High-protein and high-carbohydrate breakfasts differentially change the transcriptome of human blood cells[1–3]

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ABSTRACT

Background: Application of transcriptomics technology in human nutrition intervention studies would allow for genome-wide screening of the effects of specific diets or nutrients and result in biomarker profiles.

Objective: The aim was to evaluate the potential of gene expression profiling in blood cells collected in a human intervention study that investigated the effect of a high-carbohydrate (HC) or a high-protein (HP) breakfast on satiety.

Design: Blood samples were taken from 8 healthy men before and 2 h after consumption of an HP or an HC breakfast. Both breakfasts contained acetaminophen for measuring the gastric emptying rate. Analysis of the transcriptome data focused on the effects of the HP or HC breakfast and of acetaminophen on blood leukocyte gene expression profiles.

Results: Breakfast consumption resulted in differentially expressed genes, 317 for the HC breakfast and 919 for the HP breakfast. Immune response and signal transduction, specifically T cell receptor signaling and nuclear transcription factor κB signaling, were the overrepresented functional groups in the set of 141 genes that were differentially expressed in response to both breakfasts. Consumption of the HC breakfast resulted in differential expression of glycogen metabolism genes, and consumption of the HP breakfast resulted in differential expression of genes involved in protein biosynthesis.

Conclusions: Gene expression changes in blood leukocytes corresponded with and may be related to the difference in macronutrient content of the breakfast, meal consumption as such, and acetaminophen exposure. This study illustrates the potential of gene expression profiling in blood to study the effects of dietary exposure in human intervention studies. *Am J Clin Nutr* 2006;84:1233–41.

KEY WORDS Human blood, leukocytes, gene expression, carbohydrate, protein, acetaminophen

INTRODUCTION

Biomarker discovery can greatly benefit from genome-wide profiling techniques such as microarrays. For nutritional science, it would be interesting to apply this transcriptomics technology in human nutrition intervention studies, because this would allow for genome-wide screening in blood or tissue biopsies for the effects of specific diets or nutrients. Blood cells are of special interest because they may be the only readily available source of RNA in human intervention studies. Some studies have shown that gene expression profiles in blood leukocytes vary considerably between persons but are quite constant in time within a person (1–3). This indicates that gene expression profiles in blood could be used to study responses to environmental or dietary factors within persons.

In the present study, our aim was to evaluate the potential of gene expression profiling in blood collected in a human intervention study that investigated the effect of 2 breakfasts with different macronutrient content on hormonal regulation of satiety. Because dietary protein appears to be more satiating than dietary carbohydrate (4, 5), we tested the differential effect of a high-protein (HP) and a high-carbohydrate (HC) breakfast on satiety. The effects of the HP and the HC breakfasts on satiety-related hormones, specifically ghrelin, are described extensively by Blom et al (6).

Blood samples taken before and 2 h after breakfast consumption were used for the transcriptome analysis. Analysis of the transcriptome data focused on the effects of HP or HC breakfast consumption on blood leukocyte gene expression profiles and on the effects of acetaminophen (which was added to the breakfasts for measuring the gastric emptying rate) on blood leukocyte gene expression profiles. In addition to analyzing gene expression changes in response to breakfast consumption, we looked at intra- and interindividual differences in leukocyte gene expression profiles in our study and compared them with those of the earlier studies (1–3). Functional groups of genes or affected cellular processes were identified with pathway analysis of sets of differentially expressed genes, and these are discussed in relation to the HP, HC, or acetaminophen intake.

SUBJECTS AND METHODS

Study design and subjects

The goal of the intervention study was to investigate whether an HP breakfast was more satiating than was an HC breakfast and, if so, through what mechanisms. The concentrations of

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TABLE 1
Energy and macronutrient composition of the high-carbohydrate (HC) and high-protein (HP) breakfasts

	HC breakfast	HP breakfast
Weight (g)	400	400
Energy (MJ)	1.63	1.65
Protein (g)	18.8	57.2
Carbohydrate (g)	46.0	13.9
Fat (g)	14.4	12.2
Protein (% of energy)	19.3	58.1
Carbohydrate (% of energy)	47.3	14.1
Fat (% of energy)	33.3	27.8

several satiety-related hormones [ghrelin, glucagon, glucagon-like peptide 1, and cholecystokinin] were measured. Also, the gastric emptying rate was measured by addition of acetaminophen to the breakfasts and measuring acetaminophen concentrations in blood after consumption of the diet (6).

The study was conducted at TNO Quality of Life (Zeist, Netherlands), where subjects were recruited from a pool of volunteers. Each subject gave oral and written informed consents after being informed about the study. All subjects filled out a questionnaire on lifestyle, medical history, and dietary habits. The medical investigator physically examined each of the subjects. Blood and urine were collected from the subjects after an overnight fast for routine analysis. Each subject reported a Western lifestyle, normal Dutch dietary habits (including breakfast consumption), and a stable body weight for ≥1 mo before the study. Smokers, restrained eaters (as assessed with the Dutch Eating Behavior Questionnaire), subjects with hemoglobin concentrations <8.4 mmol/L, and subjects who reported slimming or who were on a medically prescribed diet were excluded from participation. Also, subjects who were on medication that may have influenced appetite and sensory functioning or who reported a metabolic or endocrine disease, gastrointestinal disorders, or a history of medical or surgical events that may have affected the study outcomes were not included.

Fifteen healthy, lean young men with a mean (±SD) body mass index (BMI, in kg/m^2) of 21.6 ± 1.9 (range: 19.0–25.0) and a mean (±SD) age of 20.5 ± 2.5 y (range: 18–26 y) completed the study. The experiment had a crossover design, with a washout period of 1 wk. For practical reasons, all subjects received the same treatment order. The subjects were blinded for treatment order and were informed that the treatment order was randomized.

The subjects came in after an overnight fast and consumed 1 of 2 isocaloric breakfasts, which differed in protein and carbohydrate content. The 2 breakfasts (weight: 400 g) were dairy-based, with either high carbohydrate (47% of energy) and moderate protein (19.3% of energy) content (referred to as the HC breakfast) or low carbohydrate (14.1% of energy) and high protein (58.1% of energy) content (referred to as the HP breakfast). Both breakfasts contained 1.5 g acetaminophen for measuring the gastric emptying rate. The energy and macronutrient contents of both breakfasts are described in **Table 1**. Breakfasts were kept constant in weight, volume, fat and energy content, viscosity, and taste to blind subjects to treatment order.

Blood samples

Blood samples were taken before and at several time points after the consumption of each breakfast. Blood was collected in evacuated tubes containing K_3EDTA as a coagulant, as previously described (7). Blood samples of 8 subjects taken before and 2 h after consumption of each breakfast (4 blood samples total for each subject) were used for the gene expression study.

Plasma acetaminophen was analyzed with the use of a commercially available enzyme-linked immunosorbent assay kit (Immunalysis Corporation, Pomona, CA) with an intraassay CV of 3.7% at a concentration of 5 μg/mL and 0.9% at a concentration of 25 μg/mL. Plasma glucagon concentrations were measured with a commercially available human RIA kit (Linco Research Inc, St Charles, MO) with an intraassay CV of 6.8% at a concentration of 60 pg/mL and 4.0% at a concentration of 220 pg/mL.

RNA isolation, labeling, and hybridization

RNA was isolated immediately after blood sampling by using RNeasy columns (Qiagen, Hilden, Germany). The manufacturer's protocol for RNA isolation from whole blood was followed, with the use of erythrocyte lysis buffer in the first step to isolate leukocytes. The protocol included a DNase digestion step. RNA was checked for purity and stability by gel electrophoresis and by 1 h incubation at 37 °C.

The isolated RNA samples were sent to ServiceXS BV (Leiden, Netherlands) where they were processed according to Affymetrix (Santa Clara, CA) protocols. In brief, RNA concentration was measured by absorbency at 260 nm, and RNA quality and integrity was verified by using the RNA 6000 Nano assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA).

Next, 2 μg high quality total RNA was used with the Affymetrix Eukaryotic One-Cycle Target Labeling and Control reagents to generate biotin-labeled antisense cRNA. The quality of the cRNA was checked with the use of the Agilent 2100 bioanalyzer. The labeled cRNA was further used for the hybridization to Affymetrix Human Expression U133A 2.0 Genechips (with 22215 probesets). After an automated process of washing and staining, absolute values of expression were calculated from the scanned array by using the Affymetrix GCOS software.

Data analysis

Raw signal intensities from CEL files were normalized with the use of the GCRMA algorithm. Normalized signal intensities <10 were replaced by 10, and only probesets with ≥3 signal intensity values >10 were included in further data analysis (11863 probesets).

Statistical analysis to test for differences between gene expression profiles was performed in BRB ArrayTools (software for microarray data analysis developed by Dr Richard Simon and Amy Peng Lam; http://linus.nci.nih.gov/BRB-ArrayTools.html) by using paired class comparison with multiple testing correction by estimation of false discovery rate. The thresholds for significance were set at a *P* value of 0.01 and estimated false discovery rate of 5%. Genes selected by these criteria are referred to as the "differentially expressed genes."

Subsets of differentially expressed genes were analyzed further in GenMAPP (8) to find functional groups of genes that were overrepresented in the subsets. A *z* score of 3 was used as a cutoff for identifying overrepresented groups. εAnalysis of correlation between gene expression levels and glucagon concentrations in blood was performed in GeneSpring GX 7.2 (Agilent Technologies).

Probeset annotation was updated through NetAffx on the Affymetrix website (http://www.affymetrix.com/analysis/index.affx), and additional information on gene annotation and function were retrieved through EntrezGene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db = gene) and MetaCore database (GeneGo Inc, St Joseph, MI). Detailed pathway analysis was performed in MetaCore version 3.2 (GeneGo Inc). Gene expression data will be made available through ArrayExpress, a public repository for microarray data (http://www.ebi.ac.uk/arrayexpress/).

RESULTS

Before focusing on the changes in leukocyte gene expression in response to dietary exposure, we looked at intra- and interindividual variation in leukocyte gene expression profiles. Intraindividual variation of blood cell whole-genome gene expression was studied in a comparison of the expression profiles in 2 fasting samples (ie, before breakfast consumption) from each of the 8 subjects; the 2 fasting samples were obtained with a 1-wk time interval. Within subjects, gene expression levels were relatively constant. On average, 87% (range: 74–99%) of the expressed genes showed <50% difference in expression level in the 2 fasted samples. Furthermore, a paired statistical analysis of the expression differences in the 2 samples showed only 3 differentially expressed genes. These 3 differentially expressed genes were *SMARCD1* (SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily D, member 1; involved in chromatin remodeling), *IFIT5* (interferon-induced protein 5; involved in immune responses), and *ZNF200* (zinc finger protein 200; involved in regulation of transcription).

To assess the interindividual differences between the 8 subjects in the fasting state, the range of expression among the 8 subjects was calculated for each gene by using the average expression value of a gene in the two fasted blood samples of each subject. A set of 2330 genes (≈20% of the genes included in the analysis) showed a >2-fold difference between the lowest and highest expression value among the 8 subjects. Thus, interindividual differences were clearly present in our group of subjects. A pathway analysis ranked immune response and its related biological processes as the most overrepresented functional group in this subset of genes with a >2-fold range in expression.

The present study focused on differences in blood gene expression profiles before and after breakfast consumption. A paired statistical analysis of gene expression profiles before and after consumption (as described in Subjects and methods) resulted in 317 differentially expressed genes after consumption of the HC breakfast and 919 differentially expressed genes after consumption of the HP breakfast. These differentially expressed genes were divided into 3 groups for additional analysis: *1*) genes that were differentially expressed in response to both the HC and the HP breakfasts (141 genes), *2*) genes with a significant difference in expression in response to the HC breakfast (176 genes), and *3*) genes with a significant difference in expression in response to the HP breakfast (778 genes) (**Figure 1**).

All 141 genes that were differentially expressed in response to both the HC and the HP breakfasts showed a similar response to the HC and the HP breakfasts, ie, up-regulation or down-regulation in response to both breakfasts. Both breakfast consumption as such (irrespective of the macronutrient content) and acetaminophen exposure may have contributed to the differential expression of this subset of genes. Two hours after breakfast consumption, acetaminophen concentrations in blood had increased in all subjects to between 10 and 24 μg/mL.

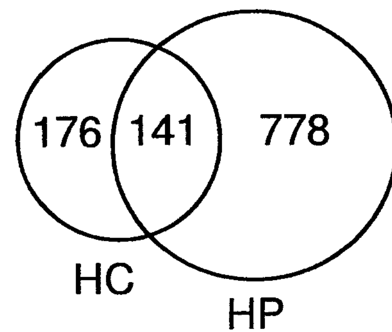

FIGURE 1. Venn diagram of the number of differentially expressed genes in human blood leukocytes after consumption of the high-protein (HP) breakfast, the high-carbohydrate (HC) breakfast, or both. Expression of these genes after consumption of the breakfasts was siginificantly different from that before breakfast consumption (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05.

The pathway analysis showed that the biological processes associated with immune response and signal transduction were overrepresented in the set of 141 genes that were differentially expressed in response to both breakfasts. A more detailed analysis of the effect on these biological processes in MetaCore (GeneGo Inc) indicated that the processes of T cell receptor–mediated signaling and nuclear transcription factor κB (NF-κB) signaling were affected in both groups. This is visualized in the pathway map in **Figure 2** (created with MetaCore from GeneGo Inc). In addition to the genes shown on this pathway map, expression of transcription factor lymphoid enhancer-binding factor 1 (*LEF1*) was also significantly lower after consumption of both breakfasts than before breakfast consumption; this transcription factor can regulate transcription of T cell receptors. Additional analysis of the subset of differentially expressed immune response genes suggested that a difference existed between the up- and down-regulated genes: most the down-regulated genes in this subset were genes expressed in T cells or natural killer cells, and most up-regulated genes in this subset were genes expressed in other leukocytes, such as B cells, monocytes, or macrophages. Other functional groups overrepresented in the subset of genes that were differentially expressed in response to both breakfasts included those involved in lipid binding, sugar binding, oxidoreductase activity, and apoptosis (**Table 2**).

In addition to an analysis based on functional groups, some interesting observations were made on the differential expression of individual genes due to breakfast consumption as such. Expression of histidine ammonia-lyase (*HAL*) was significantly increased after consumption of both the HC and the HP breakfasts (**Figure 3**). Recently, Aleman et al (9) showed that expression of this gene can be regulated by glucagon and by dietary protein content. Individual expression levels of *HAL* were significantly correlated with glucagon concentrations in blood (correlation coefficient: 0.66, $P < 0.001$). One of the most strongly down-regulated genes was DNA-damage-inducible transcript 4 (*DDIT4*, also known as *REDD1*) (Figure 3). This gene is involved in the reaction of a cell to energy depletion (10); therefore, down-regulation of its expression may be related to transition from a fasted (energy-depleted) state to a fed state.

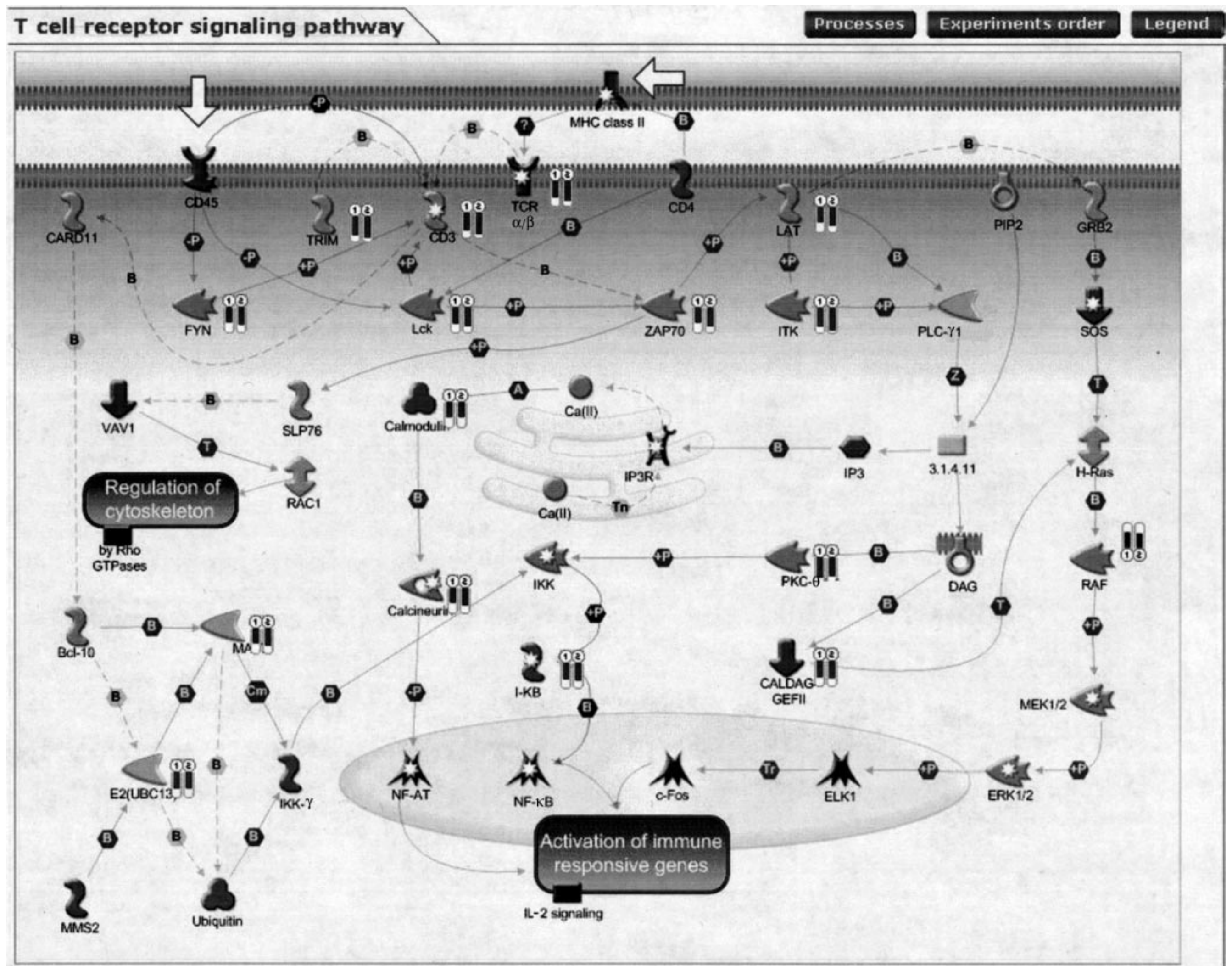

FIGURE 2. Pathway map of T cell receptor signaling pathway with visualization of mean fold change of expression in response to the high-carbohydrate breakfast (after compared with before breakfast intake, designated by thermometers labeled 1) and the high-protein breakfast (after compared with before breakfast intake, designated by thermometers labeled 2). A thermometer with the designated number on the top indicates down-regulation of expression; a thermometer with the designated number on the bottom indicates up-regulation of expression. Fold changes shown by thermometers ranged from −1.7 to 1.2. Bcl-10, B cell lymphoma-leukemia 10; CalDAG-GEFII, calcium and 1,2-diacylglycerol (DAG)-regulated guanine nucleotide exchange factor; CARD11, caspase recruitment domain-containing protein 11; c-FOS, cellular oncogene fos; E2(UBC13), ubiquitin-conjugating enzyme E2 N; ERK 1/2, extracellular signal-regulated kinase 1/2; GRB2, growth factor receptor–bound protein 2; GTP, guanosine triphosphate; I-KB, nuclear transcription factor κB (NF-κB) inhibitor; IKK, inhibitor of NF-κB kinase; IKK-γ, IκB kinase γ; IL-2, interleukin-2; IP3, inositol 1,4,5-triphosphate; IP3R, IP3 receptor; ITK, IL2-inducible T cell kinase; LAT, linker for activation of T cells family member 1; LCK, lymphocyte cell-specific protein-tyrosine kinase; MALT1, mucosa-associated lymphoid tissue lymphoma translocation protein 1; MEK1/2, mitogen activated protein/ERK kinase 1/2; MHC, major histocompatability complex; MMS2, ubiquitin-conjugating enzyme E2 variant 2; NF-AT, nuclear factor of activated T cells; PIP2, phosphoinositol biphosphate; PKC-θ, protein kinase C θ; PLC, phospholipase C; RAC1, *ras*-related C3 botulinum toxin substrate 1 precursor; SLP76, SH2 domain–containing leukocyte protein of 76 kDa; SOS, son of sevenless homolog; TCR, T cell receptor; TRIM, T cell receptor–interacting molecule; ZAP70, 70 kDa ζ-associated protein. This figure was created by using MetaCore version 3.2 (GeneGo Inc, St Joseph, MI).

Fewer genes were differentially expressed in blood after consumption of the HC breakfast than after consumption of the HP breakfast (317 compared with 919). After removal of the genes that were differentially expressed in response to both breakfasts, the subset of the remaining HC-specific genes (176) and the subset of the remaining HP specific genes (778) were submitted to pathway analysis. The most overrepresented biological process among the HC-specific genes was glycogen metabolism. Specifically, expression of 3 glycogen metabolism genes, ie, glycogen phosphorylase, glycogenin, and glucosidase α (**Table 3**), was increased after consumption of the HC breakfast. In addition to glycogen metabolism, immune response was also an overrepresented biological process among the HC-specific genes. The most overrepresented biological process among the HP-specific genes was protein biosynthesis. Consumption of the HP breakfast resulted in reduced expression of a large number of genes involved in protein biosynthesis. These included genes encoding ribosomal proteins and genes involved in translation (**Table 4**). The fold changes of these genes were quite small, ranging from 1.1- to 1.4-fold. Other overrepresented biological processes among the HP-specific genes included prostaglandin metabolism [down-regulation of aldo-keto reductase family 1

TABLE 2
Functional groups of genes overrepresented in the subset of genes that were differentially expressed in response to both the high-carbohydrate (HC) and high-protein (HP) breakfasts[1]

Functional group and gene name	Gene symbol	HC breakfast	HP breakfast
Lipid binding[2]			
Mal, T cell differentiation protein	*MAL*	−1.43 (−1.91 to 1.08)	−1.68 (−2.69 to −1.44)
Protein kinase C η	*PRKCH*	−1.39 (−1.82 to −1.09)	−1.36 (−1.91 to −1.00)
Protein kinase C θ	*PRKCQ*	−1.37 (−1.59 to −1.16)	−1.47 (−1.78 to −1.28)
RAS guanyl releasing protein 1	*RASGRP1*	−1.25 (−1.51 to −1.06)	−1.32 (−1.65 to −1.04)
Sterol carrier protein 2	*SCP2*	−1.41 (−1.69 to −1.20)	−1.43 (−1.87 to −1.09)
Prosaposin	*PSAP*	1.27 (1.08 to 1.48)	1.18 (1.05 to 1.37)
Sugar binding[2]			
CD69 antigen	*CD69*	−1.54 (−1.99 to −1.25)	−1.63 (−2.35 to −1.22)
Killer cell lectin-like receptor subfamily D member 1	*KLRD1*	−1.98 (−2.83 to −1.41)	−1.68 (−3.18 to −1.00)
Killer cell lectin-like receptor subfamily F member 1	*KLRF1*	−1.57 (−2.83 to −1.15)	−1.62 (−3.14 to −1.06)
Complement component 1 q subcomponent receptor 1	*C1QR1*	1.23 (1.11 to 1.45)	1.28 (1.08 to 1.42)
Sialic acid binding Ig-like lectin 5	*SIGLEC5*	1.23 (1.06 to 1.41)	1.42 (1.15 to 1.690)
Oxidoreductase activity, acting on CH-OH group of donors[2]			
Lactate dehydrogenase B	*LDHB*	−1.23 (−1.53 to −1.08)	−1.30 (−1.36 to −1.21)
Dehydrogenase-reductase (SDR family) member 9	*DHRS9*	1.42 (1.27 to 1.56)	1.42 (−1.06 to 1.93)
Inosine monophosphate dehydrogenase 1	*IMPDH1*	1.26 (1.14 to 1.57)	1.24 (−1.06 to 1.68)
Apoptosis[3]			
BCL2-adenovirus E1B 19 kDa interacting protein 3	*BNIP3*	−1.24 (−1.39 to 1.01)	−1.21 (−1.46 to −1.00)
CD2 antigen (p50)	*CD2*	−1.30 (−1.56 to −1.10)	−1.54 (−1.91 to −1.16)
Mal, T cell differentiation protein	*MAL*	−1.43 (−1.91 to 1.08)	−1.68 (−2.69 to −1.44)
Granzyme A	*GZMA*	−1.53 (−2.16 to −1.08)	−1.59 (−2.79 to −1.07)
Modulator of apoptosis 1	*MOAP1*	−1.14 (−1.27 to −1.09)	−1.24 (−1.75 to −1.09)
Nuclear factor of κ light polypeptide gene enhancer in B cells inhibitor α	*NFKBIA*	−1.28 (−1.57 to −1.09)	−1.39 (−2.07 to −1.12)
Programmed cell death 4	*PDCD4*	−1.17 (−1.28 to −1.04)	−1.15 (−1.28 to 1.03)
Death-associated protein kinase 1	*DAPK1*	1.39 (1.17 to 1.58)	1.39 (−1.07 to 1.78)
Tumor necrosis factor (ligand) superfamily member 13	*TNFSF13*	1.37 (1.13 to 1.58)	1.25 (1.03 to 1.41)
CD14 antigen	*CD14*	1.44 (1.22 to 2.17)	1.33 (1.01 to 1.87)

[1] Values are mean fold change; range in parentheses. $n = 8$. The functional groups in this table are those overrepresented in addition to immune response and signal transduction and were derived from the Gene Ontology annotation (www.geneontology.com). Expression of all genes after consumption of both the HC and HP breakfasts was significantly different from expression before breakfast consumption (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05. Ig, immunoglobulin.

[2] Gene Ontology functional group "molecular function."

[3] Gene Ontology functional group "biological process."

member C3 (*AKR1C3*) and macrophage migration inhibitory factor (*MIF*); up-regulation of prostaglandin-endoperoxide synthase 2 (*PTGS2*)] and immune response.

Compared with the HC breakfast, consumption of the HP breakfast resulted in significant differences in physiologic variables related to satiety, eg, delayed gastric emptying rate, decreased concentrations of ghrelin (hunger signal), and increased concentrations of glucagon-like peptide 1, cholecystokinin, and glucagon (6). Probesets for several genes encoding these satiety-related proteins (cholecystokinin, insulin, glucagon, and leptin) were present on the microarray, but expression of these genes was not detected in the human blood leukocytes. The effects of HP diets on satiety are closely linked to effects on energy intake, energy balance, and weight loss (4, 5). A detailed analysis of the genes with differential expression in response to the HP breakfast uncovered 2 genes with a role in energy homeostasis: peroxisome-proliferator activated receptor γ coactivator-related 1 (PGC-1-related coactivator *PPRC1*) and lipin 1 (*LPIN1*). Expression of these genes was down-regulated in response to the HP breakfast (**Figure 4**).

DISCUSSION

In the present study, we evaluated the potential of gene expression profiling in blood in a human nutrition intervention study. The goal of the intervention study was to investigate the mechanisms of the differential effects of an HP and an HC breakfast on satiety. Blood samples taken from 8 subjects before and 2 h after consumption of both breakfasts were used for gene expression profiling.

Our study showed that it is possible to measure responses to dietary exposure by gene expression profiling of blood cells. This is an important finding for the nutrigenomics field, because blood is the most readily available tissue for expression profiling in human intervention studies. Moreover, biological interpretation of the results through pathway analyses yielded functional groups of differentially expressed genes that, to some extent, could be linked to the nutritional intervention. Expression changes of some genes (ie, *HAL* and *DDIT4*) may have been related to nutrient intake (going from fasted to fed state), and these genes may be relevant as biomarkers of feeding. HC intake resulted in lower expression of genes involved in glycogen

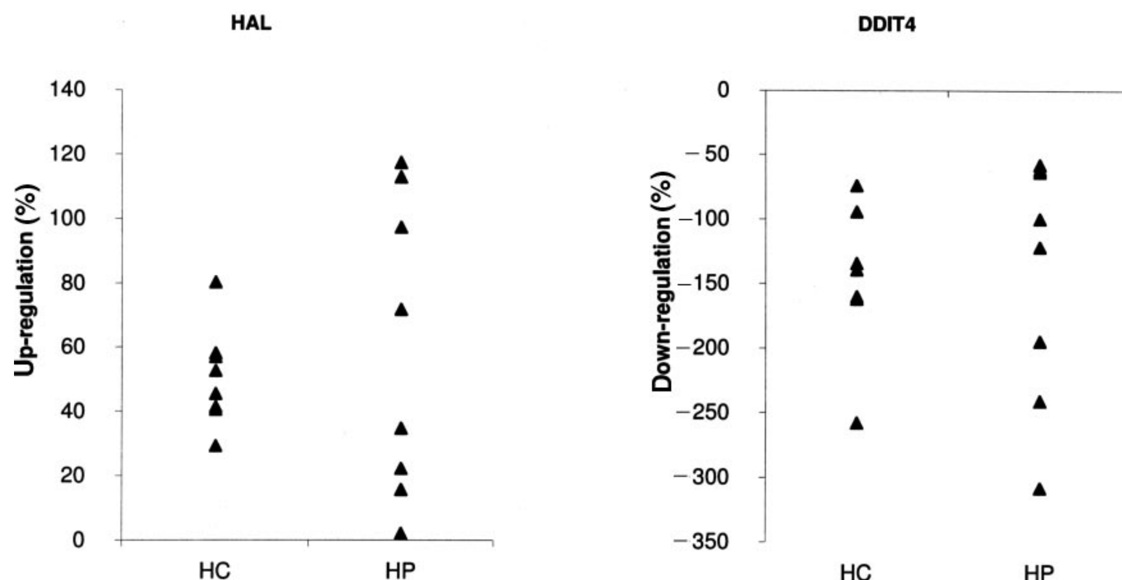

FIGURE 3. Gene expression changes of histidine ammonia-lyase (HAL) and DNA-damage-inducible transcript 4 (DDIT4) in human leukocytes of 8 subjects after consumption of a high-carbohydrate (HC) or high-protein (HP) breakfast. Expression levels of HAL and DDIT4 after consumption of both an HC and an HP breakfast were significantly different from before breakfast consumption (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05.

metabolism, and HP intake resulted in lower expression of genes involved in protein biosynthesis.

The effect of an HP diet on satiety is one of the possible mechanisms by which HP diets could lead to weight loss (5). How exactly an HP diet can affect energy homeostasis and eventually lead to weight loss remains unresolved. Research into these molecular mechanisms could benefit from the "omics" technologies. In our study, we found a slight (10–40%) down-regulation of expression of genes involved in protein biosynthesis, including genes encoding ribosomal proteins and genes involved in protein translation. One could speculate that a slight down-scaling of the protein biosynthesis process (on gene expression level) is a direct response of the body to the protein overload after consumption of the HP breakfast. Furthermore, the HP breakfast resulted in down-regulation of 2 genes that have been related to energy homeostasis. *PPRC1* belongs to the PGC-1 family of transcription coactivators, which can regulate energy homeostasis and glucose and lipid metabolism and which have been suggested to be involved in obesity (11, 12). *PPRC1* is a close homolog of *PGC-1α*, which is the most studied member of this family so far. We found no expression of PGC-1α mRNA in leukocytes (data not shown); however, *PPRC1* was expressed, and consumption of the HP breakfast significantly lowered its expression. This could be an interesting finding, considering the potential value of HP diets to weight loss and obesity. Lipin has also received attention with relation to obesity as a regulator of energy homeostasis and fat metabolism in muscle and adipose tissue (13, 14). Interestingly, consumption of the HP breakfast resulted in decreased fat intake during the subsequent ad libitum lunch (6). Considering the role of lipin and PPRC1 in energy homeostasis and fat metabolism, it could be hypothesized that their reduced expression in blood in response to the HP breakfast may somehow be related to this decreased fat intake. These 2 genes are examples of clues from gene expression studies that could lead to additional research and an increased understanding of the molecular mechanisms by which macronutrients regulate energy homeostasis.

In addition to the HC- and HP-specific effects, several genes were differentially expressed in response to both diets. As mentioned earlier, these effects could have been induced by food intake, irrespective of macronutrient content. Also, these effects could have been induced by acetaminophen exposure, because both breakfasts contained acetaminophen and resulted in increased acetaminophen concentrations in blood 2 h after breakfast consumption.

Immune response and signal transduction were the main functional groups of genes within the set of genes that responded to both the HC and HP breakfast. A large number of genes involved in T cell receptor–mediated NF-κB signaling were down-regulated in response to both breakfasts. Down-regulation of this

TABLE 3
Changes in expression of glycogen metabolism genes after the high-carbohydrate (HC) or high-protein (HP) breakfasts[1]

Gene name	Gene symbol	HC breakfast	HP breakfast
Glucosidase α	*GAA*	1.55 (1.05 to 2.13)[2]	1.06 (−2.91 to 1.49)
Glycogen phosphorylase	*PYGL*	1.27 (1.11 to 1.58)[2]	1.29 (1.06 to 2.19)
Glycogenin	*GYG*	1.22 (1.08 to 1.52)[2]	1.10 (−1.27 to 1.28)

[1] Values are mean fold change; range in parentheses. $n = 8$.

[2] Expression was significantly different from before consumption of breakfast (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05.

TABLE 4

Differential expression of genes with a role in protein biosynthesis in response to the high-protein (HP) breakfast[1]

Functional group and gene name	Gene symbol	Value
Ribosomal proteins		
Ribosomal protein L3	*RPL3*	−1.16 (−1.26 to −1.02)
Ribosomal protein L5	*RPL5*	−1.18 (−1.35 to −1.02)
Ribosomal protein L10a	*RPL10A*	−1.18 (−1.36 to −1.07)
Ribosomal protein L12	*RPL12*	−1.17 (−1.36 to −1.04)
Ribosomal protein L13a	*RPL13A*	−1.23 (−1.54 to −1.02)
Ribosomal protein L22	*RPL22*	−1.33 (−1.47 to −1.13)
Ribosomal protein L24	*RPL24*	−1.19 (−1.49 to −1.03)
Ribosomal protein L29	*RPL29*	−1.22 (−1.66 to −1.06)
Ribosomal protein L31	*RPL31*	−1.35 (−1.57 to −1.01)
Ribosomal protein L35	*RPL35*	−1.19 (−1.47 to −1.03)
Ribosomal protein L36a-like	*RPL36AL*	−1.15 (−1.28 to −1.01)
Ribosomal protein S15	*RPS15*	−1.16 (−1.38 to −1.08)
Ribosomal protein S26	*RPS26*	−1.18 (−1.38 to −1.03)
Ribosomal protein S28	*RPS28*	−1.17 (−1.40 to −1.00)
Mitochondrial ribosomal protein L9	*MRPL9*	−1.28 (−1.68 to −1.09)
Mitochondrial ribosomal protein S17	*MRPS17*	−1.25 (−1.57 to −1.02)
Mitochondrial ribosomal protein S33	*MRPS33*	−1.28 (−1.79 to −1.02)
Regulation of translation		
Eukaryotic translation initiation factor 2-α kinase 3	*EIF2AK3*	−1.30 (−1.82 to 1.04)
Ribosomal protein SA	*RPSA*	−1.19 (−1.40 to −1.09)
Putative translation initiation factor	*SUI1*	−1.22 (−1.58 to −1.01)
Eukaryotic translation initiation factor 3 subunit 5 ε	*EIF3S5*	−1.25 (−1.53 to −1.15)
Eukaryotic translation initiation factor 4A isoform 2	*EIF4A2*	−1.17 (−1.31 to −1.03)
Translational elongation		
Eukaryotic translation elongation factor 1β 2	*EEF1B2*	−1.24 (−1.39 to −1.07)
Eukaryotic translation elongation factor 1γ	*EEF1G*	−1.17 (−1.51 to −1.03)
Eukaryotic translation elongation factor 2	*EEF2*	−1.13 (−1.30 to −1.04)
Ribosomal protein, large, P0	*RPLP0*	−1.19 (−1.49 to −1.10)
Ribosomal protein, large, P1	*RPLP1*	−1.12 (−1.31 to −1.01)
Valyl-tRNA synthetase	*VARS*	−1.31 (−1.68 to 1.01)
Translational initiation		
Ligatin	*LGTN*	−1.39 (−2.19 to −1.07)
Putative translation initiation factor	*SUI1*	−1.22 (−1.58 to −1.01)
Eukaryotic translation initiation factor 3 subunit 5 ε	*EIF3S5*	−1.25 (−1.53 to −1.15)
Eukaryotic translation initiation factor 4A isoform 2	*EIF4A2*	−1.17 (−1.31 to −1.03)
Heat shock 27 kDa protein 1	*HSPB1*	−1.36 (−2.29 to −1.14)

[1] Values are mean fold change; range in parentheses. $n = 8$. Expression of all genes after consumption of the HP breakfast was significantly different from expression before consumption (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05.

signaling pathway could indicate an antiinflammatory effect, as was previously shown for acetaminophen (15). In addition to down-regulation of genes in this T cell–specific pathway, most other down-regulated immune response genes were also genes expressed in T cells. These findings could indicate a differential effect of acetaminophen on different leukocyte subpopulations.

Hepatotoxicity studies have shown that acetaminophen can cause oxidative stress in the liver and can have proinflammatory effects through NF-κB signaling (16). The antiinflammatory effects of acetaminophen (paracetamol) were in concordance with normal usage of the compound for its analgesic and antipyretic effects (15).

After an overnight fast, lipid, protein, and glucose intake all induce generation of reactive oxygen species in leukocytes for a period of several hours (17, 18). In addition, a recent study showed a similar effect after intake of a mixed meal, resulting in increased generation of reactive oxygen species and in proinflammatory changes (ie, increased NF-κB binding activity in the nucleus) in leukocytes (19). It is likely that the breakfast intake in our study also resulted in increased oxidative stress. This could mean that the down-regulation of genes involved in NF-κB signaling in our study was a net effect of proinflammatory changes in response to the meal intake and antiinflammatory changes in response to acetaminophen. This also indicates that the singular effect of acetaminophen on expression of immune response genes in blood cells may be stronger than that measured in the present study.

Differential expression of immune response genes was not restricted to the subset of genes with significant difference in expression in response to both diets; pathway analysis showed overrepresentation of the "immune response" biological process in all 3 groups of differentially expressed genes. Immune response genes with a significant difference in expression in response to just one of the diets usually showed a similar average fold change for the other diet, which was not significant. Perhaps the high-variability in expression of immune response genes makes it more difficult to find consistent and thus significant expression changes in these genes. Inclusion of >8 subjects may

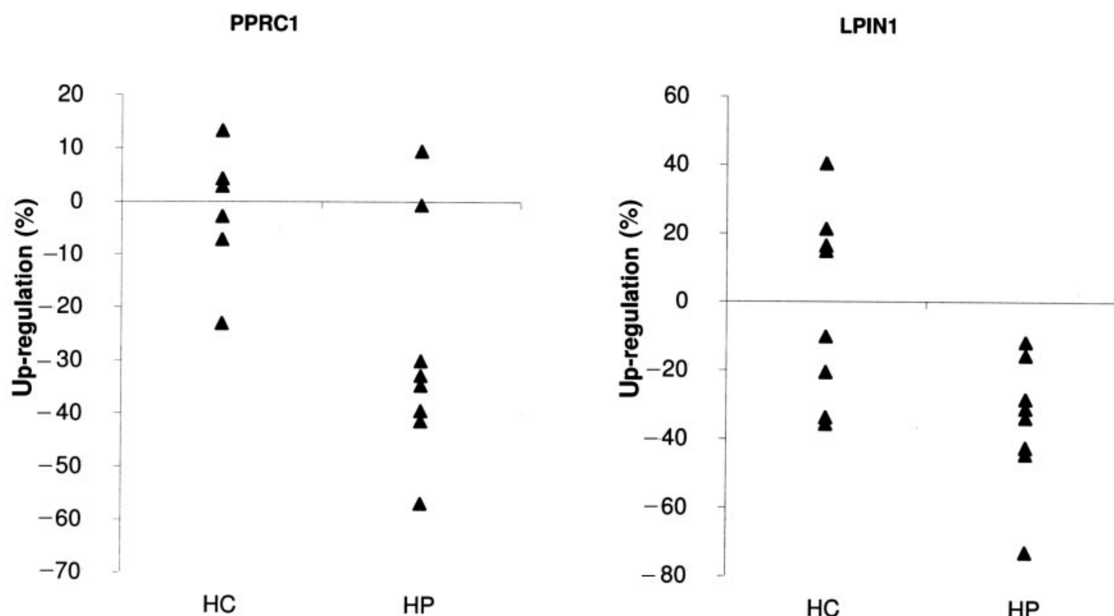

FIGURE 4. Gene expression changes of peroxisome-proliferator activated receptor γ coactivator-related 1 (PPRC1) and lipin 1 (LPIN1) in human leukocytes of 8 subjects after consumption of a high-carbohydrate (HC) or high-protein (HP) breakfast. Expression levels of PPRC1 and LPIN1 after consumption of the HP breakfast were significantly different from before breakfast consumption (paired statistical analysis), $P < 0.01$ and estimated false discovery rate <0.05.

increase the power to detect significant expression changes in high-variability genes.

Clear interindividual differences in the gene expression profiles of blood, as shown in our study and in earlier studies (1–3), emphasize that effects on gene expression profiles need to be measured within an individual in crossover studies. Comparison of the set of genes with high interindividual variation in our study with those found in earlier studies (1–3) showed a large overlap. High-variation genes present in our study and in earlier studies include the major histocompatability complex class II genes, such as *HLA-DRB1*, *HLA-DRB4*, *HLA-DQA1*, and *HLA-DQB1*; interferon-regulated genes, such as *IFIT1*, *IFIT2*, *IFIT3*, *IFI44L*, and *MX1*; killer lectin genes, such as *KLRC1*, *KLRC3*, and *KLRD1*; histone genes, such as *H1FO*, *H1FX*, *HIST1H2BD*, *HIST1H2BH*, and *HIST1H2BG*; and other genes, such as *TAP2*, *DDX17*, and *FOLR3*. In addition to stressing the importance of a crossover design for gene expression profiling in human studies, the results of our study also show the need to standardize study conditions, because not only was a difference between the fasting and fed state shown, but variations in breakfast content also resulted in differential patterns of gene expression in leukocytes.

Although it is promising that microarray technology can detect small expression changes in human blood that, to some extent, could be linked to the composition of the breakfasts, it is important to keep in mind that gene expression changes do not necessarily reflect changes in protein concentrations or activity. Also, a limitation of our study was the study of a single time point and dose. Future nutrition studies incorporating human blood cell transcriptomics will hopefully provide more insight into the time course of gene expression changes, biological processes that can be monitored in blood, effects of micronutrients, and specific (statistical) approaches for microarray data analysis in human studies. In conclusion, these results of gene expression profiling in blood illustrate the potential of blood gene expression profiling as a tool for monitoring and studying the effects of dietary exposure in human intervention studies.

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MJvE was involved in data analysis and writing of the manuscript. WAMB and HFJH (the Principal Investigator according to Good Clinical Practice guidelines) were involved in design of the protocol and data collection. WAMB, BvO, and HFJH provided significant advice during the writing of the manuscript. None of the authors had any conflict of interest.

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