Plasma fatty acid levels and gene expression related to lipid metabolism in peripheral blood mononuclear cells: a cross-sectional study in healthy subjects

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33 **Abstract**

34 Background: Solid evidence indicates that intake of marine n-3 fatty acids lower serum
35 triglycerides, and that replacing saturated fatty acids (SFA) with polyunsaturated fatty acids
36 (PUFA) reduces plasma total cholesterol and LDL-cholesterol. The molecular mechanisms
37 underlying these health beneficial effects are however not completely elucidated. The aim of
38 this study was therefore to investigate the expression of genes related to lipid metabolism in
39 peripheral blood mononuclear cells (PBMC) depending on the plasma levels of n-6 and n-3
40 fatty acids and the SFA to PUFA ratio.

41

42 Methods**:** Fifty-four healthy subjects were grouped into tertiles (n=18) based on plasma levels
43 of n-6 and n-3 fatty acids and the SFA to PUFA ratio. The PBMC gene expression levels
44 among subjects in the highest versus the lowest tertiles were compared. In total, 285 genes
45 related to cholesterol and triglyceride metabolism were selected for this explorative study.

46

47 Results: Among the 285 selected genes, 161 were defined as expressed in the PBMCs. The
48 plasma SFA to PUFA ratio was associated with the highest number of significantly different
49 expressed genes (25 gene transcripts), followed by plasma n-6 fatty acid level (15 gene
50 transcripts) and plasma n-3 fatty acid level (8 gene transcripts). In particular, genes involved
51 in cholesterol homeostasis were significantly different expressed among subjects with high
52 compared to low plasma SFA to PUFA ratio.

53

54 Conclusion: Genes involved in lipid metabolism were differentially expressed in PBMCs
55 depending on the plasma fatty acid levels. This finding may increase our understanding of
56 how fatty acids influence lipid metabolism at a molecular level in humans.

57 **Introduction**

58 Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide [1].
59 Dyslipidemia, including elevated levels of plasma total cholesterol (total-C), low density
60 lipoprotein cholesterol (LDL-C) and triglycerides (TG), is a major risk factor for CVD.
61 Dietary fatty acids play a significant role in modulating plasma lipids, thereby influencing the
62 CVD risk [2]. Solid evidence indicates that intake of marine n-3 fatty acids, and replacing
63 saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA), prevents CVD and CVD
64 mortality [3-7]. One of the CVD reducing effects of marine n-3 fatty acids is the TG lowering
65 effect, while replacing SFAs with PUFAs has been shown in several randomised controlled
66 trials to reduce plasma total- and LDL-C [8-10]. Animal studies and *in vitro* experiments have
67 demonstrated different molecular mechanisms of how marine n-3 fatty acids reduce hepatic
68 very low density lipoprotein (VLDL) production and increase the VLDL clearance [11, 12].
69 The molecular mechanisms behind the total- and LDL-C lowering effects of replacing SFAs
70 with PUFAs are however less clear. Therefore, studies investigating the molecular
71 mechanisms underlying the health effects of SFAs and PUFAs in humans are warranted.

72 In humans, linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3) are
73 not biosynthesized *de novo*. Since the conversion of these fatty acids into long chain PUFAs
74 is limited, plasma PUFA levels have been shown to be objective biomarkers of dietary intake
75 [13, 14]. Hence, using plasma fatty acids is an alternative approach to examine the association
76 between dietary fat quality and CVD risk.

77 The ability of fatty acids to regulate gene transcription may account for their effects on
78 lipid metabolism. Fatty acids regulate gene transcription directly by binding as ligands to
79 specific transcription factors, thereby controlling the activity of the transcription factor, or
80 indirectly by regulating different signalling pathways controlling the nuclear abundance of
81 transcription factors [15-17]. In particular, there is considerable evidence that PUFAs
82 modulate the transcription of genes involved in lipid metabolism by regulating the activity of
83 the nuclear receptors peroxisome proliferator activated receptors (PPAR) and liver x receptors
84 (LXR), or by suppressing the nuclear abundance of the sterol regulatory binding proteins
85 (SREBP) [17, 18]. Our understanding of how SFAs modulate the expression of genes
86 encoding proteins related to lipid metabolism is however more limited [19].

87 In order to get a comprehensive understanding of how dietary fat quality affect lipid
88 metabolism, to prevent dyslipidemia in humans, we need a suitable model system. Changes in
89 gene expression occur prior to changes in protein levels, and gene expression analysis is

90 therefore a valuable and sensitive technique measuring early changes related to diet [20, 21].
91 Peripheral blood mononuclear cells (PBMC) include lymphocytes and monocytes which
92 circulate around in the body and are exposed to both environmental factors and metabolic
93 tissues. Studies have shown that PBMCs may be used as a surrogate model for liver
94 metabolism since these cells reflect hepatic regulation of cholesterol metabolism as well as
95 metabolic and immune responses [22-26].

96 Some postprandial studies have examined the effect of fat intake on the mRNA level
97 of genes involved in lipid metabolism in PBMCs [26, 27]. To our knowledge, no studies have
98 particularly focused on the impact of plasma fatty acid levels on PBMC gene expression
99 related to lipid metabolism. The aim of the present study was therefore to investigate the
100 relation of plasma levels of n-6 and n-3 fatty acids, and SFA to PUFA ratio, to PBMC gene
101 expression specifically related to lipid metabolism using cross-sectional data from a human
102 intervention study [28].

103 **Materials and methods**

104

105 Study design and participants

106

107 Fifty-four healthy, non-smoking men and women aged 18-50 years were included in this
108 cross-sectional sub-study of a randomised controlled trial designed to investigate the health
109 effects of fish oil with different quality focusing on lipids, oxidative stress, and inflammation
110 [28, 29]. In addition, we have analysed the plasma lipidomic profile, the PBMC gene
111 expression profile, and the effects on lipoprotein subclasses from this dietary intervention
112 study [30-33]. A detailed description of the protocol, participant recruitment and enrolment,
113 inclusion and exclusion criteria, and compliance is described elsewhere [28]. In the present
114 study, data from the end of intervention was utilized.

115 The study population was grouped into tertiles three times according to the plasma
116 fatty acid levels and the SFA to PUFA ratio by arranging samples from the highest to the
117 lowest value. First, the subjects were grouped according to the plasma level of total n-3 fatty
118 acids which included ALA, eicosapentaenoic acid (EPA; 20:5n-3), docosapentaenoic acid
119 (DPA; 22:5n-3), and docosahexaenoic acid (DHA; 22:6n-3). Second, the subjects were
120 grouped according to the plasma level of total n-6 fatty acids which included LA and
121 arachidonic acid (AA; 20:4n-6). Finally the subjects were grouped according to the plasma
122 SFA to PUFA ratio, which included the SFAs myristic acid (14:0), palmitic acid (16:0) and
123 stearic acid (18:0), and the PUFA included the sum of plasma total levels of n-3 and n-6 fatty
124 acids. The subjects in the highest (n=18) and the lowest (n=18) tertile were compared.

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

130

131 Clinical and biochemical measurements

132

133 Procedures regarding clinical and biochemical measurements have been described earlier [28].
134 In brief, fasting venous blood samples were collected after an overnight fast (≥12 h). Serum
135 was obtained from silica gel tubes (Becton Dickinson Vacutainer Systems, UK) and kept at
136 room temperature for 30 min before centrifugation (1500 g, 12 min). Plasma was obtained
137 from EDTA tubes (Becton Dickinson Vacutainer Systems, UK), immediately placed on ice

and centrifuged within 10 min (1500 g, 4°C, 10 min). EDTA tubes with whole blood were kept at room temperature for a maximum of 48 h before counting the total number of lymphocytes and monocytes. Fasting serum concentrations of total-C, LDL-C, HDL-C, and TGs, as well as lymphocyte and monocyte counts, were measured by standard methods in a routine clinical laboratory (Fürst Medical Laboratory, Oslo, Norway).

Fatty acids analysis

Plasma lipids were extracted by use of the Bligh and Dyer method [34], as described by Ottestad et al [28]. Fatty acids in Bligh and Dyer extract were derivatised and analysed as methyl esters on a GC (HP 6890) equipped with a BPX-70 column (SGE Analytical Science Private Limited, Melbourne, Australia). The plasma level of the individual fatty acids is expressed as mass percentage (% wt) of total fatty acids in plasma.

PBMC and RNA isolation

PBMCs were isolated by using the BD Vacutainer Cell Preparation tubes according to the manufacturer`s instructions (Becton, Dickinson San Jose, CA, USA), as described previously [31]. This is a well-documented and standardised method to collect mononuclear cells with high purity (above 90 %), and according to the manufacturer, approximately 80 % of the cells are lymphocytes and 12 % are monocytes. Pellets were stored at -80 °C for further RNA analysis. Total RNA was isolated from the PBMC samples using RNeasy Mini Kit (Qiagen). RNA quantity and quality were measured using the ND 1000 Spectrophotometer (Seven Werner AB) and Agilent bioanalyser (Agilent Technologies Inc.), respectively.

Microarray analysis and selection of genes

Gene expression was analysed by hybridization to an Illumina HumanHT-12 v4 Expression BeadChip and scanned on an Illumina HiSCan microarray scanner (Illumina Inc., CA 92122). Illumina GenomeStudio was used to transform bead-level data to probe-level intensities and statistics, which were exported raw for bioinformatics analysis. Quantile normalization of the Illumina intensities was performed, and probes without a detectable expression (detection P >0.01) in at least 10% of the samples were excluded from further analyses. From the 48 000 probes presented on the Illumina array, 21 236 probes were defined

as expressed in the current study. A more detailed description of the protocol is given elsewhere [31]. The raw data are available from the Gene Expression Ominbus (GEO) (accession number GSE111567).

A total of 285 genes encoding proteins related to cholesterol- and TG metabolism were selected for this explorative study. The genes were selected based on relevant gene sets related to cholesterol- and TG metabolism (26 gene sets) in the Molecular Signature Database v6.0 [35] limited to collection C5 (Gene ontology, GO), as well as literature summarising loci associated with different lipid traits [36]. Out of the 285 genes, 161 genes were defined as expressed on the HumanHT-12 v4 microarray and included in the statistical analyses (Additional file 1). The list of the differentially expressed genes was based on the lowest P-values for genes containing multiple probe set. The expression levels of the differential expressed genes which were expressed by more than one probe is shown in Additional files 2-4.

Statistical analysis

Differences in Log2 gene expression between subjects in the highest and lowest tertiles were compared by Independent Samples t-test. No adjustment for multiple testing was performed because of the explorative design of the study. Significantly different expressed genes were further correlated with clinical and biochemical parameters by Pearson's correlation. Differences in subject characteristics and plasma fatty acid levels between subjects in the highest and lowest tertiles were compared by Independent Samples t-test or Mann-Whitney U test when normally and not normally distributed, respectively. All statistical analyses were performed using R open source software version 3.4.1 [37]. P-values <0.05 were considered significant.

Results

Subject characteristics and plasma fatty acid levels

The subjects included in the study were young and middle-aged adults (27 ± 7.2 years) with BMI (22.6 ± 2.6 kg/m^2) and serum lipids within the normal range as shown in table 1. There was a skewed distribution of men and women (fifteen men and thirty-nine women) among the subjects. The plasma levels of fatty acids in the study population are shown in table 1.

Insert table 1

The characteristics and plasma fatty acid levels of subjects in the highest (n=18) and lowest (n=18) tertiles based on plasma levels of n-6 and n-3 fatty acids, and SFA to PUFA ratio, are presented in table 2. The serum level of TG was significantly lower among subjects in the highest compared to subjects in the lowest plasma n-6 fatty acid tertile (P <0.01). In contrast, the serum level of TG was significantly higher among subjects in the highest compared to subjects in the lowest plasma SFA to PUFA ratio tertile (P <0.01). There were no significant differences in any markers between subjects in the highest and the lowest plasma n-3 fatty acid tertile.

As expected, the plasma levels of EPA, DPA, and DHA were significantly higher among subjects in the highest compared to subjects in the lowest plasma n-3 fatty acid tertile (P <0.01, P <0.01 and P <0.01, respectively) (Table 2). The plasma levels of myristic- and palmitic acid were significantly higher among subjects in the highest compared to subjects in the lowest plasma SFA to PUFA ratio tertile (P <0.01 and P <0.01, respectively), and significantly lower among subjects in the highest compared to subjects in the lowest plasma n-6 fatty acid tertile (P <0.01 and P <0.01, respectively). In addition, the plasma levels of LA and AA were significantly higher among subjects in the highest compared to subjects in the lowest plasma n-6 fatty acid tertile (P <0.01 and P= 0.02, respectively). The percentage of monocytes and lymphocytes did not significantly differ among subjects in the highest compared to the lowest plasma n-3 fatty acid tertile, n-6 fatty acid tertile, and the SFA to PUFA ratio tertile, respectively (Table 2).

Insert table 2

234 PBMC gene expression
235
236 Out of the 161 mRNA transcripts included in the study, 41 were significantly different
237 expressed depending on plasma fatty acid levels and the SFA to PUFA ratio (Figure 1). The
238 plasma SFA to PUFA ratio was associated with the highest number of significantly different
239 expressed genes (25 gene transcripts, P <0.05), followed by plasma n-6 fatty acid level (15
240 gene transcripts, P <0.05), and n-3 fatty acid level (8 gene transcripts, P <0.05), as shown in
241 figure 1. Seven gene transcripts were associated with both plasma n-6 fatty acid level and
242 plasma SFA to PUFA ratio, and one gene transcript was associated with both plasma n-3 fatty
243 acid level and plasma SFA to PUFA ratio (Figure 2). No gene transcripts were shared across
244 plasma n-6 and n-3 fatty acid levels and plasma SFA to PUFA ratio. Differentially expressed
245 genes associated with the plasma levels of n-6 and n-3 fatty acids and the SFA to PUFA ratio
246 are presented in table 3-5, respectively.
247
248
249 *Insert tables 3-5*
250
251 Differentially expressed genes between subjects in the highest and lowest plasma SFA
252 to PUFA ratio tertile included genes encoding proteins involved in cholesterol homeostasis
253 (table 5). The mRNA levels of *insulin induced gene 2* (INSIG2), *ER lipid raft associated 2*
254 (ERLIN2), *Caveolin 1* (CAV1) and *COPII subunit SEC24* (SEC24) were significantly higher
255 expressed among subjects in the highest compared to subjects in the lowest plasma SFA to
256 PUFA ratio tertile. The mRNA levels of *scavenger receptor class B member 1* (SCARB1,)
257 *ATP binding cassette subfamily A member 2* (ABCA2) and *3-hydroxy-3-methylglutaryl-CoA*
258 *reductase* (HMGCR) were significantly lower expressed among subjects in the highest
259 compared to subjects in the lowest plasma SFA to PUFA ratio tertile. Interestingly, several of
260 the differentially expressed genes associated with plasma SFA to PUFA ratio, including
261 CAV1 and HMGCR, were opposite differentially expressed when comparing subjects in the
262 highest versus the lowest plasma n-6 fatty acid tertile.
263 In order to examine whether the differentially expressed genes were associated with
264 BMI or serum lipids, correlation analyses were performed (table 6). BMI was significantly
265 positively correlated to the expression levels of two genes (LACTB and SNX13), and
266 significantly negatively correlated to the expression levels of five genes (ERLIN2, GSK3B,
267 KLHL8, SCARB1 and SELS). The serum level of TG was significantly negatively correlated

268 to the expression levels of five genes (DAGLB, GSK3B, KAT5, KLHL8 and SCARB1). The
269 serum levels of total-C and LDL-C were significantly negatively correlated to the expression
270 levels of two genes (FAM117B and KAT5) and one gene (LRP5), respectively.
271
272 *Insert tables 6*
273 Even if the percentage number of lymphocytes and monocytes was constant in the
274 three comparisons, we also checked if cell subset specific genes were differently expressed
275 between the groups. We did not observe any significant alterations in the gene expression
276 levels of the B-cell-specific gene *CD20*, the T helper cell specific gene *CD4* and the
277 monocyte specific gene *CD14* between the subjects in any of the groups. These findings
278 suggest that there is no indication that there is a change in B lymphocyte/monocyte ratio in
279 the comparisons. However, we observed a significant lower mRNA level of the cytotoxic T
280 lymphocyte specific gene *CD8A* in the high versus low plasma n-6 level group, and
281 significant higher mRNA levels of *CD8A* and *CD8B* in the high versus low plasma SFA to
282 PUFA group. This may indicate that plasma fatty acids may influence the T
283 lymphocyte/monocyte ratio.
284
285 **Discussion**
286
287 In this explorative study, we investigated the potential relation of plasma n-6 and n-3 fatty
288 acid levels, and plasma SFA to PUFA ratio, to PBMC gene expression related to lipid
289 metabolism in healthy subjects. The plasma SFA to PUFA ratio was associated with the
290 highest number of differentially expressed genes, followed by plasma n-6 and n-3 fatty acid
291 level. In particular genes involved in cholesterol metabolism were differentially expressed.
292 PBMCs have previously been shown to reflect hepatic lipid metabolism during fasting
293 [23]. In addition, genes related to lipid metabolism have been shown to be differentially
294 expressed in PBMCs after acute meal studies with different fat qualities [26, 27]. In the
295 present study we used a targeted approach to investigate whether 285 genes encoding proteins
296 related to cholesterol and TG metabolism were differentially expressed depending on plasma
297 fatty acid levels. Among the 285 genes, a total of 161 genes were expressed in the PBMCs.
298 Interestingly, it seems like the plasma SFA to PUFA ratio is a stronger determinant than the
299 plasma levels of n-6 and n-3 fatty acids alone regarding the potential of influencing gene
300 expression levels in PBMCs. PBMCs may therefore function as a good model system to get a

301 better understanding of how genes involved in lipid metabolism are regulated by the plasma
302 fatty acid levels and in particular the SFA to PUFA ratio.

303 Genes involved in the regulation of cholesterol homeostasis were differentially
304 expressed among subjects with high compared to low plasma SFA to PUFA ratio. ABCA2
305 encodes a member of the ATP-binding cassette (ABC) transporters, a subfamily of
306 transporters that have been functionally linked to intracellular lipid transport [38]. The mRNA
307 level of ABCA2 was significantly lower among subjects with high compared to low plasma
308 SFA to PUFA ratio. It has been demonstrated that ABCA2 positively regulates low-density
309 lipoprotein receptor (LDLR) mRNA expression and negatively regulates cholesterol
310 esterification in hamster ovary cells [39]. In addition, it has been shown that overexpression
311 of ABCA2 in neuroblastoma cells results in decreased cellular cholesterol levels [40]. Thus
312 our findings suggest that a lower ABCA2 expression level may lead to lower uptake and
313 lower synthesis of cholesterol.

314 Because most cells in the periphery of the body do not express pathways for
315 catabolizing cholesterol, efflux of cholesterol is critical for maintaining cholesterol
316 homeostasis. SCARB1 and CAV1 are genes encoding proteins involved in cholesterol efflux.
317 The mRNA level of CAV1 was significantly higher among subjects with high compared to
318 low plasma SFA to PUFA ratio. It has been shown that CAV1 regulates the ATP binding
319 cassette subfamily G member 1 (ABCG1) mediated efflux of cholesterol, probably by
320 regulating ABCG1 trafficking to the cell surface [41], and a potential increase in CAV1 will
321 subsequently lead to increased cholesterol efflux through ABCG1. Interestingly, there was
322 also a significantly lower mRNA level of CAV1 among subjects with high compared to low
323 plasma n-6 fatty acid level. As n-6 fatty acids are the majority of total plasma PUFAs, this
324 finding may suggest that SFAs and PUFAs may exert different effects on cholesterol efflux
325 pathways. We did not observe a significant difference in the mRNA level of ABCG1 among
326 subjects with high compared to low plasma SFA to PUFA ratio. However, there was a
327 significantly lower mRNA level of SCARB1, which encodes another plasma membrane
328 receptor mediating cholesterol transfer to and from HDL. In contrast to ABCG1, which
329 mediate cholesterol efflux via active transport, SCARB1 mediates cholesterol transport to and
330 from HDL via passive facilitated diffusion [42]. In cholesterol loaded mouse macrophages
331 incubated with diluted human serum it has been shown that cholesterol efflux is mainly
332 mediated by active transport [43]. We therefore speculate that there may be a higher efflux of
333 cholesterol via active transport than by passive transport in response to a high intracellular

334 cholesterol load, which may explain the lower expression level of SCARB1 in subjects with
335 high compared to low plasma SFA to PUFA ratio.

336 In addition, there was a significantly lower mRNA level of HMGCR among subjects
337 with high compared to low SFA to PUFA ratio, and the mRNA level of HMGCR was higher
338 among subjects with high compared to low plasma n-6 PUFA level. HMGCR encodes the rate
339 limiting step in the cholesterol biosynthesis pathway, and a lower expression of this gene will
340 subsequently lead to reduced synthesis of cholesterol. The finding in the present study is in
341 line with a recent postprandial study investing the effects of SFA in lean and obese subjects
342 [27]. It is interesting that although the above mentioned study investigated the acute effects of
343 a high fat meal, we were able to observe a similar change in the mRNA level of HMGCR
344 among subjects with higher plasma SFA to PUFA ratio compared to subjects with lower
345 plasma SFA to PUFA ratio. The transcription of HMGCR is regulated by sterol regulatory
346 element-binding proteins (SREBPs) [44]. SREBP2 stimulates the transcription of genes
347 involved in cholesterol biosynthesis- and uptake [45]. We did not observe a significant
348 difference in the mRNA level of SREBP2 among subjects with high compared to low SFA to
349 PUFA ratio, nor did we observe a significant difference in the expression level of the *LDL*
350 *receptor* (LDLR) which is also regulated by SREBP2. However, several genes involved in the
351 proteolytic regulation of SREBP2, including INSIG2 and ERLIN2, were significantly higher
352 expressed among subjects with high compared to low SFA to PUFA ratio. INSIG2 binds to
353 cholesterol loaded sterol binding proteins (SCAPs) in the endoplasmic reticulum (ER) and
354 prevents the movement of the SCAP-SREBP complex to the Golgi apparatus for further
355 processing and eventually transcription of HMGCR and other SREBP target genes. In
356 addition, INSIG proteins play an important role in oxysterol regulated cleavage of SREBPs
357 [46]. ERLIN2 encodes a cholesterol sensing protein which has been suggested to stabilize the
358 SREBP-SCAP-INSIG complex in the ER [47]. This gene was also significantly negatively
359 correlated with BMI, thereby implicating that BMI impact on intracellular cholesterol levels.
360 Thus, a higher mRNA level of INSIG2 and ERLIN2 is in line with the lower mRNA level of
361 HMGCR observed in the present study. Additionally, we observed a higher mRNA level of
362 SEC24 among subjects with high compared to low plasma SFA to PUFA ratio. SEC24 is a
363 component of the COP11-coated vesicles, which transport the SREBP-SCAP complex to the
364 Golgi. When ER cholesterol rises above a threshold of total lipids, the cholesterol binds to
365 SCAP, which triggers a conformational change in the protein that occludes the binding of the
366 COPII proteins [48]. We speculate that a higher expression level of SEC24 may be a response

367 to a higher intracellular cholesterol level among subjects with high compared to low plasma
368 SFA to PUFA ratio.
369 Although we did not observe differences in serum cholesterol levels among subjects
370 with high compared to low plasma SFA to PUFA ratio, the differentially expressed genes
371 observed in the present study may reflect an intracellular status of excess cholesterol. Changes
372 in the expression of genes occur prior to changes at protein level, and these findings may
373 therefore reflect early changes related to diet. The question remains how the above mentioned
374 genes are potentially regulated by changes in the plasma SFA to PUFA ratio. It is well known
375 that dietary intake PUFA, in particular of LA, AA, EPA and DHA correlate with their
376 respective percentages in plasma total fatty acids [14]. However, the total plasma fatty acid
377 profile may not reflect the total dietary intake of fat as the fatty acid composition in different
378 lipid fractions differs depending on the fat intake [49]. However, we have shown recently in a
379 dietary intervention study where SFA were replaced with PUFA that the total plasma fatty
380 acid profile reflected dietary fat intake changes [8]. It is clear that fatty acids have the ability
381 to regulate the expression of genes involved in lipid metabolism. PUFAs have been shown to
382 decrease nuclear SREBP-1 protein levels in part by inhibition of the interaction of oxysterols
383 with LXR, however this mechanism does not seem to affect SREBP2 [50]. The findings in the
384 present study may therefore be explained by the plasma levels of SFAs. Recent findings
385 suggest that SFAs may decrease SREBP activity directly, but the exact mechanisms whereby
386 SFAs may exert their effects on SREBP and its downstream targets remain to be established
387 [19].
388 The present study has several strengths. Gene expression profiling in PBMCs has been
389 shown to be more sensitive to dietary changes than the traditional biochemical parameters in
390 the circulation, and we specifically choose genes involved in biological processes related to
391 TG and cholesterol metabolism. In the present study we have compared the mRNA levels
392 with plasma levels of n-3, n-6 and the SFA to PUFA ratio. Since intake of the n-3 and the n-6
393 fatty acids are reflected in the plasma total fatty acid composition, our data indicate that intake
394 of these fatty acids may cause differences in gene expression. The major limitation of the
395 present study is the limited number of subjects. Due to the small number of subjects, the
396 subjects were separated into groups based on tertiles, which may have had an impact on the
397 number of significantly differentially expressed genes observed between the highest and
398 lowest tertiles. However, since we used end of study samples from a fish oil intervention, we
399 could at least expect a larger variation in plasma n-3 fatty acid level among the subjects.
400 Another limitation is that that there may be a different T lymphocyte/monocyte ratio in the n-

401 6 tertile groups and the SFA to PUFA tertile groups. Whether the mRNA expression levels of
402 CD8A and CD8B are linked to the plasma level of the fatty acids, or by the change in the
403 number of subset cells cannot be determined by this cross-sectional study. Another limitation is
404 the lack of PBMC material to perform protein measurements to validate our mRNA results. Since this
405 was an explorative study we did not adjust for multiple testing. Although no causal
406 relationship can be made due to the cross-sectional design of the study, the current study
407 shows that the plasma fatty acid levels can influence the PBMC expression of genes involved
408 in lipid metabolism.

409

410 **Conclusion**

411

412 In conclusion, the main findings in the present study were that PBMCs express genes
413 involved in hepatic lipid metabolism, and that the expression of several of the genes was
414 influenced by plasma fatty acid levels. This finding supports the use of PBMCs as a model
415 system for investigating the role dietary n-3 and n-6 fatty acids on gene expression related to
416 lipid metabolism. The plasma SFA to PUFA ratio seems to be more important than the plasma
417 n-6 and n-3 fatty acid level alone with regards to influencing mRNA levels. In particular,
418 genes involved in cholesterol homeostasis were significantly different expressed among
419 subjects with high compared to low plasma SFA to PUFA ratio. This may reflect an
420 intracellular status of excess cholesterol among subjects with high plasma SFA to PUFA ratio.
421 The current findings should be further studied in experimental studies and tested in well
422 controlled human dietary intervention studies.

Additional files

Additional file 1: Selection of genes related to triglyceride- and cholesterol metabolism (285 genes). Genes expressed in peripheral blood mononuclear cells are in bold (161 genes) (docx).

Additional file 2: Differentially expressed genes associated with plasma n-6 level and expressed by more than one probe (docx).

Additional file 3: Differentially expressed genes associated with plasma n-3 level and expressed by more than one probe (docx).

Additional file 4: Differentially expressed genes associated with plasma SFA to PUFA ratio and expressed by more than one probe (docx).

Declaration

Ethics approval and consent to participate

The intervention 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.clinicaltrial.gov (ID no. NCT01034423).

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Competing interests

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457

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462

463 **Authors' contributions**

464 SVL designed research, selected the genes, performed statistical analyses and analysed data,
465 and drafted the manuscript. KBH designed research, analysed data, and drafted the manuscript.
466 IO designed research, coordinated the intervention study, and drafted the manuscript. KND
467 designed research and selected the genes. MCWM designed research, performed microarray
468 analysis, analysed data and drafted the manuscript. SMU designed research, was responsible
469 for the intervention study, analysed data, and drafted the manuscript. All authors read and
470 approved the final manuscript.

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Table 1. Characteristics and plasma fatty acid profile of the study population

	n 54
Male/female (n)	15/39
Age (years)	25.0 (22.0-30.0)
BMI (kg/m^2)	22.7 ± 2.6
Total-C (mmol/l)	4.8 ± 0.9
LDL-C (mmol/l)	2.7 ± 0.8
HDL-C (mmol/l)	1.5 ± 0.4
TG (mmol/l)	0.9 (0.7-1.1)
Plasma level of fatty acids (wt %)	
Total SFA	30.7 (29.5-31.9)
Myristic acid (14:0)	0.9 (0.7-1.1)
Palmitic acid (16:0)	21.5 (20.6-23.3)
Stearic acid (18:0)	7.9 ± 1.0
Total n-6	34.7 ± 3.9
LA (18:2n-6)	28.8 ± 3.6
AA (20:4n-6)	5.9 ± 1.09
Total n-3	6.3 ± 2.4
ALA (18:3n-3)	0.5 (0.5-0.6)
EPA (20:5n-3)	1.94 (0.7-2.7)
DPA (22:5n-3)	0.6 ± 0.2
DHA (22:6n-3)	3.2 ± (2.1-4.1)
Total PUFA	40.1 (38.9-48.1)
SFA to PUFA ratio	0.8 ± (0.7-0.8)

BMI, body mass index; Total-C, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglyceride; SFA, saturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid; PUFA, polyunsaturated fatty acid. Values are presented as frequency, mean ± SD or median and 25th-75th percentiles.

Table 2. Characteristics and plasma fatty acid profile of subjects in the highest and lowest tertiles

	n-6 level			n-3 level			SFA/PUFA ratio		
	Highest tertile (n 18)	Lowest tertile (n 18)	*P*	Highest tertile (n 18)	Lowest tertile (n 18)	*P*	Highest tertile (n 18)	Lowest tertile (n 18)	*P*
Male/female	5/13	5/13		3/15	5/13		5/13	6/12	
Age (years)	25 (23-33)	25 (21-29)	0.31	25 (21-31)	24 (21-28)	0.80	25 (22-29)	28 (23-33)	0.38
BMI (kg/m^2)	22.5 ± 2.9	22.6 ± 2.6	0.94	22.2 ± 2	22.7 ± 3.3	0.57	23.3 ± 2.7	22.6 ± 2.6	0.41
Total-C (mmol/l)	4.9 ± 0.9	4.7 ± 1.1	0.65	4.9 ± 1	4.9 ± 1	0.96	4.7 ± 0.8	5.0 ± 0.9	0.25
LDL-C (mmol/l)	2.7 ± 0.8	2.6 ± 0.9	0.79	2.6 ± 0.9	2.8 ± 0.6	0.71	2.5 ± 0.6	2.8 ± 0.9	0.16
HDL-C (mmol/l)	1.6 ± 0.4	1.4 ± 0.3	0.15	1.6 ± 0.3	1.4 ± 0.4	0.28	1.5 ± 0.4	1.6 ± 0.3	0.74
TG (mmol/l)	0.8 (0.6-0.9)	1.2 (0.9-1.7)	**0.01**	0.8 (0.6-1.1)	1.0 (0.7-1.2)	0.30	1.1 (0.8-1.6)	0.8 (0.6-0.9)	**0.01**
Lymphocytes (%)	38.4 ± 6.8	43.0 ± 8.5	0.08	42.2 ± 10.0	37.6 ± 4	0.09	42.1 ± 8.7	37.4 ± 8.4	0.11
Monocytes (%)	8.5 ± 1.8	8.1 ± 1.9	0.49	8.1 ± 1.7	8.6 ± 2.3	0.42	8.8 ± 2.2	8.48 ± 2.0	0.71
Plasma level of fatty acids (wt %)									
Total SFA	29.4 (28.8-30.5)	32.1 (31.1-33.2)	**<0.01**	30.7 (29.3-32.3)	30.8 (29.8-31.8)	0.78	32.6 (31.5-33.2)	29.1 (28.7-29.5)	**<0.01**
Myristic acid (14:0)	0.6 (0.6-1.0)	1.0 (0.8-1.2)	**<0.01**	0.8 (0.6-1.1)	0.9 (0.7-1.1)	0.57	1.1 (0.9-1.2)	0.6 (0.6-0.9)	**<0.01**
Palmitic acid (16:0)	20.5 (20.2-21.6)	23.4 (21.4-24.2)	**<0.01**	20.9 (20.4-23.7)	21.5 (20.9-22.6)	0.39	23.9 (21.8-24.5)	20.4 (20.2-20.7)	**<0.01**
Stearic acid (18:0)	8.1 ± 0.7	8.1 ± 1.1	0.86	8.2 ± 1.1	8.0 ± 1.0	0.65	8.0 ± 1.1	7.7 ± 0.8	0.37
Total n-6	38.6 ± 1.3	30.3 ± 2.9	**<0.01**	31.0 ± 4.0	31.5 ± 4.5	0.74	30.8 ± 3.4	38.0 ± 2.0	**<0.01**
LA (18:2n-6)	32.3 ± 1.6	24.9 ± 2.5	**<0.01**	27.8 ± 3.8	29.2 ± 4.1	0.29	24.8 ± 2.5	31.8 ± 2.1	**<0.01**
AA (20:4n-6)	6.2 ± 1.1	5.4 ± 0.9	**0.02**	5.8 ± 0.7	6.2 ± 1.2	0.32	5.9 ± 1.3	6.2 ± 1. 1	0.58
Total n-3	5.8 ± 2.5	7.0 ± 2.3	0.12	9.0 ± 0.9	3.6 ± 0.49	**<0.01**	6.1 ± 2.5	6.8 ± 2.4	0.44
ALA (18:3n-3)	0.5 (0.5-0.6)	0.5 (0.4-0.6)	0.46	0.5 (0.5-0.6)	0.5 (0.5-0.6)	0.79	0.5 (0.5-0.6)	0.5 (0.5-0.5)	0.95

EPA (20:5n-3)	1.7 (0.6-2.3)	2.4 (1.8-2.9)	0.14	3.2 (2.8-3.7)	0.6 (0.5-0.6)	**<0.01**	2.0 (0.7-2.6)	2.2 (1.3-3.2)	0.46
DPA (22:5n-3)	0.6 ± 0.1	0.7 ± 0.2	0.26	0.8 ± 0.1	0.5 ± 0.1	**<0.01**	0.6 ± 0.2	0.6 ± 0.1	0.80
DHA (22:6n-3)	3.0 (1.9-4.1)	4.0 (3.1-4.1)	0.14	4.3 (4.1-4.7)	1.9 (1.8-2.1)	**<0.01**	3.5 (2.0-4.1)	3.3 (2.8-4.3)	0.48
Total PUFA	43.8 (42.1-47.3)	38.6 (35.8-39.7)	**<0.01**	42.2 (39.5-46.7)	40.4 (37.7-41.5)	**0.05**	37.8 (35.8-39.1)	44.6 (43.1-47.3)	**<0.01**
SFA to PUFA ratio	0.7 (0.6-0.7)	0.8 (0.8-0.9)	**<0.01**	0.7 (0.6-0.8)	0.8 (0.7-0.8)	0.13	0.9 (0.8-0.9)	0.7 (0.6-0.7)	**<0.01**

BMI, body mass index; Total-C, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; SFA, saturated fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA: docosahexaenoic acid; PUFA, polyunsaturated fatty acid.

Lymphocytes and monocytes are given as percentage of total white cell count.

Differences between tertiles were analysed using the Independent Samples t-test when normally distributed or the Mann-Whitney U-test when not normally distributed. P-values <0.05 were considered significant. Values are presented as mean ± SD or medians and 25th-75th percentile.

Table 3. Differentially expressed genes between subjects in the highest and lowest plasma n-6 tertile

Gene	Highest tertile (n 18)	Lowest tertile (n 18)	Mean difference	*P*
STAB1*	8.66 ± 0.30	8.30 ± 0.47	0.36	0.011
SORL1	**10.25 ± 0.27**	**10.06 ± 0.27**	**0.19**	**0.039**
KLHL8*	**9.13 ± 0.25**	**8.94 ± 0.19**	**0.18**	**0.020**
DAGLB	9.21 ± 0.30	9.03 ± 0.19	0.18	0.040
TOM1*	**9.78 ± 0.24**	**9.62 ± 0.20**	**0.16**	**0.037**
PXN*	8.14 ± 0.19	8.00 ± 0.18	0.14	0.030
HMGCR*	8.85 ± 0.14	8.76 ± 0.11	0.09	0.036
KAT5	**7.45 ± 0.09**	**7.39 ± 0.09**	**0.06**	**0.047**
LRP5	6.79 ± 0.09	6.87 ± 0.09	-0.08	0.013
LACTB	**7.15 ± 0.12**	**7.24 ± 0.12**	**-0.09**	**0.027**
SELS	**9.66 ± 0.12**	**9.83 ± 0.27**	**-0.18**	**0.020**
XBP1	**10.63 ± 0.23**	**10.85 ± 0.37**	**-0.22**	**0.042**
EPHX2*	8.18 ± 0.32	8.40 ± 0.30	-0.22	0.043
CAV1*	6.88 ± 0.11	7.13 ± 0.32	-0.25	0.005
COBLL1	7.98 ± 0.23	8.28 ± 0.49	-0.30	0.028

Expression of genes is given as mRNA level. Values are presented as mean ± SD and are log2 transformed. Differences between tertiles were analysed using the Independent Samples t-test. *P*-values <0.05 were considered significant.

* Genes differentially expressed between subjects in the highest and lowest n-6 tertile and between subjects in the highest and lowest SFA to PUFA ratio tertile. The genes marked in bold are expressed by more than one probe (shown in Supplementary Table S2).

Table 4. Differentially expressed genes between subjects in the highest and lowest plasma n-3 tertile

Gene	Highest tertile (n 18)	Lowest tertile (n 18)	Mean Difference	*P*
SNX5	**8.53 ± 0.13**	**8.43 ± 0.14**	**0.10**	**0.029**
AMPD3	6.94 ± 0.06	6.88 ± 0.06	0.06	0.012
LCAT	6.93 ± 0.07	6.99 ± 0.07	-0.06	0.013
RORA	**7.11 ± 0.13**	**7.21 ± 0.10**	**-0.10**	**0.018**
FAM13A	**7.33 ± 0.14**	**7.43 ± 0.15**	**-0.10**	**0.049**
UBE2L3*	**7.16 ± 0.16**	**7.28 ± 0.17**	**-0.12**	**0.035**
ANKRA2	9.10 ± 0.20	9.25 ± 0.17	-0.15	0.017
THBS1	7.29 ± 0.32	7.62 ± 0.43	-0.32	0.016

Expression of genes is given as mRNA level. Values are presented as mean ± SD and are log2 transformed. Differences between tertiles were analysed using the Independent Samples t-test. *P*-values <0.05 were considered significant.

* Genes differentially expressed between subjects in the highest and lowest n-3 tertile and between subjects in the highest and lowest SFA to PUFA ratio tertile. The genes marked in bold are expressed by more than one probe (shown in Supplementary Table S3).

Table 5. Differentially expressed genes between subjects in the highest and lowest plasma SFA to PUFA ratio tertile

Gene	Highest tertile (n 18)	Lowest tertile (n 18)	Mean difference	*P*
EPHX2*	8.41 ± 0.33	8.18 ± 0.31	0.23	0.036
FAM117B	**8.58 ± 0.33**	**8.36 ± 0.09**	**0.22**	**0.012**
CAV1*	7.09 ± 0.27	6.87 ± 0.23	0.22	0.014
SEC24A	7.72 ± 0.26	7.56 ± 0.17	0.16	0.038
UBE2L3**	**7.24 ± 0.18**	**7.09 ± 0.13**	**0.15**	**0.010**
ARV1	8.07 ± 0.16	7.95 ± 0.11	0.12	0.011
NFKB1	11.09 ± 0.10	11.00 ± 0.15	0.09	0.037
SNX13	7.49 ± 0.15	7.40 ± 0.10	0.09	0.045
INSIG2	8.14 ± 0.09	8.07 ± 0.07	0.06	0.025
TMEM188	7.01 ± 0.07	6.95 ± 0.09	0.06	0.034
ERLIN2	**6.99 ± 0.06**	**6.93 ± 0.06**	**0.05**	**0.034**
CPS1	6.84 ± 0.07	6.79 ± 0.07	0.05	0.033
TOM1*	**7.04 ± 0.08**	**7.13 ± 0.10**	**-0.09**	**0.006**
POR	7.75 ± 0.14	7.85 ± 0.11	-0.10	0.037
GSK3B	8.22 ± 0.15	8.32 ± 0.13	-0.10	0.021
HMGCR*	8.76 ± 0.19	8.88 ± 0.13	-0.12	0.015
RBM5	10.81 ± 0.12	10.93 ± 0.15	-0.12	0.033
ERGIC3	**10.54 ± 0.18**	**10.67 ± 0.13**	**-0.12**	**0.031**
PXN*	8.00 ± 0.14	8.13 ± 0.15	-0.13	0.014
SCARB1	7.49 ± 0.23	7.62 ± 0.12	-0.14	0.025
APOBR	8.11 ± 0.18	8.25 ± 0.17	-0.14	0.038
KLHL8*	**8.96 ± 0.21**	**9.13 ± 0.25**	**-0.17**	**0.042**
ABCA2	7.41 ± 0.25	7.61 ± 0.30	-0.20	0.042
PNPLA2	8.28 ± 0.28	8.48 ± 0.30	-0.20	0.050
STAB1*	8.31 ± 0.50	8.67 ± 0.38	-0.35	0.023

Expression of genes is given as mRNA level. Values are presented as mean ± SD and are log2 transformed. Differences between tertiles were analysed using the Independent Samples t-test. *P*-values <0.05 were considered significant.

* Genes differentially expressed between subjects in the highest and lowest n-6 tertile and between subjects in the highest and lowest SFA to PUFA ratio tertile.

** Genes differentially expressed between subjects in the highest and lowest n-3 tertile and between subjects in the highest and lowest SFA to PUFA ratio tertile. The genes marked in bold are expressed by more than one probe (shown in Supplementary Table S4).

Table 6. Correlations between subject characteristics and differentially expressed genes associated with plasma n-6 level and/or SFA to PUFA ratio (n 54)

	BMI	TG	Total-C	LDL-C
DAGLB		- 0.31 (*P* 0.020)		
ERLIN2	- 0.31 (*P* 0.021)			
FAM117B			- 0.29 (*P* 0.034)	
GSK3B	- 0.28 (*P* 0.042)	- 0.31 (*P* 0.024)		
KAT5		- 0.33 (*P* 0.016)	- 0.32 (*P* 0.017)	
KLHL8	- 0.29 (*P* 0.034)	- 0.30 (*P* 0.026)		
LACTB	0.31 (*P* 0.023)			
LRP5				- 0.30 (*P* 0.027)
SCARB1	- 0.26 (*P* 0.059)	- 0.27 (*P* 0.045)		
SELS	- 0.32 (*P* 0.033)			
SNX13	0.42 (*P* <0.01)			

BMI, body mass index; TG, triglycerides; Total-C, total cholesterol; LDL-C, low density lipoprotein cholesterol.
Correlations were analysed using Pearson`s r. *P*-values <0.05 were considered significant.

Figure legend

Figure 1: Flowchart of gene selection and number of differentially expressed genes between subjects in the highest and lowest tertile of plasma n-6 level, plasma n-3 level and plasma SFA to PUFA ratio.

Differences in Log2 gene expression between subjects in the highest and lowest tertiles were compared by Independent Samples t-test. P-values <0.05 were considered significant.

Figure 2: Differently expressed genes associated with plasma fatty acid levels and SFA to PUA ratio.

Number of differentially expressed genes associated with n-3 level (8 gene transcripts), n-6 level (15 gene transcripts), and SFA to PUFA ratio (25 gene transcripts). Seven differentially expressed genes were associated with both n-6 level and SFA to PUFA ratio, and one differently expressed gene was associated with both n-3 level and SFA to PUFA ratio. Differences in Log2 gene expression between subjects in the highest and lowest tertiles were compared by Independent Samples t-test. P-values <0.05 were considered significant.