Daily intake of cod or salmon for two weeks decreases 18:1n-9/18:0 ratio and serum triacylglycerols in healthy subjects

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ABSTRACT

Intake of fish and omega-3 (n-3) fatty acids is associated with reduced concentration of plasma triacylglycerols (TAG) but the mechanisms are not fully clarified. Stearoyl-CoA desaturase-1 (SCD1) activity, governing TAG synthesis, is affected by n-3 fatty acids. Peripheral blood mononuclear cells (PBMC) display expression of genes involved in lipid metabolism. The aim of the present study was to estimate whether intake of lean and fatty fish would influence n-3 fatty acids composition in plasma phospholipids (PL), serum TAG, 18:1n-9/18:0 ratio in plasma PL, as well as PBMC gene expression of SCD1 and fatty acid synthase (FAS). Healthy males and females (n = 30), aged 20—40, consumed daily for 15 days either 150 g of cod, salmon, or potato (control). During intervention docosahexaenoic acid (DHA, 22:6n-3) increased in the cod group ($P < 0.05$), while TAG concentration decreased ($P < 0.05$). In the salmon group both eicosapentaenoic acid (EPA, 20:5n-3) and DHA increased ($P < 0.05$) whereas TAG concentration and the 18:1n-9/18:0 ratio decreased ($P < 0.05$). Reduction of the 18:1n-9/18:0 ratio was associated with a corresponding lowering of TAG ($P < 0.05$) and an increase in EPA and DHA ($P < 0.05$). The mRNA levels of SCD1 and FAS in PBMC were not significantly altered after intake of cod or salmon when compared with the control group. In conclusion, both lean and fatty fish may lower TAG, possibly by reducing the 18:1n-9/18:0 ratio related to allosteric inhibition of SCD1 activity, rather than by influencing the synthesis of enzyme protein.

Keywords

Fish · 18:1n-9/18:0 ratio · Omega 3 · Triacylglycerols · Gene expression · PBMC · Humans

Abbreviations

CE	Cholesterol esters
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
FAS	Fatty acid synthase
GUSβ	Glucuronidase-beta
HDL-C	High density lipoprotein cholesterol
LA	Linoleic acid
LDL-C	Low density lipoprotein cholesterol
n-3	Omega-3
PBMC	Peripheral blood mononuclear cells
PL	Phospholipids
PPARα	Peroxisome-proliferator activated receptor alpha
PUFA	Polyunsaturated fatty acids
SCD1	Stearoyl-CoA desaturase-1
SREBP1	Sterol regulatory element-binding protein 1
TBP	TATA binding protein
TAG	Triacylglycerols
VLDL	Very low density lipoprotein

INTRODUCTION

Numerous epidemiological studies have demonstrated reduced risk of cardiovascular diseases (CVD) in response to increased intake of fish or fish oils [1-3]. Especially fatty fish is a major source of long chain omega-3 polyunsaturated fatty acids (n-3 PUFA), such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Many of the beneficial health effects of fish and fish oils have been linked to intake of these fatty acids [4-10]. Serum triacylglycerols (TAG) is recognized as an independent risk factor of CVD [11] and marine n-3 fatty acids in high doses (>3g/day) have been shown to reduce serum TAG by 25—30 % [12-15]. The magnitude of the TAG reducing effect seems to be dependent on n-3 fatty acid dose and baseline TAG concentrations [14, 16]. The American heart association (AHA) recommend the consumption of a variety of fish (preferably fatty fish) at least twice a week as guidance for healthy people [17]. However, intake of lean fish is also known to provide health benefits [18-20] in which one effect is reduced serum TAG [20]. Whether the health beneficial effects of lean fish are related to the n-3 fatty acids content, or to other bioactive components, is not known.

The molecular mechanisms involved in the hypotriglyceridemic effect of marine n-3 fatty acids are not clarified. In general, the effects could be due to reduced production, and/or to increased elimination of TAG [2]. In the present work we have focused upon some aspects of the synthesis of TAG. In the fasted state serum TAG is mainly carried in very low density lipoproteins (VLDL), which are synthesized and secreted in the liver. TAG, cholesterol esters (CE) and phospholipids (PL) in VLDL preferably contain monounsaturated fatty acids, i.e. palmitoleic (16:1n-7) and oleic (18:1n-9) acid. The rate limiting enzyme for the synthesis of these fatty acids is stearoyl-CoA desaturase-1 (SCD1 or Δ9 desaturase). Mice lacking SCD1 have reduced hepatic lipogenesis and lower plasma TAG concentration [21, 22]. Accordingly, one mechanism by which fish intake decreases serum TAG could be inhibition of desaturase

activities in the liver, caused directly or indirectly by some of the constituents in fish. In general, the rate of an enzyme catalyzed reaction may be influenced by the amount of enzyme protein, by phosphorylation and dephosphorylation of the enzyme, or by allosteric regulation [23]. Oleic acid is a major constituent of TAG produced by *de novo* lipogenesis, and therefore the 18:1n-9/18:0 ratio, i.e. a product/precursor ratio, in plasma may be used to estimate SCD1 activity [22, 24-26]. Animal studies have demonstrated that marine n-3 fatty acids can suppress hepatic lipogenesis [4], and one regulatory mechanism may be inhibition of SCD1 activity [27] or by transcriptional regulation of sterol regulatory element-binding protein 1 (SREBP1) [28]. SREBP1 is regulating the expression of lipogenic genes such as SCD1 and fatty acid synthase (FAS) [28], and an inhibition of SREBP1 by n-3 fatty acids will cause a down-regulation of these genes [29]. To study whether n-3 fatty acids might influence human hepatic gene expression *in vivo* is challenging. However, since human PBMC can display the expression of genes involved in lipid metabolism [30-35] we have chosen PBMC as a test system.

The aim of the present exploratory study was accordingly to investigate whether daily intake of lean and fatty fish for two weeks would influence n-3 fatty acids composition in plasma PL, serum TAG levels, 18:1n-9/18:0 ratio in plasma PL (an estimated indication of SCD1 activity), and to assess SCD1 and FAS by their mRNA levels in PBMC, in healthy subjects.

SUBJECTS AND METHODS

This study is an extension of a previous study involving 38 healthy subjects randomized to four different intervention groups consuming either salmon (*n* = 11), cod (*n* = 9), blue mussel (*n* = 8) or potato (control) (*n* = 10) in order to study arsenic metabolism (Molin et al., manuscript in preparation). The opportunity was taken to investigate lean versus fatty fish in this exploratory study (*n* = 30).

Subjects Thirty subjects (7 men and 23 women) aged 20—40 were recruited from Akershus University College, Lillestrøm, Norway. Healthy subjects with C-reactive protein (CRP) <10 mg/L using no medication, except for oral contraceptives (female subjects (*n* = 11), all subjects maintained the use throughout the study, except for one subject in the control group), were included in this study. Smoking, pregnancy and lactation were exclusion criteria. Additionally, subjects who had a habitual seafood consumption of more than three servings per week were excluded. All subjects were compliant with the protocol throughout the study. Compliance was assessed based on observations of the participants during the test meals, served at the University College and the amount of leftovers after the experimental period. Compliance was estimated to be 95—100 % in all three groups. The study protocol was approved by Regional Committee of Medical Ethics in Norway. Written informed consent for participation was obtained from each subject and it complied with the Declaration of Helsinki.

Study design A fifteen days randomized controlled parallel-group study was conducted. The participants were randomized but not stratified by gender, and therefore by chance all the subjects in the salmon group were females. The subjects received a daily test meal of 150 g of either farmed salmon (*Salmo salar*) (*n* = 11), cod (*Gadus morhua*) (*n* = 9) or potato (control) (*n* = 10) for 15 consecutive days. The subjects were carefully instructed not to eat any seafood except the seafood provided in the study, and not to take any dietary supplements during the intervention period. Marine n-3 supplements (including cod liver oil) were prohibited 5 weeks

prior to and during the study. Each subject was requested not to change dietary and exercise habits during the study.

Test meals A homogenous mixture of cod or salmon fillets was prepared as fish puddings and cut into cubes. Potatoes were cooked and cut into cubes. The test meal menu was a 7-day menu which was served hot and repeated twice and all the dishes were similar for all intervention groups except for the fish/potato. The test meal was served at Akershus University College Monday—Friday, and lunch boxes to bring home were provided for the weekend.

Blood sampling and biochemical analysis Blood samples were collected from fasting subjects (minimum12 h) at the same time (between 8 a.m. and 10 a.m.) at baseline and at end of study. PBMC were isolated using cell preparation tubes (CPT) according to the manufacturer's instructions (Becton, Dickinson and Company, NJ 07417, USA). Determination of serum total cholesterol (total-C), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), TAG and CRP was performed using routine laboratory methods (Fürst Medical laboratory, Norway). Plasma was obtained from EDTA tubes and kept frozen (-70 ^{o}C) until analysis.

Fatty acid composition in fish Homogenous mixture of cod or salmon fillets were kept frozen (-20 ºC) until analysis and total lipids were extracted by adding chloroform/methanol (2:1, vol/vol), and nonadecylic acid (19:0) was added as internal standard. The samples were filtered, saponified, and methylated using 12% BF_3 in methanol. Fatty acid composition of total lipids was analyzed using methods as described earlier [36, 37].

Fatty acid composition in plasma PL was determined as previously reported [38]. The 18:1n-9/18:0 ratio was calculated from the fatty acid composition in plasma PL and used as an estimate of desaturase activity.

RNA isolation and Quantitative Real-time Polymerase Chain Reaction (Q-RT-PCR)

Total RNA was extracted from PBMC using a combination of TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and RNeasy mini kit (Qiagen, Hilden, Germany) purified with RNAse-free DNAse (Invitrogen). Subsequently the samples were stored in RNAse-free water at -80°C. RNA quality was measured on an Agilent Bioanalyser 2100 system (Agilent Technologies, Santa Clara, CA, USA) and showed RNA Integrity Numbers (RIN) between 8.7 and 9.8. Total RNA yield was measured on a Nanodrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA). For cDNA synthesis, 500 ng RNA of each total RNA sample was reverse-transcribed by Super Script (Invitrogen) according to the manufacturer's protocol, using oligo dT as primers. The Taqman real-time polymerase chain reaction (RT-PCR) technique was used to quantify the mRNA expression of each gene. cDNA corresponding to fifteen ng RNA was applied to each well and each sample was run in triplets. Quantification was performed using the relative standard curve method. A combination of aliquots from all cDNA samples was made and diluted in order to make a dilution curve that was included on each plate. The points on the standard curve corresponded to 50, 25, 12.5 and 6.25 ng RNA. The average of the three values measured per gene per

sample were divided by the average of the corresponding combined Glucuronidase-beta (GUSβ) and TATA binding protein (TBP) values, generating a normalized value used to compare the relative amount for each gene in the different samples. GUSβ and TBP were chosen as endogenous genes due to the results from running a TaqMan Human Endogenous Control Plate-test from Applied Biosystems (data not shown). The primers and probes for GUSβ and TBP were initially designed as three assays per gene, and validated for efficiency and specificity. The best of the three was then chosen. The primers and probe for the GUSβ assay were: forward primer: 5'-GAAAATATGTGGTTGGAGAGCTCATT-3', probe: 5'-CCAGCACTCTCGTCGGTGACTGTTCA-3' and reverse primer: 5'-CCGAGTGAAGATCCCCTTTTTA-3'. TBP forward primer: 5'-CTGGAAAAGTTGTATTAACAGGTGC-3', probe: 5'-AGCAGAAATTTATGAAGCATTTGAAAACATCTACCCTATT-3' and reverse primer: 5'-CATTACGTCGTCTTCCTGAATC-3'. All other genes were measured by "single tube" assays, which are a premade combination of primers and probe, specific for the gene to be determined (Applied Biosystems, Foster City, CA) and utilized according to the manufacturer's protocol. SCD1 (Δ-9 desaturase): no. Hs01682761_m1 and FAS: no. Hs00188012_m1. RT-PCR was carried out on a 7900HT real time PCR machine from Applied Biosystems (Applied Biosystems, Foster City, CA, USA).

Statistical analysis Probability values (asymptotic) were considered statistically significant at a value of $P \leq 0.05$. Nonparametric tests were used due to the small sample size and values are given as median (25—75 percentile). Percent change is calculated from median values. Differences between the randomisation groups were analysed at end of study (baseline adjusted values). Delta values refer to values at end of study minus baseline values for the plasma parameters, while gene expression delta values refer to values at end of study divided with baseline values (fold change). The present study is considered an exploratory study and

therefore no adjustment for multiple testing was performed. Mann-Whitney U test and Wilcoxon matched-pair signed-rank test were used to either compare changes between groups or within-groups, respectively. Coefficients of correlation were calculated by the Spearman's rho test. The SPSS for Windows (version 18.0) was used for all statistical analyses.

RESULTS

Baseline characteristics There were no significant differences in baseline characteristics between the study groups **(Table 1).**

Intake of n-3 fatty acids from the intervention meals Daily intake of EPA and DHA provided from the seafood lunch meal were 1.4 and 1.7 g/day in the salmon group while the total daily n-3 fatty acids intake was 5.4 g **(Table 2).** Corresponding intake were 0.048 and 0.086 g/day in the cod group with a total n-3 intake of 0.15 g/day. The potatoes did not contain any marine n-3 fatty acids, but total intake of n-3 fatty acids (α-linolenic acid, 18:3n-3) was 0.14 g/day (Table 2).

Fatty acid profile in plasma PL Daily intake of 150 g fish for 15 days significantly increased the amount of total n-3 fatty acids (EPA and DHA) in plasma PL in both the cod ($P = 0.008$) and the salmon ($P < 0.001$) groups, compared to the control group (**Table 3**). DHA increased in the cod group ($P = 0.003$) while both EPA and DHA increased in the salmon group ($P < 0.001$ and $P = 0.001$, respectively), compared to the control group. The baseline values of EPA and DHA were not different between the groups, nor were any of the other fatty acids in the plasma PL.

Serum concentration of TAG, total-C, HDL-C and LDL-C As shown in **Table 4**, the serum concentration of TAG was significantly decreased both in the cod group ($P = 0.02$) and in the salmon group ($P = 0.003$) as compared with the control group. The reduction was significant within the cod ($P = 0.05$) and the salmon ($P = 0.008$) groups, corresponding to a reduction of 11 % and 22 %, respectively. No significant alteration in the TAG concentration was seen within the control group. Serum HDL-C was significantly increased after intake of salmon compared with the control group ($P = 0.009$), and the within-group increase was 5 % ($P = 0.02$). Serum total-C and serum LDL-C was not changed between or within any of the groups (Table 4).

The change in both EPA and DHA in plasma PL correlated negatively with the change in TAG (n = 30) (r = -0.5, P = 0.007 and r = -0.4, P = 0.04, respectively) (Data not shown).

Effects on 18:1n-9/18:0 ratio in plasma PL As shown in **Figure 1**, there was a significant reduction in the 18:1n-9/18:0 ratio in plasma PL in the salmon group compared with the control group (P = 0.004)**,** and a significant within-group reduction after intake of cod (P = 0.04) and salmon (P = 0.003) for two weeks. In contrast, there was no significant within-group change in the control group during the experiment (Figure 1).

Relationship between serum TAG, marine n-3 fatty acids and 18:1n-9/18:0 ratio in plasma PL As illustrated in **Figure 2**, there was a positive correlation (r = 0.5, P = 0.01) between change in the 18:1n-9/18:0 ratio and change in serum TAG (n = 30) (Figure 2). Since previous studies suggest that SCD1 might be regulated by EPA and DHA, we investigated whether the 18:1n-9/18:0 ratio was related to n-3 fatty acids in plasma PL. Indeed, as shown in **Figure 3**, there was a highly significant negative correlation between the increase in marine n-3 fatty acids in plasma PL and reduction in the 18:1n-9/18:0 ratio (n = 30) (r = -0.7, $P < 0.001$).

Effects on mRNA expression in PBMC There was no significant change in mRNA level of the two selected lipogenesis related genes in the two intervention groups when compared to the control group **(Figure 4)**. However, mRNA level of FAS was significantly increased within the salmon group (P = 0.008) (Figure 4).

DISCUSSION

In this exploratory study in healthy subjects we found that a short term intake of both lean and fatty fish decreased serum TAG levels (Table 4) and 18:1n-9/18:0 ratio in plasma PL (an estimated indication of SCD1 activity) (Figure 1). The marine n-3 fatty acid DHA in plasma PL increased significantly after intake of lean and fatty fish, while EPA increased only after fatty fish intake (Table 3).

Most studies have investigated health effects after intake of relatively high doses of n-3 fatty acids, often administered as supplements. In the present study n-3 fatty acids were provided as regular fish meals. The hypotriglyceridemic effect of fatty fish has been largely attributed to n-3 fatty acids, and our finding of a decrease in serum TAG in the salmon group is consistent with previous reports [12]. However, as shown in the present study also cod significantly reduced serum TAG. The main carriers of TAG in fasting plasma are VLDL, and in general the hypotriglyceridemic effect of fish could be related to reduced production, and/or to increased elimination of these lipoproteins. The present work focused only upon some aspects of TAG synthesis. TAG, CE and PL in VLDL preferably contain monounsaturated fatty acids, i.e. palmitoleic and oleic acid. Since SCD1 is the rate limiting enzyme for the synthesis of these fatty acids, one mechanism by which fish intake decreases serum TAG could be a reduced hepatic desaturase activity, caused directly or indirectly by some of the constituents in fish. Fish intake might influence the amount of SCD1 enzyme protein and during the past decade, several human intervention studies have focused on diet-induced gene interactions using PBMC as a model system [33, 34, 39, 40]. From the present work it would appear that fish intake might not affect the amount of enzyme protein in PBMC, since there was no alteration in mRNA levels of SCD1 in response to fish intake. Even so, this observation does not rule out the possibility that fish/n-3 fatty acids intake affect the hepatic SCD1 mRNA levels, which have been reported by others [25, 41-45]. Human PBMC display the expression

of genes involved in lipid metabolism [30-35, 46, 47] and recently we showed that n-3 fatty acids regulate lipid gene expression in *ex vivo* PBMC [48]. Human research has limitations in tissue availability except for blood samples which is readily and easily obtained. It has been shown that the expression of genes involved in lipid metabolism are regulated in PBMC in a similar pattern as in liver upon fasting [33] and several dietary intervention studies have shown that expression of genes involved in lipid metabolism is altered in PBMC after intervention [34, 35, 46, 47]. This indicates that PBMC is a potentially good model system in dietary intervention studies to study genes related to lipid metabolism. However, liver cells and PBMC do have different biochemical properties and the use of PBMC as a model system of hepatic activity is at its very beginning and weaknesses with this model system may exist. It is also likely that due to the small sample size in the present study, type 2 errors are liable to occur and a false effect on gene expression cannot be ruled out. The increase in FAS mRNA in PBMC after intake of salmon in the present work (Figure 4) is in contrast to previous observations from *in vitro* and mice studies where it has been shown that PUFA suppress the hepatic expression of FAS [25, 28, 29, 49-51]. Even though the present mRNA level of FAS in the salmon group has two outliers, the within-group change is still significant ($P = 0.02$) when these are removed from the analysis. However, Knight et al. showed that the gene expression of FAS and SCD1 was increased in mice liver injected with a synthetic activator of β-oxidation (a peroxisome-proliferator activated receptor alpha (PPARα) agonist) [52]. Thus, discrepancy exists regarding the effect of PUFA in the hepatic regulation of lipogenesis. Even though fish intake did not influence mRNA levels involved in synthesis of SCD1, the data seem to fit the hypothesis that n-3 fatty acids can reduce the *activit*y of SCD1, indirectly estimated in the present study by 18:1n-9/18:0 ratio, as has been previously reported by others [27]. However, this “desaturase index” approach is an indirect method to assess whether desaturases are inhibited, in lack of a more direct biochemical measure which is required to

demonstrate an inhibiting effect. Nevertheless, we suggest that fatty acids like EPA and DHA might serve as allosteric inhibitors. In support of this suggestion are the lowered 18:1n-9/18:0 ratio after intake of fatty fish (Figure 1), the positive correlation between the 18:1n-9/18:0 ratio and serum TAG (Figure 2), as well as the inverse relationship between the 18:1n-9/18:0 ratio and EPA and DHA (Figure 3). It would appear that various fatty acids compete for being incorporated into PL. Although, monounsaturated fatty acids seem to be the preferred ones for PL formation [53], both EPA and DHA can be incorporated into the same position in plasma PL. By mass action it is assumed that the level of plasma PL 18:1n-9 should decrease as the levels of EPA and DHA increase. Hence, other unsaturated fatty acids in plasma PL, in addition to EPA and DHA, should be inversely associated with the 18:1n-9/18:0 ratio. This is not the case in the present study. The change in linoleic acid (18:2n-6) is in fact positively associated with the change in 18:1n-9/18:0 ratio ($r = 0.5$, $P = 0.003$), while the change in arachidonic acid (20:4n-6) shows no association at all. It is however hard to appreciate the magnitude of this possible mass effect, as compared with the other suggested explanations for obtaining reduced desaturase indexes after fish intake. Even though the intake of cod contributed to only 0.13 g of marine n-3 fatty acids per day, the level of DHA (but not EPA) in plasma PL in this group increased significantly compared to the control group (Table 3), and both the level of DHA and EPA in plasma PL correlated positively with the treatment effect on TAG ($n = 30$) (data not shown). The n-3 fatty acids in cod are largely incorporated into PL, in contrast to fatty fish which mainly have the fatty acids incorporated in TAG in adipose tissue. We recently demonstrated that the bioavailability of n-3 fatty acids from krill oil (also mainly incorporated into PL [54]) is more efficient than n-3 fatty acids from fish oil (TAG) [55]. This may partly be the reason why we find positive health effects (reduced TAG concentration) also after cod intake, despite low levels of n-3 in cod. Previously, DHA has been demonstrated to have similar TAG-lowering effects as EPA [56-58] and the increase in

DHA observed in the cod group in the present study may at least partly explain the reduction in serum TAG.

Also other bioactive molecules in fish, like taurine, have been suggested to have TAG reducing effects [59]. Yanagita et al. found that when HepG2 cells were stimulated with taurine, there was a reduction in TAG in both cells and medium. They also found that taurine reduced the incorporation of [^{14}C]-labelled oleic acid into cellular TAG, suggesting the inhibition of TAG synthesis [59]. Or there may be a synergistic effect of taurine and marine n-3 fatty acids which have the reducing effect on TAG [18]. Since our data suggest that also lean fish may reduce serum TAG, it would appear that the TAG lowering effect of lean fish is relevant to reduce CVD risk, thus supporting a previous study by Leaf and Hatcher [20]. In addition to increased TAG levels being a risk factor of CVD, HDL-C levels correlate inversely with cardiovascular risk [60]. In the present study HDL-C levels are increased after intervention with fatty fish (Table 4), which is in line with a previous report [61]. There is a well-known inverse relationship between plasma TAG levels and HDL-C [62], and this may be the reason why salmon intake might increase HDL-C. However, there is a discrepancy in the n-3 fatty acids effect on HDL-C as reviewed by Harris [13].

In conclusion, both lean and fatty fish can increase the level of marine n-3 fatty acids in plasma PL, and reduce the serum TAG levels in healthy subjects after short term intervention. The hypotriglyceridemic action of dietary marine n-3 fatty acids can be related to a reduced 18:1n-9/18:0 ratio in plasma PL, but whether gene expression in PBMC is influenced is not clarified. Our results would seem to fit the hypothesis that components in both lean and fatty fish may lower serum TAG possibly by reducing the 18:1n-9/18:0 ratio related to allosteric inhibition of SCD1 activity, rather than by influencing the synthesis of enzyme protein.

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FIGURE LEGENDS

Figure 1: 18:1n-9/18:0 ratio in plasma phospholipids in the three intervention groups. Values are given as median with 25—75 percentiles. * $P < 0.05$ within-groups.

Figure 2: Relationship between the change in serum triacylglycerols concentration and corresponding change in the 18:1n9/18:0 ratio in plasma phospholipids in response to the intervention (n = 30). r = 0.453, $P = 0.001$.

Figure 3: Relationship between the increase in omega-3 fatty acids in plasma phospholipids and reduction in 18:1n-9/18:0 ratio ($n = 30$). r = -0.7, $P < 0.001$.

Figure 4: The fold change PBMC mRNA levels of lipogenetic enzymes in the three intervention groups (control ($n = 10$), cod ($n = 9$), or salmon ($n = 11$)). Target genes are related to the mean value of the endogenous controls TBP and GUSβ. Values are given as median with 25—75 percentiles. * $P < 0.05$ within-groups. FAS, Fatty acid synthase; GUSβ, Glucuronidase β; PBMC, peripheral blood mononuclear cells; SCD1, Stearoyl-CoA desaturase-1; TBP, TATA binding protein.