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Basic nutritional investigation

A low-protein, high-carbohydrate diet increases browning in perirenal adipose tissue but not in inguinal adipose tissue

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ABSTRACT

Objective: The aim of this study was to evaluate the browning and origin of fatty acids (FAs) in the maintenance of triacylglycerol (TG) storage and/or as fuel for thermogenesis in perirenal adipose tissue (periWAT) and inguinal adipose tissue (ingWAT) of rats fed a low-protein, high-carbohydrate (LPHC) diet.
Methods: LPHC (6% protein, 74% carbohydrate) or control (C; 17% protein, 63% carbohydrate) diets were administered to rats for 15 d. The tissues were stained with hematoxylin and eosin for histologic analysis. The content of uncoupling protein 1 (UCP1) was determined by immunofluorescence. Levels of T-box transcription factor (TBX1), PR domain containing 16 (PRDM16), adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase, lipoprotein lipase (LPL), glycerokinase, phosphoenolpyruvate carboxykinase (PEPCK), glucose transporter 4, β_3-adrenergic receptor (AR), β_1-AR, protein kinase A (PKA), adenosine-monophosphate-activated protein kinase (AMPK), and phospho-AMPK were determined by immunoblotting. Serum fibroblast growth factor 21 (FGF21) was measured using a commercial kit (Student's *t* tests, $P < 0.05$).
Results: The LPHC diet increased FGF21 levels by 150-fold. The presence of multilocular adipocytes, combined with the increased contents of UCP1, TBX1, and PRDM16 in periWAT of LPHC-fed rats, suggested the occurrence of browning. The contents of β_1-AR and LPL were increased in the periWAT. The ingWAT showed higher ATGL and PEPCK levels, phospho-AMPK/AMPK ratio, and reduced β_3-AR and PKA levels.
Conclusion: These findings suggest that browning occurred only in the periWAT and that higher utilization of FAs from blood lipoproteins acted as fuel for thermogenesis. Increased glycerol 3-phosphate generation by glyceroneogenesis increased FAs reesterification from lipolysis, explaining the increased TG storage in the ingWAT.

Introduction

The presence of multilocular adipocytes, which contain a greater number of mitochondria and express uncoupling protein 1 (UCP1), in white adipose tissue (WAT) was firstly reported in 1984 by Young et al. [1]. These "brown-like" adipocytes were termed *brite* or *beige* [2], and the process of differentiation to brite cells was called the *browning process* [3–8]. The browning process is

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normally increased in the context of cold stimulation in inguinal and perirenal white adipose tissue (ingWAT and periWAT, respectively) [8,9] and is rarely observed in epididymal WAT (epiWAT) [4]. UCP1 is located in the inner mitochondrial membrane, promoting proton leakage and uncoupling of the electron transport chain and oxidative phosphorylation in the mitochondrial respiratory chain. Thus, proton flux through ATP synthase is reduced, and some of the potential energy produced by the electrochemical proton gradient is dissipated as heat [10]. This uncoupling also increases the rate of electron transport in the mitochondrial respiratory chain and the consumption of oxygen and fatty acids (FAs) as FAs are the main fuel for thermogenesis [11]. For this reason, attempts to decrease overweightness and obesity have included strategies to increase energy expenditure by increasing in the number of brite cells [12].

We have been steadily focusing on the effects of administering a low-protein, high-carbohydrate (LPHC) diet to rats, introduced during the early phase of life [13–22] because children in occidental societies are often fed diets with these characteristics, introduced soon after weaning [23]. Fifteen days after introducing this diet, alterations in the energy balance occur, along with increased food intake and energy gain; these changes increase the amount of body lipids [13]. Adaptation to the LPHC diet increases plasma norepinephrine, epinephrine, corticosterone, tumor necrosis factor (TNF)-α, leptin, and insulin sensitivity [14,15,18]. Additionally, the LPHC diet increases energy expenditure by increasing thermogenesis in the interscapular brown adipose tissue (iBAT), enhancing UCP1 content, and resulting in a higher response to adrenaline stimulation; this attenuates the energetic gains in these animals as they ingest 18% more calories than rats fed a control (C) diet [13]. Elucidation of major changes in the hormonal profiles and specific responses from these tissues have improved the understanding of the mechanisms involved in thermogenesis in iBAT and other alterations and processes in the adipose tissues, liver, and muscles of these animals [13–22].

To our knowledge, the effects of the LPHC diet on browning in the WAT have not yet been studied, although this issue is particularly interesting to researchers studying obesity control. Although many studies of browning have used cold as a stimulus, the main environmental factors triggering human obesity are a sedentary lifestyle and inadequate diet. Thus, the objective of this study was to investigate the development of brite cells in the periWAT and ingWAT and the origin of FAs in the maintenance of triacylglycerol (TG) storage, as fuel for thermogenesis, or both. Improving the understanding of these mechanisms and the effects of nutritional factors will contribute to the development of strategies for the prevention and treatment of human obesity [24].

Materials and methods

Animals and treatment

Male Wistar rats weighing approximately 100 g (30 to 32 d old) were randomized into either the C group or the LPHC group. Rats in the C group were fed a diet containing 17% protein, 63% carbohydrates, and 7% lipids [25], and those in the LPHC group were fed a diet containing 6% protein, 74% carbohydrates, and 7% lipids for 15 d. The diets were isocaloric (16.3 kJ·g^{-1}), with the energy difference from the reduction in dietary protein compensated for by increasing the carbohydrate content [13–23,25] (Table 1). The rats were individually housed in metabolic cages in an environmentally controlled room (lights on from 0600 to 1800; temperature 22 ± 1°C). The experimental protocol was approved by the Ethics Committee of the Federal University of Mato Grosso. Samples for all determinations were obtained from rats in the fed state.

Immunoblotting

Segments of the WAT were coarsely minced and immediately homogenized in 50 mmol·L^{-1} Tris-HCl buffer (pH 7.4; 4°C) containing 150 mmol·L^{-1} sodium chloride (NaCl), 1 mmol·L^{-1} ethylenediaminetetraacetic acid, 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 10 mmol·L^{-1} sodium orthovanadate, 100 mmol·L^{-1} sodium fluoride, 5 μg·mL^{-1} of aprotinin, and 1 mmol·L^{-1} phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 10 000 *g* for 40 min at 4°C, and the supernatant was used to determine the protein content [26], with bovine serum albumin (BSA) as the standard. Samples containing 100 μg of protein from each experimental group were separated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. The levels of T-box transcription factor (TBX1), PR domain containing 16 (PRDM16), adipose triacylglycerol lipase (ATGL), hormone-sensitive lipase (HSL), lipoprotein lipase (LPL), glycerokinase (GyK), phosphoenolpyruvate carboxykinase (PEPCK), glucose transporter 4 (GLUT4), β_3-adrenergic receptor (AR), β_1-AR, protein kinase A (PKA), adenosine-monophosphate-activated protein kinase (AMPK), and phospho-AMPK were detected after overnight incubation of the membrane at 4°C with primary antibodies that had been diluted in TBS-T containing 5% dry albumin. α-Tubulin was used as an internal control. The primary antibodies were detected using a peroxidase-conjugated secondary antibody and were then visualized with chemiluminescence reagents. The band intensities were quantified using ImageJ software (version 1.38), and the results are expressed as the relative ratio using the internal control as the baseline. Phospho-AMPK was expressed as the ratio relative to the level of total AMPK.

Table 1
Composition (g·kg^{-1}) of the control (C) and low-protein, high-carbohydrate (LPHC) diets

Ingredient	Control diet	LPHC diet
Casein (84% protein)	202	71.5
Cornstarch	397	480
Dextrinized cornstarch	130.5	159
Sucrose	100	121
Soybean oil	70	70
Fiber (cellulose)	50	50
Mineral mix (AIN 93 G)*	35	35
Vitamin mix (AIN 93 G)*	10	10
L-cystine	3	1
Choline bitartrate	2.5	2.5

* For detailed composition, see Reeves et al. [25].

Immunofluorescence

Immunofluorescence was performed as described by Lemes da Silva et al. [27]. We used epiWAT as a negative control and iBAT as a positive control. Samples were incubated overnight in a fixative solution (4% paraformaldehyde in 0.8 mol·L^{-1} phosphate buffer), followed by dehydration through a gradient of increasing ethanol concentrations. Samples were then clarified in xylene and embedded in paraffin. Samples were cut into 3-μm-thick sections using a HYRAXM60 microtome (Carl Zeiss, Germany), deparaffinized, and stained with hematoxylin and eosin. To determine UCP1 protein content, histologic sections were placed on slides with a biologic adhesive, deparaffinized, and incubated for 1 h at 70°C in 0.21% sodium citrate solution (pH 6.0). Samples were then incubated for 30 min with 3% hydrogen peroxide in 70% methanol to block endogenous peroxidase, permeabilized in 0.4% Tween 20 in phosphate-buffered saline (PBS) for 15 min, and then blocked for 30 min with 5% BSA (Sigma-Aldrich, Rio de Janeiro, Brazil) in PBS. Sections were then incubated with rabbit anti-UCP1 (Abcam, Inc., Cambridge, MA, USA; 1:1000) for 18 h at 4°C in a humidified chamber. Thereafter, sections were incubated with a goat antirabbit immunoglobulin G (IgG) secondary antibody conjugated to AlexaFluor 488 (Invitrogen, Eugene, OR, USA; 1:50). Antibodies were diluted with 1% BSA in PBS. Sections also were stained with the nuclear fluorescent dye 4′,6-diamidino-2-phenylindole (Sigma-Aldrich, St. Louis, MO, USA) to facilitate morphologic characterization. Finally, slices were washed in PBS, mounted in 1:1 glycerin:PBS, and examined under an AxioScope.A1 microscope (Carl Zeiss, Germany). We quantified UCP1 expression using AxioVision software (version 4.8.1, 2009), with values presented as median optical density in arbitrary units (AU; scale value range 0–255). For densitometry analysis, images were obtained with a 20 × objective lens, and readings were reported as means ± SEMs.

Lipid content in the white adipose tissue

The lipid content in the WAT was determined by gravimetric methods after extraction, according to the protocol published by Folch et al. [28]. The tissue aliquot (about 1 g) was homogenized in chloroform-methanol (2:1), and the volume was brought to 10 mL. After an overnight rest, the material was filtered, and NaCl 0.9% was added (20% of the volume after filtration). After slight shaking, the mixture was centrifuged. The superior aqueous phase was aspirated with a vacuum pump, and

one aliquot of the chloroform phase (~3 mL) was transferred to a preweighed vial. The material was evaporated until a constant weight was reached.

Determination of serum fibroblast growth factor 21 levels

Fibroblast growth factor (FGF21) levels were assessed in serum using a commercial FGF21 immunoassay (MF2100 Quantikine ELISA kit products; R&D Systems, Minneapolis, MN, USA).

Statistical analysis

The data are presented as means ± SEMs. Statistical analysis was performed using GraphPad Prism software (version 5.01). Independent samples *t* tests was used to compare the data from the two groups, which were found to be normally distributed. Differences were considered statistically significantly at $P < 0.05$.

Results

Rats receiving the LPHC diet had lower body weights at the end of 15 d of treatment (~22%; $P < 0.001$), and the mass of the ingWAT was 78% higher without any changes in periWAT mass when compared with those in rats fed the C diet. The lipid content per gram of ingWAT and periWAT was similar between groups (Table 2).

In histologic and immunofluorescence analyses, we usedepiWAT as a negative control and iBAT as a positive control. The periWAT of LPHC diet-fed rats showed the presence of multilocular adipocytes (Fig. 1) and increased (176%; $P = 0.02$) UCP1 content (Fig. 2) when compared with that of C diet-fed rats. There were no alterations in adipocytes or UCP1 contents in the ingWAT of LPHC diet-fed rats.

The levels of TBX1 and PRDM16 were increased by 128% ($P = 0.01$) and 110% ($P = 0.02$), respectively, in the periWAT of LPHC diet-fed rats compared with that of C diet-fed rats (Fig. 3). The contents of these proteins in the ingWAT were similar between groups.

Serum concentrations of FGF21 (Fig. 4) were increased ~150-fold in LPHC diet-fed rats (C: 41.43 ± 8.04 $pg \cdot mL^{-1}$; LPHC: 6346.00 ± 574.70 $pg \cdot mL^{-1}$; $P < 0.0001$). The contents of β_3-AR and PKA were reduced by 50% in the ingWAT of LPHC diet-fed rats compared with that in C diet-fed rats ($P = 0.04$), and β_1-AR levels were similar between groups ($P > 0.05$; Fig. 5). In the periWAT, β_3-AR and PKA were similar between groups ($P > 0.05$), but β_1-AR levels were increased by 44% in LPHC diet-fed rats when compared with that in C diet-fed rats ($P = 0.02$; Fig. 5).

The LPHC diet induced an increase in ATGL contents by ~72% ($P = 0.04$); however, there were no changes in HSL or lipoprotein lipase (LPL) contents in the ingWAT ($P > 0.05$; Fig. 6). In contrast, the LPHC diet induced an increase in LPL content in the periWAT by about 61% ($P < 0.009$), without any changes in ATGL or HSL ($P > 0.05$; Fig. 6). The LPHC diet induced a 131% increase in the level of PEPCK, a key enzyme involved in glyceroneogenesis, in the ingWAT ($P = 0.004$); however, no changes in the periWAT were observed ($P > 0.05$; Fig. 7). The levels of GLUT4 and GyK, which catalyzes the phosphorylation of glycerol to glycerol 3-phosphate (G3 P), were similar between LPHC and C diet–fed rats in both tissues ($P > 0.05$; Fig. 7).

Figure 8 shows AMPK and phospho-AMPK levels. No diet-related changes were observed in AMPK contents in either tissue ($P > 0.05$); however, the LPHC diet induced a 51% increase ($P = 0.01$) and 62% reduction ($P = 0.002$) in the phospho-AMPK/AMPK ratio in the ingWAT and periWAT, respectively.

Table 2
Initial and final body weight, relative mass, and lipid and protein contents in inguinal white adipose tissue (ingWAT) and perirenal white adipose tissue (periWAT) of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 d

Variable	Control group	LPHC group
Initial body weight, g	91.99 ± 1.67	91.90 ± 1.39
Final body weight, g	190.50 ± 5.63	148.50 ± 5.75*
Weight of ingWAT, $g \cdot 100g^{-1}$	1.31 ± 0.09	2.33 ± 0.10*
Weight of periWAT, $g \cdot 100g^{-1}$	0.115 ± 0.011	0.137 ± 0.008
Content of lipid in ingWAT, $g \cdot g$ tissue^{-1}	0.54 ± 0.05	0.48 ± 0.04
Content of lipid in periWAT, g.g tissue^{-1}	0.56 ± 0.01	0.53 ± 0.02
Content of protein in ingWAT, $\mu g \cdot mL^{-1}$	1.17 ± 0.16	1.05 ± 0.16
Content of protein in periWAT, $\mu g \cdot mL^{-1}$	1.02 ± 0.06	1.04 ± 0.10

Data presented as the mean ± standard error of the mean (N = 5–12 rats)
* $P < 0.01$ versus control group (Student's *t* test).

Discussion

We previously studied the energy balance in rats treated with an LPHC diet, and showed that these animals had higher energy intake, energy gain, and energy expenditure (data are shown in Supplementary Table 1) [13]. The increase in energy expenditure is interpreted as a consequence of diet-induced adaptive thermogenesis related to higher food intake associated, at least partly, with the increased activity of iBAT, the mechanisms of which we also investigated in previous studies [13,21].

The present study was designed to evaluate the development of brite cells in the periWAT and ingWAT and the origin of FAs in the maintenance of TG storage, as fuel for thermogenesis, or both, as the periWAT and ingWAT are the main tissues in which browning has been observed [8,9]. The presence of multilocular adipocytes and increased levels UCP1, TBX1, and PRMD16 in periWAT from LPHC diet-fed rats when compared with that of C diet-fed rats supported the hypothesis that the browning process was increased in this tissue. TBX1 is a member of the T-box family, which is expressed only in brite cells; TBX1 is not associated with cells of the myogenic lineage, including brown adipocytes [7,29]. Despite the morphologic similarities between brite and brown adipocytes, increased content of TBX1 protein triggers different gene expression profiles in brite cells than in classical brown adipocytes [29]. Furthermore, PRDM16 triggers the expression of genes involved in the synthesis of proteins associated with the characteristics of brown adipocytes [30–32]. Both beige and classical brown fat cells express PRDM16 [4]. The high levels of serum FGF21 observed in LPHC diet-fed rats were consistent with increased in brite cells in the periWAT. FGF21 is a member of the FGF family and is expressed in the liver, as well as other tissues, including the WAT, brown adipose tissue (BAT), skeletal muscle, and pancreatic β-cells [33,34]. This factor induces the browning process in the WAT [8,35,36]. Moreover, fasting [37,38] and leucine restriction [39] are associated with high levels of FGF21 in the blood. Sousa-Coelho et al. [39] reported that male C57 BL/6 J mice consuming a diet lacking leucine showed increased blood FGF21 levels. We have previously shown that rats from the LPHC group have higher levels of nonessential amino acids (data are shown in Supplementary Table 2) [19] can be synthesized from glycolysis (pyruvate) and Krebs cycle (alpha ketoglutarate) intermediaries [19]. Additionally, other studies show that the protein synthesis in the soleus [19] and EDL muscles is reduced [22]. So, an important pathway that uses amino acids is inhibited in LPHC rats.

However, the levels of essential amino acids, including leucine, are reduced in the blood [19].

In contrast to our findings in periWAT, the LPHC diet did not induce significant changes in ingWAT adipocytes or in the contents of UCP1, TBX1, and PRMD16 in ingWAT signaling; thus, browning was not observed in this tissue. Previous studies using different stimuli have reported browning in the ingWAT

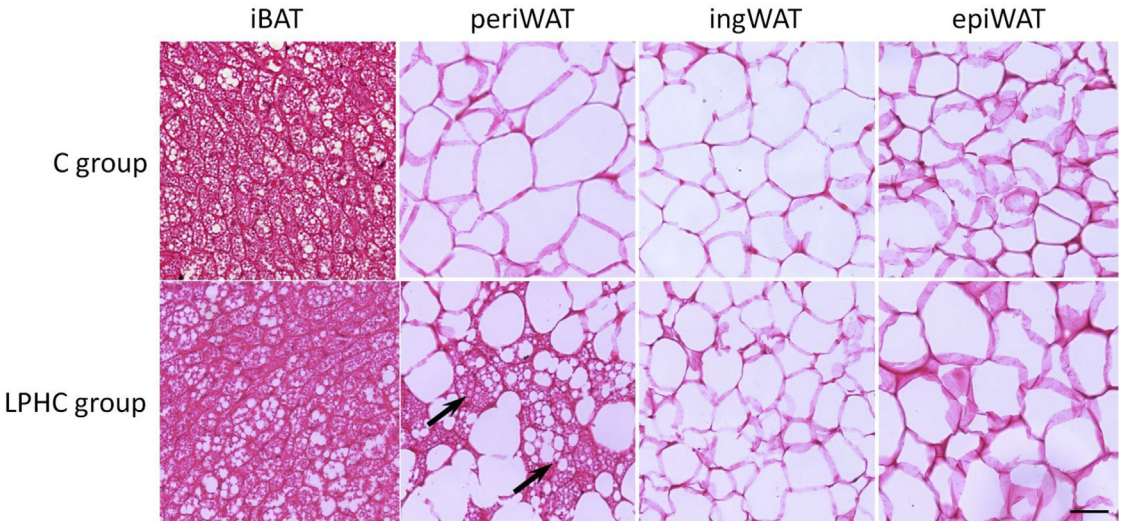

Fig. 1. Hematoxylin and eosin-stained sections of interscapular brown adipose tissue (iBAT) and inguinal (ingWAT), perirenal (periWAT), and epididymal (epiWAT) white adipose tissues of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. The scale bar represents 40 μm. Arrows indicate multilocular adipocytes.

[8,35,40]. Véniant et al. [35] demonstrated that browning is stimulated by FGF21 when administered exogenously in lean but not obese mice at 21°C but not at 30°C. These results showed that the effects of FGF21 on browning in the tissues depended on other stimuli; this could explain why the LPHC diet, despite increasing FGF21 levels and stimulating browning in the periWAT, did not result in generation of brite cells in the ingWAT.

We evaluated β_1-AR and β_3-AR contents in the ingWAT and periWAT since serum cathecolamines in LPHC diet-fed rats are higher than those in C diet-fed rats (data shown in Supplementary Table 3) [14] and adrenegic stimulation also is positively associated with brite cells [8,40,41]. In the periWAT of LPHC diet-fed rats, the content of β_3-AR did not change, whereas the content of β_1-AR was increased. In contrast, we observed the same β_1-AR content and a reduction in β_3-AR in the ingWAT when comparing corresponding tissues from C diet-fed rats. The levels of PKA were reduced in the ingWAT and remained unchanged in the periWAT of LPHC diet-fed rats, confirming that the periWAT showed higher activation of the adrenergic system via β-AR. Thus, high levels of serum FGF21 and increased β_1-AR contents in the periWAT of LPHC diet-fed rats, regardless of whether there was a change in β_3-AR or PKA, could stimulate the browning process.

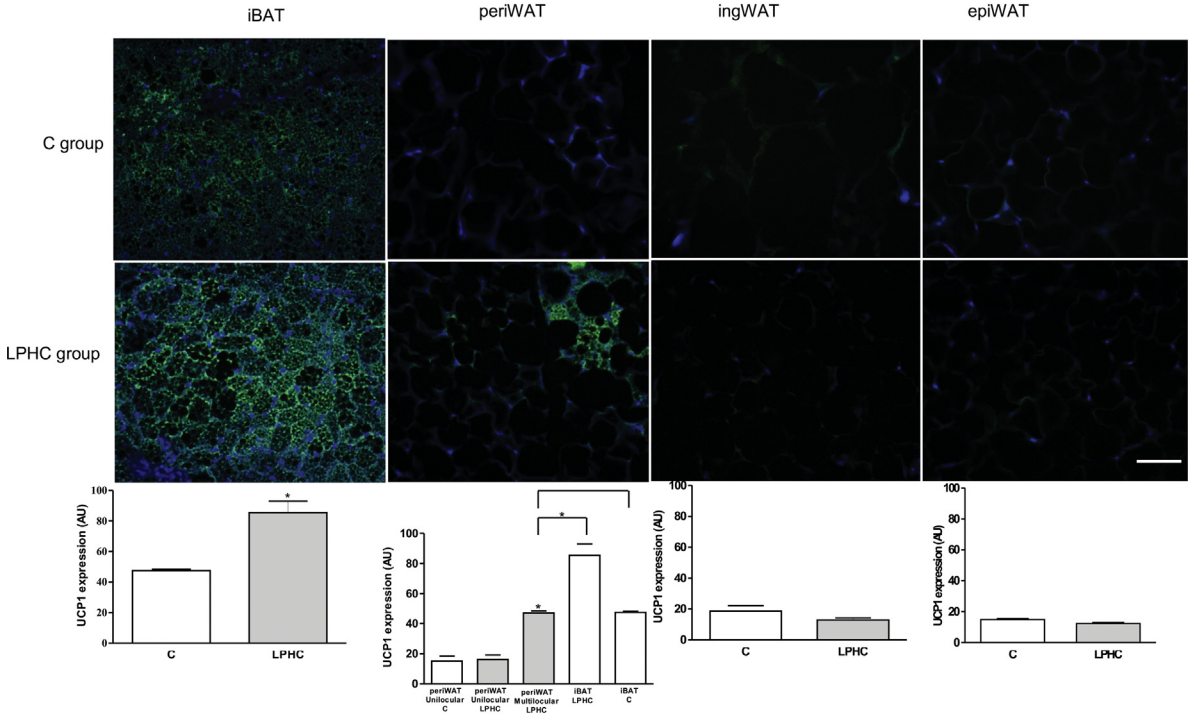

Fig. 2. Immunostaining for UCP1 in interscapular brown adipose tissue (iBAT) and perirenal (periWAT), inguinal (ingWAT), and epididymal (epiWAT) white adipose tissues of rats fed a control (C) or low-protein, high-carbohydrate diet (LPHC) for 15 days. The scale bar represents 100 μm. Graphs display UCP1 protein content in the iBAT, periWAT, epiWAT, and ingWAT from C and LHPC rats. UCP1 levels in the periWAT was compared with UCP1 levels in different cell types. Data are presented as means ± SEMs (n = 4–6 rats). *$P < 0.05$ versus the control group (Student's t test).

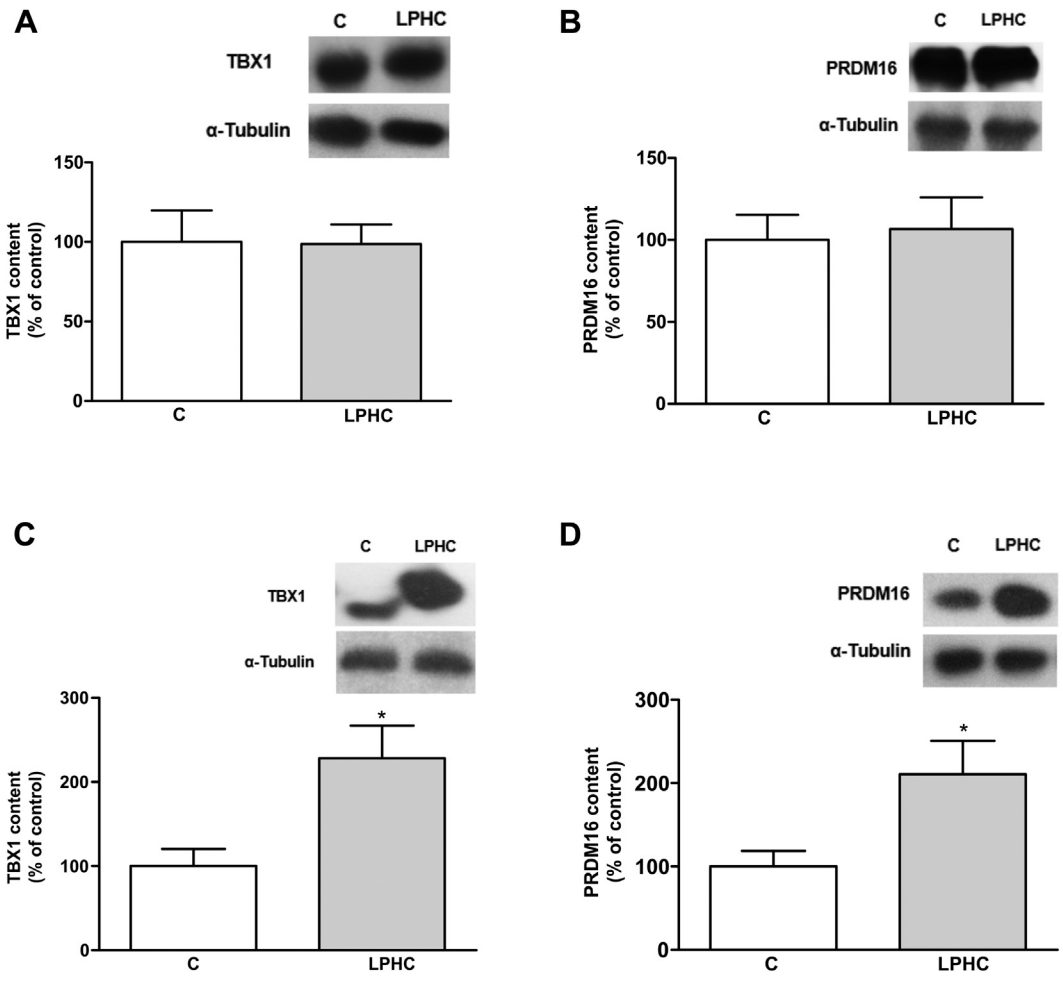

Fig. 3. Contents of T-box transcription factor (TBX1) and PR domain containing 16 (PRDM16) protein in the inguinal white adipose tissue (ingWAT) (A and B, respectively) and perirenal white adipose tissue (periWAT) (C and D, respectively) of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the means ± SEMs (n = 5–8 rats). $^{*}P < 0.05$ versus the control group (Student's *t* test).

Douris et al. [42] showed that central or peripheral FGF21 administration did not initiate browning in mice lacking β-AR, suggesting a relationship with the adrenergic system [42]. β_3-AR is thought to play a role in the browning process [43]; however, studies also have demonstrated that overexpression of β_1-AR in the WAT of mice induces the differentiation of brite adipocytes [44].

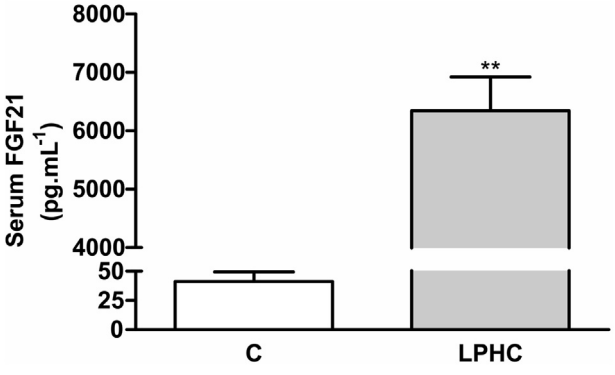

Fig. 4. Serum Fibroblast growth factor 21 (FGF21) level in rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the means ± SEMs (n = 6–8 rats). $^{**}P < 0.01$ versus the control group (Student's *t* test).

Thus, our data show that the stimulation of the browning process in tissues does not depend solely on high serum levels of FGF21. In LPHC rats, the results suggest that differences in adrenergic stimulation may be one of the causes of the different responses observed in ingWAT and periWAT.

For evaluation of FAs generation for TG storage or thermogenesis, we determined the levels of the enzymes ATGL and HSL, which are involved in lipolysis, and for proteins in the G3 P generation pathway (GyK, PEPCK, and GLUT4). The phospho-AMPK/AMPK ratio also was determined because of the importance of AMPK as a regulator of some of these proteins. The ingWAT from LPHC diet-fed rats showed a significant increase in relative mass and lipid contents, without evidence of browning. The increase in ATGL and the phospho-AMPK/AMPK ratio in the ingWAT of LPHC diet-fed rats suggested increased lipolysis in the tissue, although no changes were observed in HSL level. These enzymes act sequentially; although ATGL has a high affinity for TG, HSL has a high affinity for diacylglycerol (DAG) [16]. Studies have shown that the activity of ATGL is increased following phosphorylation at Ser406 by AMPK [45] and that depletion of AMPK promotes a reduction in the phosphorylation and activity of ATGL [46]. In contrast, HSL is activated by PKA-dependent phosphorylation. However, HSL phosphorylation at Ser565 by AMPK prevents phosphorylation at Ser563 and Ser660 by PKA [46]. Thus, the

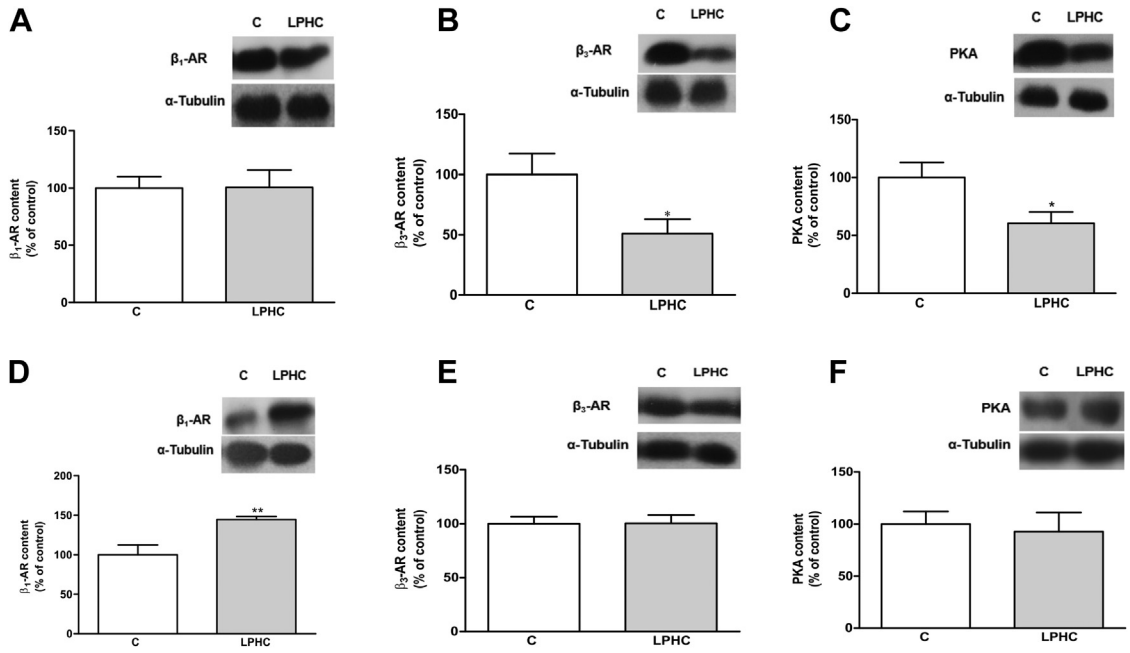

Fig. 5. Contents of β_1 adrenergic receptor (β_1-AR), β_3 adrenergic receptor (β_3-AR), and protein kinase A (PKA protein) in the inguinal white adipose tissue (ingWAT) (A, B, and C, respectively) and perirenal white adipose tissue (periWAT) (D, E, and F, respectively) of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the means ± SEMs (n = 5–8 rats). $^*P < 0.05$ versus the control group; $^{**}P < 0.01$ versus the control group (Student's *t* test).

increased ratio of phospho to AMPK/AMPK suggested higher activity of ATGL and lower activity of HSL. The inhibition of HSL and simultaneous activation of ATGL by AMPK is a metabolic mechanism through which adipose cells preserve fat storage and maintain their main functions [46]. The increased PEPCK content observed in the ingWAT of LPHC diet-fed rats suggested an increase in the rate of G3 P generation by glyceroneogenesis, increasing the reesterification of FAs into TG [47]. The other proteins involved in G3 P synthesis (i.e., GyK and GLUT4) were not altered by the LPHC diet. As observed previously, the consumption of an LPHC diet induces increases in several substrates for glyceroneogesis, such as serum L-lactate, L-alanine, and L-glutamine [18] (data are shown in Supplementary Table 4) [18]. The levels of LPL in the ingWAT were similar in LPHC and C diet-fed rats, suggesting

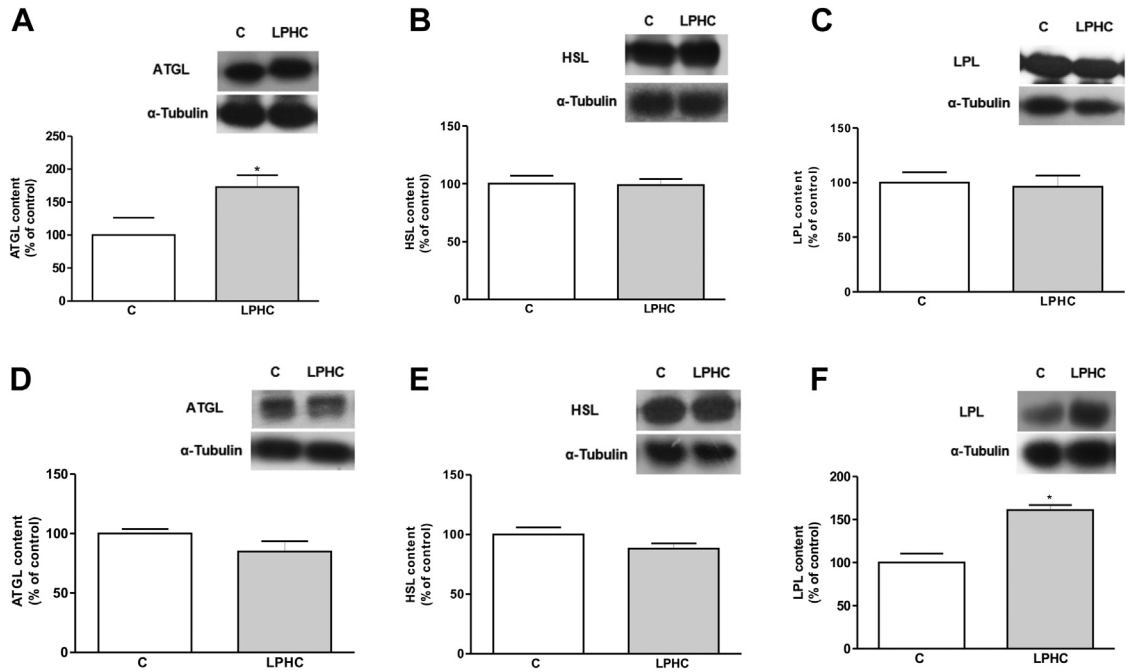

Fig. 6. Content of adipose triacylglycerol lipase (ATGL), hormone sensitive lipase (HSL), and lipoprotein lipase (LPL) protein in the inguinal white adipose tissue (ingWAT) (A, B, and C, respectively) and perirenal white adipose tissue (periWAT) (D, E, and F, respectively) of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the means ± SEMs (n = 5–8 rats). $^*P < 0.05$ versus the control group (Student's *t* test).

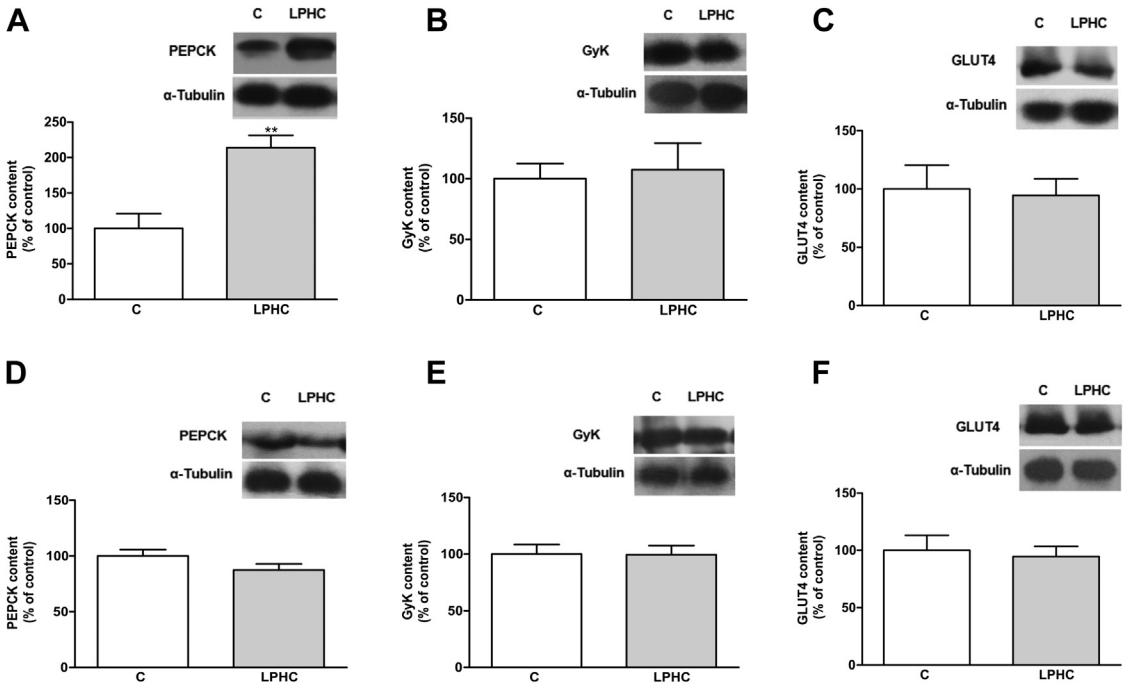

Fig. 7. Content of phosphoenolpyruvate carboxykinase (PEPCK), glycerokinase (GyK), and glucose transporter (GLUT4) proteins in the inguinal white adipose tissue (ingWAT) (A, B, and C, respectively) and perirenal white adipose tissue (periWAT) (D, E, and F, respectively) of rats fed a control (C) or low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the means ± SEMs (n = 5–8 rats). $^{**}P < 0.01$ versus the control group (Student's *t* test).

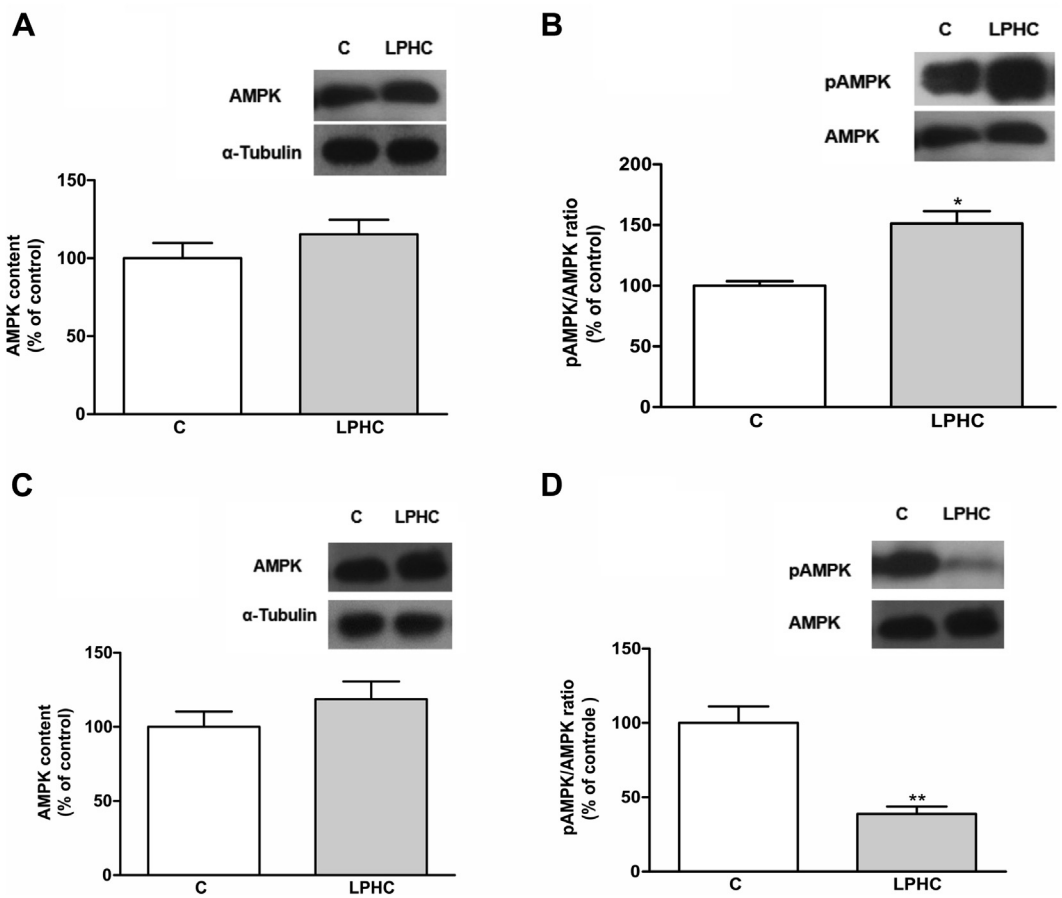

Fig. 8. Content of adenosine-monophosphate-activated protein kinase (AMPK) protein and pAMPK/AMPK ratio in inguinal white adipose tissue (ingWAT) (A and B, respectively) and in perirenal white adipose tissue (periWAT) (D and E, respectively) of rats fed a control (C) or a low-protein, high-carbohydrate (LPHC) diet for 15 days. Data are presented as the mean ± SEM (n = 5–8 rats). $^{*}P < 0.05$ vs. control group; $^{**}P < 0.01$ vs. control group (Student's *t* test).

that the contributions of FAs uptake from blood lipoprotein were similar. These findings suggest that the higher relative mass of the ingWAT were due, at least in part, to higher reesterification of FAs from lipolysis with increases in G3 P content from glyceroneogenesis. Although the greater contribution of *de novo* FA synthesis could not be excluded, the high phospho-AMPK/AMPK ratio could signal the reduction of *de novo* FAs synthesis by inhibition of the acetyl-coenzyme (CoA) carboxylase enzyme [48,49].

In the periWAT from LPHC diet-fed rats, the mass and lipid contents in the tissues were maintained at levels similar to those in the periWAT from C diet-fed rats, even with the additional use of FAs as a fuel for thermogenesis. The levels of ATGL and HSL also were similar between groups, accompanied by decreased phospho-AMPK/AMPK ratio. These findings suggested that the mechanism of activation of ATGL through the phospho-AMPK/AMPK ratio in the periWAT could be reduced and the phosphorylation of HSL, induced by adrenergic stimuli, could be maintained at the same level as that in C diet-fed rats. These data indicated that alterations in the cycle of lipolysis–FA reesterification were not the source of additional FAs. However, the increase in LPL content induced by the LPHC diet suggested an increase in FAs uptake from circulating lipoproteins. Thus, these findings suggested that the additional FAs involved in thermogenesis in the periWAT of LPHC diet-fed rats were obtained from circulating lipoproteins.

The data set showing the browning process in periWAT suggests that this tissue contributes to the increase in energy expenditure in LPHC rats in conjunction with thermogenesis in iBAT. This may have important clinical implications because most studies on browning focus on cold despite that the main environmental factors triggering human obesity are a sedentary lifestyle and inadequate diet. The present findings that the LPHC diet leads to browning in the periWAT, increased thermogenesis, and attenuated body weight may suggest that when this diet is provided to newborns, it should be accompanied by instructions for increased food intake. In the ingWAT, where browning seems not to be stimulated, the FAs–TG cycle is increased. When FAs are converted into CoA derivatives and TG storage, and in another moment TG are hydrolyzed and the FAs oxidize, there is an energy loss of about 2% [50]. Although the loss of energy by this process can be limited, the adaptation to LPHC diet increases the energetic expenditure for actions in several tissues and for different thermogenic processes activated simultaneously.

Conclusion

The increase in FGF21 after administration of the LPHC diet was accompanied by an increase in the browning process in the periWAT, but not in the ingWAT, likely because of reduced adrenergic stimulation. The present data suggested that additional FAs for thermogenesis in the periWAT originate from circulating lipoprotein, whereas FAs involved in increased TG storage in the ingWAT originate from higher reesterification of FAs from lipolysis.

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Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.nut.2017.05.007.

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