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Basic nutritional investigation

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

Suélem Aparecida de França M.Sc. [a], Maísa Pavani dos Santos M.Sc. [a], Roger Vinícius Nunes Queiroz da Costa [a], Mendalli Froelich M.Sc. [a], Samyra Lopes Buzelle M.Sc. [b], Valéria Ernestânia Chaves Ph.D. [c], Morenna Alana Giordani [a], Mayara Peron Pereira M.Sc. [a], Edson Moleta Colodel Ph.D. [d], Cláudia Marlise Balbinotti Andrade Ph.D. [a], Nair Honda Kawashita Ph.D. [a,*]

[a] *Department of Chemistry, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil*
[b] *Department of Biochemistry and Immunology, School of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil*
[c] *Laboratory of Physiology and Pharmacology, Federal University of São João Del Rei, Divinópolis, Minas Gerais, Brazil*
[d] *Department of Veterinary Science, Federal University of Mato Grosso, Cuiabá, Mato Grosso, Brazil*

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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 (~100 g) for 15 d. Total FA and G3P synthesis and the synthesis of FA and G3P from glucose were evaluated in vivo by $^{3}H_2O$ and ^{14}C-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; $P \leq 0.05$).
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.

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

Researchers SAF and MPS. analyzed fatty acid and glycerol synthesis and carried out Western blot analyses. RVNQC, MF, and MAG carried out Western blot analyses. SLB and MPP determined glucose uptake in brown adipose tissue, and EMC assisted in experimental procedures. Researchers VEC and CMBA read the manuscript and contributed to the discussion. NHK designed the experiment, helped analyze the data, wrote the manuscript, and supervised the project. None of the authors have financial or non-financial competing interests.

* Corresponding author: Tel.: +55 to 65 to 3615 to 8765; Fax: +55 to 65 to 3615 to 8005.
E-mail address: Nairhonda@terra.com.br (N. H. Kawashita).

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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 $^{3}H_2O$ (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 $kJ.g^{-1}$) (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 hemidenervation 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 ^{14}C from glucose and ^{3}H from $^{3}H_2O$ (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-^{14}C]glucose (10 μCi) and $^{3}H_2O$ (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-^{14}C]glucose-specific activity (SpA). Each animal was sacrificed by cervical dislocation immediately after obtaining the

Table 1
Compositions ($g \cdot kg^{-1}$) of the control and LPHC diets

Ingredient	Control diet (17%)	LPHC diet (6%)
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

LPHC, low-protein, high-carbohydrate
* For detailed composition, see [23].