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Differential Expression of Oxidative Stress and Inflammation Related Genes in Peripheral Blood Mononuclear Cells in Response to a Low-Calorie Diet: A Nutrigenomics Study

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Abstract

Nutrigenomics is a new application of omics technologies in nutritional science. Nutrigenomics aims to identify molecular markers of diet-related diseases and mechanisms of interindividual variability in response to food. The aim of this study was to evaluate peripheral blood mononuclear cells (PBMC) as a model system and readily available source of RNA to discern gene expression signatures in relation to personalized therapy of obesity. PBMC were collected from obese men before and after an 8-week low-calorie diet (LCD) to lose weight. Changes in gene expression before and after the LCD were initially screened using a DNA-microarray platform and validated by qRT-PCR. Global gene expression analysis identified 385 differentially expressed transcripts after the LCD. Further analyses showed a decrease in some specific oxidative stress and inflammation genes. Interestingly, expression of these genes was directly related to body weight, while a lower IL8 gene expression was associated with higher fat mass decrease. Collectively, these observations suggest that PBMCs are a suitable RNA source and model system to perform nutrigenomics studies related to obesity and development of personalized dietary treatments. IL8 gene expression warrant further research as a putative novel biomarker of changes in body fat percentage in response to an LCD.

Introduction

Understanding the molecular basis involved in obesity and weight homeostasis is a crucial first step in developing therapeutic strategies against excess in body weight gain (Sun, 2007; Viguerie et al., 2005a). Based on this, the study of gene expression at the level of mRNA in response to changing conditions, such as in nutritional intervention studies (Kussmann et al., 2006), is being carried out by transcriptomics, mainly using arrays (Heidecker and Hare, 2007; Moreno-Aliaga et al., 2001). This new field, known as nutrigenomics, attempts to elucidate the impact of diet on gene expression, whose long-term aim is personalized nutrition for health maintenance and disease prevention (Kaput, 2008; Mariman, 2006; Vakili and Caudill, 2007).

In this context, most gene expression studies in human obesity are often focused on adipocytes, mainly on biopsed subcutaneous adipose tissue (Hernandez-Morante et al., 2008; Marrades et al., 2006; Viguerie et al., 2005a). Analysis of visceral adipose tissue is basically restricted to morbid patients, whose tissue samples can be easily obtained during surgery (Baranova et al., 2005). However, nutrigenomics studies in humans have remained clearly insufficient to date because it is difficult to obtain biopsies from every volunteer involved in a nutritional study due to the invasive sample collection procedure (Kussmann et al., 2006).

Molecular mechanisms underlying several human diseases have been investigated by gene expression analysis of peripheral blood mononuclear cells (PBMC) as surrogates for predicting potential effects in tissues that are not easily accessible (Eady et al., 2005). These cells have been proposed as a useful tool to better understand multiple sclerosis related pathways (Achiron and Gurevich, 2006), the pathophysiology of Alzheimer's disease (Maes et al., 2006), hepatic diseases (Chiappini et al., 2006; Patalay et al., 2005), oncology (DePrimo et al., 2003; Twine et al., 2003), and cardiovascular research (Horwitz et al., 2004). Also, peripheral blood mononuclear cells (PBMC) were suggested as a surrogate tissue to muscle biopsies to study mitochondrial dysfunction (Abu-Amero and Bosley, 2005; Marriage et al.,

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2003). The suitability of PBMCs for nutrigenomics studies is in part related to their active metabolism (Kussmann et al., 2006) and accessibility by a simple venipuncture (Liew et al., 2006; Marriage et al., 2003), which may also permit repeated time-series analysis of changes in gene expression in response to food-related interventions. Interestingly, a remarkable concordance (>80%) of gene expression profiles between PBMC and different tissues has been demonstrated (Liew et al., 2006). Therefore, transcriptome analysis of peripheral blood is a promising approach for determining disease and treatment outcome (Debey et al., 2006). Based on these considerations, the aim of the present study was to investigate whether PBMC gene expression is a suitable model system to carry out nutrigenomics studies in human obesity. Moreover, we evaluated PBMC gene expression signatures to identify biomarkers that could predict individual differences in weight loss after a low-calorie diet (LCD).

Subjects and Methods

Subjects

The study was carried out in Caucasian obese men (n = 9) carefully recruited to participate as volunteers (Table 1). Initial screening evaluations included a medical history, physical examination, and fasting blood profile to exclude subjects with clinical evidence of diabetes, hypertension, liver, renal or hematological disease, or other disorders that could interfere with the weight-loss process. Such features were tested by a specifically trained physician. Other exclusion criteria were weight change ±3 kg within the 3 months before the start of the study, participation in another scientific study up to 1 month before, drug administration, pregnancy, surgical or drug-obesity treatment, or alcohol or drug abuse.

After a clear explanation of the study, all subjects gave their written informed consent to participate, which was previously approved by the Ethics Committee of the University of Navarra (54/2006), in agreement with the Helsinki Declaration.

Study design

The nutritional treatment involved an 8-week LCD, in which the energy restriction was set up at −35% with respect to the total energy expenditure of each participant calculated by Harris-Benedict equation after applying the WHO's correction factor on physical activity [OMS (WHO), 2000]. The LCD was balanced for macronutrients (55% of energy as carbohydrates, 15% as proteins, and 30% as fat). The menus were fixed following a dietary framework based on a food exchange system (Crujeiras et al., 2006, 2007; Tremblay et al., 1983). Each subject was provided with a detailed diet plan to follow for the 8 weeks and met with a dietician periodically. Weight loss was monitored weekly by a dietician, and the intake was controlled by 3-day weighted food records (2 weekdays and 1 weekend day). Foods records were performed during the week before the beginning of the intervention (week 0), and during the week before the end of the nutritional trial (week 8). These data provided information about baseline intake and the adherence to the prescribed diets. All subjects lost at least 5% of body weight as a result of the hypocaloric diet. At baseline (day 0) and at the end of the nutritional intervention (day 56), anthropometry was measured, and blood samples were collected with EDTA as anticoagulant to obtain the PBMC for gene expression analyses.

Blood pressure and nutritinal status assessment

Blood pressure was measured with a standard mercury sphygmomanometer (Minimus II, Riester, Germany) after the subject was quietly sitting for 5 min following OMS criteria. Plasma levels of glucose and total cholesterol were measured by specific colorimetric assays (Horiba ABX Diagnostics, Montpellier, France) using an automatized system (COBAS MIRA, Roche, Basel, Switzerland).

Isolation of RNA from PBMC

The PBMC were isolated by differential centrifugation using the PMN medium (Axis Shield PoC AS, Oslo, Norway). After PBS washing, the total RNA was extracted from PBMC by the Trizol reagent method (Invitrogen, Carlsbad, CA). Quantitation and purity of the RNA obtained were assessed by UV spectrophotometry (Multiskan Spectrum, Thermo Electron Corp., Finland). The RNA integrity was assesed using a 2100 Bioanalyzer instrument (Agilent Technologies, Palo Alto, CA). Additionaly, RNA quality was also checked on agarose gel electrophoresis.

Synthesis and labeling of cDNA

Fluorescently labeled cDNA was synthesized from 10 μg of each total RNA sample. Anchored oligo(dT)20 and amino–allyl adducts (Sigma-Aldrich, St. Louis, MO) were used in the first strand cDNA synthesis reaction. The resulting amino modified cDNA was divided into two tubes, and each replicate was labeled using either Cy3 or Cy5 fluorescent dyes (Amersham Biosciences, Piscataway, NJ), according to manufacturers protocols for appropriate dye-swap hybridizations.

Microarray hybridization and image acquisition

Samples were hybridized to the Human 1A Oligo Microarray Kit (V2) as described by the supplier (Agilent Technologies, Santa Clara, CA). Each single oligo microarray comprises over 22,575 probes and spans conserved exons across the targeted human full-length gene transcripts. Specifically, this array represents about 20,000 well-characterized, full-length human genes. This probe set is sourced from the Incyte Foundation Database, RefSeq, and GenBank databases.

RNA samples coming from PBMC that were taken before and after the nutritional intervention were differentially labeled and cohybridized on microarray slides. Appropriate dye-swap hybridizations (dye reversal) were also carried out to minimize potential biases arising from differences in the dyes. Microarrays were hybridized overnight at 60°C in hybridization chambers (Genetix, Boston, MA). After hybridizations slides were washed and dried prior to scanning.

Microarray images were obtained by scanning each slide in a Gene Pix 4100A scanner (Axon Instruments, Union City, CA). Image quantitation was performed using associated software GenePiX Pro 6.0 and median intensity background

Table 1. Volunteers' characteristics before and after the low calorie diet

Subjects	*Age, y*	*BW, kg (day 0)*	*BMI, kg/m^2 (day 0)*	*SBP/DBP, mmHg (day 0)*	*Glucose, mg/dL (day 0)*	*Total cholesterol, mg/dL (day 0)*	*BW loss, %*	*BFM loss, %*
1	39	104.5	31.9	114/71	94.3	214.4	−9.47	−12.13
2	40	86.6	29.3	122/67	85.8	210.1	−8.31	−14.18
3	51	93.6	32.4	143/91	88.9	232.6	−5.45	nd
4	49	105.8	34.5	116/71	78.5	198.7	−9.64	−13.78
5	40	91.9	31.4	141/85	92.6	250.4	−8.71	−14.15
6	46	104.9	33.8	126/79	89.7	224.9	−9.82	−18.43
7	39	144.9	38.9	138/83	109.4	266.3	−8.42	−10.48
8	50	89.7	32.5	134/79	90.5	211.2	−9.14	−8.96
9	49	157.3	40.1	123/87	107.1	207.2	−10.93	−11.58
Mean ± SD	44.8 ± 5.2	108.8 ± 25.2	33.9 ± 3.5	128 ± 11/79 ± 8	94.0 ± 9.9	224.0 ± 22.2	−8.9 ± 1.5[a]	−13.0 ± 2.9[a]

BW, body weight; BMI, body mass index; BFM, body fat mass; SBP/DBP, systolic blood pressure/diastolic blood pressure; nd, not determined

[a] $p < 0.001$, when comparing day 56 vs day 0 by Wilcoxon-paired test.

subtracted values for each spot were used in subsequent analysis.

Normalization and data analysis

Scanned microarray images were first examined for visible defects and then checked for the fitness of the gridding. When passed, the image file was analyzed to generate composite data files. From this point on, analyses were carried out using the GeneSpring GX software v 7.3.1 (Agilent Technologies).

To normalize data, intensity-dependent (LOWESS) normalization was used to eliminate dye-related artefacts. Once data were normalized, consecutive filtering steps were performed to remove noise derived from absent genes, background, and nonspecific hybridizations.

A Student's *t*-test was applied to select differentially expressed genes between both conditions (before and after nutritional intervention). Comparisons were performed for each gene, and genes with the most significant differential expression (p-value cutoff < 0.05) were returned. Additionally, statistically selected genes were assigned into specific Gene Ontology categories, as well as in KEGG associated pathways in order to extract maximum biological information. The GO ontologies provide a systematic language for the consistent description of attributes of genes and gene products in three key biological domains that are shared by all organisms: molecular function, biological process, and cellular component (The Gene Ontology Consortium, 2008).

MIAME ArrayExpress

The current microarray data have been curated and accepted in the ArrayExpress database. This is a public repository for microarray data, which is aimed at storing MIAME (*Minimum Information About Microarray Experiments*) compliant data in accordance with MGED (*Microarray Gene Expression Data*) recommendations. The ArrayExpress Data Warehouse stores gene-indexed expression profiles from a curated subset of experiments in the repository. Access to data concerning thus study may be found at ArrayExpress Web page (http://www.ebi.ac.uk/arrayexpress), with experiment accession number: E-MEXP-1055.

Quantitative real-time PCR

Quantitation of mRNA was performed using quantitative real-time polymerase chain reaction (qRT-PCR) to confirm microarray data. Extracted total RNA from all the subjects was purified with DNase treatment by means of DNA-free kit (Ambion, Austin TX) used as a template to generate first-strand cDNA synthesis using M-MLY reverse transcriptase as described by the manufacturer (Invitrogen). Quantitative real-time PCR was performed using an ABI PRISM 7000 HT Sequence Detection System as described by the provider (Applied Biosystems, Foster City, CA). Taqman probes for genes were supplied by Applied Biosystems and gene expression levels were normalized using *18S* rRNA as internal control, because this housekeeping gene does not change after the 8 weeks of low caloric diet, and it has been proved in previous studies with PBMC (Ghanim et al., 2004).

Eleven genes were selected for PCR quantificantion according to their association with the proinflammatory and/or oxidative stress processes. These genes were selected because of their importance in the respective metabolic pathways. Thus, regarding inflammation-related processes, all the genes selected (*TANK*, *TNFAIP8L1*, *TRIAD3*, *NKRF*, *TNIP1*, and *RIPK3*) are related to TNFα/NF-κB signalling pathway, in addition to *IL8*. Regarding oxidative stress-related genes, some of them are involved in the mitochondrial function (*NDUFS2*, *COX15*, and *ACAA2*), whereas others are related to antioxidant defenses (*MGST2*).

Statistical analysis

The fold change in gene expression was calculated using the $2^{-\Delta\Delta Ct}$ relative quantitation method, using the average of *Ct* values after subtraction with *Ct* value of *18S* from baseline (Livak and Schmittgen, 2001; Milagro et al., 2007) according to the manufacturer`s guidelines (Applied Biosystems). The nonparametric Wilcoxon-paired test was applied to detect differences before and after weight loss in anthropometric measurements, as well as the expression levels of selected genes. Data are reported as the mean $\pm$ SD for anthropometric measurements and geometric mean (SEM as range) for qRT-PCR results. Correlation analysis was performed with Spearman rank order correlation. A p-value ≤ 0.05 was considered as statistically significant and a p-value ≤ 0.1 as a trend for significance. Statistical analysis was performed by SPSS 13.0 software (SPSS Inc. Chicago, IL) for Windows XP (Microsoft, Redmond, WA).

Results

Owing to the energy restriction, all volunteers lost body weight ($-8.9 \pm 1.5\%$), which was accompanied by a marked decrease in body mass index (Table 1). The microarray comparison between before and after the nutritional intervention resulted in 385 differentially expressed genes, with 158 genes overexpresed and 227 genes downregulated after the LCD treatment with respect to baseline before following the LCD. As shown in Figure 1, genes encoding factors involved in nucleotide, DNA, and chromatin metabolism, as well as in cellular biosynthetic and regulation of metabolic processes, including protein and lipid metabolic pathways, were mostly upregulated ($p \leq 0.05$), whereas those related to signal transduction, cell communication, transport, immune response, and carbohydrate metabolism were mostly downregulated ($p \leq 0.05$).

Gene ontology and pathway analysis (Table 2) showed that the biological functions associated with carbohydrate, lipid, and protein metabolism, oxidative phosphorylation, immune response, and coagulation were affected by the nutritional intervention. Moreover, after weight loss, other genes involved in relevant metabolic pathways, such as *SIRT2*, *GHRL*, *RBP2*, and *SRF*, were differentially expressed (Table 2).

After analyzing microarray data, 11 oxidative stress and inflammation-related genes were selected for qRT-PCR quantification. Thus, although some genes related to the TNFα/NF-κB signaling pathway did not change their expression, such as *TANK*, *TNFAIP8L1*, *TRIAD3*, and *NKRF*, others, such as *TNIP1* and *RIPK3*, as well as *IL8*, significantly decreased after the LCD treatment (Table 3). Regarding oxidative stress related genes, *NDUFS2* significantly decreased the expression levels in PBMC, while no changes in *MGST2*,

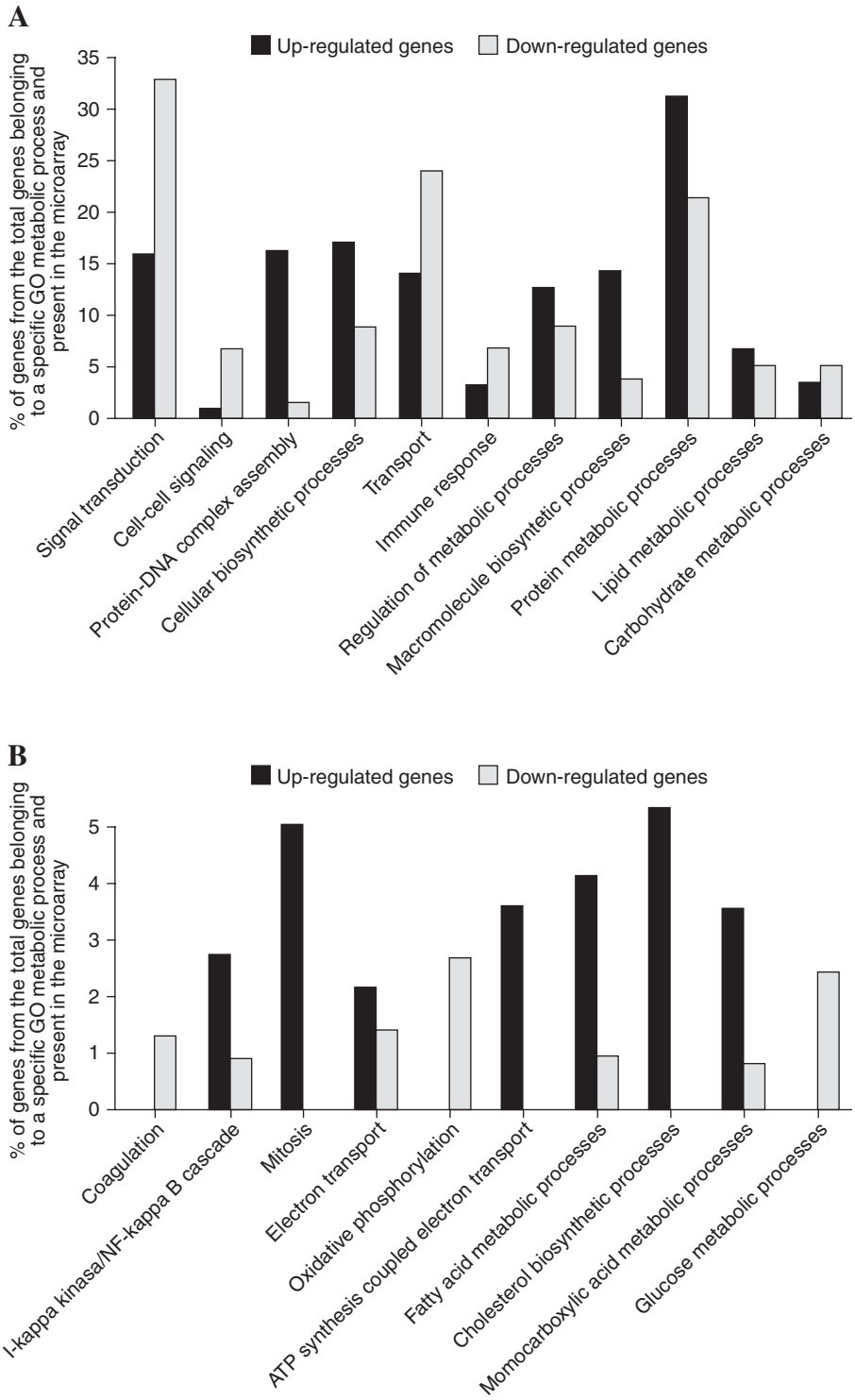

FIG. 1. Histograms showing some biological processes differentially regulated by weight loss in PBMC of obese subjects treated by an 8-week low-caloric diet. (**A**) Some metabolic processes from levels 4 and 5 of Gene Ontology, which are stated in the X-axis. (**B**) Other specific molecular functions belonging to levels 3 to 7 of Gene Ontology (GO), which are stated in the X axis. In the Y-axis, the percentage of genes belonging to each biological process showed in the X-axis that are down- or upregulated by the caloric restriction treatment, is represented compared to the total number of genes of each biological process that are present in the microarray.

TABLE 2. SELECTED GENES AND PATHWAYS OF INTEREST CONCERNING CHANGES IN PBMC GENE EXPRESSION PROFILING LIST AFTER WEIGHT LOSS

Pathways and Genes	*Gene symbol*	*GenBank ID*	*Fold change*
Protein metabolism			
Solute carrier family 7	SLC7A4	NM_004173	0.78 ± 0.05
Gamma-glutamyltransferase-like activity 1	GGTLA1	NM_004121	0.71 ± 0.16
Homogentisate 1,2-dioxygenase	HGD	NM_000187	0.89 ± 0.05
Acireductone dioxygenase 1	MTCBP-1	NM_018269	1.30 ± 0.27
Glutaminase 2	GLS2	NM_013267	1.31 ± 0.34
Nitrogen fixation 1 homolog	NFS1	NM_021100	1.50 ± 0.41
Carbohydrate metabolism			
ST6 beta-galactosamide alpha-2,6-sialyltranferase 2	ST6GAL2	NM_032528	0.58 ± 0.18
Alcohol dehydrogenase IB	ADH1B	NM_000668	0.73 ± 0.19
Ribose 5-phosphate isomerase A	RPIA	NM_144563	0.84 ± 0.11
Glycogenin 2	GYG2	NM_003918	0.77 ± 0.19
Insulin receptor substrate 2	IRS2	NM_003749	0.87 ± 0.11
Xylosyltransferase II	XYLT2	NM_022167	1.11 ± 0.05
Hyaluronoglucosaminidase 1	HYAL1	NM_007312	1.21 ± 0.16
Fucosyltransferase 8	FUT8	NM_178154	1.28 ± 0.24
Lactate dehydrogenase D	LDHD	NM_153486	1.27 ± 0.31
Lipid metabolism			
Apolipoprotein C-I	APOC1	NM_001645	0.84 ± 0.08
Very low density lipoprotein receptor	VLDLR	NM_003383	0.88 ± 0.06
Acetyl-Coenzyme A acyltransferase 2	ACAA2	NM_006111	1.26 ± 0.15
Acyl-CoA synthetase long-chain family member 5	ACSL5	NM_203380	1.24 ± 0.23
Phospholipase A2, group XIIB	PLA2G12B	NM_032562	1.42 ± 0.41
Patatin-like phospholipase domain containing 3	ADPN	NM_025225	0.66 ± 0.21
Gamma-glutamyltransferase-like activity 1	GGTLA1	NM_004121	0.71 ± 0.16
Pancreatic lipase-related protein 1	PNLIPRP1	NM_006229	0.75 ± 0.18
Apolipoprotein L, 1	APOL1	NM_145344	0.84 ± 0.12
24-dehydrocholesterol reductase	DHCR24	NM_014762	1.05 ± 0.05
Sphingosine kinase 1	SPHK1	NM_021972	1.27 ± 0.28
Fatty acid transport protein 4	SLC27A4	AF055899	0.98 ± 0.61
Microsomal glutahione S-transferase 2	MGST2	NM_002413	1.28 ± 0.32
Oxidative Phosphorilation			
ATPase, H+ transporting, lysosomal V0 subunit a4	ATP6V0A4	NM_020632	0.74 ± 0.10
Cytochrome *c* oxidase assembly protein	COX15	NM_078470	0.68 ± 0.21
NADH-coenzyme Q reductase	NDUFS2	NM_004550	0.77 ± 0.18
Cytochrome b-561	CYB561	NM_001017916	1.15 ± 0.12
Immune Response			
T-cell activation antigen CD26	DPP4	NM_001935	0.79 ± 0.10
Tumor necrosis factor related protein 4	C1QTNF4	NM_031909	0.88 ± 0.04
TNFAIP3 interacting protein 1	TNIP1	NM_006058	0.92 ± 0.06
Tumor necrosis factor (ligand) superfamily, member 8	TNFSF8	NM_001244	0.86 ± 0.12
T-cell surface glycoprotein CD8 beta chain	CD8B1	NM_172099	1.09 ± 0.10
Rho-related GTP-binding protein	RHOH	NM_004310	1.14 ± 0.13
Tumor necrosis factor, alpha-induced protein 8-like1	TNFAIP8L1	NM_152362	1.33 ± 0.37
Bloom syndrome protein	BLM	NM_000057	1.54 ± 0.72
Interferon, alpha 16	IFNA16	NM_002173	1.85 ± 0.85
Interleukin-1 receptor-associated kinase 4	IRAK4	NM_016123	0.68 ± 0.16
Coagulation factor III (thromboplastin, tissue factor)	F3	NM_001993	0.71 ± 0.13
Prostate associated gene 1	PAGE1	NM_003785	0.67 ± 0.18
Killer cell lectin-like receptor subfamily A	KLRA1	NM_006611	0.68 ± 0.20
T-cell receptor T3 gamma chain	CD3G	NM_000073	0.78 ± 0.15
B-lymphocyte antigen CD19	CD19	NM_001770	0.77 ± 0.16
ATP-binding cassette transporter	TAP1	NM_000593	0.84 ± 0.09
CD24 molecule	CD24	NM_013230	0.88 ± 0.07
Adenosine deaminase, RNA-specific	ADAR	NM_001111	0.82 ± 0.15
Interferon, alpha-inducible protein 6	G1P3	NM_022873	1.12 ± 0.11
Oral-facial-digital syndrome 1	OFD1	NM_003611	1.23 ± 0.24
Microsomal glutahione S-transferase 2	MGST2	NM_002413	1.28 ± 0.32
Bradykinin receptor B1	BDKRB1	NM_000710	1.52 ± 0.64
Interleukin 8	IL8	NM_000584	1.32 ± 1.07[a]

TABLE 2. SELECTED GENES AND PATHWAYS OF INTEREST CONCERNING CHANGES IN PBMC GENE EXPRESSION PROFILING LIST AFTER WEIGHT LOSS (CONT'D)

Pathways and Genes	*Gene symbol*	*GenBank ID*	*Fold change*
NF-kappa-B-responsive genes			
TRIAD 3 protein	TRIAD3	NM_207111	0.77 ± 0.10
Receptor-interacting serine-threonine kinase 3	RIPK3	NM_006871	0.68 ± 0.22
NF-kappaB repression factor	NKRF	NM_017544	0.84 ± 0.10
TNFAIP3 interacting protein 1	TNIP1	NM_006058	0.92 ± 0.06
TRAF (tumor necrosis factor receptor-associated factor) family member-associated NF-kappaB activator	TANK	NM_004180	1.09 ± 0.08
Coagulation			
Coagulation factor III (thromboplastin, tissue factor)	F3	NM_001993	0.71 ± 0.13
Vitamin K-dependent gamma-carboxylase	GGCX	NM_000821	0.68 ± 0.22
Other Genes			
Ghrelin	GHRL	NM_016362	0.79 ± 0.15
Sirtuin	SIRT2	NM_012237	1.18 ± 0.11
c-Fos serum response element-binding transcription factor	SRF	NM_003131	1.37 ± 0.37
Retinol binding protein 2	RBP2	NM_004164	1.51 ± 0.69

All the genes in this list were significantly up- or down-regulated ($p < 0.05$) when analyzing the microarray by Student's t-test except for those marked with (a).

COX15, and *ACAA2* were found after the weight loss induced by a LCD (Table 3).

A consistent positive correlation was detected among mRNA levels of some of the genes studied (Table 4). Thus, *RIPK3* was highly associated with *TANK*, *TNIP1*, and *TRIAD3*, all of them in the TNF α/NF-κB pathway. *TNIP* was also related to other genes of this pathway, such as *TANK* and *TRIAD3*, while *TNFAIP8L1* was correlated with *NKRF* and *TANK*, suggesting that these genes share the same regulatory pathway.

On the other hand, the oxidative stress-related genes were also associated each other and with the inflammation-related genes (Table 4). Finally, significant correlations were found between body weight and mRNA expression of *TNIP* ($r = 0.62$; $p = 0.006$) and *TANK* ($r = 0.53$; $p = 0.023$), and *NDUFS2* tended to be correlated ($r = 0.40$; $p = 0.098$), before and after caloric restriction treatment ($n = 18$). While, *TRIAD3* mRNA levels ($r = 74$; $p = 0.037$) after the intervention were associated with body weight at this point, together a trend for a correlation in the *ACAA2* transcripts ($r = 0.64$; $p = 0.066$). Interestingly, a lower *IL8* gene expression after LCD was associated with higher fat mass decrease (Fig. 2).

Discussion

The impact of nutrition on gene expression is being explored in the obesity domain because nutrigenomics approach could be applied to personalize nutrition (Kaput, 2008; Ronteltap et al., 2008). In this context, technologies involved in DNA microarrays make it possible to assess the effect of

TABLE 3. EXPRESSION CHANGES FROM SELECTED GENES IN PBMC ASSESSED BY QRT-PCR AFTER WEIGHT LOSS INDUCED BY A LCD IN OBESE VOLUNTEERS.

Gene symbol	*GenBank ID*	*qRT-PCR*	p-*value*
Inflammation-related genes			
RIPK3	NM_006871	0.72 (0.62–0.85)	**0.086**
TNIP1	NM_006058	0.70 (0.63–0.78)	**0.021**
NKRF	NM_017544	0.90 (0.74–1.09)	0.314
TANK	NM_004180	0.85 (0.71–1.01)	0.441
TRIAD3	NM_207111	0.85 (0.72–0.99)	0.263
TNFAIP8L1	NM_152362	0.87 (0.70–1.09)	0.314
IL8	NM_000584	0.46 (0.29–0.73)	**0.066**
Oxidative stress-related genes			
ACAA2	NM_006111	1.03 (0.78–1.20)	0.678
COX15	NM_078470	0.91 (0.73–1.14)	0.678
NDUFS2	NM_004550	0.71 (0.63–0.80)	**0.021**
MGST2	NM_002413	1.19 (1.02–1.39)	0.374

mRNA levels were normalized to 18S. Fold changes were evaluated using the $2^{-\Delta\Delta Ct}$ method of relative quantification above the expression levels of before low calorie treatment state (set at unity). Data are reported as geometric mean (S.E.M as a range).

Table 4. Spearman rank order correlation analysis between the expression of the evaluated genes

	Inflammation-related genes						*Oxidative stress-related genes*			
	TNIP1	*NKRF*	*TANK*	*TRIAD3*	*TNFAIP8L1*	*IL8*	*ACAA2*	*COX15*	*NDUFS2*	*MGST2*
RIPK3	0.68[c]	0.38	0.83[b]	0.71[c]	0.55	0.43	0.80[c]	0.69[c]	0.80[c]	0.79[c]
TNIP1		0.60	0.82[b]	0.76[c]	0.58	0.30	0.73[c]	0.38	0.60	0.60
NKRF			0.32	0.83[c]	0.93[a]	0.33	0.72[c]	0.56	0.27	0.18
TANK				0.79[c]	0.48	0.17	0.73[c]	0.59	0.93[b]	0.88[b]
TRIAD3					0.90[b]	0.48	0.90[b]	0.76[c]	0.68[c]	0.50
TNFAIP8L1					0.28	0.85[b]	0.74[c]	0.50	0.35	
IL8							0.48	0.33	0.033	−0.08
ACAA2								0.83‡	0.68[c]	0.53
COX15									0.73[c]	0.59
NDUFS2										0.93[a]

Data showed the Spearman correlation coefficient (r).
[a]$p < 0.001$; [b]$p < 0.01$; [c]$p < 0.05$.

a specific diet or nutrient on the expression of a large number of genes (Afman and Muller, 2006; Kussmann et al., 2006; Puskas et al., 2006). Recent examples of this approach show differential gene expression profiling during nutritional intervention both in animal models (Campion et al., 2006; Lopez et al., 2003) or in human biopsies of adipose tissue (Baranova et al., 2005; Clement et al., 2004; Viguerie et al., 2005a). However, few studies have been performed in humans, where an important barrier to identify molecular biomarkers is the inaccessibility to tissue samples (Muller and Kersten, 2003). It would be useful to find a noninvasive source of RNA to explore the role of gene expression in the context of human diet intervention studies. Thus, the gene expression differences in normal individuals has suggested the power of the gene expression in PBMC to study the variation in populations that may account for differences in disease incidence and treatment response (Radich et al., 2004). Accordingly, some authors have recently studied the responses to dietary exposure by analyzing the gene expression profile in blood cells, suggesting that this kind of sample could be applied to perform nutrigenomics studies (van Erk et al., 2006). Moreover, a previous study showed 35 common genes downregulated both in adipose tissue and PBMC from controls compared to obese (García-Amigot et al., 2005). In this sense, the current experimental study has specifically utilized PBMC samples, collected by simple blood extraction,

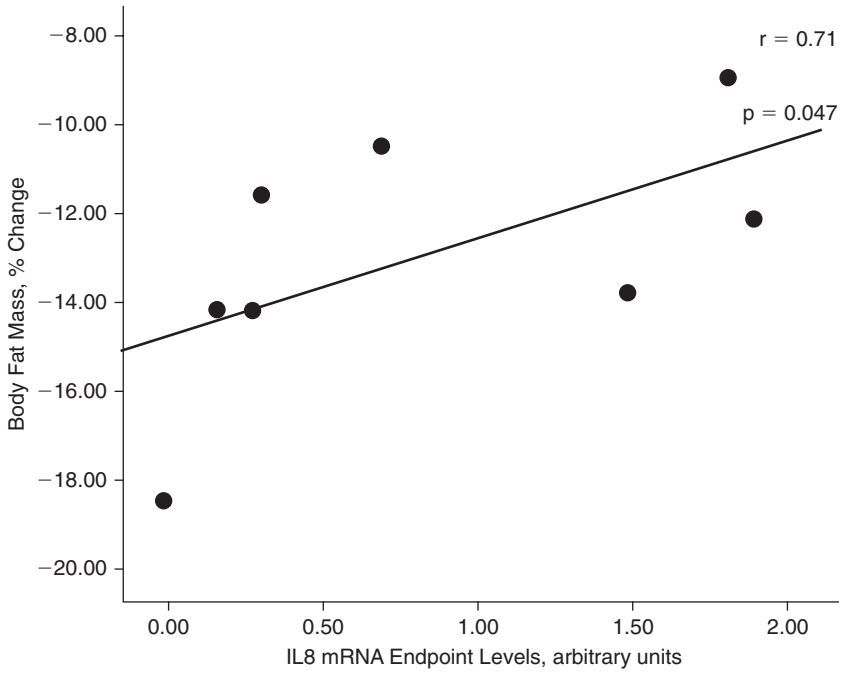

FIG. 2. Correlation between IL8 gene expression in blood peripheral mononuclear cells after nutritional intervention and changes in body fat mass after weight loss. Data show that lower IL8 transcripts were related to higher decrease in body fat mass induced by LCD.

instead of fat biopsies, to perform nutrigenomics studies in obese subjects, and with the additional advantage that this sample type could be a source of information about the oxidative stress and inflammatory status, which are both increased in obesity and its complications (Vincent and Taylor, 2006).

In the context of obesity treatment, dietary interventions such as caloric restriction diets remain the most efficient therapeutic approach to promote weight loss in obese subjects (Abete et al., 2006). Moreover, hypocaloric diets have been proposed to empower the oxidative stress improvement related to weight loss through a reduction in lipid peroxidation (Crujeiras et al., 2006, 2007). Global gene expression profiling in PBMC, performed in the current work by using DNA microarrays, revealed that a 8-week LCD devised to lose weight is able to modulate a wide variety of biological processes, such as macronutrient metabolism, coagulation, immune response, and oxidative phosphorylation. The first step in this study shows that several PBMC biological processes could be regulated in the same direction that in adipose tissue after nutritional intervention in obesity (Viguerie et al., 2005a, 2005b). For example, the genes related to signal transduction, cell–cell signaling, and immune response, together with genes associated with insulin sensitivity such as TNF-α/NF-κB signal cascade pathway were mostly downregulated by caloric restriction. Conversely, protein–DNA assembly complex and biosynthetic and metabolic regulation processes were upregulated (Clement et al., 2004; Higami et al., 2004; Viguerie et al., 2005a, 2005b). In addition, most of changes in gene expression found in this study, were between of the range (overexpressed: 1.2–2.9; downregulated: 0.8–0.4) previously reported in adipose tissue after 28 day very LCD (Clement et al., 2004).

Some genes also modulated by the nutritional intervention, such as *SIRT2* (sirtuin), *GHRL* (ghrelin), and *RPB2* (retinol binding protein 2) are, as far as we know, described in PBMC for the first time.

Further validation by qRT-PCR confirmed that some key genes involved in the TNF-α and the nuclear factor NF-κB signal cascade pathway, such as *RIPK3* (receptor-interacting serine–threonine kinase 3) and *TNIP1* (TNF-α-induced protein 3 interacting protein 1) were downregulated by a LCD. *RIPK3* is a family member of the receptor-interacting proteins implicated in activation of NF-κB and induction of apoptosis (Yang et al., 2005) and *TNIP1*, also known as *Naf1*, can inhibit NF-κB-dependent gene expression (Gallagher et al., 2003). These findings are in agreement with reports from human adipose tissue, which showed an improvement in the inflammatory profile induced by caloric restriction (Clement et al., 2004).

Also, other important genes related to oxidative phosphorylation, the main source of free radicals (Finkel and Holbrook, 2000), such as *NDUFS2* (NADH-coenzyme Q reductase), were downregulated by the nutritional intervention, suggesting a possible mitochondrial adaptation that could trigger a decrease in reactive oxygen species production, as has been shown after caloric restriction in rat liver studies (Ayala et al., 2007) and human muscle biopsies (Civitarese et al., 2007).

Therefore, our results suggest a beneficial effect induced by weight loss or negative energy balance on the TNF-α/NF-κB signaling cascade and in oxidative stress, which are both associated with obesity and insulin resistance (Dandona et al., 2005). Reinforcing these findings, the body weight appears to regulate the expression of some genes, such as *TNIP*, *TANK*, *TRIAD3*, *ACAA2*, and *NDUFS2*, as shown by the correlation analyses, suggesting that these genes could be good markers of body weight in PBMC, despite the fact that a correlation between the gene expression and the diet-induced changes in the body weight was not detected. However, it should not be discarded that not only weight loss but also the negative energy balance could have influenced in the regulation of these genes. Caloric restriction by itself has been suggested to regulate adipose tissue gene expression independently of the fat mass loss (Viguerie et al., 2005a).

Interestingly, the expression of *IL8* (Interleukin 8), a proinflammatory cytokine regulated by reactive oxygen intermediates (DeForge et al., 1993; Lekstrom-Himes et al., 2005), tended to be downregulated in PBMC after weight loss induced by caloric restriction. This decrease in *IL8* expression was closely associated to the LCD-induced reduction in body fat mass, suggesting that it could be a good indicator of variations in body fat percentage. This finding supports previous work indicating a upregulation of IL8 gene expression in preadipocytes of obese subjects (Nair et al., 2005), as well as studies that show a decrease in the adipose tissue IL8 secretion in humans treated following a hypocaloric diets (Arvidsson et al., 2004).

Summing up, the current study demonstrates the applicability of circulating mononuclear cells in the search of transcriptional biomarkers in the context of body weight regulation and nutritional treatments. Moreover, we report for the first time, the differential expression profile of oxidative stress and inflammation related genes in these cells under a model of weight loss induced by an LCD, which is of particular relevance given the current view of obesity as a low-degree proinflammatory disease (Moreno-Aliaga et al., 2005; Vincent and Taylor, 2006). In addition, the *IL8* gene expression in PBMC could be proposed as a possible biomarker for obesity treatment outcome, given the relationship found between the decrease in body fat mass and *IL8* transcripts. However, this finding should be corroborated in a future studies.

Therefore, gene expression in PBMC could be used for diagnosis and development of therapeutic strategies in obesity, and could be applied in a more personalized nutritional approach based on nutrigenomics studies.

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Author Disclosure Statement

The author declares there are no conflicts of interest.

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