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Intake of Fatty Fish Alters the Size and the Concentration of Lipid Components of HDL Particles and Camelina Sativa Oil Decreases IDL Particle Concentration in Subjects with Impaired Glucose Metabolism

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Intake of fish alters the size and composition of HDL particles and camelina sativa oil decreases IDL particle concentration in subjects with impaired glucose metabolism

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Keywords: α-linolenic acid, docosahexaenoic acid, eicosapentaenoic acid, lipoprotein subclasses, n-3 fatty acids

Abbreviations: ALA, alpha-linolenic acid; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; NMR, nuclear magnetic resonance.

Clinical Trial Registry: Study is registered in Clinicaltrials.gov (NCT01768429).

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**Scope**: Intake of long-chain n-3 PUFAs affects the lipoprotein subclass profile, whereas the effect of shorter chain n-3 PUFAs remains unclear. We investigated the effect of fish and camelina sativa oil (CSO) intakes on lipoprotein subclasses.

**Methods and results**: Altogether 79 volunteers with impaired glucose metabolism were randomly assigned to CSO, fatty fish (FF), lean fish (LF) or control group for 12 weeks. Nuclear magnetic resonance spectroscopy was used to determine lipoprotein subclasses and their lipid components. The average HDL particle size increased in the FF group (overall $p=0.032$) as compared with the control group. Serum concentrations of cholesterol in HDL and HDL$_2$ (overall $p=0.024$ and $p=0.021$, respectively) and total lipids and phospholipids in large HDL particles (overall $p=0.012$ and $p=0.019$, respectively) increased in the FF group, differing significantly from the LF group. The concentration of intermediate-density lipoprotein (IDL) particles decreased in the CSO group (overall $p=0.033$) as compared with the LF group.

**Conclusion**: Our study suggests that FF intake causes a shift towards larger HDL particles and increases the concentration of lipid components in HDL, which may be associated with the antiatherogenic properties of HDL. Furthermore, CSO intake decreases IDL particle concentration. These changes may favorably affect cardiovascular risk.
1 Introduction

Intake of long-chain n-3 polyunsaturated fatty acids (PUFA) is known to have cardiovascular benefits [1,2]. The mechanisms behind the beneficial effects of n-3 PUFA on the risk factors of cardiovascular disease (CVD) are not fully understood, but their effects on lipoprotein metabolism are potential contributing factors [3,4]. The main long-chain n-3 PUFAs are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are primarily derived from fish [1]. Alpha-linolenic acid (ALA) is a shorter chain n-3 fatty acid from plant sources and a precursor for EPA and DHA synthesis [5]. However, the degree of conversion of ALA to EPA and DHA varies extensively. The effects of ALA intake on CVD risk factors remains unclear, but it may decrease the risk of coronary heart disease (CHD) when long-chain n-3 PUFA intake is low [6].

High serum concentrations of LDL cholesterol and low serum concentrations of HDL cholesterol are known risk factors for CVD [7]. However, HDL and LDL are not homogeneous groups of particles, but have subfractions differing in size and density as well as in lipid and apolipoprotein compositions [8]. These subfractions have different impact on the risk of CVD; large HDL particles may be more atheroprotective than small HDL particles [9-11]. Conversely, small LDL particles may be more atherogenic than large LDL particles [12,13].

There are only a few intervention studies that have examined the effect of fish intake on lipoprotein subclasses, especially in individuals at high risk of developing CVD [14-21]. In these studies, fish intake has been reported to decrease the size and concentrations VLDL particles and increase the size and concentrations of HDL particles and lipid components in the particles as compared with control diets. Furthermore, associations of higher intake of dietary n-3 PUFA with particle concentrations and size of HDL and VLDL have been found in cross-sectional studies [22-24].
Studies suggest that especially fatty fish intake favorably affects the lipoprotein subclass profile [15-17], but beneficial effects have been found also during lean fish intake [18-20]. Earlier intervention studies investigating the effects of ALA intake on lipoprotein characteristics have reported mixed results [25-29], mostly no effects [25-27].

To this end, the aim of this study was to investigate how intakes of fatty and lean fish and camelina sativa oil (CSO), a source of ALA, affect lipoprotein subclasses in subjects with impaired glucose metabolism.

2 Materials and methods

2.1 Subjects

Altogether 96 Caucasian volunteers aged 40 to 75 years were recruited in Kuopio area to the study (Figure 1) via advertisements in newspapers, noticeboards and intranet of the university and from previous clinical trials at our Department. The main inclusion criterion was fasting plasma glucose concentration 5.6 - 6.9 mmol/l. The 2-h glucose concentration in the oral glucose tolerance test (OGTT) had to be < 11.0 mmol/l. Other inclusion criteria were: BMI 25 - 36 kg/m², concentrations of fasting serum total cholesterol < 7 mmol/l, LDL-cholesterol < 5.0 mmol/l and total triglycerides < 4.0 mmol/l. The main exclusion criteria included any chronic disease, a condition hampering the ability to follow the dietary intervention protocol, alcohol abuse (> 40 g/d), and weight loss of > 5 % during the preceding 6 months. Altogether 79 subjects completed the study (Figure 1). There were no differences in age, sex, fasting plasma glucose or serum lipids between the dropouts and the subjects who completed the study. The power calculation was based on differences in DHA in plasma phospholipids, a valid biomarker of dietary intake [30] (n = 18 per group, total n = 72, difference of 1.2 mol %, when alpha < 0.05 and beta > 0.9), because at the time of the onset of this
study there was not enough data on lipoprotein particle size and composition to be used for power calculations.

### 2.2 Study design

Recruitment for AlfaFish study started in autumn 2012 and the intervention was completed in June 2014. In the run-in phase of the intervention, the subjects followed their conventional diet for four weeks and were not allowed to use any oil supplements or products enriched in plant stanols or sterols. After the run-in period, the subjects were randomly assigned into CSO, lean fish (LF), fatty fish (FF) or control group for 12 weeks. Randomization was stratified by sex and use of statins and performed by the study nurse. The subjects visited the study clinic at 0 and 12 weeks and the blood samples were drawn after 10-hour overnight fasting. The serum lipid profile was analyzed by the methodology in use at the UEF and the Eastern Finland Laboratory Center (ISLAB). Details of the methodology of lipid analyses have been described earlier [15]. Physical activity, alcohol intake, smoking, body weight and use of medication known to affect the measures of lipid metabolism were kept constant during the study.

The study diets were isocaloric and current nutrient recommendations [31] were followed except for fish and ALA intakes. Subjects in the fish groups were instructed to consume 4 meals of fish (100–150 g per meal) per week as lunch or dinner: in the FF group e.g. salmon, rainbow trout, Baltic herring, vendace, whitefish and mackerel to provide around 1 g of EPA+DHA per day and in the LF group e.g. tuna, pike, perch, pike-perch, saithe and cod. The tuna fish consumed by the subjects was canned tuna (data not shown) and it did not have the same content of EPA and DHA as fresh tuna (fineli.fi). The CSO group ingested camelina sativa oil (27 g) in order to get 10 g of ALA per day. The control and CSO groups were allowed to eat 1 fish meal per week and were instructed to consume lean meat and chicken. Compliance with the study diets was assessed with the fatty acid
composition of plasma phospholipids determined by gas chromatography as previously described [32], with food records and with daily consumption records: in the fish groups regarding the intake of fish (number of meals and type of fish) and in the CSO group regarding the intake of CSO.

The study was approved by the Ethical committee of the Hospital District of Northern Savo (55/2012). The subjects received both oral and written information and gave their written informed consent.

### 2.3 Lipoprotein subclass and serum lipid analysis by NMR spectroscopy

Lipoprotein particle concentration and size were measured from fasting serum samples using high-throughput proton NMR spectroscopy. The details of the methodology have been described previously [33,34]. The lipoprotein subclasses were defined by particle diameter as follows: chylomicrons and largest VLDL particles (average particle diameter at least 75 nm); five different VLDL subclasses: very large VLDL (average particle diameter of 64.0 nm), large VLDL (53.6 nm), medium VLDL (44.5 nm), small VLDL (36.8 nm), and very small VLDL (31.3 nm); IDL (28.6 nm); three LDL subclasses: large LDL (25.5 nm), medium LDL (23.0 nm), and small LDL (18.7 nm); and four HDL subclasses: very large HDL (14.3 nm), large HDL (12.1 nm), medium HDL (10.9 nm), and small HDL (8.7 nm). The following components of the lipoprotein particles were quantified: phospholipids, triglycerides, cholesterol, free cholesterol, and cholesterol esters. The mean size for VLDL, LDL and HDL particles were calculated by weighting the corresponding subclass diameters with their particle concentrations. Serum lipid extract analyses were performed as described earlier [33,34].

### 2.4 Statistical analyses
Statistical analyses were performed using the IBM SPSS statistical software (v. 21, IBM Corp., Armonk, NY). The normality of the variables was tested with the Kolmogorov-Smirnov test. The variables with abnormal distribution were normalized with logarithmic transformation. A non-parametric test was used if normal distribution was not achieved with transformation. The paired samples t-test or Wilcoxon signed ranks test was used to compare baseline and endpoint values within the groups. Analysis of covariance (ANCOVA), followed by multiple comparisons test with Bonferroni correction was used to test the changes in lipoprotein particles during intervention. Analyses of covariance were performed using fold changes. Fold changes were calculated by dividing the endpoint values of the variable by their baseline values. Sex, age, use of statins and baseline values were included in the ANCOVA models. Spearman rank correlation was used to calculate correlation coefficients for the baseline associations. P-value < 0.05 was considered as statistically significant. R Project for Statistical Computing version 3.2.2 was used to calculate Benjamini-Hochberg false discovery rate (FDR) to adjust results for multiple comparisons. None of the results remained statistically significant after correction (FDR-p < 0.05).

3 Results

Characteristics of the participants and compliance

Mean (± SD) age of the study subjects was 58.9 ± 6.5 years. There were no differences in baseline characteristics among the study groups (Table 1). Reported physical activity of the subjects did not change during the study [35]. The average number of fish meals per week was 4.4 ± 0.4 in the FF, 4.3 ± 0.5 in the LF, 0.9 ± 0.4 in the CSO and 0.9 ± 0.4 in the control group during the study. The camelina oil consumption was 25.7 ± 2.7 g per day in the CSO group. Dietary intakes have been reported in detail elsewhere [35]. The proportion of ALA (18:3n-3) in plasma phospholipids increased in CSO group (p < 0.001) and differed significantly from the other groups. Furthermore, the proportion of EPA (20:5n-3) increased (p < 0.001) in the FF group as compared with the LF and
control groups and the proportion of DHA (22:6n-3) increased in the FF group ($p < 0.001$) as compared with the CSO and control groups.

*The effects of fatty fish intake on lipoprotein particles*

Mean HDL particle diameter (overall difference among the groups $p=0.032$) increased in the FF group as compared with the control group (Figure 2, Supporting Information Table S1). The changes in the mean particle size for VLDL and LDL did not differ among the groups (Table 2).

Serum concentrations of cholesterol in HDL and HDL$_2$ (overall differences among the groups $p=0.024$ and $p=0.021$, respectively) and total lipids and phospholipids in large HDL particles (overall differences among the groups $p=0.012$ and $p=0.019$, respectively) increased in the FF group, differing significantly from the LF group (Figure 2, Supporting Information Table S1). Furthermore, serum concentration of phospholipids in very large HDL particles increased in the FF group as compared with the LF group when adjusted for baseline values (overall difference among the groups $p=0.048$). The significance level also remained after further adjustment for sex, age and use of statins ($p=0.050$).

*The effects of ALA intake on lipoprotein particles and serum lipids*

Serum concentration of IDL particles decreased in the CSO group (overall difference among the groups $p=0.033$), differing significantly from the LF group ($p=0.043$) (Table 2). Furthermore, serum concentration of total lipids in IDL particles and serum concentration of total cholesterol decreased in the CSO group when adjusted for baseline values (overall difference among the groups $p=0.030$, $p=0.042$, respectively) but after further adjustment for sex, age and use of statins there were no significant differences among the groups in the Bonferroni-corrected pairwise comparison. A decrease was also observed in the concentration of esterified cholesterol in the CSO group (overall difference among the groups $p=0.020$).
Correlations of DHA and n-3 PUFA and habitual fish consumption with HDL particles

Serum concentrations of DHA and total n-3 PUFA correlated with serum concentrations of triglycerides in very large ($r=0.293, p=0.009$; $r=0.352, p=0.001$, respectively), large ($r=0.287, p=0.01$; $r=0.228, p=0.043$, respectively), medium ($r=0.226, p=0.045$; $r=0.298, p=0.008$, respectively) and total HDL particles ($r=0.321, p=0.004$; $r=0.353, p=0.001$, respectively) at the baseline (Supporting Information Table S2). Furthermore, DHA and total n-3 PUFA correlated with the concentration and lipid components of medium HDL particles at the baseline. Habitual fish consumption was positively associated with average particle size of HDL, concentrations of very large and large HDL particles and their lipid components at the baseline (Supporting Information Table S2).

4 Discussion

In this study we investigated the effect of fish and ALA intake on the size and composition of lipoprotein particles. We showed that intake of fatty fish 4 times a week significantly increased the average particle size of HDL which is consistent with our earlier studies [15,16]. In addition to our studies there are only three previous trials investigating the effect of fish intake using NMR lipoprotein data. In these studies, high fish intake had no significant effect on HDL particle size [14,20,21]. However, these studies had small samples sizes and in two of these studies the fish consumed was either partly [14] or entirely lean fish [20]. In the present study, an increase in HDL particle size was found only in the FF group whereas in the other groups, particle size decreased, although non-significantly (Figure 2). This may be due to the high DHA content of fatty fish, which has been reported to increase HDL particle size [36].

Recent studies on the effects of n-3 fatty acids on lipoproteins have focused on the use of supplements, mostly fish oil supplements. Earlier trials using fish oil, EPA or DHA supplements
have reported an increase in HDL particle size [37] and an increase in large HDL particles [37,38] compared with control treatments. Studies have also found that n-3 PUFA supplements result in lower concentrations and smaller size of VLDL particles [37-40] and decreased concentrations of IDL and LDL particles [41]. However, these results are not fully comparable with studies investigating dietary intakes of n-3 PUFA since doses of n-3 PUFA in most of the studies using supplements are greater than from dietary sources [42]. Furthermore, there are other nutrients in fish not found in fish oil that may have an atheroprotective effect [43].

In addition to particle size, HDL lipid content has an essential part in the atheroprotective functions of HDL [44]. The HDL lipidome favorably affect cholesterol efflux, inflammation, oxidative damage and potentially also antithrombotic and vasodilatory activities. We found, that the concentration of phospholipids in very large and large HDL particles increased in the FF group. These changes may be explained by the observed increase in HDL particle size. We have previously shown that the concentration of phospholipids in large HDL particles increases within the tertile with the greatest increase in fish intake [15]. Earlier studies have proposed an inverse relationship between phospholipid content of HDL and CHD [45] and vascular stiffness [46]. Moreover, HDL phospholipids affect cholesterol efflux capacity [47,48] and possibly contribute to the anti-inflammatory activities of HDL [49].

The concentration of total cholesterol in HDL increased in the FF group. Similar findings have previously been reported in normolipidemic to mildly hyperlipidemic subjects [50] and in our previous study in patients with CHD [16]. Moreover, we found an increase in the concentration of total cholesterol in HDL$_2$. Similarly, Lindqvist et al. [17] found an increase in HDL$_2$ cholesterol after intake of herring 5 times a week for 6 weeks. However, we did not observe change in the HDL$_2$ cholesterol concentration in the LF group in contrast with earlier studies [18,19].
Our secondary analyses of the baseline associations of habitual fish consumption and HDL particles are in line with the changes found in HDL particles during the intervention. The subjects consumed mainly fatty fish or equally fatty and lean fish before the intervention (data not shown). This further confirms our results that fatty fish intake has favorable effects on the size and composition of HDL particles.

The effects of marine n-3 PUFA on CVD risk factors have been widely investigated, whereas the knowledge of the effects of ALA intake on CVD is limited [51]. In this study, we found a decrease in the serum concentration of IDL particles in the CSO group. There are only a few intervention studies investigating the effects of ALA on lipoprotein subclass profile [25-29], and to our knowledge there is only one previous study using NMR data [29]. In that study margarines containing sunflower oil, olive oil and rapeseed oil were used in order to get 1.1 % of energy from ALA. There was no significant change in the IDL particle concentration in that study. However, increased ALA intake decreased total and small VLDL particle concentrations as compared with the control diet. It has been shown that high concentrations of VLDL and IDL cholesterol are associated with the increased risk of CHD [52]. Consequently, our results suggest that ALA intake may decrease the risk of CVD, but further studies are needed to investigate the potential mechanisms.

In previous studies, impaired glucose metabolism has been observed to have an adverse effect on the lipoprotein particle profile [53,54]. VLDL particles have been larger and concentrations of larger VLDL particles higher. Furthermore, a shift in the concentrations of LDL and HDL particles towards smaller particles and decreased concentrations of large LDL and HDL particles have been reported in these studies. Impaired glucose metabolism may also affect also the lipid components of
lipoproteins [54]. The concentration of lipids has been found to increase in VLDL particles and decrease in HDL particles. The effects of diets high in fatty fish and ALA on lipoprotein subclasses seen in these overweight subjects with impaired glucose metabolism may therefore not apply to lean individuals with normal glucose metabolism.

The strength of the current study is good compliance with the study diets. Furthermore, the sample size of this study is comparable with earlier studies [16,17]. However, further studies are needed to investigate the long-term effects of fish and ALA intake on lipoprotein particles. There are also some limitations to consider in our study. Power calculations were based on differences in DHA in plasma phospholipids, and it is possible that there was not enough power to see all changes in these secondary outcome variables. Moreover, Benjamini-Hochberg false discovery rate was also used to adjust results for multiple comparisons, but after using this conservative adjustment our results were no longer statistically significant. Furthermore, possible confounding due to medication, alcohol consumption and physical activity should be considered since statin [55] and alcohol use [56,57] and exercise [58] have been shown to affect lipoprotein particles. However, the changes in the size and composition of HDL and in the concentration of IDL particles were independent of statin use in our study. Adjustment for alcohol use had no effect on the results either (data not shown).

Furthermore, the subjects were asked to keep their physical activity constant and according to the reports from the subjects, there were no significant changes in physical activity during the study.

In conclusion, fatty fish intake 4 times a week alters the size and composition of HDL towards larger and lipid-rich particles. These changes may be associated with the atheroprotective properties of HDL and therefore partly explain the beneficial effects of fish consumption on the prevention of CVD. Furthermore, ALA intake of 10 g per day decreases IDL particle concentration and may therefore have a favorable effect on the risk of CVD.
Author contributions
U.S.S and A.T.E are the principal investigators in the Alpha-fish study. S.M.M. analyzed the data and wrote the article with the help of U.S.S., A.T.E. and M.A.L. who planned and conducted the study together with V.D.M. D.E.L. had the medical charge of the study.

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Conflict of interest statement
None of the authors have a conflict of interest.
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**Figure legends**

**Figure 1.** Flow chart of the study.

CSO, camelina sativa oil; OGTT, oral glucose tolerance test.

**Figure 2.** Changes in mean values (12 wk – 0 wk) for the average diameter of HDL particles (A), serum concentration of total lipids in large HDL (B), serum concentration of total cholesterol in HDL (C), serum concentration of total cholesterol in HDL2 (D), serum concentration of phospholipids in very large (E) and large HDL (F). Changes among the groups were tested using analysis of covariance adjusted for age, sex, use of statins and baseline values followed by Bonferroni’s post hoc tests. The p-value in the box represents the overall difference among the groups. CSO, camelina sativa oil.
Table 1. Characteristics of the subjects at the baseline of the intervention

<table>
<thead>
<tr>
<th></th>
<th>CSO (n=18)</th>
<th>Fatty fish (n=20)</th>
<th>Lean fish (n=21)</th>
<th>Control (n=20)</th>
<th>p value a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>58.0 ± 5.6</td>
<td>59.0 ± 6.1</td>
<td>58.1 ± 7.8</td>
<td>60.6 ± 6.2</td>
<td>0.590</td>
</tr>
<tr>
<td>Sex, female/male, n/n</td>
<td>10/8</td>
<td>10/10</td>
<td>10/11</td>
<td>9/11</td>
<td>0.928</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.7 ± 2.2</td>
<td>29.3 ± 2.0</td>
<td>29.6 ± 3.0</td>
<td>29.3 ± 2.6</td>
<td>0.736</td>
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<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>5.3 ± 1.0</td>
<td>5.1 ± 1.1</td>
<td>5.4 ± 1.1</td>
<td>5.3 ± 0.9</td>
<td>0.897</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.2 ± 0.9</td>
<td>3.0 ± 0.9</td>
<td>3.3 ± 0.8</td>
<td>3.2 ± 0.9</td>
<td>0.746</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>1.4 ± 0.5</td>
<td>1.3 ± 0.3</td>
<td>0.463</td>
</tr>
<tr>
<td>Triglycerides (mmol/l) b)</td>
<td>1.6 ± 0.6</td>
<td>1.6 ± 0.8</td>
<td>1.3 ± 0.5</td>
<td>1.6 ± 0.7</td>
<td>0.426</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126 ± 12</td>
<td>131 ± 13</td>
<td>129 ± 10</td>
<td>133 ± 11</td>
<td>0.411</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85 ± 7</td>
<td>85 ± 7</td>
<td>83 ± 8</td>
<td>86 ± 5</td>
<td>0.663</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l) b)</td>
<td>6.1 ± 0.4</td>
<td>5.9 ± 0.4</td>
<td>6.1 ± 0.4</td>
<td>6.1 ± 0.6</td>
<td>0.589</td>
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<tr>
<td>Use of statins, n</td>
<td>4</td>
<td>5</td>
<td>4</td>
<td>5</td>
<td>0.966</td>
</tr>
<tr>
<td>Habitual fish consumption (meals/week) b)</td>
<td>1.9 ± 0.9</td>
<td>1.9 ± 0.7</td>
<td>2.0 ± 1.0</td>
<td>1.6 ± 0.9 c)</td>
<td>0.580</td>
</tr>
</tbody>
</table>

Values are means ± SD unless otherwise indicated. CSO, camelina sativa oil.

a) p-values were determined by analysis of variance or χ² test.
b) Variables were log-transformed.
c) n=18
<table>
<thead>
<tr>
<th></th>
<th>CSO (n=18)</th>
<th>Fatty fish (n=20)</th>
<th>Lean fish (n=21)</th>
<th>Control (n=20)</th>
<th>p values ANCOVA</th>
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<tr>
<td></td>
<td>0 wk</td>
<td>12 wk</td>
<td>0 wk</td>
<td>12 wk</td>
<td>Model 1</td>
</tr>
<tr>
<td>Chylomicrons and largest VLDL (nmol/l)</td>
<td>0.13 ± 0.10</td>
<td>0.09 ± 0.08</td>
<td>0.16 ± 0.14</td>
<td>0.11 ± 0.07</td>
<td>0.11 ± 0.07</td>
</tr>
<tr>
<td>Very large VLDL particles (nmol/l)</td>
<td>0.77 ± 0.59</td>
<td>0.64 ± 0.49</td>
<td>0.87 ± 0.82</td>
<td>0.58 ± 0.39</td>
<td>0.56 ± 0.45</td>
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<tr>
<td>Large VLDL particles (nmol/l)</td>
<td>5.63 ± 3.34</td>
<td>5.14 ± 3.15</td>
<td>5.61 ± 4.36</td>
<td>4.28 ± 2.16</td>
<td>4.21 ± 2.74</td>
</tr>
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<td>Medium VLDL particles (nmol/l)</td>
<td>19.7 ± 9.03</td>
<td>19.0 ± 8.49</td>
<td>18.6 ± 10.6</td>
<td>15.4 ± 5.10</td>
<td>15.6 ± 7.16</td>
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<tr>
<td>Small VLDL particles (nmol/l)</td>
<td>31.9 ± 10.1</td>
<td>30.5 ± 9.02</td>
<td>30.0 ± 11.8</td>
<td>26.7 ± 5.87</td>
<td>27.9 ± 8.32</td>
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<tr>
<td>Very small VLDL particles (nmol/l)</td>
<td>38.3 ± 9.63</td>
<td>34.0 ± 7.26</td>
<td>38.1 ± 11.3</td>
<td>35.8 ± 8.46</td>
<td>38.3 ± 7.86</td>
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<tr>
<td>Mean diameter for VLDL particles (nm)</td>
<td>37.2 ± 1.03</td>
<td>37.3 ± 1.20</td>
<td>37.1 ± 1.58</td>
<td>36.9 ± 0.98</td>
<td>36.6 ± 1.30</td>
</tr>
<tr>
<td>Total cholesterol in VLDL (mmol/l)</td>
<td>0.70 ± 0.27</td>
<td>0.61 ± 0.23</td>
<td>0.68 ± 0.30</td>
<td>0.59 ± 0.18</td>
<td>0.64 ± 0.17</td>
</tr>
<tr>
<td>Triglycerides in VLDL (mmol/l)</td>
<td>1.00 ± 0.43</td>
<td>0.96 ± 0.39</td>
<td>0.96 ± 0.54</td>
<td>0.80 ± 0.26</td>
<td>0.80 ± 0.37</td>
</tr>
<tr>
<td>IDL particles (nmol/l)</td>
<td>100 ± 26.1</td>
<td>84.3 ± 18.7</td>
<td>99.1 ± 27.5</td>
<td>95.9 ± 28.2</td>
<td>104 ± 22.0</td>
</tr>
<tr>
<td>Total lipids in IDL (mmol/l)</td>
<td>1.01 ± 0.27</td>
<td>0.85 ± 0.19</td>
<td>1.00 ± 0.28</td>
<td>0.97 ± 0.29</td>
<td>1.06 ± 0.23</td>
</tr>
<tr>
<td>Large LDL particles (nmol/l)</td>
<td>167 ± 45.3</td>
<td>141 ± 31.3</td>
<td>159 ± 45.8</td>
<td>157 ± 48.7</td>
<td>172 ± 38.8</td>
</tr>
<tr>
<td>Medium LDL particles (nmol/l)</td>
<td>138 ± 38.1</td>
<td>118 ± 25.3</td>
<td>126 ± 37.0</td>
<td>127 ± 39.8</td>
<td>140 ± 32.5</td>
</tr>
<tr>
<td>Phospholipids in medium LDL (mmol/l)</td>
<td>0.19 ± 0.04</td>
<td>0.17 ± 0.03</td>
<td>0.18 ± 0.04</td>
<td>0.18 ± 0.04</td>
<td>0.19 ± 0.03</td>
</tr>
<tr>
<td>Small LDL particles (nmol/l)</td>
<td>163 ± 41.3</td>
<td>141 ± 27.2</td>
<td>149 ± 40.3</td>
<td>151 ± 41.8</td>
<td>164 ± 35.6</td>
</tr>
<tr>
<td>Mean diameter for LDL particles (nm)</td>
<td>23.5 ± 0.4</td>
<td>23.4 ± 0.10</td>
<td>23.6 ± 0.10</td>
<td>23.5 ± 0.09</td>
<td>23.5 ± 0.07</td>
</tr>
<tr>
<td>Total cholesterol in LDL (mmol/l)</td>
<td>1.56 ± 0.46</td>
<td>1.30 ± 0.32</td>
<td>1.43 ± 0.45</td>
<td>1.44 ± 0.50</td>
<td>1.60 ± 0.42</td>
</tr>
<tr>
<td>Triglycerides in LDL (mmol/l)</td>
<td>0.16 ± 0.04</td>
<td>0.15 ± 0.03</td>
<td>0.16 ± 0.06</td>
<td>0.16 ± 0.04</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>4.11 ± 0.84</td>
<td>3.65 ± 0.65</td>
<td>4.04 ± 0.92</td>
<td>4.03 ± 0.92</td>
<td>4.23 ± 0.78</td>
</tr>
<tr>
<td>Esterified cholesterol (mmol/l)</td>
<td>2.90 ± 0.59</td>
<td>2.55 ± 0.45</td>
<td>2.84 ± 0.64</td>
<td>2.85 ± 0.64</td>
<td>2.98 ± 0.57</td>
</tr>
<tr>
<td>Free cholesterol (mmol/l)</td>
<td>1.21 ± 0.26</td>
<td>1.10 ± 0.20</td>
<td>1.21 ± 0.29</td>
<td>1.17 ± 0.28</td>
<td>1.24 ± 0.23</td>
</tr>
<tr>
<td>Serum total triglycerides (mmol/l)</td>
<td>1.39 ± 0.49</td>
<td>1.33 ± 0.43</td>
<td>1.37 ± 0.64</td>
<td>1.18 ± 0.30</td>
<td>1.20 ± 0.40</td>
</tr>
</tbody>
</table>

Values are means ± SD. Differences in fold changes among the groups were tested using ANCOVA and Bonferroni’s post hoc tests. Variables in ANCOVA were log-transformed. ANCOVA: Model 1 no adjustments, Model 2 adjusted for baseline value, Model 3 adjusted for baseline value, age, sex and use of statins. a) Change within the group, p < 0.05; b) CSO group vs. lean fish group, p < 0.05; c) CSO group vs. fatty fish group, p < 0.05. Benjamini-Hochberg false discovery rate (FDR) was used to adjust results for multiple comparisons. After FDR-adjustment p-values were no longer statistically significant. CSO, camelina sativa oil; ANCOVA, analysis of covariance.