipid in small HDL (mmol/L)	0.019	-0.022	0.046	0.07	-0.038	0.024	0.042	-0.009	0
Phospholipid in small HDL (mmol/L)	0.033	0.033	-0.014	0.026	-0.045	0	-0.027	-0.022	-0.006
Total cholesterol in small HDL (mmol/L)	0.006	-0.072	0.083	0.061	-0.031	-0.002	**.109***	-0.003	-0.006
Cholesterolester in small HDL (mmol/L)	0.001	-0.077	0.079	0.058	-0.024	-0.001	**.105***	-0.002	-0.011
Free cholesterol in small HDL (mmol/L)	0.02	0.016	0.026	0.038	-0.043	0.015	0.018	-0.013	-0.002
Triglycerides in small HDL (mmol/L)	-0.009	-0.014	-0.042	-0.014	0.063	-0.011	-0.029	-0.065	-0.082
Mean diameter of VLDL, LDL and HDL									
Mean diameter of VLDL (nm)	0.005	0.013	0.01	0.055	0.014	**.087***	-0.017	**-.086***	-0.081
Mean diameter of LDL (nm)	0.028	0.062	**-.112****	**-.119****	0.056	**-.097***	-0.08	0.032	0.033
Mean diameter of HDL (nm)	-0.015	-0.028	-0.004	-0.037	0.015	-0.034	0.004	0.03	0.043

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).
MUFA= monounsaturated fatty acids, PUFA = polyunsaturated fatty acids and SFA= saturated fatty acids.

The Pearson correlation coefficient is reported for correlations between normally distributed variables.
The Spearman correlation coefficient is reported for correlations between variables that either one of them or both are not normally distributed.

Although total fat (E %), PUFA (E %), MUFA (E %), EPA and DHA had some correlations with different subclasses of lipoprotein, most of the significant correlations were observed between SFA (E %) and lipoprotein subclasses. In addition, subclasses of VLDL, LDL and IDL had the higher amount of significant correlations with dietary fatty acids in comparison to subclasses of HDL. Therefore, tertiles of SFA intake (E%) were made to evaluate the relation of SFA and subclasses of VLDL, LDL and IDL. The result of this ANCOVA test with adjustment for physical activity, intake of lipid lowering drugs, smoking, age and BMI is shown in Table 10. After these adjustments, two significant and not linear relations between intake of SFA (E%) and subclasses of LDL and VLDL were found. The higher intake of SFA significantly associated with the smaller size of LDL particles ($p=0.04$) (Figure 1). Post hoc analysis showed that the difference in the mean diameter of LDL is significant between the middle and highest tertiles categories of SFA ($P= 0.035$). In addition, higher intake of SFA significantly related to the lower amount of triglyceride in small VLDL ($p=0.046$). The differences between the mean of triglycerides in small VLDL was significant between lowest and middle tertiles of SFA (E%) ($P= 0.039$). Higher intake of SFA tended to be in significant relation with higher amount of phospholipid in small LDL, as well ($p=0.06$).

Table 10. Concentration of lipoprotein particles in tertiles of saturated fatty acid intake (E%).

	First tertile (3.6-10.8)	Second tertile (10.8-13.3)	Third tertile (13.3-24.9)	P trend
N	185	185	185	-
Total lipid in chylomicron and extremely large VLDL (mmol/L)	0.029± 0.027	0.026± 0.019	0.030± 0.028	0.372
Phospholipid in chylomicron and extremely large VLDL (mmol/L)	0.003± 0.003	0.003± 0.002	0.003± 0.003	0.309
Total cholesterol in chylomicron and extremely large VLDL (mmol/L)	0.005± 0.005	0.005± 0.004	0.006± 0.005	0.359
Cholesterol ester in chylomicron and extremely large VLDL (mmol/L)	0.003± 0.003	0.003± 0.002	0.003± 0.003	0.485
Free cholesterol in chylomicron and extremely large VLDL (mmol/L)	0.002± 0.002	0.002± 0.002	0.002± 0.002	0.249
Concentration of very large VLDL (nmol//l)	0.688± 0.727	0.565± 0.498	0.702± 0.688	0.564
Total lipid in very large VLDL (mmol/L)	0.068± 0.071	0.056± 0.049	0.069± 0.067	0.550
Phospholipid in very large VLDL (mmol/L)	0.011± 0.012	0.009± 0.008	0.011± 0.011	0.585
Total cholesterol in very large VLDL (mmol/L)	0.015± 0.015	0.013± 0.010	0.015± 0.014	0.528
Cholesterol ester in very large VLDL (mmol/L)	0.008± 0.008	0.007± 0.005	0.008± 0.007	0.685
Free cholesterol in very large VLDL (mmol/L)	0.007± 0.007	0.006± 0.005	0.007± 0.007	0.442
Triglyceride in very large VLDL (mmol/L)	0.042± 0.045	0.034± 0.031	0.043± 0.043	0.596
Concentration of large VLDL (nmol/L)	4.527± 3.875	3.736± 2.649	4.512± 3.671	0.467
Total lipid in large VLDL (mmol/L)	0.262± 0.225	0.217± 0.154	0.262± 0.214	0.468
Phospholipid in large VLDL (mmol/L)	0.047± 0.41	0.39± 0.028	0.047± 0.038	0.420
Total cholesterol in large VLDL (mmol/L)	0.061± 0.053	0.051± 0.037	0.061± 0.052	0.555
Cholesterol ester in large VLDL (mmol/L)	0.032± 0.026	0.028± 0.019	0.033± 0.026	0.755
Free cholesterol in large VLDL (mmol/L)	0.028± 0.027	0.023± 0.019	0.028± 0.026	0.511
Triglyceride in large VLDL (mmol/L)	0.154± 0.133	0.126± 0.091	0.153± 0.125	0.467
Phospholipid in medium VLDL (mmol/L)	0.114± 0.061	0.100± 0.043	0.114± 0.062	0.165
Total cholesterol in medium VLDL (mmol/L)	0.160± 0.085	0.145± 0.062	0.162± 0.085	0.224
Cholesterol ester in medium VLDL (mmol/L)	0.094± 0.046	0.088± 0.036	0.095± 0.046	0.397
Free cholesterol in medium VLDL (mmol/L)	0.067± 0.040	0.057± 0.028	0.066± 0.041	0.138
Phospholipid in small VLDL (mmol/L)	0.155± 0.042	0.144± 0.034	0.153± 0.049	0.099
Triglyceride in small VLDL (mmol/L)	0.250± 0.097	0.220± 0.071	0.246± 0.113	0.046

Concentration of very small VLDL (nmol/L)	47.635± 10.287	46.317± 8.953	48.054± 11.394	0.282
Total lipid in very small VLDL (mmol/L)	0.607± 0.132	0.594± 0.115	0.614± 0.143	0.377
Free cholesterol in very small VLDL (mmol/L)	0.097± 0.024	0.095± 0.021	0.099± 0.026	0.554
Triglyceride in large LDL (mmol/L)	0.121± 0.033	0.115± 0.027	0.122± 0.036	0.146
Triglyceride in medium LDL (mmol/L)	0.056± 0.017	0.053± 0.014	0.057± 0.019	0.141
Concentration of small LDL (nmol/L)	170.823± 46.934	170.440± 43.127	181.791± 52.546	0.182
Total lipid in small LDL (mmol/L)	0.479± 0.132	0.479± 0.122	0.511± 0.149	0.189
Phospholipid in small LDL (mmol/L)	0.143± 0.028	0.142± 0.025	0.150± 0.030	0.066

The Multivariate ANOVA model was adjusted for BMI, age, physical activity, lipid lowering drugs and smoking.
Unadjusted mean ± SD was reported for all of the variables.
Log transformed variables were used for analysis.

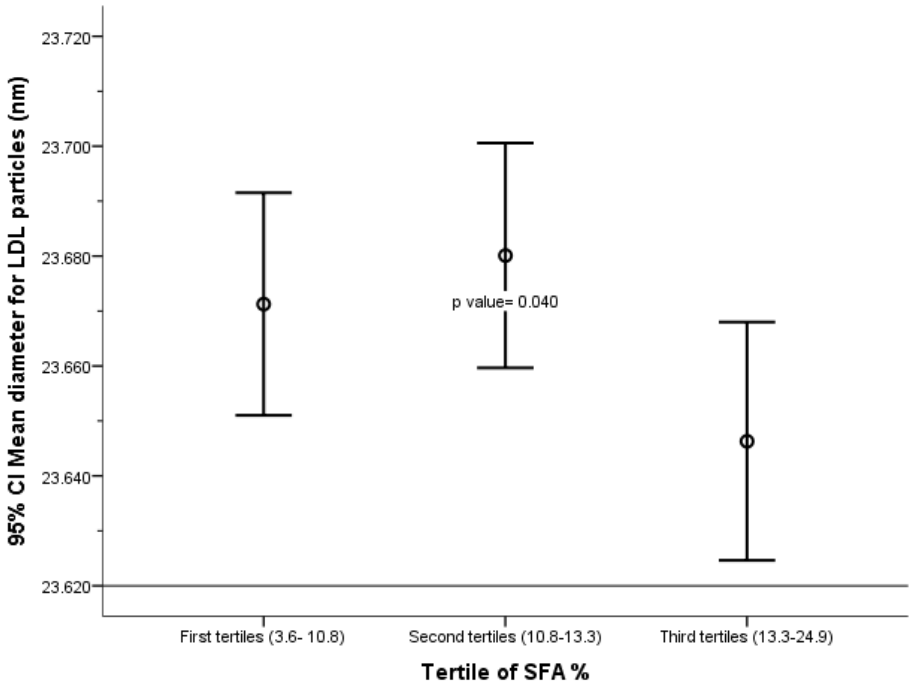


Figure 1. Error bar graph showing mean diameter of LDL particles and 95% confidence intervals by tertiles of SFA (E %)

6. DISCUSSION

This is the first study that showed the significant non-linear correlation between the intake of SFA and subclasses of LDL and VLDL defined by the NMR method. We found that the mean diameter of LDL is significantly higher in the middle tertile categories of SFA in comparison to the highest tertile of SFA. In addition, lowest tertile of SFA intake had significantly higher mean triglycerides in small VLDL than the middle tertile of SFA. It should be noted, that the mentioned non-linear and negative correlation was found after adjustment for BMI, age, physical activity, lipid lowering drugs and smoking in postmenopausal women.

6.1 Intake of SFA and Mean Size of LDL Particles

Previous Studies

The result of the present study is consistent with an interventional study involving ten mildly hypercholesterolemic men. In this study, participants were fed different kinds of hamburgers (5 patties per week) in a crossover design. The intervention started with the consumption of hamburger rich in SFA (MUFA/ SFA= 0.95) for 5 weeks, then followed by usual diet for 3 weeks. Afterwards, participants consumed hamburger rich in MUFA (MUFA/ SFA= 1.31) for another 5 weeks. It should be mentioned that the 114 g hamburger patties which were made from beef were provided for participants. The result of mentioned study showed that the intake of hamburger rich in SFA decreased the LDL particle diameter. In addition, this study found that the decline of LDL diameter remained even after 3 week washout period or after intake of hamburger rich in MUFA (Adams et al. 2010). Furthermore, in another interventional study, Smith and his colleague reported that the intake of hamburger rich in SFA (MUFA/ SFA= 0.83-0.96) increased the apo B/ LDL cholesterol (Smith et al 2002). Since according to the result of present study and another study conducted by Hayashi and his colleagues, the apoB negatively correlated with the mean size of LDL (Hayashi et al. 2006), higher apoB/ LDL might be related to decreased size and increased density of LDL.

Moreover, in an interventional study low carbohydrate, high SFA diet (15% of energy from SFA) decreased the mean diameter of LDL as compared to low carbohydrate, low SFA diet (8% of energy from SFA), but this reduction was not significant. The main source of SFA in this

study was dairy products (Mangravite et al. 2011). It should be noted that different kinds of SFA might have various effects on subclasses of LDL. For example, while it is found that intake of total SFA increased the LDL cholesterol, the intake of stearic acid (one of the main sources of stearic acid is dairy products) decreased the LDL cholesterol in a dose- response relation (Hunter et al. 2010). In addition, a cross-sectional study found that SFA derived from milk products had negative correlation with concentration of small LDL particles (Sjogren et al. 2004). Therefore, not finding a significant relation in this study might be due to not having control over the sources of SFA.

Based on a review which was done by Hunter and his colleagues in most of the studies that aimed to find the effects of SFA intake on risk of CHD, a positive correlation between intake of SFA and risk of CHD or other coronary events was found (Hunter et al. 2010). Numerous studies reported that the predominance of small and dense LDL is related to two or three times increased risk of CVD (Krauss 2001, Gerber & Berneis 2012). Therefore, the intake of SFA might increase the risk of CHD through decreasing the mean diameter of LDL.

On the contrary, in an interventional study Dreon and his colleagues reported that intake of SFA increased the mean diameter of LDL particles (Dreon et al. 1998). In this study, healthy men consumed either a high fat (46% of energy from fat, 18% of energy from SFA, and 39 % of energy from carbohydrate) or a low fat diet (24% of energy from fat, 6% of energy from SFA, and 59% of energy from carbohydrate) in a crossover design. Although the intake of MUFA and PUFA were similar in both diets, the intake of carbohydrate differed greatly and carbohydrate was replaced with SFA in the low fat diet. It is reported that dietary carbohydrate did not have a linear correlation with plasma lipoprotein, but it might have a non-linear correlation with lipoprotein particles. In addition, based on a result of a review article, recent studies demonstrated that the high intake of carbohydrate decreased the size of LDL (Gerber & Bernis 2012). Therefore, lower consumption of carbohydrate in this study might be the reason of increasing the mean size of LDL, not higher intake of SFA.

Probable Mechanism

Some probable mechanisms for the relation between intake of SFA and size of LDL particles can be discussed, but further studies are needed to find the mechanism. ApoCIII exist on the surface of VLDL, LDL and HDL particles. This apolipoprotein has a strong impact on the metabolism of VLDL, LDL and HDL particles. It is reported that apoCIII significantly increased the level of small LDL and decreased the mean diameter of LDL particles independent of TG level. This effect might happen due to increasing the secretion, and decreasing the catabolism of TG- rich lipoproteins. ApoCIII can be the reason of the mentioned changes through inhibiting the lipoprotein lipase activity and decreasing uptake of apoCIII enriched VLDL that follow with higher circulation of VLDL and greater exchange of VLDL triglyceride for the cholesterol esters of LDL and HDL. Consequently, produced LDL is denser and smaller (Shin & Krauss 2010). However, a kinetic study showed that apoCIII increased the production of dense LDL by accelerating the action of hepatic lipase and not inhibiting lipoprotein lipase activity. In addition, it has been found that apoCIII decreased the liver clearance of all lipoproteins enriched in apoB that lead to increased formation of LDL (Mendivil et al. 2010). Although there is not consistency about the impact of apoCIII on lipoprotein lipase activity, both theories reached to the same result about the effect of apoCIII on the formation of small and dense LDL.

On the other hand, an interventional study showed that diet rich in SFA (15% of energy from SFA, 15% of energy from MUFA) significantly increased the amount of apoCIII in LDL by 33.5% in comparison to diet low in SFA and rich in MUFA (8% of energy from SFA and 21% of energy from MUFA). It should be mentioned that other macronutrients were kept constant in this study, the intake of carbohydrate was moderate and the changes were independent of the TG level (Faghihnia et al. 2012). In addition, another interventional study that used two different diets, including low SFA diet (8% of energy from SFA) and high SFA diet (15% energy from SFA with replacement of SFA by MUFA) showed that lower intake of SFA decreased the activity of hepatic lipase (Mangravite et al. 2011). Therefore, SFA might increase the formation of small LDL or decrease the mean diameter of LDL by increasing the concentration of apoCIII and decreasing the hepatic lipase activity.

Furthermore, there were several negative correlations between protein intake and lipid content of LDL, IDL and HDL particles. In addition, we found that the intake of protein had negative correlation with intake of SFA and total fat ($p< 0.001$). Therefore, lower concentration of the lipid component of LDL, HDL and IDL subclasses might be due to lower intake of SFA and total fat and not higher intake of protein. However, based on the result of a review, the effect of protein on lipid profile of plasma remained unclear (El Khoury & Anderson 2013). Therefore, further studies are needed to investigate the relation between intake of protein and lipid profile of plasma.

6.2 Intake of SFA and Concentration of TG in Small VLDL

To our knowledge in this study the negative correlation between SFA intake and concentration of triglycerides in small VLDL was found for the first time. Therefore, more studies are needed to confirm the result and investigate the potential mechanism of this association.

6.3 Intake of Unsaturated Fatty Acids and Lipoprotein Subclasses

Although there is lots of evidence about the effect of unsaturated fatty acids, especially PUFA on lipoprotein subclasses (Almario 2001, Goyens & Mensink 2005, Bos et al. 2007, Neff et al. 2011, Perez-Martinez et al. 2011, Annuzzi et al. 2012, Parlesak et al. 2014), we only found a few relations between unsaturated fatty acids and lipoprotein subclasses. In addition, in contrast to the several studies that found the significant relations between intake of fish and lipoprotein subclasses mainly HDL (Li et al. 2004, Erkkila et al. 2014, Lankinen et al. 2014), we did not find any significant differences in distribution of VLDL and HDL subclasses through categories of fish intake. This contrast might be due to some factors. First, intakes of fish and unsaturated fatty acids among participants were low in the current study as compared to other studies. Second, variations in intakes of fish and unsaturated fatty acids were not large enough to find an association.

6.4 Strengths and Weaknesses

Using the large population is one strength of this study, but the non-response rate of 36.5% decreased the benefit of that. Since the 750 participants were randomly selected from 3432 volunteered women, the sample is representative of cohort population and the result of study is

generalizable to the population. Moreover, availability of diet records and lipoprotein subclasses defined by NMR is another strength of this study. However, micro and macro nutrients are used instead of food so this study ignored the special characteristics of food, which might be the reason of found correlations. Using the cross-sectional design that cannot find the causal relation is another weakness of this study.

7. CONCLUSION

Our result suggests that the higher intake of SFA might be related to the unfavorable size of LDL particles which has been related to increased risk of heart diseases. In addition, we found that higher intake of SFA correlated with lower amount of triglyceride in small VLDL for the first time. This result supports the need for further longitudinal and interventional studies to elucidate the mechanism of the relation between SFA intake and lipoprotein subclasses and to clarify the clinical meaning of this associations.

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