Peripheral blood mononuclear cells: a potential source of homeostatic imbalance markers associated with obesity development

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Abstract Peripheral blood mononuclear cells (PBMC) have a great potential for nutrition and obesity studies. PBMC reflect the nutritional response of key organs involved in energy homeostasis maintenance, which is altered in the obese state. Here, we aimed to determine the usefulness of PBMC as a source of early markers of obesity. To that purpose, we analysed whether PBMC could reflect the insensitivity to changes in feeding conditions associated with obesity during the development of this pathology. Expression of key genes central to energy metabolism was measured by Q-PCR in PBMC samples of normoweight (control) and cafeteria-fed (obese) rats in feeding, fasting and refeeding conditions. Samples were obtained monthly from 2 (beginning of cafeteria diet-feeding) to 6 months of age. In general terms, expression of genes related to fatty acid synthesis (Fasn, Srebp1) and adipogenesis (Pparg) decreased with fasting and increased with refeeding. Conversely, the expression of a key gene regulating beta-oxidation (Cpt1a) and the gene for an orexigenic neuropeptide (Npy)—in accordance with their metabolic role—increased with fasting and decreased with refeeding. This expression pattern disappeared in obese rats, in which insensitivity to feeding conditions was observed after only 1 month of cafeteria diet-feeding. Thus, during development, PBMC accurately reflect nutritional regulation of energy homeostatic genes and the insensitivity to feeding associated with obesity, even in the earlier stages with a low degree of overweight. For this reason, this set of blood cells could constitute a potential source of biomarkers of early homeostatic imbalance which would be useful in nutrition studies that could help prevent the occurrence of obesity.

Keywords Blood cells · Obesity · Nutrition · Energy homeostasis · Biomarkers

Introduction

In response to changes in food intake, adaptations in energy expenditure and fat storage occur to help maintain energy homeostasis and body weight [21, 31]. However, in the obese state, mechanisms involved in homeostatic control are impaired and insensitivity in the response to feeding conditions is observed [9, 10, 33]. Therefore, proper maintenance of energy homeostasis is of extreme importance for survival, and its deregulation can be related to the appearance of obesity [31]. Due to the multiple health problems related to increased body weight, it would be highly relevant to be able to detect early markers of disturbances in energy homeostasis which could help prevent obesity. In fact, there is increasing interest in the identification of early determinants of adult obesity [6]. Here, our objective was to determine the usefulness of a set of blood cells, peripheral blood mononuclear cells (PBMC), as a source to detect early biomarkers of obesity based on their ability to reflect alterations in energy metabolism related to this pathology [8, 11].

PBMC, which include lymphocytes and monocytes, constitute interesting biological material: they travel through the
body reflecting gene expression changes that occur in different tissues in response to internal or external stimuli and can be collected easily and repeatedly with minimum invasion in comparison to sampling of other tissues [22]. The use of PBMC for the development of diagnostic tools is growing [4, 6, 7, 13, 24, 30, 42], and they have also been shown to be useful for nutritional studies, reflecting specific effects of diets or nutrients [1, 5, 12, 27, 34, 40], response to fasting [3, 8, 11] and weight loss due to hypocaloric diets [14–16]. Moreover, there is a clear association between changes in PBMC gene expression and obesity [2, 12, 16, 18, 19, 28, 29, 32, 36, 39], and we have previously demonstrated that PBMC can reflect the energy homeostasis adaptations to acute changes in feeding conditions (fasting and refeeding) which occur in key tissues such as liver or adipose tissue, and that this response is altered in obese animals [8, 11].

In this study, we used PBMC to identify potential biomarkers related to energy homeostasis whose nutritional regulation could be altered during obesity development. To that purpose, we analysed alterations in the response of key energy metabolism-related genes to different feeding conditions (fasting and refeeding) in PBMC of rats fed a cafeteria diet as body weight increased. Specifically, we analysed the expression of genes (mainly involved in lipid metabolism homeostasis) which we have previously described to be regulated in response to acute fasting and refeeding in a similar manner as happens in important energy homeostatic tissues [8]. We selected two genes related to fatty acid synthesis, fatty acid synthase (Fasn) and sterol regulatory element-binding protein 1 (Srebp1), a gene involved in adipogenesis and lipogenesis, peroxisome proliferator activated receptor gamma (Pparg), and a key gene regulating beta-oxidation, carnitine palmitoyltransferase 1a (Cpt1a). We also studied a gene involved in food intake regulation, neuropeptide Y (Npy), coding for an orexigenic signal. Characterization of impairment in the apparent energy homeostatic control in PBMC, as reflected by the insensitivity to nutritional changes in the energy-related genes studied, could be used to intervene in obesity-prone subjects in an early pre-obese state and thereby prevent the development of obesity.

Material and methods

Animals, diets and experimental design

The animal protocol followed in this study was reviewed and approved by the Bioethical Committee of the University of the Balearic Islands, and University guidelines for the use and care of laboratory animals were followed. Two-month-old male Wistar rats (Charles River Laboratories España, SA, Barcelona, Spain) were divided into two dietary groups for 4 months: control group (n=15) fed with a standard chow diet (Panlab, Barcelona, Spain) and cafeteria group (n=15) fed with a cafeteria diet in addition to the standard chow. Cafeteria diet is a fat-rich hypercaloric diet containing the following foodstuffs: cookies with liver pate and sobrassada (a typical Majorcan sausage), candies, fresh bacon, biscuits, chocolate, salted peanuts, cheese, milk containing 20 % (w/v) sucrose and ensaimada (a typical Majorcan pastry). The cafeteria diet is a well-established model to study obesity; it is a highly palatable hyperlipidic diet that mimics Western diet, inducing voluntary hyperphagia and rapid weight gain in rodents [12, 37]. The gross composition of the diets was as follows: for the standard chow diet, 60.5 % carbohydrate, 2.9 % lipid, 15.4 % protein, 12 % water, 5.3 % minerals and 3.9 % fibre, and for the cafeteria diet, 35.2 % carbohydrate, 23.4 % lipid, 11.7 % protein, 28.4 % water and 1.31 % fibre. The animals were single housed at 22 °C with 12 h periods of light or dark.

Each group of rats was divided into three subgroups submitted to different feeding conditions (n=5 for each condition): feeding (animals provided with ad libitum access to diet), fasting (animals deprived of food for 14 h) and refeeding (fasted animals with subsequent free access to diet for 6 h). Coprophagy was prevented by changing the cage immediately prior to food deprivation. Water was offered ad libitum and body weight was followed twice a week.

Prior to administering the cafeteria diet (age 2 months) and thereafter every month until rats were 6 months old, blood samples (1.5–2.5 ml) were collected in the different feeding conditions (feeding, fasting and refeeding) from the saphena vein, using heparin in NaCl (0.9 %) as anticoagulant. Immediately after blood collection, PBMC were isolated by Ficoll gradient separation, according to the instructions indicated by the manufacturer (GE Healthcare Bio Sciences, Barcelona, Spain), with some modifications [12].

At the age of 6 months, rats were killed in the experimental feeding conditions studied (feeding, fasting and refeeding), and different white adipose tissue depots—epididymal, inguinal, mesenteric and retroperitoneal—were removed and weighed to determine the adiposity index, and then immediately frozen in liquid nitrogen and stored at −70 °C for analysis. Liver samples were also harvested and stored at −70 °C for gene expression studies. However, liver mRNA was degraded and, for that reason, the liver gene expression data presented in this work come from another set of animals submitted to the same experimental protocol (control and cafeteria group) and which were sacrificed under two different feeding conditions: ad libitum feeding and 14-h fasting (n=6 animals per condition); refeeding conditions were not studied in this case. Truncal blood was collected from the neck, stored at room temperature for 1 h and overnight.
at 4 °C, and was then centrifuged at 1,000×g for 10 min to collect the serum.

Adiposity index

Adiposity was determined as an adiposity index computed for each rat as the sum of epididymal, inguinal, mesenteric and retroperitoneal white adipose tissue depot weight and expressed as a percentage of total body weight.

Quantification of circulating insulin, leptin and glucose levels

Serum insulin and leptin levels were measured using enzyme-linked immunosorbent assay kits (from DRG Instruments, Marburg, Germany, and R&D Systems, Minneapolis, MN, USA), and blood glucose using an Accu-Chek Glucometer (Roche Diagnostics, Barcelona, Spain).

Homeostatic model assessment for insulin resistance analysis

Insulin resistance was assessed by the homeostatic model assessment for insulin resistance (HOMA-IR) in rats submitted to overnight (14 h) fasting (n=5 for all groups). HOMAR-IR score was calculated from fasting insulin and glucose concentrations using the formula of [25]. HOMA-IR=fasting glucose (millimoles per liter)×fasting insulin (milliunits per liter).

Total RNA isolation

Total RNA from PBMC samples was extracted using TriPure reagent (Roche Diagnostics Barcelona, Spain) and purified with Qiaqen RNeasy Mini Kit spin columns (Izasa, Barcelona, Spain). RNA yield was quantified on a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Total RNA from retroperitoneal adipose tissue was extracted using TriPure reagent, and RNA from liver samples was extracted using Eazzy Nucleic Acid Isolation Kit E.Z.N.A.ATM (Omega Bi-Tek, Vermont, USA). RNA integrity was confirmed using agarose gel electrophoresis.

Real-time quantitative reverse transcriptase PCR analysis

Quantitative PCR (Q-PCR) was performed to determine mRNA expression levels of key energy metabolism genes: fatty acid synthase (Fasn), sterol regulatory element-binding protein 1 (Srebp1), peroxisome proliferator activated receptor gamma (Pparg), carnitine palmitoyltransferase 1a (Cpt1a) and neuropeptide Y (Npy), in PBMC from blood collected in control and cafeteria-fed Wistar rats at different ages (from 2 to 6 months) and in the different feeding conditions studied (feeding, fasting and refeeding). In addition, at the age of 6 months, mRNA expression of these genes (except for Npy) was also measured in samples of retroperitoneal adipose tissue in feeding, fasting and refeeding conditions and in liver samples of fed and fasted animals. For adipose tissue and liver, we only analysed gene expression at the end of the experimental period (6 months of age) because doing it at different times would require the use of a high number of animals.

An amount of 0.25 μg of total RNA was denatured at 90 °C for 1 min and then reverse transcribed to cDNA using MuLV reverse transcriptase (Applied Biosystems, Madrid, Spain) at 42 °C for 1 h, with a final step of 5 min at 99 °C in an Applied Biosystems 2720 Thermal Cycler (Applied Biosystems, Madrid, Spain). Each PCR was performed from diluted (1/5 for PBMC, 1/20 for retroperitoneal adipose tissue and liver) cDNA template, forward and reverse primers (10 μM each) and Power SYBER Green PCR Master Mix (Applied Biosystems, Madrid, Spain) in a total volume of 11 μl, with the following profile: 10 min at 95 °C, followed by a total of 40 temperature cycles (15 s at 95 °C and 1 min at 60 °C) with a final cycle of 15 s at 95 °C, 1 min at 60 °C and 15 s at 95 °C. In order to verify the purity of the products, a melting curve was produced after each run according to the manufacturer’s instructions (Applied Biosystems, Madrid, Spain). The threshold cycle (Ct) was calculated by the instrument’s software (StepOne Software v2.0, from Applied Biosystems), and the relative expression of each mRNA was calculated as a percentage of control rats, using the 2^[-ΔΔCt] method [23]. Data were normalized against the housekeeping gene guanosine diphosphate dissociation inhibitor 1 (Gdi1) which we have previously identified as a good constitutive gene based on microarray studies [8]. Gdi1 was used as a reference gene for all the samples except for PBMC from 2-month-old rats, a time point at which β-actin was a better constitutive gene. Primers for the different genes are described in Table 1, and all were obtained from Sigma Genosys (Sigma Aldrich Quimica SA, Madrid, Spain).

Statistical analysis

All data are expressed as the mean±SEM. Differences between groups were analysed using two- or one-way ANOVA or Student's t test. LSD post hoc test was used after ANOVA analysis. The specific statistical analysis used for each comparison is specified in the footnotes of the Tables and Figures. Analyses were performed with SPSS® for windows (SPSS, Chicago, IL). Threshold of significance was defined at p<0.05 and is indicated when different.
Results

Body weight, adiposity and circulating parameters

Data of body weight, adiposity and circulating parameters have been previously described in the same cohort of animals [8]. Briefly, a progressive increase in body weight was observed in the cafeteria-fed animals: 13, 20 and 26 % increase in weight compared to controls after 1, 2 and 3 months of cafeteria diet feeding. At the end of the experiment (4 months of diet), cafeteria-fed animals attained 29 % increased body weight (645±20 vs. 500±15 g in cafeteria-obese vs. control rats) and a higher adiposity index compared with animals of the control group (17.5±0.9 vs. 7.95±0.86 %). These animals were hyperleptinemic (38.0±4.9 vs. 10.7±1.8 pg/μl) and had greater blood glucose levels in the fed state (6.41±0.39 vs. 5.18±0.22 mM), although circulating insulin levels were not affected (3.44±0.98 vs. 3.23±0.46 μg/l). HOMA-IR score was increased in cafeteria-obese rats suggesting reduced insulin sensitivity, although the increase was non-significant due to inter-individual variations in the cafeteria group (11.0±4.9 vs. 5.25±1.52 %). Feeding conditions (feeding, fasting and refeeding) affected circulating insulin and leptin levels, but not glucose levels. As a general tendency, circulating insulin and leptin levels decreased after 14-h fasting and were recovered after refeeding, although the decrease in insulin levels with fasting was only significant in control animals and the recovery of leptin after refeeding in cafeteria-fed animals [8].

The liver samples used for gene expression analysis present in this work were from another set of animals submitted to the same experimental protocol (control and cafeteria group) and sacrificed under two different feeding conditions: ad libitum feeding and 14-h fasting. These animals presented similar characteristics to the previous animals: cafeteria-fed rats attained 32 % body weight at the end of the experiment (677±50 vs. 512±18 g in cafeteria-obese vs. control rats) and a higher adiposity index (18.5±2.0 vs. 8.96±0.95 %), and were hyperleptinemic (46.7±2.9 vs. 16.5±3.0 μg/l) and had greater circulating glucose (6.68±0.10 vs. 6.01±0.18 mM) and insulin levels (5.63±0.18 vs. 3.28±0.36 μg/l) in the fed state. Glucose and insulin levels decreased as a result of 14-h fasting, both in control and cafeteria-fed animals (data not shown).

Nutritional response of key genes involved in energy homeostasis in PBMC during obesity development due to the intake of a cafeteria diet

At the age of 2 months, when animals were fed a standard chow diet, all the studied genes were regulated in response to fasting and refeeding as expected according to their metabolic role: there was a decrease in Fasn and Srebp1 expression with 14-h fasting and an increase with 6-h refeeding after fasting, also observed for Pparγ, while there was an increase with fasting for Cpt1a and Npy (Fig. 1). At the age of 3 months, in control animals, gene expression of the studied genes followed the same pattern as the previous month (although statistical significance was not reached in some cases due to greater variability). However, in cafeteria-diet fed animals, after 1 month of cafeteria-diet feeding (13 % increased body weight), nutritional regulation was

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5′-3′)</th>
<th>Reverse primer (5′-3′)</th>
<th>Amplicon size (bp)</th>
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<td>222</td>
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<tr>
<td>Pparγ</td>
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<tr>
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<tr>
<td>Npy</td>
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</tr>
<tr>
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<td>GCATCTGAAACGCTACAA</td>
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<tr>
<td>β-actin</td>
<td>TACAGCTTCCACCACACGC</td>
<td>TCTCCAGGGAGGAAGGAT</td>
<td>164</td>
</tr>
</tbody>
</table>

**Table 1** Nucleotide sequences of primers and cycling conditions used for Q-PCR amplification in PBMC

**Fig. 1** Expression of genes involved in energy homeostasis (Fasn, Srebp1, Pparγ, Cpt1a and Npy) in PBMC of control and obese Wistar rats at different ages submitted to ad libitum feeding (fed), 14-h fasting (fasted) and 6-h refeeding after fasting (refed), measured by Q-PCR. Rats were fed a standard chow diet (control) or a cafeteria diet (obese) from 2 to 6 months of age. At 3, 4, 5 and 6 months of age, the animals had 13, 20 and 29 % increased body weight (overweight), respectively, compared to controls. Results represent mean±SEM (n=5) of ratios of specific mRNA levels relative to Gdi or β-actin, expressed as a percentage of the value of fed animals in the control group that was set to 100 %. A one-way ANOVA taking into account control and obese animals independently was performed. Within control and obese animals, data not sharing a common letter (a, b, c) are significantly different (p<0.05 and indicated when different). LSD post hoc test was used after ANOVA analysis. Additionally, a two-way ANOVA (p<0.05) was performed considering all the animals and taking into account feeding conditions (F) and obesity (O). For this statistical analysis, F indicates effect of feeding conditions, O effect of obesity induced by cafeteria-diet feeding, and F×O interaction of feeding conditions and obesity induced by cafeteria-diet feeding.
not observed, indicating insensitivity to feeding conditions at this early stage of overweight. A clear increase in Srebp1 gene expression after refeeding in cafeteria-obese animals that was not observed in control animals is worth noting. The same nutritional behaviour was observed at the ages of 4 and 6 months, when animals had 20 and 29% increased...
body weight, respectively: there was a decrease with fasting and an increase with refeeding in the expression of the genes involved in fatty acid synthesis (Fasn and Srebp1), and an increase with fasting in the expression of the key gene in beta-oxidation, Cpt1a and of the orexigenic gene Npy, a regulation that was lost in the cafeteria-obese animals. At 6 months of age and contrary to what happened in younger rats, Fasn nutritional regulation was not observed in control animals, which could be indicating impairment in response to feeding conditions associated with ageing [9, 35, 43]. No difference was evident in mRNA levels of the studied genes in PBMC when comparing ad libitum fed control and cafeteria-obese animals.

Nutritional response of key genes involved in energy homeostasis in liver and adipose tissue of cafeteria-obese rats

Liver and adipose tissue play a key role in energy homeostasis maintenance with well-known nutritional regulation. We were interested in studying whether the gene expression changes in response to fasting and refeeding observed in PBMC in control and cafeteria-obese animals reflect the nutritional adaptations that take place in liver and adipose tissue. We have previously compared nutritional response between PBMC and mesenteric adipose tissue (reported to be especially sensitive to changes in feeding patterns) in 6-month-old control rats [8]. Here, we decided to select the retroperitoneal adipose tissue. In liver, we only studied response to 14-h fasting (not refeeding) because problems with RNA integrity made it necessary to use samples from another set of animals (see “Material and methods” section).

Nutritional regulatory pattern of the studied genes in PBMC was similar to that observed in liver and adipose tissue (retroperitoneal depot). As expected, in control rats there was a decrease with fasting in gene expression of the two lipogenic genes, Fasn and Srebp1, both in liver and in the retroperitoneal adipose tissue, which was not recovered with 6-h refeeding (studied in adipose tissue). Fasn and Srebp1 nutritional response was lost or impaired in obese animals. In cafeteria-obese rats, no decrease was observed with fasting in liver for Fasn, and the decrease of Srebp1 was impaired (57 % decrease in obese vs. 97 % in control rats), neither was a decrease for Srebp1 observed in adipose tissue (Figs. 2 and 3). On the other hand, fasting produced a remarkable increase of Cpt1a mRNA levels in liver of control animals (260 %) which was not observed in liver of obese rats (Figs. 2 and 3). Cpt1a was not regulated by fasting in the retroperitoneal adipose tissue. In control rats, gene expression of the adipogenic Pparg did not respond to fasting either in liver or in adipose tissue (Figs. 2 and 3). However, in adipose tissue of obese animals, an increase in Pparg expression was observed in the refed condition which was also present in PBMC of the same animals (Figs. 1 and 3).

Gene expression of some of the studied genes was altered in ad libitum fed animals when comparing control and cafeteria groups. In adipose tissue, Fasn and Srebp1 expression was lower, while Cpt1a was higher in cafeteria-fed than in control animals (Fig. 3). In liver, lower mRNA levels for Srebp1 were also observed in cafeteria-obese rats (Fig. 2).

Discussion

There is a large amount of research in recent years focusing on the identification of early makers of obesity development [6]. For studies using rodents as an animal model, the identification of these biomarkers using blood as biological material would simplify a future transference of the obtained knowledge to humans. Here, we studied alterations in nutritional response of genes central to energy homeostasis in a subset of blood cells, PBMC, during a process of dietary-induced obesity development in order to evaluate them as an easily accessible source of markers of early homeostatic imbalance which could be used as part of tools to prevent obesity. Our results indicate that PBMC are highly valuable to detect early changes associated with insensitivity to feeding conditions due to the intake of a high fat diet (cafeteria diet).

Physiologically relevant metabolic conditions such as fasting are reflected by PBMC gene expression profiles [3, 8, 11]. We previously observed by microarray analysis that PBMC obtained from 6-month-old rats express genes involved in energy homeostasis, and their expression is affected by acute changes in feeding conditions in a similar manner to what is expected in tissues involved in homeostatic control [8]. Using the same animals, here we found that the expression in PBMC of selected genes with a key role in energy metabolism (Fasn, Srebp1, Pparg, Cpt1a and Npy) reflects the expected metabolic adaptations of the organism to acute changes in feeding conditions (feeding, fasting and refeeding) at different time points during rat development, from 2 to 6 months of age. As a general trend, at the different ages studied (2, 3, 4 and 6 months) the expression of Fasn, Srebp1 and Pparg in PBMC decreased with fasting and increased with refeeding, in accordance with their role in fatty acid synthesis and adipogenesis. A decrease of Fasn and Srebp1 mRNA levels with fasting was also observed for liver and adipose tissue in 6-month-old animals. Fasn codes for fatty acid synthase, the enzyme directly involved in fatty acid synthesis, and Srebp1 for SREBP1, a critical transcription factor required for the expression of numerous genes involved in fatty acid synthesis, among them fatty acid synthase [20]. Lipid synthesis in liver and adipose tissue is regulated by food consumption, and a
Fig. 2 Expression of genes involved in energy homeostasis (Fasn, Srebp1, Pparg and Cpt1a) in liver of 6-month-old control Wistar rats and Wistar rats made obese by cafeteria-diet feeding submitted to ad libitum feeding (fed) and 14-h fasting (fasted), measured by Q-PCR. Results represent mean±SEM (n=6) of ratios of specific mRNA levels relative to Gdi, expressed as a percentage of the value of fed animals in the control group that was set to 100 %. Asterisk effect of fasting (fasted animals vs. their respective controls; Student’s t test, p<0.05), number sign effect of obesity induced by cafeteria-diet feeding (obese fed animals vs. their respective controls; Student’s t test, p<0.05).

Fig. 3 Expression of genes involved in energy homeostasis (Fasn, Srebp1, Pparg and Cpt1a) in retroperitoneal white adipose tissue of 6-month-old control Wistar rats and Wistar rats made obese by cafeteria-diet feeding submitted to ad libitum feeding (fed), 14-h fasting (fasted) and 6-h refeeding after fasting (refed), measured by Q-PCR. Results represent mean±SEM (n=5) of ratios of specific mRNA levels relative to Gdi, expressed as a percentage of the value of fed animals in the control group that was set to 100 %. A one-way ANOVA taking into account control and obese animals independently was performed. Within control and obese animals, data not sharing a common letter (a, b, c) are significantly different (p<0.05 and indicated when different). LSD post hoc test was used after ANOVA analysis. Number sign indicates the effect of obesity induced by cafeteria-diet feeding (obese fed animals vs. their respective controls; Student’s t test, p<0.05). Additionally, a two-way ANOVA (p<0.05) was performed considering all the animals and taking into account feeding conditions (F) and obesity (O). For this statistical analysis, F indicates effect of feeding conditions and O effect of obesity induced by cafeteria-diet feeding.
decrease in Fasn and Srebp1 expression with fasting, which is reflected in PBMC, may explain, in part, the decreased lipogenesis in a situation of lack of energetic substrates and of insulin, while refeeding reestablishes lipogenic capacity [20]. Notably, Fasn nutritional regulation was not observed in PBMC of the 6-month-old rats, maybe indicating an insensitivity to feeding conditions related to ageing [9, 35, 43]. Regarding Pparg, it codes for a transcription factor, PPARg, which is highly expressed in adipose tissue and regulates adipogenesis by promoting the expression of specific adipocyte markers [38]. Moreover, PPARg also plays an important role in fatty acid uptake and posterior storage into triacylglycerides [38]. Thus, in accordance with its role in adipogenesis and lipogenesis, Pparg expression in adipose tissue is inhibited in fasted animals and is activated with refeeding to allow adipocyte maturation and lipogenesis [41], and this same pattern was observed in PBMC at the different ages studied. Meanwhile, gene expression in PBMC of Cpt1a, with a role in fatty acid beta-oxidation, and of the orexigenic peptide Npy, increased with fasting and decreased with refeeding. Cpt1a codes for carnitine palmitoyl-transferase 1a, a mitochondrial enzyme (liver form) that is involved in the transport of long-chain fatty acids across the inner membrane by binding them to carnitine for subsequent beta-oxidation in the mitochondrial matrix. As expected [44], our results in liver of 6-month-old animals indicate an increase in Cpt1a expression in fasted rats to allow mobilization of fat energy stores, and a decrease with refeeding. The same nutritional profile was evident in PBMC obtained in fasted and refed conditions during development (2, 3, 4 and 6 months). As we previously demonstrated [8], PBMC express the gene coding for the orexigenic peptide NPY, a well-known potent regulator of food intake, with an important function in energy homeostasis [17]. Npy gene expression was also nutritionally regulated in PBMC and—in accord with its role stimulating food intake, and similar to what happens in hypothalamus [26]—an increase with fasting and a decrease with refeeding, which were mainly evident at 6 months of age, was observed. In this way, we provide information indicating that gene expression studied in PBMC provides a representative view of the homeostatic status of the organism at different ages and, as such, represents an interesting tool to obtain biomarkers for nutritional studies and body weight control studies.

Obesity is associated with an insensitivity in the response to feeding conditions [9, 10, 33]. In our previous study, a transcriptomic analysis revealed that nutritional regulation was impaired in PBMC of obese cafeteria rats (29 % increased body weight) [8]. Here, we analysed the nutritional response of our selected genes in PBMC of rats made obese by the intake of a cafeteria diet at different stages of obesity development. Interestingly, we observed a general lack of response to acute fasting and refeeding in comparison to control-fed animals that was evident after only 1 month of intake of the high fat diet, when animals had 13 % increased body weight (3 months of age), and subsequently, after 2 and 4 months of cafeteria-diet feeding, when animals had 20 and 29 % increase in body weight compared to controls, respectively. This general impairment in nutritional response observed in key homeostatic regulatory genes was similar to that observed in liver and adipose tissue (retroperitoneal depot) and would be showing impairment in homeostatic control and, thus, an alteration in the capacity to regulate energy balance which is a characteristic of obese animals. More specifically, during obesity development, PBMC would be reflecting problems in lipid homeostasis (mobilization and storage) by liver and adipose tissue in acute periods of fasting and refeeding and also impairment in hypothalamic regulation of food intake which could be contributing to increased fat deposition.

PBMC are therefore easily obtainable biological material which can be periodically collected that properly reflect the metabolic adaptations to acute changes in fasting and refeeding that take place in liver or adipose tissue. Moreover, the increased adiposity due to the intake of a hyperlipidic diet like the cafeteria diet produces metabolic alterations (such us insulin resistance) which are related to an impairment in the response to feeding [8, 37]. These alterations in energy homeostatic control are reflected in PBMC from early stages of weight gain. Studies of changes in gene expression pattern in PBMC can help identify early determinants of the pre-obese state (early markers of obesity) as well as to go further into understanding the molecular mechanisms involved in insensitivity to feeding.

In conclusion, here we show that changes in gene expression in PBMC can be used as early predictors of disease. In particular, alterations in the expression of key energy homeostatic genes in PBMC could indicate metabolic alterations which lead to obesity and, therefore, these cells constitute an easily obtainable source of biomarkers related to early dysregulation of energy homeostasis, which is potentially useful for interventions aimed at preventing obesity.

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Ethical standards The experiments followed in this study were reviewed and approved by the Bioethical Committee of the University of the Balearic Islands.
Conflict of interest  The authors declare that they have no conflict of interest.

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