Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 1701 – 1715

Journal of Nutritional Biochemistry

Postprandial blood cell transcriptomics in response to the ingestion of dairy products by healthy individuals☆

Francina M. Sagaya[a,b], Richard F. Hurrell[b], Guy Vergères[a,*]

[a]Agroscope Liebefeld-Posieux Research Station ALP, Berne, Switzerland
[b]Human Nutrition Laboratory, Institute of Food, Nutrition and Health, ETH Zurich, Zurich, Switzerland

Received 18 August 2011; accepted 27 January 2012

Abstract

The aim of this intervention study was to measure genome-wide postprandial gene expression in human blood cells after the ingestion of a single serving of milk, to identify the downstream physiological processes regulated by the differentially expressed genes, and to use this gene expression signature as a reference to compare it with the response following the ingestion of a transformed dairy product, namely, yogurt. We conducted a randomized, controlled, single-blinded, crossover study on six healthy male individuals. After an overnight fast, 540 g of milk or yogurt was ingested by the subjects. Blood samples were collected before (0 h) and after (2 h/4 h/6 h) ingestion, and the blood cell transcriptome was analyzed using a linear kinetic analysis that increases the statistical power of the study. The differentially expressed transcripts identified after the ingestion of milk (575 transcripts) and yogurt (625 transcripts) modulated similar biological processes. In particular, genes involved in protein biosynthesis and mitochondrial activities followed biphasic kinetics being down-regulated at 2 h and more pronouncedly up-regulated at 6 h. The opposite kinetics were observed for inflammatory and apoptotic processes during the same time frame. The human blood cell transcriptome appeared to be specifically modulated by specific nutrients present in bovine milk, a property that was further modified when milk was fermented to yogurt. The coordinated changes in postprandial expression of genes involved in basic biological processes suggest that postprandial blood cell transcriptomics may allow insight into the nutritional effects of selected foods in the prevention or development of chronic metabolic and inflammatory disorders.

Keywords: Dairy products; Nutrition; Postprandial metabolism; Inflammation; Transcriptomics

1. Introduction

Milk not only provides essential macronutrients, vitamins, trace elements and minerals but also contains bioactive components that may act synergistically to modulate metabolic and immunomodulatory processes that are important to the maintenance of health [1,2]. The impact of dairy products has, therefore, been discussed and debated in relation to the development or the prevention of cancer [3], allergy [4], hypertension [5], infection [6] and obesity [7]. Milk is easily amenable to technological and microbiological transformation. The large spectrum of marketed dairy products resulting from these technologies has significantly contributed to stimulate research on the nutritional properties of milk-based products [2]. However, measuring the impact of processing on the nutritional properties of food, in this particular case, dairy products, is a challenging task.

Postprandial blood cell transcriptomics is emerging as a powerful tool that can complement classical analytics in nutritional intervention studies by capturing coordinated and subtle changes induced by nutrients in humans, in particular metabolic and immunological processes [8–11]:

First, the identification of clinical endpoints or biomarkers that change in a statistically significant manner often requires relatively long-term interventions that are often incompatible with the limited resources usually available for exploratory research. As cells in the human organism respond specifically and sensitively to the ingestion of food by inducing gene expression within a few hours of the nutritional stimuli, postprandial gene expression could deliver preliminary information on the early molecular events induced by nutrients.

Second, insight into the metabolic networks affected by the nutrients is hampered due to the restricted access of researchers to cellular material, in particular tissue biopsies, in human subjects. White blood cells can easily be recovered from human subjects, respond metabolically to nutritional stimuli and are important mediators of the immune system what makes them attractive cellular targets for preventive nutritional research, particularly in the context of chronic inflammatory diseases.

Third, most of the information obtained so far in classical nutrition intervention studies has been gathered using a hypothesis-driven analytical strategy that focuses on the measurement of a restricted set of preselected biomarkers, thus precluding a comprehensive analysis

☆ This work was supported by Agroscope Liebefeld-Posieux Research Station ALP internal funding.
* Corresponding author. Tel.: +41 31 323 81 54.
E-mail address: guy.vergeres@alp.admin.ch (G. Vergères).

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jnutbio.2012.01.001

of the underlying physiological processes. Genome-wide transcriptomics now allow a holistic investigation of the effects of bioactive components, including complex food matrices, on cellular processes.

Dairy products are ideal candidates to be investigated using a postprandial blood cell transcriptomic strategy. In order to get a mechanistic insight into the nutritional properties of dairy products, we have thus conducted a nutritional human intervention study in which we measured the postprandial transcriptome of blood cells of humans having ingested whole milk and yogurt. The milk group was first measured, and the differentially expressed pathways were identified. We also used this gene expression signature as a reference to identify biological pathways that were either commonly or uniquely regulated after the ingestion of yogurt, thus allowing first insights, using blood cell transcriptomics, into the physiological consequences of processing food on human physiology.

2. Methods and materials

2.1. Study design and subjects

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the ethical committee of the ETH Zurich, Switzerland.

Interested healthy male volunteers from the student population filled a questionnaire on lifestyle, medical history and dietary habits and reported a Western lifestyle. Smokers and subjects who were on a medically prescribed diet were excluded from participation. Also, subjects who were on medication or who reported metabolic, gastrointestinal disorders or a history of medical or surgical events that could affect the study outcome were excluded, as well as subjects who had donated blood less than 4 months before the beginning of the study. Eight healthy young males were recruited, and written consent was obtained from all subjects. The numbers of subjects were kept small, as this was an initial exploratory study. Two subjects had to be excluded during the study because of the abnormal neutrophil count and low RNA yield (see below). Six subjects completed the study. They had a mean (±S.D.) body mass index of 22.7±1.3 kg/m^2 (range: 20.9–24.3 kg/m^2) and a mean (±S.D.) age of 26.6±3.1 years (range: 23–32 years).

The experiment had a randomized, controlled, single-blinded, crossover design. The duration of the study was 8 days and included a run-in period of 3 days, 2 single days of intervention on day 4 and day 8, separated by 3 days of washout period. During the run-in period on days 1–3 and the washout period on days 5–7, the volunteers had a controlled diet for breakfast, lunch and dinner. This diet was devoid of dairy products, fermented products and minimized for food with potentially high levels of bioactive nutrients, in particular fruits and vegetables (Table 1). The subjects were advised not to drink more than one cup of coffee a day. Apart from consuming the controlled meals under supervision, no additional control for dietary compliance was made. On days 4 and 8 after an overnight fast, the subjects ingested 540 g of either milk or yogurt for breakfast in a randomized order. In transcriptomics studies involving humans, particularly in blood cell transcriptome, interindividual variations are significant and intraindividual variations are relatively small [9,10]. Our study, thus, had a crossover design so that each subject can serve as his/her own control, thereby minimizing variations.

Table 1
Composition of the diet served to the subjects from days 1 to 8 of the study

Day	Meal	Food
1	Breakfast	White bread toast[a], apricot jam, omelette, carbonated water
	Lunch	French fries, turkey schnitzel, puff pastry, syrup
	Dinner	Couscous (wheat semolina), meat sauce, syrup
2	Breakfast	White bread toast, apricot jam, omelette, carbonated water
	Lunch	Parboiled rice, lentils, ice sorbet, syrup
	Dinner	Spätzle with meat sauce, cake[a], carbonated water
3	Breakfast	Potato pancakes, scramble egg, carbonated water, sweet
	Lunch	Fried grated potatoes, fish finger, syrup
	Dinner	Macaroni with meat sauce, cake, carbonated water
4	Breakfast	Test dairy product — milk or yogurt
	Lunch	French fries, turkey schnitzel, syrup
	Dinner	Couscous with meat sauce, cake, carbonated water
5	Breakfast	White bread toast, apricot jam, omelette, carbonated water
	Lunch	French fries, turkey schnitzel, puff pastry, syrup
	Dinner	Couscous (wheat semolina), meat sauce, syrup
6	Breakfast	White bread toast, apricot jam, omelette, carbonated water
	Lunch	Parboiled rice, lentils, ice sorbet, syrup
	Dinner	Spätzle with meat sauce, cake, carbonated water
7	Breakfast	Potato pancakes, scramble egg, carbonated water, sweet
	Lunch	Fried grated potatoes, fish finger, syrup
	Dinner	Macaroni with meat sauce, cake, carbonated water
8	Breakfast	Test dairy product — yogurt or milk
	Lunch	End of the study
Snacks[b]		Potatoes chips, cake

[a] Bread and cake were made without yeast and dairy products.
[b] Snacks were provided every day between 4 and 5 PM.

Table 2
Protocol used for the preparation of yogurt and GDL milk

Processing	GDL milk	Yogurt
Whole milk	97%	97%
Fat-free powder	3%	3%
Homogenization 1	65°C/150 bar	65°C/150 bar
Homogenization 2	65°C/30 bar	65°C/30 bar
Pasteurization	92°C/5 min	92°C/5 min
Cooling	30°C	42°C
GDL	2%	
Starter culture[a]		3%
Incubation	30°C/up to pH 4.34/~7 h	42°C/up to pH 4.5/~6 h
Cooling	5°C, 1 day	5°C, 1 day
Storage	<5°C	<5°C
Shelf-life	21 days	21 days

[a] The starter culture was composed of *L. delbrueckii* subsp. *bulgaricus* (>10^9 CFU ml^{-1}) and *S. salivarius* subsp. *thermophilus* (>10^9 CFU ml^{-1}).

2.2. Test meals

We used homogenized pasteurized whole milk to prepare two dairy products for the study. One half of the milk was acidified using 2% glucono delta-lactone (GDL) (Jungbunzlauer AG, Basel, Switzerland). GDL is used in dairy industries for controlled acidification in milk and yogurt preparation [12,13]. In this study, acidified milk using GDL is referred to as milk. The other half was used to make conventional yogurt using 3% of starter culture comprising of *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus salivarius* subsp. *thermophilus*. The detailed procedure used for the preparation of milk and yogurt is described in Table 2. Both milk and yogurt were kept similar in weight, volume, energy content, appearance and pH (4.27 for yogurt; 4.12 for milk) to blind the subjects to treatment order. An informal sensory panel composed of six trained persons did not identify significant differences in the texture or the appearance of the two products. A routine microbiological analysis of both products revealed the absence of pathological microbiological contaminants. The macronutrient composition of the milk and yogurt products is described in Table 3.

2.3. Blood samples

On days 4 and 8, an intravenous catheter was installed in the forearm of the volunteers by trained nurses to facilitate multiple blood sampling. For gene expression analysis, the first blood samples defined as the 0-h sample were drawn immediately prior to the ingestion of the dairy products, which was completed within a period of 20–30 min. Samples were collected at 2, 4 and 6 h after the ingestion of the dairy products. Blood was collected in triplicate into PAXgene tubes (Preanalytical Systems, Basel, Switzerland) according to the manufacturer's instructions. The samples were incubated at room temperature for 9 h [14], placed at −20°C for a period of 24 h, and finally transferred at −80°C until use for further processing. The PAXgene system with RNA-stabilizing solution offers several advantages for collection, storage and processing of whole blood samples in multicentre trials [15]. In particular, the isolation of leucocyte subtypes requires immediate processing of the samples on the site of collection, a shortcoming in a multicenter setup such as in our study. Moreover, *ex vivo* changes in gene expression profiles are induced during the separation of leucocyte subtypes [16]. Hence, in order to be able to measure a transcriptional signature that reflects the sum of the changes induced by dairy products, a whole blood analysis was preferred over an analysis restricted to a subset of cells.

In order to identify subjects with subclinical signs of infection or inflammation blood samples collected at 0 and 6 h on the intervention days were analyzed for C-reactive protein using immunoturbidimetry method and differential leukocyte count using automatic differentiation method. Based on this analysis, one subject was excluded from the microarray analysis as he had an abnormal neutrophil count at one 0-h time point.

2.4. RNA isolation, labeling and hybridization

Prior to RNA isolation, PAXgene tubes were removed from −80°C and allowed to thaw at room temperature overnight. RNA was extracted according to the manufacturer's instructions. Extracted RNA was stored at −80°C. RNA quality and integrity was

Table 3
Macronutrient composition of milk and yogurt

Composition (per 100 ml)	Milk	Yogurt
Energy (kJ)	284.5	263.6
Water (ml)	83.2	86.2
Protein (g)	4.4	4.1
Fat (g)	3.6	3.3
Lactose (g)	4.3	3.7
Other carbohydrates (g)	–	1.1
GDL (g)	2	–
Starter culture (ml)[a]	–	3

The values for lactose and carbohydrates were not measured and are taken from [75].
[a] The starter culture was composed of *L. delbrueckii* subsp. *bulgaricus* and *S. salivarius* subsp. *thermophilus*.

verified using the RNA 6000 Nano assay on the Agilent 2100 Bioanalyzer (Agilent Technologies, Basel, Switzerland). RNA concentrations were determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Littau, Switzerland). All RNA samples used for microarray analysis had a 260:280 absorbance ratio between 1.9 and 2.1.

Globin messenger RNA was depleted from a portion of each total RNA sample (1.0 ± 0.2 μg) using the GLOBINclear-Human kit (Ambion, Huntingdon, UK). A second subject was also excluded from further analysis as he had an RNA yield that was insufficient for microarray hybridization. Globin-depleted RNA samples (200 ng) were amplified using Agilent low RNA input linear amplification kit and control reagents to generate Cy3-labeled antisense cRNA. The labeled cRNA samples were hybridized to Agilent 4×44 k Whole Human Genome (G4112F) Oligo Microarray (Agilent Technologies). The hybridized slides were washed, stabilized, dried and immediately scanned (Agilent Technologies Microarray Scanner; Agilent Technologies) according to a standard protocol (Agilent Technologies). Microarray hybridization was performed at the Functional Genomics Center Zurich microarray core facility (ETH Zurich, Switzerland).

2.5. Identification of differentially expressed genes

Image processing was performed using Agilent's *feature extraction* software version 9.5.3.1. The resulting output file contains the intensity of each spot, which is summarized by the mean or the median pixel intensity of the spot. The normalization of the median (raw) intensity was carried out using the standardization and normalization of microarray data (SNOMAD) method [17] and R statistical language. Briefly, the SNOMAD approach first performs global normalization and then local mean normalization across each microarray surface to reduce spatial bias if any, followed by log2 transformation of normalized values. The log2 signal ratios (using the 0-h time point as the reference) and log2 mean intensities were estimated. The log2 ratios are normalized across local log2 mean intensities using the "loess" function of R. SNOMAD then finally provides the normalized standardized local z score ratio for each analyzed gene. SNOMAD z score ratios for the following comparisons were estimated: M2/M0, M4/M0, M6/M0, Y2/Y0, Y4/Y0, and Y6/Y0, where M represents milk, Y represents yogurt, and the digits 0, 2, 4, and 6 refer to the time points, in hours, at which the blood samples were taken. SNOMAD z score ratio comparing gene expression at days 4 and 8 before the ingestion of milk and yogurt, respectively, were also estimated (Y0/M0).

For each of the dairy product, an analysis of variance (ANOVA; SYSTAT version 12.0), more specifically ANOVA repeated measures was performed for each gene using the normalized z scores ratio to identify the differentially expressed genes. In addition, linear contrasts and quadratic contrasts were tested to identify temporal trends in gene expression over time. In each of the three analyses, a stringent estimate of Benjamini–Hochberg false discovery rate (FDR) [18] was obtained using the entire gene data set. FDR threshold for significance was set at an FDR of 12.5%. Differentially expressed probe sets in the groups of samples from subjects having ingested milk are, therefore, referred as the "M group" and yogurt as "Y group." Information on gene annotation and function was retrieved through EntrezGene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db_gene). In order to ascertain that the genes selected as differentially expressed are not due to spatial bias, we performed χ^2 goodness-of-fit tests using FDR thresholds of 10%, 15% and 20%, respectively, with the null hypothesis of uniform distribution of significant genes across array surface. We found the null hypothesis to be confirmed for all the FDR thresholds for both milk and yogurt data sets.

Spearman rank correlation analysis between gene expression levels in the M and Y groups was performed using R environment. Pathway analysis was performed on M group and Y group by using GENMAPP 2.0 [19] and MAPPfinder [20] to find functional groups of genes (based on Gene Ontology using gene database — Hs-Std_20070817.gdb) that were overrepresented in the subsets. Criteria where z score >2, permuted P value <.05 and more than 3 genes changed in a functional group were used as a threshold for identifying overrepresented functional groups. Additional information on gene function and pathway analysis was retrieved through MetaCore version 5.4 (GeneGo, St. Joseph, MI, USA). The gene expression data in a MIAME format will be made available through ArrayExpress, a public repository for microarray data, accession number E-MEXP-2571 (http://www.ebi.ac.uk/arrayexpress/).

2.6. Identification of differentially expressed human genes that are homolog to proteins present in bovine milk

A list of 183 proteins present in bovine milk was identified according to published references [21,22]. One hundred thirty-two of these have homologues in humans according to the Homologene database. The corresponding gene is present on the Agilent 4×44 k Whole Human Genome array G4112F for 112 of them (Additional file 1). Using this set of 112 homologous genes, we first checked, using the gene set enrichment analysis (GSEA) algorithm, whether these 112 proteins were randomly distributed throughout the list of genes for the M group ranked according to the FDR values of their linear contrasts.

In a second analysis, we first separated the genes according to the sign of their linear contrasts (positive or negative) before ranking the entire list according to the FDR values of the linear contrasts. In a separate analysis, we also identified differentially expressed human genes in the M and Y groups, for example, genes with FDR <12.5% for the linear contrast analysis, that are also present as homologues in the list of 112 bovine proteins.

2.7. GSEA for comparison between the M and Y groups

In addition, GSEA was carried out independently on the entire gene list from the M and Y groups to compare and identify pathways differently modulated in these two groups. The gene lists of the M and Y groups were ranked based on FDR and linear contrast values. Molecular databases from Biocarta comprising 246 gene sets were used for enrichment analysis. An FDR of <5% was used to identify pathways with significant enrichment.

3. Results

3.1. Identification of differentially expressed genes in the M and Y groups

This transcriptome study assesses kinetic changes in gene expression in the blood cells of human subjects after the ingestion of two dairy products, namely, milk and yogurt.

A repeated-measures ANOVA, considering the gene expression analyses at the time points 2, 4 and 6 h relative to 0 h as repeated measures, identified 70 genes after the ingestion of milk (M group) and 5 genes after the ingestion of yogurt (Y group) that are differentially expressed using a 12.5% FDR threshold. A quadratic contrast, assuming a quadratic function for the changes in gene expression between time points 2, 4 and 6 h, did not lead to the identification of differentially expressed genes. However, a linear contrast, assuming a linear change of gene expression between 2 and 6 h, identified 495 differentially expressed genes (576 transcripts, $P \leq .002$) in the M group and 499 differentially expressed genes (625 transcripts, $P \leq .002$) in the Y group. Both groups shared 11% of their differentially expressed genes. Of 70 significant genes selected from the repeated-measures ANOVA, 65 genes were also found significant in the linear contrast test. The list of all differentially expressed genes is available in Additional file 2.

3.2. Kinetic analysis of differentially expressed genes in the M and Y groups

Fig. 1 illustrates the average kinetics of differential gene expression in the form of box plots showing the average SNOMAD z scores as a function of time for the genes in the M and Y groups that had either a positive or a negative linear contrast value. For all four groups of genes, the kinetics showed a linear trend between the time points 2 and 6 h in agreement with the identification of these genes by significant linear contrasts. Despite this linear trend between 2 and 6 h, the kinetics of gene expression is not linear throughout the entire postprandial phase observed in our study, that is, between 0 and 6 h. Indeed, one-sample Wilcoxon signed rank tests on the median z scores of the four groups of genes at the time points 2 and 6 h confirmed the visual observation that the median

values at 2 h (M2/M0, Y2/Y0) were either negative (for the group of genes with positive linear contrasts) or positive (for the group of genes with negative linear contrasts) and significantly different from zero ($P<.05$), whereas the median values at 6 h (M6/M0, Y6/Y0) followed the inverse pattern and were either positive (for the group of genes with positive linear contrasts) or negative (for the group of genes with negative linear contrasts) and significantly different from zero ($P<.05$).

Taken together, the statistical analysis of the data at the various time points showed that each of the four sets of genes followed biphasic kinetics. For the set of genes with a positive linear contrast in the M and Y groups, we observed an initial transient down-regulation between 0 and 2 h, followed by a more pronounced and sustained up-regulation between 2 and 6 h, with the 6-h values being higher than the 0-h values (Fig. 1A). We refer to this biphasic kinetics in the text as "downUP kinetics." The opposite effect was observed for the set of genes with a negative linear contrast in the M and Y groups, as these genes first showed a transient increase in expression between 0 and 2 h, followed by a more pronounced and sustained decrease between 2 and 6 h, with the 6-h values being lower than the 0-h values (Fig. 1B). We refer to this biphasic kinetics later in the text as "upDOWN kinetics."

A comparison of the linear contrast values of the significant genes in the M group to the linear contrast values of the same genes after the ingestion of yogurt showed a statistically significant positive correlation of 0.90 between the two data sets (Fig. 2A). Similarly, the linear contrast values of the significant genes in the Y group, when compared to the linear contrast values of the same genes after the ingestion of milk, also showed a statistically significant positive correlation of 0.80 between the two data sets (Fig. 2B). Finally, the correlation between the linear contrast values of all the genes differentially expressed in either the M or the Y group reached a value of 0.79 (Fig. 2C).

3.3. Identification of differentially expressed pathways in the M and Y groups

Fig. 3 shows a process network analysis of the genes differentially expressed in the blood cells of human subjects following the ingestion of milk and yogurt. Overall, most of the biological processes were identified within the group of genes having a negative linear contrast. In particular, processes mediating the immune response, signal transduction, apoptosis and the inflammatory response contained genes with negative linear contrasts. Analysis of the genes having a positive linear contrast identified only two major processes, namely, transcription and translation.

The shape of the functional profiles of the M and Y groups of genes in Fig. 3 was globally similar, suggesting that the ingestion of milk and yogurt is followed by a common postprandial response in humans. Interestingly, despite the fact that the M and Y groups contained a similar number of differentially expressed genes, a larger number of processes reached statistical significance in the M group than in the Y group. This finding suggests a quantitative, rather than a qualitative, difference in the postprandial response of the subject following the ingestion of milk and yogurt.

We also conducted a pathway enrichment analysis of the set of genes with statistically significant linear contrasts in the M and Y groups using GenMAPP (Table 4). At the level of the biological processes, this analysis provided us with similar results than obtained with the process network analysis shown in Fig. 3. In addition, the GenMAPP analysis with the genes having a positive linear contrast revealed an enrichment of cellular components associated with ribosomal and mitochondrial activity, while the processes containing genes with negative linear contrasts were localized to the extracellular matrix and to cytoplasmic membrane-bound vesicles. The data, thus, indicate that the protein biosynthesis machinery and the mitochondrial functions are up-regulated at 6 h, whereas immunomodulatory functions involving signal transduction are down-regulated at 6 h.

Fig. 4 shows the heat map for genes belonging to the four functional processes overrepresented in the GenMAPP pathway analysis that are shared by the M and Y groups (protein biosynthesis, oxidative phosphorylation, apoptosis, inflammatory response), again highlighting the segregation between upDOWN immunomodulatory genes and downUP metabolic genes.

Fig. 5 graphically presents the linear contrasts of the genes identified in Table 4 that are differentially expressed in either the M or the Y group and that belong to the functional groups "mitochondrial part," "ribosomes" and "inflammatory response." This graph further highlights the similarity in the postprandial response of the subjects following the ingestion of milk and yogurt.

3.4. Kinetic analysis of functional groups of genes

The left panels of Fig. 6A (M group) and 6B (Y group) illustrate the downUP kinetics of genes involved in protein biosynthesis, a group of genes almost exclusively composed of genes coding for ribosomal proteins. The largest difference in relative gene expression was observed between time points 2 and 6 h. An analysis of the data by hierarchical clustering of the time-point comparisons also showed, for both sets of ribosomal genes in the M (right panel of Fig. 6A) and Y (right panel of Fig. 6B) groups, that the 2- and 6-h time points maximized the differences in relative gene expression.

The group of proteins with a mitochondrial function (e.g., genes involved in oxidative phosphorylation: *NDUFB7*, *NDUFB9*, *NDUFS3* and *NDUFS4*, which are subunits of complex I, *ATP synthase*; *UQCR*; cytochrome *c*) also follows downUP kinetics (not shown).

The left panels of Fig. 7A (M group) and 7B (Y group) show the upDOWN kinetics of genes involved in the inflammatory response in the form of box plots (average kinetics for the group of genes) and illustrates that the largest difference in relative gene expression was observed between the time points 2 and 6 h. An analysis of the data by hierarchical clustering of the time-point comparisons also showed, for both sets of inflammatory genes in the M (right panel of Fig. 7A) and Y (right panel of Fig. 7B) groups, that the 2- and 6-h time points maximized the differences in relative gene expression. Of note, the upDOWN kinetics of the inflammatory group of genes appeared to be more pronounced after the ingestion of milk than of yogurt.

Key players in the induction of inflammation, such as *Rel-A* [p65 component of nuclear factor (NF) κB heterodimer], *IL6*, *IL1β*, *TLR2*, *TLR4* and *IL8RB*, were differentially regulated after the ingestion of dairy products. Fig. 8 shows a GeneGO Metacore map illustrating the toll-like receptor (TLR) signaling cascade and all the genes in these pathways that were differentially expressed with a P value $<.05$ in either the M or the Y group. With the exception of *TRAF6*, all other genes had a negative value in the linear contrast indicating down-regulation at 6 h. These genes code for proteins that cover most of the signaling cascade from cell surface receptors (*CD14*; *TLR2*, *TLR4*, *TLR6*), intracellular mediators (*IRAK1*, *IRAK1/2*, *MEK3*, *NIK*, *TLR8*, *TOLLIP*, *TRAF6*), nuclear transcription factors (*NF-κB*), and finally extracellular effectors (*IL6*, *IL8*).

The group of genes involved in the apoptotic process (e.g., *BCL10*, *TP53BP2*, *PDCD6IP*, *BAG5* and *PTEN*) also follows average upDOWN kinetics (not shown).

3.5. Specificity of the postprandial transcriptomic profile

The biological pathways identified in this study could be attributed either to the direct effects of the dairy products on the

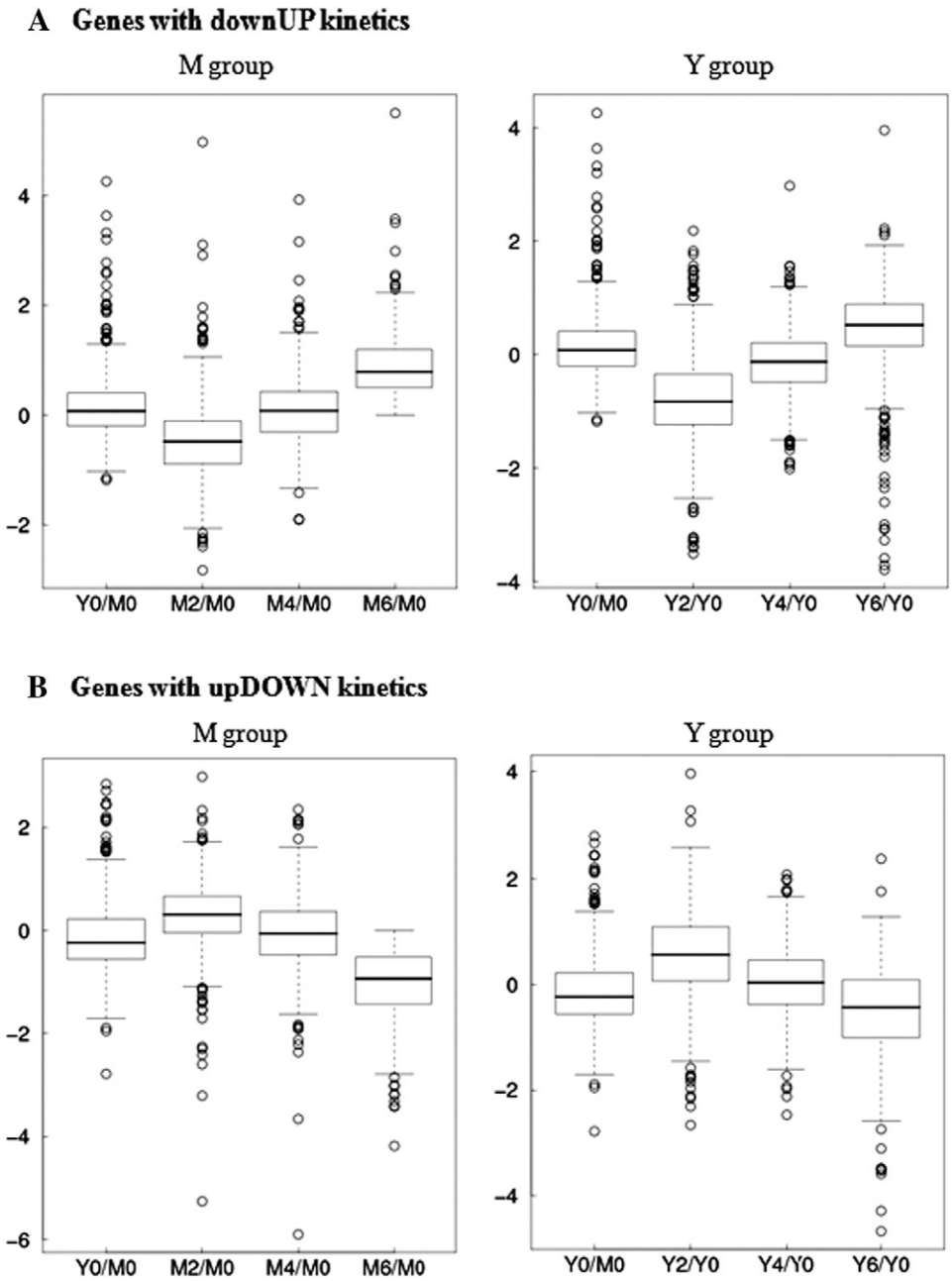


Fig. 1. Box plot of the downUP and upDOWN genes in the M and Y groups. Analysis of the SNOMAD *z* score ratio for the time-point comparisons of all genes in the M (M2/M0, M4/M0, M6/M0) and Y (Y2/Y0, Y4/Y0, Y6/Y0) groups. Y0/M0 represents the *z* score ratio of two 0-h times from the crossover design, which is used as a reference in this analysis. The relative SNOMAD *z* score ratio is shown on the *y*-axis. The box plots show the maximum value, lower quartile, median, upper quartile and minimum values observed for each time point in the groups indicated. (A) Box plot of downUP genes in the M (307 transcripts) and Y (394 transcripts) groups. (B) Box plot of upDOWN genes in the M (269 transcripts) and Y (231 transcripts) groups.

blood cell transcriptome of the organism or to endogenous effects that are independent of the ingested dairy products.

First, the gene expression program induced during the fasting phase (e.g., the circadian rhythm or fasting metabolism) may overlap with the feeding phase of the study. However, we found that only ~7% of the blood cell genes that are differentially expressed after 6 h in response to the dairy products in our study were also reported to change their expression during a 48-h fasting period [9]. The contribution of the fasting and circadian metabolic processes to our sets of differentially expressed genes is, thus, limited.

Second, the acid or volume load resulting from the ingestion of 540 g of dairy products may also trigger gene expression. To check this possibility, we performed a text-based search using PubMatrix [23] on the literature database (NCBI PubMed). We uploaded the M and Y groups of genes and searched against the following keywords: acid load, acid overload, acidosis, lactic acid, acid challenge, acid–base equilibrium, acid–base imbalance and gluconic acid. The results from this search (data not shown) did not provide evidence that an acid overload was responsible for the gene expression profile observed in our study.

In conclusion, the functional analyses of our data strongly indicate that the observed postprandial changes in gene expression directly result from the ingestion of the nutrients present in the dairy products.

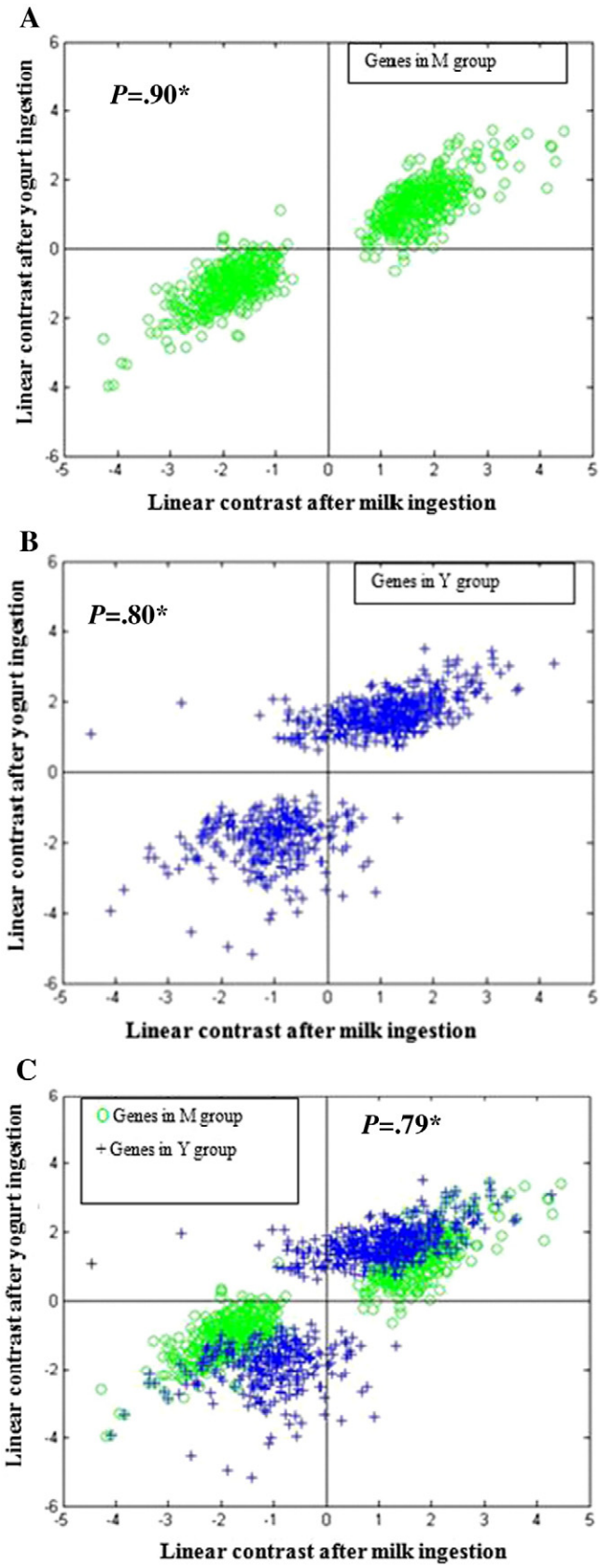


Fig. 2. Spearman rank correlation analysis using linear contrast values. (A) Linear contrast values of all genes of the M group plotted against the corresponding linear contrast values for the same genes after ingestion of yogurt (green open circles). (B) Linear contrast values of all genes of the Y group plotted against the corresponding linear contrast values for the same genes after ingestion of milk (blue crosses). (C) Combined panels A and B. *Spearman rank correlation coefficient.

The extent to which proteins or bioactive peptides (after milk processing, digestion and absorption into the circulation) in dairy products, milk in particular, may express their specific biological activity in humans is an intriguing question. Whereas such effects are clearly important in breast-fed babies [24], their relevance in human adults is clearly less evident and subject of more debate. In order to address this point, we investigated whether the postprandial genes differentially expressed in the blood cells of our adult subjects were indicative of biological properties that may be specifically attributed to the ingested bovine milk proteins:

First, we used the GSEA algorithm [25] to evaluate the distribution of human genes — that are homolog to proteins present in bovine milk — in the list of genes ranked by the FDR values associated with their postprandial linear contrasts. We observed that the 112 gene products identified in bovine milk (see Additional file 1) clustered toward the top of the milk gene list (e.g., the most differentially expressed genes), with a corresponding P value of .0024. In other words, these 112 genes tended to be more differentially expressed than other genes in the 4×44 k array. The statistical relevance of this analysis was further increased when the GSEA analysis was performed, with the list sorted first according to the direction of differential expression and second according to the FDR values associated with their postprandial linear contrasts (up-regulated genes with positive linear contrast at the top; down-regulated genes with negative linear contrast at the bottom). The GSEA indicated that milk genes tended to cluster toward the end of the list (down-regulated genes, $P=.0094$) rather than toward the top ($P=.9925$).

Second, we have identified eight human genes coding for homolog gene products present in bovine milk that have a statistically significant linear contrast in human blood cells after the ingestion of the dairy products (*A1BG*, *ALDH2*, *HPSE*, *SERPING1*, *TLR4* and *YWHAB* in the M group; *HPSE*, *TLR2* and *SERPINA1* in the Y group). Strikingly, genes belonging to the same protein families were separately identified in the M and Y groups (toll-like receptors: *TLR2*, *TLR4*; serpins: *SERPING1*, *SERPINA1*; heparanase: *HPSE*), a finding that is highly unlikely to occur by chance. Also, seven of eight genes showed a negative linear contrast (only *A1BG*, whose function is unknown, had a positive linear contrast).

3.6. Comparative analysis of the blood cell transcriptomes of the M and Y groups

Milk and yogurt share more similarities in their chemical composition than they differ, which is reflected in the observations that the data presented in Figs. 1–7 as well as in Table 4 globally reveal similar postprandial transcriptomic profiles for the M and Y groups. On the other hand, numerous quantitative differences that are statically significant can also be observed between these two groups, in particular at the level of single genes (see Fig. 2) and single pathways (see Fig. 3). The biological significance of these differences can, however, not be inferred from our data set and requests additional studies.

Keeping in mind this limitation, the potential of using comparative postprandial blood cell transcriptomic to investigate the biological properties of a particular food compared to a reference, can, nonetheless, be illustrated by the following two analyses.

First, Fig. 2 revealed that a lower correlation coefficient was obtained when the linear contrasts of the differentially expressed genes of the Y group were correlated to their respective values in the M group ($\rho=0.8$), compared to when the linear contrasts of the differentially expressed genes of the M group were correlated to their respective values in the Y group ($\rho=0.9$). As the microbiological transformation of milk to yogurt increases the complexity of its

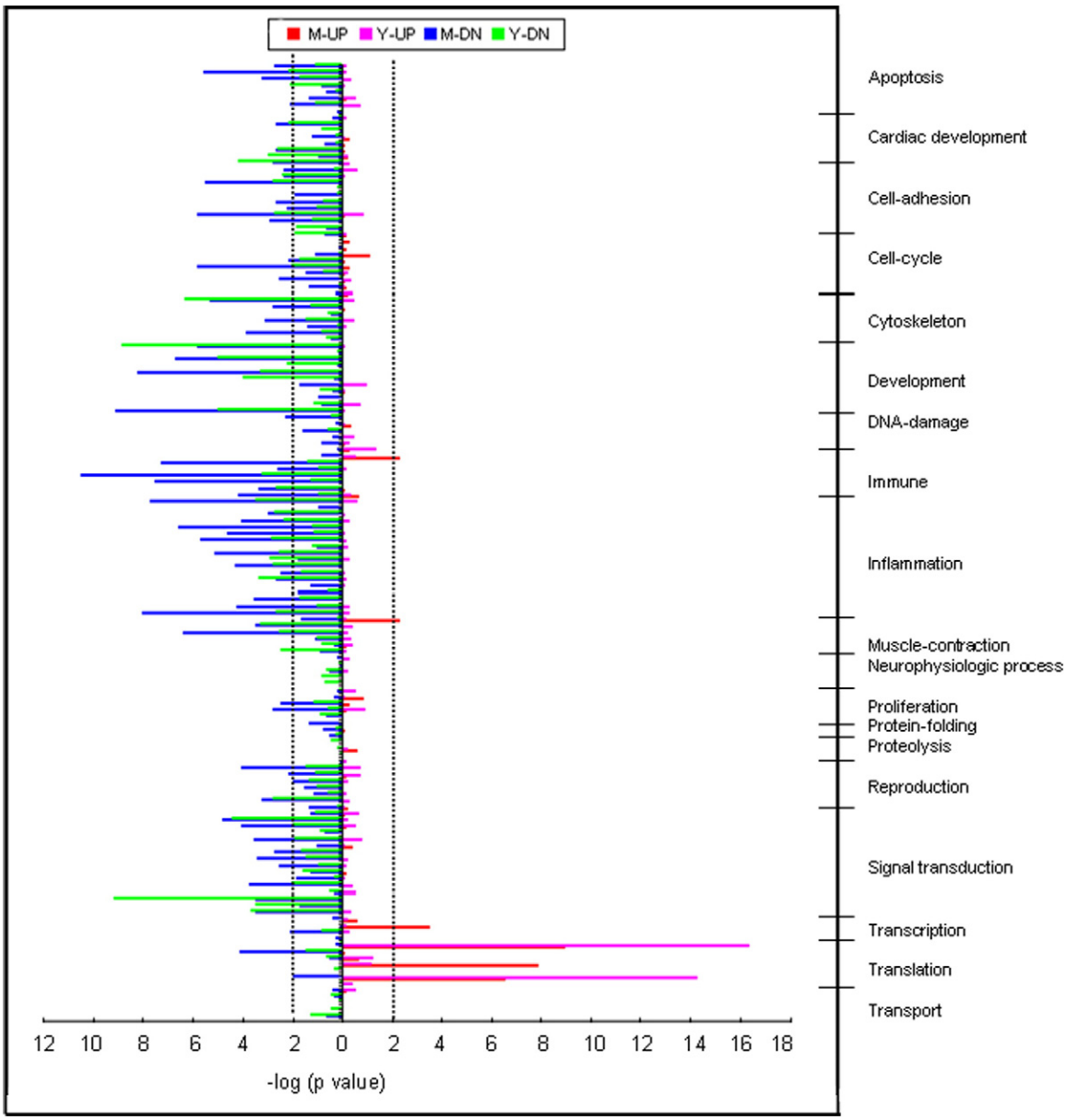


Fig. 3. Overview of biological processes in blood cell gene expression profiles induced by milk and yogurt ingestion. Bar plot showing the processes with negative and positive linear contrasts for the M and Y groups resulting from a network analysis performed using MetaCore version 5.4. (A) $-\log(P\text{ value}) > 2$ is considered as statistically significant (dotted lines).

chemical composition, the quantitative difference in these correlation coefficients may reflect changes in the nutritional properties of this product. This conclusion must clearly be strengthened by additional studies. Meanwhile, the potential use of this methodology for conducting comparative nutritional genomics on processed food appears appealing.

Second, another GSEA analysis was performed to compare the biological pathways modulated in the M and Y groups (Fig. 9). This analysis confirmed our conclusion that most of the biological processes regulated in the Y group are shared by the M group. However, despite these similarities, GSEA revealed eight significantly changed pathways that were unique to the Y group. These pathways were angiotensin-converting enzyme 2 (ACE2); alpha-hemoglobin-stabilizing protein (AHS); macrophage differentiation (ETS); intrinsic prothrombin activation pathway (INTRINSIC); metastasis-associated 1 family, member 3 pathway (MTA3); p53 signaling (P53); vitamin C in the brain pathway (VITCB); and wnt signaling (WNT).

4. Discussion

4.1. Statistical validity and representativeness of the study

In contrast to drugs that usually contain a single compound exerting a high pharmacological activity, the action of food on gene expression is more complex and difficult to measure as it is characterized by the induction of a broad arrays of genes with modest changes in their intensity [10,11]. Particular care must, therefore, be attributed to the statistical analysis of transcriptomic data in nutrition research in order to obtain meaningful data.

Despite the modest sample size (the transcriptome of six subjects was finally measured) and the small changes in expression (the fold changes can indirectly be deduced from Additional file 2 and Fig. 1), we have identified several hundred genes that are differentially expressed after consumption of the dairy products. We attribute this positive outcome to our kinetic strategy in the analysis

Table 4
Overrepresented functional groups in the total set of differentially expressed genes in the M and Y groups

GO	Functional groups containing genes with positive linear contrasts	A	B	Milk		Yogurt	
				C	D	C	D
BP	Biosynthetic process	1261	1358	42	6.4	48	7.1
BP	RNA processing	403	424	17	5.1	13	2.9
BP	Lymphocyte differentiation	86	93	5	3.6	4	2.4
MF	RNA Binding	636	673	23	5.1	36	8.9
CC	Ribosome	217	249	27	14.3	34	17.3
CC	Mitochondrial part	359	342	24	8.9	13	3.4
CC	Spliceosome	128	132	6	3.3	5	2.3
CC	Ribonucleoprotein complex	446	485	37	13.0	41	13.6
CC	Structural constituents of ribosome	195	226	26	14.7	34	18.5
CC	Small ribosomal subunit	59	64	8	8.2	13	13.0
CC	Large ribosomal subunit	65	68	10	9.9	12	11.3
GO	Functional groups containing genes with negative linear contrast	A	B	C	D	C	D
BP	Signal transduction	3006	3464	71	4.9	54	4.9
BP	Apoptosis	715	716	19	2.9	16	3.4
BP	Inflammatory response	284	303	12	4.1	11	4.9
BP	Immune response	590	714	17	3.1	11	2.1
BP	Cell motility	420	416	14	3.4	13	4.3
BP	Acute inflammatory response	67	75	4	3.2	3	2.9
BP	Cytokine production	111	110	6	3.6	8	6.6
BP	Cell surface receptor-linked signal transduction	1393	1745	30	2.5	35	5.9
BP	Cell adhesion	657	688			13	2.5
BP	Angiogenesis	139	131			5	3.1
CC	Cytoplasmic vesicle	256	256	9	2.9	9	4.1

Overrepresentation analysis was performed in GenMAPP 2.0 by comparing differentially expressed genes in the M and Y groups to all genes on the microarray. GO, gene ontology functional groups; CC, cellular component; BP, biological process; MF, molecular function; A, number of genes measured; B, number of genes in the GO group; C, number of genes changed; D, z score with $P \leq .05$ (permuted P).

of the data. Indeed, the power of the F test on linear trends is higher than the power of the overall repeated-measures ANOVA F test because of the bigger noncentrality parameters of the noncentral F distribution used in power calculation. A more direct consideration with the same conclusion is the comparison of the error variance used to calculate the F test statistics for the repeated-measures ANOVA with the error variance used for the F test statistics of the linear contrasts: in our case, the latter is only one third of the former. Thus, this reduction of variance clearly means more power for the F tests of the linear contrasts compared to the repeated-measures ANOVA F tests.

In light of the low percentage of differentially expressed genes shared between the M and Y groups (11%), the significant correlation between the linear contrasts of both data sets suggests that the Benjamini–Hochberg calculation overestimates the true FDR (therefore, a cutoff value of 12.5% was selected). Despite this limitation, the sets of differentially expressed genes used for the functional analyses in the M and Y groups globally lead to the identification of similar physiological processes and pathways for both dairy products. This finding, therefore, validates the selection criteria used for the statistical cutoff values.

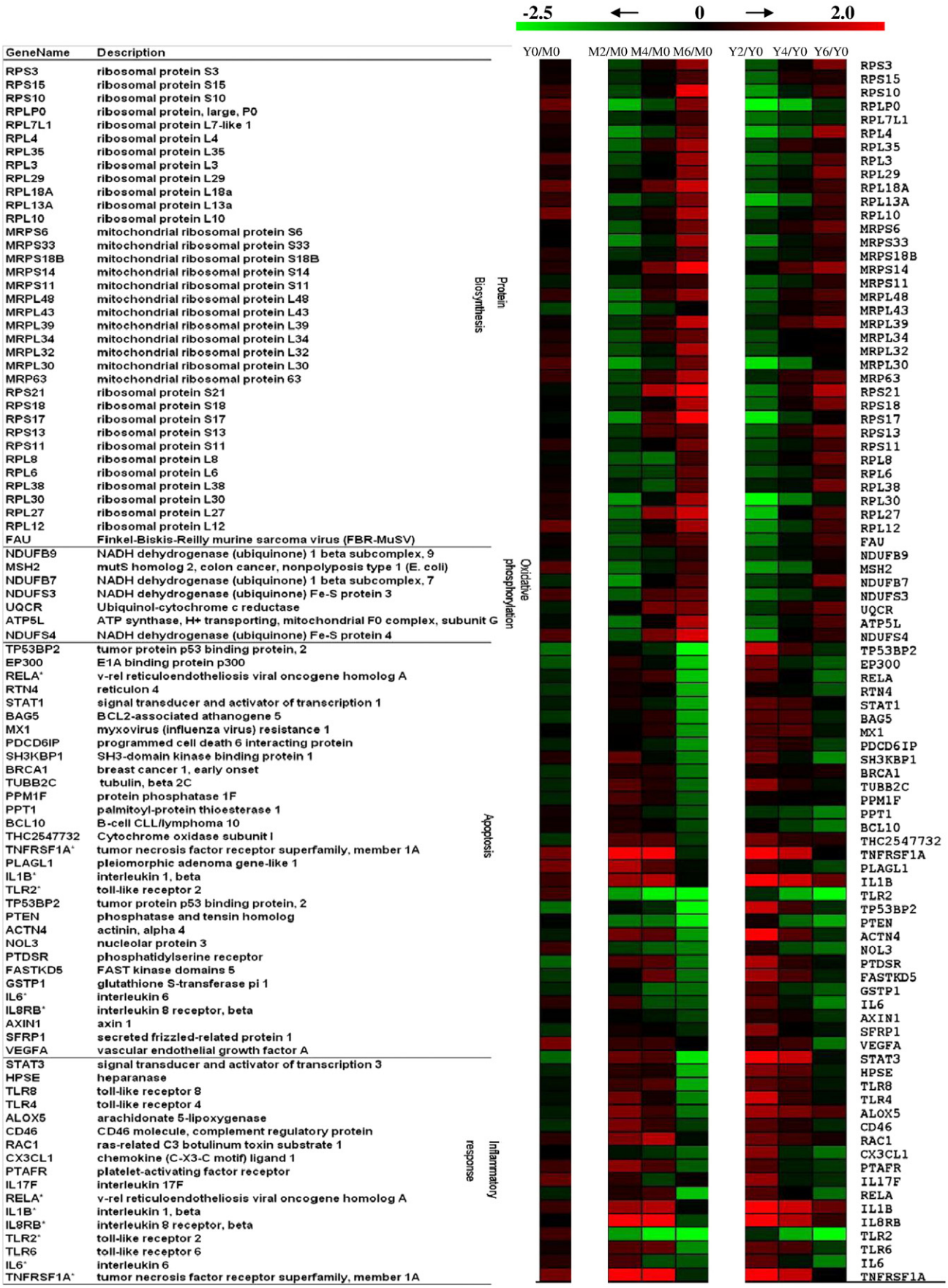
Intraindividual and interindividual variations between the two starting points, though statistically not important for the study of linear contrasts, were assessed by the z scores of the Y0/M0 comparison. Intraindividual variation was considered significant for absolute z scores ≥ 1.96. Proportions of about 17% of the whole 43,376 transcripts and about 16% of the 650 differentially expressed transcripts exceeded this limit. The proportions of significant interindividual variation were estimated by comparing the means of the six z scores to the 0.025 and 0.975 quantiles of the expected distribution. About 18% and 14% for the whole and differentially expressed transcripts, respectively, were outside these significance limits; a finding similar to other studies [9,10]. The similar proportions of significant intraindividual and interindividual variation for differentially expressed transcripts and the whole transcripts indicate the increased sensitivity of the kinetic analysis by linear contrasts compared to common pairwise t tests.

A definite asset of the study is the crossover design in which each subject served as his own control. A combined effect of the kinetic and crossover design is the fact that the error term in the denominator of the F tests is the mean intraindividual variance, which does not depend on the interindividual variability and, therefore, allowed us to identify the genes changing their expression after the ingestion of the dairy products with accuracy. The box plots in Fig. 1 illustrate this conclusion for all four panels, as the mean values of the SNOMAD z score ratios for the comparisons M6/M0 and Y6/Y0 were all statistically different from zero, whereas those for the Y0/M0 were not. In other words, despite the known interindividual variability in human transcriptomic analyses [9,10], we can identify genes that change their expression within a few hours of the ingestion of dairy products and that do not vary over a time frame of 4 days when measured under fasting conditions. Interestingly, as the mean values of the SNOMAD z score ratios for the two Y0/M0 comparisons were not significantly different from zero, we also conclude that a carryover effect is not observed and that our study was appropriately designed in that regard.

The postprandial response is characterized by modest changes in the intensity of the expressed genes in human blood cells. Consequently, we observed that the housekeeping genes usually selected for conducting confirmatory reverse transcriptase polymerase chain reaction (RT-PCR) analytics vary in their intensity values on the microarrays in a range that is close to the fold change identified for the sets of differentially expressed genes (data not shown). The normalization procedures used with microarrays are more comprehensive and robust than RT-PCR [26,27]. Also, the correlation between microarray and RT-PCR data is no longer satisfactory in cases where the fold change in gene expression is below 1.4 [26], a situation that is typically seen in gene expression studies in nutrition research [9,10] and that is also observed for the vast majority of the differentially expressed genes identified in our study (data not shown). As the analysis of our data clearly points to biologically relevant phenomena (see, in particular, Fig. 5), we did not use RT-PCR as a control method to confirm the validity of the microarray data. To take this limitation into account, we conducted most of the discussion of the microarray data at the level of biological processes and pathways being careful not to overinterpret results from the finding of single genes. In that context, more experimental data should be generated before attempting a biologically relevant functional analysis of the blood cell transcriptomes following the ingestion of milk and yogurt at the gene-by-gene level.

Several, nonexclusive explanations can be put forward to account for the biphasic kinetic pattern: (a) homeostatic response of the organism that responds to the initial diet-induced stress by restoring the original equilibrium of the biological system (of note, in apparent disfavor to this hypothesis, the second phase of the kinetics proceeds beyond the equilibrium point at time 0 h); (b) superimposition of different kinetics of gene expression arising from different types of blood cells; (c) synergistic and antagonistic

Fig. 4. Genes statistically changed (FDR < 12.5%) after ingestion of milk and yogurt in various overrepresented functional processes. Heat map of genes associated with multiple groups is indicated with an asterisk (*) appended to the gene name. Compared to 0 h, the green and red colors represent down-regulated and up-regulated genes, respectively. The colored-graded bar gives the z score ratio. The distribution of the genes into the functional processed was obtained by GenMAPP pathway analysis.



GeneName	Description	
RPS3	ribosomal protein S3	Protein Biosynthesis
RPS15	ribosomal protein S15	
RPS10	ribosomal protein S10	
RPLP0	ribosomal protein, large, P0	
RPL7L1	ribosomal protein L7-like 1	
RPL4	ribosomal protein L4	
RPL35	ribosomal protein L35	
RPL3	ribosomal protein L3	
RPL29	ribosomal protein L29	
RPL18A	ribosomal protein L18a	
RPL13A	ribosomal protein L13a	
RPL10	ribosomal protein L10	
MRPS6	mitochondrial ribosomal protein S6	
MRPS33	mitochondrial ribosomal protein S33	
MRPS18B	mitochondrial ribosomal protein S18B	
MRPS14	mitochondrial ribosomal protein S14	
MRPS11	mitochondrial ribosomal protein S11	
MRPL48	mitochondrial ribosomal protein L48	
MRPL43	mitochondrial ribosomal protein L43	
MRPL39	mitochondrial ribosomal protein L39	
MRPL34	mitochondrial ribosomal protein L34	
MRPL32	mitochondrial ribosomal protein L32	
MRPL30	mitochondrial ribosomal protein L30	
MRP63	mitochondrial ribosomal protein 63	
RPS21	ribosomal protein S21	
RPS18	ribosomal protein S18	
RPS17	ribosomal protein S17	
RPS13	ribosomal protein S13	
RPS11	ribosomal protein S11	
RPL8	ribosomal protein L8	
RPL6	ribosomal protein L6	
RPL38	ribosomal protein L38	
RPL30	ribosomal protein L30	
RPL27	ribosomal protein L27	
RPL12	ribosomal protein L12	
FAU	Finkel-Biskis-Reilly murine sarcoma virus (FBR-MuSV)	
NDUFB9	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 9	Oxidative phosphorylation
MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	
NDUFB7	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7	
NDUFS3	NADH dehydrogenase (ubiquinone) Fe-S protein 3	
UQCR	Ubiquinol-cytochrome c reductase	
ATP5L	ATP synthase, H+ transporting, mitochondrial F0 complex, subunit G	
NDUFS4	NADH dehydrogenase (ubiquinone) Fe-S protein 4	
TP53BP2	tumor protein p53 binding protein, 2	Apoptosis
EP300	E1A binding protein p300	
RELA*	v-rel reticuloendotheliosis viral oncogene homolog A	
RTN4	reticulon 4	
STAT1	signal transducer and activator of transcription 1	
BAG5	BCL2-associated athanogene 5	
MX1	myxovirus (influenza virus) resistance 1	
PDCD6IP	programmed cell death 6 interacting protein	
SH3KBP1	SH3-domain kinase binding protein 1	
BRCA1	breast cancer 1, early onset	
TUBB2C	tubulin, beta 2C	
PPM1F	protein phosphatase 1F	
PPT1	palmitoyl-protein thioesterase 1	
BCL10	B-cell CLL/lymphoma 10	
THC2547732	Cytochrome oxidase subunit I	
TNFRSF1A*	tumor necrosis factor receptor superfamily, member 1A	
PLAGL1	pleiomorphic adenoma gene-like 1	
IL1B*	interleukin 1, beta	
TLR2*	toll-like receptor 2	
TP53BP2	tumor protein p53 binding protein, 2	
PTEN	phosphatase and tensin homolog	
ACTN4	actinin, alpha 4	
NOL3	nucleolar protein 3	
PTDSR	phosphatidylserine receptor	
FASTKD5	FAST kinase domains 5	
GSTP1	glutathione S-transferase pi 1	
IL6*	interleukin 6	
IL8RB*	interleukin 8 receptor, beta	
AXIN1	axin 1	
SFRP1	secreted frizzled-related protein 1	
VEGFA	vascular endothelial growth factor A	
STAT3	signal transducer and activator of transcription 3	Inflammatory response
HPSE	heparanase	
TLR8	toll-like receptor 8	
TLR4	toll-like receptor 4	
ALOX5	arachidonate 5-lipoxygenase	
CD46	CD46 molecule, complement regulatory protein	
RAC1	ras-related C3 botulinum toxin substrate 1	
CX3CL1	chemokine (C-X3-C motif) ligand 1	
PTAFR	platelet-activating factor receptor	
IL17F	interleukin 17F	
RELA*	v-rel reticuloendotheliosis viral oncogene homolog A	
IL1B*	interleukin 1, beta	
IL8RB*	interleukin 8 receptor, beta	
TLR2*	toll-like receptor 2	
TLR6	toll-like receptor 6	
IL6*	interleukin 6	
TNFRSF1A*	tumor necrosis factor receptor superfamily, member 1A	

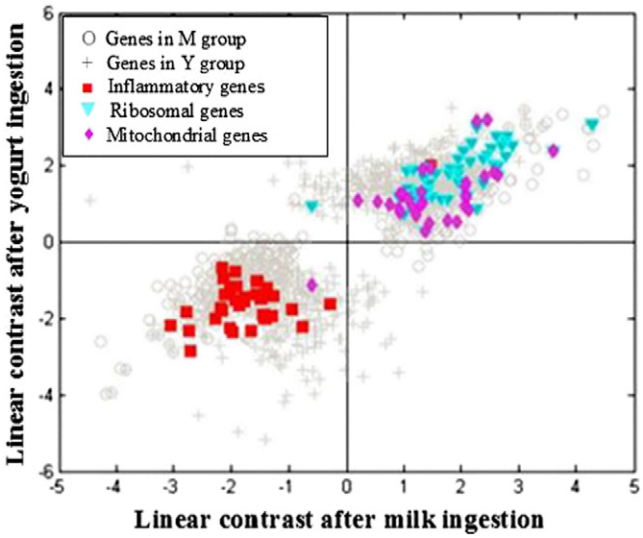


Fig. 5. Linear contrasts in the M and Y groups for genes belonging to selected functional groups. All genes of the M (gray circles) and Y (gray crosses) groups; all mitochondrial genes in the M and Y groups are shown as pink rhombus (24 of 25 genes and 13 of 14 genes have positive linear contrasts in the M and Y groups, respectively); all ribosomal genes are shown as cyan triangles (27 of 28 genes and 34 of 35 genes have positive linear contrasts in the M and Y groups, respectively); all inflammatory genes are shown as red squares (12 of 13 genes and 11 of 13 genes have a negative linear contrast in the M and Y groups, respectively).

properties of different nutrients in dairy products; and (d) kinetic effects resulting from the digestion and absorption processes of the dairy products in the gastrointestinal tract. Further experiments need to be conducted to differentiate between these hypotheses. Meanwhile, our findings highlight the importance of conducting kinetic studies, while looking at the expression of genes resulting from the ingestion of nutrients as the measurement of single time points (e.g., the comparisons between M4/M0 and Y4/Y0) only identifies a limited number of genes and leads to an incomplete assessment of the biological response.

Taking a heterogeneous population of blood cells for a transcriptomic analysis of the data restricts the interpretation of the data as it is not possible to differentially evaluate the specific contribution of each type of blood cells to the postprandial response. On the other hand, this disadvantage can be turned into an advantage as a transcriptomic analysis of whole blood may fulfil the needs of nutrition research by selectively allowing the detection of universal metabolic processes that are common to many cell types and that will eventually be physiology relevant, ignoring more specific effects. The relevance of this statement is strikingly illustrated by Fig. 5 as the linear contrasts of the genes belonging to the same functional group almost exclusively segregate in the same quadrants, that is, positive values for ribosomal and mitochondrial genes and negative values for inflammatory genes.

The representativeness of our study may be questioned due to the small number of subjects. However, this restriction is induced by the selection criteria (gender, age, body mass index) and much less by the number of individuals. In fact, since we have identified many genes that are differentially expressed after the ingestion of dairy products in a statistically significant and biologically meaningful manner with a small sample size, this effect is most probably important. The striking functional clustering of the differentially expressed genes in the negative (inflammatory genes) or positive (ribosomal genes; mitochondrial genes) quadrants of the linear contrasts shown in Fig. 5 again illustrates this conclusion in a dramatic manner. This finding can only be explained by a biological interpretation of the data that is likely to be universal and not restricted to the six individuals tested.

4.2. Physiological relevance of the postprandial blood cell transcriptome

4.2.1. Protein biosynthesis

Down-regulation of ribosomal genes has been observed in healthy humans 2 hours after the consumption of a protein-rich breakfast [10]. This down-regulation has been interpreted as a response of the organism to protein overload. In our study, we extend these findings by observing biphasic downUP kinetics for the ribosomal genes and the decrease at 2 h being transient, followed by a pronounced up-regulation at 6 h.

Proteins in dairy products are efficient in delivering essential amino acids for the synthesis of endogenous proteins [28]. In particular, whey proteins are rich sources of branched chain amino acids, such as leucine, and play a pivotal role in the initiation of protein synthesis in muscle [29–32]. Also, dairy proteins are more efficient in sustaining protein synthesis in comparison to soy proteins in human muscle cells [33]. Dairy products may, therefore, contribute to the management of muscle mass in physically active persons and the elderly [34,35]. In that context, the extent to which specific nutrients in dairy products, in particular whey proteins, contribute to the up-regulating phase of the downUP kinetics remains to be established.

4.2.2. Oxidative phosphorylation

Interestingly, the enzymatic activity of complex I in blood cells responds within a few days to the refeeding of malnourished patients in a more sensitive manner than validated nutritional markers such as albumin [36]. Our observation that changes in the expression of 33 mitochondrial genes, including complex 1, can be measured within a few hours of feeding, therefore, substantiates the use of mitochondrial nutritional markers in clinical nutrition.

The similarity in downUP kinetic patterns observed with ribosomal and mitochondrial genes is in line with the role of oxidative phosphorylation as a provider of metabolic energy for the protein biosynthetic process. The extent to which specific nutrients in dairy products, for example, carnitine and calcium that are known to stimulate oxidative phosphorylation, contribute to the up-regulating phase of the downUP kinetics remains to be established [37-39].

4.2.3. Inflammation

We observed coordinated changes in the expression of inflammatory genes, in particular genes of the TLR pathway, after the ingestion of milk and yogurt. TLRs are major mediators of the inflammatory signaling pathways [40]. Although TLRs are primarily involved in the recognition of pathogenic motifs [e.g., recognition of lipopolysaccharide (LPS) from gram-negative bacteria by TLR4], studies suggest that TLR4 is activated by saturated fat and antagonized by unsaturated fat thereby linking nutrition to inflammation and chronic diseases [41]. A nutritional intervention study in healthy human subjects has demonstrated postprandial activation of TLR2 and TLR4 genes and proteins in mononuclear cells in response to a diet enriched in fat and carbohydrates. Furthermore, the addition of orange juice to the diet counteracted these effects [42].

Together with other components of the TLR signaling pathway, the activities of TLR2 and TLR4 are modulated by the addition of milk to epithelial cells in culture [43]. Also, several dairy components, among these conjugated linoleic acid (CLA) [44], lactoferrin and tumor growth factor β [45,46], posses anti-inflammatory properties that are enhanced by the milk matrix. More importantly, an anti-inflammatory activity of dairy products in human adults has already been documented in intervention studies showing that the ingestion of milk is associated with a decrease in the fasting inflammatory

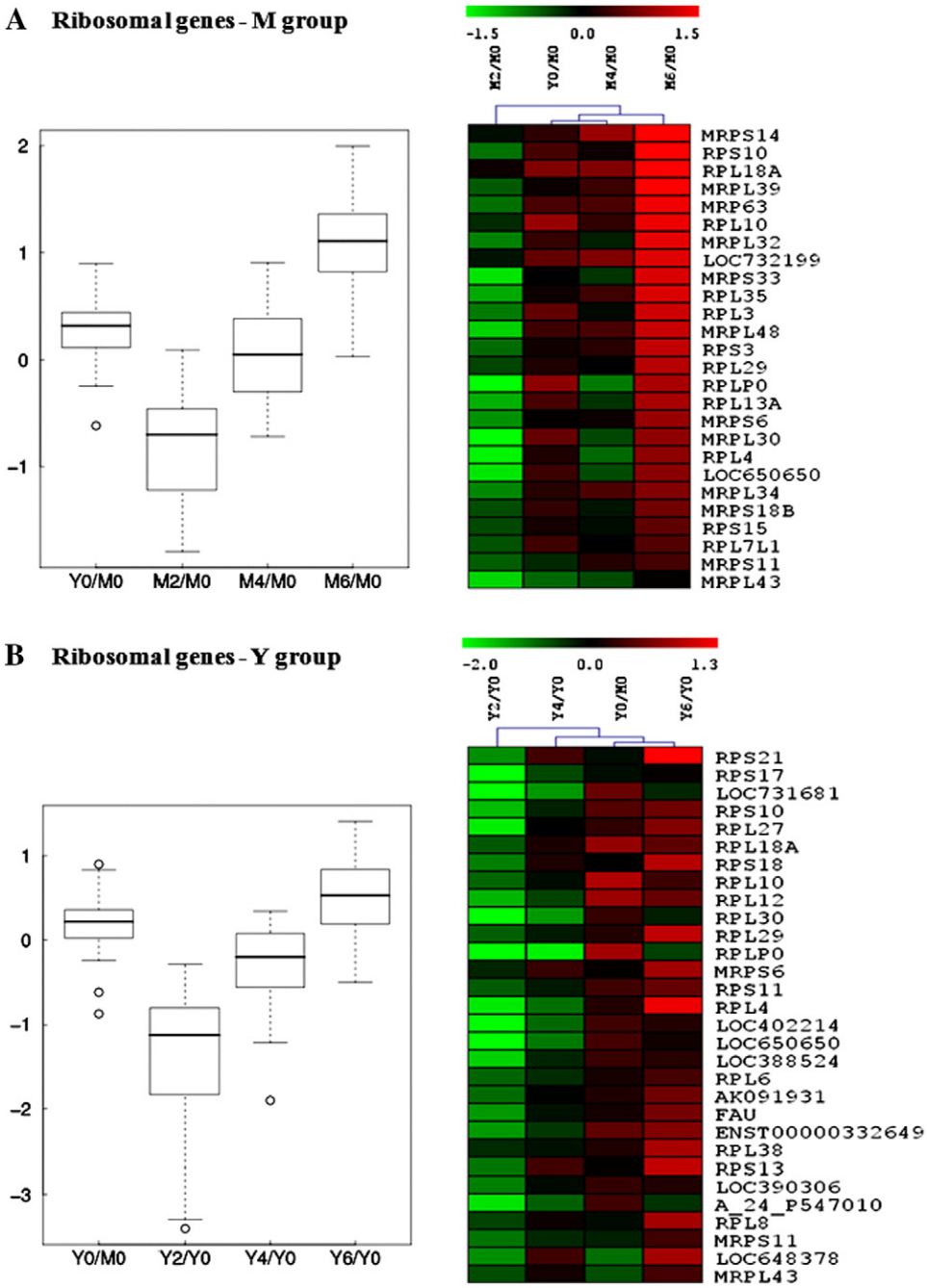


Fig. 6. Box plot and hierarchical clustering of the ribosomal genes in the M and Y groups. Analysis of the SNOMAD *z* score ratio for the time-point comparisons of the 26 ribosomal genes in the M group (M2/M0, M4/M0, M6/M0) and of the 31 ribosomal genes in the Y group (Y2/Y0, Y4/Y0, Y6/Y0). Y0/M0 represents the *z* score ratio of two 0-h times from the crossover design. Left panel: a box plot showing the distribution of the *z* score ratio for each time-point comparison. Right panel: hierarchical clustering of the time-point comparisons using Euclidean distance measure. The red color indicates up-regulation of gene expression relative to 0 h. The green color indicates down-regulation of gene expression relative to 0 h. The dendrogram illustrates that 2 and 6 h are the two farthest clusters.

markers interleukin-6, interleukin-1β and tumour necrosis factor α [47,48].

Our finding that the ingestion of dairy products is accompanied by an overall down-regulation of inflammatory genes at 6 h is, thus, in line with these studies and suggests that the biological activity of nutrients in dairy products with a potential for anti-inflammatory activity (e.g., unsaturated fat [8] or whey proteins) prevails over the activity of nutrients with proinflammatory activity (e.g., saturated fat) [49,50]. A stronger support for this conclusion will come from the comparison of the postprandial kinetics of expression of inflammatory genes after the ingestion of a diet known to promote the postprandial release of inflammatory markers, for example, a high-fat meal [51].

4.2.4. Inflammation and apoptosis

Inflammation and apoptosis are highly regulated processes that play an important role in the maintenance of tissue homeostasis. These processes are coordinately activated in response to cellular stress [52–56]. For example, NF-kB participates in the activation of

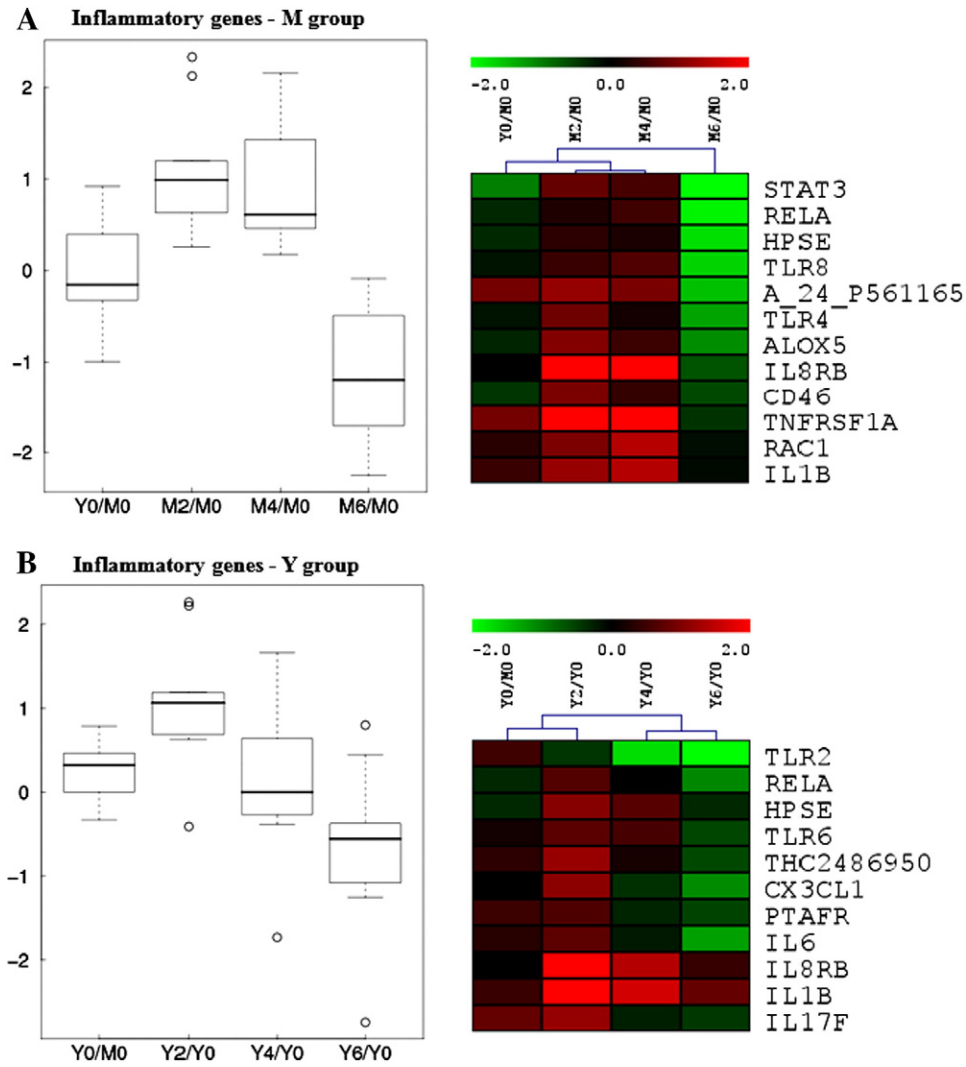


Fig. 7. Box plot and hierarchical clustering of the inflammatory genes in the M and Y groups. Analysis of the SNOMAD *z* score ratio for the time-point comparisons of the 12 inflammatory genes in the M group (M2/M0, M4/M0, M6/M0) and of the 11 inflammatory genes in the Y group (Y2/Y0, Y4/Y0, Y6/Y0). Y0/M0 represents the *z* score ratio of two 0-h times from the crossover design. Left panel: a box plot showing the distribution of the *z* score ratio for each time-point comparison. Right panel: hierarchical clustering of the time-point comparisons using Euclidean distance measure. The red color indicates up-regulation of gene expression relative to 0 h. The green color indicates down-regulation of gene expression relative to 0 h. The dendrogram illustrates that 2 and 6 h are the two farthest clusters.

inflammation and apoptosis in the presence of stress factors, including reactive oxygen species (ROS).

The ingestion of lipids and proteins as well as of a mixed meal after an overnight fast induces the generation of ROS [57,58]. On the other hand, dietary calcium and dairy products possess antioxidant properties that suppress ROS generation in humans [48,59] and that may modulate apoptotic processes [60]. In line with a coordinated modulation of genes involved in inflammatory, apoptotic and oxidative processes, we also observed an increased expression at 6 h of the genes coding for the antioxidant proteins thioredoxin 2 (M: $P \leq .002$; Y: $P \leq .02$) and superoxide dismutase 1 (M: $P \leq .03$; Y: $P \leq .0003$), an enzyme involved in the suppression of both inflammation and apoptosis [61]. In that context, the extent to which antioxidants in dairy products contribute to the down-regulating phase of the upDOWN kinetics of apoptotic and inflammatory genes remains to be established.

4.3. Specificity of the postprandial transcriptomic profile

The results presented in Section 3.5 identify a statistically relevant correlation between the identity of proteins present in bovine milk and the corresponding homologous genes differentially expressed in humans after the ingestion of milk. The most straightforward and provocative interpretation for the above results is a negative feedback postprandial regulation of human blood cell genes by bioavailable bovine milk proteins or digested peptides derived from these proteins. Keeping in mind the sequence homology between the human and bovine genomes, our findings lead us to propose that the bioactivity of dairy products may cross the barriers of species (*Homo sapiens* — *Bos taurus*) and life cycles (suckling offspring — adult consumer) to deliver nutritional properties that extend beyond their macronutrient composition.

The presence of membrane-bound and soluble forms of TLR2 and TLR4 is well documented in bovine and human milk [43,62,63]. Also, soluble TLR2 in human breast milk modulates the cellular response to bacterial components by regulating the activity of the TLR coreceptor CD14 [63]. As TLR2 and TLR4 are postprandially down-regulated at 6 h in our study (see Fig. 8), these proteins are interesting candidates as a source of bioactive molecules in bovine milk that may retain biological activity after digestion in humans.

Our results show that a similar postprandial response is measured after the ingestion of milk and yogurt by healthy individuals, an

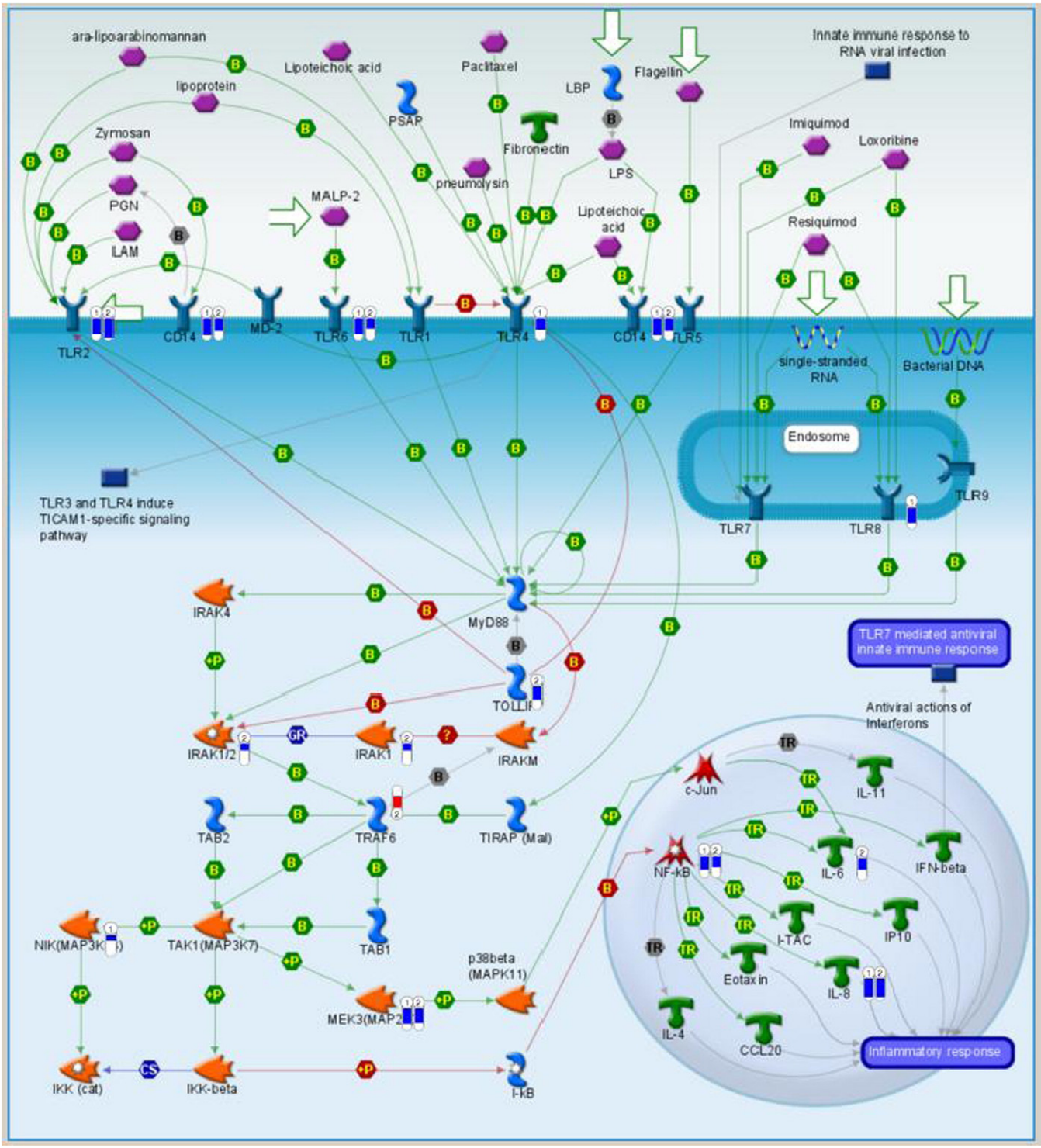


Fig. 8. Genes in TLR signaling pathway that are differentially expressed after the ingestion of milk and yogurt. All genes indicated with the thermometer symbol represent genes that are significantly expressed with linear contrasts, with *P* values < .05 in milk and yogurt data set. Black arrows on the gene name indicate that the gene is differentially expressed in the M and Y groups (FDR < 12.5%). The thermometers labeled "1" indicate genes in milk data set. The thermometers labeled "2" indicate the yogurt data set. A thermometer with the designated number on the top and blue color indicates genes with a negative linear contrast; a thermometer with the designated number on the bottom and red color indicates genes with a positive linear contrast. The magnitude of expression is indicated by the height of the colored column in each thermometer. Abbreviations for the differentially expressed genes: CD14, CD14 antigen; IL6, interleukin 6; IL8, interleukin 8; IRAK1, interleukin-1 receptor-associated kinase 1; IRAK1/2, interleukin-1 receptor-associated kinase 1; MEK3, mitogen-activated protein kinase kinase 3; NF-κB, nuclear factor of kappa light polypeptide gene enhancer in B cells; NIK, NF-kappa beta-inducing kinase; TLRs, toll-like receptors; TOLLIP, toll-interacting protein; and TRAF6, TNF receptor-associated factor 6.

expected result given the fact that the two food products are based on the same raw material. Despite overall similarities between the M and Y groups, the Y group showed a few specific differences in comparison to the M group. One striking difference, however, was a significant down-regulation of the ACE2 pathway in the Y group. The identification of this pathway in the Y group suggests a specificity

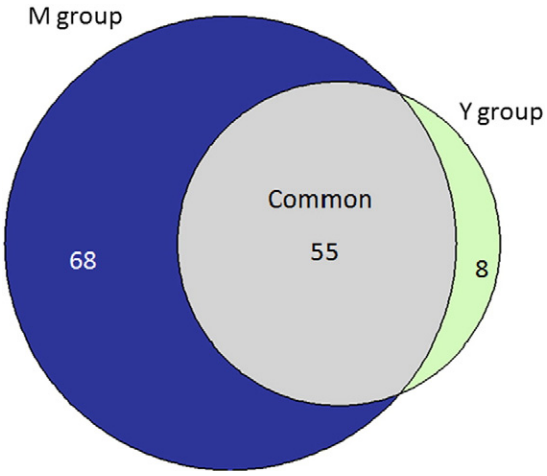


Fig. 9. Venn diagram showing common and differently regulated pathways in the Y group in comparison to the M group. Of 63 pathways in the Y group, 55 were shared with the M group and only 8 were unique.

in the response of the blood cells transcriptome to the ingestion of dairy products as the fermentation of milk by lactic acid bacteria releases peptides from casein that inhibit the rennin–angiotensin system and may, thus, reduce blood pressure in humans [64–67].

Our report provides preliminary information suggesting a specific response of the human blood cell transcriptome to specific nutrients in food. Further evidence in support of this conclusion will come from the comparison of the postprandial transcriptomes resulting from the ingestion of a larger spectrum of foods, including various dairy products and other food types.

4.4. Conclusions

Dairy products appear to have a beneficial role in the dietary management of chronic metabolic and inflammatory diseases [68,69]. Recent interventional [47,48] and epidemiological [70] studies showed that the consumption of dairy products is associated with lower levels of fasting systemic inflammatory markers in human adults. These observations raise the exciting possibility that postprandial blood cell transcriptomic data may be indicative of changes in clinical biomarkers that are otherwise only expressed and detected after long-term dietary interventions. Establishing a causality between the postprandial modulation of the inflammatory genes observed in our work, the long-term intervention studies and the epidemiological data is challenging. As such, further efforts on the development of postprandial blood cell transcriptomics as a key tool in human nutrition research are granted.

The biological processes identified in our study point to the concept of postprandial stress, a normal response of the organism to the ingestion of food that expresses itself in the activation of metabolic, inflammatory and oxidative pathways [71]. Lipids, glucose and insulin [72,73] are classical examples of postprandial blood parameters that are widely used in the evaluation of the long-term risk associated with the development of chronic diseases. However, the limitation of using limited sets of parameters is dramatically illustrated by the increasing evidence available on the detrimental contribution of fructose to the development of obesity, with the low glycemic index of this carbohydrate having played a key role in the promotion of its consumption worldwide [74]. In this context, postprandial blood cell transcriptomics has a clear potential for the identification of physiologically meaningful sets of biomarkers that will complement the already established clinical chemistry of lipid and carbohydrate metabolism to holistically and sensitively measure the impact of food ingestion on the plasticity of human metabolism.

Supplementary materials related to this article can be found online at http://dx.doi.org/10.1016/j.jnutbio.2012.01.001.

Acknowledgments

We thank all the volunteers and nurses who took part in the study; Prof. N. Shah (Melbourne, Australia), Prof. I. Johnson (Norwich, UK), Prof. T. Walczyk (Singapore city, Singapore), Prof. M. Zimmermann (Zurich, Switzerland) and Dr. R. Elliott (Norwich, UK) for helpful discussions on the study design; Dr. S. Storcksdieck genannt Bonsmann, Dr. I. Aeberli, Dr. S. Good and Dr. S. Mallia for their assistance during the trial; Dr. D. Thommen and his team (Berne, Switzerland) for technical advice on blood sampling; W. Strahm and C. Hegel (Berne, Switzerland) for the preparation of milk and yogurt; Dr. B. Walther, Dr. R. Sieber, A. Schmid, D. Gille and M. Chollet at ALP (Berne, Switzerland) for commenting on the manuscript; A. Patrignani (Functional Genomics Center Zurich, Zurich, Switzerland) for his assistance on the microarray experiment; and Dr. W. Luginbühl (ChemStat, Berne, Switzerland) and Dr. F. Schütz (SIB, Lausanne, Switzerland) for statistical analyses and consultation.

References

[1] Lopez-Exposito I, Pellegrini A, Amigo L, Recio I. Synergistic effect between different milk-derived peptides and proteins. J Dairy Sci 2008;91(6):2184–9.
[2] Ebringer L, Ferencik M, Krajcovic J. Beneficial health effects of milk and fermented dairy products — review. Folia Microbiol (Praha) 2008;53(5):378–94.
[3] Papenburg R, Bounous G, Fleiszer D, Gold P. Dietary milk proteins inhibit the development of dimethylhydrazine-induced malignancy. Tumour Biol 1990;11(3):129–36.
[4] Cross ML, Stevenson LM, Gill HS. Anti-allergy properties of fermented foods: an important immunoregulatory mechanism of lactic acid bacteria? Int Immunopharmacol 2001;1(5):891–901.
[5] Jauhiainen T, Vapaatalo H, Poussa T, et al. Lactobacillus helveticus fermented milk lowers blood pressure in hypertensive subjects in 24-h ambulatory blood pressure measurement. Am J Hypertens 2005;18(12 Pt 1):1600–5.
[6] van Hooijdonk AC, Kussendrager KD, Steijns JM. In vivo antimicrobial and antiviral activity of components in bovine milk and colostrum involved in non-specific defence. Br J Nutr 2000;84(Suppl 1):S127–34.
[7] Zemel MB. The role of dairy foods in weight management. J Am Coll Nutr 2005;24(6 Suppl):537S–46S.
[8] Bouwens M, van de RO, Dellschaft N, et al. Fish-oil supplementation induces antiinflammatory gene expression profiles in human blood mononuclear cells. Am J Clin Nutr 2009;90(2):415–24.
[9] Bouwens M, Afman LA, Muller M. Fasting induces changes in peripheral blood mononuclear cell gene expression profiles related to increases in fatty acid beta-oxidation: functional role of peroxisome proliferator activated receptor alpha in human peripheral blood mononuclear cells. Am J Clin Nutr 2007;86(5):1515–23.
[10] van Erk MJ, Blom WA, van Ommen B, Hendriks HF. High-protein and high-carbohydrate breakfasts differentially change the transcriptome of human blood cells. Am J Clin Nutr 2006;84(5):1233–41.
[11] van Erk MJ, Pasman WJ, Wortelboer HM, van Ommen B, Hendriks HF. Short-term fatty acid intervention elicits differential gene expression responses in adipose tissue from lean and overweight men. Genes Nutr 2008;3(3-4):127–37.
[12] Banon S, Hardy J. A colloidal approach of milk acidification by glucono-delta-lactone. J Dairy Sci 1992;75:935–41.
[13] Lee WJ, Lucey JA. Structure and physical properties of yogurt gels: effect of inoculation rate and incubation temperature. J Dairy Sci 2004;87(10):3153–64.
[14] Thach DC, Lin B, Walter E, et al. Assessment of two methods for handling blood in collection tubes with RNA stabilizing agent for surveillance of gene expression profiles with high density microarrays. J Immunol Methods 2003;283(1–2):269–79.
[15] Debey S, Zander T, Brors B, et al. A highly standardized, robust, and cost-effective method for genome-wide transcriptome analysis of peripheral blood applicable to large-scale clinical trials. Genomics 2006;87(5):653–64.
[16] Hartel C, Bein G, Muller-Steinhardt M, Kluter H. Ex vivo induction of cytokine mRNA expression in human blood samples. J Immunol Methods 2001;249(1–2):63–71.
[17] Colantuoni C, Henry G, Zeger S, Pevsner J. SNOMAD (Standardization and NOrmalization of MicroArray Data): Web-accessible gene expression data analysis. Bioinformatics 2002;18(11):1540–1.
[18] Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple rating testing. J R Stat Soc Ser B 1995;57:289–300.

[19] Dahlquist KD, Salomonis N, Vranizan K, Lawlor SC, Conklin BR. GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. Nat Genet 2002;31(1):19–20.
[20] Doniger SW, Salomonis N, Dahlquist KD, et al. MAPPFinder: using Gene Ontology and GenMAPP to create a global gene-expression profile from microarray data. Genome Biol 2003;4(1):R7.
[21] Boehmer JL, Bannerman DD, Shefcheck K, Ward JL. Proteomic analysis of differentially expressed proteins in bovine milk during experimentally induced Escherichia coli mastitis. J Dairy Sci 2008;91(11):4206–18.
[22] Gagnaire V, Jardin J, Jan G, Lortal S. Invited review: proteomics of milk and bacteria used in fermented dairy products: from qualitative to quantitative advances. J Dairy Sci 2009;92(3):811–25.
[23] Becker KG, Hosack DA, Dennis Jr G, et al. PubMatrix: a tool for multiplex literature mining. BMC Bioinformatics 2003;4:61.
[24] Bernt KM, Walker WA. Human milk as a carrier of biochemical messages. Acta Paediatr Suppl 1999;88(430):27–41.
[25] Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA 2005;102(43):15545–50.
[26] Morey JS, Ryan JC, Van Dolah FM. Microarray validation: factors influencing correlation between oligonucleotide microarrays and real-time PCR. Biol Proced Online 2006;8:175–93.
[27] Rockett JC, Hellmann GM. Confirming microarray data — is it really necessary? Genomics 2004;83(4):541–9.
[28] Dangin M, Boirie Y, Guillet C, Beaufrere B. Influence of the protein digestion rate on protein turnover in young and elderly subjects. J Nutr 2002;132(10):3228S–33S.
[29] Garlick PJ. The role of leucine in the regulation of protein metabolism. J Nutr 2005;135(6 Suppl):1553S–6S.
[30] Haug A, Hostmark AT, Harstad OM. Bovine milk in human nutrition — a review. Lipids Health Dis 2007;6:25.
[31] Norton LE, Layman DK. Leucine regulates translation initiation of protein synthesis in skeletal muscle after exercise. J Nutr 2006;136(2):533S–7S.
[32] Proud CG. Regulation of mammalian translation factors by nutrients. Eur J Biochem 2002;269(22):5338–49.
[33] Phillips SM, Tang JE, Moore DR. The role of milk- and soy-based protein in support of muscle protein synthesis and muscle protein accretion in young and elderly persons. J Am Coll Nutr 2009;28(4):343–54.
[34] Ha E, Zemel MB. Functional properties of whey, whey components, and essential amino acids: mechanisms underlying health benefits for active people (review). J Nutr Biochem 2003;14(5):251–8.
[35] Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. Am J Physiol Endocrinol Metab 2006;291(2):E381–7.
[36] Briet F, Twomey C, Jeejeebhoy KN. Effect of feeding malnourished patients for 1 mo on mitochondrial complex I activity and nutritional assessment measurements. Am J Clin Nutr 2004;79(5):787–94.
[37] Marriage B, Clandinin MT, Glerum DM. Nutritional cofactor treatment in mitochondrial disorders. J Am Diet Assoc 2003;103(8):1029–38.
[38] Territo PR, Mootha VK, French SA, Balaban RS. Ca(2+) activation of heart mitochondrial oxidative phosphorylation: role of the F(0)/F(1)-ATPase. Am J Physiol Cell Physiol 2000;278(2):C423–35.
[39] Bruckbauer A, Gouffon J, Rekapalli B, Zemel MB. The effects of dairy components on energy partitioning and metabolic risk in mice: a microarray study. J Nutrigenet Nutrigenomics 2009;2(2):64–77.
[40] Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001;2(8):675–80.
[41] Wolowczuk I, Verwaerde C, Viltart O, et al. Feeding our immune system: impact on metabolism. Clin Dev Immunol 2008:639803.
[42] Ghanim H, Sia CL, Upadhyay M, et al. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and toll-like receptor expression. Am J Clin Nutr 2010;91(4):940–9.
[43] LeBouder E, Rey-Nores JE, Raby AC, et al. Modulation of neonatal microbial recognition: TLR-mediated innate immune responses are specifically and differentially modulated by human milk. J Immunol 2006;176(6):3742–52.
[44] Loscher CE, Draper E, Leavy O, et al. Conjugated linoleic acid suppresses NF-kappa B activation and IL-12 production in dendritic cells through ERK-mediated IL-10 induction. J Immunol 2005;175(8):4990–8.
[45] Donnet-Hughes A, Duc N, Serrant P, Vidal K, Schiffrin EJ. Bioactive molecules in milk and their role in health and disease: the role of transforming growth factor-beta. Immunol Cell Biol 2000;78(1):74–9.
[46] Doring F, Will J, Amasheh S, et al. Minimal molecular determinants of substrates for recognition by the intestinal peptide transporter. J Biol Chem 1998;273(36):23211–8.
[47] Hunter DC, Brown R, Green T, et al. Changes in markers of inflammation, antioxidant capacity and oxidative stress in smokers following consumption of milk, and milk supplemented with fruit and vegetable extracts and vitamin C. Int J Food Sci Nutr 2011.
[48] Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. Am J Clin Nutr 2010;91(1):16–22.
[49] Weatherill AR, Lee JY, Zhao L, et al. Saturated and polyunsaturated fatty acids reciprocally modulate dendritic cell functions mediated through TLR4. J Immunol 2005;174(9):5390–7.
[50] Wong SW, Kwon MJ, Choi AM, et al. Fatty acids modulate toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner. J Biol Chem 2009;284(40):27384–92.
[51] Nappo F, Esposito K, Cioffi M, et al. Postprandial endothelial activation in healthy subjects and in type 2 diabetic patients: role of fat and carbohydrate meals. J Am Coll Cardiol 2002;39(7):1145–50.
[52] Fadeel B, Orrenius S. Apoptosis: a basic biological phenomenon with wide-ranging implications in human disease. J Intern Med 2005;258(6):479–517.
[53] Ren G, Su J, Zhao X, et al. Apoptotic cells induce immunosuppression through dendritic cells: critical roles of IFN-gamma and nitric oxide. J Immunol 2008;181(5):3277–84.
[54] Rossi AG, Hallett JM, Sawatzky DA, Teixeira MM, Haslett C. Modulation of granulocyte apoptosis can influence the resolution of inflammation. Biochem Soc Trans 2007;35(Pt 2):288–91.
[55] Savill J. Apoptosis in resolution of inflammation. Kidney Blood Press Res 2000;23(3-5):173–4.
[56] Voll RE, Herrmann M, Roth EA, et al. Immunosuppressive effects of apoptotic cells. Nature 1997;390(6658):350–1.
[57] Aljada A, Mohanty P, Ghanim H, et al. Increase in intranuclear nuclear factor kappaB and decrease in inhibitor kappaB in mononuclear cells after a mixed meal: evidence for a proinflammatory effect. Am J Clin Nutr 2004;79(4):682–90.
[58] Mohanty P, Ghanim H, Hamouda W, et al. Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. Am J Clin Nutr 2002;75(4):767–72.
[59] Zemel MB, Sun X. Dietary calcium and dairy products modulate oxidative and inflammatory stress in mice and humans. J Nutr 2008;138(6):1047–52.
[60] Spagnuolo PA, Bird RP, Hoffman-Goetz L. Effect of short-term dietary intake of bovine lactoferrin on intestinal lymphocyte apoptosis in healthy mice. Nutrition 2007;23(11-12):812–7.
[61] Yasui K, Kobayashi N, Yamazaki T, et al. Superoxide dismutase (SOD) as a potential inhibitory mediator of inflammation via neutrophil apoptosis. Free Radic Res 2005;39(7):755–62.
[62] Reinhardt TA, Lippolis JD. Bovine milk fat globule membrane proteome. J Dairy Res 2006;73(4):406–16.
[63] LeBouder E, Rey-Nores JE, Rushmere NK, et al. Soluble forms of toll-like receptor (TLR)2 capable of modulating TLR2 signaling are present in human plasma and breast milk. J Immunol 2003;171(12):6680–9.
[64] Beierwaltes WH. The role of calcium in the regulation of renin secretion. Am J Physiol Renal Physiol 2010;298(1):F1–F11.
[65] Crackower MA, Sarao R, Oudit GY, et al. Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002;417(6891):822–8.
[66] FitzGerald RJ, Meisel H. Milk protein-derived peptide inhibitors of angiotensin-I-converting enzyme. Br J Nutr 2000;84(Suppl 1):S33–7.
[67] Hayes M, Stanton C, Slattery H, et al. Casein fermentate of *Lactobacillus animalis* DPC6134 contains a range of novel propeptide angiotensin-converting enzyme inhibitors. Appl Environ Microbiol 2007;73(14):4658–67.
[68] Fumeron F, Lamri A, Abi KC, et al. Dairy consumption and the incidence of hyperglycemia and the metabolic syndrome: results from a french prospective study. Data from the Epidemiological Study on the Insulin Resistance Syndrome (DESIR). Diabetes Care 2011;34(4):813–7.
[69] Pereira MA, Jacobs Jr DR, Van Horn L, et al. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. JAMA 2002;287(16):2081–9.
[70] Panagiotakos DB, Pitsavos CH, Zampelas AD, Chrysohoou CA, Stefanadis CI. Dairy products consumption is associated with decreased levels of inflammatory markers related to cardiovascular disease in apparently healthy adults: the ATTICA study. J Am Coll Nutr 2010;29(4):357–64.
[71] Margioris AN. Fatty acids and postprandial inflammation. Curr Opin Clin Nutr Metab Care 2009;12(2):129–37.
[72] Su JW, Nzekwu MM, Cabezas MC, Redgrave T, Proctor SD. Methods to assess impaired post-prandial metabolism and the impact for early detection of cardiovascular disease risk. Eur J Clin Invest 2009;39(9):741–54.
[73] Brand-Miller J, McMillan-Price J, Steinbeck K, Caterson I. Dietary glycemic index: health implications. J Am Coll Nutr 2009;28(Suppl):446S–9S.
[74] Tappy L, Le KA. Metabolic effects of fructose and the worldwide increase in obesity. Physiol Rev 2010;90(1):23–46.
[75] Souci S, Fachmann W, Kraut H. Food composition and nutrition tables. 6th ed. Stuttgart: Medpharm Scientific Publications, CRC Press; 2000.