Basic nutritional investigation

Low-protein, high-carbohydrate diet increases glucose uptake and fatty acid synthesis in brown adipose tissue of rats

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ABSTRACT

Objective: The aim of this study was to evaluate glucose uptake and the contribution of glucose to fatty acid (FA) synthesis and the glycerol-3-phosphate (G3P) of triacylglycerol synthesis by interscapular brown adipose tissue (IBAT) of low-protein, high-carbohydrate (LPHC) diet-fed rats.

Methods: LPHC (6% protein; 74% carbohydrate) or control (17% protein; 63% carbohydrate) diets were administered to rats (w100 g) for 15 d. Total FA and G3P synthesis and the synthesis of FA and G3P from glucose were evaluated in vivo by3H2O and 14C-glucose. Sympathetic neural contribution for FA synthesis was evaluated by comparing the synthesis in denervated (7 d before) IBAT with that of the contralateral innervated side. The insulin signaling and β3 adrenergic receptor (β3-AR) contents, as well as others, were determined by Western blot (Student’s t test or analysis of variance; P < 0.05).

Results: Total FA synthesis in IBAT was 133% higher in the LPHC group and was reduced 85% and 70% by denervation for the LPHC and control groups, respectively. Glucose uptake was 3.5-fold higher in the IBAT of LPHC rats than in that of the control rats, and the contribution of glucose to the total FA synthesis increased by 12% in control rats compared with 18% in LPHC rats. The LPHC diet increased the G3P generation from glucose by 270% and the insulin receptor content and the p-AKT insulin stimulation in IBAT by 120% and reduced the β3-AR content by 50%.

Conclusions: The LPHC diet stimulated glucose uptake, both the total rates and the rates derived from glucose-dependent FA and G3P synthesis, by increasing the insulin sensitivity and the sympathetic flux, despite a reduction in the β3-AR content.

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Introduction

Brown adipose tissue (BAT) is the main site of non-shivering thermogenesis and it has been recognized since the 1960s as an important component of the energetic balance in small mammals [1,2]. In recent years, functional BAT has been identified in adult humans [3–5], and observations have been made that prolonged cold exposure or the β-adrenergic agonist can convert white adipose tissue (WAT) to a “brown-like” state [6]. Because
of this finding, the significance of BAT in the energetic balance in humans has been reviewed [7–9]. These findings have renewed an interest in studies that may contribute to a better understanding of the role and metabolism of this tissue.

In BAT, the storage of triacylglycerol (TAG) is fundamental to its physiological role because the fatty acids (FA) from intracellular lipolysis are the main substrates for oxidation while simultaneously activating uncoupling protein 1 (UCP1). Activation of UCP1 leads to the dissipation of the proton gradient and the uncoupling of the oxidative phosphorylation, thus increasing heat production by mitochondrial respiratory chain [10]. The FA for TAG synthesis in BAT may be preformed FA or de novo FA synthesized within tissue. Preformed FA can originate from lipolysis in the brown adipocytes or taken up efficiently by lipoprotein lipase (LPL), which mainly occurs in lipoproteins in the blood that are rich in TAG. So, the BAT LPL action is important for blood lipid clearance [11–13]. It is also well established that BAT is important for blood glucose clearance [14,15]. Studies show significant amounts of glucose in BAT are used for the production of adenosine triphosphate (ATP) by anaerobic glycolysis, thus supplying the lower ATP production induced by mitochondrial uncoupling [15], and by replacement of the citric acid cycle intermediates that are important for maintaining the high levels of FA oxidation when thermogenesis is activated [16]. Glucose may also be an important carbon source for FA and glycerol-3-phosphate synthesis (G3P) [17] and an essential metabolite for TAG synthesis. Glyceroneogenesis and glycerol phosphorylation by glycerokinase (GyK) are the other two pathways responsible for the continuous generation of G3P in BAT. In BAT, the insulin and sympathetic nervous systems stimulate the glucose utilization and FA synthesis [18].

Previously, our research group used a low-protein, high-carbohydrate (LPHC) diet to investigate the nutritional, hormonal and neural control of energy-linked metabolic processes in adipose tissue and liver from younger rats [19–22]. After 15 d of being fed an LPHC diet, rats showed an increase in energetic gain and energy expenditure, noticeable changes in body chemical composition, and an increase both in lipid content and in the sympathetic flux to interscapular brown adipose tissue (IBAT) suggesting that the thermogenesis can be activated in this tissue [19].

Several tissue-specific alterations were observed in LPHC diet-fed rats, including lower insulin sensitivity in retroperitoneal [21] and epididymal adipose tissues with similar glucose uptake when compared with control rats (not-verbal) and a higher insulin sensitivity in the liver [22]. We have also demonstrated that FA synthesis from 3H2O (total) and from glucose is increased in the liver of LPHC-fed rats and glycerol is more important than glucose for the increase in the levels of de novo FA and glyceride-glycerol synthesis [22]. Additionally, we observed that these animals showed lower serum postprandial insulin and similar glycemia relative to the control diet-fed rats despite a higher intake of calories from lipids and carbohydrates (54%) [19].

Due to the high metabolic activity of IBAT and consequently high ATP production by anaerobic glycolysis, our hypothesis in this work was that the uptake of glucose and the FA and glycerol-glyceride synthesis from glucose are increased in IBAT of these animals, thus contributing to the maintenance of the postprandial glycemia in LPHC diet-fed rats. The possible preservation in the insulin signaling in IBAT and the increase in sympathetic flux may be contributing to the increase in TAG storage. To test our hypothesis, we evaluated the following in IBAT from control and LPHC diet-fed rats:

1. insulin signaling pathway protein content;
2. rate of glucose uptake;
3. rate of in vivo FA and glycerol synthesis from glucose and from all sources;
4. sympathetic contribution of total FA synthesis and levels of the β3 adrenergic receptor (β3-AR);
5. enzyme activity involved in de novo FA synthesis;
6. contribution of preformed FA for TAG synthesis;
7. content of the GyK enzyme; and
8. levels of the peroxisome proliferator-activated receptor gamma (PPARγ) transcription factor that is involved in FA metabolism.

Materials and methods

Animals and treatment

Male Wistar rats (7–12 animals) with an initial body weight of ~100 g (30 d) were randomly divided into two groups: Controls and LPHC. Control rats were fed a diet composed of 17% protein, 63% carbohydrate, and 7% lipids, whereas the LPHC rats were fed a diet composed of 6% protein, 74% carbohydrate, and 7% lipids for 15 d. The decrease in dietary protein was compensated for by an increase in dietary carbohydrates to keep the diets isocaloric (16.3 kkal g–1)(Table 1). Rats were kept in individual metabolic cages at 22 ± 1°C with a 12-h light–dark cycle. Rats received water and food ad libitum. Body weight and food intake of each rat were recorded daily. All of the experiments were performed between 08:00 and 10:00 h, and all of the rats were euthanized on day 15 of treatment. Rats were housed according to the Brazilian College of Animal Experimentation Rules, and the experiments were approved by the Ethics Committee of the Federal University of Mato Grosso (protocol no. 23108.033936/08-3).

Unilateral sympathetic denervation of IBAT

While the rats were under anesthesia, a careful dissection of the IBAT from the surrounding muscle and WAT was performed. Then, five branches of the right intercostal nerve bundles were isolated, and ~5-mm sections were removed from these nerves. Surgical hemi-denervation was performed 7 d before the use of the animals for the experiments. After this period, the norepinephrine content of the denervated side was reduced to ~2% of the values of the control, innervated side [24].

In vivo lipogenesis

Experimental approach

The rate of conversion of 14C from glucose and 3H from 3H2O (which estimates the total synthesis from all carbon sources) in two fractions (glyceride-FA and glyceride-glycerol) in the IBAT were determined simultaneously using the same animal, as previously described [20].

The labeled molecules [U-14C]glucose (10 μCi) and 3H2O (3 mCi) were dissolved in 0.5 mL of saline and injected into non-anesthetized rats, which were freely moving in their cages, using a catheter inserted under anesthesia into the right jugular vein 2 d before the experiments. After flushing the catheter with saline, 0.2 mL blood samples were taken at 1.5, 15, 30, and 60 min after injection of the label for determination of [U-14C]glucose-specific activity (S/μA). Each animal was sacrificed by cervical dislocation immediately after obtaining the

<table>
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<th>Table 1</th>
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<tr>
<td><strong>Compositions (g kg–1) of the control and LPHC diets</strong></td>
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<tr>
<td>Ingredient  </td>
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<tr>
<td>Cornstarch  </td>
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<td>Dextrose cornstarch  </td>
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<tr>
<td>Sucrose  </td>
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<tr>
<td>Soybean oil  </td>
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<tr>
<td>Fiber (cellulose)  </td>
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<tr>
<td>Mineral mix (AIN 93 G)*  </td>
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<tr>
<td>Vitamin mix (AIN 93 G)*  </td>
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<tr>
<td>L-cystine  </td>
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<td>Choline bitartrate  </td>
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* For detailed composition, see [23].
The in vivo rates of tissue glucose uptake and the concentration of the homogenates used in the enzymatic assays was determined by measuring the rate of oxidation of NADH. The composition of the assay mixture included NADPH, ATP-CLY was assayed as described previously [38], which involved supernatants obtained after the suspension of IBAT in 0.25 mol L\(^{-1}\) sucrose dissolved in SX20 to 5 mL scintillation liquid. In both the LPHC diet-fed and control periods, which was expected for the technique used. The results of glyceride-glycerol synthesis from glucose were calculated as described previously [25,26] using a factor of 0.036 to convert from µg of glucose carbon to nmol of synthesized glycerol. Two studies [27,28] previously presented the assumptions and supportive arguments behind the adequacy of using \(^{3}H_{2}O\) for measuring lipid synthesis from all carbon sources. Rates of glyceride-glycerol synthesis from \(^{3}H_{2}O\) were estimated by assuming that each glycolate incorporated into glyceride contains 3.3 atoms of \(^{3}H\) when glycerol is formed from glucose via glycolysis [28] and 5 atoms of \(^{3}H\) when glycerol is formed from non-glucose substrates via glycero genesis [29].

Isolation of tissue glyceride-FA and glyceride-glycerol

Total lipids from the IBAT were extracted with a 2:1 chloroform to methanol solution using the procedure described previously [30]. Labeled \(^{3}H_{2}O\) was removed from the inferior phases (predominantly chloroform) by washing three times with a saline solution. After each wash, the tubes were briefly centrifuged to sharpen the phase boundary, and the superior phase was then aspirated and discarded. The chloroform phase was evaporated to dryness, and the saponifiable lipids were hydrolyzed with ethanolic KOH for 1 h at 70 °C. After extraction of the non-saponifiable lipids and acidification with 6% \(HClO_4\), the \(^{14}C\)-\(^{3}H\)-FA was extracted with petroleum ether, the extract was evaporated to dryness in a scintillation vial, and the resulting product was dissolved in SX20 to 5 mL scintillation liquid. The \(^{14}C\)-\(^{3}H\)-glycerol value was obtained by subtracting the \(^{14}C\)-\(^{3}H\)-FA value from the \(^{14}C\)-\(^{3}H\)-total lipid values.

Determination of plasma glucose and water-specific radioactivity

Plasma was deproteinized with 6% \(HClO_4\), and after neutralization, the supernatant was applied to a Dowex column (1 x 8; 100-200 mesh; formate form) to separate \([U-^{14}C]\)glucose from \([^{13}C]^{2}\)pyruvate and \([^{13}C]citrate\). The compounds that were retained in the column were recovered by thoroughly washing the column with 1 N NaOH. The concentration of plasma glucose was determined enzymatically using a commercial kit from Labtest (Lagoa Santa, Brazil). Water SpA was determined directly from aliquots of diluted (1:50) plasma dissolved in SX20 to 5 mL scintillation liquid. In both the LPHC diet-fed and control rats, the plasma glucose concentration did not change significantly during the experimental period [20], which was expected for the technique used.

Radioactivity measurements

The degree of quenching in each sample was obtained to enable the amount of radioactivity in disintegrations per minute. Simultaneous liquid scintillation counting of the \(^{3}H\) and \(^{14}C\) contents of FA and glycerol was performed using a channel ratio method [31] on a Packard Tri-Carb 2100 TR spectrometer.

Enzyme activity measurement

Lipoprotein lipase (LPL) activity was assayed as described previously [32] in supernatants obtained after the suspension of IBAT in 0.25 mol L\(^{-1}\) sucrose, 1 mol L\(^{-1}\) EDTA, and 20 kU L\(^{-1}\) heparin, pH 7.4, and centrifugation at 17 000 g. Both the assay mixture containing glycolate tri[1 to \(^{14}C\)]-oleate and the protocol for isolating \(^{14}C\)-FA were previously described [33]. Glucose-6-phosphate dehydrogenase (G6PDH), malic enzyme (ME), and ATP-citrate lyase (ATP-CLY) activities were determined in 100 000 g supernatants of tissue homogenates prepared as previously described [44]. G6PDH was assayed as described in terms of radioactivity. After the last blood sample, the rats were sacrificed, and IBAT was removed to measure the content of 2-deoxy-\(^{14}C\)-glucose-6-phosphate (2-DG-P) [41]. The plasma glucose concentration was determined with the glucose oxidase method. The rates of glucose uptake were calculated from the 2-DG/glucose ratios versus time curves and tissue 2-DG-P using an equation derived from a two-compartment (plasma and tissue) mathematical model [40].

Western blot for protein analysis

To detect GyK, \(\beta\)-AR, and \(\alpha\)-tubulin contents in total extracts, fragments from IBAT were collected, and the tissue samples were immediately homogenized in 100 mmol L\(^{-1}\) Tris-HCl buffer, pH 7.4, containing 1% Triton X-100, 100 mmol L\(^{-1}\) sodium pyrophosphate, 100 mmol L\(^{-1}\) sodium fluoride, 10 mmol L\(^{-1}\) EDTA, 10 mmol L\(^{-1}\) sodium orthovanadate, 2.0 mmol L\(^{-1}\) phenylmethylsulfonyl fluoride, and 0.1 mg mL\(^{-1}\) aprotinin at 4 °C. The IBAT nuclear extract was obtained as previously described [42] and was used to evaluate the expression of PPAR\(\gamma\) and histone. The total protein concentration was determined by the Bradford method [43]. Samples containing 100 µL of total extract or nuclear extract from each experimental group were incubated for 5 min at 80 °C with 4 \(\times\) concentrated Laemmli sample buffer (0.25 mmol L\(^{-1}\) Tris-HCl buffer at pH 6.8, 0.5% bromophenol blue, 50% glycerol, 10% sodium dodecyl sulfate, and 50 mmol L\(^{-1}\) dithiothreitol) (4:1, v/v). IBAT proteins (100 µg for the detection GyK, \(\beta\)-AR, and \(\alpha\)-tubulin and 40 µg for the detection of PPAR\(\gamma\) and histone) were separated by 10% SDS-PAGE, transferred to nitrocellulose membranes and blotted with antibodies against GyK, \(\beta\)-AR, \(\alpha\)-tubulin, PPAR\(\gamma\), and histone. Specific bands were detected with the Super Signal West Pico chemiluminescent substrate, and the band intensity was quantified using the Scion Image Program (version 4.03, Frederick, MD, USA). The GyK and \(\beta\)-AR band intensities were normalized by the \(\alpha\)-tubulin band intensity, and the PPAR\(\gamma\) band intensity was normalized by the histone band intensity. There was no difference between the \(\alpha\)-tubulin band intensity and the histone band intensity of the different groups. The results were expressed as a relative ratio.

Insulin signaling studies

To examine the changes in the intracellular insulin signaling pathway in the IBAT from the control and LPHC groups, rats from both groups were fasted for 5 h and were then intraperitoneally injected with saline or insulin (10 µg/kg body weight). After 10 min, the animals were sacrificed and the IBAT was quickly removed. The protein levels of the insulin signaling cascade components insulin receptor (IR\(\beta\)) and AKT and the phosphorylation levels of AKT (serine-473 residue) were determined through Western blotting, as described above.

Statistical analysis

Levene’s test for the homogeneity of variances was initially used to determine whether the data satisfied the assumptions for a parametric analysis of variance. When necessary, the data were log transformed to correct for variance in heterogeneity or non-normality [44]. The sympathetic contribution in the FA synthesis data was analyzed using a two-way variance analysis followed by Tukey’s post hoc test. The statistical significances of all of the other experiments were analyzed using Student’s t test for independent samples. The statistical analysis was performed using the Stata software package (Tulsa, OK, USA). All data were expressed as the mean \(\pm\) SE for the number of rats indicated. P < 0.05 was taken as the criterion of significance.

Results

Administration of the LPHC diet induced an increase of 26% in relative daily food ingestion, followed by similar increases in weight of the IBAT after 15 d (Table 2), as has been observed previously [19]. Our data also confirmed that the LPHC diet-fed rats had a lower body weight (13%) and alterations in the chemistry of body composition relative to the control rats. The absolute lipid content in the IBAT was approximately 38% higher than that of the control diet-fed rats (Table 2).

The evaluation of the insulin signaling pathway showed that the administration of an LPHC diet resulted in an increase in the IR\(\beta\) content by approximately 120% (Fig. 1A) but did not alter the
Table 2
Initial and final body weight, relative daily food intake, IBAT weight, and lipid content of rats fed control or LPHC diet for 15 d

<table>
<thead>
<tr>
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<th>Control diet</th>
<th>LPHC</th>
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<tr>
<td>Initial body weight (g)</td>
<td>100.8 ± 1.3</td>
<td>99.4 ± 1.9</td>
</tr>
<tr>
<td>Final body weight (g)</td>
<td>148.3 ± 3.4</td>
<td>129.0 ± 3.1</td>
</tr>
<tr>
<td>Food intake (g 100 g bw⁻¹ df⁻¹)</td>
<td>10.5 ± 0.2</td>
<td>13.2 ± 0.4</td>
</tr>
<tr>
<td>IBAT weight (g)</td>
<td>0.369 ± 0.023</td>
<td>0.456 ± 0.031</td>
</tr>
<tr>
<td>IBAT lipid content (g tissue⁻¹)</td>
<td>0.148 ± 0.014</td>
<td>0.204 ± 0.014</td>
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IBAT, interscapular brown adipose tissue; LPHC, low-protein, high-carbohydrate diet.

* Values are expressed as the means ± SE of five to seven animals per diet group.

1 P < 0.01 versus control, as determined by Student’s t test.

IBAT glucose uptake from LPHC diet-fed rats, as estimated in vivo by 2-deoxy-[¹⁴C] glucose, was 3.5-fold higher than control diet-fed rats and directly reflected the FA synthesis from glucose (Figs. 4 and 5). In Figure 5, we can observe that the LPHC diet induced an increase in the de novo total FA synthesis and an increase in the contribution of glucose to the total FA synthesis. The contribution of glucose to the total FA synthesis is approximately 12% in the IBAT of the control diet-fed rats and 18% in the LPHC diet-fed rats. The increase in FA synthesis was accompanied by higher (42%) ATP-CLY activity in the IBAT from LPHC rats without changes in the activities of G6PDH and ME, two enzymes that are involved in the generation of reducing equivalents necessary for biosynthetic processes in the cell. ATP-CLY is responsible for the production of activated acetyl units (acyl-CoA) from several substrates for de novo FA synthesis. LPL activity also did not differ between the two groups (Table 3), suggesting that the increase in FA uptake from circulating lipoproteins in the IBAT was not determinant on the increase in lipid content in this tissue. The increase in ATP-CLY activity reinforces the importance of de novo FA synthesis, more than that of preformed FA, to TAG synthesis. In the same way, the protein content of the transcription factor PPARγ is 120% higher in the IBAT of LPHC compared with that of the control diet-fed rats (Fig. 6). This result appears to be associated with the increase in FA synthesis in the IBAT of LPHC diet-fed rats.

The rates of G3P generation from glucose and from non-glucose substrates, as evaluated by the simultaneous administration of [³H₂O] and [¹⁴C]-glucose, demonstrate adaptation to the diet after 15 d. LPHC diet-fed rats did not change in vivo glyceride-glycerol synthesis from non-glucose substrates (glyceroneogenesis) in the IBAT of LPHC diet-fed rats; however, the
IBAT of LPHC diet-fed rats had approximately 270% faster rates of in vivo glyceride-glycerol synthesis from glucose than did control diet-fed rats (Fig. 7). The GyK content in the IBAT, which is responsible for G3P generation from glycerol phosphorylation, was not altered by the LPHC diet (Fig. 8).

Discussion

This investigation was conducted to better understand the adaptive mechanisms developed by rats subjected to the LPHC diet in the growing phase for maintenance of the energetic and nutrient homeostasis. As previously demonstrated, total FA synthesis and FA synthesis from glucose was increased in the liver (an important organ in the reduction of postprandial glucose levels) of LPHC diet-fed rats; however, glycogen content was not increased. Glycerol seems to contribute more than glucose to increases in FA and G3P synthesis [22]. Due to the importance of the BAT, together with liver and muscle in the glucose clearance [10] in fed animals, we proposed in this work to study the utilization of the diet glucose by IBAT. The increase in the glucose uptake by IBAT of LPHC diet-fed rats (~3.5-fold) compared with the uptake by IBAT of control diet-fed rats shows the relevance of the IBAT for reduction of postprandial glucose levels in plasma in these animals. Recent research has shown that the glucose uptake by BAT is 20-fold greater than that by visceral WAT (by mg of tissue) and 4-fold greater than that of gastrocnemius and tibialis anterior muscles in mice in basal conditions [14]. These findings, together with our observations, reveal an important role for BAT in glucose metabolism.

The increase of IRβ content and AKT phosphorylation-insulin stimulated suggest a higher sensitivity of the tissue to insulin according to the higher glucose uptake in IBAT. However, in BAT, some processes are regulated by the interplay between insulin and adrenergic stimulation [45]. Studies show that electrical stimulation of the sympathetic nerves entering BAT or norepinephrine infusion produced a marked increase in 2-DG uptake in BAT. The impairment in the 2-DG uptake in BAT by β3-AR blockade, but not by α-adrenergic receptor blockade, shows that β3-AR is more involved in this action in BAT. Currently, it is clear that glucose uptake is activated through two different pathways:
1) via insulin increasing the expression and translocation of the glucose transporter (GLUT) 4 isoform and 2) via the sympathetic nervous system increasing GLUT1 isoform [10,15,46,47]. We previously demonstrated that the sympathetic flux to IBAT is increased in the LPHC fed rats [19]. However, our results showed that β3-AR content in the IBAT of LPHC diet-fed rats is reduced (50%) relative to that of the control diet-fed rats. However, even considering the reduction in the levels of the β3-AR, the increase of de novo FA synthesis in the IBAT of LPHC rats suggests a higher adrenergic stimulation. Additionally, the FA synthesis in the denervated side of IBAT of the LPHC diet-fed rats is reduced by 85%, whereas the FA synthesis in the denervated side of IBAT of the control diet-fed rats is reduced 70% compared with the contralateral innervated side. The reduction in β3-AR can be explained by increases in the insulin sensitivity in the IBAT of LPHC diet-fed rats because its expression is down-regulated by insulin. Long-term insulin exposure is associated with a reduction in β3-AR mRNA in WAT and BAT. The reduction in transcription β3-AR was also observed in adipose tissues after 6 h for euglycemic hyperinsulinemic glucose clamp [48]. It was previously demonstrated that the fasted/fed transition has been accompanied by decreases within a few hours in β3-AR mRNA and in the adrenergic response from a direct effect of the insulin [48]. This adaptive mechanism is essential to the control of lipid storage. The results discussed up until now have showed the importance of the de novo FA synthesis for increases of the TAG storages in the IBAT of LPHC diet-fed animals. This hypothesis is corroborated by an increase in the expression of the PPARγ, which is directly related to the expression of the lipogenic proteins [11] and an increase in the ATP-CLY activity that provides acetyl-CoA for de novo FA synthesis. The similar LPL activity in the IBAT of LPHC rats and of control rats also supports this hypothesis because this finding suggests that the use of preformed FA was not altered by the LPHC diet. Studies have demonstrated the importance of the LPL activity and the ability of the BAT in the reduction of elevated triacylglycerol concentrations in bloodstream [13]. However, the LPL activity may be altered in several situations; for example, as a result of alteration in diet composition [49,50].

In the same way as FA synthesis, our results showed that the generation of G3P from glucose is activated to produce G3P in a rate adequate for the esterification of FA. The unaltered content of GyK and the similar synthesis of G3P from non-glucose substrates suggest that glycerol phosphorylation and glyceroenogenesis are not determinants in the increase of TAG in the IBAT of LPHC diet-fed rats. Despite the direct relation of the GyK activity with sympathetic flux in the IBAT in animals subjected to different conditions that have been observed [24,51], the positive

![Fig. 6. The effect of the low-protein, high-carbohydrate diet on the peroxisome proliferator-activated receptor gamma (PPARγ) content in interscapular brown adipose tissue. The results are expressed as the means ± SE; control (n = 6) and LPHC (n = 5). *P < 0.05 versus control group (Student’s t test). C, control diet; LPHC, low-protein, high-carbohydrate diet.](Image 60x92 to 267x249)

![Fig. 7. The effect of the low-protein, high-carbohydrate diet on in vivo TAG-glycerol synthesis from glucose via glycolysis and non-glucose substrates via glyceroenogenesis in interscapular brown adipose tissue. The results are expressed as the means ± SE; 6–7 rats per diet group. *P < 0.05 versus control group (Student’s t test). C, control diet; LPHC, low-protein, high-carbohydrate diet.](Image 40x92 to 267x249)

![Fig. 8. The effect of the low-protein, high-carbohydrate diet on the glycerokinase (GyK) content in interscapular brown adipose tissue. The results are expressed as the means ± SE; control (n = 6) and LPHC (n = 6). P < 0.05 versus control group (Student’s t test). C, control diet; LPHC, low-protein, high-carbohydrate diet.](Image 40x92 to 267x249)

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Malic enzyme, glucose-6-phosphate dehydrogenase, ATP-citrate lyase, and lipoprotein lipase activity in the IBAT of rats fed control or LPHC diet for 15 d</th>
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<tbody>
<tr>
<td>Malic enzyme (nmol·mg prot.⁻¹·min⁻¹)</td>
<td>723.3 ± 97.3 681.4 ± 85.2</td>
</tr>
<tr>
<td>G6PDH (nmol·mg prot.⁻¹·min⁻¹)</td>
<td>1338.8 ± 150.6 1800.9 ± 140.0</td>
</tr>
<tr>
<td>ATP-citrate lyase (nmol·mg prot.⁻¹·min⁻¹)</td>
<td>564.8 ± 43.0 803.6 ± 64.3</td>
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<tr>
<td>LPL (nmol FA·g⁻¹·min⁻¹)</td>
<td>365.2 ± 54.2 292.8 ± 47.2</td>
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ATP, adenosine triphosphate; FA, fatty acid; G6PDH, glucose-6-phosphate dehydrogenase; IBAT, interscapular brown adipose tissue; LPHC, low-protein, high-carbohydrate; LPL, lipoprotein lipase; prot, protein

* Values are expressed as the means ± standard error of six to seven animals per diet group.

1 P < 0.05 versus control, as determined by Student’s t test.
relating these two parameters was not observed in the IBAT of LPHC diet-fed rats. Reports in the literature show other situations in which this relation was also not observed [52]. Thus, future research will be needed to clarify other factors involved in GyK expression in BAT.

Finally, we concluded that the increase in the adrenergic stimulation and the higher sensitivity to insulin in the IBAT resulting from the LPHC diet administration led to a higher glucose uptake by IBAT, and, consequently, to a higher clearance of glucose with reduced glycaemia of LPHC diet-fed rats compared with that of control diet-fed rats. The FA and G3P synthesis are glucose utilization pathways that are activated in the IBAT by a LPHC diet.

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