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A low-protein, high-carbohydrate diet increases de novo fatty acid synthesis from glycerol and glycerokinase content in the liver of growing rats

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ABSTRACT

We had previously shown that adipose tissue increased in rats fed a low-protein, high-carbohydrate (LPHC) diet (6% protein, 74% carbohydrate) without a simultaneous increase in the de novo fatty acids (FA) synthesis. In addition, impairment in insulin signaling in adipose tissues was observed in these rats. For this study, we hypothesized that the insulin signaling pathway is preserved in the livers from these rats, which contributes to an increase in liver lipogenesis and, consequently, an increase in the weight of the adipose tissue. We also hypothesized that glycerol from triacylglycerol is an important substrate for FA synthesis. Our results showed that administration of the LPHC diet induced an increase in the in vivo rate of total FA synthesis (150%) as well as FA synthesis from glucose (270%) in the liver. There were also increased rates of [U-^{14}C]glycerol incorporation into glyceride-FA (15-fold), accompanied by increased glycerokinase content (30%) compared with livers of rats fed the control diet. The LPHC diet did not change the glycerol-3-phosphate generation from either glucose or glyceroneogenesis. There was an increase in the insulin sensitivity in liver from LPHC-fed rats, as evidenced by increases in IR_{β} (35%) levels and serine/threonine protein kinase (AKT) levels (75%), and basal (95%) and insulin-stimulated AKT phosphorylation (105%) levels. The LPHC diet also induced an increase in the liver sterol regulatory element-binding protein–1c content (50%). In summary, these data confirmed the hypothesis that lipogenesis and insulin signaling are increased in the livers of LPHC-fed rats and that glycerol is important not only for FA esterification but also for FA synthesis.

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Abbreviations: ACC, acetyl-CoA carboxylase; AKT, Serine/threonine protein kinase; C, control; EWAT, epididymal white adipose tissue; FA, fatty acids; G3P, glycerol 3-phosphate; glyceride-GLY, glyceride-glycerol; GyK, glycerokinase; IR, insulin receptor; LPHC, low-protein, high-carbohydrate diet; PEPCK, phosphoenolpyruvate carboxykinase; RWAT, retroperitoneal white adipose tissue; SREBP, sterol regulatory element-binding proteins; TAG, triacylglycerol.

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1. Introduction

Liver and adipose tissues are directly involved in the maintenance of glucose and lipid homeostasis. In a fed state, the liver stores glucose as glycogen and excess nutrients are converted into triacylglycerol (TAG), which is secreted into the bloodstream as TAG-enriched lipoproteins (very low-density lipoprotein) [1]. Adipose tissue plays a crucial role in buffering the TAG level in circulation in the postprandial period [2] by increasing TAG clearance [2] by uptake of FA from lipoproteins very low-density lipoprotein and quilomicrons, although only a small quantity of glycerol from TAG hydrolysis is used by the adipose tissue. Our recent findings support this hypothesis since they show that the use of preformed FA for glyceride synthesis is high in the adipose tissue of control rats [3].

For many years, our research group has used diets with different macronutrient compositions to investigate the nutritional and hormonal control of energy-linked metabolic processes. We had previously confirmed [4] that body lipid content and food intake increase in rats fed a low-protein, high-carbohydrate (LPHC) diet (6% protein, 74% carbohydrate, 7% lipid) for 15 days compared to rats fed a control diet (C) (17% protein, 63% carbohydrate, 7% lipids) [4]. Despite an increase in the lipid content in the adipose tissues and a higher caloric intake from carbohydrates and lipids by LPHC-fed rats, de novo fatty acids (FA) synthesis in the epididymal white adipose tissue (EWAT) was similar to that of the control diet-fed rats [5]; although, the rate was reduced in the retroperitoneal white adipose tissue (RWAT) [6]. Other experiments indicated that these findings may be related to the impairment in insulin signaling in EWAT (unpublished data) and RWAT [6]. Since LPHC diet-fed rats showed lower insulinemia and similar post-prandial glycemia [4] compared to control-diet fed rats, we considered the possibility of an increase in the sensitivity to insulin in other tissues such as liver, muscle, and brown adipose tissue in the LPHC-fed rats. Studies showing that insulin resistance does not develop simultaneously in all tissues corroborate this possibility [7]. One of the pathways stimulated by insulin to buffer blood glucose in the liver of fed rats is FA synthesis, in which acetyl groups from several substrates (amino acids, glucose, glycerol) are used to form the carbon chain of FA. In the rats fed either a balanced diet or a hyperproteic, carbohydrate-free diet, non-glucose substrates are more important for de novo FA synthesis than glucose, and glucose is more frequently used for glycerol-3-phosphate (G3P) generation [8]. For both normoproteic and hyperproteic-diet fed rats, amino acids, in addition to glucose, are important precursors for FA synthesis. However, it seems unlikely that the amino acids are converted to fat in rats given an LPHC diet.

We proposed that the high glycerokinase (GyK) activity in the liver compared to other tissues [5,9] allowed for most of the glycerol available from TAG hydrolysis (from diet or liver) to be phosphorylated to G3P and its use for FA esterification as well as for either the formation of glucose or the repurposing to other metabolic destinations such as FA synthesis, while glyceroneogenesis [8] and glucose would complement each other in providing the G3P required for TAG synthesis [3]. Thus, we hypothesized that despite the impairment in insulin signaling in RWAT and EWAT, the insulin signaling in the livers of LPHC-fed rats will not be impaired. The insulin signaling preservation is essential to the increase in body TAG levels and the energetic gain in LPHC-fed rats. We also believe that glycerol in livers (more than glucose) is an important substrate for FA esterification and FA synthesis. To test this hypothesis, we evaluated the following: (i) the rate of in vivo FA and glycerol synthesis using [U-^{14}C]glucose and $^{3}H_2O$; (ii) incorporation of [1-^{14}C]pyruvate into glyceride-glycerol (glyceride-GLY) and [U-^{14}C]glycerol into glyceride-fatty acids (glyceride-FA); and (iii) the content of the proteins involved in the insulin signaling pathway, as well as the contents of the enzymes GyK and phosphoenolpyruvate carboxykinase (PEPCK); and of isoform 1c of the transcription factor sterol regulatory element-binding protein (SREBP-1c).

We believe that understanding the mechanisms that lead to lipid accumulation in the rats that consumed the LPHC diet can provide insight into the prevention of diseases, since the administration of this type of diet in growing children is associated with obesity and metabolic syndrome in adults.

2. Methods and materials

2.1. *Animals and treatment*

The experiments conducted for this study were approved by the Ethics Committee of the Federal University of Mato Grosso (protocol no. 23108.029611/09-7). Male Wistar rats (7-12 animals), with an initial body weight of approximately 90 to 100 g (30 days of age), were randomly divided into two groups and received their respective diets for 15 days. The control rats were fed a diet comprised of 17% protein, 63% carbohydrates, and 7% lipids, whereas the LPHC-fed rats received a diet comprised of 6% protein, 74% carbohydrates, and 7% lipids [10]. The decrease in dietary protein was compensated with an increase in carbohydrates to maintain isocaloric diets (16.3 kJ g^{-1}, Table 1). The rats were maintained in individual metabolic cages at 22ºC ± 1ºC with a 12-hour light:dark cycle. The rats received water and food ad libitum. The body weight and food intake of each rat was recorded daily. The experiments were performed between 08:00 and 10:00 AM, and all of the rats were euthanized on day 15 of treatment. The rats were housed according to the Brazilian College of Animal Experimentation Rules.

Table 1 – Ingredient composition (g kg^{-1}) of the control and 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-cysteine	3	1
Choline bitartrate	2.5	2.5

* For a more detailed composition, Ref. see [13].