Postprandial dietary lipid–specific effects on human peripheral blood mononuclear cell gene expression profiles1–3

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ABSTRACT

Background: Dietary polyunsaturated fatty acids (PUFAs) have a variety of beneficial effects, and immune cells play an important role in these effects. The mechanisms of action of PUFAs are still not completely understood, but it is known that PUFAs can influence the expression of a broad set of genes.

Objective: The objective was to determine the postprandial effects of intake of different fatty acids on the gene expression profiles of peripheral blood mononuclear cells (PBMCs).

Design: In a single-blind crossover study, 21 healthy male volunteers consumed shakes enriched in PUFAs, monounsaturated fatty acids (MUFAs), or saturated fatty acids (SFAs) in random order. Blood samples were collected before and at several time points after intake. Whole-genome gene expression profiles of PBMCs were examined before and 6 h after intake of the PUFA and SFA shakes. In addition, ex vivo incubation of human PBMCs with different fatty acids was performed.

Results: Whole-genome expression analysis showed distinct differences between PUFA and SFA consumption. PUFA intake decreased the expression of genes in liver X receptor signaling, whereas SFA intake increased the expression of these genes. PUFA intake also increased the expression of genes related to cellular stress responses. MUFA intake had an intermediate effect on several genes. Ex vivo experiments showed a direct effect of free fatty acids on PBMC gene expression.

Conclusion: This study showed that PBMCs can reveal fatty acid–specific gene expression profiles in young healthy men after the consumption of different fatty acids, as evidenced by the opposite effects of PUFA and SFA intake on the expression of genes involved in liver X receptor signaling. This trial was registered at www.clinicaltrials.gov as NCT01000194. Am J Clin Nutr 2010;91:208–17.

INTRODUCTION

Dietary polyunsaturated fatty acids (PUFAs), such as omega-3 (n-3) fatty acids, are suggested to have a variety of beneficial effects, of which the effects on inflammation (1) and cardiovascular diseases (2) are the most generally accepted. Immune cells play an important role in inflammation and the development of cardiovascular disease. Several long-term intervention trials have examined the effects of PUFAs on human immune cells (3), and the immunologic function of these cells has also been studied. However, the mechanism of action of PUFAs on the function of immune cells is not completely understood (4). It is known that PUFAs can mediate their effects through changes in gene expression, mainly regulated via activation of transcription factors, such as peroxisome proliferator–activated receptors (PPARs) and liver X receptor (LXR) (5, 6). We recently published a study that used whole-genome expression to explore the effects of long-term supplementation of PUFAs on immune cells and found that n-3 fatty acids induce antinflammatory gene expression profiles in human peripheral blood mononuclear cells (PBMCs) (7). Measurements in this study, as in most long-term studies, were performed by using fasted samples. However, most individuals are in a postprandial state for the greater part of every 24-h period, and it is known that consumption of a meal rich in fatty acids leads to postprandial inflammation (8). It is unknown whether different fatty acids elicit differential postprandial effects on the gene expression of immune cells in humans, and what these effects will encompass is also unknown. Therefore, we examined the postprandial effects of consumption of a shake containing 70% of energy from fat in the form of PUFAs (65% of total fat), saturated fatty acids (SFAs; 70% of total fat), or monounsaturated fatty acids (MUFAs; 80% of total fat) on gene expression changes in PBMCs of 21 healthy men according to a crossover design. Whole-genome microarray analyses were performed before and 6 h after the consumption of PUFA- and SFA-enriched shakes. To explore the response kinetics, quantitative real-time polymerase chain reaction (Q-PCR) analysis was performed before and at 5 time points after the intake of all shakes.

SUBJECTS AND METHODS

Recruitment of subjects

Twenty-one healthy male white volunteers aged 19–27 y were recruited from the Wageningen University student population. Exclusion criteria were as follows: body mass index (BMI; in kg/m2) <18 or >27, urine protein or glucose concentrations outside

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normal ranges, fasting blood glucose outside the normal range, tobacco smoking, regular use of prescribed medication, receipt of inoculations within 2 mo of starting the study or the intention to receive such during the study, donation of or intention to donate blood within 8 wk of the first and last study samples, diagnosis of long-term medical condition (eg, diabetes, hemo-
philia, cardiovascular disease, anemia, or gastrointestinal dis-
ease), symptoms of allergy, or vegetarianism. The subjects were
informed about the design and purpose of the study, and all
subjects provided written informed consent.

Study design

At a screening visit, glucose was measured in urine and fasting venous blood samples, and protein concentrations were measured in urine. Twenty-one men randomly consumed 3 shakes enriched with PUFAs, MUFAs, or SFAs in a crossover design, on 3 differ-
ent days, with ≥1 wk between each study day. Before each
study day, the subjects received identical meals containing <10 g fat, which they were told to consume before 2000 h the previous
evening. On the morning of each study day, the subjects came
to the University in a fasted condition, and a canula was placed in
their forearm. At the start of the study, 6 mL blood was drawn into
EDTA-containing tubes for plasma isolation and 24 mL into BD
Vacutainer Cell Preparation Tubes containing sodium citrate (BD, Breda, Netherlands) for PBMC isolation. Directly after the first
blood sampling, the subjects were given a shake, which they had
to consume within 10 min. Subsequently, blood was drawn every
2 h, until 8 h after intake of the shake. During a study day, the
subjects were not allowed to eat or drink anything except water,
which they were advised to drink regularly (∼150 mL each hour).
After 8 h, the volunteers received a meal. The subjects were asked
to keep a record of their physical condition during the complete
study period, which lasted from 14 January 2008 until 7 March
2008. The study protocol was approved by the Medical Ethical
Committee of Wageningen University.

Shake composition

The shakes contained 350 mL low-fat yogurt, 100 mL skim
milk, 7.5 g sugar, and 55 g of the test fatty acids. Vitamin E (165
mg) was added as an antioxidant. The test fatty acids were 65% PUFA,
of which ≈40% was docosahexaenoic acid (DHA) (PUFA shake, Marinol D-40; Lipid Nutrition, Wormerveer, Netherlands); 80% MUFAs, predominantly oleic acid (MUFA shake, high-oleic acid sunflower oil; Aldoc BV, Schiedam, Netherlands); or 70% SFAs (SFA shake, butter). The macronu-
trient composition of the shakes was calculated by using the
computer package KOMEET (9). This program is based on the
database of the Dutch Nutrient Databank (10). The fatty acid
composition of the shakes was measured by gas-liquid chro-
matography (GLC) (11).

Blood glucose and plasma free fatty acids, triglycerides,
and cholesterol

Blood glucose concentrations were measured with Accu-Chek
Compact blood glucose meters (Roche Applied Science, Almere,
Netherlands). Immediately after blood was drawn, blood in
the EDTA-containing tubes was centrifuged (750 × g, 4°C, 10 min),
and the plasma was stored at −80°C. Plasma free fatty acids
(FFAs) and triglycerides were measured by GLC. Plasma cho-
lesterol was measured by using a cholesterol liquicolor kit (In-
struchemie, Delftziejl, Netherlands)

PBMC isolation and flow cytometry analysis

Immediately after blood collection, PBMCs were isolated by
using the BD Vacutainer Cell Preparation Tubes according to the
manufacturer’s instructions. Cells were counted at baseline and
6 h after intake of the shakes and were tested for viability by
trypan blue exclusion. Flow cytometry analysis was performed on
1.5 × 10⁶ cells with a FACS Canto II flow cytometer (BD Bio-
sciences). Cells surface markers were examined with labeled
anti-CD3 (for T lymphocytes), anti-CD14 (for monocytes), and
anti-CD19 (for B lymphocytes). Data were analyzed by using
BD FACSDiva software (BD Biosciences).

Total RNA isolation

PBMC RNA was isolated from all PBMC samples by using the
Qiagen RNeasy Micro kit (Qiagen, Venlo, Netherlands). The
RNA yield was quantified with a Nanodrop ND 1000 spectrophot-
ometer (Nanodrop Technologies, Wilmington, DE), and
integrity was measured with an Agilent 2100 Bioanalyzer with
RNA 6000 Nano chips (Agilent Technologies, South Queen-
sferry, United Kingdom). Samples were only accepted if the RNA
integrity number (RIN) was >8.

Microarray processing

PBMC samples from baseline and 6 h after intake of the PUFA
and the SFA shakes were used for microarray analysis, which
resulted in a total of 84 arrays. Total RNA from PBMCs from
all subjects was labeled by using a one-cycle cDNA labeling
kit (MessageAmpTM II-Biotin Enhanced Kit; Ambion Inc,
Nieuwekerk a/d IJssel, Netherlands) and hybridized to human
whole-genome NuGO GeneChip arrays encoding 17,699 genes,
designed by the European Nutrigenomics Organization (NuGO)
and manufactured by Affymetrix (Affymetrix Inc, Santa Clara,
CA). Sample labeling, hybridization to chips, and image scanning
were performed according to the manufacturer’s GeneChip
Expression Analysis Technical Manual (Affymetrix).

Microarray analysis

All microarrays fulfilled our quality control criteria. Micro-
arrays were analyzed using the reorganized oligonucleotide
probes, as described by Dai et al in 2005 (12). Dai et al combined
all individual probes for a gene, enabling the possibility of detecting the overall transcription activity of a gene, based on the latest genome and transcriptome information instead of the Affymetrix probe set annotation. Expression values were calculated by using the Robust Multichip Average (RMA) method. RMA signal value estimates are based on a robust average of background-corrected perfect match intensities, and normalization was performed by using quantile normalization (13, 14).

Only genes with normalized signals at least 20 on C21 arrays were defined as “expressed” and were selected for further analysis. Genes were defined as “changed within shakes” when a comparison of the normalized signal intensities showed a false discovery rate (FDR) Q value (15), 0.05 in a 2-tailed paired t test with Bayesian correction (Limma) (16). Genes were defined as “different between shakes” when the signal log ratios of the genes showed an FDR Q value, 0.05 in a 2-tailed paired t test between the samples after intake of the PUFA shake and the SFA shake.

Pathway analysis was performed by using Ingenuity Pathway Analysis 5.5 (Ingenuity Systems, Redwood City, CA). Array data were submitted to the Gene Expression Omnibus, accession number GSE13466.

cDNA synthesis and real-time PCR

RNA from all subjects and shakes was reverse transcribed with the use of a cDNA synthesis kit (Promega, Leiden, Netherlands). Standard Q-PCR was performed with the use of Platinum Taq DNA polymerase and SYBR Green on an iCycler PCR machine (Bio-Rad Laboratories BV, Veenendaal, Netherlands) and duplicated at least twice. Primer sequences used in the PCRs were chosen based on the sequences available in PRIMERBANK (http://pga.mgh.harvard.edu/primerbank/index.html). Q-PCR data were normalized by measuring cycle threshold ratios between candidate genes and a housekeeping gene, human ribosomal protein LP0, which was shown to be consistent within PBMCs (17).

PBMC incubation studies

Blood from 4 healthy white male donors, aged 26–42 y was obtained from the blood bank (Sanquin, Nijmegen, Netherlands), and PBMCs were isolated directly after arrival by using Ficoll-Paque Plus density gradient centrifugation (Amersham Biosciences, Roosendaal, Netherlands). PBMCs were incubated in RPMI1640 medium with 2 mmol L-glutamine/L, 10% fetal bovine serum, and antibiotics (penicillin and streptomycin) in the presence of 5% CO2 at 37°C at 1.0 · 10^6 cells/mL for 12 h. Bovine serum albumin was added to bind fatty acids, and the following fatty acids were tested: palmitic acid (16:0, 200 μmol/L), oleic acid (18:1, 500 μmol/L), and DHA (22:6n-3, 10 μmol/L).

### TABLE 2

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<th>PUFAs</th>
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<td>71.5</td>
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*As described in reference 11. PUFAs, polyunsaturated fatty acids; MUFAs, monounsaturated fatty acids; SFAs, saturated fatty acids.

"TABLE 2" Percentages of fatty acids as measured by gas-liquid chromatography

![Flow cytometric analysis results](https://example.com/flow-cytometry)

**FIGURE 1.** Flow cytometric analysis results. A: A representative flow cytometry plot showing lymphocytes and monocytes. B: Quantity of lymphocyte and monocyte subsets on the different test days (n = 21). No differences were found by ANOVA. C: Quantity of lymphocyte and monocyte subsets before and after intake of the polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA), or saturated fatty acid (SFA) shakes. Note the different number of subjects tested per shake. *Significantly different from baseline, P < 0.01 (one-factor ANOVA). PBMCs, peripheral blood mononuclear cells; Monos, monocytes; SSC-A, side scatter; FSC-A, forward scatter.
Concentrations were based on physiologically normal concentrations of the different fatty acid types found in the plasma of volunteers in a previous study (18). Fatty acids were dissolved in a potassium hydroxide solution, which was used as control. All donors gave full written informed consent.

Statistical methods

In this crossover study, each subject consumed the 3 shakes and was used as their own reference. Data are provided as means ± SDs (n = 21). Incremental shake responses (means over 21 determinations) are expressed as delta concentrations or the relative gene expression values to baseline values (fasting baseline values being zero) per person and shake. The 0–8-h incremental area under the curve (AUC) was calculated by the trapezoidal method. The significance of the differences between experimental shakes, the 0–8-h time points for a given shake and the difference between shake AUCs, were assessed by using a repeated-measures analysis of variance (ANOVA) with Bonferroni correction. A one-factor ANOVA was used to determine significant differences in FFA concentrations and in percentages of blood cells in PBMCs between baseline and 6 h after intake of the shakes. Statistical significance was accepted at P < 0.05. All calculations were performed by using the SPSS-software package 15.0.1 (SPSS Inc, Chicago, IL).

RESULTS

Subject characteristics

Twenty-one healthy men were included in the study, who returned to the university on 3 separate study days to consume a shake enriched with PUFAs, MUFAs, or SFAs. Microarray analyses were performed for the PUFA- and SFA-enriched shakes. Subject characteristics, as measured during the screening visit, are shown in Table 1. The shakes contained 2824 kJ energy and consisted of 18.4 g protein, 34 g carbohydrates, and 55 g fat. The fatty acid composition was determined by GLC and is shown in Table 2. Flow cytometric analysis showed complete removal of neutrophils on PBMC isolation (Figure 1A). No differences were observed in blood cell subpopulations between the study days (Figure 1B), and no changes were found after intake of the PUFA or SFA shakes, although the latter was tested.
in 5 individuals only. There was a significant decrease in T lymphocytes 6 h after intake of the MUFA shake (Figure 1C).

Blood glucose and plasma FFAs, triglycerides, and cholesterol

Individual plasma FFAs of 18 subjects were measured at baseline and 6 h after intake of all shakes. PUFA shake intake resulted in a significant increase in plasma DHA concentration of 201 ± 80 μg/mL (P < 0.01), from a baseline concentration of 37 ± 12 μg/mL; whereas intake of the MUFA and SFA shakes resulted in a nonsignificant increase in oleic acid (by 81 ± 184 μg/mL; baseline: 533 ± 130 μg/mL; P = 0.08) and palmitic acid concentrations (by 68 ± 157 μg/mL; baseline: 602 ± 120 μg/mL; P = 0.07), respectively (Figure 2D). Plasma total FFAs increased only after intake of the PUFA shake (by 356 ± 482 μg/mL, baseline 2129 ± 353 μg/mL; P < 0.01), and was largely explained by the PUFA increase (data not shown).

No significant differences in total response, as measured by incremental AUC, of cholesterol, triglyceride, and glucose responses were observed between the shakes (Figure 2, A–C). However, the peak response in triglycerides was later after the PUFA shakes than after both other shakes. All shakes significantly increased plasma triglycerides, but the PUFA shake resulted in significantly higher triglyceride concentrations at 6 and at 8 h compared with the SFA shake at the same time points. Intake of all shakes resulted in a similar significant decrease in blood glucose with time. No changes in cholesterol concentrations were found, except for a slight significant decrease 6 h after intake of the MUFA shake (Figure 2).

Microarray analysis

Microarray hybridization was performed on PBMC RNA collected from all 21 subjects at baseline and 6 h after consumption of the PUFA and SFA shakes. All arrays passed the quality control criteria. Changes in gene expression were determined by comparison of the microarray results of the samples from 6 h with those from 0 h within all individuals in both intervention groups. From the 17,699 genes present on the microarray, 13,365 genes were defined as expressed in PBMCs (Figure 3). Consumption of the PUFA shake resulted in changes in the expression of 437 genes, whereas consumption of the SFA shake resulted in the change in expression of 297 genes (Figure 3). Of these genes, 146 genes overlapped between the groups, of which all except 2 genes changed in the same direction. These 2 genes, ABCG1 and SREBF1, were down-regulated after intake of the PUFA shake and up-regulated after intake of the SFA shake. Besides determining which genes were significantly changed within the shakes, we also elucidated whether gene expression was changed significantly between the shakes. Strict FDR Q-values <0.05 indicated changes in expression between shakes for 3 genes: ABCG1, SREBF1, and ABCA1.

Gene expression changes

To elucidate the role of the various genes for which expression changed after consumption of the shakes, pathway analysis was performed. This analysis indicated that these genes were linked to processes related to LXR signaling, oxidative stress, inflammation, carbohydrate metabolism, and a variety of other processes (Figure 4).

A heat map of the genes involved in LXR signaling, of which expression was changed on intake of at least one of the shakes, is shown in Figure 5. In most individuals, SFA shake consumption resulted in an increase in expression of genes involved in LXR signaling, such as LXR (NR1H3), ABCG1, SREBF1, and ABCA1, whereas PUFA shake consumption resulted in a down-regulation of the same genes. Moreover, PUFA shake consumption resulted in a decrease in SCD—a rate-limiting enzyme in the cellular synthesis of MUFAs.

PUFA shake consumption resulted in gene expression changes indicating both an increase and a decrease in oxidative stress.

![Gene selection procedures and criteria. PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; FDR Q, false discovery rate Q value.](https://academic.oup.com/ajcn/article-abstract/91/1/208/4597083/BOUWENS-ET-AL)
The genes responsible for the down-regulation of this pathway were glutathione metabolism genes (e.g., GSTK1, GSTP1, and MGST3), whereas the genes responsible for the up-regulation of this pathway were involved in activation of inflammatory and cellular stress pathways (e.g., IL-8, OSM, and JUN). SFA shake consumption did not show a strong effect in stress responses on pathway level (Figure 4). However, a variety of proinflammatory and stress-related genes were also up-regulated after intake of the SFA shake, including MAP3K1, CD40, and ICAM1 (data not shown). In addition, we examined the changes in expression of previously described PPAR-α target genes in human PBMCs (19). We found that, of the total

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<table>
<thead>
<tr>
<th>LXR Signaling</th>
<th>LPS/IL-1 Mediated Inhibition of RXR Function</th>
<th>LXR/RXR Activation</th>
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<td>NRF2-mediated Oxidative Stress Response</td>
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<td>IL-4 Signaling</td>
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of 106 PPAR response element–containing PPARζ target genes, 6 genes changed after intake of the PUFA shake and 5 genes changed after intake of the SFA shake, of which 4 were overlapping between shakes, including PDK4 (data not shown).

To elucidate the response in gene expression in time and on the MUFA shake, Q-PCR was performed for all time points after intake of the 3 shakes on a selection of genes. Genes were selected out of the pathways changed after shake intake and included LXR, ABCA1, SREBF1, cJUN, GSTP1, and the PPARζ target gene PDK4. These genes showed that consumption of the shakes enriched with PUFA, MUFA, and SFA resulted in different gene expression responses with time, but also confirmed the microarray results, as reflected by the same direction of gene expression changes 6 h after intake of the PUFA and SFA shakes. Kinetics of the changes in expression of SREBF1 and ABCA1 in the hours after shake consumption showed that differences between the 2 shakes were already present 2 and 4 h, respectively, after consumption of the shakes and lasted up to 8 h (Figure 6). This indicated a rapid response after intake of the fatty acids that disappeared within several hours. Compared with the PUFA shake, the MUFA shake resulted in a lower but significant effect on ABCA1 gene expression and similar effects on SREBF1 and LXR gene expression 6 h postprandially. For the stress-related genes, MUFA intake had effects similar to those of SFA shake.

Ex vivo incubation experiments

To explore whether the observed in vivo effects on gene expression changes were direct effects of the increased concentrations of different FFAs in plasma, we performed ex vivo incubation of PBMCs with different fatty acids representing the main fatty acid types in the 3 shakes, ie, palmitic acid as SFA, oleic acid as MUFA, and DHA as PUFA. Q-PCR of ABCA1 in all 4 subjects showed increases after SFA and decreases after PUFA incubation. In 3 of 4 subjects ABCA1 expression increased after MUFA incubations. SREBF1 showed a similar decrease after PUFA incubation and increases in 3 of 4 subjects after SFA and MUFA incubations. These changes were comparable with changes in gene expression observed after consumption of the SFA and PUFA shakes (Figure 7).

DISCUSSION

This study described the results of a single-blind crossover trial and showed that consumption of high-fat shakes varying in dietary fatty acid composition results in postprandial fatty acid–specific changes in gene expression profiles in PBMCs. The differences in the gene expression profiles were characterized by differential effects on LXR signaling, glutathione metabolism, oxidative stress, and inflammation.

PUFAs are known to be better ligands for PPARs than SFAs; therefore, we expected that intake of the PUFA shake would result in higher PPAR target gene expression than would consumption of the SFA shake. However, only a few PPARz target genes, including PDK4, ADFP, and SLC25A20 were found to be increased, and no differences were observed between the different shakes. In a previous study we observed that many more PPARz target genes were increased after 24 h of fasting than after shake intake, but we also found that endogenous-derived plasma total FFAs increased more after fasting than 6 h after shake consumption. It seems that postprandial gene expression changes in human PBMCs are not mainly regulated through PPARz or that other regulatory processes, such as NF-E2-related factor 2 (NRF2)–mediated gene transcription, are more important in these cells.

For genes involved in LXR-signaling, opposite effects of PUFA and SFA shakes were found. The LXR target genes ABCA1 and ABCG1 play a major role in reverse cholesterol transport in macrophages (20) and in macrophages (21), our data are in line with these findings, because PUFAs shake intake in our in vivo study down-regulated the expression of ABCA1 and ABCG1, and, similarly, our PBMC ex vivo incubation with DHA decreased ABCA1 expression. However, the effect of dietary PUFA or SFA consumption on PBMC whole-genome gene expression in humans has never been studied before; therefore, the observed fatty acid–dependent response of ABCA1 and ABCG1 expression are new in that respect. Another target gene of LXR, SREBF1, was also down-regulated after PUFA intake and encodes a transcription factor involved in fatty acid synthesis and cholesterol metabolism (23). Also for this transcription factor, only in vitro studies have shown that PUFAs inhibit expression of SREBF1 in hepatocytes (24) and in embryonic kidney cells (25) which were also showed in our ex vivo PBMC experiments after DHA incubation. Besides down-regulation of SREBF1 on PUFA shake intake, its target gene, SCD, was also down-regulated. SCD is the rate-limiting enzyme in the cellular synthesis of MUFAs and expression of SCD was shown to be decreased by PUFAs through regulation of SREBF1 in animal and in vitro studies (26), which is clearly in accordance with our in vivo study findings. Yoshikawa et al (25) showed that PUFAs inhibit binding of LXR to LXR response elements in the promoter of LXR target genes such as SREBF1. This repression of LXR target genes might also have happened in PBMCs in our in vivo study after consumption of the PUFA-enriched shakes with a possible inhibition of cholesterol efflux. Conversely, the

FIGURE 5. Heat map of genes changed after consumption of the shakes enriched in polyunsaturated fatty acids (PUFAs) or saturated fatty acids (SFAs) within the process liver X receptor signaling, with a false discovery rate Q value <0.05, in all 21 subjects. Red indicates up-regulation, green indicates down-regulation, and black indicates no change.

FIGURE 6.
increase in LXR target gene expression after SFA shake consumption might result in an increased cholesterol efflux out of the cells. However, it is hard to conclude whether SFAs directly induced LXR activation or whether cholesterol derivatives such as oxysterols served as ligands for LXR in these cells (27). Because the SFA shake was made with butter fat, it

**FIGURE 6.** Mean (±SD) effects of consumption of 3 shakes enriched in polyunsaturated fatty acids (PUFAs), monounsaturated fatty acids (MUFAs), or saturated fatty acids (SFAs) on the gene expression of several genes that were selected because they changed 6 h after intake of the PUFA and/or the SFA shakes as assessed by microarray analysis. A: Liver X receptor (LXR)–related genes: ABCA1 (ATP-binding cassette A1), SREBF1 (sterol regulatory element binding protein-1). B: oxidative stress–related genes: GSTP1 (glutathione S-transferase P1), JUN (c-Jun oncogene). C: Peroxisome proliferator–activated receptor α target genes: PDK4 (pyruvate dehydrogenase kinase 4). n = 21. The incremental areas under the curve (AUC) are shown in insets. Different letters indicate a difference (P < 0.05) between shakes at a given time. For a given test shake, a filled symbol indicates that the corresponding value differs (P < 0.05) from baseline (0 h) value. Differences were assessed by using a repeated-measures ANOVA with Bonferroni correction.
SREBF1 binding cassette A1) and contained more cholesterol than the PUFA shake. On the other hand, ex vivo PBMC incubation with solely SFA resulted in a consistent increase in expression of the LXR target gene ABCA1.

Another difference observed between consumption of both fatty acids was the response in cellular stress. PUFA consumption induced both an increased and decreased expression of genes involved in cellular stress response, as illustrated by the up- and down-regulation of genes involved in NRF2-mediated metabolism, whereas no effects were observed after SFA consumption. The down-regulated genes were involved in glutathione coupled metabolism, and mainly encoded glutathione S-transferases, whereas the up-regulated genes were related to inflammation and cellular stress, such as cJUN, a component of activator protein 1, a transcription factor that regulates gene expression in response to a broad variety of stress stimuli. This bidirectional regulation of cellular stress may point toward an initial triggering of the cells and work as a protective response mechanism of the cell. It is becoming more evident that improvement of cellular capacity (eg, by exercise or healthy food components) is accompanied by induction of low-grade stress that leads to the activation of sets of sensing transcription factors governing transcriptional regulation of genes involved in metabolism and cell-protection pathways (28). In contrast, the PBMCs seemed to be capable of dealing with the high dose of the more commonly consumed SFAs with a much lower cellular stress response likely linked to differences in beneficial health properties of these fatty acids. In addition, unlike the results of our present postprandial study, long-term high PUFA intake is resulting in reduced expression of cellular stress and proinflammatory pathways (7, 29, 30). However, whereas long-term interventions provide information about the nutritional effects on whole-body homeostasis, postprandial fat intake will provide information about the actual metabolic capacity of cells to deal with this increased concentration of different dietary lipids. For instance, prolonged intake of lower doses of DHA will most likely increase the capacity of metabolic organs, such as intestine and liver, to more efficiently handle these potential stressors. This may result in an overall increased capability of the body to handle these lipids and consequently in a decreased expression of stress- and inflammation-related pathways.

The comparable but lower effects observed after MUFA than after PUFA intake for some but not all genes have been described before after a long-term intervention of 6 mo (7). However, because whole-genome microarray analyses were not performed for the MUFA shake, conclusions regarding the total effects of MUFA on the whole transcriptome cannot be drawn. However, it has been shown that MUFA consumption can change expression of other genes in PBMCs, postprandially (31, 32).

In this study we showed that PBMC gene expression profiles cannot only be used to differentiate between health and disease, as has been elucidated by previous whole-genome gene expression studies (33, 34), or to show effects of long-term supplementation with n–3 fatty acids, as we showed previously (7), but these profiles are also able to reflect postprandial changes that differ by type of fatty acid. Changes in gene transcription of a cell will only be induced by sensing signaling and transcription factor–mediated mechanisms when cells can respond to environmental challenges in an appropriate and timely fashion. Therefore, challenge strategies combined with gene expression profiling of PBMC, as used within this study, can elucidate the capacity of cell systems to deal with healthy food bioactives, such as the PUFAs studied, in a comprehensive manner. This makes these cells highly interesting for future exploitation for elucidating the influences of optimized nutrition on various phenotypic aspects of human homeostasis. In summary, our study showed that PBMCs are sensitive enough to reveal fatty acid–specific gene expression profiles in young healthy men after the consumption of different fatty acids. This is nicely illustrated by opposite effects of PUFA and SFA on expression of genes involved in LXR signaling.

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