Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid β-oxidation: functional role of peroxisome proliferator–activated receptor α in human peripheral blood mononuclear cells1–3

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ABSTRACT

Background: Peripheral blood mononuclear cells (PBMCs) are the only readily available cells in healthy humans. Various studies showed disease-characteristic gene expression patterns in PBMCs. However, little is known of nutritional effects on PBMC gene expression patterns. Fatty acids are nutrients that regulate gene expression by activating the nuclear receptor peroxisome proliferator–activated receptor α (PPARα). PBMCs express PPARα, making these cells interesting to study FA-dependent gene expression.

Objective: The aim of this study was to elucidate whether PBMC gene expression profiles also reflect nutrition-related metabolic changes. Furthermore, we focused on the specific role of PPARα in regulation of PBMC gene expression during fasting, when plasma free fatty acids are elevated.

Design: Four healthy male volunteers fasted for 48 h. PBMC RNA was hybridized on Affymetrix whole genome microarrays. To elucidate the role of PPARα, PBMCs of 9 blood donors were incubated with the specific PPARα ligand Wy14643.

Results: After 24 and 48 h of fasting, 1200 and 1386 genes were changed >1.4-fold, respectively. Many of those genes were involved in fatty acid β-oxidation and are known PPARα target genes. Incubation of PBMCs with Wy14643 resulted in up-regulation of genes that were also up-regulated during fasting.

Conclusions: We conclude that PBMC gene expression profiles reflect nutrition-related metabolic changes such as fasting and that part of the fasting-induced changes are likely regulated by PPARα. Am J Clin Nutr 2007;86:1515–23.

KEY WORDS Nutrigenomics, microarray, gene expression profiles, PPARα, peroxisome proliferator–activated receptor α, peripheral blood mononuclear cell, PBMC, beta oxidation, fasting, humans

INTRODUCTION

White blood cells travel through the entire body and respond to various internal and external signals. A subset of white blood cells known as peripheral blood mononuclear cells (PBMCs) receives increased interest for diagnostic purposes since the development of sophisticated transcriptomics techniques such as whole genome microarray analysis. The advantage of PBMCs above other cells is that blood is the most readily accessible cellular material in humans, and PBMCs can be isolated from whole blood relatively easily. Various studies have shown that PBMCs can display gene expression patterns characteristic for certain diseases, such as acute myeloid leukemia, atherosclerosis, and autoimmune diseases (1–5).

From physiologic studies we know that nutritional effects on indicators of human physiology are generally smaller than pathophysiologic changes. Similarly, nutritional-induced changes in PBMC gene expression are expected to be smaller than the described effects observed in various diseases. Characterization of nutrient-specific effects on cellular metabolism and homeostatic control could in the end lead to evidence-based dietary advice to prevent disease or to intervene in a early predisease state. The comprehensive understanding of the mechanisms involved in nutritional effects on gene expression is of considerable importance in this respect. This is especially the case in PBMCs, because obtaining alternative cellular material, through invasive tissue biopsies, is not always feasible or even possible in healthy volunteers for ethical reasons. Therefore, the ability to detect diet-induced changes in gene expression profiles of relatively easily obtainable PBMCs could not only be highly valuable for the identification of nutrient-related molecular mechanism but will also be of pivotal importance to study the development of diet-related chronic disorders.

It is known that several nutrients are able to activate gene expression by binding to transcription factors (6). This is quite well understood for fatty acids (FAs) (7), which mediate their modulatory effects on changes of expression of specific genes by a group of nuclear receptors, the peroxisome proliferator–activated receptors (PPARs). These ligand-activated transcription factors (PPARα, PPARβ/δ, PPARγ) bind FAs, in particular if they are unsaturated, which results in transcriptional activation.

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Activation of PPARα occurs especially when plasma free FA (FFA) concentrations are increased in situations such as fasting and heavy exercise and postprandially and in conditions such as diabetes type 2 and visceral obesity. Fasting results in the most pronounced elevation in plasma FFAs, because of a breakdown of triacylglycerols stored in the adipose tissue (8). Studies in liver from mice deprived of food showed that PPARα transcriptionally activates genes involved in processes essential for the use of increased FA concentrations for energy, such as β-oxidation (9). Because previous studies showed that PPARα is expressed in human PBMCs (10–12) and FAs are abundantly present in blood plasma of fasting humans, these nutrients are excellent candidates to study the feasibility of detecting and analyzing nutrient-induced changes in PBMC gene expression profiles. To assess this, we conducted a study in which 4 healthy men fasted for 48 h, changes in PBMC gene expression were monitored during fasting by whole genome microarray analysis, and the role of PPARα in this respect was examined.

SUBJECTS AND METHODS

Recruitment of subjects

Four healthy male white volunteers, between 19 and 22 y of age, were recruited from the Wageningen student population. Physiologically measured exclusion criteria were a body mass index (in kg/m²) <19 or >25, urine protein or glucose concentrations outside normal ranges, or fasting blood glucose outside the normal range. Other exclusion criteria were tobacco smoking, taking regular prescribed medication, having received administrations within 2 mo of starting the study or planned to during the study, having donated or intending to donate blood within 8 wk of the first and last study samples, having a diagnosis of any long-term medical condition (eg, diabetes, hemophilia, cardiovascular disease, anemia, gastrointestinal disease), experiencing symptoms of allergy, or being a vegetarian. Subjects were informed about the design and purpose of the study and provided full informed written consent.

Study design of the intervention study

At a screening visit, urine and fasting venous blood samples were taken to measure glucose concentrations in both urine and blood and protein concentrations in urine. Four healthy male subjects who fulfilled selection criteria were included in the study. Volunteers received an identical meal at 1700, before the start of a 48-h fasting period. During the fasting period, the subjects were not allowed to eat or drink anything except water, which they could consume ad libitum. After 48 h, the volunteers received a light meal. At baseline and after 24 and 48 h of fasting, 45 mL of blood was drawn into EDTA-coated tubes from the antecubital vein in each volunteer’s forearm. Volunteers were asked to keep a record of their physical condition, and after 15 h of fasting they remained under constant supervision. The total study was repeated 2 mo later with the same subjects. The study protocol was approved by the Medical Ethical Committee of Wageningen University.

Blood glucose and plasma FFAs

Blood glucose concentrations were measured with Accu-Chek Compact blood glucose meters (Roche Applied Science, Almere, Netherlands). Immediately after blood drawing, blood was centrifuged (750 × g, 4 °C, 10 min), and plasma was stored at −80 °C. Plasma FFAs were measured by gas–liquid chromatography (13, 14). Briefly, plasma was incubated with a modified Dole reagent (2-propanol/heptane/2 M orthophosphoric acid, 40:10:1) for 10 min at room temperature. Heptane was added, and the tubes were mixed (5 min) and centrifuged at 1600 × g and 20 °C for 5 min. The upper heptane layer was transferred to another tube and evaporated under nitrogen. The residue was solved in chloroform, and FFAs were separated from the triacylglycerols and phospholipids on amino propyl columns. The FFAs were hydrolyzed in 0.5 N methanolic sodium-hydroxide and methylated with boron trifluoride in methanol. The methyl esters were separated on a glass capillary column and measured with a flame ionization detector. Identification of individual methyl esters was performed by comparisons with authentic standard mixtures. Heptadecanoic acid (C17:0) was used as an internal standard for quantification of individual FFAs.

PBMC isolation for the intervention study

Immediately after blood collection, PBMCs were isolated from 36 mL of blood, from all time points and subjects for both study periods. Optiprep (Axis-shield, Oslo, Norway) was used according to the manufacturer’s manual.

PBMC isolation for cell culture

Buffy coats from 9 blood donors were acquired through the blood bank (Sanquin, Nijmegen, the Netherlands). Blood donors were healthy white men, aged between 18 and 40 y, who gave informed consent. PBMCs were isolated directly after arrival of theuffy coat (≤8 h after donation) by Ficoll-paque Plus density centrifugation (Amersham Biosciences, Roosendaal, the Netherlands). Cells were washed twice in RPMI1640 medium, and isolated cells were counted and tested for viability by trypan blue exclusion before culture.

PBMC cell culture

PBMCs were cultured with the use of RPMI1640 medium with 2 mmol/L L-glutamine, 10% fetal bovine serum, and antibiotics (penicillin and streptomycin) in the presence of 5% CO₂ at 37 °C. Cell culture medium containing glutamine and serum were purchased from Gibco BRL (Grand Island, NY). PBMCs were incubated at 1.0 × 10⁶ cells/mL with 50 μmol/L Wy14643, or 0.1% dimethyl sulfoxide (vehicle), for 12 h. After incubation, the cells were collected, washed twice in phosphate-buffered saline, counted, and tested for viability by trypan blue exclusion.

Flow cytometric analysis of PBMC vitality

Viability of the cells after incubation with 50 μmol/L Wy14643 was determined by staining with annexin and propidium iodide. Flow cytometry analysis with a Coulter Epics XL-MCL flow cytometer (Beckman Coulter, Mijdrecht, the Netherlands) followed.

Total RNA isolation

PBMC RNA was isolated from all PBMC samples with the use of Qiagen RNeasy Micro kit (Qiagen, Venlo, the Netherlands). RNA yield was quantified on a Nanodrop ND 1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE), and integrity was measured on an Agilent 2100 Bioanalyzer with RNA
Microarray processing

From the 12 PBMC RNA samples from the first study, 11 samples were used for microarray processing. One sample from the first study was excluded because of low RNA quality. Total RNA (1 μg/sample) was labeled with the use of a 1-cycle cDNA labeling kit (Affymetrix Inc, Santa Clara, CA) and hybridized to Affymetrix Human whole genome U133 plus 2.0 arrays (Affymetrix). Sample labeling, hybridization to chips, and image scanning were performed according to the manufacturer’s GeneChip Expression Analysis Technical Manual (Affymetrix).

Microarray data analysis

Quality control was performed and fulfilled the criteria for array hybridization suggested by the Tumor Analysis Best Practices Working Group (15). Expression values were calculated with the use of the Robust Multichip Average method. Robust Multichip Average signal value estimates are based on a robust average of background-corrected perfect match intensities, and normalization was done with the use of quantile normalization (16, 17). Probe sets were filtered with the use of the interquartile range (18), using a cutoff value of 0.3. Only probe sets with normalized signals >20 on ≥2 arrays were defined as expressed and selected for further analysis. Individual probe sets were defined as “changed” when comparison of the normalized signal intensities showed a \( P \) value < 0.05 in a 2-tailed paired \( t \) test. For pathway analysis, only probe sets with an average fold change of >1.4 or \(<-1.4\) between baseline and 24 h of fasting or between baseline and 48 h of fasting selected. Conversion of number of probe sets to amount of genes was performed by using the DAVID Gene ID conversion tool (DAVID Bioinformatic Resources, 2006, http://niaid.abc.ncifcrf.gov/conversion.jsp). Pathway analysis was performed with the use of GENMAPP 2.0 (19) (http://www.genmapp.org/). The MAPPFINDER software was used to rank the GenMAPP local pathways by \( z \) score during fasting. The \( z \) scores in this software program are calculated by subtracting the expected number of genes meeting the criterion from the observed number, divided by the SD of the observed number of genes.

cDNA synthesis and quantitative real-time PCR

RNA was reverse transcribed with the use of the iScript cDNA synthesis kit (Bio-Rad Laboratories BV, Veenendaal, the Netherlands). Standard quantitative real-time polymerase chain reaction (Q-PCR) was performed with the use of Platinum Taq DNA polymerase and SYBR Green on an iCycler PCR machine (Bio-Rad Laboratories BV) 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 acid ribosomal phosphoprotein PO, which was shown to be consistent within PBMCs (20).

Statistical methods

A 2-tailed paired \( t \) test was used to determine significant differences in plasma metabolite concentrations and Q-PCR gene expression values between baseline and 24 h of fasting and between baseline and 48 h of fasting. We applied Bonferroni corrections as an adjustment for multiple testing and defined statistical significance at \( P < 0.025 \). For the ex vivo experiments statistical significance was accepted at \( P < 0.05 \). All calculations were performed with the use of SPSS (version 12.0.1; SPSS, Chicago, IL).

RESULTS

Volunteer characteristics

All 4 volunteers completed both 48-h fasting periods without any problems. The characteristics of the subject population are presented in Table 1, in which the glucose concentrations are measured during the screening after an overnight fast. Fasting during the study resulted in a decrease in glucose concentrations, from an average of 4.2 mmol/L directly after the meal to an average of 3.6 mmol/L after 48 h (data not shown). Changes in individual FFAs are shown in Figure 1. All plasma FFA concentrations were strongly elevated after 24 h of fasting and remained elevated after 48 h of fasting. The mean increases after 48 h of fasting in monounsaturated FAs, saturated FAs, and polyunsaturated FAs (PUFAs) were 10, 7, and 6 times the basal concentration, respectively (Figure 1A). Within the PUFAs the \( n-3 \) and \( n-6 \) FAs increased 4 and 6 times, respectively (Figure 1B).

Differential gene expression on microarray

Fasting for 24 and 48 h resulted in a differential expression of 3582 of a total 54 675 tested probe sets in PBMCs (Figure 2). These changed probe sets encoded for 3030 genes. After selection of probe sets with a signal >20 in ≥2 arrays and a fold change >1.4 or \(<-1.4\), 1200 genes were changed after 24 h of fasting and 1386 genes after 48 h of fasting. Most of those genes showed an up-regulation in expression, ie, 74% after 24 h and 78% after 48 h. Of the genes meeting the selection criteria >74% were changed at both time points.

Pathway analysis

Subsequent pathway analysis of the microarray data showed that several genes that exhibited increased expression on fasting were involved in FA metabolism and, more specifically, in FA \( \beta \)-oxidation (Figure 3). Furthermore, pyruvate metabolism and most of the RNA-DNA metabolism pathways were up-regulated on fasting in PBMCs. A decreased expression of genes involved in the tricarboxylic acid (TCA) cycle was observed. Most pathways were already changed after 24 h, which sustained until 48 h. Exceptions were regulation of viral genome replication and B

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**Table 1**

<table>
<thead>
<tr>
<th>Characteristics of participants (n = 4 men)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>20.6 (19–22)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.84 (1.80–1.87)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72 (68–79)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.3 (20.4–22.6)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)²</td>
<td>5.2 (4.9–5.7)</td>
</tr>
</tbody>
</table>

¹ All values are \( \bar{x} \); range in parentheses.

² Determined after an overnight fast during the screening visit.
cell differentiation, which only showed up-regulation after 48 h, and heme biosynthesis, which only showed down-regulation at 48 h. Another exception was negative regulation of transcription, which showed up-regulation and down-regulation of genes in the same “pathway,” leading to both an increase and decrease at 24 h.

Specific gene expression

Of all the genes changed after 24 and 48 h of fasting, pyruvate dehydrogenase kinase isoform 4 (PDK4) showed the largest changes in gene expression with increases of 7.2 and 7.4 after 24 and 48 h of fasting, respectively (See Table S1 under “Supplemental Data” in the online issue). This gene is known to be regulated by PPARα. The genes that we found to change expression after fasting and that are known PPARα target genes from mice studies are listed in Table 2. Interestingly, expression of all these PPARα target genes was up-regulated, either at 24 h or 48 h of fasting or at both time points. Many of these genes are involved in FA β-oxidation, such as carnitine palmitoyltransferase 1 (CPT1); acetyl-Coenzyme A acyltransferase 2 (AACA2), solute carrier family 25 (carnitine/acyl/carnitine translocase), member 20 (SLC25A20); acyl-Coenzyme A dehydrogenase very long chain; and acyl-Coenzyme A thioesterase 2.

Confirmation of gene expression data

To confirm the data found with microarray analysis and to observe whether the changes were consistent, Q-PCR was performed on RNA from the first and the second study (Figure 4). Genes chosen for confirmation were PDK4 and several genes involved in FA β-oxidation. For all genes the mode of change observed with microarray analysis could be confirmed by Q-PCR, although changes were smaller. Q-PCR changes after 48 h of fasting, but not after 24 h, were significant for PDK4 and SLC25A20. In the second study, all genes were significantly changed at 48 h, except for CPT1, which showed no significant change. Changes at 24 h in the second study were increased significantly for PDK4 and SLC25A20 (Figure 4).

Cell culture experiments

Several of the genes changed on fasting are known PPARα target genes. To show whether those genes can be activated by PPARα in PBMCs, freshly isolated PBMCs from 9 healthy male donors were used in cell-culture experiments. Incubation of these PBMCs with 50 μmol/L of the highly specific PPARα ligand Wy14643 showed an increase in expression of PDK4, CPT1, ACAA2, and SLC25A20 compared with the control cells, which were incubated with vehicle (Figure 5). PPARα expression itself was not changed on incubation with Wy14643. PBMCs showed no loss of viability after incubation with 50 μmol/L of Wy14643 for 12 h (data not shown).

DISCUSSION

Here, we show that elevated concentrations of plasma FFAs during fasting have a pronounced effect on PBMC gene expression, in particular on expression of PPARα target genes. Correspondingly, pathway analysis showed a strong increase in genes involved in FA metabolism, and, more specifically, of the FA β-oxidation in PBMCs. The functionality of PPARα in PBMCs was shown in an ex vivo study in which we showed that PPARα is responsible for an increased transcription of genes involved in FA β-oxidation when these cells are incubated with the highly specific PPARα ligand Wy14643.

So far, mainly disease-related PBMC genomic fingerprints have been shown with transcriptomics techniques (1–5). We observed that, even during a metabolic condition such as fasting when FFAs are elevated, microarray analyses can show many differentially expressed genes in PBMCs. These gene expression changes are not only observed after 48 h but also are already present after 24 h of fasting. The clear overlap in genes changed at both time points is also reflected in a similar change in pathways after 24 and 48 h of fasting. Interestingly, the number of genes changed in PBMCs is quite large, especially if the low number of 4 subjects included in the study is taken into account. To assure that the gene expression changes were truly due to fasting, the whole study was repeated 2 mo later with the same persons. In this second study, we observed that several of the genes, which expression was changed in the first study, were also changed, showing the consistency of our microarray findings. In addition, the high number of genes changed and the consistency of the findings indicate that fasting has a profound effect on gene expression in PBMCs. These promising results implicate that PBMCs may also be used to monitor more subtle effects associated with dietary intervention, providing that sufficient numbers of subjects are included.
Although a large number of genes were changed after fasting, many of the genes changed play a role in metabolic pathways normally regulated during fasting in the liver, such as an increase in FA β-oxidation and a decrease in the TCA cycle (8). During fasting, when FFAs are used as the main fuel source, FA β-oxidation will be up-regulated to provide acetyl coenzyme A (CoA), subsequently used in the TCA cycle. Our study showed that genes involved in FA β-oxidation were also up-regulated in PBMCs after fasting. Interestingly, as known from mice studies, the expression of several of these genes is regulated by the FA-activated nuclear receptor PPARα. In these mice studies, fasting was shown to activate PPARα in liver, resulting in up-regulation of processes such as FA β-oxidation (9). Moreover, the gene with the most profound up-regulation, PDK4, is also known to be regulated by PPARα during fasting in rat skeleton muscle and heart (21, 22). PDK4 executes its role during fasting by inhibiting the pyruvate dehydrogenase complex, thereby decreasing conversion of pyruvate to acetyl CoA, leading to conservation of glucose. PDK4 is expressed at high concentrations in various tissues, predominantly in heart and skeletal muscle but also in liver and kidney (23). The role of human PDK4 during fasting was mainly studied in skeletal muscle, in which, similar to our study, PDK4 expression was increased (24–26). The functionality of PPARα, with respect to its capability to increase the expression of genes involved in FA β-oxidation and PDK4 in PBMCs, was proven by the ex vivo incubation with the specific PPARα ligand Wy14643. We showed that these PPARα target genes were up-regulated in PBMCs from 9 different donors after incubation with the ligand. This indicates that PPARα is functional in PBMCs and likely mediates the FA-dependent gene expression in these cells. It was previously reported that PPARα-controlled genes in mice T cells could only be up-regulated by ligand-induced activation of PPARα when histone deacetylase inhibitors are used (27). Interestingly, we found an up-regulation of PPARα target genes in freshly isolated human PBMCs, consisting of roughly 60% T cells, without the addition of histone deacetylase inhibitors. In addition, it was also reported that activation of PPARα during fasting or with the PPARα ligand Wy14643 results in an up-regulation in expression of PPARα itself in liver tissue of mice studies and from in vitro studies with human primary hepatocytes (9, 28). We however did not observe such autoregulation of PPARα in human PBMCs, neither during fasting nor after incubation of the cells with Wy14643. Both studies showed no change in gene expression of PPARα. We speculate that this autoregulation is less pronounced in or not present in PBMCs, which may explain the discrepancies between the studies performed in either mouse or other human tissues. We found in our study a sizable variation in both basal expression of the genes, as in gene expression changes, between the subjects. This not only points toward a large variation between persons but also to a large variety in the gene expression response toward a metabolic change. From microarray studies it is reported that not only variation in PBMC basal gene expression between human subjects can be large, but also that there is a
sizable variation in proportions of PBMC subpopulations between persons, which might explain the large variation in individual response (29–31). However, all studies report that within one person, variety is relatively small, both in PBMC gene expression and subpopulations, and has little effect on PBMC gene transcription profiles. Therefore, dietary intervention studies with multiple measurements within the same person are necessary for the detection of gene expression changes in PBMCs.

<table>
<thead>
<tr>
<th>Affymatrix identification</th>
<th>Gene symbol</th>
<th>Gene name</th>
<th>Mean signal</th>
<th>24 h</th>
<th>48 h</th>
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<tbody>
<tr>
<td>225207_at</td>
<td>PDK4</td>
<td>Pyruvate dehydrogenase kinase, isoenzyme 4</td>
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<td>7.19</td>
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<td>7.10</td>
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<td>Carnitine palmitoyltransferase 1A (liver)</td>
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<td>203658_at</td>
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<td>Acyl-CoA thioesterase 2</td>
<td>20–50</td>
<td>NC</td>
<td>1.53</td>
</tr>
</tbody>
</table>

1 NC, not changed significantly.
2 All fold changes were significant: \( P < 0.05 \).
3 All genes were up-regulated.
Concisely, our study shows that during fasting, blood FFAs are elevated, activating PPARα in the blood cells, i.e., the PBMCs, thereby inhibiting the conversion of pyruvate to acetyl CoA to conserve glucose, by up-regulating PDK4 and at the same time increasing FA-oxidation to provide energy by metabolizing the released FFAs. This elevated FA β-oxidation increases the lipid handling capacity of PBMCs. From all FAs, PUFAs were shown to activate PPARα most profoundly (32, 33). We therefore hypothesize that these FAs will have the largest effect on PPARα-dependent gene transcription and subsequently increase lipid-handling capacity in PBMCs to the highest extent. Moreover, it was found that activation of T cells after preincubation with a PPARα ligand reduces expression of proinflammatory cytokines (12). Both the cellular lipid handling and the balanced inflammatory response capacity are of pivotal importance in the development of diet-related chronic diseases such as metabolic syndrome, diabetes type 2, and atherosclerosis. Illustrative for this is that PPARα-specific agonists strongly inhibited atherosclerosis in mice studies (34, 35). In addition, several clinical trials have shown that synthetic agonists of PPARα have beneficial, antiatherogenic effects in humans (36). We hypothesize that replacing dietary saturated FAs with dietary PUFAs will activate PPARα, leading to an increased fat-handling capacity and decreased proinflammatory response in PBMCs, ultimately resulting in a decreased cell activation and consequent prevention of the onset of diet-related disorders such as atherosclerosis and metabolic syndrome. These results not only imply that PBMCs can be used for further characterization of human PPARα, in vivo and ex vivo, but also that PBMCs are good candidate cells to study other molecular mechanism of nutrients.

In summary, we have shown that physiologically relevant metabolic conditions such as fasting are reflected by PBMC gene expression profiles. Therefore, these profiles show great potential to be used in the detection of even more subtle changes in gene expression as expected in nutritional intervention studies. In addition, our intervention study showed that during fasting, when blood FFAs are increased, the fat-handling capability of PBMCs was augmented by an increase in FA β-oxidation.
Finally, our ex vivo study showed a functional role of PPARα in human PBMCs, suggesting that this nuclear factor regulates the lipid-handling capacity of these cells, which probably can be regulated by the type of dietary fat intake.

The author’s responsibilities were as follows—MB: collected and analyzed the data and wrote the manuscript; LAA and MM: designed the studies and interpreted the data. All authors revised the manuscript.

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30. Radich JP, Mao M, Stepaniants S, et al. Individual-specific variation of gene expression profiles in peripheral blood mononuclear cells of 9 donors after incubation with 50 μmol/L Wy14643 for 12 h. Dots represent different donor; lines represent mean values. Significantly changed compared with vehicle (P < 0.01) as determined with a paired t test. PDK4, pyruvate dehydrogenase kinase isoform 4; CPT1, carnitine palmitoyltransferase 1; SLC25A20, solute carrier family 25 (carnitine/acylcarnitine translocase), member 20; ACAA2, acetyl-coenzyme A acyltransferase 2.

