

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

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- 26 Reprints are not available.
- 27 Running title: Fish intake, desaturase activity and triacylglycerols.

28 **ABSTRACT**

29 Intake of fish and omega-3 (n-3) fatty acids is associated with reduced concentration of  
30 plasma triacylglycerols (TAG) but the mechanisms are not fully clarified. Stearoyl-CoA  
31 desaturase-1 (SCD1) activity, governing TAG synthesis, is affected by n-3 fatty acids.  
32 Peripheral blood mononuclear cells (PBMC) display expression of genes involved in lipid  
33 metabolism. The aim of the present study was to estimate whether intake of lean and fatty fish  
34 would influence n-3 fatty acids composition in plasma phospholipids (PL), serum TAG,  
35 18:1n-9/18:0 ratio in plasma PL, as well as PBMC gene expression of SCD1 and fatty acid  
36 synthase (FAS). Healthy males and females ( $n = 30$ ), aged 20—40, consumed daily for 15  
37 days either 150 g of cod, salmon, or potato (control). During intervention docosahexaenoic  
38 acid (DHA, 22:6n-3) increased in the cod group ( $P < 0.05$ ), while TAG concentration  
39 decreased ( $P < 0.05$ ). In the salmon group both eicosapentaenoic acid (EPA, 20:5n-3) and  
40 DHA increased ( $P < 0.05$ ) whereas TAG concentration and the 18:1n-9/18:0 ratio decreased  
41 ( $P < 0.05$ ). Reduction of the 18:1n-9/18:0 ratio was associated with a corresponding lowering  
42 of TAG ( $P < 0.05$ ) and an increase in EPA and DHA ( $P < 0.05$ ). The mRNA levels of SCD1  
43 and FAS in PBMC were not significantly altered after intake of cod or salmon when  
44 compared with the control group. In conclusion, both lean and fatty fish may lower TAG,  
45 possibly by reducing the 18:1n-9/18:0 ratio related to allosteric inhibition of SCD1 activity,  
46 rather than by influencing the synthesis of enzyme protein.

47

48 **Keywords**

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

50 **Abbreviations**

51	CE	Cholesterol esters
52	CVD	Cardiovascular disease
53	DHA	Docosahexaenoic acid
54	EPA	Eicosapentaenoic acid
55	FAS	Fatty acid synthase
56	GUS $\beta$	Glucuronidase-beta
57	HDL-C	High density lipoprotein cholesterol
58	LA	Linoleic acid
59	LDL-C	Low density lipoprotein cholesterol
60	n-3	Omega-3
61	PBMC	Peripheral blood mononuclear cells
62	PL	Phospholipids
63	PPAR $\alpha$	Peroxisome-proliferator activated receptor alpha
64	PUFA	Polyunsaturated fatty acids
65	SCD1	Stearoyl-CoA desaturase-1
66	SREBP1	Sterol regulatory element-binding protein 1
67	TBP	TATA binding protein
68	TAG	Triacylglycerols
69	VLDL	Very low density lipoprotein

## 70 INTRODUCTION

71 Numerous epidemiological studies have demonstrated reduced risk of cardiovascular diseases  
72 (CVD) in response to increased intake of fish or fish oils [1-3]. Especially fatty fish is a major  
73 source of long chain omega-3 polyunsaturated fatty acids (n-3 PUFA), such as  
74 eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Many of  
75 the beneficial health effects of fish and fish oils have been linked to intake of these fatty acids  
76 [4-10]. Serum triacylglycerols (TAG) is recognized as an independent risk factor of CVD [11]  
77 and marine n-3 fatty acids in high doses (>3g/day) have been shown to reduce serum TAG by  
78 25—30 % [12-15]. The magnitude of the TAG reducing effect seems to be dependent on n-3  
79 fatty acid dose and baseline TAG concentrations [14, 16]. The American heart association  
80 (AHA) recommend the consumption of a variety of fish (preferably fatty fish) at least twice a  
81 week as guidance for healthy people [17]. However, intake of lean fish is also known to  
82 provide health benefits [18-20] in which one effect is reduced serum TAG [20]. Whether the  
83 health beneficial effects of lean fish are related to the n-3 fatty acids content, or to other  
84 bioactive components, is not known.

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

95 activities in the liver, caused directly or indirectly by some of the constituents in fish. In  
96 general, the rate of an enzyme catalyzed reaction may be influenced by the amount of enzyme  
97 protein, by phosphorylation and dephosphorylation of the enzyme, or by allosteric regulation  
98 [23]. Oleic acid is a major constituent of TAG produced by *de novo* lipogenesis, and therefore  
99 the 18:1n-9/18:0 ratio, i.e. a product/precursor ratio, in plasma may be used to estimate SCD1  
100 activity [22, 24-26]. Animal studies have demonstrated that marine n-3 fatty acids can  
101 suppress hepatic lipogenesis [4], and one regulatory mechanism may be inhibition of SCD1  
102 activity [27] or by transcriptional regulation of sterol regulatory element-binding protein 1  
103 (SREBP1) [28]. SREBP1 is regulating the expression of lipogenic genes such as SCD1 and  
104 fatty acid synthase (FAS) [28], and an inhibition of SREBP1 by n-3 fatty acids will cause a  
105 down-regulation of these genes [29]. To study whether n-3 fatty acids might influence human  
106 hepatic gene expression *in vivo* is challenging. However, since human PBMC can display the  
107 expression of genes involved in lipid metabolism [30-35] we have chosen PBMC as a test  
108 system.

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

## 113 **SUBJECTS AND METHODS**

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

119 **Subjects** Thirty subjects (7 men and 23 women) aged 20—40 were recruited from Akershus  
120 University College, Lillestrøm, Norway. Healthy subjects with C-reactive protein (CRP)  $<10$   
121 mg/L using no medication, except for oral contraceptives (female subjects ( $n = 11$ ), all  
122 subjects maintained the use throughout the study, except for one subject in the control group),  
123 were included in this study. Smoking, pregnancy and lactation were exclusion criteria.

124 Additionally, subjects who had a habitual seafood consumption of more than three servings  
125 per week were excluded. All subjects were compliant with the protocol throughout the study.  
126 Compliance was assessed based on observations of the participants during the test meals,  
127 served at the University College and the amount of leftovers after the experimental period.  
128 Compliance was estimated to be 95—100 % in all three groups. The study protocol was

129 approved by Regional Committee of Medical Ethics in Norway. Written informed consent for  
130 participation was obtained from each subject and it complied with the Declaration of Helsinki.

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

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

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

146 **Blood sampling and biochemical analysis** Blood samples were collected from fasting  
147 subjects (minimum 12 h) at the same time (between 8 a.m. and 10 a.m.) at baseline and at end  
148 of study. PBMC were isolated using cell preparation tubes (CPT) according to the  
149 manufacturer's instructions (Becton, Dickinson and Company, NJ 07417, USA).  
150 Determination of serum total cholesterol (total-C), HDL cholesterol (HDL-C), LDL  
151 cholesterol (LDL-C), TAG and CRP was performed using routine laboratory methods (Først  
152 Medical laboratory, Norway). Plasma was obtained from EDTA tubes and kept frozen (-70  
153 °C) until analysis.



154 **Fatty acid composition in fish** Homogenous mixture of cod or salmon fillets were kept  
155 frozen (-20 °C) until analysis and total lipids were extracted by adding chloroform/methanol  
156 (2:1, vol/vol), and nonadecylic acid (19:0) was added as internal standard. The samples were  
157 filtered, saponified, and methylated using 12% BF<sub>3</sub> in methanol. Fatty acid composition of  
158 total lipids was analyzed using methods as described earlier [36, 37].

159 **Fatty acid composition in plasma PL** was determined as previously reported [38]. The  
160 18:1n-9/18:0 ratio was calculated from the fatty acid composition in plasma PL and used as an  
161 estimate of desaturase activity.

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

163 Total RNA was extracted from PBMC using a combination of TRIzol Reagent (Invitrogen,  
164 Carlsbad, CA, USA) and RNeasy mini kit (Qiagen, Hilden, Germany) purified with RNase-  
165 free DNase (Invitrogen). Subsequently the samples were stored in RNase-free water at -  
166 80°C. RNA quality was measured on an Agilent Bioanalyser 2100 system (Agilent  
167 Technologies, Santa Clara, CA, USA) and showed RNA Integrity Numbers (RIN) between  
168 8.7 and 9.8. Total RNA yield was measured on a Nanodrop ND-1000 Spectrophotometer  
169 (NanoDrop Technologies, Wilmington, Delaware, USA). For cDNA synthesis, 500 ng RNA  
170 of each total RNA sample was reverse-transcribed by Super Script (Invitrogen) according to  
171 the manufacturer's protocol, using oligo dT as primers. The Taqman real-time polymerase  
172 chain reaction (RT-PCR) technique was used to quantify the mRNA expression of each gene.  
173 cDNA corresponding to fifteen ng RNA was applied to each well and each sample was run in  
174 triplets. Quantification was performed using the relative standard curve method. A  
175 combination of aliquots from all cDNA samples was made and diluted in order to make a  
176 dilution curve that was included on each plate. The points on the standard curve corresponded  
177 to 50, 25, 12.5 and 6.25 ng RNA. The average of the three values measured per gene per

178 sample were divided by the average of the corresponding combined Glucuronidase-beta  
179 (GUS $\beta$ ) and TATA binding protein (TBP) values, generating a normalized value used to  
180 compare the relative amount for each gene in the different samples. GUS $\beta$  and TBP were  
181 chosen as endogenous genes due to the results from running a TaqMan Human Endogenous  
182 Control Plate-test from Applied Biosystems (data not shown). The primers and probes for  
183 GUS $\beta$  and TBP were initially designed as three assays per gene, and validated for efficiency  
184 and specificity. The best of the three was then chosen. The primers and probe for the  
185 GUS $\beta$  assay were: forward primer: 5'-GAAAATATGTGGTTGGAGAGCTCATT-3', probe:  
186 5'-CCAGCACTCTCGTCGGTGACTGTTCA-3' and reverse primer: 5'-  
187 CCGAGTGAAGATCCCCTTTTAA-3'. TBP forward primer: 5'-  
188 CTGGAAAAGTTGTATTAACAGGTGC-3', probe: 5'-  
189 AGCAGAAATTTATGAAGCATTTGAAAACATCTACCCTATT-3' and reverse primer: 5'-  
190 CATTACGTCGTCTTCCTGAATC-3'. All other genes were measured by "single tube"  
191 assays, which are a premade combination of primers and probe, specific for the gene to be  
192 determined (Applied Biosystems, Foster City, CA) and utilized according to the  
193 manufacturer's protocol. SCD1 ( $\Delta$ -9 desaturase): no. Hs01682761\_m1 and FAS: no.  
194 Hs00188012\_m1. RT-PCR was carried out on a 7900HT real time PCR machine from  
195 Applied Biosystems (Applied Biosystems, Foster City, CA, USA).

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

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

## 207 **RESULTS**

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

210 **Intake of n-3 fatty acids from the intervention meals** Daily intake of EPA and DHA  
211 provided from the seafood lunch meal were 1.4 and 1.7 g/day in the salmon group while the  
212 total daily n-3 fatty acids intake was 5.4 g (**Table 2**). Corresponding intake were 0.048 and  
213 0.086 g/day in the cod group with a total n-3 intake of 0.15 g/day. The potatoes did not  
214 contain any marine n-3 fatty acids, but total intake of n-3 fatty acids ( $\alpha$ -linolenic acid, 18:3n-  
215 3) was 0.14 g/day (Table 2).

216 **Fatty acid profile in plasma PL** Daily intake of 150 g fish for 15 days significantly  
217 increased the amount of total n-3 fatty acids (EPA and DHA) in plasma PL in both the cod ( $P$   
218 = 0.008) and the salmon ( $P < 0.001$ ) groups, compared to the control group (**Table 3**). DHA  
219 increased in the cod group ( $P = 0.003$ ) while both EPA and DHA increased in the salmon  
220 group ( $P < 0.001$  and  $P = 0.001$ , respectively), compared to the control group. The baseline  
221 values of EPA and DHA were not different between the groups, nor were any of the other  
222 fatty acids in the plasma PL.

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

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

234 **Effects on 18:1n-9/18:0 ratio in plasma PL** As shown in **Figure 1**, there was a significant  
235 reduction in the 18:1n-9/18:0 ratio in plasma PL in the salmon group compared with the  
236 control group ( $P = 0.004$ ), and a significant within-group reduction after intake of cod ( $P =$   
237  $0.04$ ) and salmon ( $P = 0.003$ ) for two weeks. In contrast, there was no significant within-  
238 group change in the control group during the experiment (Figure 1).

239 **Relationship between serum TAG, marine n-3 fatty acids and 18:1n-9/18:0 ratio in**  
240 **plasma PL** As illustrated in **Figure 2**, there was a positive correlation ( $r = 0.5$ ,  $P = 0.01$ )  
241 between change in the 18:1n-9/18:0 ratio and change in serum TAG ( $n = 30$ ) (Figure 2).

242 Since previous studies suggest that SCD1 might be regulated by EPA and DHA, we  
243 investigated whether the 18:1n-9/18:0 ratio was related to n-3 fatty acids in plasma PL.

244 Indeed, as shown in **Figure 3**, there was a highly significant negative correlation between the  
245 increase in marine n-3 fatty acids in plasma PL and reduction in the 18:1n-9/18:0 ratio ( $n =$   
246  $30$ ) ( $r = -0.7$ ,  $P < 0.001$ ).

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

## 251 **DISCUSSION**

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

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

276 of genes involved in lipid metabolism [30-35, 46, 47] and recently we showed that n-3 fatty  
277 acids regulate lipid gene expression in *ex vivo* PBMC [48]. Human research has limitations in  
278 tissue availability except for blood samples which is readily and easily obtained. It has been  
279 shown that the expression of genes involved in lipid metabolism are regulated in PBMC in a  
280 similar pattern as in liver upon fasting [33] and several dietary intervention studies have  
281 shown that expression of genes involved in lipid metabolism is altered in PBMC after  
282 intervention [34, 35, 46, 47]. This indicates that PBMC is a potentially good model system in  
283 dietary intervention studies to study genes related to lipid metabolism. However, liver cells  
284 and PBMC do have different biochemical properties and the use of PBMC as a model system  
285 of hepatic activity is at its very beginning and weaknesses with this model system may exist.  
286 It is also likely that due to the small sample size in the present study, type 2 errors are liable to  
287 occur and a false effect on gene expression cannot be ruled out. The increase in FAS mRNA  
288 in PBMC after intake of salmon in the present work (Figure 4) is in contrast to previous  
289 observations from *in vitro* and mice studies where it has been shown that PUFA suppress the  
290 hepatic expression of FAS [25, 28, 29, 49-51]. Even though the present mRNA level of FAS  
291 in the salmon group has two outliers, the within-group change is still significant ( $P = 0.02$ )  
292 when these are removed from the analysis. However, Knight et al. showed that the gene  
293 expression of FAS and SCD1 was increased in mice liver injected with a synthetic activator of  
294  $\beta$ -oxidation (a peroxisome-proliferator activated receptor alpha (PPAR $\alpha$ ) agonist) [52]. Thus,  
295 discrepancy exists regarding the effect of PUFA in the hepatic regulation of lipogenesis.  
296 Even though fish intake did not influence mRNA levels involved in synthesis of SCD1, the  
297 data seem to fit the hypothesis that n-3 fatty acids can reduce the *activity* of SCD1, indirectly  
298 estimated in the present study by 18:1n-9/18:0 ratio, as has been previously reported by others  
299 [27]. However, this “desaturase index” approach is an indirect method to assess whether  
300 desaturases are inhibited, in lack of a more direct biochemical measure which is required to

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



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

328 Also other bioactive molecules in fish, like taurine, have been suggested to have TAG  
329 reducing effects [59]. Yanagita et al. found that when HepG2 cells were stimulated with  
330 taurine, there was a reduction in TAG in both cells and medium. They also found that taurine  
331 reduced the incorporation of [<sup>14</sup>C]-labelled oleic acid into cellular TAG, suggesting the  
332 inhibition of TAG synthesis [59]. Or there may be a synergistic effect of taurine and marine n-  
333 3 fatty acids which have the reducing effect on TAG [18]. Since our data suggest that also  
334 lean fish may reduce serum TAG, it would appear that the TAG lowering effect of lean fish is  
335 relevant to reduce CVD risk, thus supporting a previous study by Leaf and Hatcher [20]. In  
336 addition to increased TAG levels being a risk factor of CVD, HDL-C levels correlate  
337 inversely with cardiovascular risk [60]. In the present study HDL-C levels are increased after  
338 intervention with fatty fish (Table 4), which is in line with a previous report [61]. There is a  
339 well-known inverse relationship between plasma TAG levels and HDL-C [62], and this may  
340 be the reason why salmon intake might increase HDL-C. However, there is a discrepancy in  
341 the n-3 fatty acids effect on HDL-C as reviewed by Harris [13].

342 In conclusion, both lean and fatty fish can increase the level of marine n-3 fatty acids in  
343 plasma PL, and reduce the serum TAG levels in healthy subjects after short term intervention.  
344 The hypotriglyceridemic action of dietary marine n-3 fatty acids can be related to a reduced  
345 18:1n-9/18:0 ratio in plasma PL, but whether gene expression in PBMC is influenced is not  
346 clarified. Our results would seem to fit the hypothesis that components in both lean and fatty  
347 fish may lower serum TAG possibly by reducing the 18:1n-9/18:0 ratio related to allosteric  
348 inhibition of SCD1 activity, rather than by influencing the synthesis of enzyme protein.  
349

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562

## 563 FIGURE LEGENDS

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

566

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

570

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

573

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