The postprandial effect of a single high-fat meal with different fat quality on lipoprotein subclasses and their lipid composition

Master thesis by
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UNIVERSITY OF OSLO

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Abstract

Background: Cardiovascular disease (CVD) is the number one cause of death worldwide. Postprandial lipemia is a risk factor for CVD. Fatty acids have been demonstrated to affect CVD risk and postprandial lipemia differently. However, little is known about the change in lipoprotein subclasses in the postprandial phase, after consumption of high-fat meals with different fat quality.

Objectives: We aimed to elucidate the acute effect of a single high-fat meal with different fat quality on lipoprotein subclasses and their lipid constituents in healthy women.

Method: A cross-over designed study was conducted with 14 healthy women, with a median (25th-75th percentile) age of 24 y (22-25) and body mass index of 22 kg/m^2 (21-25). All subjects were served three isocaloric cakes with 70 E % from fat, enriched with either coconut oil (43 E% saturated fat (SFA), 1 E% α-linolenic acid (ALA)) or linseed oil (30 % SFA, 14 % ALA) or cod liver oil (31 E% SFA, 5 E% ALA, 2 E% eicosapentaenoic acid and 3 E% docosahexaenoic acid), resulting in different fatty acid composition in all three meals. The concentration of lipoprotein subclasses, their lipid constituents and circulating metabolites were analyzed in fasting and 3h and 6h postprandial plasma samples using Nuclear magnetic resonance-spectroscopy.

Results: After 3h, compared to fasting, both the XXL VLDL particle- and the TG concentration increased after intake of the coconut cake (p= 0.002 for both). This increase was also significantly higher compared to intake of both linseed- and cod liver cake (p= 0.007 and p= 0.006, respectively for XXL VLDL-particle, and p= 0.005 and p= 0.008, respectively for XXL VLDL-TG). Intake of the coconut cake induced a significantly lower IDL-TG concentration increase compared to after intake of the cod liver cake (p= 0.015). At 3h compared with fasting, S HDL-TG concentration declined after intake of coconut cake, which was significantly different from the increase after intake of linseed cake (p= 0.004). There was only a significant time effect after intake of linseed cake (p= 0.009). At 3h compared with fasting, lactate concentration decreased after intake of coconut cake and increased after intake of linseed and cod liver cake, pairwise comparisons showed that the coconut and linseed cakes differed significantly (p= 0.001). At 6h compared with fasting, lactate concentration declined after

intake of coconut- and cod liver cake and increased after intake of linseed cake, and the changes after intake of the coconut and cod liver cakes were significantly different ($p \leq 0.012$).

Conclusion: A high fat meal with different fat quality resulted in different postprandial response on lipoprotein subclasses, their lipid compounds as well as circulating metabolites. Intake of the coconut cake caused the highest increase in particle- and TG concentration of the largest VLDL subclasses (XXL, XL, L), which is thought to be adverse in relation to CVD risk. The clinical relevance of the findings remains to be investigated. Further research is needed on the effect of fat quality on lipoprotein subclasses in the postprandial phase.

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Abbreviations

ABCA1	ATP-binding cassette A1
ABCG1	ATP-binding cassette G1
ALA	Alpha-linolenic acid
Apo	Apolipoprotein
BMI	Body mass index
BOHBut	3-hydroxybutyrate
C	Cholesterol
CE	Cholesteryl ester
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CM	Chylomicron
CMR	Chylomicron remnant
CPT1	Carnitine palmitoyltransferase 1
CRP	C-reactive protein
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
E%	Energy percent
EPA	Eicosapentaenoic acid
FA	Fatty acid
FH	Familial hypercholesterolemia

FTT	Fat tolerance test
Gp:	Glycoprotein acetyl
HDL-C	High-density lipoprotein-cholesterol
iAUC	Incremental area under the curve
IDL-C	Intermediate-density lipoprotein-cholesterol
L	Large
LA	Linoleic acid
LCAT	Lecithin-cholesterol acyltransferase
LDL-C	Low-density lipoprotein-cholesterol
LPL	Lipoprotein lipase
M	Medium
MUFA	Monounsaturated fatty acid
NMR	Nuclear magnetic resonance
OFTT	Oral fat tolerance test
P	Particle concentration
PL	Phospholipid
PPAR	Peroxisome proliferator activated receptor
PUFA	Polyunsaturated fatty acid
RCT	Randomized controlled trial
S	Small
SFA	Saturated fatty acid

SR-BI	Scavenger receptor class B type I
SREBP	Sterol regulatory element binding protein
TG	Triacylglycerol
TRL	Triglyceride-rich lipoprotein
TRLR	Triglyceride-rich lipoprotein remnant
VLDL	Very low-density lipoprotein
WHO	World Health Organization
XL	Extra large
XS	Extra small
XXL	Extremely large

1 Introduction

1.1 Cardiovascular disease: A worldwide perspective

Cardiovascular disease (CVD) is a group of disorders that includes diseases of the heart, blood vessels and vascular diseases in the brain (1). CVD includes coronary heart disease (CHD) (heart attack), cerebrovascular disease (stroke), heart failure and hypertension (2). CVD is the number one cause of death worldwide (1, 2). The World Health Organization (WHO) estimated that 17.7 million people died from CVDs in 2015 (1). Even though deaths caused by these diseases have decreased in high-income countries, including Norway, an increase is seen in middle- and low-income countries (3, 4). In Norway, a reduction is observed in all parts of the country, over the last 40 years (5).

1.2 CVD and the role of atherosclerosis

Atherosclerosis is the major cause of CVD (6). The atherosclerotic process is initiated by lipid retention in the artery wall and lipid oxidation and modification (7). Proliferation of fibrous tissues and smooth muscle cells causes fatty streaks to evolve into fibrous plaques, which can rupture, cause stenosis or thrombosis (7, 8). The atherosclerotic process develops over a period of 40-50 years, as a natural aging process, beginning already in early childhood and adolescence (7). Formation of atherosclerotic lesions is the same for everyone, however, the speed of progression depends on exposure to several risk factors (7, 9). Physical inactivity, tobacco use, unhealthy diet and harmful use of alcohol are some major behavioral risk factors of CVD (1, 4). The long-term exposure of behavioral risk factors might affect metabolic risk factors, resulting in hypertension, diabetes, hyperlipidemia, overweight and obesity (1, 4). According to WHO, a diet high in saturated fatty acid (SFA), trans-fat, cholesterol and salt, as well as low in fruits, vegetables and fish is associated with increased risk of developing CVD (4). Furthermore, genetic predisposition, gender, age (4) and ethnicity (10) are important non-behavioral risk factors. **Figure 1** illustrates the atherosclerotic development over time.

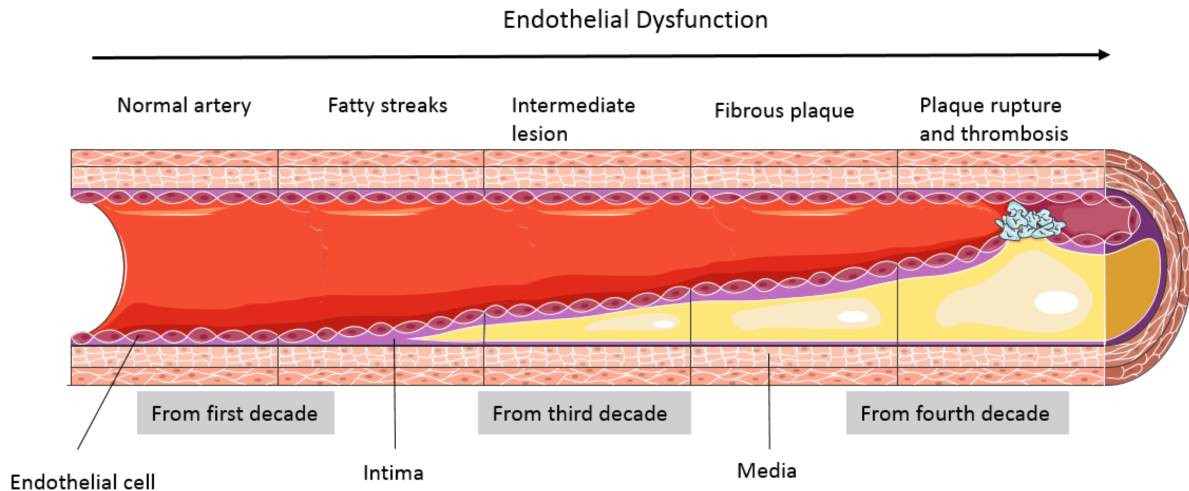

Figure 1: Simplified illustration of the atherosclerotic development over time. Inspired by (11) and based on free images from Servier Medical Art (Creative Commons Attribution License, http://creativecommons.org/licenses/by/3.0/).

The initiation of lipid retention and fatty streak development begins when circulating low-density lipoprotein (LDL) particles and other apolipoprotein B (ApoB) containing lipoproteins <70 nm in diameter flux through the endothelial barrier, to the intima layer of the artery (7-9, 12, 13). The increase of plasma LDL will result in an increase in intracellular LDL concentration (9). LDL particles become trapped in the intima due to increased extracellular proteoglycans, which has a high affinity to LDL molecules (9). Within the intima, LDL particles become oxidized and modified to pro-inflammatory inducing particles (7). Oxidized LDL particles can secrete cytokines (9). Furthermore, endothelial cells are activated and also secrete adhesion molecules. As a result, monocytes, lymphocytes, mast cells and neutrophils are dragged into the arterial wall (7). Monocytes are transformed into macrophages that take up lipids and oxidized LDL molecules and become foam cells. Accumulation of foam cells leads to the formation of fatty streaks (9, 14). Lesion development includes the migration of smooth muscle cells from the media layer to the intima. These cells secrete chemokines and chemoattractants (14), as well as, extracellular matrix proteoglycans, collagen and elastic fibers, which increase retention of LDL particles (7, 14). Additionally, the lesions in the intima might progress to affect the entire blood vessel (7). Fatty streaks build up in the artery wall and form plaques that can grow into the blood vessels and cause the blood flow to decrease. The plaque might also cause stenosis or thrombosis or they might rupture in major arteries to the heart, brain and other organs (7) and lead to coronary heart- and cerebrovascular diseases (1).

1.3 Fat quality and CVD risk

1.3.1 Chemical structure of fatty acids and sources in the diet

Dietary fat and fat stored in the body are mostly in the form of triacylglycerol (TG). Fatty acids (FAs) are the building blocks of lipids and TG consists of three FAs, esterified to a glycerol-molecule (15). All FAs consist of a hydrocarbon chain, with a methyl end (omega) and a carboxylic acid end (alpha). The hydrocarbon chain length, as well as the number and placement of double bonds differ, allowing FAs to have different chemical- and physiological properties (16).

SFA includes FAs between 6 to 24 carbon atoms (17) that contain no double bonds between the carbon atoms (16). SFAs are primarily found in dairy products, red meat and in great proportions in some plant oils, such as coconut- and palm oil (18). Monounsaturated fatty acids (MUFAs) contain one double bond, while polyunsaturated fatty acids (PUFAs) have more than one double bond (16). PUFAs include omega-3 and -6 FAs. Classification of PUFAs depend on the location of the first double bond from the methyl group (16, 17). Eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6, n-3) are two omega-3 FAs, mostly found in fish (17). A small proportion of EPA and DHA can be synthesized in the body from the essential omega-3 FA, alpha-linolenic acid (ALA, 18:3 n-3) (17). ALA is found in e.g. vegetable oils such as in linseed and canola, as well as green leafs, walnuts and beans (19).

1.3.2 Intake of SFA and PUFA and CVD risk

Several national dietary guidelines (18-20), as well as WHO (4) recommend reducing saturated fat in the diet, in favor of unsaturated fatty acids, due to cardiac health. These recommendations are related to the negative effect some SFAs have on LDL-cholesterol (C) (21), which is one of the major players in the development of atherosclerosis (8, 13, 18). The negative effect on LDL-C is especially linked to three SFAs: Lauric- (C12:0)-, myristic- (C14:0)- and palmitic acid (C16:0) (18, 22, 23). These three SFAs cause a significant increase in total serum cholesterol, LDL-C and high-density lipoprotein (HDL)-C levels as well as a reduction in TG levels, compared with carbohydrates (21-23). There is great evidence in the literature that replacing SFA with PUFA reduces plasma LDL-C levels. This is well documented in several systematic reviews and meta-analyses of randomized controlled trials (RCTs) (18, 21), systematic review of RCTs and observational studies (23) and other systematic reviews (22). The greatest

beneficial effect is seen when SFA is replaced with PUFA, rather than MUFA or carbohydrates (18, 21, 24). It is especially linoleic acid (LA, 18:2n-6) and ALA that have the most favorable effects on LDL-C (18, 21). According to Ference et al., a diet rich in unsaturated fat, plant based protein, plant phytosterols and fiber, as well as low in SFA and refined carbohydrates could reduce plasma LDL levels with 0.75-1 mmol/l (13). In addition to lowering LDL-C, a diet rich in PUFA reduces TG levels and increases HDL-C (21). Zong and colleagues found that replacing 1 E% of lauric-, myristic-, palmitic- and stearic acid (C18:0) with PUFA reduced CHD risk with 8 % in two large American prospective cohorts (25).

Omega-3 fatty acids and CVD risk

Omega-3 FAs have been shown to have beneficial health effects, especially on cardiac health (26-28). The positive effects are particularly linked to the long chain marine omega-3 FAs, EPA and DHA (19, 29), that are mainly found in fish and fish oil (17). The Norwegian Ministry of Health recommends consuming 300-450 g fish per week, where at least 200 g should be from fatty fish (30). Alexander et al. did a meta-analysis of RCTs and prospective cohorts of intake of EPA and DHA on CHD in 2017. The authors found a significant reduction of CHD in patients with higher intake of EPA and DHA, and that had higher TG- and LDL-C levels (26). Other systematic reviews have shown that EPA and DHA supplements reduce TG levels and increase LDL-C and HDL-C levels (27, 28). The reduction of TG concentration is dose-dependent (27, 28) and related to reduction of very low-density lipoprotein (VLDL) particles (31).

Furthermore, ALA has been shown to have beneficial effects on CVDs. However, results about ALA's effect on cardiac health are still inconclusive (17). Observational studies suggest that consumption of ALA might reduce frequency of coronary- (32) and all-cause mortality (33). It is also suggested that ALA should be recommended for people that do not consume fish and fish oils (32).

However, a recent Cochrane review of RCTs questioned the protective effects of omega-3 on CVD health. The studies included in the review were mainly supplement trials. There was no effect of EPA, DHA or ALA on CVD related mortality. The authors concluded that ALA might reduce the risk of CVD events (34). In contrast, in the REDUCE-IT trial, 4g/day of an EPA ethyl ester was found to significantly lower CVD events, including CVD death in patients with established CVD or diabetes or other risk factors and who had elevated LDL-C or TG levels, that were statin treated (35). The reduction of CHD risk by fish consumption is affected by the

habitual fish intake (17). In subgroup analysis of participants in the VITAL study, subjects whom had low consumption fish had a 19 % reduction of CVD events after supplementation of vitamin-D and marine omega-3, while the overall analyses did not show any effect on CVD events (36).

1.4 Transport of lipids and lipid metabolism

Lipids are water-insoluble molecules, and include a number of organic compounds, such as TGs, FAs, phospholipids (PLs), eicosanoids and sterols (16). Because they are water-insoluble they require a transport system in the blood circulation. Lipoproteins are responsible for the transport of hydrophobic compounds in water, such as in blood and extracellular fluid (37). Each lipoprotein consists of a lipid core with cholesteryl ester (CE) and TG, and a hydrophilic surface with a single layer of PL, free cholesterol and apolipoproteins (37) (**Figure 2**).

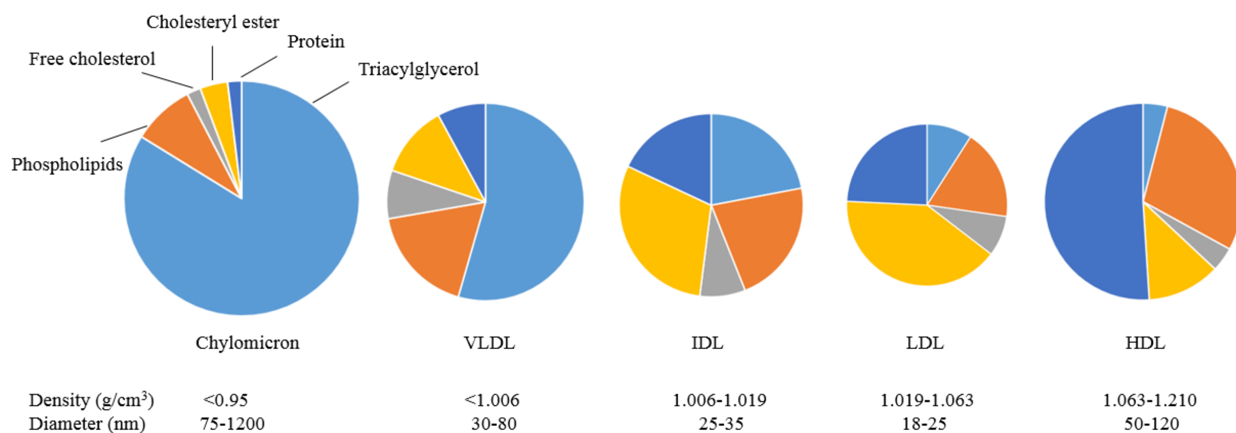

Figure 2: The major classes of lipoproteins and their constituents, divided by size and density (38).

Lipoproteins are normally defined according to their size, density, lipid and apolipoprotein composition (37). They are usually divided into five main classes: Chylomicron (CM), VLDL, intermediate-density lipoprotein (IDL), LDL, and HDL (37, 39, 40). However, lipoproteins can be further divided into several subclasses. Subdivision of lipoproteins depends on the method used for analysis. This is because the density of lipoproteins is a continuum and not clearly distinguished subclasses (40).

CMs are assembled in the intestine from dietary fat, ApoB-48 and other apolipoproteins. The particles are secreted via the lymph, into the circulation, to peripheral tissues (37). CMs carry most of the TGs found in the circulation, as well as some cholesterol and CE. The size of CMs depends on the amount of fat ingested (37). Lipoprotein lipase (LPL) is an enzyme found in

adipose- and muscle tissue. It is responsible for the uptake of TG to latter tissues, by hydrolysis of TG to free fatty acids (37). Apo C-II, on CMs acts as a cofactor for LPL. As CMs are depleted of TGs, they shrink in size and lose unesterified cholesterol, PL and some apolipoproteins from the surface that are taken up by HDL particles (15). Chylomicron remnants (CMRs) are then transported to and taken up by the liver. These particles are enriched in CE (37).

VLDL particles are synthesized in the liver and secreted into the circulation and further to peripheral tissue. They are assembled from mostly TG, some cholesterol and CE, as well as Apo B-100 and a small amount of other apolipoproteins. CM and VLDL are known as TG-rich lipoproteins (TRLs) due to the amount of TG in these particles (41). VLDL size depends on the amount of TG in the core, hence the amount of TG synthesized by the liver. However, they are smaller than CMs (37). The uptake in peripheral tissues is facilitated in the same way as for CMs. As CMs, VLDL particles shrink in size and lose surface coat after hydrolysis of TG to FAs. The surface coat is mainly taken up by HDL (15). VLDL remnants are enriched with CE. The particles are either taken up by the liver or remain in the circulation, where they keep shrinking and eventually forming IDL and later LDL particles (15). LDL carry most of the cholesterol found in the circulation (37). LDL deliver CE to peripheral tissue through uptake by LDL receptor (15).

HDL particles are assembled and matured in the circulation (15). Firstly, ApoA1 is secreted from the liver and intestine with some PLs. Secondly, nascent HDL particles acquire cholesterol from other tissues and lipoproteins. HDL particles can either interact with cells and collect excess cholesterol, or take up cholesterol from the surface of other lipoproteins (15). Unesterified cholesterol is esterified in the HDL particles by the enzyme lechitin-cholesterol acyl transferase (LCAT) (15). Furthermore, HDL particles transfer their cholesterol to the liver, either directly by interacting with scavenger receptor (SR-B1) (15), or CE can be transferred to ApoB containing particles in exchange for TGs (37). This process is facilitated by the enzyme cholesteryl ester transfer protein (CETP) (37). The HDL can then return to circulation and repeat the process (15, 42). Transport of cholesterol to the liver by HDL is called reversed cholesterol transport (15). **Figure 3** shows an overview of lipoprotein metabolism.

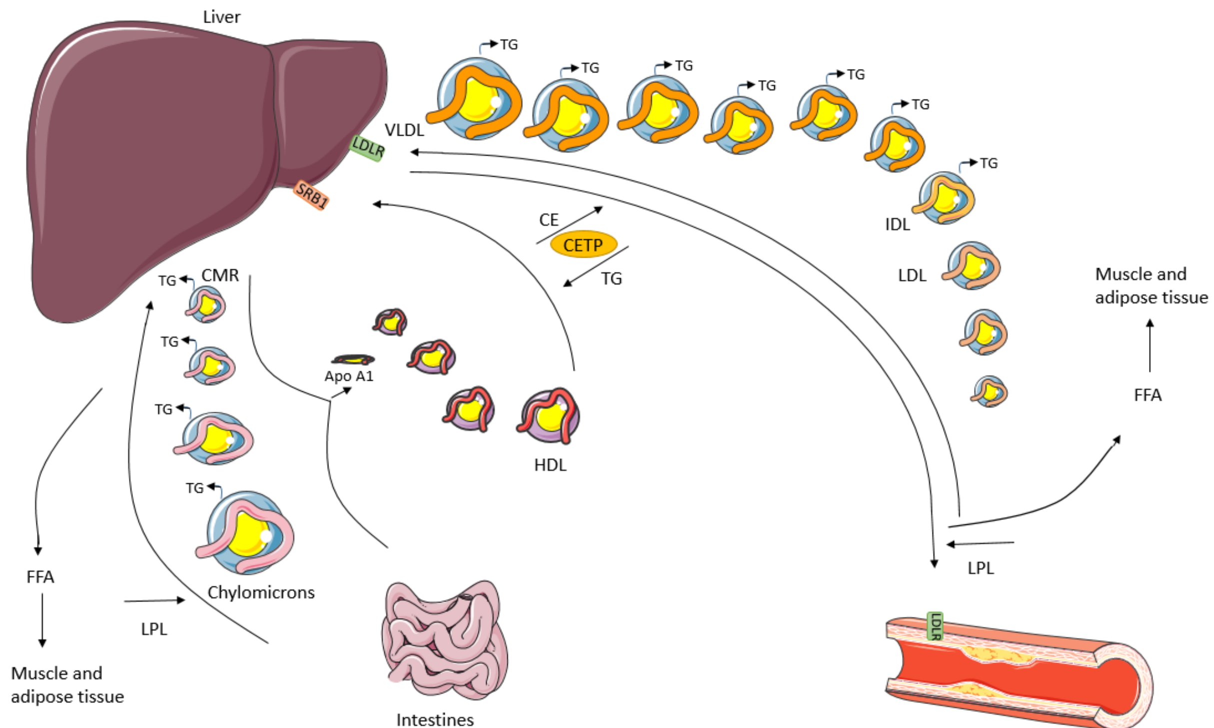

Figure 3: Overview of lipoprotein metabolism. See text for details. Inspired by (43) and based on free images from Servier Medical Art (Creative Commons Attribution License, http://creativecommons.org/licenses/by/3.0/).

1.5 Postprandial lipemia and lipoproteins

Postprandial lipemia is characterized by elevated levels of TG-rich lipoproteins after consumption of food or drinks (44). There is today increased evidence that postprandial lipemia plays an important role in the process of atherosclerosis (45), and is an important predictor of CVD risk (46). This is most likely because most people spend more time in the postprandial phase rather than fasting (46). The increase of lipids, both duration and extent in the blood is affected by genetic and physiological factors, such as isoforms of different apolipoproteins, age, gender and menopausal status. Lifestyle factors like exercise also modulate postprandial lipemia. In addition, disease states such as obesity, diabetes and dyslipidemia have a negative effect on postprandial lipemia (44, 47). Moreover, the increase of TG concentration after a meal is associated with the type and amount of fat consumed. Øyri et al. found that subjects with familial hypercholesterolaemia (FH) and healthy controls had a delayed TG peak after intake of SFA, compared with PUFA (48). Furthermore, dietary factors such as fructose, sucrose and alcohol will increase postprandial TG response, while dietary fiber, glucose and proteins together with fat reduce postprandial lipemia (44, 47). Studies show that the intake of long chain omega-3 PUFAs is associated with lower TG concentrations and lower postprandial lipemia

(44). The mechanisms of how PUFAs lower postprandial lipemia are not clarified with certainty (49), but may involve activation of transcription factors and nuclear receptors (50).

1.6 Lipoproteins: Atherogenic or anti-atherogenic?

Lipoprotein metabolism is linked to CHD (15). Some lipoproteins have been shown to be more atherogenic than others (9). CMR, VLDL, IDL and LDL are some of these (37). TRL and their remnants (TRLRs) are responsible for the elevated TG concentrations in the postprandial period (48). TRLs are too big to flux through the endothelial barrier. However, lipolysis of TRL by LPL results in smaller remnants that may penetrate through the barrier, however, in a less efficient manner than small LDL particles (51). Additionally, prolonged elevation TRL results in increased exchange of CE and TG between lipoproteins, facilitated by CETP (44, 49). TRL become enriched with cholesterol (44, 51) and contain 40 times more CE than LDL particles (51). Elevated TRL levels and prolonged postprandial lipemia may therefore contribute to the atherosclerotic process, by accumulation of remnant particles in the artery wall (51). TRLR might also cause increased CVD risk through endothelial dysfunction and enhanced inflammatory response (44, 51, 52).

Furthermore, small LDL particles are shown to be more atherogenic than large LDL particles (37). Small LDL particles enter the arterial wall more easily, as well as being more prone to oxidation, resulting in increased uptake by macrophages. In addition, small LDL have less affinity to LDL receptor, causing a longer time in circulation (37). Moreover, both large and small VLDL particles have been shown to be atherogenic (53). However, large VLDL particles are shown to give rise to the smallest LDL particles (49, 54).

HDL is important for reverse cholesterol transport from peripheral tissues to the liver, hence might act anti-atherogenic (37). It is suggested that HDL also has an anti-oxidant, anti-inflammatory, anti-thrombotic and anti-apoptotic role (37, 55). Studies have demonstrated that high levels of LDL and low levels of HDL in the blood have a negative effect on the atherosclerotic development (7). On the other hand, the causality between plasma HDL-C and CVD risk has not been confirmed (56).

1.7 Metabolic profiling and postprandial lipoprotein subclasses

Analysis of lipoprotein subclasses is suggested to improve CVD risk assessment, however these results are not clarified yet (57). Metabolic profiling, is the measurement of small molecules within an organism, including lipoproteins (58). Studying the metabolome makes it possible to describe the physiological condition of an organism (59), thereby get a deeper insight in molecular mechanisms (60) and understand in depth health and disease causes (61). One method used to study the metabolome is quantitative Nuclear Magnetic Resonance (NMR)-spectroscopy (53, 61, 62). This method has been used in several studies to understand CVD risk and CVD events further (39, 48, 53, 63-65). Moreover, there is also an increase in evidence that postprandial plasma lipid and lipoprotein concentrations are important in evaluating coronary risk (44). Furthermore, it is proposed that meals with the same amount of total fat, but different fat quality may affect the underlying mechanisms of atherosclerosis differently (66). However, the literature is scarce regarding the effect of different FAs on postprandial lipemia and lipoprotein subclasses (66, 67).

We have previously demonstrated that postprandial plasma TG levels increased 3h after a single meal supplemented with coconut oil, linseed oil or cod liver oil. There was no significant effect at 6h, or between the test meals (68). We aimed to further elucidate this effect by using NMR-spectroscopy as a method to get a deeper understanding of the change in lipoproteins in the postprandial phase.

2 Aim

The main objective of this thesis is to investigate whether postprandial lipoprotein subclass distribution and composition is different 3h and 6h after a single high-fat meal with different fat quality, in healthy, young, Norwegian females. Secondly, we want to investigate whether there is any difference in circulating metabolites between meals with different fat quality.

The specific aims are:

I. To investigate the effect of high-fat meals with different fat quality on VLDL subclasses and their lipid components 3h and 6h after intake

II. To investigate the effect of high-fat meals with different fat quality on IDL subclass and its lipid components 3h and 6h after intake

III. To investigate the effect of high-fat meals with different fat quality on LDL subclasses and their lipid components 3h and 6h after intake

IV. To investigate the effect of high-fat meals with different fat quality on HDL subclasses and their lipid components 3h and 6h after intake

V. To investigate the effect of high-fat meals with different fat quality on variety of circulating metabolites 3h and 6h after intake

3 Method

3.1 Study population and design

All the participants were recruited among nutrition students at OsloMet - Oslo Metropolitan University, formerly called Akershus University College, in October 2008 (68). Inclusion criteria were healthy females and males (CRP<10 mg/L), between 20-50 y. Pregnant or lactating subjects were excluded from the trial.

Study design

This study was a postprandial trial where the participants were given three different test meals in a fixed order, unaware of which meal they were given at each occasion. All test days were separated by two weeks. Participants were instructed to maintain their regular diet during the study period. Before each test day, subjects were served a standardized low-fat meal as dinner at OsloMet. The meal consisted of 22 % energy (E%) from fat. In addition, they were told to eat a low-fat meal at home and not to eat after 8 PM. The participants were instructed to eat the same supper meal prior to each test day. Subjects had to avoid alcohol for 24h before each test day and did not eat anything 12h before blood sampling (68).

At each test day, body weight and fasting blood samples (0h) were taken. The test meals were served between 8-9 AM and had to be eaten within 20 min. Postprandial blood samples were taken 3h and 6h after each test meal. During the test day, subjects were only allowed to consume water, perform minimum of physical activity and had to stay at the university area. Subjects were served dinner after the last blood sample was taken (68).

3.1.1 Test meals

The three test meals consisted of a 150 g chocolate cake, with the same amount of energy (1923-1977 kJ/100 g) and same percentage of energy from protein (14 E%), total fat (67-70 E%) and carbohydrates (16-19 E%). The cakes had different amount of coconut fat, soy bean oil, linseed oil and cod liver oil, thereby resulting in different FA composition. The cakes were made and then stored at -20 °C until consumption day (68, 69). Coconut fat was used as a source of SFA. In the coconut cake 43 E% was SFA, 11 E% PUFA, where only 1 E% was *n-3* from ALA. In the linseed cake, some of the coconut fat was replaced with fat from linseed oil as a vegetable

n-3 FA source, where ALA was the main FA. The linseed cake contained 30 E% SFA, 22 E% PUFA, where 14 E% was from *n-3* ALA. In the cod liver cake, some of the linseed oil was replaced with cod liver oil as a source of marine *n-3* FA. The cake contained 31 E% SFA, 14 E% PUFA, where 10 E% was *n-3* FA; 5 E% from ALA, 2 E% from EPA and 3 E% DHA (68). See **Table 1** for further nutrient values of the test meals. The FA composition of the cakes was analyzed at Eurofins Norsk Matanalyse (Oslo, Norway) in duplicate portions of each test meal (68).

Table 1: Nutrient values of the three test meals

	Coconut cake		Linseed cake		Cod liver cake	
	E%	g/100 g	E%	g/100 g	E%	g/100 g
Energy content (kJ/100g)	1973		1977		1923	
Carbohydrate	16	19	17	20	19	22
Protein	14	16	14	16	14	16
Fat	70	37	69	37	67	35
SFA	43	23	30	16	31	16
MUFA	10	5.5	13	7.2	18	9.4
PUFA	11	6.1	22	12	14	7.4
n-6 Fatty acids	10	5.4	7	4	4	2
n-3 Fatty acids	1	0.7	14	7.7	10	5.3
ALA	1	0.6	14	7.6	5	2.7
EPA	0	0	0	0	2	0.9
DHA	0	0	0	0	3	1.3

SFA: Saturated fatty acids, MUFA: Mono unsaturated fatty acids, PUFA: Polyunsaturated fatty acids, ALA: alpha-linolenic acid, EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid. Based on (68), with permission from Br. J. Nutr.

3.1.2 Blood sampling and standard biochemical analysis

Plasma was obtained from EDTA tubes (BD Vacutainer; Becton, Dickinson and Co.). Samples were immediately placed on ice and centrifuged within 12 min at 1500 g for 10 min at 10 °C. Serum was obtained from sicilia gel tubes (BD Vacutainer: Becton, Dickinson and Co. Franklin Lakes, NJ, USA) (68). They were kept at room temperature at least 30 min and maximum 2h, before centrifugation at 1300 g for 12 min. Standard blood chemistry was measured in serum or plasma at Oslo University Hospital (Rikshospitalet) (68).

NMR-spectroscopy of lipoprotein subclasses and metabolites

The plasma concentration of lipoprotein subclasses, their lipid composition and circulating metabolites were analyzed and quantified using a commercial NMR-metabolomics platform, at Nightingale Health Ltd (www.nightingalehealth.com), in Vantaa, Finland (39, 65). Lipoprotein

subclasses were defined according to their average size in diameter (39, 65). The fourteen lipoprotein subclasses were defined as: extremely large VLDL (XXL VLDL) with a possible contribution of chylomicrons (>75 nm), five VLDL subclasses (extra large (XL), large (L), medium (M), small (S) and extra small (XS), with a particle diameter of 64.0 nm, 53.6 nm, 44.5 nm, 36.8 nm and 31.3 nm, respectively), IDL (28.6 nm), three LDL subclasses (L, M and S, with a diameter of 25.5 nm, 23.0 nm and 18.7 nm, respectively) and four HDL subclasses (XL, L, M and S, with a diameter of 14.3 nm, 12.1 nm, 10.9 nm and 8.7 nm, respectively). Additionally, several metabolites, such as amino acids, ketone bodies and one inflammatory marker were measured.

3.2 Statistical analysis

In this postprandial study, each subject was used as its own control since the subjects ate all three test meals. All statistical analyses were performed in IBM SPSS Statistics 25. Non-parametric tests were used throughout the study due to a low number of subjects. Data is given as median and 25 and 75 percentiles. No power calculation was performed and the number of subjects were based on previous studies using TG as primary outcome (68). Within each test meal, the significance between all three time points were analyzed using exact plasma. The significance between test meals were analyzed by comparing delta plasma changes between different time points (0h-3h, 3h-6h and 0h-6h). All analyses were assessed with Friedman's ANOVA, and significant findings were further tested with a Wilcoxon matched-pairs test. To adjust for multiple testing, Bonferroni correction of p-value was performed and significance level was therefore set to be $p \leq 0.017$. Missing values in the Friedman's ANOVA were excluded listwise, while missing values in the Wilcoxon matched-pairs test were excluded test-by-test.

3.3 Ethics

The participation was voluntary. Participants signed a written consent form prior to the trial and could withdraw from the study at any time point. Withdrawal from the study would not affect the assessment of the students at the end of the semester. Each participant was given an ID number. Biomaterial was stored in a biobank at OsloMet. Prof. Stine Marie Ulven is responsible for the biobank. Subjects were informed that collaborating institutions, such as the University of Oslo, could use collected data. The study was conducted in accordance to the Declaration of

Helsinki and approved by the Regional Committee of Medical Ethics, south-east region of Norway (08/338b, Omega-3 and HDL-1). The study was also reported to the Norwegian Social Science Data Service AS and to the Directorate of Health and Social Affairs. It was approved with a condition that there was a clear distinction between research and teaching.

4 Results

4.1 Study population

Sixteen females were recruited to the postprandial study. Two participants dropped out after the first day and were not included in the analyses. Among the 14 participants, three participants completed two of the three test days, but all 14 were included in the statistical analyses (68). The baseline characteristics of the participants included in the study are given in **Table 2.** The values are based on analyses from the first test day. All subjects were young healthy normal weight with no infectious diseases. Subjects had a median age of 24 y (22-25) and median body mass index (BMI) of 22 kg/m^2 (21-25). The fasting cholesterol- and glucose levels were within reference values.

Table 2: Baseline characteristics of the participants

	Median (25 and 75 percentiles)	Reference values[a]
		Women
Subjects (n)		
Female	14	
Male	0	
Age	24 (22-25)	
Omega-3 supplement		
Yes	0	
No	14	
Body mass index (kg/m^2)	22 (21-25)	18.5-24.9[b]
Glucose (mmol/l)	4.6 (4.3-4.9)	3.95-6.04
Triglycerides (mmol/l)	0.8 (0.7-1.1)	<2.60
Total cholesterol (mmol/l)	4.8 (3.6-5.2)	2.9-6.1
HDL-cholesterol (mmol/l)	1.6 (1.4-1.9)	0.95-2.74
LDL-cholesterol (mmol/l)	2.7 (1.9-3.4)	1.15-4.34
Apolipoprotein A1 (g/l)	1.9 (1.7-2.0)	1.1-2.0
Apolipoprotein B1 (g/l)	0.7 (0.5-0.8)	0.50-1.4
C-reactive protein (mg/l)	1.5 (0.7-3.0)	<5
Systolic blood pressure (mmHg)	118 (111-121)	<120[c]
Diastolic blood pressure (mmHg)	76 (73-81)	<80[c]

[a] Reference values are based on (70), [b] based on (71), [c] based on (72).

4.2 Postprandial and meal effects on lipoprotein subclasses

We have previously shown that the TG concentration increased after 3h, but not 6h after intake of all three test meals (68). To further elucidate the effects of meals with different fat quality

on postprandial lipids, we analyzed the particle concentration and the concentration of TG, C and CE in fourteen different lipoprotein subclasses.

4.2.1 Particle, TG, CE and C concentration of VLDL subclasses

Between the meals, intake of the coconut cake caused a significantly higher increase of particle- and TG concentration of XXL VLDL from 0h to 3h compared to the intake of both linseed and cod liver cake ($p = 0.007$ and $p = 0.006$, respectively for particle concentration (**Figure 4**) and $p = 0.005$ and $p = 0.008$, respectively for TG concentration (**Figure 5**)).

There was a significant difference between the cakes on XL VLDL-particle concentration. Pairwise comparisons revealed that the increase in particle concentration of XL VLDL after intake of coconut cake was significantly higher than after intake of linseed cake at 3h compared with fasting ($p \leq 0.05$). However, this difference was not significant after Bonferroni correction of p-value ($0.017 \leq p \leq 0.05$) (data not shown).

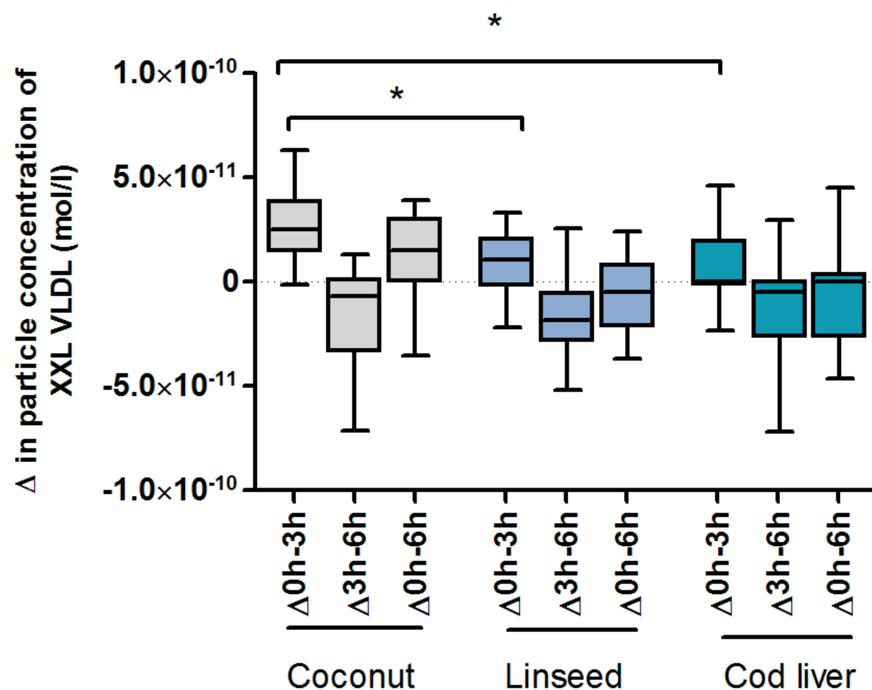

Figure 4: Change in particle concentration of XXL VLDL after intake of the three test meals. * Significant differences between the cakes were tested with Friedman's ANOVA, followed by post hoc analysis with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied ($p \leq 0.017$).

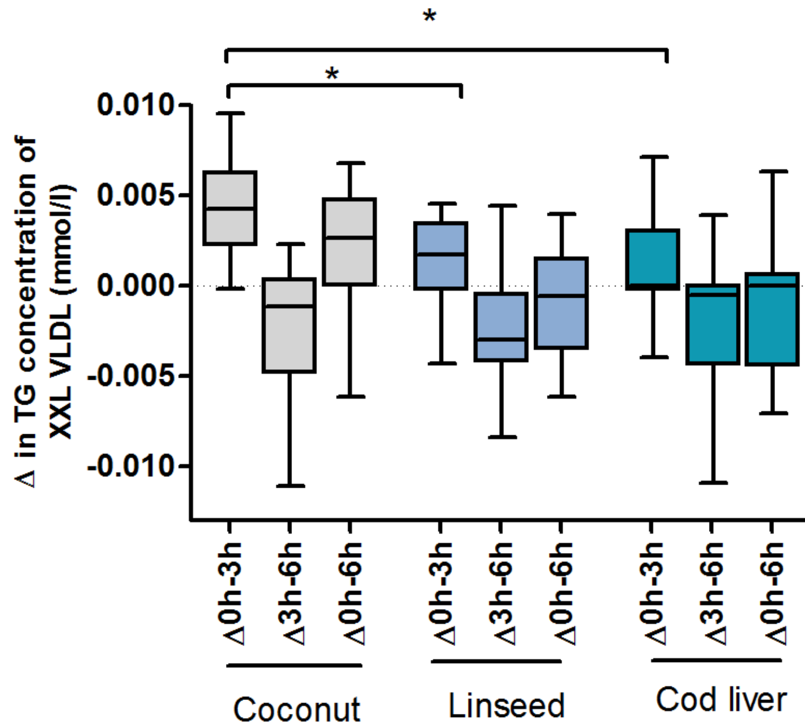

Figure 5: Change in TG concentration of XXL VLDL after intake of the three test meals. * Significant differences between the cakes were tested with Friedman's ANOVA, followed by post hoc analysis with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied (p≤ 0.017).

Within the meals, intake of the coconut cake significantly increased the XXL, XL and L VLDL subclass particle concentration (p= 0.002, p = 0.004 and p= 0.015, respectively) and TG concentration (p= 0.002, p= 0.004 and p= 0.015, respectively) from 0h to 3h (**Table 3**). The increase was 17 %, 21 % and 18 % for the three different VLDL particle concentrations and 17 %, 35 % and 25 % for the TG concentration in the three different VLDL particles. At 6h compared with 3h, intake of the coconut cake reduced the XL, L, M and S VLDL particle concentration (p = 0.005, p= 0.003, p= 0.002 and p= 0.003, respectively) and TG concentration (p= 0.006, p= 0.003, p= 0.002 and p= 0.003, respectively). From 0h to 6h, the coconut cake significantly reduced the M and S VLDL particle concentration (p= 0.004 and p= 0.001, respectively) and TG concentration in the M and S VLDL particle (p= 0.004 for both).

The increases in VLDL subclass particles from 0h to 3h after intake of the linseed cake were not significant. On the other hand, intake of linseed cake significantly increased TG concentration of M and S VLDL from 0h to 3h, with 3 % and 4.6 %, respectively (p= 0.013 and p= 0.011, respectively). There was a significant decrease in particle- (p= 0.004, p= 0.003, p= 0.001 and p= 0.002) and TG concentration (p= 0.004, p= 0.003, p= 0.001 and p= 0.003) of XL, L, M and S VLDL respectively, from 3h to 6h. XL, L and M VLDL-particle- (p= 0.010, p=

0.006 and p= 0.002, respectively) and TG concentration (p= 0.013, p= 0.006 and p= 0.003, respectively) declined significantly from 0h to 6h.

Intake of cod liver cake did not significantly change any VLDL subclass particle concentration at any time point, however, the M VLDL-TG concentration significantly decreased from 3h to 6h (p= 0.015). Interestingly, it seems to be greater individual differences after intake of cod liver cake compared with after intake of coconut and linseed cake (**Figure 6**). A similar pattern was observed in several lipoprotein subclasses (data not shown).

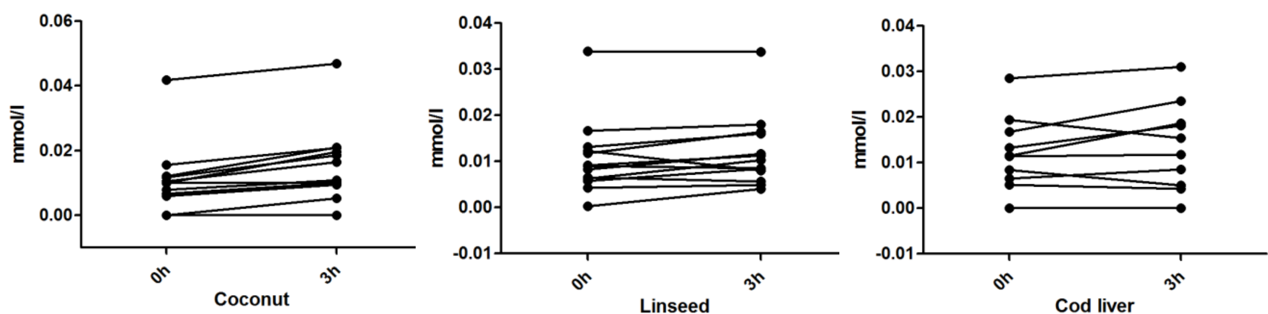

Figure 6: Individual changes in TG concentration of XXL VLDL from baseline to 3h after intake of the three test meals

Intake of coconut cake caused a significant increase in CE concentration of XXL and XL VLDL from baseline to 3h (p= 0.003 and p= 0.005, respectively) (**Table 3**). There was a decrease in XXL, XL, L and M VLDL-CE and an increase in XS VLDL-CE from 3h to 6h (p= 0.013, p= 0.002, p= 0.003, p= 0.005 and p= 0.011, respectively). At 6h compared with baseline, there was a significant decrease in L, M and S VLDL and an increase in XS VLDL-CE (p= 0.001, p= 0.006, p= 0.004 and p= 0.004, respectively).

Intake of the linseed cake caused a significant increase in CE concentration of L VLDL from baseline to 3h (p= 0.006). Moreover, there was a decrease in XXL, XL, L and M VLDL and an increase in XS VLDL from 3h to 6h (p= 0.004, p= 0.003, p= 0.003 and p= 0.005, respectively). There was also a significant decrease in XL, L and M VLDL 6h after the linseed cake compared to fasting (p= 0.003, p= 0.004 and p= 0.013, respectively).

Intake of cod liver cake did not cause any significant change in CE concentration of any VLDL subclasses.

Changes in C concentration of VLDL subclasses followed an almost similar pattern as CE concentration of VLDL classes (data not shown).

Table 3: Particle, TG and CE concentration of VLDL subclasses measured by NMR-spectroscopy after intake of coconut-, linseed- and cod liver oil cake. Data presented as median and 25 and 75 percentiles.

	Baseline (0h)	3h	6h	p value between time points
	Median (25-75 percentiles)	Median (25-75 percentiles)	Median (25-75 percentiles)	
VLDL-P				
XXL (nmol/l)				
Coconut	0.06 (0.03-0.07)	0.07 (0.05-0.13)	0.07 (0.03-0.10)	≤ 0.017§
Linseed	0.06 (0.04-0.08)	0.07 (0.04-0.10)	0.05 (0.05-0.08)	NS
Cod liver oil	0.07 (0.01-0.10)	0.07 (0.01-0.13)	0.06 (0.00-0.10)	NS
XL (nmol/l)				
Coconut	0.29 (0.09-0.32)	0.35 (0.22-0.55)	0.29 (0.00-0.36)	≤ 0.017†§
Linseed	0.21 (0.17-0.44)	0.31 (0.21-0.52)	0.19 (0.04-0.30)	≤ 0.017‡†
Cod liver oil	0.34 (0.00-0.50)	0.40 (0.04-0.69)	0.28 (0.00-0.46)	NS
L (nmol/l)				
Coconut	2.38 (1.58-2.81)	2.81 (1.84-3.55)	2.19 (1.10-2.48)	≤ 0.017†§
Linseed	2.30 (1.62-3.49)	2.54 (1.97-4.22)	1.74 (0.86-2.47)	≤ 0.017‡†
Cod liver oil	2.76 (0.44-3.92)	3.13 (0.41-4.47)	2.33 (0.11-3.57)	NS
M (nmol/l)				
Coconut	10.26 (8.63-12.83)	11.56 (8.67-13.75)	8.93 (5.75-11.10)	≤ 0.017‡†
Linseed	11.45 (8.72-14.20)	11.38 (10.06-15.53)	8.69 (6.63-10.74)	≤ 0.017‡†
Cod liver oil	11.94 (7.57-14.12)	12.52 (7.51-16.09)	10.33 (4.98-14.22)	NS
S (nmol/l)				
Coconut	22.06 (19.44-26.68)	22.21 (17.31-25.29)	19.34 (14.71-23.60)	≤ 0.017‡†
Linseed	23.78 (19.46-28.65)	23.97 (22.21-27.67)	20.80 (18.82-26.11)	≤ 0.017†
Cod liver oil	23.52 (17.53-27.35)	23.68 (17.91-29.43)	22.42 (14.21-32.01)	NS
XS (nmol/l)				
Coconut	32.24 (25.03-37.71)	31.78 (24.34-35.66)	31.05 (26.18-39.76)	NS
Linseed	34.27 (26.88-38.48)	33.92 (27.29-39.62)	35.41 (28.48-43.83)	NS
Cod liver oil	29.43 (25.57-36.91)	29.39 (26.17-39.38)	30.52 (26.15-43.52)	NS

VLDL-TG				
XXL (µmol/l)				
Coconut	8.90 (4.45-11.78)	10.39 (8.40-19.84)	11.69 (5.10-15.29)	≤ 0.017§
Linseed	9.05 (6.02-12.75)	10.24 (6.85-16.18)	8.27 (5.85-12.13)	NS
Cod liver oil	9.89 (1.30-15.90)	10.16 (1.07-18.50)	7.68 (0.00-13.61)	NS
XL (µmol/l)				
Coconut	17.33 (6.65-20.87)	23.42 (14.50-34.18)	18.91 (0.00-22.83)	≤ 0.017†§
Linseed	13.92 (11.05-25.83)	20.30 (12.81-31.86)	13.71 (2.80-19.99)	≤ 0.017‡†
Cod liver oil	21.54 (0.00-28.81)	26.33 (2.19-43.14)	17.40 (0.00-28.07)	NS
L (µmol/l)				
Coconut	78.81 (55.04-94.52)	98.51 (64.41-120.73)	75.87 (41.59-84.69)	≤ 0.017†§
Linseed	78.40 (55.42-115.70)	87.88 (65.26-139.15)	60.55 (30.00-83.20)	≤ 0.017‡†
Cod liver oil	93.30 (16.93-126.30)	108.11 (13.19-152.75)	76.93 (5.37-117.07)	NS
M (µmol/l)				
Coconut	188.50 (155.43-227.00)	207.15 (158.15-245.10)	167.80 (114.70-187.00)	≤ 0.017‡†
Linseed	197.10 (155.45-243.25)	203.20 (171.65-279.75)	154.60 (111.10-187.75)	≤ 0.017‡†§
Cod liver oil	211.95 (148.48-254.25)	231.00 (135.53-287.45)	182.75 (95.24-234.33)	≤ 0.017†
S (µmol/l)				
Coconut	173.95 (159.10-204.33)	183.70 (156.45-213.13)	166.95 (126.53-182.48)	≤ 0.017‡†
Linseed	187.30 (150.40-214.65)	196.00 (177.10-236.25)	168.00 (136.35-199.90)	≤ 0.017†§
Cod liver oil	193.95 (151.98-219.45)	205.45 (148.33-242.20)	190.95 (113.20-245.73)	NS
XS (µmol/l)				
Coconut	90.03 (78.56-101.82)	93.76 (76.84-101.22)	88.01 (73.56-107.48)	NS
Linseed	91.83 (76.09-105.85)	101.50 (87.59-107.40)	98.95 (82.33-107.35)	NS
Cod liver oil	92.85 (75.55-114.15)	98.54 (74.62-124.95)	98.82 (69.64-139.25)	NS
VLDL-CE				
XXL (µmol/l)				
Coconut	1.13 (0.18-1.61)	1.41 (0.39-2.26)	0.80 (0.04-1.79)	≤ 0.017†§

Linseed	1.06 (0.31-2.00)	1.09 (0.52-2.23)	0.38 (0.10-1.62)	≤ 0.017†
Cod liver oil	1.41 (0.07-2.15)	1.29 (0.13-3.02)	0.89 (0.00-2.44)	NS
XL (µmol/l)				
Coconut	4.09 (1.60-4.84)	5.02 (2.74-7.38)	3.45 (0.00-4.69)	≤ 0.017†§
Linseed	4.03 (2.22-6.22)	4.62 (2.93-6.67)	2.65 (0.40-4.12)	≤ 0.017‡†
Cod liver oil	4.93 (0.00-7.22)	5.47 (0.52-9.27)	3.67 (0.00-6.43)	NS
L (µmol/l)				
Coconut	20.44 (15.25-25.80)	23.45 (14.73-30.28)	16.89 (7.57-22.65)	≤ 0.017‡†
Linseed	20.44 (16.72-29.69)	22.29 (18.58-32.12)	16.59 (7.86-21.16)	≤ 0.017‡†§
Cod liver oil	24.81 (1.89-30.94)	26.02 (3.75-35.88)	19.79 (0.02-31.07)	NS
M (µmol/l)				
Coconut	53.93 (37.34-69.37)	54.00 (27.4-69.78)	38.27 (22.52-66.72)	≤ 0.017‡†
Linseed	57.15 (43.42-73.31)	55.75 (48.02-84.95)	40.38 (32.22-67.79)	≤ 0.017‡†
Cod liver oil	61.68 (28.56-70.92)	56.47 (30.78-80.67)	39.95 (15.32-87.81)	NS
S (µmol/l)				
Coconut	84.30 (66.59-114.98)	78.04 (46.3-95.68)	58.78 (40.17-96.12)	≤ 0.017‡
Linseed	101.10 (69.58-112.95)	82.25 (67.81-115.75)	88.96 (53.55-117.10)	NS
Cod liver oil	82.16 (61.70-101.51)	74.39 (60.69-105.50)	61.37 (37.66-122.97)	NS
XS (µmol/l)				
Coconut	126.50 (103.32-146.63)	127.60 (96.07-145.90)	133.25 (112.18-162.33)	≤ 0.017‡†
Linseed	139.20 (110.10-145.05)	128.20 (111.45-162.75)	157.20 (119.75-183.10)	≤ 0.017†
Cod liver oil	118.35 (100.55-142.13)	123.45 (99.48-151.83)	120.80 (111.58-175.95)	NS

Differences between time points were tested with Friedman's ANOVA, and p-values of the overall time-effect is given. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. Significant results of pairwise comparisons are indicated with symbols.

P ≤ 0.017: § 0h vs. 3h, † 3h vs. 6h, ‡ 0h vs. 6h

P: Particle; TG: Triacylglycerol; CE: Cholesteryl ester; VLDL: Very low-density lipoprotein; XXL: Extremely large; XL: Extra large; L: Large; M: Medium; S: Small; XS: Extra small

4.2.2 Particle, TG, CE and C concentration of IDL subclass

Between the meals, the increase in TG concentration of IDL was significantly lower after intake of coconut cake compared with the increase after intake of cod liver cake from 0h to 3h ($p = 0.015$) (**Figure 7**). The difference between the coconut cake and the linseed cake from 0h to 3h was significant, however, not after Bonferroni correction of p-value ($0.017 \leq p \leq 0.05$) (data not shown). Within the meals, TG concentration of IDL increased significantly at 6h compared with both 3h ($p = 0.001$, $p = 0.009$ and $p = 0.004$) and fasting ($p = 0.001$, $p = 0.007$ and $p = 0.004$) after intake of coconut-, linseed and cod liver cake respectively (**Table 4**). The increase in TG-concentration from baseline to 6h was 12 %, 20 % and 21 % for the three different cakes. There was no significant change in neither particle-, C- nor CE concentration of IDL at any time point after intake of the three test meals.

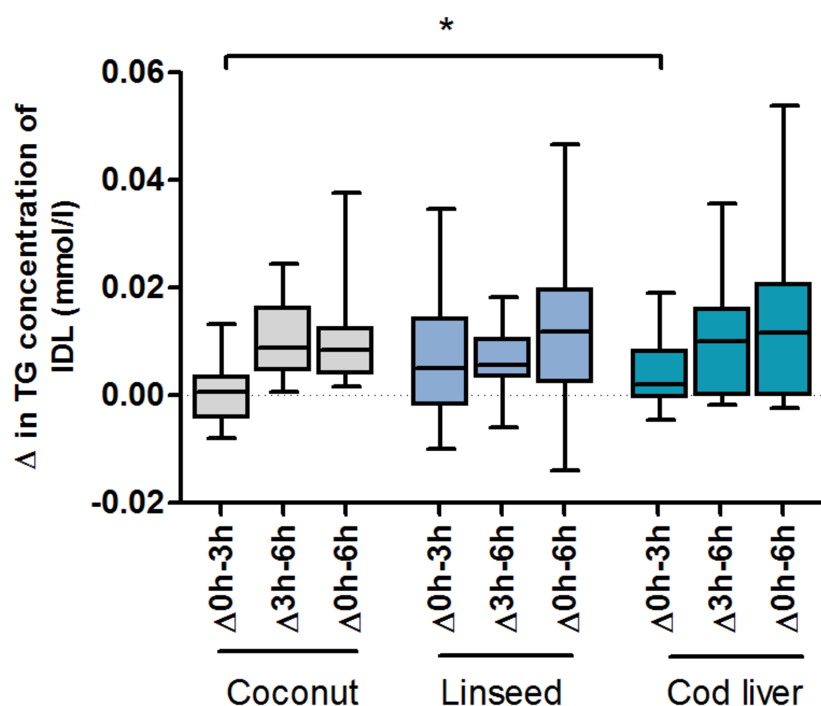

Figure 7: Change in TG concentration of IDL after intake of the three test meals. * Significant differences between the cakes were tested with Friedman's ANOVA, followed by post hoc analysis with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied ($p \leq 0.017$).

Table 4: Particle, TG and CE concentration of IDL subclass measured by NMR-spectroscopy after intake of coconut-. linseed- and cod liver oil cake. Data presented as median and 25 and 75 percentiles.

	Baseline (0h)	3h	6h	p value between time points
	Median (25-75 percentiles)	Median (25-75 percentiles)	Median (25-75 percentiles)	
IDL-P (nmol/L)				
Coconut	79.19 (59.76-95.22)	78.32 (57.65-89.17)	77.03 (62.16-106.35)	NS
Linseed	86.13 (66.43-94.40)	79.34 (65.39-103.90)	87.59 (65.94-109.30)	NS
Cod liver oil	76.74 (58.26-93.74)	75.67(58.44-93.88)	68.44(63.03-101.48)	NS
IDL-TG (µmol/L)				
Coconut	90.85 (77.90-114.85)	94.10 (77.49-111.98)	101.83 (86.32-128.05)	≤0.017‡†
Linseed	95.02 (78.42-115.25)	103.50 (89.90-116.00)	113.80 (93.97-125.50)	≤0.017‡†
Cod liver oil	90.89 (75.91-127.70)	99.02 (79.24-128.53)	110.80 (84.93-143.70)	≤0.017‡†
IDL-CE (mmol/L)				
Coconut	0.32 (0.24-0.40)	0.34 (0.23-0.38)	0.31 (0.25-0.46)	NS
Linseed	0.37 (0.27-0.40)	0.31 (0.26-0.43)	0.38 (0.27-0.46)	NS
Cod liver oil	0.32 (0.23-0.40)	0.32 (0.21-0.40)	0.29 (0.22-0.44)	NS

Differences between time points were tested with Friedman's ANOVA, and p-values of the overall time-effect is given. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. Significant results of pairwise comparisons are indicated with symbols.

$P \leq 0.017$: § 0h vs. 3h, † 3h vs. 6h, ‡ 0h vs. 6h

P: Particle; TG: Triglyceride; CE: Cholesteryl ester; IDL: Intermediate-density lipoprotein

4.2.3 Particle, TG, CE and C concentration of LDL subclasses

Between the three test meals, there was no differences in LDL subclass particle concentration or the concentration of lipid components in LDL subclasses. Within the meals, there was no significant change in particle, C or CE concentration of any of the LDL subclasses at any time point after intake of the three test meals. However, intake of the coconut cake significantly increased the concentration of L LDL-TG from 3h to 6h ($p = 0.001$) and L- and M LDL-TG from 0h to 6h ($p = 0.001$ and $p = 0.016$, respectively) (**Table 5**). Intake of linseed cake caused a significant increase in TG concentration of L and M LDL at 6h compared with both 3h ($p = 0.003$ and $p = 0.004$) and baseline ($p = 0.009$ for both). Intake of cod liver cake caused a significant increase in TG concentration of L and M LDL at 6h compared with 3h ($p = 0.004$ for both) and an increase in all LDL subclasses at 6h compared with baseline ($p = 0.004$ for L LDL, $p = 0.005$ for M LDL, $p = 0.008$ for S LDL). The largest change in TG concentration of LDL subclasses was found after intake of cod liver cake, where L-, M- and S-LDL TG concentration increased by 26 %, 26.8 % and 16 % from 0h to 6h.

Table 5: Particle, TG and CE concentration of LDL subclasses measured by NMR-spectroscopy after intake of coconut-. linseed- and cod liver oil cake. Data presented as median and 25 and 75 percentiles.

	Baseline (0h)	3h	6h	p value between time points
	Median (25-75 percentiles)	Median (25-75 percentiles)	Median (25-75 percentiles)	
LDL-P				
L (nmol/l)				
Coconut	125.35 (93.22-152.08)	124.65 (88.27-141.05)	115.90 (91.72-165.73)	NS
Linseed	142.20 (105.75-151.95)	124.80 (103.03-164.85)	139.00 (98.74-169.2)	NS
Cod liver oil	122.30 (86.58-150.30)	120.25 (83.28-146.20)	109.40 (88.09-154.35)	NS
M (nmol/l)				
Coconut	97.20 (73.37-122.40)	95.97 (66.9-111.38)	86.27 (66.47-124.75)	NS
Linseed	113.50 (83.16-124)	97.71 (79.86-126.90)	108.60 (73.38-128.9)	NS
Cod liver oil	94.49 (69.84-120.08)	96.46 (62.02-113.63)	86.68 (63.61-115.50)	NS
S (nmol/l)				
Coconut	118.45 (94.26-147.80)	117.80 (86.11-136.73)	109.40 (85.06-146.25)	NS
Linseed	135.00 (101.85-149.35)	121.00(98.98-150.70)	130.50 (92.07-152.20)	NS
Cod liver oil	115.65 (87.61-144.73)	118.05 (82.53-137.43)	106.85 (84.70-140.58)	NS
LDL-TG				
L (µmol/l)				
Coconut	74.47 (61.05-97.51)	77.67 (62.60-96.13)	84.28 (72.85-110.23)	≤ 0.017‡†
Linseed	80.32 (64.08-98.64)	81.28 (74.29-97.41)	91.58 (78.27-110.40)	≤ 0.017‡†
Cod liver oil	73.11 (63.23-107.25)	82.21 (62.95-108.23)	92.15 (71.39-120.15)	≤ 0.017‡†
M (µmol/l)				
Coconut	37.07 (29.33-48.02)	38.22 (29.98-46.78)	38.91 (31.49-52.84)	≤ 0.017‡
Linseed	39.75 (31.48-47.97)	40.47 (32.04-47.59)	44.24 (37.9-55.15)	≤ 0.017‡†
Cod liver oil	35.85 (31.65-52.93)	39.71 (31.41-53.12)	45.44 (34.17-58.59)	≤ 0.017‡†
S (µmol/l)				
Coconut	23.75 (18.5-28.21)	24.94 (18.93-28.06)	25.09 (20.36-30.69)	NS
Linseed	25.60 (20.41-30.12)	25.56 (19.77-29.98)	25.95 (22.41-33.63)	NS

Cod liver oil	24.06 (18.69-33.68)	26.14 (19.20-34.17)	27.92 (19.08-37.99)	$\leq$ 0.017‡
LDL-CE				
L (mmol/l)				
Coconut	0.39 (0.27-0.50)	0.39 (0.25-0.46)	0.34 (0.25-0.53)	NS
Linseed	0.46 (0.32-0.51)	0.37 (0.31-0.53)	0.45 (0.28-0.54)	NS
Cod liver oil	0.37 (0.25-0.49)	0.38 (0.21-0.46)	0.34 (0.21-0.48)	NS
M (mmol/l)				
Coconut	0.20 (0.14-0.28)	0.20 (0.12-0.25)	0.17 (0.10-0.28)	NS
Linseed	0.25 (0.17-0.30)	0.19 (0.16-0.29)	0.24 (0.13-0.29)	NS
Cod liver oil	0.19 (0.13-0.26)	0.20(0.09-0.25)	0.18 (0.08-0.25)	NS
S (mmol/l)				
Coconut	0.13 (0.09-0.18)	0.13 (0.08-0.16)	0.11 (0.07-0.17)	NS
Linseed	0.16 (0.11-0.19)	0.12 (0.10-0.18)	0.15 (0.09-0.18)	NS
Cod liver oil	0.12 (0.09-0.16)	0.12 (0.06-0.16)	0.12 (0.06-0.16)	NS

Differences between time points were tested with Friedman's ANOVA, and p-values of the overall time-effect is given. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. Significant results of pairwise comparisons are indicated with symbols.

$P \leq 0.017$: § 0h vs. 3h. † 3h vs. 6h. ‡ 0h vs. 6h

P: Particle; TG: Triacylglycerol; CE: Cholesteryl ester; LDL: Low-density lipoprotein; L: Large; M: Medium; S: Small

4.2.4 Particle, TG, CE and C concentration of HDL subclasses

Between the meals, the reduction in TG concentration of S HDL from baseline to 3h after intake of coconut cake was significantly different from the increase after intake of the linseed- and cod liver cake. However, after Bonferroni adjustment only the effect between the coconut and linseed cakes remained significantly different (p= 0.004) (**Figure 8**). Furthermore, there was a significant baseline difference in TG concentration of S HDL (data not shown).

The increase in cholesterol concentration of XL HDL after intake of coconut cake at 3h compared with fasting was significantly higher than after intake of both linseed and cod liver cake, (p≤ 0.05). However, these results were not significant after Bonferroni correction of p-value (0.017≤ p≤ 0.05) (data not shown).

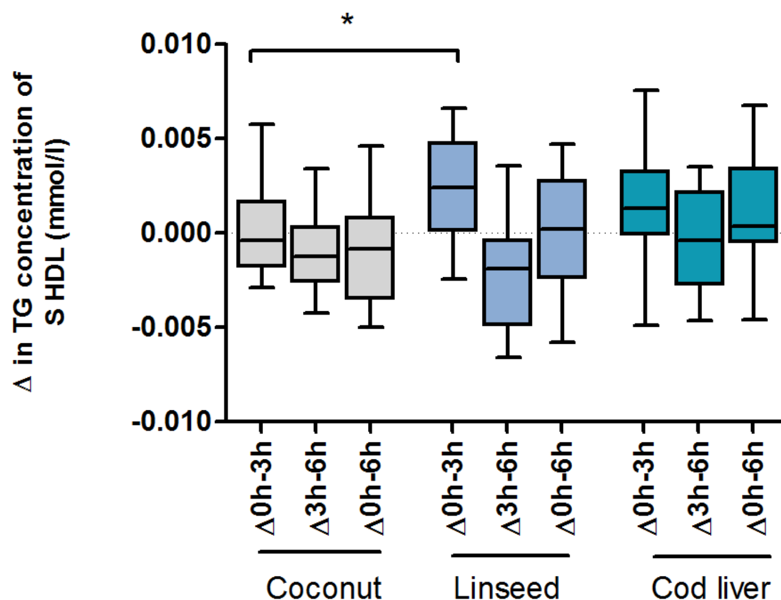

Figure 8: Change in TG concentration of S HDL after intake of the three test meals.
* Significant differences between the cakes were tested with Friedman's ANOVA, followed by post hoc analysis with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied (p≤ 0.017).

Within the meals, intake of the coconut, linseed and cod liver cakes significantly increased XL HDL particle concentrations from 0h to 3h (p=0.001, p=0.001 and p=0.004, respectively). In addition, the coconut cake significantly increased L HDL particle concentration from 0h to 3h (p=0.017) (**Table 6**). Furthermore, all three cakes significantly increased the particle concentration of XL, L and M HDL from 3h to 6h (coconut p≤0.004, linseed p≤0.006 and cod liver p≤ 0.015) and from 0h to 6h (coconut p≤0.004, linseed p≤0.006 and cod liver p≤0.012).

Intake of all three meals increased XL HDL-TG concentration from 0h to 3h (coconut p= 0.002, linseed p= 0.007 and cod liver p= 0.005, respectively) (**Table 6**). In addition, intake of the linseed cake increased TG concentration of S HDL from 0h to 3h (p= 0.009). L HDL-TG concentration increased from 3h to 6h after intake of coconut-, linseed- and cod liver cake (p= 0.006, p= 0.011, p= 0.012, respectively). Furthermore, intake of linseed cake caused a significant decrease in S HDL-TG from 3h to 6h (p= 0.013). All three test meals increased TG concentration of XL- and L HDL from 0h to 6h (coconut p= 0.001, linseed p≤ 0.009 and cod liver p≤ 0.003, respectively). The linseed cake also increased TG concentration of M HDL from 0h to 6h (p= 0.013). The greatest change in TG concentrations in HDL subclasses was seen after intake of linseed cake, where XL-, L- and M-HDL TG concentration increased by 37 %, 36.9 % and 8.7 % from 0h to 6h.

In regard of CE concentration of HDL subclasses, intake of the coconut-, linseed- and cod liver cake significantly increased CE concentration of XL HDL (p= 0.001, p= 0.001 and p= 0.002, respectively) from 0h to 3h (**Table 6**). Additionally, intake of the coconut cake significantly increased L HDL-CE concentration from 0h to 3h (p= 0.003). All test meals caused a significant increase in XL- and L HDL-CE from 3h to 6h (coconut p≤0.002, linseed p= 0.001 and cod liver p≤0.003, respectively) and from 0h to 6h (coconut p= 0.001, linseed p≤0.002 and cod liver p= 0.002, respectively). M HDL-CE concentration increased significantly from 3h to 6h after intake of both coconut- and linseed cake (p= 0.016 and p= 0.013, respectively) and from 0h to 6h after intake of linseed cake (p= 0.011). The CE concentration of S HDL decreased significantly after intake of all test meals from 0h to 3h (coconut p= 0.001, linseed p= 0.007 and cod liver p= 0.005, respectively) and from 0h to 6h (coconut p= 0.001, linseed p= 0.002 and cod liver p= 0.015, respectively). The greatest change in CE-concentration of HDL-subclasses was in the two largest HDL subclasses after all three test meals. XL HDL increased with 29 %, 10 % and 23.5 % and L HDL with 26 %, 26 % and 20 % after intake of coconut-, linseed- and cod liver cake, respectively. To summarize, the concentration of CE in the largest HDL subclasses increased, while the concentration of CE in the smallest HDL subclass decreased during the postprandial period after intake of all three cakes. The changes in C concentration of HDL subclasses followed almost the same pattern as CE concentrations of HDL classes (data not shown).

Table 6: Particle, TG and CE concentration of HDL subclasses measured by NMR-spectroscopy after intake of coconut-. linseed- and cod liver oil cake. Data presented as median and 25 and 75 percentiles.

	Baseline (0h)	3h	6h	p value between time points
	Median (25-75 percentiles)	Median (25-75 percentiles)	Median (25-75 percentiles)	
HDL-P				
XL (µmol/l)				
Coconut	0.50 (0.36-0.59)	0.56 (0.42-0.65)	0.64 (0.56-0.72)	≤ 0.017‡†§
Linseed	0.55 (0.39-0.61)	0.59 (0.44-0.62)	0.67 (0.5-0.72)	≤ 0.017‡†§
Cod liver oil	0.49 (0.40-0.57)	0.53 (0.46-0.59)	0.62 (0.52-0.65)	≤ 0.017‡†§
L (µmol/l)				
Coconut	1.40 (1.13-1.65)	1.43 (1.27-1.65)	1.75 (1.57-1.88)	≤ 0.017‡†§
Linseed	1.37 (1.23-1.56)	1.44 (1.25-1.73)	1.75 (1.62-1.92)	≤ 0.017‡†
Cod liver oil	1.39 (1.32-1.48)	1.47 (1.36-1.54)	1.68 (1.49-1.92)	≤ 0.017‡†
M (µmol/l)				
Coconut	2.02 (1.74-2.37)	1.99 (1.83-2.35)	2.38 (2.01-2.53)	≤ 0.017‡†
Linseed	2.08 (1.74-2.39)	2.11 (1.90-2.33)	2.33 (2.07-2.59)	≤ 0.017‡†
Cod liver oil	2.28 (1.7-2.41)	2.32 (1.77-2.42)	2.43(1.97-2.55)	≤ 0.017‡†
S (µmol/l)				
Coconut	4.68 (4.39-5.04)	4.60 (4.34-4.96)	4.74 (4.53-4.89)	NS
Linseed	4.65 (4.24-4.99)	4.78 (4.27-4.93)	4.73 (4.35-5.11)	NS
Cod liver oil	4.85 (4.13-5.27)	4.82 (4.29-5.18)	4.93 (4.64-5.08)	NS
HDL-TG				
XL (µmol/l)				
Coconut	11.50 (4.93-15.18)	13.80 (7.00-16.51)	15.09 (8.33-17.12)	≤ 0.017‡§
Linseed	10.89 (7.60-16.28)	13.35 (9.87-19.75)	14.92 (10.15-19.74)	≤ 0.017‡§
Cod liver oil	12.43 (6.70-17.35)	14.29 (7.58-19.83)	14.21 (8.91-19.19)	≤ 0.017‡§
L (µmol/l)				
Coconut	27.63 (17.94-35.72)	30.11 (20.69-37.25)	34.07 (28.29-44.49)	≤ 0.017‡†
Linseed	27.73 (21.62-37.83)	34.98 (24.26-38.19)	37.99 (31.30-51.70)	≤ 0.017‡†

Cod liver oil	31.02 (25.74-37.67)	32.32 (24.14-43.79)	38.47 (31.26-47.65)	≤ 0.017‡†
M (µmol/l)				
Coconut	39.08 (28.72-45.69)	39.31 (29.31-46.63)	41.12 (33.35-49.47)	NS
Linseed	41.05 (30.51-47.17)	40.20 (37.69-48.92)	44.63 (36.82-50.75)	≤ 0.017‡
Cod liver oil	40.62 (28.89-49.48)	40.19 (34.79-53.06)	44.38 (32.57-58.68)	NS
S (µmol/l)				
Coconut	43.59 (39.29-47.25)	41.67 (39.06-48.92)	40.75 (37.89-48.63)	NS
Linseed	43.56 (36.66-48.52)	44.28 (40.35-51.35)	41.98 (37.26-49.22)	≤ 0.017†§
Cod liver oil	43.36 (38.54-56.83)	45.26 (39.42-60.50)	44.95 (36.88-57.47)	NS
HDL-CE				
XL (mmol/l)				
Coconut	0.17 (0.13-0.20)	0.20 (0.15-0.22)	0.22 (0.19-0.25)	≤ 0.017‡†§
Linseed	0.18 (0.14-0.21)	0.19 (0.16-0.22)	0.20 (0.17-0.25)	≤ 0.017‡†§
Cod liver oil	0.17 (0.14-0.20)	0.19 (0.15-0.21)	0.21 (0.17-0.23)	≤ 0.017‡†§
L (mmol/l)				
Coconut	0.34 (0.28-0.39)	0.35 (0.32-0.41)	0.43 (0.39-0.46)	≤ 0.017‡†§
Linseed	0.34 (0.30-0.38)	0.37 (0.31-0.41)	0.43 (0.39-0.47)	≤ 0.017‡†
Cod liver oil	0.35 (0.31-0.36)	0.35 (0.35-0.38)	0.42 (0.35-0.47)	≤ 0.017‡†
M (mmol/l)				
Coconut	0.35 (0.30-0.39)	0.34 (0.32-0.38)	0.40 (0.34-0.42)	≤ 0.017†
Linseed	0.35 (0.30-0.41)	0.35 (0.31-0.38)	0.37 (0.34-0.42)	≤ 0.017‡†
Cod liver oil	0.38 (0.29-0.38)	0.38 (0.29-0.40)	0.39 (0.34-0.40)	NS
S (mmol/l)				
Coconut	0.28 (0.27-0.31)	0.25 (0.23-0.28)	0.24 (0.22-0.28)	≤ 0.017‡§
Linseed	0.30 (0.27-0.32)	0.28 (0.24-0.30)	0.28 (0.24-0.29)	≤ 0.017‡§
Cod liver oil	0.28 (0.26-0.32)	0.25 (0.23-0.30)	0.27 (0.24-0.30)	≤ 0.017‡§

Differences between time points were tested with Friedman's ANOVA, and p-values of the overall time-effect is given. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. Significant results of pairwise comparisons are indicated with symbols.
P ≤ 0.017: § 0h vs. 3h. † 3h vs. 6h. ‡ 0h vs. 6h
P: Particle; TG: Triacylglycerol; CE: Cholesteryl ester; HDL: High-density lipoprotein; XL: Extra large; L: Large; M: Medium; S: Small

4.3 Postprandial and meal effects on circulating metabolites

4.3.1 Glycolysis related metabolites

Between the meals, the reduction in lactate concentration from 0h to 3h after intake of the coconut cake was significantly different from the increase after intake of linseed cake, (p = 0.001). The reduction in lactate levels from 0h to 6h after the coconut cake was significantly larger than after intake of cod liver cake, (p= 0.012) (**Figure 9**). Within the meals, intake of coconut cake caused a significant 28.7 % reduction in lactate from 0h to 3h, and a 28.4 % reduction from 0h to 6h (p = 0.001 and p = 0.002, respectively). After the cod liver oil cake, lactate significantly decreased with 24 % from 0h to 3h (p = 0.012). Lactate did not change after intake of the linseed cake (**Table 7**).

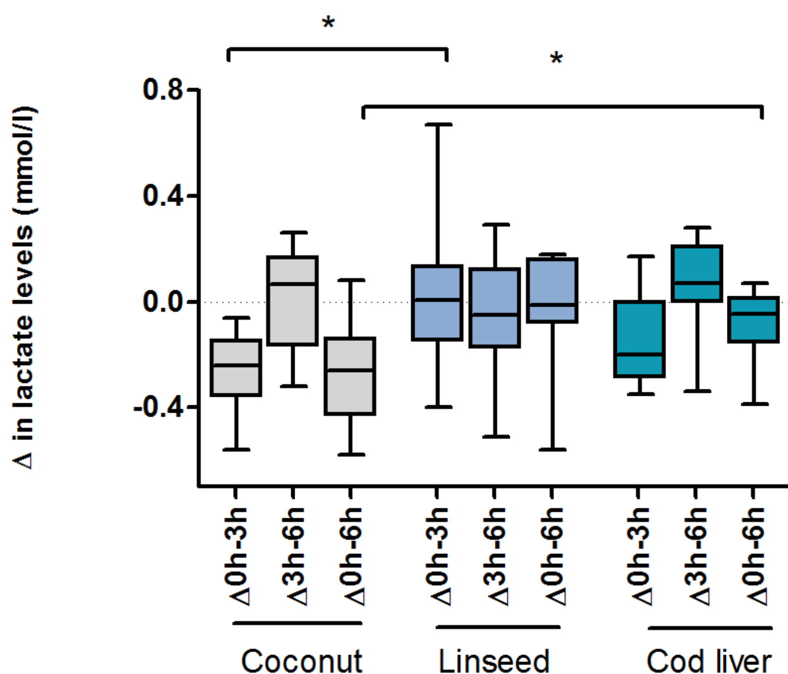

Figure 9: Change in lactate concentration after intake of the three test meals. * Significant differences between the cakes were tested with Friedman's ANOVA, followed by post hoc analysis with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied (p≤ 0.017).

Furthermore, the increase in lactate concentration 3h after intake of the linseed cake was significantly different from the reduction in lactate concentration 3h after intake of the cod liver cake, but not before Bonferroni correction (0.017≤ p≤ 0.05) (data not shown). There was a significant difference in the change from 3h to 6h between coconut and linseed cake (0.017≤ p≤ 0.05), but after Bonferroni this difference was not significant (data not shown). There was a

significant baseline difference in fasting lactate levels. The baseline level of lactate was significantly higher before intake of cod liver cake compared to linseed cake (data not shown).

There were no significant changes in glucose and citrate concentrations between or within any of the test meals (**Table 7**).

4.3.2 Branched chain amino acids

There was no significant difference in any of the branched chain amino acids between the test meals. Intake of the coconut cake caused a significant increase in isoleucine, leucine and valine concentrations from 0h to 3h (p= 0.001, p= 0.003 and p= 0.003, respectively) and 6h in isoleucine and leucine (p= 0.001 and p= 0.004, respectively). The linseed cake increased the concentration of isoleucine and leucine significantly from baseline to both 3h (p= 0.001 and p= 0.002, respectively) and 6h (p= 0.002 and p= 0.007, respectively), whereas valine increased significantly from baseline to 3h (p= 0.002) and from 3h to 6h (p= 0.016). After intake of cod liver cake, isoleucine concentration significantly increased from 0h to 3h (p= 0.002), from 3h to 6h (p= 0.015) and from 0h to 6h (p= 0.003), while leucine and valine concentration increased from fasting to 3h (p= 0.002 for both). In addition, intake of cod liver cake decreased valine concentrations at 6h compared with 3h (p= 0.015) (**Table 7**).

4.3.3 Aromatic amino acids

There was no significant difference in any of the aromatic amino acids between the test meals. After intake of coconut cake, phenylalanine levels decreased at 6h compared with 3h (p= 0.009). Intake of linseed and cod liver cake did not cause any significant change in phenylalanine levels. All test meals caused an increase in tyrosine levels from baseline to 3h, with 22 % after coconut and linseed cake and 15.6 % after cod liver cake (p= 0.016, p= 0.011 and p= 006, respectively). There was a decrease in tyrosine concentration from 3h to 6h after all three test meals (p= 0.001, p= 002 and p= 0.006, respectively) (**Table 7**).

4.3.4 Other amino acids

The reduction in histidine concentration from 3h to 6h after intake of coconut cake was significantly higher than after intake of both linseed and cod liver cake, (p≤ 0.05), but after Bonferroni correction none of these differences were significant (0.017≤ p≤ 0.05). Within the meals, intake of the coconut cake caused a decrease in alanine and histidine concentrations at

6h compared with 3h (p= 0.013 and p= 0.004, respectively) and from 0h to 6h (p= 0.005 and p= 0.008, respectively) (alanine -13.9 %, histidine -16.5 %). Intake of linseed cake caused a decline in alanine levels at 6h compared with 3h (p= 0.009), but no significant change in histidine levels. Intake of the cod liver cake did not cause any significant change in alanine concentrations, but reduced histidine concentration at 6h compared with 3h (p= 0.005). There was no significant change in glutamine levels after the three test meals (**Table 7**).

4.3.5 Ketone bodies

There was no significant difference in any of the ketone bodies between the test meals. All test meals caused a significant increase in acetoacetate and 3-hydroxybutyrate levels at 6h compared with both 3h and fasting (p= 0.001 for all after coconut and linseed cake; p= 0.002 for all after cod liver cake). The increase in acetoacetate concentrations from fasting to 6h were 132.7 % after the coconut cake, 230.7 % after the linseed cake and 151.6 % after the cod liver cake. The increases in 3-hydroxybutyrate concentrations were respectively 155.5 %, 252 % and 186.2 %. There was no significant change in acetate levels after intake of the three test meals (**Table 7**).

4.3.6 Other metabolites

There was no significant difference in creatinine, glycoprotein acetyl or albumin between the test meals. Intake of the coconut cake and cod liver cake significantly decreased creatinine concentration with 10 % from 0h to 3h (p = 0.002 and p =0.004) and with 11.4% and 5.7 % from 0h to 6h (p = 0.002 and p = 0.012). The linseed cake did not cause any significant change in creatinine levels. There was no significant change in Glycoprotein acetyls or albumin after the three test meals (**Table 7**).

Table 7: Concentration of circulating metabolites after intake of coconut-, linseed- and cod liver cake. Data presented as median and 25-75 percentiles.

	Baseline (0h)	3h	6h	p value between time points
	Median (25-75 percentiles)	Median (25-75 percentiles)	Median (25-75 percentiles)	
Glycolysis Related Metabolites				
Glucose (mmol/l)				
Coconut	3.35 (3.27-3.56)	3.46 (3.27-3.61)	3.41 (3.23-3.58)	NS
Linseed	3.51 (3.30-3.74)	3.64 (3.41-3.78)	3.37 (3.32-3.61)	NS
Cod liver oil	3.60 (3.32-3.88)	3.66 (3.36-3.74)	3.51 (3.45-3.78)	NS
Lactate (µmol/l)				
Coconut	976.9 (856.45-1086)	696.45 (548.25-909.23)	699.40 (613.58-779.33)	≤ 0.017‡§
Linseed	681.00 (630.05-921.35)	747.40 (598.10-934.05)	763.40 (604.45-859.15)	NS
Cod liver oil	942.75 (721.33-1043.75)	716.35 (606.70-861.18)	820.55 (694.40-870.35)	≤ 0.017§
Citrate (µmol/l)				
Coconut	112.60 (95.7-115.85)	106.00 (99.03-112.45)	109.90 (102.23-117.60)	NS
Linseed	101.90 (97.16-112.60)	111.70 (99.65-115.40)	110.20 (99.77-114.00)	NS
Cod liver oil	106.30 (98.82-115.15)	108.90 (102.5-114.33)	111.25 (98.93-118.78)	NS
Amino acids				
Alanine (µmol/l)				
Coconut	344.05 (298.48-355.10)	324.50 (292.60-349.23)	296.00 (274.20-317.70)	≤ 0.017‡†
Linseed	332.60 (303.25-343.75)	334.90 (299.05-356.95)	309.60 (290.35-326.50)	≤ 0.017†
Cod liver oil	337.75 (296.10-394.28)	351.15 (278.85-380.60)	313.30 (297.68-317.98)	NS
Glutamine (µmol/l)				
Coconut	449.20 (426.60-493.33)	480.05 (401.15-527.15)	453.40 (415.98-496.75)	NS
Linseed	464.90 (400.95-510.95)	455.30 (378.05-511.15)	439.00 (413.8-476.90)	NS
Cod liver oil	458.70 (405.70-516.53)	463.20 (411.38-516.98)	440.45 (393.55-505.05)	NS
Histidine (µmol/l)				
Coconut	84.79 (72.69-88.07)	82.66 (77.98-87.51)	70.84 (67.14-79.79)	≤ 0.017‡†
Linseed	81.43 (69.81-87.09)	76.24 (73.05-85.13)	75.14 (69.93-82.30)	NS
Cod liver oil	82.01 (72.95-90.39)	83.52 (80.11-90.86)	72.38 (68.21-80.63)	≤ 0.017†

Branched chain				
Isoleucine (µmol/l)				
Coconut	41.43 (38.05-46.56)	59.83 (51.07-73.52)	56.57 (49.67-61.62)	≤ 0.017‡§
Linseed	40.92 (38.19-54.97)	70.83 (56.66-80.46)	57.28 (52.76-63.42)	≤ 0.017‡§
Cod liver oil	44.76 (38.11-48.46)	68.87 (60.25-75.04)	56.62 (47.73-62.87)	≤ 0.017‡†§
Leucine (µmol/l)				
Coconut	66.36 (61.51-72.22)	78.87 (70.55-90.8)	76.23 (67.16-80.38)	≤ 0.017‡§
Linseed	62.77 (58.91-80.85)	92.75 (76.09-100)	77.67 (71.32-86.90)	≤ 0.017‡§
Cod liver oil	66.21 (58.64-71.86)	86.48 (74.79-99.31)	73.69 (66.50-83.74)	≤ 0.017§
Valine (µmol/l)				
Coconut	143.05 (130.45-156.53)	174.40 (158.60-181.70)	152.40 (144.68-164.60)	≤ 0.017§
Linseed	139.60 (132.15-169.95)	178.10 (162.95-194.40)	155.20 (151.8-165.55)	≤ 0.017†§
Cod liver oil	126.60 (114.68-138.83)	167.95 (159.15-191.53)	143.05 (136.2-158.18)	≤ 0.017†§
Aromatic				
Phenylalanine (µmol/l)				
Coconut	66.87 (60.95-71.18)	70.31 (60.44-76.21)	61.97 (58.29-66.94)	≤ 0.017†
Linseed	65.88 (59.77-73.32)	70.27 (64.67-78.01)	67.00 (61.20-71.78)	NS
Cod liver oil	67.11 (60.76-75.83)	71.10 (66.64-79.76)	71.00 (56.85-76.05)	NS
Tyrosine (µmol/l)				
Coconut	39.20 (35.64-43.20)	47.83 (40.30-52.19)	33.61 (28.71-41.87)	≤ 0.017†§
Linseed	35.99 (31.27-44.93)	44.03 (36.77-50.28)	35.22 (27.04-42.42)	≤ 0.017†§
Cod liver oil	40.42 (34.92-48.10)	46.71 (41.14-57.78)	37.45 (30.92-42.53)	≤ 0.017†§
Ketone Bodies				
Acetate (µmol/l)				
Coconut	44.32 (39.22-46.19)	40.31 (36.61-44.34)	41.81 (34.15-46.83)	NS
Linseed	40.23 (33.51-43.36)	41.31 (36.41-49.20)	40.83 (37.36-46.61)	NS
Cod liver oil	43.44 (34.65-51.31)	40.44 (34.67-42.74)	42.28 (38.95-46.83)	NS
Acetoacetate (µmol/l)				
Coconut	30.69 (24.68-37.32)	35.72 (28.56-42.68)	71.41 (58.43-82.55)	≤0.017‡†
Linseed	28.08 (19.08-46.40)	42.32 (29.95-56.33)	92.86 (63.49-109.45)	≤0.017‡†

Cod liver oil	28.11 (23.21-46.21)	31.86 (26.82-42.40)	70.72 (49.75-84.57)	≤ 0.017‡†
bOHBut (µmol/l)				
Coconut	129.25 (85.61-151.15)	117.20 (97.47-174.70)	330.20 (278.6-448.63)	≤ 0.017‡†
Linseed	109.50 (95.27-156.25)	180.60 (130.65-208.80)	385.50 (311.35-491.70)	≤ 0.017‡†
Cod liver oil	117.65 (95.33-145.53)	122.70 (98.13-162.33)	336.75 (236.98-402.05)	≤ 0.017‡†
Inflammation				
Gp (mmol/l)				
Coconut	1.19 (1.10-1.32)	1.22 (1.10-1.32)	1.24 (1.03-1.34)	NS
Linseed	1.27 (1.10-1.46)	1.28 (1.19-1.46)	1.33 (1.22-1.48)	NS
Cod liver oil	1.29 (1.16-1.43)	1.26 (1.15-1.40)	1.35 (1.14-1.50)	NS
Other metabolites				
Creatinine (µmol/l)				
Coconut	56.34 (53.92-64.28)	50.53 (47.58-52.33)	49.93 (47.15-57.33)	≤ 0.017‡§
Linseed	57.74 (51.51-63.88)	53.81 (51.07-55.96)	54.56 (52.08-59.58)	NS
Cod liver oil	58.23 (56.68-63.22)	52.38 (48.06-55.13)	54.91 (49.71-57.18)	≤ 0.017‡§
Albumin (signal area)				
Coconut	0.09 (0.08-0.09)	0.09 (0.08-0.09)	0.09 (0.08-0.10)	NS
Linseed	0.08 (0.08-0.11)	0.09 (0.08-0.09)	0.09 (0.09-0.10)	NS
Cod liver oil	0.09 (0.09-0.10)	0.09 (0.09-0.10)	0.09 (0.09-0.10)	NS

Differences between time points were tested with Friedman's ANOVA, and p-values of the overall time-effect is given. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. Significant results of pairwise comparisons are indicated with symbols.

$P \leq 0.017$: § 0h vs. 3h, † 3h vs. 6h, ‡ 0h vs. 6h

bOHBut: 3-hydroxybutyrate

Gp: Glycoprotein acetyls, mainly a1-acid glycoprotein

5 Discussion

In present study, the effect of a single high fat meal with different fat quality on lipoprotein subclasses, their lipid constituents and circulating metabolites was elucidated. In the first section, the methodological considerations will be discussed, followed by a discussion of the results in the second section.

5.1 Discussion of the study design, subjects and method

Subjects and study design

The sample size in the present study was small and the study population was relatively homogenous, which is important regarding the interpretation of the findings. Since only women were recruited to this study, the existing sex differences in the dietary and postprandial response were ruled out (46, 73-75). Men are demonstrated to have a larger increase in TG and VLDL after an acute fat load than women (73, 76). In contrast, women are shown to have lower (46) and more rapid (74) postprandial response, possibly due to higher LPL activity, in particular before menopause (73). The lipoprotein response after menopause is similar to what is observed in middle-aged men (73). Additionally, estrogen is shown to promote LDL receptor gene expression, thereby increasing the number of LDL receptors (77), and increasing cholesterol clearance (74). Moreover, there are fluctuations in hormones and metabolites, including lipids, in premenopausal women related to different phases of the menstrual cycle. The greatest increase in lipids is seen in the follicular phase, while TG concentrations are reduced in the luteal phase of the cycle (78). We separated the test days only by two weeks. Observed differences between the meals in present trial might be partly related to the fact that the test meals were ingested at different time points in the menstrual cycle.

Since the study population was young, the increase in plasma TG after all meals was lower than what most likely would have been observed in an older population, as tolerance to a fatty meal decreases with increased age (44). In addition, total C and LDL-C increase, while HDL-C declines with age (79). A mild to moderat increase in TG is reported to be between 2-10 mmol/L (80). The median increase after intake of the test meals in present population did not exeed 1.3 mmol/L (68). Additionally, all of the participants were healthy, normal weight, with biochemical values within reference area, which may explain why the TG response observed in the present study (68) was lower compared to other studies. RCTs show that the postprandial

TG response is greater in obese and overweight individuals (81), as well as in people with dyslipidemia (76) and different diseases, such as diabetes type 2 (82) and established cardiovascular disease (83). Moreover, the habitual diet also affects postprandial lipemia (44). All of the participants in the trial were nutrition students. It is therefore reasonable to assume that their habitual diet was relatively healthy and that the postprandial response may have been different from unhealthy individuals.

The study was designed as a cross-over study. Each subject ate all three meals, and the participants were used as their own control, which exclude variability between individuals (84). This is in contrast to a parallel RCT were subjects are randomized to different groups, and inter-individual variations could affect the results (84). Additionally, a crossover design requires less participants than parallel designs, due to no control group (85). Moreover, the test meals in present study were served in a fixed order, separated by two weeks of wash out. This is probably sufficient time to rule out any carry-over effect of the meals, which means that the effect of the first meal would most likely have disappeared before ingestion of the second meal (85). However, we did not measure if there was any carry-over effect. Furthermore, a great strength in this study design is that the participants were served the same dinner prior to each day and were asked to consume the same low-fat supper each evening before blood sampling, as the pre-meal condition is proposed to affect the postprandial response as well (73).

The high fat meal

Assessing the magnitude of postprandial lipemia after ingestion of fat is complex. Firstly, there is no consistency in the literature regarding the method used to evaluate postprandial TG response (51, 73). This is in opposed to postprandial glycemia that is mostly determined by a standardized oral glucose tolerance test. Oral fat tolerance tests (OFTTs) are not standardized in terms of duration of the test, amount or type of fat, nor meal preparations. Secondly, it is unclear how different FAs affect postprandial TG response (66). Additionally, factors such as which nutrients that are ingested at the same time (15) and meal-preparation (66) are also important contributors.

TG elevation after a fatty meal might be long lasting and result in raised plasma TG concentration several times. Peak in plasma TG might occur 3h to 5h after a fatty meal (15), and could be elevated for 5h to 8h after a typical meal with 30-60 g fat (73). It is also evidence that postprandial lipemia occurs 2h to 12h after a meal (76). According to a systematic review, an ideal fat tolerance test (FTT) should last for 8h to 10h (66), while other studies have found

that TG concentration at 4h is reliable to use as a marker for postprandial lipemia (46, 86). In the present study, the participants were followed for 6h, and it is therefore unsure if that is sufficient time to reveal all postprandial differences. An important aspect to take into concern regarding the duration of a FTT is the participant's compliance (66). A trial that lasts too long could possibly have a high number of drop outs that would lead to underpowered analyses (87). Moreover, according to an expert panel, a single FTT meal should be given after an 8h fast and consist of 75 g (62.5 E%) fat, 25 g (24.1 E%) carbohydrates and 10 g (13.4 E%) protein (86). Subjects in present study were asked to fast for 12h before blood sampling. During the test day, they ingested around 55 g fat, which accounted for around 70 E%. The amount of fat in g is less than the recommendations, in contrast to the amount of fat in E%. It might be that the postprandial alterations would have been greater with a higher amount of fat in g. On the other hand, a smaller amount of fat might reflect a more comparable daily meal and the normal metabolic capacity of each subject (73).

5.1.1 Use of NMR-spectroscopy to measure lipoprotein subclasses and metabolites

Quantitative NMR-spectroscopy is a method that can be used to provide quantitative data on lipoprotein subclasses, hence, their size, density, concentration and lipid composition, as well as data on low molecular weight metabolites (53, 61, 62). This is in contrast to routine lipid measurements in clinics, where a standard lipid panel is used and includes only the concentration of plasma TG, total cholesterol, LDL-C and HDL-C (40).

In the present study, NMR analysis was performed at Nightingale health, a commercial laboratory in Finland. Furthermore, there is a number of methods that can be used to identify lipoprotein subfractions, e.g. by size, density, number, electrophoretic mobility, etc., which makes it difficult to compare the literature and be sure that it is the same lipoprotein particle that is being measured (88). Additionally, there are several existing commercial high-throughput proton NMR-metabolomics platforms that divide lipoprotein subclasses differently, as illustrated in **Figure 10** (62, 76, 89).

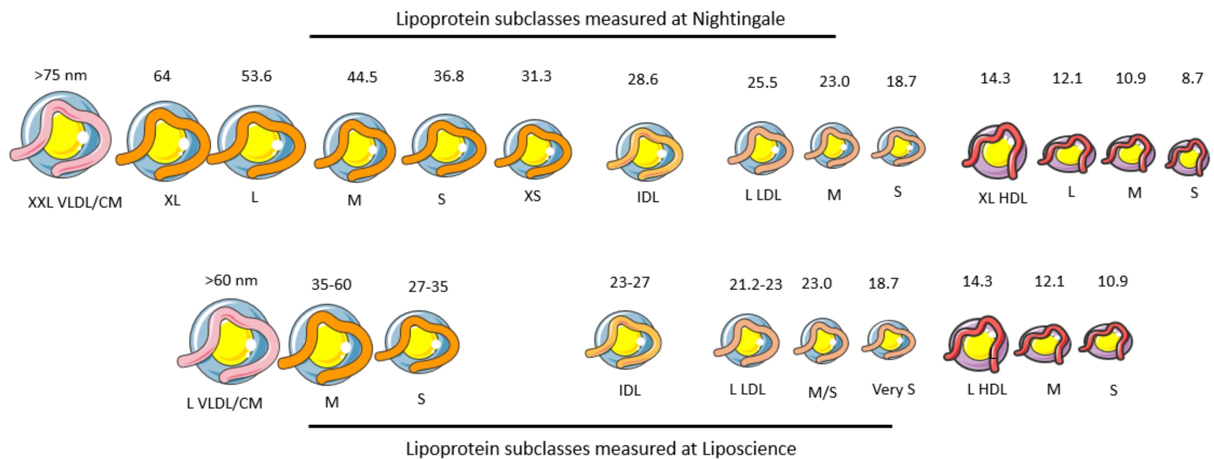

Figure 10: Classification of lipoprotein subclasses based on two commercial high-throughput serum nuclear magnetic resonance (NMR) metabolomics platforms. Based on free images from Servier Medical Art (Creative Commons Attribution License, http://creativecommons.org/licenses/by/3.0/).

5.1.2 Statistical considerations

Non-parametric tests were used in all the analyses in present study. Non-parametric tests might be better to use than parametric tests when there is a small sample size (90), as in present study. These tests are less sensitive to outliers. In contrast to parametric test, there are no assumptions of the study population in non-parametric tests (90). We adjusted for multiple testing by performing a Bonferroni correction of p-value, which could reduce the chances of having Type I errors, false positive. On the other hand, adjustment of p-value for multiple comparisons increases the chances of Type II errors, false negative, which could be the case with a small sample size (91). Furthermore, another method that could have been used to analyze the data in this thesis is the linear mixed model. Observed baseline differences in some of the data could have been adjusted for with this method (92), which would have strengthen the results. However, this statistical method was considered to be too extensive to learn for this master thesis.

5.2 Discussion of the results

5.2.1 Postprandial effect on lipoprotein subclasses

In the present study, the effect of three different meals with different fat quality on lipoprotein subclasses was conducted. Summary of our findings on lipoprotein particles within the meals is shown in **Table 8.**

Effect of a meal rich in fat on VLDL subclasses

In present study, the particle- and TG concentration of XXL VLDL were significantly higher after intake of coconut cake compared to both linseed- and cod liver cake, from 0h-3h. In agreement with our findings, Øyri and colleagues found a significantly higher incremental area under the curve (iAUC) of XXL VLDL after intake of a SFA rich meal (mainly palm- and coconut oil) compared to a PUFA rich meal (mainly rapseed- and sunflower oil) (48). Additionally, the authors found a delayed peak of the largest VLDL subclasses after the SFA meal compared to the PUFA meal. In the present study, a non-significant delayed peak of XXL VLDL-TG was observed after the coconut cake. The test meals in Øyri et al.'s study consisted of 60 g fat, close to the amount of fat in the present study. The control group consisted of both men and women, but the participants had the same median age as in the present study, which could explain why the findings were similar. Tholstrup et al. demonstrated that in men, CM-TG and VLDL-TG peaked 4h after intake of 6 test meals with either stearic-, palmitic, palmitic + myristic, oleic (C18:1), trans 18:1 and linoleic acid (93). There was no significant effect between the meals, but the authors showed that the highest VLDL-TG iAUC was after intake of the palmitic and myristic meal. A large amount of myristic acid is also found in coconut oil, which was used as a source of SFA in the present study (94).

In vitro studies have demonstrated that SFA and PUFA might affect LPL activity differently (95). In unpublished results by Rundblad et al., the change in mRNA expression of LPL was measured to be close to significant after intake of the three test meals. The greatest increase in the LPL mRNA expression level was seen 3h after intake of linseed cake. The level of LPL could therefore to some extent explain the observed differences between the linseed and coconut cake. It is likely that the study did not have enough power to detect significant differences in LPL mRNA expression level. Furthermore, marine omega-3 FAs have been shown to lower TG levels (96, 97). One possible mechanism is that the Peroxisome proliferator activated receptors (PPARs), which are nuclear receptors, can be activated by FAs and

Table 8: Simplified table of the significant results on lipoprotein subclasses within the meals

Particle	Coconut			Linseed			Cod liver oil		
	0t-3t	3t-6t	0t-6t	0t-3t	3t-6t	0t-6t	0t-3t	3t-6t	0t-6t
XXL VLDL P	↑								
XL VLDL P	↑	↓			↓	↓			
L VLDL P	↑	↓			↓	↓			
M VLDL P		↓	↓		↓	↓			
S VLDL P		↓	↓		↓				
XXL VLDL TG	↑								
XL VLDL TG	↑	↓			↓	↓			
L VLDL TG	↑	↓			↓	↓			
M VLDL TG		↓	↓	↑	↓	↓		↓	
S VLDL TG		↓	↓	↑	↓				
XXL VLDL CE	↑	↓			↓				
XL VLDL CE	↑	↓			↓	↓			
L VLDL CE		↓	↓	↑	↓	↓			
M VLDL CE		↓	↓		↓	↓			
S VLDL CE			↓						
XS VLDL CE		↑	↑		↑				
IDL TG		↑	↑		↑	↑		↑	↑
L LDL TG		↑	↑		↑	↑		↑	↑
M LDL TG			↑		↑	↑		↑	↑
S LDL TG									↑
XL HDL P	↑	↑	↑	↑	↑	↑	↑	↑	↑
L HDL P	↑	↑	↑		↑	↑		↑	↑
M HDL P		↑	↑		↑	↑		↑	↑
XL HDL TG	↑		↑	↑		↑	↑		↑
L HDL TG		↑	↑		↑	↑		↑	↑
M HDL TG						↑			
S HDL TG				↑	↓				
XL HDL CE	↑	↑	↑	↑	↑	↑	↑	↑	↑
L HDL CE	↑	↑	↑		↑	↑		↑	↑
M HDL CE		↑			↑	↑			
S HDL CE	↓		↓	↓		↓	↓		↓

Differences between time points were tested with Friedman's ANOVA. Post hoc analysis of significant results was conducted with Wilcoxon signed-rank test. A Bonferroni correction of p-value was applied. The arrows indicate whether there was an increase or a decrease in the concentration of the specified particle.
P: Particle concentration; TG: Triacylglycerol; CE: Cholesteryl ester; XXL: Extremely large; XL: Extra large; L:

Large; M: Medium; S: Small; XS: Extra small; VLDL: Very low-density lipoprotein; IDL: Intermediate-density lipoprotein; LDL: Low-density lipoprotein; HDL: High-density lipoprotein

compounds derived from FAs (15). PUFAs in general are more potent to activate PPARs than SFAs (15). Omega-3 FAs have been shown to be potent PPAR-α ligands, in the liver, which increase FAs beta-oxidation, and results in less TG formation and less TG available for VLDL synthesis (96, 97). The mRNA level of CPT1 has previously been shown to be increased 6h after intake of the cod liver oil cake compared to after 3h (68). The gene encodes the protein, carnitine palmitoyl transferase I, which is mitochondrial enzyme responsible for the formation of acyl carnitines by catalyzing the transfer of the acyl group of a long-chain fatty-acyl-CoA from coenzyme A to 1-carnitine (15). An increase in this enzyme may lead to increased b-oxidation (50). Another mechanism is the inhibition of sterol regulatory element binding protein (SREBP)-1c, a hepatic gene transcription factor, by omega-3 FAs, which causes a suppression of *de novo lipogenesis*, also resulting in less TG formation in the liver (96). All of these mechanisms could have contributed to the lower increase in XXL VLDL particle- and TG concentration that was observed after both PUFA meals, compared to after intake of the coconut cake. FAs could also affect the secretion of TRL. A high-fish diet caused a decrease in TRL-ApoB-48 concentration by reducing the secretion from the intestine (67). We did not measure ApoB-48 to confirm this.

We further found that only the coconut cake caused a significant increase in particle- and TG concentration of the largest VLDL subclasses from 0h to 3h. The linseed cake increased the TG concentration of M and S VLDL at the same time point. Since larger VLDL particles have been shown to be atherogenic (49, 76, 98, 99), we therefore find the increase in the largest VLDL particles by coconut very interesting. A raise in the largest VLDL subclass was observed in another postprandial study (76). Wojczynski et al. showed that the postprandial particle concentration of L VLDL increased with a greater magnitude in hyper-triglyceridemic, than normo-triglyceridmic men (76). Another postprandial study found that increase in larger VLDL contributed to the formation of small, dense LDL in CHD patients after an acute fat load (100). Our findings indicate that also fat quality intake in lean, healthy women is important and that a meal rich in SFA (coconut oil) may have a more atherogenic effect.

Furthermore, we did not find any significant time effect on the concentration of any of the VLDL constituents following intake of the cod liver cake, enriched with marine omega-3 FAs. This is in opposed to another study, where acute consumption of fish oil increased VLDL

particle concentration and reduced VLDL particle size in the postprandial period. However, the study population was older than in present study and consisted only of men (49), which could explain differences in the findings. Moreover, we found a significant reduction in M VLDL-TG from 3h-6h after the cod liver cake. It is unsure of this finding is of clinical relevance. Interestingly, there were greater individual differences after the cod liver cake, compared to the other two cakes, that could explain the lack of effect after intake of this cake. This in accordance with findings in an eight weeks intervention study, where the participants who consumed fish or krill oil had greater individual variations in TG response, than in the control group (65). The large individual differences could possibly be explained by genetic variations (101), which we did not measure to support this assumption. Other researchers have found that the postprandial CM-TG response after intake of dietary fat could be explained by a combination of 42 single nucleotide polymorphisms in 23 genes (102).

The lack of effect of marine omega-3 FAs could also be related to the fact that the participants in present study were only females. Some researchers have suggested that the reduction of plasma TG is higher in men than in women after EPA and DHA supplementation (103). Additionally, the time frame of this trial might not have been long enough to cause any significant change in VLDL particles. Studies with longer duration have demonstrated that long-term consumption of fish oil decreases VLDL particle size (98, 104), which are some of the suggested beneficial anti-atherogenic effects of consuming fish and fish oil (31). Finally, the reduction of TG is dependent on the dose of EPA and DHA, as well as levels of fasting TG. A meta-analysis of RCTs indicated that the TG lowering effect of EPA and DHA supplementation is larger at doses >3g/day^{-1} and higher in people with elevated TG levels (27). Participants in our study had fasting TG levels within reference area. The cod liver cake contained only 0.9 g EPA and 1.3 g DHA and the meal was only given at one occasion.

Few studies have investigated the acute effect of fat quality on VLDL subclasses (66, 67). Most studies have measured postprandial lipemia in regard of change in plasma TG levels (66). Plasma TG reflects the total amount of TG found in CM, VLDL and their remnants (105). In previous analyses of the present study population, plasma TG increased from 0h-3h, and not from 0h-6h after all test meals. There was no significant difference between the meals at any time point (68). This is in agreement with findings in a systematic review and meta-analysis of the postprandial response after different FAs. However in the review, the TG response was significantly higher 8h after intake of SFA compared with PUFA (66). We might have detected

differences in plasma TG between the different FAs if the participants were followed for a longer duration. The postprandial effect of SFAs could also be affected by chain length of SFAs (106). Coconut oil contains 92 % SFA, where 0.5 % is of C6:0, 7.6 % of C8:0, 48 % of C12:0 and 20 % C14:0 (94). The C-increasing FAs, lauric- and myristic acid are found in great proportions in coconut oil (107). The origin of SFAs is also important regarding the postprandial effect (18). Hansson et al. found that sour cream induced larger TG-iAUC compared to whipped cream, two dairy products rich in SFA, with similar FA composition and same amount of fat (18).

IDL and LDL

We found a significantly higher increase in IDL-TG after intake of the cod liver cake, compared to the coconut cake. The relevance of this finding is uncertain. We further found that all three test meals altered TG concentration of IDL and M and L LDL. The raise in LDL-TG is suggested to be a result of increased CETP activity (106). Increased activity of CETP facilitates the exchange of CE from LDL and HDL particles to VLDL, which exchange TG to LDL and HDL particles (108). The exchange of CE and TG could also be between HDL and LDL particles (55). These TG-enriched LDL and HDL particles become substrate for hepatic lipase (HL), resulting in smaller and more dense particles (15). We do not know if this mechanism is responsible for the observed effect on TG-enrichment in LDL and IDL particles.

Furthermore, there was no significant effect of time or meal on neither IDL- nor LDL particle-, C and CE-concentration after any of the test meals. Some researchers have indicated that LDL size is not acutely affected by a high fat meal (109), while others have found that higher postprandial TG levels are associated with smaller LDL particles, which are thought to be the most atherogenic lipoprotein (99, 108, 110) and most susceptible to oxidation (99). Jackson and colleagues isolated TRLs from plasma after a meal rich in SFA (palm oil and cocoa butter), *n-6* PUFA (safflower oil) or MUFA (olive oil) in middle aged men. The authors showed that SFA reduced the expression of LDL receptor-related protein 1 (111). In agreement with our findings, Sabaka and colleagues reported no significant effect on particle concentration of LDL subclasses in women after intake of a test meal with 52 g fat (20 g SFA). On the other hand, they found a significant decline in LDL-C concentration in men and women after the acute fat load and a significant decrease in the particle concentration LDL subclasses, in men. They used red meat as a source of fat, in contrast to plant oil in present study (112). Koba et al. found that an OFTT (64.3 % SFA) increased total C, not LDL-C, but the LDL particle size decreased in

myocardial infarction patients (100). Another postprandial study of a meal with a low PUFA to SFA ratio found an increase in L LDL particle concentration and a decrease in S LDL in men and women with normal fasting TG levels (76).

In regard of the postprandial response after intake of the cod liver cake, our findings are in accordance with the study by Burdge and colleagues. They did not either find any significant effect of marine omega-3 FAs and particle concentration of LDL subclasses (measured by Liposcience), even though the study population was not similar to ours, as already discussed in the VLDL section. The authors suggested that the effect of some of the FAs could have occurred after blood sampling or after repeated exposures (49). Similarly, Dias et al. did not find any difference in postprandial iAUC of total C and LDL-C between a meal rich in SFA or *n*-6 PUFA after enrichment of marine omega-3 FAs, in healthy women and men (74).

HDL

In the present study, the change in HDL particles and their compounds followed a similar pattern after all test meals, with increased particle and lipid concentrations in all HDL subclasses, except reduced CE concentrations in the smallest HDL subclass (**Table 8**). Similarly, in the Genetics of Lipid-Lowering drugs and diet network (GOLDN) study, particle concentration of the largest HDL subclass (measured by Liposcience) increased in women with normal TG levels after an acute fat load, with 83 % fat and PUFA/SFA-ratio of 0.06. In contrast, the authors found a decrease in L HDL in women with elevated TG levels (76), which was also seen in another postprandial study (113). Sabaka and colleagues did not find any significant change in the largest HDL particles in men and women with normal waist circumference (112). Differences in the results between our findings and Sabaka's study could be related to different fat sources in the OFTT or the unlike methods used to measure lipoprotein subclasses, or that we only included women in our study (112).

There was a time effect on XL and L HDL-TG after all test meals, with the greatest increase observed after intake of linseed cake. In contrast to our findings, Tholstrup and colleagues demonstrated that the meal rich in myristic + palmitic acid caused the highest iAUC of HDL-TG 4h after the fat load, followed by the linoleic acid meal (93). Differences in the results could be caused by the gender differences or differences in fat amount in the OFTT (93).

TG-enrichment of HDL particles could be related to increased CETP activity in the postprandial phase (113). Some postprandial studies have demonstrated that there is a

correlation between the magnitude of postprandial lipemia and the increase in HDL-TG in expense of HDL-CE (113, 114). Nordestegaard suggested that low HDL-C is a marker of raised TG and remnant cholesterol (80). Moreover, we observed a reduction in S HDL-CE 3h and 6h after all test meals, which together with the increase in HDL-TG, may imply an altered CETP activity. There was an increase in CE concentration of the three largest HDL subclasses. The shift in CE concentration could also be related to increased LCAT activity and increased esterification of C (115), which we did not measure. Rundblad et al. found that the mRNA expression of ATP-binding cassette A1 (ABCA1) and -G1 (ABCG1) from the same study, two proteins responsible for the transport of cholesterol from cells to HDL particles (15), were altered differently after the test meals (unpublished results). This removal of excess cholesterol is suggested to be one of the most important anti-atherogenic mechanisms of HDL particles (116). Rundblad and colleagues found that intake of the coconut cake increased mRNA expression of ABCA1 significantly from 0h-3h, and ABCG1 from 0h-6h. The increase in mRNA expression of ABCG1 from 0h-6h after intake of the coconut cake seemed to be significantly different from the decrease after intake of cod liver cake (unpublished results). Alteration in these two proteins could possibly explain why there was an increase in CE concentration in the largest HDL subclasses, however, only after intake of coconut cake. Furthermore, there was no significant difference in HDL-C, nor CE between the test meals. Findings in other postprandial studies have been divided regarding postprandial change in HDL-C after intake of different FAs. Some trials have reported a decrease in HDL-C after a fat load (93, 112, 117). The fat content in these studies have consisted mostly of SFA. In contrast, Dias et al did not find any significant difference in iAUC over 6h of total HDL-C after consumption of butter or *n-6* rich oil, supplemented with marine omega-3 FAs (74). Another study that used marine omega-3 FAs did also not find an effect on postprandial HDL-C (49). All these studies differed from our in terms of study population, fat load and measurement of HDL-particles.

5.2.2 Postprandial effect on metabolites

We found that some of the metabolites were differently altered after intake of the three test meals, indicating that the FAs result in varying postprandial response. However, changes in metabolites in the postprandial state as well as in relation to a high fat meal is poorly studied and the clinical relevance of our findings remains to be elucidated.

Between the three test meals, only lactate differed significantly among all the metabolites. Intake of the coconut cake caused the greatest reduction in lactate concentration. There was a significant difference in fasting lactate levels, which could have affected the findings. The present study is to our knowledge the first to report the effect on lactate after acute consumption of FAs with different quality. Moreover, in a postprandial study by Silberbauer et al., 28 lean men with a habitual diet high or low in fat were served a high-fat meal (71 g fat). The authors demonstrated that lactate concentration increased in both groups after the meal and decreased close to baseline level after around 4.5h. There was no significant difference between the groups (118). A postprandial increase in lactate is suggested to be a result of enhanced glucose utilization (15), in insulin sensitive subjects (118, 119). Glucose is stored as glycogen in some cells after intake of carbohydrates. Glucose also enters glycolysis and could thereby cause an increase in lactate levels (119). The amount of carbohydrates were similar in all three test meals. We did not measure insulin to see if FAs affected insulin sensitivity (119). The relevance of our findings is uncertain. Moreover, increased lactate concentration is demonstrated to be associated with increased CVD risk in several large cohorts, both when profiling with NMR- and mass-spectroscopy (60, 62), as well as with reduced insulin sensitivity (120). If the change in lactate concentrations observed in present study could affect CVD risk or insulin sensitivity is yet to be studied and determined.

5.3 Strengths and limitations

Our study has several strengths including that we elucidated the effect of different FAs on lipoprotein subclasses that could give us a deeper understanding about changes in the postprandial phase. The trial was designed as a cross-over study. Inter-individual differences were ruled out, because each subject was used as its own control. Another strength is that the participants consumed a standardized fat load, as well as the same dinner prior to each test day. The study population was homogenous, which eliminates factors that could have affected the findings, compared to a heterogeneous study population (121). However, this is also a limitation, because the findings can not be generalized to the entire population. Another limitation in this study is the small sample size. No power calculation was made and the number of participants included in present study is not based on the outcomes of this thesis (68). Moreover, we did not measure carry-over effect of the meals or were able to adjust for baseline differences. The postprandial response after the three test meals were not measured at the same time in the menstrual cycle, which also could affect the findings (78). The postprandial measurements were only up to 6h. Studies indicate that the postprandial phase might last for a longer duration. There could also be greater differences after a second meal, compared to the first meal of the day. We did not measure all the enzymes and proteins activated in the postprandial period that could have given us a deeper understanding of the results. Another possible limitation in this study is that both the linseed and cod liver cake contained 30 E% of SFA, which may have masked some of the postprandial effect of linseed- and cod liver oil, compared to if they were given as e.g. supplements. Additionally, the amount of ALA in g in the cod liver cake is similar to the total amount of EPA and DHA in g that also could have masked some of the possible effect of marine omega-3.

6 Conclusion and future perspectives

In conclusion, we demonstrated that a high-fat meal with different fat quality altered lipoprotein subclasses, their lipid components and metabolites differently at both 3h and 6h. The clinical relevance of the findings needs to be further investigated. More specifically and in line with the aims the conclusions are;

I. The coconut cake increased particle- and TG concentration of the largest VLDL subclasses, while the linseed cake increased TG concentration of M and S VLDL. The cod liver cake did not alter VLDL particle- or lipid concentration, but induced greater postprandial individual changes.

II. The change in IDL-particle and lipid components were similar after intake of the three test meals. However, intake of the cod liver cake induced a larger increase in IDL-TG concentration compared to the coconut cake.

III. Particle concentration and lipid compounds of LDL subclasses changed similarly after intake of all three test meals, with no significant difference between the meals.

IV. HDL-particle- and lipid concentration increased after intake of the three test meals, except HDL-CE concentration, which declined in the smallest particle. The increase in HDL-TG was greatest after intake of linseed cake.

V. Lactate concentration reduced after intake of coconut cake. There was no significant difference in any of the other metabolites between the test meals.

Further research is needed to understand the role of lipoprotein subclasses after intake of FAs with different quality in relation to CVD risk or other metabolic disorders. In order to be able to compare studies, the measurement of lipoprotein subclasses should be more standardized. Future projects should involve a greater number of participants in order to increase the statistical power. It would be interesting to investigate the postprandial response after a second meal of the day, as it can differ from the first meal of the day. The postprandial response in women should be elucidated at the same time in the menstrual cycle. Future projects should also involve more gene expression analyses and measurements of enzyme activity to understand the postprandial mechanisms in depth. Individual variation in TG response after intake of marine omega-3 needs to be further studied.

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Appendix

Appendix 1: Approval from the Regional Committee of Medical Ethics, south-east region of Norway (08/338b, Omega-3 and HDL-1)

UNIVERSITETET I OSLO

DET MEDISINSKE FAKULTET

Førsteamanuensis Stine Marie Ulven
Høgskolen i Akershus
Instituttveien 24
2007 Kjeller

Regional komité for medisinsk og helsefaglig forskningsetikk Sør-Øst B (REK Sør-Øst B)
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NO-0318 Oslo

Telefon: 22 85 06 70
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Dato: 26.05.2008
Deres ref.:
Vår ref.: 08/338b

08/338b **Omega-3 og HDL-1**

Komiteen behandlet søknaden i sitt møte den 15. mai 2008. Prosjektet er vurdert etter lov om behandling av etikk og redelighet i forskning av 30. juni 2006, jfr. Kunnskapsdepartementets forskrift av 8. juni 2007 og retningslinjer av 27. juni 2007 for de regionale komiteer for medisinsk og helsefaglig forskningsetikk.

Saksframstilling
Formålet med studien er å studere betydningen av high-density lipoprotein (HDL)s beskyttende egenskaper for utvikling av aterosklerose og mulige mekanismer for denne effekten.

Forskningsetisk vurdering
Deltakerne skal rekrutteres fra 3. klasse på samfunnsernæringsstudiet på Høyskolen i Akershus. Komiteen er av den oppfatning at det i søknaden ikke skilles tydelig mellom deltakelse i undervisning og i selve forskningsprosjektet siden oppgitt antall deltakere synes å stemme overens med antall studenter. Komiteen forutsetter at det er frivillig å delta i forskningsdelen av prosjektet og har for øvrig ingen forskningsetiske betenkeligheter til studien.

Forskningsbiobank
Komiteen har ingen merknader til søknad om opprettelse av forskningsbiobank og tilrår at denne opprettes.

Informasjonsskriv/samtykkeerklæring
Komiteen har ingen merknader til informasjonsskrivet

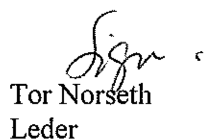
Vedtak
Prosjektet godkjennes under forutsetning av at man i prosjektet skiller tydelig mellom forskning og undervisning slik at frivilligheten ved deltakelse ivaretas. Komiteen videresender skjema for opprettelse av forskningsbiobank, informasjonsskriv samt komiteens vedtak til Helsedirektoratet for endelig behandling av spørsmålet om opprettelse av forskningsbiobank.

Komiteens avgjørelse var enstemmig.

Komiteens vedtak kan påklages (jfr. Forvaltningslovens § 28) til Den nasjonale forskningsetiske komité for medisin og helsefag. Klagen skal sendes til REK Sør-Øst B (jfr. Forvaltingslovens § 32). Klagefristen er tre uker fra den dagen du mottar dette brevet (jfr. Forvaltningslovens § 29). Det bes

presisert hvilke vedtak/vilkår som påklages og den eller de endringer som ønskes. Se informasjon om klageadgang og partsinnsynsrett på http://www.etikkom.no/REK/klage

Med vennlig hilsen



Tor Norseth
Leder

Julianne Krohn-Hansen
Komitésekretær

Kopi: Helsedirektoratet