

## The effect of intakes of fish and *Camelina sativa* oil on atherogenic and anti-atherogenic functions of LDL and HDL particles: A randomized controlled trial



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### HIGHLIGHTS

- Camelina oil intake decreases the binding of lipoproteins to aortic proteoglycans.
- Intakes of Camelina oil or fish have no effect on LDL aggregation.
- Intake of fatty fish has no effect on cholesterol efflux capacity of HDL.

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### ABSTRACT

**Background and aims:** Omega-3 fatty acids are known to have several cardioprotective effects. Our aim was to investigate the effects of intakes of fish and *Camelina sativa* oil (CSO), rich in alpha-linolenic acid, on the atherogenic and anti-atherogenic functions of LDL and HDL particles.

**Methods:** Altogether, 88 volunteers with impaired glucose metabolism were randomly assigned to CSO (10 g of alpha-linolenic acid/day), fatty fish (4 fish meals/week), lean fish (4 fish meals/week) or control group for 12 weeks. 79 subjects completed the study. The binding of lipoproteins to aortic proteoglycans, LDL aggregation and activation of endothelial cells by LDL and cholesterol efflux capacity of HDL were determined *in vitro*.

**Results:** Intake of CSO decreased the binding of lipoproteins to aortic proteoglycans in a non-normalized model ( $p = 0.006$ ). After normalizing with serum concentrations of non-HDL cholesterol, apolipoprotein B (apoB) or LDL cholesterol, which decreased in the CSO group, the change was no longer statistically significant. In the fish groups, there were no changes in the binding of lipoproteins to proteoglycans. Regarding other lipoprotein functions, there were no changes in any of the groups.

**Conclusions:** Intake of CSO decreases the binding of lipoproteins to aortic proteoglycans by decreasing serum LDL cholesterol concentration, which suggests that the level of apoB-containing lipoproteins in the circulation is the main driver of lipoprotein retention within the arterial wall. Intake of fish or CSO has no effects on other lipoprotein functions.

### 1. Introduction

Progression of atherosclerosis consists of several molecular and inflammatory processes, taking place in the arterial wall [1–3]. The initiating process in atherogenesis is retention of lipoproteins, mainly LDL particles, in the arterial intima. Lipoproteins containing apolipoprotein

B (apoB) and apoE bind to proteoglycans of the intimal layer and can be modified, e.g. by oxidation and lipolysis. Modified lipoproteins may further aggregate or fuse [4], which increases their binding strength to proteoglycans [5,6] and prevents their exit back to the bloodstream [7]. The aggregation susceptibility of LDL varies among individuals and was recently shown to associate with cardiovascular deaths [8]. LDL,

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particularly when modified, also stimulate several atherogenic processes, such as interleukin-8 (IL-8) secretion of endothelial cells, which induce the chemotactic recruitment of monocytes to the arterial intima [1,9,10]. Modified lipoproteins are taken up by macrophages, which leads to foam-cell formation. Retained and modified lipoproteins and foam cell formation leads to the release of proinflammatory cytokines and bridging molecules (e.g. lipoprotein lipase), smooth muscle cell proliferation, induced synthesis of proteoglycans and eventually further entrapment of lipoproteins [4,11]. These reactions trigger a self-accelerating process that eventually leads to progression of atherosclerosis.

Cholesterol efflux from macrophage foam cells has a central role in the reverse cholesterol transport, which is considered the primary anti-atherogenic mechanism of HDL [12]. HDL particles, however, possess also several other atheroprotective activities, such as anti-inflammatory and antioxidative functions [12,13]. The profile and structure of HDL particles are key mediators of their atheroprotective functions. Examples of structural components, which can mediate HDL function, are apoE and serum amyloid A (SAA) [14,15]. ApoE is known to have several anti-atherogenic functions such as mediating plasma lipoprotein clearance by the liver [14]. Furthermore, apoE has been shown to contribute to lipid retention via HDL [16]. HDL particles containing apoE can bind to proteoglycans and, therefore, interfere with the formation of LDL-proteoglycan complex. This competition of binding sites could potentially prevent LDL accumulation in the arterial wall. Another component which could impair the atheroprotective functions of HDL is SAA, which is carried primarily in HDL particles in plasma [15]. SAA is considered a potential mediator of atherosclerosis by acting as a bridging protein between lipoproteins and proteoglycans and impairing cholesterol efflux capacity.

Diet is an important modifiable lifestyle factor in the prevention of CVD [17,18]. Among the beneficial dietary factors, fish has been considered as an important component in a cardioprotective diet. Fish, especially fatty fish, is a rich source of *n*-3 polyunsaturated fatty acids (*n*-3 PUFAs), which have been shown to affect several molecular mechanisms and pathways related to CVD, such as physical and chemical properties of cellular membranes, inflammation and regulation of gene expression [19]. Previous studies have shown that intake of *n*-3 PUFAs from fish increases HDL cholesterol level but has no effect on LDL cholesterol [20] whereas evidence on the effects of plant-derived *n*-3 fatty acid, alpha-linolenic acid (ALA), on serum lipids is inconsistent [21]. In addition to fatty fish, also lean fish intake has been found to have cardioprotective effects [22–24]. This suggests that the beneficial effects of fish consumption on CVD risk factors may, in addition to *n*-3 PUFAs, be mediated through other components in the fish, such as protein and other bioactive compounds or their interactions.

Although the effects of *n*-3 PUFAs on CVD have been extensively studied, little is known about the effects of dietary intake of these fatty acids on lipoprotein functions related to atherosclerosis. To this end, the aim of our study was to investigate the effect of Camelina sativa oil (CSO), high in ALA, and fish intake on the pro- and anti-atherogenic functions of LDL and HDL particles, such as binding of lipoproteins to aortic proteoglycans, LDL aggregation, cholesterol efflux capacity and activation of endothelial cells in subjects with impaired glucose metabolism.

## 2. Materials and methods

### 2.1. Subjects

Details of the subjects, study design and recruitment were described previously [25]. Briefly, volunteers aged 40–75 years and with impaired glucose metabolism were recruited from Kuopio area. Glucose metabolism was one of the primary endpoints in this study and, therefore, 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 had to be  $\leq$  11.0 mmol/l. A total of 79

subjects completed the study. Subjects were randomly assigned into four parallel groups for 12 weeks: CSO, lean fish, fatty fish or control group. Randomization was stratified by sex and use of statins. The subjects received both oral and written information and informed consent was obtained from all of the subjects. The study was approved by the Ethical committee of the Hospital District of Northern Savo (55/2012) and ethical guidelines were followed throughout the study.

### 2.2. Study design

The study diets were isocaloric and current nutrient recommendations [26] were followed except for fish and ALA intakes. Fish diets included four fish meals per week, e.g. salmon, Baltic herring and vendace in the fatty fish group providing around 1 g of EPA + DHA per day and e.g. pike, perch, pike-perch and saithe in the lean fish group. The CSO group ingested 30 ml of CSO (27 g) in order to get 10 g of ALA per day. Intake of ALA > 4.5 g/day has been shown to result in increased levels of EPA in the plasma phospholipids [27]. The control and CSO groups were allowed to eat one fish meal per week and were instructed to consume lean meat and chicken. Subjects kept daily consumption records regarding the intake of fish (number of meals and type of fish) and subjects in the CSO group recorded also the intake of CSO. Food records from four consecutive days, including one weekend day, were collected and checked at return by clinical nutritionists at baseline and during the study in weeks 3, 7 and 11. In the CSO group, intakes of total fat, PUFA and ALA increased as compared with the control group. In the fatty fish group, intakes of EPA, DHA and vitamin D increased and intake of carbohydrates decreased as compared with the control group. In the lean fish group intake of vitamin D increased as compared with the control group. Detailed dietary intakes have been reported previously [25].

The blood samples were drawn after 10-h overnight fasting at baseline (0 wk) and at the end of the study (12 wk). Physical activity, alcohol intake, smoking, body weight and use of medications known to affect the measures of lipid metabolism were to be kept constant during the study. As an objective measure of compliance, the proportions of plasma fatty acids in phospholipids were measured using gas chromatography as previously described [28] with an exception of using C19:0 as an internal standard instead of C17:0.

### 2.3. Analytical methods

#### 2.3.1. Binding of lipoproteins to proteoglycans

Proteoglycan binding was assessed in 96-well plates coated with proteoglycans isolated from human aortas as previously described [29]. 1  $\mu$ l plasma was added to the wells and incubated for 1 h. The amount of cholesterol bound to the wells was measured with Amplex Red cholesterol assay kit.

#### 2.3.2. Isolation of LDL and HDL

LDL ( $d = 1.019$ – $1.063$  g/ml) and HDL ( $d = 1.063$ – $1.210$  g/ml) were prepared from isolated plasma by sequential flotation ultracentrifugation using  $D_2O$  for density adjustment as described previously [30].

#### 2.3.3. LDL aggregation

LDL was isolated from the plasma and LDL stability was determined by inducing LDL aggregation by human recombinant sphingomyelinase as previously described [8]. LDL aggregation was followed by measuring the particle size using dynamic light scattering.

#### 2.3.4. Culture of human coronary artery endothelial cells (HCAECs) and analysis of LDL effect on endothelial cell activation

HCAECs (PromoCell) were cultured in Endothelial Cell Growth Medium MV supplemented with 5% fetal calf serum, 0.4% endothelial cell growth supplement, 10 ng/ml epidermal growth factor, 90  $\mu$ g/ml

heparin, 1 µg/ml hydrocortisone (Supplement pack, Catalog Number C-39220, PromoCell), 100 U/ml penicillin streptomycin solution, and 50 ng/ml amphotericin B to yield Complete Medium in T-75 flask according to the manufacturer's instructions. Confluent HCAECs were washed with 15 ml of PBS, trypsinized, and replated in complete medium, as described below. Endothelial cell activation was measured in HCAECs between passages 4 and 7. HCAECs were seeded at a density of  $1\text{--}2 \times 10^4$  cells/well in 96-well plate and cultured for 2–3 days until the cells reached 90–95% confluency. To determine the effect of LDL on IL-8 secretion, HCAECs grown in Complete Medium were washed, placed in serum-free medium and incubated with LDL (50 µg protein/ml) for 6 h. The amount of IL-8 released into the media was measured by an IL-8 ELISA Kit (R & D Systems, Minneapolis, Minnesota, USA).

### 2.3.5. Culture of primary human monocyte-derived macrophages

Human monocytes were isolated from buffy coats (Finnish Red Cross Blood Transfusion Center, Helsinki, Finland) by centrifugation in Ficoll-Paque gradient as described [31]. Washed cells were suspended in DMEM supplemented with 100 U/ml penicillin and 100 µg/ml streptomycin, counted, and seeded on 24 well-plates (1.5 million cells per well). After 1 h incubation, non-adherent cells were removed, and the medium was replaced with macrophage-SFM medium (Gibco) supplemented with 1% penicillin-streptomycin and 10 ng/ml of granulocyte macrophage colony-stimulating factor (GM-CSF) (Biosite, San Diego, USA). The cells were cultured for 7 days in the presence of GM-CSF to allow them to differentiate into GM-CSF macrophages. The medium was then changed every 2–3 days throughout the culture period.

### 2.3.6. Cholesterol efflux from macrophage foam cells

The monocyte-derived macrophages were incubated in DMEM (pH 7.4) containing 25 µg/ml of [3H]CE-acetyl-LDL for 24 h to induce the formation of foam cells. To measure cholesterol efflux, macrophages were washed with PBS, and fresh media containing the various HDL (25 µg protein/ml) were added. After incubation for 16 h, the media were collected, centrifuged at 300 g for 10 min to remove cellular debris, and the radioactivity in the supernatants was determined by liquid scintillation counting. Cells were solubilized with 0.2 M NaOH and analyzed for radioactivity. Cholesterol efflux was expressed as the percentage radioactivity in the medium relative to the sum of total radioactivity present in the medium and the cells. Cholesterol efflux to the incubation medium in the absence of HDL was considered as basal efflux and was subtracted from the efflux values obtained in the presence of HDL. In an initial experiment using THP-1 cells, a small increase in cholesterol efflux was observed in the fatty fish group but not in other groups (data not shown). Therefore, the fatty fish group was selected to determine cholesterol efflux capacity using human primary macrophages, a relevantly physiological cell type.

### 2.3.7. Analyses of SAA, apoE, serum lipids and lipoprotein subclasses

Plasma apoE was assessed using ELISA Development Kit (catalog number 3712-1A-6; Mabtech, Nacka Strand, Sweden). SAA in serum was measured using Invitrogen SAA ELISA Kit (Cat nr. KHA0011, KHA0012 or KHA0011C; Thermo Fisher Scientific). Concentrations of serum total, LDL and HDL cholesterol were analyzed as previously described [25]. Serum concentrations of lipoprotein subclasses were measured using high-throughput proton NMR spectroscopy, method has been described in detail previously [32].

## 2.4. Statistical analyses

IBM SPSS statistical software (v. 24, IBM Corp., Armonk, NY) was used for statistical analyses. The Kolmogorov-Smirnov test was used to test the normality of the variables and logarithmic transformation was performed for skewed variables. Non-parametric test was used if normality was not achieved with logarithmic transformation. For the

comparison of baseline and endpoint values within the groups, paired samples *t*-test or Wilcoxon signed ranks test were used. The amount of cholesterol bound to aortic proteoglycans were normalized with LDL-, non-HDL cholesterol or apoB concentrations. Changes among the groups were tested with the analysis of variance (ANOVA) or Kruskal-Wallis -test. Analyses were performed using fold changes. Fold changes were calculated by dividing the endpoint values of the variable by their baseline values. Differences between genders in LDL aggregation were tested with Mann-Whitney's U -test. Change in the cholesterol efflux capacity was tested with independent samples *t*-test. Associations between lipoprotein subclasses and concentrations of apoE and SAA were tested using Spearman rank correlation.  $p < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Characteristics of the participants and compliance

Mean ( $\pm$  SD) age of the study subjects was  $58.9 \pm 6.5$  years. Baseline characteristics of the study subjects are shown in [Supplementary Table 1](#). Compliance with the study diets was good according to food records and consumption records regarding intakes of fish and CSO [25]. The proportion of ALA in plasma phospholipids increased in the CSO group ( $p < 0.001$ ) and differed significantly from the other groups. Furthermore, the proportion of EPA increased ( $p < 0.001$ ) in the fatty fish group as compared with the lean fish and control groups. The proportion of DHA increased in the fatty fish group ( $p < 0.001$ ) as compared with the CSO and control groups. Similar changes were also observed in the proportions of these fatty acids in plasma cholesteryl esters, triglycerides and erythrocyte membranes [33].

### 3.2. Binding of lipoproteins to proteoglycans

Binding of lipoproteins to proteoglycans decreased in the CSO group compared with the fatty fish group in a non-normalized model (overall difference among the groups  $p = 0.006$ , [Fig. 1A](#)). After normalizing to serum LDL cholesterol, non-HDL cholesterol or apoB concentrations, the change was no longer statistically significant ( $p = 0.771$ ,  $p = 0.625$  and  $p = 0.267$ , respectively), indicating that the level of apoB-containing lipoproteins in the circulation is the main driver of LDL retention to proteoglycans.

### 3.3. LDL aggregation

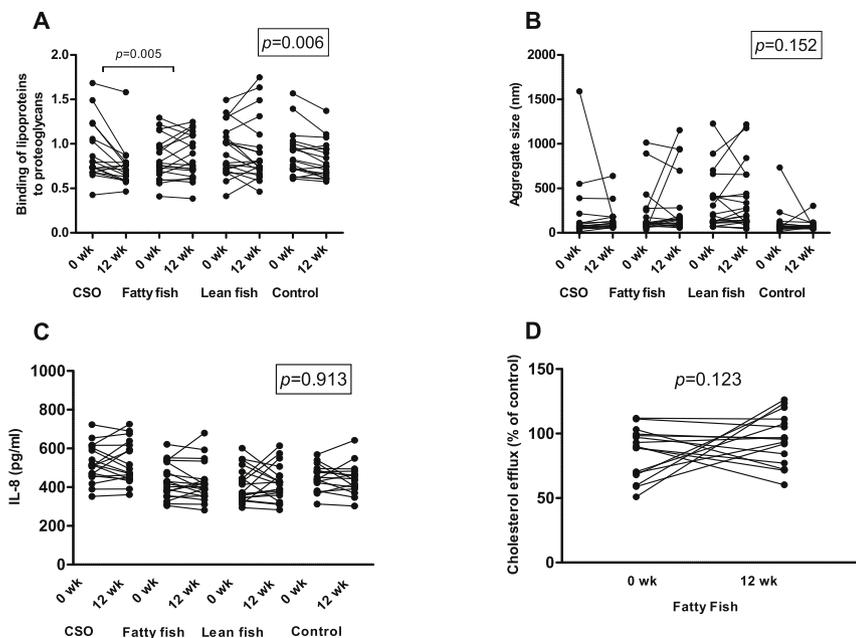
The susceptibility of LDL to aggregation has recently been shown to differ significantly among human donors and to be associated with cardiovascular deaths [8]. Here, we again found significant individual differences in LDL aggregation, but found no statistically significant change in LDL aggregation among the groups (overall difference among the groups  $p = 0.152$ , [Fig. 1B](#)) or between genders ( $p = 0.505$ ).

### 3.4. Activation of endothelial cells

A previous study has shown that modulation of LDL lipid composition influences the ability of LDL to induce secretion of IL-8 from endothelial cells [10]. We determined the IL-8 level upon treatment of human coronary artery endothelial cells with LDL (50 µl/ml) isolated from 0-week and 12-week samples. We found that dietary intervention did not influence the ability of LDL to stimulate IL-8 release ([Fig. 1C](#)), indicating that diet intervention does not affect the pro-inflammatory properties of LDL particles as determined by IL-8 secretion.

### 3.5. Cholesterol efflux capacity

We examined whether diet intervention would modulate the ability



**Fig. 1.** (A) Changes in the binding of lipoproteins to proteoglycans (n = 79), (B) LDL aggregation (n = 79), (C) activation of endothelial cells (n = 16–19/group) and (D) cholesterol efflux capacity in the fatty fish group (n = 16). Changes among groups in the binding of lipoproteins to proteoglycans, LDL aggregation and IL-8 were tested using Kruskal-Wallis test or analysis of variance followed by Bonferroni's *post hoc* tests. The *p*-value in the box represents the overall difference among groups. Change in cholesterol efflux capacity in the fatty fish group was tested with independent samples *t*-test. CSO, Camelina sativa oil.

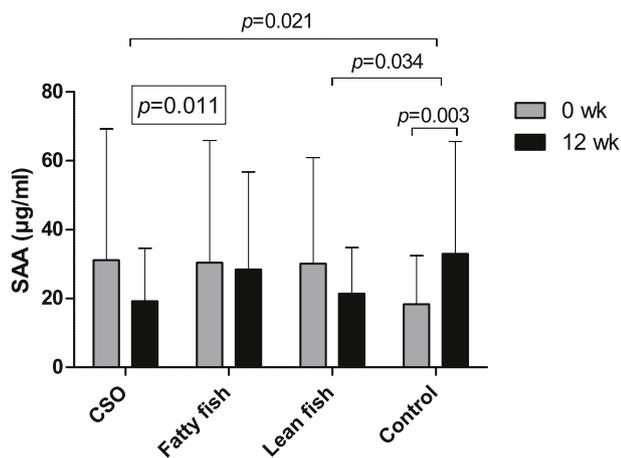
of HDL particles to promote cholesterol efflux. To this end, we isolated HDL particles from plasma in the same subjects before and after 12 weeks of fatty fish intervention and determined their cholesterol efflux from human primary foam cells. As shown in Fig. 1D, fatty fish treatment after 12 weeks did not significantly affect the cholesterol efflux capacity of HDL.

### 3.6. Concentrations of apoE and SAA

There were no statistically significant changes in plasma concentration of apoE among groups (fold changes for CSO group 1.05, fatty fish group 0.92, lean fish group 1.05 and control group 0.97, overall difference among the groups *p* = 0.322). Serum concentration of SAA decreased in the CSO and lean fish groups compared with the control group (overall difference among the groups *p* = 0.011, Fig. 2).

### 3.7. Correlations between lipoprotein subclasses, SAA and apoE

Correlations between lipoprotein subclasses, SAA and apoE are



**Fig. 2.** Changes in the concentration of SAA. Changes within groups were tested with Mann-Whitney's *U* test and changes among groups were tested using Kruskal-Wallis test. The *p*-value in the box represents the overall difference among the groups. CSO, Camelina sativa oil; SAA, serum amyloid A.

shown in Supplementary Tables 2–4. We found weak correlations between SAA and HDL subclasses (*r* = 0.245–0.416, *p* < 0.05) and apoE and HDL subclasses (*r* = 0.223–0.306, *p* < 0.05). HDL<sub>3</sub> also correlated with LDL subclasses (*r* = 0.383–0.629, *p* < 0.01).

## 4. Discussion

In this study, we investigated the effect of fish and CSO intakes on binding of lipoproteins to proteoglycans, LDL aggregation, cholesterol efflux capacity and activation of endothelial cells. This is the first dietary intervention study to investigate all these lipoprotein functions in humans. We showed that intake of 30 ml (27 g) of CSO rich in ALA decreases the binding of lipoproteins to aortic proteoglycans compared with the fatty fish group. Previously, Jones et al. [34] found that corn/safflower oil and high-oleic canola oil decreased slightly the binding of LDL to biglycan. In the present study, we used whole plasma samples instead of isolated LDL particles. In this way, we were able to assess the effect of all plasma lipoproteins on cholesterol accumulation to proteoglycans. We have previously shown that intake of CSO decreases serum LDL cholesterol compared with the fatty fish and lean fish groups and IDL particle concentration compared with the lean fish group [25,32]. Taken together, our findings show a clear relation between plasma apoB containing lipoprotein levels and the binding of lipoprotein-derived cholesterol to human aortic proteoglycans, a finding highlighting the importance of plasma lipoprotein levels in the accumulation of cholesterol within the arterial proteoglycans.

The effect of a healthy Nordic diet has recently been shown to decrease LDL aggregation in most donors, the decrease associating with an increased consumption of poly- and monounsaturated fatty acids [8]. Here, we found no change in the LDL aggregation among the groups, but it should be noted that the individual differences in LDL aggregation were large, as also reported earlier [8]. In that study, the diet-induced differences in LDL aggregation were shown to be due to differences in the surface lipids of LDL, so that a decrease in LDL-sphingomyelins was associated with decreased LDL aggregation. Neither CSO, fatty fish nor lean fish induced changes in the proportion of LDL-sphingomyelins in this study (unpublished data by Manninen et al.). Furthermore, it has been previously shown that plasma sphingomyelins are affected by genetic factors, which may partly explain the inter-individual variance found in LDL aggregation [35]. These differences in LDL aggregation related to individual differences and the potential

underlying mechanisms require further investigation.

Modulation of LDL lipid composition has previously been shown to induce the release of IL-8 from endothelial cells [10,36]. In these studies, changes in the LDL lipidome were induced enzymatically and increased content of free fatty acids and lysophosphatidylcholine was found to enhance IL-8 release. Instead, studies investigating dietary effects on LDL lipidome are scarce [8,37], and to our knowledge, there are no previous studies investigating the effects of dietary intervention on inflammatory properties of LDL particles. Padro et al. [37] found that milk enriched with long-chain *n*-3 PUFA (0.375 g/250 ml milk) did not induce changes among lipid species of LDL. *N*-3 enriched milk, however, increased long-chain *n*-3 PUFA content in cholesteryl esters, phosphatidylcholine and PC36:5/lysoPC16:0 ratio. Authors concluded that these changes may be associated with reduced inflammatory activity of LDL particles. Here, we found no change in the ability of LDL to release IL-8.

Previous research on the effects of *n*-3 fatty acids on cholesterol efflux has been controversial [38–41]. In experimental models, where macrophages have been directly exposed to EPA, cholesterol efflux capacity has been reduced [38,39], whereas EPA-supplementation with a high dose (1.8 g/day) has been shown to enhance cholesterol efflux capacity of HDL from THP-1 macrophages in patients with coronary risk factors [40]. In the present study, the dietary intake of EPA in the fatty fish group was considerably lower: approximately 0.5 g/day [25]. Here, we found that intake of fatty fish had no effect on the cholesterol efflux capacity of HDL particles isolated from plasma. Furthermore, ALA-treatment has been found to increase cholesterol efflux capacity in foam cells by decreasing stearoyl CoA desaturase 1 expression [41]. Thus, it appears that ALA-treatment may be able to influence the properties of macrophages rather than that of HDL particles. Overall, further studies are needed to elucidate the role of dietary *n*-3 PUFAs on cholesterol efflux capacity.

SAA and apoE are known to contribute to atherosclerosis [14,15]. SAA has been shown to have several atherogenic functions whereas apoE is best recognized for its anti-atherogenic properties. Both SAA and apoE are also potential mediators in the binding of HDL to proteoglycans [16,42]. HDL<sub>2</sub> has been found to inhibit the formation of the LDL-proteoglycan complex more efficiently than HDL<sub>3</sub>. This is explained by the higher apoE content in large HDL particles compared with small HDL particles [43]. Here, we found no changes in plasma concentration of apoE in any of the groups, but there was a decrease in the serum concentration of SAA in the CSO and lean fish groups as compared with the control group. After excluding individuals with hs-CRP > 10 mg/l at baseline or at the end of the study (*n* = 3), there were no differences between the groups in the pairwise comparison (data not shown). Moreover, we found weak correlations between SAA and HDL subclasses and apoE and HDL subclasses. HDL<sub>3</sub> also correlated with LDL subclasses, which may be an indication of dyslipidemic profile including small HDL particles [44].

The strengths of the current study include a randomized controlled study design and good compliance with study diets, as previously reported [25]. However, there are also some limitations to consider. Power calculations were based on differences in DHA in plasma phospholipids, and it is likely that there was not enough power to see all changes in these secondary outcome variables. Baseline omega-3 index of subjects was relatively high (> 8% for all groups) [33], which may have attenuated the effects. Furthermore, subjects in this study were overweight and had impaired glucose metabolism. Therefore, the results of this study may not apply to individuals with normal body weight and glucose metabolism.

In conclusion, intake of CSO, fatty fish or lean fish has no effect on LDL aggregation or activation of endothelial cells, and fatty fish intake has no effect on cholesterol efflux capacity in subjects with impaired glucose metabolism. Intake of CSO, however, decreases the binding of lipoproteins to aortic proteoglycans by decreasing serum apoB-containing lipoprotein concentrations. This finding highlights the

importance of plasma lipoprotein levels in the accumulation of cholesterol within the arterial wall.

### Clinical trial registry

The study is registered in [Clinicaltrials.gov](https://clinicaltrials.gov) (NCT01768429).

### Conflicts of interest

K.Ö. and M.R. have applied a patent for the measurement of LDL aggregation. All other authors declare no conflict of interest.

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### Author contributions

U.S. and A.E. are the principal investigators in the AlfaFish –study and they planned and conducted the study together with M.L. and V.M. S.M. analyzed the data, wrote the article and had primary responsibility for final content. S.D.N. and M.R. isolated plasma LDL and HDL particles. S.D.N. performed cholesterol efflux and endothelial cell activation assays, analyzed the data and interpreted the results. M.R. performed LDL aggregation analyses, analyzed the data and interpreted the results. K.Ö. performed plasma proteoglycan binding assays, analyzed the data, and interpreted the results. U.S., A.E., M.L. K.Ö. and S.D.N. participated in writing and editing the manuscript. All authors have read and approved the final manuscript.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.atherosclerosis.2018.12.017>.

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