British Journal of Nutrition (2017), **117**, 1291–1298
doi:10.1017/S0007114517001167

High-quality fish oil has a more favourable effect than oxidised fish oil on intermediate-density lipoprotein and LDL subclasses: a randomised controlled trial

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(Submitted 10 February 2017 – Final revision received 28 March 2017 – Accepted 21 April 2017 – First published online 31 May 2017)

Abstract

Fish oil (FO) supplementation reduces the risk of CVD. However, it is not known if FO of different qualities have different effects on lipoprotein subclasses in humans. We aimed at investigating the effects of oxidised FO and high-quality FO supplementation on lipoprotein subclasses and their lipid concentrations in healthy humans. In all, fifty-four subjects completed a double-blind randomised controlled intervention study. The subjects were randomly assigned to receive high-quality FO (*n* 17), oxidised FO (*n* 18) or high-oleic sunflower oil capsules (HOSO, *n* 19) for 7 weeks. The concentration of marine *n*-3 fatty acids was equal in high-quality FO and oxidised FO (1·6 g EPA + DHA/d). The peroxide value (PV) and anisidine value (AV) were 4 mEq/kg and 3 in high-quality FO and HOSO, whereas the PV and AV in the oxidised FO were 18 mEq/kg and 9. Blood samples were collected at baseline and end of study. NMR spectroscopy was applied for the analysis of lipoprotein subclasses and their lipid concentrations. High-quality FO reduced the concentration of intermediate-density lipoprotein (IDL) particles and large, medium and small LDL particles, as well as the concentrations of total lipids, phospholipids, total cholesterol, cholesteryl esters and free cholesterol in IDL and LDL subclasses compared with oxidised FO and HOSO. Hence, high-quality FO and oxidised FO differently affect lipid composition in lipoprotein subclasses, with a more favourable effect mediated by high-quality FO. In future trials, reporting the oxidation levels of FO would be useful.

Key words: Fish oil: Oxidised fish oil: Lipoprotein subclasses: LDL

CVD is the main cause of death and disability worldwide[1]. Intake of fish oil (FO) reduces all-cause mortality in patients with chronic heart failure[2], death and CVD death in secondary prevention[3] as well as major coronary events in primary prevention[4]. The CVD-reducing effect of FO is mainly attributed to the marine *n*-3 fatty acids EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3). These fatty acids are suggested to reduce CVD risk by lowering TAG levels, reduction of blood pressure and platelet aggregation and prevention of arrhythmia[5–8]. The effect of FO on cholesterol levels, however, is less clear. A review of the early studies on FO consumption concluded that *n*-3 fatty acids from FO reduce serum cholesterol as effectively as *n*-6 fatty acids from plant oils[9]. In contrast, two recent reviews conclude that FO consumption does not have an effect on total cholesterol levels, but results in a modest increase in LDL-cholesterol and HDL-cholesterol[5,10]. The inconsistency in the effect of FO on LDL-cholesterol may be due to genetic variants in the study populations. It has previously been reported that the apoE4 genotype is associated with a more pronounced increase in LDL-cholesterol, especially in response to supplementation with DHA[11]. In contrast, Minihane *et al.*[12] reported that supplementation with marine *n*-3 fatty acids decreased the level of LDL-cholesterol to a greater extent in apoE4 individuals, suggesting that other factors might mediate the inconsistency of FO effects on LDL-cholesterol.

EPA and DHA are prone to oxidation because they contain a high number of bisallylic carbon atoms. Lipid peroxides of these fatty acids are formed in the presence of initiators[13]. The degree of oxidation in commercially available FO supplements has raised concern because of possible adverse effects on human health[14–17]. The peroxide value (PV) and the anisidine value (AV) are used as a measure of primary and secondary oxidation products in oils[18]. Different maximum lipid peroxide levels have been suggested to ensure high-quality oil, but these

Abbreviations: AV, anisidine value; CETP, cholesteryl ester transfer protein; E%, percentage of energy; FO, fish oil; HOSO, high-oleic sunflower oil capsules; IDL, intermediate-density lipoprotein; PBMC, peripheral blood mononuclear cells; PV, peroxide value.

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limits are based on palatability and not on adverse health effects in humans[18,19]. The Global Organization for EPA and DHA (GOED) Voluntary Monograph has set maximum limits for oxidation of FO to PV = 5 mEq/kg and AV = 20. Other international and regional limits vary between 5 and 10 mEq/kg for PV and between 10 and 30 for AV[20]. Evaluation of the oxidation levels of over-the-counter *n*-3 supplements yields highly variable results, with between 11 and 100 % of products tested exceeding one or more of the oxidation limits set by GOED[14–17,21,22]. We have previously shown that intake of oxidised FO (PV = 18, AV = 9) had no effect on different *in vivo* markers of oxidative stress, inflammation, lipid peroxidation or on the level of oxidised LDL after 7 weeks, compared with a control oil and a high-quality FO (PV = 4, AV = 3) in healthy adults[23,24]. In contrast, Garcia-Hernandez *et al.*[25] found that intake of a less oxidised FO (PV = 15), but not a highly oxidised FO (PV = 47) or a control, lowered fasting cholesterol after a 4 week intervention in dyslipidaemic subjects not taking lipid-lowering drugs.

Lipoproteins of intestinal and hepatic origin are divided into five classes; TAG-rich chylomicrons and VLDL and cholesterol-rich intermediate-density lipoprotein (IDL), LDL and HDL. These lipoprotein classes can be further divided into subclasses, based on their size and density. However, these classifications are determined by the method used to measure lipoproteins, as they exist in a continuum of sizes and densities and not as distinct classes[26]. The use of NMR spectroscopy enables quantification of lipoprotein subclasses and their protein and lipid constituents[27]. Knowledge about the lipoprotein subclass distribution might give a more precise risk assessment for CVD than only measuring total cholesterol, LDL-cholesterol and HDL-cholesterol[28].

The effect of FO supplementation on lipoprotein subclasses have previously been studied in type II diabetic patients. FO reduced VLDL size and particle concentrations of large VLDL and small HDL compared with maize oil[29]. Another trial investigated the effects of lean and fatty fish intake on lipoprotein subclasses in CHD patients. They found an increase in cholesterol, cholesteryl esters and total lipids in the largest HDL subclass as well as an increase in mean size of HDL particles after consumption of fatty fish[30]. Similar results were found in subjects with the metabolic syndrome that had a high increase in fish consumption[31]. Small dense LDL particles have been suggested to be the most atherogenic of the LDL particles as they enter the arterial wall more easily than larger LDL particles, they are more easily oxidised and are less efficiently cleared from blood because of their reduced affinity for the LDL receptor[32]. FO intake has been shown to cause a shift from small dense to large buoyant LDL particles, which is associated with a reduced risk of CVD[33].

Even though we did not observe any changes in serum lipids in our previous study[23], we wanted to do an in-depth analysis of lipoprotein subclasses and their constituents to study the effects of FO with different qualities on lipoproteins in more detail. The aim of the present exploratory study was to investigate the effect on lipoprotein subclasses and their lipid concentrations, plasma lipids and apolipoproteins after supplementation with high-quality FO and oxidised FO in healthy humans. The hypothesis was that high-quality FO and oxidised FO differently affects lipoprotein subclasses. We have previously investigated the effect of the different oils on the gene expression profiles in peripheral blood mononuclear cells (PBMC)[34]. We therefore wanted to correlate the gene expression of specific genes with any observed changes in plasma lipoprotein subclasses in the current study.

Methods

Subjects

Healthy, non-smoking men and women aged 18–50 years who met the eligibility criteria were included in this study. In brief, the exclusion criteria were chronic illness, pregnancy, lactation, BMI ≥ 30 kg/m^2, hypertension (≥160/100 mmHg) and fasting serum levels of total cholesterol > 7·5 mM, TAG > 4·0 mmol/l, glucose > 6·0 mmol/l and C-reactive protein > 10 mg/l, as well as serum levels of thyroxine stimulating hormone, free T3 and free T4 outside the reference ranges. A more detailed description of participant recruitment and enrolment, inclusion and exclusion criteria, protocol, study products and compliance is given elsewhere[23].

Ethics statement

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Committee of Medical Ethics (approval no. 6.2008.2215) and the Norwegian Social Science Data Services (approval no. 21924). Written informed consent was obtained from all participants. The study was registered at www.clinicaltrials.gov (ID no. NCT01034423).

Study design

This study was a part of a 7-week double-blind, randomised, controlled parallel-group study with three intervention arms, conducted Akershus University College in 2009. The primary aim of this study was to investigate the health effects of intake of high-quality FO and oxidised FO[23]. Participants were randomised in a 1:1:1 ratio, and stratified by sex, using Microsoft Excel's random generator. Randomisation was performed by LINK Medical Research AS. The three intervention groups received sixteen capsules per day containing in total 8 g/d of high-quality FO, or 8 g/d of oxidised FO or 8 g/d of high-oleic sunflower oil (HOSO). The EPA + DHA content was equal in the high-quality FO and oxidised FO. The daily dose of EPA + DHA was 1·6 g, with EPA and DHA intake corresponding to 0·7 and 0·9 g, respectively[23]. The total oxidation values (2PV + AV) of the HOSO, oxidised FO and high-quality FO oils were 11, 45 and 11, respectively. The fatty acids in the oils were analysed on a GC (Agilent GC-6890N; Agilent Technologies) equipped with an autosampler (Agilent G-2614A; Agilent Technologies) and flame ionisation detector. The fatty acid content and the level of oxidation of the oils have previously been described[23]. The capsules and containers had identical appearance to ensure blinding for the subjects and investigators. Compliance was assessed by counting the remaining capsules after the intervention period and the mean compliance was calculated to be

97 % for HOSO, 100 % for oxidised FO and 96 % for high-quality FO. The high compliance estimated by capsule count was supported by a similar increase in plasma EPA and DHA in both FO groups, and no increase in the HOSO group[23]. Adverse effects were reported by two participants in each of the FO groups (belching with fishy taste) and by three participants in the HOSO group (burping, constipation and urgent to stool). No reports of off-taste of the oxidised FO were given. In all, fifty-eight participants received allocated intervention, three subjects discontinued intervention and one was excluded because of low compliance. Flow chart of the study is given elsewhere[23]. Participants were instructed to avoid consumption of fish, fish products and other products enriched with marine *n*-3 fatty acids 4 weeks before baseline and during the intervention. The first 3 weeks of the intervention was a fully controlled isoenergetic diet period that provided 24 % of energy (E%) from fat (8 E% SFA, 5 E% MUFA and 6 E% PUFA) 20 E% protein and 57 E% carbohydrates. A more detailed description of the fully controlled diet period is given elsewhere[23]. During the remaining 4 weeks of the intervention, the participants returned to their habitual diet, still omitting food items containing marine *n*-3 fatty acids. Blood samples were collected at 0, 3 and 7 weeks. The relatively short length of the intervention period was set because of unknown adverse effect of oxidised FO on human health. Samples from 0 and 7 weeks were used for lipoprotein subclass analyses.

Blood sampling

Subjects were instructed to avoid consumption of alcohol and doing vigorous physical activity the day before blood sampling. Blood samples were collected after an overnight fast (≥12 h). Serum was kept at room temperature for 30 min before centrifugation (1500 ***g***, 12 min) and plasma was obtained from EDTA-tubes immediately placed on ice and centrifuged within 10 min (1500 ***g***, 4 °C, 10 min). Plasma samples were stored at −80°C, thawed before aliquoting for NMR analyses and stored again at −80°C before shipping on dry ice.

Lipoprotein subclass and metabolite analyses

Metabolic biomarkers were quantified from EDTA plasma using a commercial high-throughput proton NMR metabolomics platform (Brainshake Ltd; www.brainshake.fi). This method quantifies routine lipids, lipoprotein subclass profiling with lipid concentrations within fourteen subclasses, fatty acid composition, abundant proteins and various low-molecular-weight metabolites. Details of the experimentation and applications of the NMR metabolomics platform have been described previously[35]. The fourteen lipoprotein subclass sizes were defined as follows: extremely large VLDL with particle diameters from 75 nm upwards and a possible contribution of chylomicrons, five VLDL subclasses (average particle diameters of 64·0, 53·6, 44·5, 36·8 and 31·3 nm), IDL (28·6 nm), three LDL subclasses (25·5, 23·0 and 18·7 nm), and four HDL subclasses (14·3, 12·1, 10·9 and 8·7 nm). The following components of the lipoprotein subclasses were quantified: phospholipids, cholesterol, cholesteryl esters, free cholesterol and TAG. The mean size for VLDL, LDL and HDL particles was calculated by weighting the corresponding subclass diameters with their particle concentrations.

Microarray analysis of RNA

In a previous study, PBMC were isolated using BD Vacutainer Cell Preparation tubes (Becton, Dickinson and Co.) according to the manufacturer's instructions. Total RNA was isolated from the PBMC using RNeasy Mini Kit (Qiagen) and gene expression was analysed by hybridisation to an Illumina HumanHT-12 v4 Expression BeadChip (Illumina Inc.). A detailed protocol is given elsewhere[36]. We chose to study gene expression in PBMC because they are readily available and can serve as a model system for less accessible tissue, such as liver and adipose tissue. Altered expression of genes related to cholesterol and fatty acid metabolism, as well as inflammation, has been observed in PBMC in response to dietary interventions[37].

Statistical analyses

Sample size calculation was based on expected changes in *n*-3 fatty acids in plasma, as previously described[23]. The lipoprotein subclass measurements were *post hoc* analyses not specified *a priori*, and an explorative part of the study. Thus, the study was not powered for these outcomes. Statistical analyses were performed with SPSS 22 (IBM SPSS Statistics; IBM Corp.). Normality of variables was checked, and all lipoprotein subclass variables were log2-transformed to account for non-normal distributions. One-way ANOVA was used to analyse if the change from baseline to end of study differed between the three intervention groups. $P \leq 0{\cdot}05$ was considered statistically significant. If there was an overall significant result, pairwise comparisons were further investigated with Tukey's *post hoc* analysis to examine where the difference between groups occurred. Correlation of log2-transformed data was tested with Pearson's correlation after removal of one outlier in the HOSO group.

Results

In total fifty-four subjects completed the intervention, nineteen in the HOSO group, eighteen in the oxidised FO group and seventeen in the high-quality FO group. Data from all fifty-four participants were used for lipoprotein subclass analyses. The subjects had a mean age of 27 (SD 7) years and a BMI of 22·6 (SD 2·6) kg/m^2. There were no significant differences between the groups at baseline on age, BMI or serum glucose or serum lipids, as previously reported[23].

The particle concentrations and the concentrations of lipid constituents in fourteen lipoprotein subclasses were analysed to investigate the effect of FO of different quality. Intake of high-quality FO caused a significant reduction in particle concentrations of IDL and large, medium and small LDL compared with oxidised FO and HOSO ($P = 0{\cdot}023$, 0·018, 0·020 and 0·018, respectively) (Fig. 1). Pairwise comparisons revealed that the reduction in IDL and large LDL particle concentrations in the high-quality FO group were significantly different compared with the increase in the oxidised FO group and HOSO group. The reduction in medium and small LDL particle concentrations

in the high-quality FO group was significantly different from the increase in the oxidised FO group (online Supplementary Table S1). The median changes in particle concentrations of IDL and LDL subclasses were on average +5, +11 and −3 %, for HOSO, oxidised FO and high-quality FO, respectively (not shown).

Furthermore, intake of high-quality FO significantly reduced the concentrations of total lipids, phospholipids, total cholesterol, cholesteryl esters and free cholesterol in IDL and large, medium and small LDL compared with oxidised FO and HOSO (Fig. 2 and online Supplementary Table S1). Pairwise comparisons showed that the reduction in these lipid constituents in IDL and LDL subclasses in high-quality FO were significantly different to the increase in oxidised FO and HOSO in most cases. Otherwise, the reduction in the high-quality FO group was significantly different to the increase in the oxidised FO group (online Supplementary Table S1). The changes in cholesteryl esters within IDL and large, medium and small LDL differed most between the three intervention groups. The median changes in cholesteryl esters in IDL and LDL subclasses were on average +4, +13 and −5 % for HOSO, oxidised FO and high-quality FO, respectively (not shown).

There was no difference between the intervention groups in TAG in IDL and LDL subclasses, or in any lipid constituent in VLDL and HDL subclasses, except for total cholesterol and cholesteryl esters in the smallest VLDL subclass and cholesteryl esters in the largest HDL subclass (results not shown).

Intake of high-quality FO resulted in a significant reduction in LDL-cholesterol, remnant-cholesterol and non-HDL-cholesterol measured with NMR spectroscopy, compared with oxidised FO and HOSO ($P=0{\cdot}019$, 0·050 and 0·018, respectively) (Fig. 3). Pairwise comparisons showed that the reduction in non-HDL-cholesterol in the high-quality FO group was significantly different from the increase in the oxidised FO group and HOSO group, whereas the reduction in LDL-cholesterol in the high-quality FO group was significantly different from the increase in the oxidised FO group. However, the change in remnant-cholesterol was not significantly different between the groups after pairwise comparisons. There were no differences between the groups in VLDL-cholesterol and HDL-cholesterol (Fig. 3 and online Supplementary Table S2). The changes in LDL-cholesterol were +15, +19 and −6 % after intake of HOSO, oxidised FO and high-quality FO, respectively (not shown).

The concentration of apoB was reduced in the high-quality FO group and increased in the HOSO and oxidised FO groups after 7 weeks of intervention. The overall difference was significant, but pairwise comparisons showed that high-quality FO was significantly different from HOSO only. There was no difference in apoA1 concentration between the groups (Fig. 4 and online Supplementary Table S2).

We have previously analysed the expression of genes in PBMC, and there was no difference in the gene expression of cholesteryl ester transfer protein (*CETP*) between the groups

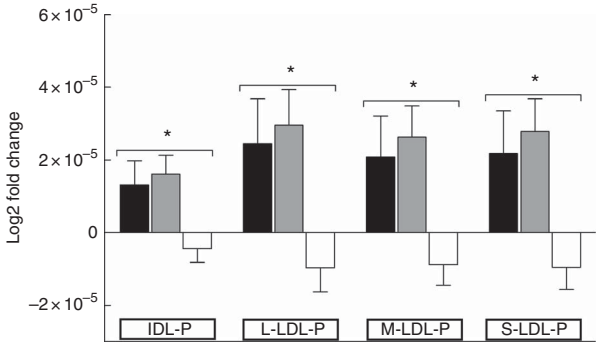

Fig. 1. Log2-transformed change in particle (P) concentration (mmol/l) from baseline to end of study for intermediate-density lipoprotein (IDL), large (L-LDL), medium (M-LDL) and small LDL (S-LDL) in healthy subjects receiving capsules with high-oleic sunflower oil (■), oxidised fish oil (FO, ▒) and high-quality FO (□). Overall difference between groups was tested with one-way ANOVA. Values are means, with standard errors represented by vertical bars. * $P \leq 0{\cdot}05$.

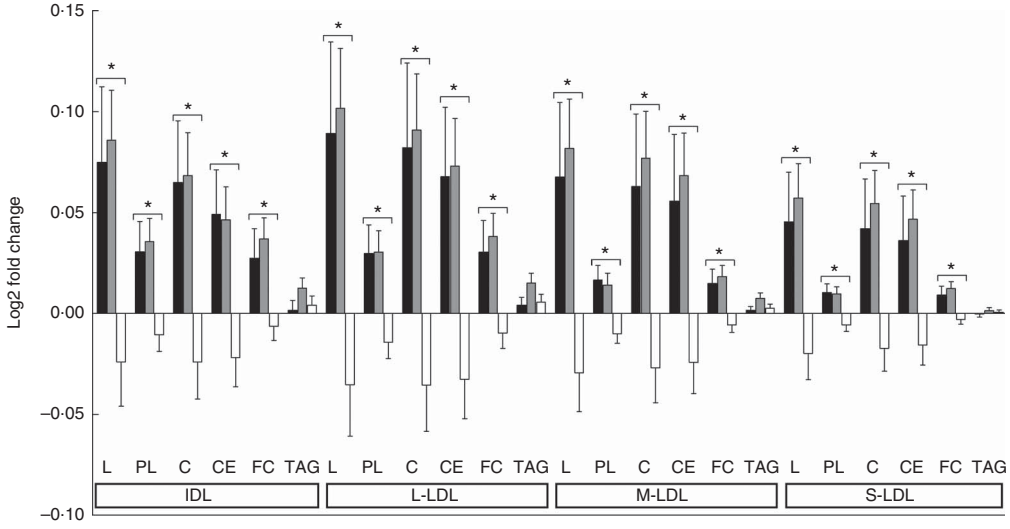

Fig. 2. Log2-transformed change in lipid concentration (mmol/l) from baseline to end of study for total lipids (L), phospholipids (PL), total cholesterol (C), cholesteryl esters (CE), free cholesterol (FC) and TAG within intermediate-density lipoprotein (IDL) and large (L-LDL), medium (M-LDL) and small LDL (S-LDL) in healthy subjects receiving capsules with high-oleic sunflower oil (■), oxidised fish oil (FO, ▒) and high-quality FO (□). Overall difference between groups was tested with one-way ANOVA. Values are means, with standard errors represented by vertical bars. * $P \leq 0{\cdot}05$.

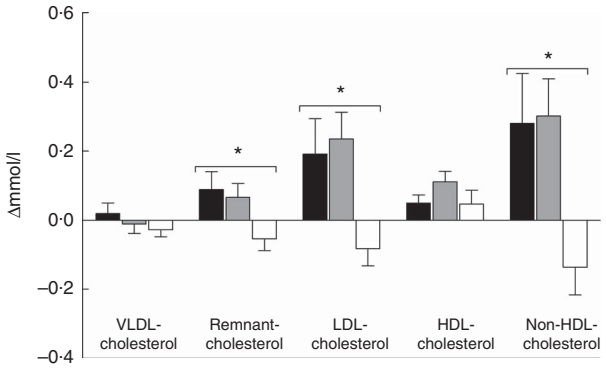

Fig. 3. Change in lipid concentrations (mmol/l), from baseline to end of study, measured with NMR in healthy subjects receiving capsules with high-oleic sunflower oil (■), oxidised fish oil (FO, ▩) and high-quality FO (□). Overall difference between groups was tested with one-way ANOVA. Values are means, with standard errors represented by vertical bars. * $P \leq 0{\cdot}05$.

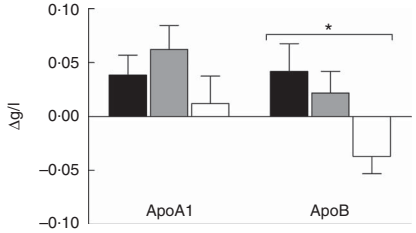

Fig. 4. Change in apoA1 and apoB concentrations (g/l) from baseline to end of study in healthy subjects receiving capsules with high-oleic sunflower oil (■), oxidised fish oil (FO, ▩) and high-quality FO (□). Overall difference between groups was tested with one-way ANOVA. Values are means, with standard errors represented by vertical bars. * $P \leq 0{\cdot}05$.

after the intervention[36]. However, there was a significant ($P < 0{\cdot}016$) positive correlation between the change in *CETP* gene expression and the change in cholesteryl esters in IDL (r 0·41), large LDL (r 0·36), medium LDL (r 0·33) and small LDL (r 0·33) (Fig. 5).

Discussion

In this study, detailed lipoprotein subclass analysis revealed differences in particle concentrations of IDL and large, medium and small LDL as well as total lipids, phospholipids, cholesterol, cholesteryl esters and free cholesterol in IDL and the three LDL subclasses after intake of high-quality FO compared with oxidised FO and HOSO. To our knowledge, this is the first randomised controlled trial investigating the effects of supplementation with FO of different quality in healthy, normolipidaemic subjects on distribution and composition of lipoprotein subclasses measured with NMR spectroscopy.

The results from this study may indicate that FO with different qualities elicit different effects on blood lipids, as previously suggested[38,39]. This can potentially explain why some studies find reduced total cholesterol and LDL-cholesterol after intake of FO[9], whereas other studies show no change in total cholesterol and an increase in LDL-cholesterol after intake of FO[5,10]. In line with the results in this study, one other study that examined the effects of FO with different qualities on blood lipids found that only the less oxidised oil reduced cholesterol levels, and not the oxidised oil or the control[25]. In addition, FO consumption reduced all lipid components in LDL particles, except TAG, in a study with non-human primates[40].

We showed a 0·1 mmol/l reduction and a 0·2 mmol/l increase in LDL-cholesterol following intake of high-quality FO and oxidised FO, respectively. As the subjects had low baseline levels of LDL-cholesterol, this change corresponds to −6 and +19 % from baseline. LDL-cholesterol is the most established risk factor for CVD[41], and reducing cholesterol levels at an early age provides additional benefit to CVD risk reduction[42]. A 10 % reduction in LDL-cholesterol in 40-year-old men has been associated with a 54 % reduced risk of ischaemic heart disease[43]. Thus, the results on LDL-cholesterol might have a clinical relevance.

We have previously reported that intake of high-quality FO, oxidised FO and HOSO did not result in a change in serum lipids[23]. When lipids are measured with NMR spectroscopy, however, the difference between the groups was significant. With clinical routine measurement of LDL-cholesterol, some cholesterol from the smallest VLDL subclasses as well as IDL-cholesterol and Lp(a)-cholesterol is included in this measure[44]. LDL-cholesterol measured with NMR spectroscopy is based on cholesterol in lipoprotein particles with average size between 18·7 and 25·5 nm, and is thus more specific[35]. As a result, the within group changes we observed in LDL-cholesterol measured with NMR were smaller and the overall difference between the groups was statistically significant. It is important to note that the changes in routine clinical measurement we previously reported were of the same magnitude and direction as observed in this study.

The concentration of TAG did not change from baseline to end of study in the young healthy participants in this study. The baseline level of TAG was 1·05 mmol/l in the oxidised FO group, and 0·86 mmol/l in the high-quality FO group. It has been reported that the TAG lowering effect of EPA and DHA is dose dependent and is influenced by baseline TAG levels[10]. Thus, a dose of 1·6 g EPA + DHA/d might not be enough to further lower TAG in these participants.

The concentration of apoB was reduced after intake of high-quality FO compared with oxidised FO and HOSO. This reduction argues that the lowering of lipid constituents in LDL subclasses did not lead to a reduction of LDL size. Indeed, the average size of LDL particles tended to increase more for high-quality FO compared with oxidised FO and HOSO (results not shown). This finding is in line with studies showing that intake of marine *n*-3 fatty acids reduce the level of small dense LDL[33,45]. Hence, the combined results on lipoprotein subclasses and apoB after intake of high-quality FO could be considered antiatherogenic.

CETP transfers cholesteryl esters into VLDL and LDL particles from HDL in the exchange for TAG. The changes in cholesteryl esters in IDL and LDL subclasses correlated with the change in *CETP* expression in all groups combined. Hence, changes in *CETP* expression might be one of the underlying mechanisms behind the changes in lipoprotein subclasses. As we did not observe an increase in HDL-cholesterol after intake of

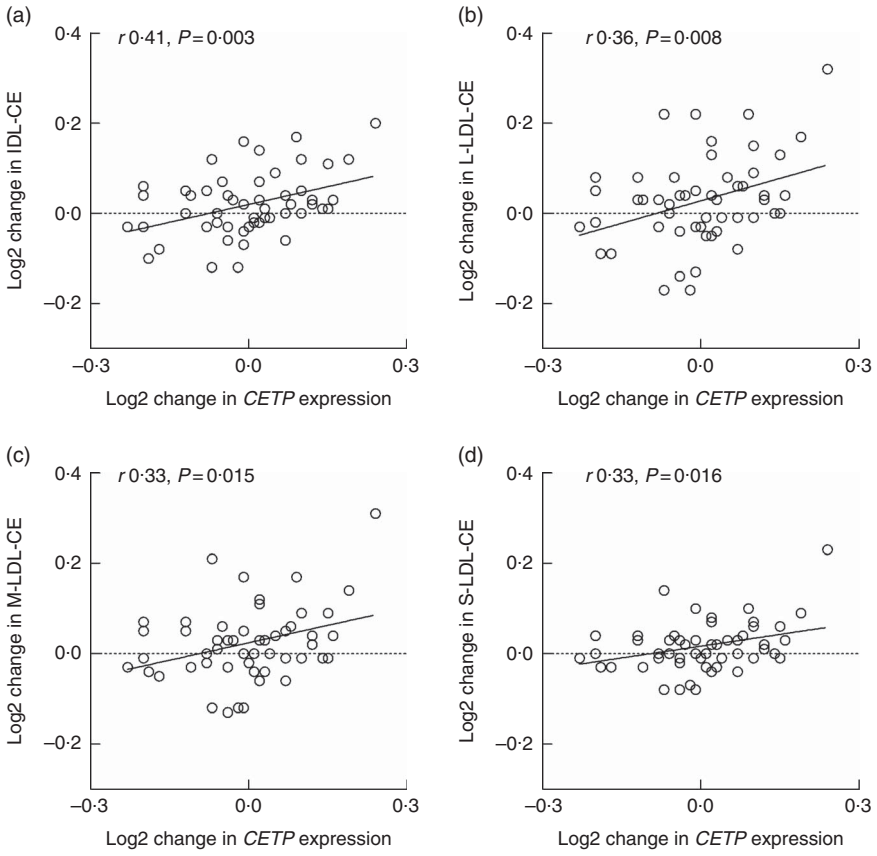

Fig. 5. Scatter plot of log2-transformed change from baseline to end of study of mRNA level of cholesteryl ester (CE) transfer protein (*CETP*) analysed with microarray and CE in (a) intermediate-density lipoprotein (IDL), (b) large (L-LDL), (c) medium (M-LDL) and (d) small LDL (S-LDL).

high-quality FO, this might suggest improved reverse cholesterol transport. A study with hamsters fed a high-fat diet and injected with radiolabelled cholesterol demonstrated that EPA and DHA improve reverse cholesterol transport from macrophages to faeces. This improved reverse cholesterol transport was related to increased expression of scavenger receptor-B1 in liver[46]. Furthermore, in a study by Bagdade *et al.*[47] an 8 week intervention with *n*-3 fatty acids in type 1 diabetes patients reduced cholesteryl ester transfer in serum *in vitro*, even though the amount of CETP was increased. This suggests that marine *n*-3 fatty acids may inhibit CETP protein activity.

Although the high-quality FO group had a decrease in cholesteryl esters in IDL and LDL subclasses, there was an increase in these lipids in the oxidised FO group. Cell studies have shown that the addition of oxidised lipids, that have similar chemical structures to bile acids, enhance the solubility of cholesterol and incorporation of cholesterol into micelles. Similarly, mice fed oxidised lipids had higher serum cholesterol, probably due to increased uptake of dietary cholesterol[48,49]. This might explain the increase in total cholesterol and cholesteryl esters in IDL and LDL in the oxidised FO group. This increase in uptake of dietary cholesterol might have masked the beneficial effect of EPA and DHA on cholesterol levels in the oxidised FO group. We did not see an increase in VLDL cholesteryl esters, except for the smallest VLDL subclass, after intake of oxidised FO. One possible explanation for this observation is that blood samples were drawn when subjects were fasting.

In the HOSO group, the increase of lipid constituents in IDL and LDL subclasses might be explained by the relatively lower content of PUFA in the HOSO capsules (8·8 g/100 g) compared with the oxidised FO capsules (28·4 g/100 g) and high-quality FO capsules (28·3 g/100 g)[23].

The results in the present study are important and relevant as it has been shown that as much as 50 % or more of the *n*-3 supplements on the market exceed one or more of the limits set by the GOED (PV < 5mEq/kg and AV < 20)[14–16,21,22]. The high-quality FO (PV = 4 mEq/kg and AV = 3) used in this study thus represents an oil of good quality compared with the available over-the-counter supplements and the GOED limits. Because the oxidised FO (PV = 18 mEq/kg and AV = 9) used in this study was made from a very pure food grade oil, it was difficult to achieve a high AV. Nevertheless, the oxidised FO used in the present study is comparable to many commercially available *n*-3 supplements.

One weakness of this explorative study might be the lack of adjustment for multiple comparisons. However, as the variables are not independent, but measure a continuum of dependent variables, not adjusting for multiple comparisons might be reasonable. In addition, as this study was not powered to detect changes in lipoprotein subclasses, there is a risk for type II errors. Moreover, we cannot exclude the possibility that the

frequency of the apoE4 genotype, reported to affect the LDL-cholesterol response after FO supplementation[11], differed by random between the intervention groups. This might have caused the differences we observed in IDL and LDL subclasses, as it is hypothesised that individuals with the apoE4 genotype have a lower hepatic uptake of IDL and LDL because of competition with VLDL for the LDL receptor[11]. The results in this study are strengthened by similar effects of the intervention across all IDL and LDL subclasses. In addition, the changes on lipoprotein subclass level sum up to a clinically relevant change in LDL-cholesterol.

Conclusion

Intake of oxidised FO and high-quality FO differently affect lipid composition in lipoprotein subclasses, with a more favourable effect mediated by high-quality FO. Hence, in future trials investigating the health beneficial effects of FO supplementation, it would be useful to report the oxidation levels of the oils consumed.

Acknowledgements

The authors thank Gjermund Vogt, Nofima Mat, Norway, for oxidising the fish oil, and Kirsti W. Brønner, TINE AS, Norway and Grete I. Borge, Nofima Mat, Norway for participating in development of the overall research plan of the main project.

This study received financial support from Oslo and Akershus University College of Applied Sciences, the University of Oslo, the Throne Holst Foundation for Nutrition Research and the Research Council of Norway (184813/I10).

The authors' contributions are as follows: A. R., K. B. H., M. C. M. and S. M. U. designed the research (project conception, development of overall research plan and study oversight) and analysed data or performed statistical analysis. A. R., K. B. H., I. O., M. C. M. and S. M. U. conducted the research (hands-on conduct of the experiments and data collection) and wrote the paper (only authors who made a major contribution). A. R., K. B. H. and S. M. U. had primary responsibility for the final content.

The authors declare that there are no conflicts of interest.

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517001167

References

1. Mozaffarian D, Benjamin EJ, Go AS, *et al.* (2016) Heart disease and stroke statistics-2016 update: a report from the American Heart Association. *Circulation* **133**, e38–e360.
2. Tavazzi L, Maggioni AP, Marchioli R, *et al.* (2008) Effect of *n*-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet* **372**, 1223–1230.
3. Gruppo Italiano Per Lo Studio Della Sopravvivenza Nell'Infarto Miocardio - Heart Failure (GISSI-HF) Investigators (1999) Dietary supplementation with *n*-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico. *Lancet* **354**, 447–455.
4. Yokoyama M, Origasa H, Matsuzaki M, *et al.* (2007) Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* **369**, 1090–1098.
5. Harris WS (1997) *n*-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr* **65**, 1645s–1654s.
6. Morris MC, Sacks F & Rosner B (1993) Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* **88**, 523–533.
7. Knapp HR (1997) Dietary fatty acids in human thrombosis and hemostasis. *Am J Clin Nutr* **65**, 1687s–1698s.
8. Cheng JW & Santoni F (2008) Omega-3 fatty acid: a role in the management of cardiac arrhythmias? *J Altern Complement Med* **14**, 965–974.
9. Goodnight SH Jr, Harris WS, Connor WE, *et al.* (1982) Polyunsaturated fatty acids, hyperlipidemia, and thrombosis. *Arteriosclerosis* **2**, 87–113.
10. Balk EM, Lichtenstein AH, Chung M, *et al.* (2006) Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis* **189**, 19–30.
11. Olano-Martin E, Anil E, Caslake MJ, *et al.* (2010) Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. *Atherosclerosis* **209**, 104–110.
12. Minihane AM, Khan S, Leigh-Firbank EC, *et al.* (2000) ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arterioscler Thromb Vasc Biol* **20**, 1990–1997.
13. Shahidi F & Zhong Y (2010) Lipid oxidation and improving the oxidative stability. *Chem Soc Rev* **39**, 4067–4079.
14. Albert BB, Derraik JG, Cameron-Smith D, *et al.* (2015) Fish oil supplements in New Zealand are highly oxidised and do not meet label content of *n*-3 PUFA. *Sci Rep* **5**, 7928.
15. Jackowski SA, Alvi AZ, Mirajkar A, *et al.* (2015) Oxidation levels of North American over-the-counter *n*-3 (omega-3) supplements and the influence of supplement formulation and delivery form on evaluating oxidative safety. *J Nutr Sci* **4**, e30.
16. Opperman M & Benade S (2013) Analysis of the omega-3 fatty acid content of South African fish oil supplements: a follow-up study. *Cardiovasc J Afr* **24**, 297–302.
17. Halvorsen BL & Blomhoff R (2011) Determination of lipid oxidation products in vegetable oils and marine omega-3 supplements. *Food Nutr Res* **55**, 10.3402/fnr.v55i0.5792.
18. European Food Safety Authority (2010) Scientific opinion on fish oil for human consumption. Food hygiene, including rancidity. *EFSA J* **8**, 1874.
19. Health Canada (2013) Monograph: fish oil. http://webprod.hc-sc.gc.ca/nhpid-bdipsn/monoReq.do?id=88 (accessed February 2017).
20. Ismail A, Bannenberg G, Rice HB, *et al.* (2016) Oxidation in EPA- and DHA-rich oils: an overview. *Lipid Technol* **28**, 55–59.
21. Mason RP & Sherratt SC (2017) Omega-3 fatty acid fish oil dietary supplements contain saturated fats and oxidized lipids that may interfere with their intended biological benefits. *Biochem Biophys Res Commun* **483**, 425–429.
22. Nichols PD, Dogan L & Sinclair A (2016) Australian and New Zealand fish oil products in 2016 meet label omega-3 claims and are not oxidized. *Nutrients* **8**, 703.
23. Ottestad I, Vogt G, Retterstol K, *et al.* (2012) Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *Br J Nutr* **108**, 315–326.
24. Ottestad I, Retterstol K, Myhrstad MC, *et al.* (2013) Intake of oxidised fish oil does not affect circulating levels of oxidised

LDL or inflammatory markers in healthy subjects. *Nutr Metab Cardiovasc Dis* **23**, e3–e4.
25. Garcia-Hernandez VM, Gallar M, Sanchez-Soriano J, *et al.* (2013) Effect of omega-3 dietary supplements with different oxidation levels in the lipidic profile of women: a randomized controlled trial. *Int J Food Sci Nutr* **64**, 993–1000.
26. Mallol R, Rodriguez MA, Brezmes J, *et al.* (2013) Human serum/plasma lipoprotein analysis by NMR: application to the study of diabetic dyslipidemia. *Prog Nucl Magn Reson Spectrosc* **70**, 1–24.
27. Soininen P, Kangas AJ, Wurtz P, *et al.* (2009) High-throughput serum NMR metabonomics for cost-effective holistic studies on systemic metabolism. *Analyst* **134**, 1781–1785.
28. Krauss RM (2010) Lipoprotein subfractions and cardiovascular disease risk. *Curr Opin Lipidol* **21**, 305–311.
29. Mostad IL, Bjerve KS, Lydersen S, *et al.* (2008) Effects of marine *n*-3 fatty acid supplementation on lipoprotein subclasses measured by nuclear magnetic resonance in subjects with type II diabetes. *Eur J Clin Nutr* **62**, 419–429.
30. Erkkila AT, Schwab US, Lehto S, *et al.* (2014) Effect of fatty and lean fish intake on lipoprotein subclasses in subjects with coronary heart disease: a controlled trial. *J Clin Lipidol* **8**, 126–133.
31. Lankinen M, Kolehmainen M, Jaaskelainen T, *et al.* (2014) Effects of whole grain, fish and bilberries on serum metabolic profile and lipid transfer protein activities: a randomized trial (Sysdimet). *PLOS ONE* **9**, e90352.
32. Packard CJ (2006) Small dense low-density lipoprotein and its role as an independent predictor of cardiovascular disease. *Curr Opin Lipidol* **17**, 412–417.
33. Davidson MH & Benes LB (2016) The future of *n*-3 polyunsaturated fatty acid therapy. *Curr Opin Lipidol* **27**, 570–578.
34. Myhrstad MC, Ottestad I, Gunther CC, *et al.* (2016) The PBMC transcriptome profile after intake of oxidized versus high-quality fish oil: an explorative study in healthy subjects. *Genes Nutr* **11**, 16.
35. Soininen P, Kangas AJ, Wurtz P, *et al.* (2015) Quantitative serum nuclear magnetic resonance metabolomics in cardiovascular epidemiology and genetics. *Circ Cardiovasc Genet* **8**, 192–206.
36. Myhrstad MC, Ulven SM, Gunther CC, *et al.* (2014) Fish oil supplementation induces expression of genes related to cell cycle, endoplasmic reticulum stress and apoptosis in peripheral blood mononuclear cells: a transcriptomic approach. *J Intern Med* **276**, 498–511.
37. de Mello VD, Kolehmanien M, Schwab U, *et al.* (2012) Gene expression of peripheral blood mononuclear cells as a tool in dietary intervention studies: What do we know so far? *Mol Nutr Food Res* **56**, 1160–1172.
38. Albert BB, Cameron-Smith D, Hofman PL, *et al.* (2013) Oxidation of marine omega-3 supplements and human health. *Biomed Res Int* **2013**, 464921.
39. Turner R, McLean CH & Silvers KM (2006) Are the health benefits of fish oils limited by products of oxidation? *Nutr Res Rev* **19**, 53–62.
40. Parks JS & Bullock BC (1987) Effect of fish oil versus lard diets on the chemical and physical properties of low density lipoproteins of nonhuman primates. *J Lipid Res* **28**, 173–182.
41. Taylor F, Huffman MD, Macedo AF, *et al.* (2013) Statins for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, issue 1, CD004816.
42. Ridker PM (2014) LDL cholesterol: controversies and future therapeutic directions. *Lancet* **384**, 607–617.
43. Law MR, Wald NJ & Thompson SG (1994) By how much and how quickly does reduction in serum cholesterol concentration lower risk of ischaemic heart disease? *BMJ* **308**, 367–372.
44. Yeang C, Witztum JL & Tsimikas S (2015) 'LDL-C' = LDL-C + Lp(a)-C: implications of achieved ultra-low LDL-C levels in the proprotein convertase subtilisin/kexin type 9 era of potent LDL-C lowering. *Curr Opin Lipidol* **26**, 169–178.
45. Baumstark MW, Frey I, Berg A, *et al.* (1992) Influence of *n*-3 fatty acids from fish oils on concentration of high- and low-density lipoprotein subfractions and their lipid and apolipoprotein composition. *Clin Biochem* **25**, 338–340.
46. Kasbi Chadli F, Nazih H, Krempf M, *et al.* (2013) Omega 3 fatty acids promote macrophage reverse cholesterol transport in hamster fed high fat diet. *PLOS ONE* **8**, e61109.
47. Bagdade JD, Ritter M & Subbaiah PV (1996) Marine lipids normalize cholesteryl ester transfer in IDDM. *Diabetologia* **39**, 487–491.
48. Khan-Merchant N, Penumetcha M, Meilhac O, *et al.* (2002) Oxidized fatty acids promote atherosclerosis only in the presence of dietary cholesterol in low-density lipoprotein receptor knockout mice. *J Nutr* **132**, 3256–3262.
49. Penumetcha M, Khan-Merchant N & Parthasarathy S (2002) Enhanced solubilization and intestinal absorption of cholesterol by oxidized linoleic acid. *J Lipid Res* **43**, 895–903.