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A low-protein, high carbohydrate diet induces increase in serum adiponectin and preserves glucose homeostasis in rats

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Abstract: The aim of this study was investigate the effects of a low-protein, high-carbohydrate (LPHC) diet introduced to rats soon after weaning. The animals were distributed in the following groups: $LPHC_{45}$: fed an LPHC diet (6%-protein, 74%-carbohydrate) for 45 days; C_{45}: fed a control (C) diet (17%-protein, 63%-carbohydrate) for 45 days; R (Reverse): fed with LPHC for 15 days followed by C diet for 30 days. The $LPHC_{45}$ group showed alterations in the energetic balance with an increase in brown adipose tissue, and in glucose tolerance, and lower final body weight, muscle mass and total protein in blood when compared with C_{45} group. The HOMA-IR index was similar between $LPHC_{45}$ and C_{45} groups, but this parameter was lower in $LPHC_{45}$ compared with R groups. Serum adiponectin was higher in $LPHC_{45}$ group than C_{45} and R groups. The R group presented higher fed insulin than C_{45} and $LPHC_{45}$ and higher T4 compared with C_{45} group. Total cholesterol in R group was higher when compared with $LPHC_{45}$ group. Thus, the data show that the change of the diet LPHC for a balanced diet led to different metabolic evolution and suggest that the different response can be due to different levels of adiponectin.

Key words: low-protein, high-carbohydrate diet, post-weaning period, HOMA-IR, adiponectin.

INTRODUCTION

Studies have shown that the composition of nutrients consumed by mothers during pregnancy or infants during the first year of life can exert permanent and powerful effects on developing tissues and their function (Langley-Evans 2015). In several occidental societies, during the transition between breastfeeding and weaning, children intake a higher amount of carbohydrate and a lower amount of protein than is recommended for this stage of life (Ramalho et al. 2013).

Aparecida de França et al. (2009), in previous studies in our laboratory, observed that rats maintained on a low-protein, high-carbohydrate (LPHC) diet (6%-protein, 74%-carbohydrate) for

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15 days, introduced soon after weaning, ingested a greater amount of food and calories and showed an increase in the energy gain when compared with rats fed on a normal (control) diet (17%-protein and 63%-carbohydrate). Reduction in the body weight was also observed, although there was an increase in the body lipids in those animals characterizing an adiposity state. Along with these alterations, rats treated with LPHC diet had higher levels of leptin (Aparecida de França et al. 2009), corticosterone and tumor necrosis factor-alpha (TNFα) in the blood (Santos et al. 2012). Leptin, a hormone primarily synthesized in adipose tissues, normally exhibits a direct relationship with adipose mass, and its action on the hypothalamic neurons inhibits the secretion of orexigenic peptides while increasing secretion of anorexigenic peptides, resulting in reduced food intake (Jequier 2002, Valassi et al. 2008). An increase in serum leptin along with hyperphagia suggests leptin resistance in rats fed an LPHC diet, a condition frequently seen in several types of obesity in humans and rats (Myers et al. 2008, 2010). In turn, high levels of TNFα and corticosterone are associated with insulin resistance due to impairments in the intracellular signaling cascade (Piroli et al. 2007). Although hyperglycemia was not observed in rats fed an LPHC diet, there was a reduction in IRS-1, AKT content and in insulin-stimulated AKT phosphorylation in the retroperitoneal adipose tissue suggesting impaired insulin signaling (Santos et al. 2012). The prevalence of obesity and its association with metabolic syndrome has increased its importance to public health over the last several decades. Metabolic syndrome refers to a group of risk factors associated with overweight and obesity (Grundy 2005, Tkac 2005, Ginsberg and MacCallum 2009, Singh et al. 2010) and is defined as a combination of any three of these disorders: high blood pressure, central adiposity, high serum triglycerides, low serum HDL-cholesterol, and high fasting glycemia (Armitage et al. 2004). Other authors have suggested the inclusion of additional criteria such as oxidative stress, leptin resistance (Arch et al. 1998) and an index of inflammation as TNFα or Interleukin (Reilly and Rader 2003). Based on these references, rats in the growing stage fed with the LPHC diet for 15 days showed several alterations associated with obesity and metabolic syndrome.

Thus, the objective of this investigation was to verify the effects of an LPHC diet introduced soon after weaning, for a longer period (45 days) and rats fed on an LPHC diet for 15 days and then fed a balanced diet for further 30 days, in this case, the objective was to compare the effects of the change for a balanced diet, after a short period on LPHC diet.

MATERIALS AND METHODS

ANIMALS AND TREATMENT

Male Wistar rats weighing approximately 100 g (30 days old) were randomly divided into three groups: 1) a control (C_{45}) group, fed a control diet containing 17%-protein and 63%-carbohydrate (AIN-93G; Reeves et al. 1993) for 45 days; 2) a low-protein, high-carbohydrate ($LPHC_{45}$) group, fed a diet containing 6%-protein and 74%-carbohydrate for 45 days; and 3) a reverse (R) group, fed an LPHC diet for 15 days followed by a control diet for 30 days. The diets were isocaloric (16.3 $kJ \cdot g^{-1}$) in that the calories lost to reduced protein in the LPHC diet were replaced by the calories added by increased carbohydrates (Aparecida de França et al. 2009, Buzelle et al. 2010, Santos et al. 2012, Menezes et al. 2013, Pereira et al. 2017, Silva et al. 2018). Rats were housed individually in metabolic cages in an environmentally-controlled room (light from 6 AM to 6 PM; 23 ± 1°C) and had free access to food and water. Body weight and water and food intake were recorded daily for each rat. Animals were maintained according to the Brazilian College of Animal Experimentation, and

the study was approved by the Ethics Committee of the Federal University of Mato Grosso (Protocol No. 23108.045355/12-7).

SAMPLE COLLECTION AND BIOCHEMICAL ANALYSES

All rats were killed by decapitation between 7 AM and 10 AM on 45th day of treatment. Blood samples were collected into anticoagulant-containing tubes to determine, using commercial kits, glucose (Labtest®), urea (Labtest®), corticosterone (Cayman Chemical Company, Ann Arbor, MI, USA), glucagon (Wako Chemicals, Inc., Bellwood Road Richmond, VA, USA), leptin (Société de Pharmacologie et d'Immunologie – BIO. Montigny le Bretonneux, France), triiodothyronine (T3), thyroxine (T4) (Interkit, Inc., Vintage Park Drive Foster City, CA, USA), adiponectin (R&D Systems, Minneapolis, MN, USA) and TNFα (Pierce Biotechnology, Rockford, IL, USA); serum were obtained to determine the concentrations of protein (Labtest®), triglycerides (Labtest®), total cholesterol (Labtest®), aspartate aminotransferase (AST) (Labtest®), and alanine aminotransferase (ALT) (Labtest®). The liver, extensor digitorum longus (EDL) and soleus muscles, interscapular brown adipose tissue (IBAT), epididymal, retroperitoneal and perirenal white adipose tissues were removed and weighed. Groups of rats were fasted for either 12 h or 14 h and sample of blood were collected for determination, using commercial kits, concentration of insulin (Millipore Res., St. Charles, MO, USA), glucose (Labtest®), and free fatty acids (FFA) (Wako Chemical®, VA, USA).

The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated for each rat as follows:

HOMA-IR = [fasting insulin ($\mu U \cdot mL^{-1}$) x fasting glucose ($mmol \cdot L^{-1}$)] / 22.5

This index is a predictor of insulin resistance (Bowe et al. 2014).

BIOMETRIC PARAMETERS

On day 45, the body mass index (Lee Index) was calculated using the formula (Lee 1928):

$$\text{Lee Index} = \frac{\sqrt[3]{\text{body weight (g)}} \times 1000}{\text{ANL (cm)}}$$

where ANL is the anus-nasal length. The Lee Index is used as an index of obesity in rodents (Myers et al. 2010).

CARCASS COMPOSITION AND ENERGY INTAKE

Carcasses were eviscerated, weighed and stored at -20ºC. They were used for determination of the body chemical composition and energetic balance.

Carcass composition was determined as described by Aparecida de França et al. (2009). Water content was measured as the difference between wet and dry weights, the latter obtained by oven-drying the carcass to a constant weight. Fat content was calculated by subtracting the fat-free dry mass after extraction with petroleum ether from the dry carcass weight. Ash content was estimated following combustion to a constant weight. Protein content was determined by subtracting the water, fat, and ash contents from the wet carcass weight. To determine the energy gain, an energy baseline was assessed in a group of weaned rats just before introduction to the assigned diet. Energy gain was calculated as the difference between the carcass energy at the end of the experiment and the carcass energy on the first day (baseline), as previously described (Ferreira et al. 2007, Aparecida de França et al. 2009). Carcass energy was calculated as previously described (Aparecida de França et al. 2009).

LIPID AND GLYCOGEN DETERMINATION

Hepatic lipid content was determined using gravimetric methods after chloroform-methanol (2:1) extraction according to Folch et al. (1957). The method of Carroll et al. (1956) was used to determine hepatic and muscle glycogen content.

ORAL GLUCOSE TOLERANCE TESTE (OGTT)

The OGTT was determined as described by Pereira et al. (2014). Rats received, after 15 h of fasting, a load of 2.5 g of glucose·kg^{-1} (orogastric gavage). Plasma glucose was measured in blood withdrawn from the tip of the tail using an Accu-Chek II blood glucose monitor, before load (t=0), and 15, 30, 60, 90, and 120 min after glucose administration.

STATISTICAL ANALYSIS

Data were collected, and were subjected to statistical analysis using the Statistics for Windows program (StatSoft, USA) and the GraphPad Prism program. Levene's test for homogeneity of variances was initially used to determine whether the data complied with the assumptions required for parametric analyses of variance. Between-group differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Log transformations were performed to correct heterogeneity in the data of insulin serum in the fed state (Sokal and Rohlf 1995). Food intake data were analyzed by repeated-measures one-way ANOVA. Significance was recognized when $p < 0.05$. The data are presented as mean ± standard error of the mean.

RESULTS

FOOD AND PROTEIN INTAKE, BODY WEIGHT GAIN, LEE INDEX

The data on food intake was normalized by body weight and are presented as g·100g^{-1} b.w. The $LPHC_{45}$ rats showed higher food ingestion than the C_{45} rats, since the first day until one week before the end of the treatment. However, in the last week, the difference started reducing and, on the 45th day of treatment, the food intake of rats of the $LPHC_{45}$ group was similar to C_{45} group. In the first 15 days, when they received the LPHC diet, R rats also had higher food intake, but after 5 days on C diet, they reduced food intake to levels similar to C_{45} rats (Figure 1). $LPHC_{45}$ and R rats ingested about 50% and 20% less protein than C_{45} rats, respectively, although they had ingested more food in the period (27% and 19% respectively) when compared with C_{45} rats (Table I).

The daily body weight gain in C_{45} rats was ~7 g·day^{-1} in the first 15 days and ~5 g·day^{-1} after this period. Rats of $LPHC_{45}$ group showed a body weight gain of about 4 g·day^{-1}. The lower daily body weight gain in $LPHC_{45}$ rats resulted in a lower total body weight than in C_{45} group at the end of the study. The daily body weight gain in rats of R group was from 4 g·day^{-1} to 6 g·day^{-1} when the LPHC diet was changed to C diet (Supplementary Material – Figure S1). At the end of the study, the R rats reached the same body weights as C_{45} rats (Table II). The Lee index was similar among all groups at 45th day (Table II).

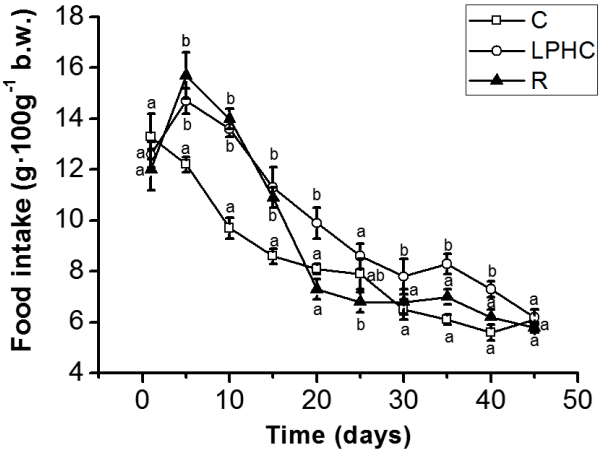

Figure 1 - Relative food intake (g · 100g^{-1}·b.w.) of rats fed with the control diet (C_{45}= □), low-protein, high-carbohydrate diet ($LPHC_{45}$= ○) for 45 days and rats treated with the LPHC diet for 15 days, followed by the control diet – reverse (R) group (R= ▲) until the 45th day. The results are expressed as the means ± standard error of the mean every five days; C_{45} (n=11), $LPHC_{45}$ (n=10) and R (n=10). Different letters represent significant differences among groups. Repeated measures one-way ANOVA. Treatment (between columns) F= 23.54; Individual (between rows) F= 5.94 ($p<0.05$).

TABLE I
Relative values of daily food intake, total food intake, and total protein intake in C_{45}, $LPHC_{45}$, and R rats.*

Variable	C_{45}	$LPHC_{45}$	R	F
Daily food intake ($g \cdot 100g^{-1}$ b.w.)				
(1-15 days)	11.7±0.3[a]	14.9±0.5[b]	14.9±0.5[b]	21.33
(16-45 days)	6.7±0.2[a]	8.6±0.2[b]	7.4±0.3[a]	21.38
Total food intake ($g \cdot 100g^{-1}$ b.w.)				
(1-45 days)	376.4±4.8[a]	479.8±6.9[b]	446.7±7.6[c]	72.36
Total protein intake ($g \cdot 100g^{-1}$ b.w.)				
(1-45 days)	64.0±0.8[a]	28.9±0.4[b]	51.3±1.0[c]	525.3

C_{45}, control group; $LPHC_{45}$, low-protein, high-carbohydrate group; R, reverse group; F values for ANOVA. *Values are expressed as the means ± standard error of the mean of 10-11 animals per diet group. Mean values with different lowercase letters are significantly different $p<0.05$, as determined by one-way ANOVA.

WEIGHT OF THE TISSUES

The $LPHC_{45}$ group showed a reduction in the relative weight ($g \cdot 100g^{-1}$ b.w.) of the EDL muscle and an increase in the weight of IBAT when compared with C_{45} and R rats. The relative weight of the white adipose tissues (perirenal, epididymal and retroperitoneal) and liver were not different among groups. All the tissues evaluated in R group showed relative weights similar to C_{45} rats (Table III).

LIPID AND GLYCOGEN CONTENT

The lipid content in the liver was similar in C_{45} and R rats, but it was higher in $LPHC_{45}$ rats, when compared with R group. Additionally, the hepatic glycogen content was four-fold higher in $LPHC_{45}$ rats than in C_{45} and R rats. Glycogen contents in the soleus and in the EDL muscles were similar among groups (Table III).

CHEMICAL COMPOSITION AND ENERGETIC BALANCE

The weight of the carcass was similar among groups (Table IV), but the chemical composition, in percentage, was altered in the $LPHC_{45}$ group when compared with C_{45} group. The proteins constituted 21.1%, the fat 14.0% and ashes 6.2% in rats treated with C diet and those percentages were altered in $LHPC_{45}$ group, respectively: 16.5%, 21.7% and 7.6%. In rats of R group, the percentages were: 20.0% protein, 16.9% fat and 6.3% ash. R groups showed higher content of protein and lower content of ashes than $LPHC_{45}$ group. The water content in the carcass was similar among the groups. (Table IV).

The $LPHC_{45}$ rats ingested higher total energy than C_{45} rats in the period of 45 days, and showed an increase in the energy expenditure (Table IV). Neither the waste energy nor energy gain were statistically different in R when compared with C_{45} rats. They ingested higher total calories than C_{45} rats but lower than $LPHC_{45}$ rats.

METABOLITES, HORMONES AND CYTOKINES IN THE BLOOD AND HOMA-IR INDEX

$LPHC_{45}$ rats showed lower total protein (22%), urea (55%), cHDL (44%) and FFA (35%) concentration in the blood when compared with C_{45} rats. The level of total triglycerides and total cholesterol were not altered by LPHC diet. All these parameters in R rats were similar to C_{45} rats (Table V). However, when the R group is compared with $LPHC_{45}$ group, the total cholesterol and cHDL were 27% and 47% higher in R rats (Table V).

The fasting (Figure 2) and fed glycemia (Table V) were alike among the groups and only serum insulin in fed rats of the R group was higher when

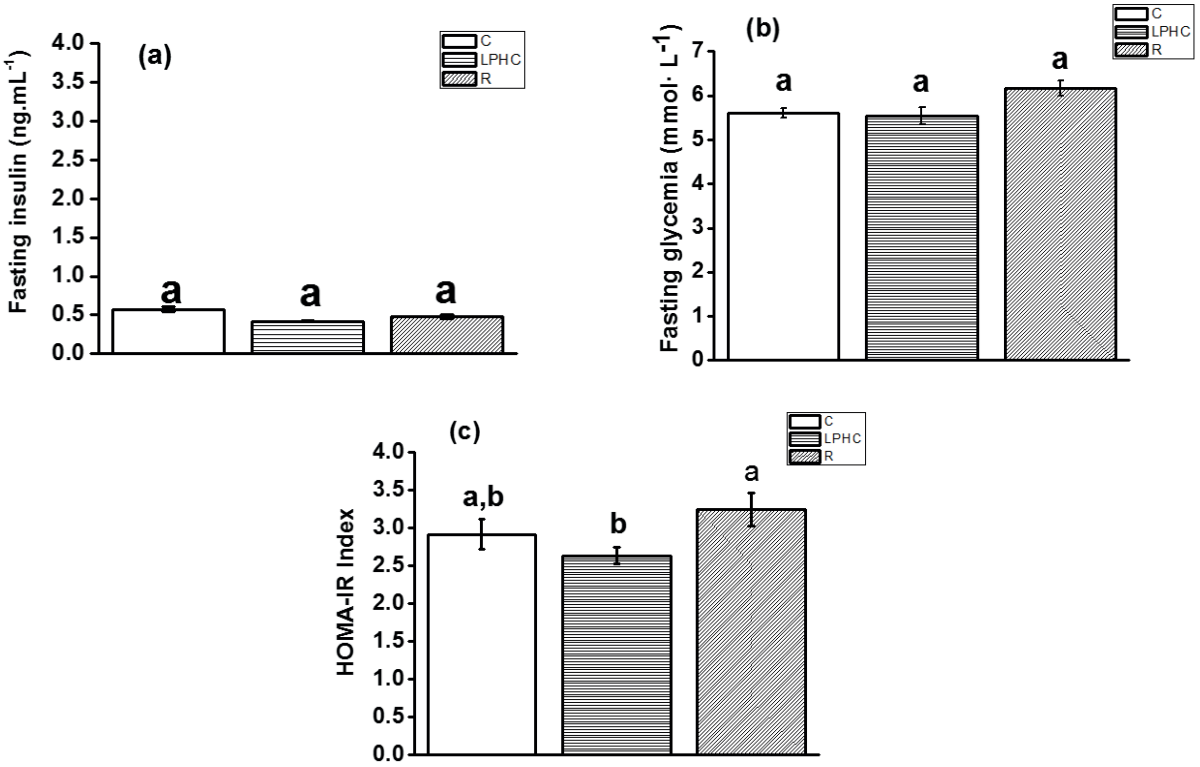

Figure 2 - Serum fasted insulin (**a**), glycemia (**b**) and HOMA-IR index (**c**) of rats fed a control diet or a low-protein, high-carbohydrate (LPHC) diet for 45 days (C_{45} and $LPHC_{45}$, respectively) and rats fed an LPHC diet for 15 days followed by the control diet for 30 days (R). Results are expressed as means ± standard errors of the means across eight animals per group. Letters indicate a significant difference among groups. One-way ANOVA, F = 2.19 for insulin; F = 1.04 for glycemia; F = 4.40 for HOMA-IR Index ($p < 0.05$).

compared with C_{45} and $LPHC_{45}$ group (128% and 289%, respectively).

HOMA-IR Index in $LPHC_{45}$ and R groups was similar to C group, but R group showed a higher Index than $LPHC_{45}$ group (Figure 2).

The level of glucagon was similar between $LPHC_{45}$ and C_{45} group, but the rats of R group had lower glucagon level than rats of C_{45} group. No difference was observed between $LPHC_{45}$ and R groups in this parameter. The concentration of adiponectin in the plasma of $LPHC_{45}$ rats was 2-fold higher when compared with C_{45} and R rats; however, it was similar between C_{45} and R groups themselves (Figure 3). The level of T3 was increased in $LPHC_{45}$ group and T4 in R group when compared with C_{45} group, however, there was no difference between $LPHC_{45}$ and R neither in T3 nor in T4 (Table V).

The level of leptin, TNFα and corticosterone were similar among all the groups, as well as the AST and ALT transaminase measured in the blood (Table V).

ORAL GLUCOSE TOLERANCE TEST (OGTT)

Glucose tolerance in animals was evaluated by Area Under the Curve (AUC) distribution, determined by glucose concentration in the blood at different times, after overload of glucose. Rats of $LPHC_{45}$ group showed lower AUC than C. The AUC of R and C_{45} rats was similar. Therefore, comparing the three groups, only the treatment with LPHC diet for 45 days increased glucose tolerance (Figure 4).

TABLE II
The initial and final body weights and Lee indices for C_{45}, $LPHC_{45}$, and R rats.*

Variable	C_{45}	$LPHC_{45}$	R	F
Initial body weight (g)	98.5±3.2[a]	96.8±2.2[a]	95.9±1.6[a]	0.78
Final body weight (g)	368.5±3.1[a]	303.2±6.5[b]	350.0±8.9[a]	27.24
Lee index	299.7±3.2[a]	297.5±2.6[a]	294.6±2.5[a]	0.80

C_{45}, control group; $LPHC_{45}$, low-protein, high-carbohydrate group; R, reverse group; F values for ANOVA. *Values are expressed as the means ± standard error of 10-11 animals per diet group. Mean values with different lowercase letters are significantly different ($p < 0.05$), as determined by one-way ANOVA.

TABLE III
Tissue weights, liver lipid and glycogen contents, retroperitoneal, epididymal, perirenal, and interscapular brown adipose tissue (IBAT) weights, and soleus and extensor digitorum longus (EDL) muscle weights in C_{45}, $LPHC_{45}$, and R rats.*

	Tissue weights ($g \cdot 100g^{-1}$ b.w.)				Lipids ($mg \cdot g^{-1}$ of tissue)			
Variable	C_{45}	$LPHC_{45}$	R	F	C_{45}	$LPHC_{45}$	R	F
Liver	3.61±0.09[a]	3.88±0.15[a]	3.77±0.14[a]	1.10	19.1±2.1[ab]	29.5±5.7[a]	15.5±2.3[b]	3.57
Retroperitoneal	1.97±0.12[a]	2.09±0.11[a]	1.86±0.14[a]	0.86	-	-	-	-
Epididymal	2.11±0.13[a]	2.26±0.09[a]	2.07±0.19[a]	0.61	-	-	-	-
Perirenal	0.35±0.04[a]	0.35±0.02[a]	0.35±0.02[a]	1.00	-	-	-	-
IBAT	0.16±0.01[a]	0.25±0.01[b]	0.18±0.01[a]	22.33	-	-	-	-
					Glycogen ($mg \cdot g^{-1}$ of tissue)			
Liver	3.61±0.09[a]	3.88±0.15[a]	3.77±0.14[a]	1.10	21.5±5.9[a]	90.3±16.1[b]	29.7±7.2[a]	13.81
Soleus	0.083±0.003[a]	0.077±0.004[a]	0.081±0.002[a]	0.96	1.07±0.42[a]	0.82±0.30[a]	1.62±0.48[a]	0.49
EDL	0.080±0.002[a]	0.070±0.001[b]	0.077±0.002[a]	8.77	0.70±0.42[a]	0.44±0.12[a]	0.23±0.13[a]	0.97

C_{45}, control group; $LPHC_{45}$, low-protein, high-carbohydrate group; R, reverse group; EDL, extensor digitorum longus muscle; F values for ANOVA.* Values are expressed as the means ± standard error of 5-7 animals per diet group. Mean values with different lowercase letters are significantly different ($p < 0.05$), as determined by one-way ANOVA.

DISCUSSION

Our objective in this work was to verify the effects of the LPHC diet when administered to growing rats for 45 days and the effects of the exchange by C (balance) diet after a short period on LPHC diet.

Rats treated with an LPHC diet for 45 days consumed 22% more calories than the rats of the C_{45} group. However, they showed similar energy gain during this period due to the increase in energy expenditure. Thermogenesis in BAT is an important component of the energetic balance in rodents. Thermogenesis induced by a low-protein diet has previously been described and involves an increase in BAT mass, a rise in the sympathetic flux, and higher energy dissipated as heat (Stirling and Stock 1968, Rothwell et al. 1983). Consistent with that hypothesis, $LPHC_{45}$ rats showed, in addition to increased T_3 levels, higher relative food intake, higher energy expenditure, and increased IBAT weights than C_{45} rats. A previous study conducted at our laboratory (Aparecida de França et al. 2009) showed that rats fed on an LPHC diet for 15 days had a higher sympathetic flux to IBAT, activating thermogenesis and increasing UCP1 expression in the tissue via p38 MAPK and ATF2. Thus, we concluded that the adaptation to the LPHC diet reduced food efficiency in the rats and contributed

TABLE IV
Carcass composition and energetic balance in C_{45}, $LPHC_{45}$, and R rats.*

Carcass composition				
Variable	**C_{45}**	**$LPHC_{45}$**	**R**	**F**
Carcass weight (g)	271.5±9.2[a]	247.7±12.3[a]	281.5±8.9[a]	2.85
Water (%)	58.7±1.0[a]	54.2±1.2[a]	56.8±1.7[a]	2.74
Fat (%)	14.0±1.4[a]	21.7±1.9[b]	16.9±2.2[a,b]	4.36
Protein (%)	21.1±0.7[a]	16.5±0.7[b]	20.0±0.7[a]	11.78
Ash (%)	6.2±0.3[a]	7.6±0.1[b]	6.3±0.3[a]	9.63
Energetic balance				
Variable	**C_{45}**	**$LPHC_{45}$**	**R**	**F**
Daily energy intake (kJ·100g^{-1}b.w.)	83.7±1.1[a]	102.9±1.3[b]	88.1±0.4[c]	106.32
Total energy intake (kJ·100g^{-1}b.w.)	3766.8±46.9[a]	4617.1±53.6[b]	3962.5±17.4[c]	110.49
Energy gain (kJ·100g^{-1}b.w.)	827.0±47.7[a]	1000.0±67.2[a]	907.5±86.2[a]	1.58
Energy expenditure (kJ·100g^{-1}b.w.)	4188.8±80.5[a]	5185.0±129.3[b]	4394.0±77.7[a]	28.40
Carcass energy (kJ·100g^{-1}b.w.)	1055.9±41.7[a]	1252.0±59.3[a]	1128.1±81.9[a]	2.47
Baseline carcass (kJ)	618.4±29.1[a]	618.4±29.1[a]	618.4±29.1[a]	2.86

C_{45}, control group; $LPHC_{45}$, low-protein, high-carbohydrate group; R, reverse group; F values for ANOVA. *Values are expressed as the means ± standard error of the mean of 5 animals per diet group. Mean values with different lowercase letters are significantly different ($p < 0.05$), as determined by one-way ANOVA.

to the maintenance of the adipose tissue depots and Lee index similar to C_{45} rats.

The increase in food intake is an adaptation of the organism to reach its protein requirement when species are fed with a diet with low protein content (Whitaker et al. 2011). However, the lower content of total protein and urea in the blood, of protein content in the carcass and the impaired development of the muscles in rats of the $LPHC_{45}$ group show that the higher food intake was not enough to supply the animals with the requirement of proteins necessary in this phase of life. The protein intake in $LPHC_{45}$ group was 55% lower than in C_{45} rats. Miñana-Solis and Escobar (2008) found similar results with post-weaned rats (25 days old) receiving a low-protein (6%) diet for 30 days. They had lower body mass gains, resulting in lower final body weights, compared with controls. Aparecida de França et al. (2009) also observed a lower intake of protein in animals submitted to an

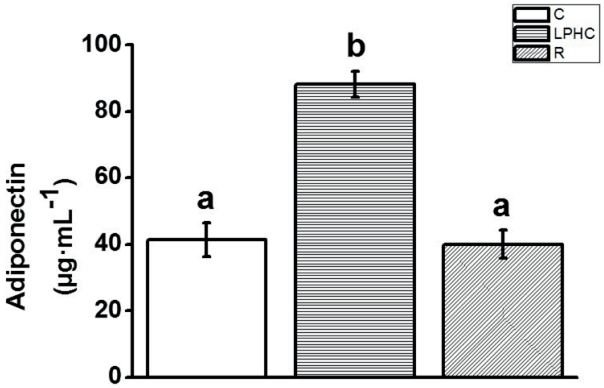

Figure 3 - Serum adiponectin levels in rats fed a control diet for 45 days (C_{45}), a low-protein, high-carbohydrate (LPHC) diet for 45 days or an LPHC diet for 15 days followed by a control diet for 30 days (R). Results are expressed as means ± standard errors of the means across eight animals per group. Statistical analysis was performed using ANOVA (one-way ANOVA, $F = 8.65$). Letters indicate significant differences among groups ($p < 0.05$).

LPHC diet for 15 days (about 60%), despite the 14% increase in food intake.

Besides higher food intake by rats of the $LPHC_{45}$ group, the LPHC diet is a hyperglycemic

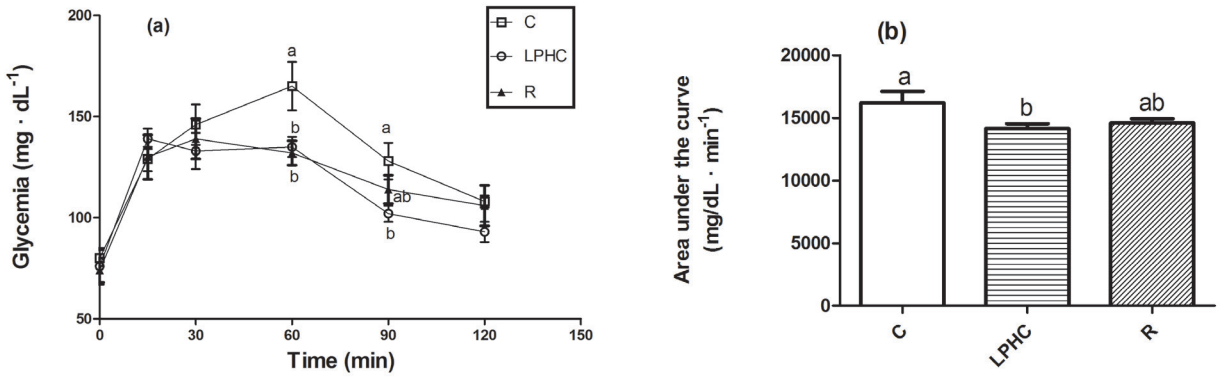

Figure 4 - Serum glucose (mg/dL) (**a**) and area under the curve (**b**) of the oral glucose-tolerance test (OGTT) of rats treated with the control diet (C group) or low-protein, high-carbohydrate diet (LPHC group) for 45 days and rats treated with the LPHC diet for 15 days followed by the control diet until the 45th day (R group). The results are expressed as the means ± standard error of the mean of seven animals per group. Different letters represent significant differences among groups. One-way ANOVA ($p<0.05$).

TABLE V
Blood biochemical, hormone, and cytokine analyses in C_{45}, $LPHC_{45}$, and R rats.*

Biochemical analyses				
Variable	**C_{45}**	**$LPHC_{45}$**	**R**	**F**
Fed glycemia ($mg \cdot dL^{-1}$)	129 ± 7^{a}	120 ± 4^{a}	130 ± 8^{a}	0.64
Total protein ($g \cdot dL^{-1}$)	6.4 ± 0.2^{a}	5.0 ± 0.2^{b}	7.0 ± 0.2^{a}	22.82
Urea ($mg \cdot dL^{-1}$)	38 ± 2^{a}	17 ± 1^{b}	38 ± 1^{a}	96.74
ALT ($U \cdot dL^{-1}$)	30 ± 3^{a}	34 ± 3^{a}	33 ± 23^{a}	0.48
AST ($U \cdot dL^{-1}$)	113 ± 11^{a}	91 ± 7^{a}	86 ± 3^{a}	3.38
Triglycerides ($mg \cdot dL^{-1}$)	139 ± 9^{a}	123 ± 6^{a}	150 ± 13^{a}	1.92
Total cholesterol ($mg \cdot dL^{-1}$)	143 ± 7^{ab}	127 ± 8^{a}	162 ± 10^{b}	4.51
HDL-cholesterol ($mg \cdot dL^{-1}$)	105 ± 8^{a}	59 ± 7^{b}	87 ± 8^{a}	23.56
Free fatty acid ($Eq \cdot mL^{-1}$)	222 ± 22^{a}	144 ± 9^{b}	189 ± 11^{ab}	6.33
Hormones and cytokines analyses				
Variable	**C_{45}**	**$LPHC_{45}$**	**R**	**F**
Fed insulin ($ng \cdot mL^{-1}$)	1.260 ± 0.235^{a}	0.738 ± 0.049^{a}	2.874 ± 0.745^{b}	9.92
Glucagon ($ng \cdot mL^{-1}$)	0.321 ± 0.016^{a}	0.257 ± 0.027^{ab}	0.245 ± 0.014^{b}	4.69
Leptin ($ng \cdot mL^{-1}$)	5.51 ± 1.87^{a}	5.68 ± 1.38^{a}	6.33 ± 0.95^{a}	0.09
T3 ($ng \cdot mL^{-1}$)	2.48 ± 0.02^{a}	2.65 ± 0.06^{b}	2.62 ± 0.03^{ab}	4.19
T4 ($ng \cdot mL^{-1}$)	3.08 ± 0.01^{a}	3.10 ± 0.01^{ab}	3.12 ± 0.01^{b}	6.23
TNFα ($pg \cdot mL^{-1}$)	248.51 ± 32.93^{a}	257.37 ± 16.52^{a}	276.17 ± 38.00^{a}	0.21
Corticosterone ($ng \cdot mL^{-1}$)	20.27 ± 3.05^{a}	24.56 ± 5.50^{a}	12.85 ± 3.50^{a}	2.03

C_{45}, control group; $LPHC_{45}$, low-protein, high-carbohydrate group; R, reverse group; ALT, alanine aminotransferase; AST, aspartate aminotransferase; F values for ANOVA. *Values are expressed as the means ± standard error of the mean of 8 animals per diet group. Mean values with different lowercase letters are significantly different ($p < 0.05$), as determined by one-way ANOVA.

diet (LPHC - 74%-carbohydrate and C diet-63%-carbohydrate). Therefore, the animals of $LPHC_{45}$ group intake 47% more carbohydrate than C_{45} group. The storage of glycogen per gram of tissue, was maintained in the muscles of the $LPHC_{45}$ group; but the glycogen content in the liver was about 4-fold higher, when compared with C_{45} group. The different effects obtained on glycogen depot

in the liver (increased) and in the muscle (similar among groups) can be explained by presence of different enzymatic isoforms. Glycogen synthase (GS) catalyses the incorporation of glucose to a growing glycogen molecule via α-1,4-glycosidic bonds. Mammals have two main GS isoforms designated as muscle GS (MGS) and liver GS (LGS). The two isoforms have different intracellular distribution and molecular mechanisms involved in their controls. Both Hexokinase I and MGS are sensitive to the low concentrations of glucose. In the liver, only when blood glucose concentration increases above a threshold level the Glucokinase (hexoquinase isoform in the liver) increases the glucose phosphorylation to glucose-6-phosphate, thus giving the signal that triggers the synthesis of hepatic glycogen. It seems that LGS is one way for the hepatocyte to ensure hepatic glycogen synthesis when blood glucose levels are high (Gomis et al. 2002).

It is also presumable that the $LPHC_{45}$ rats use part of the glucose excess from the diet for the synthesis of non-essential amino acids and lipids. We observed a 270% increase in the rate of fatty acid synthesis from glucose in rats which received the LPHC diet for 15 days (Menezes et al. 2013). Levels of non-essential amino acids were preserved or even increased in the plasma of those rats probably due the Carbons from glycolysis and Krebs Cycle intermediates (Batistela et al. 2014). The increase in glucose tolerance and similar HOMA-IR index in $LPHC_{45}$ rats suggest that the increase occurred without altering in insulin resistance. The higher tolerance to glucose overload was also observed in animals fed an LPHC diet for 15 days (Pereira et al. 2014). The increase in the adiponectin level may have contributed for a better response of $LPHC_{45}$ rats to glucose overload. Yamauchi et al. (2001) observed that adiponectin replacement in adiponectin deficiency mouse model increased PPAR-alpha expression, fatty acid oxidation, and energy consumption, causing a reduction of triglyceride content in muscle and liver. In the skeletal muscle, the decrease in triglyceride content was associated with increased GLUT-4 translocation, which led to improved insulin sensitivity. The adiponectin gene expression in adipose tissue significantly correlates with plasma levels and higher sensitivity to insulin action, higher glucose uptake and higher fatty acid oxidation (Bollen et al. 1998, Yamauchi et al. 2001, 2002).

The rats of the R group showed a slight increase in total food and calories intake in the experimental period in consequence of the higher intake of the diet when the group was submitted to 15 days on LPHC diet. The body weight, body chemistry composition and the other energetic parameters were similar to rats of the C_{45} group. At the end of the 45 days, we observed that the change for C diet in R rats was efficient in compensating the consequences of the lower content of protein in the LPHC diet administered for 15 days. Only T4 and fed insulin were increased in the blood of R rats as compared with C_{45} rats. The low protein content in the diet has already been associated with low serum thyroid hormones in other several studies (Gão et al. 2013, Palkowaska-Gozdzik et al. 2017).

The hyperinsulinaemia observed in R rats is supported by other investigations in animals submitted to a diet poor in protein. Studies using 30-d-old C57BL/6 mice exposed to a protein-restricted diet for 14 weeks showed that they produce and secrete less insulin, but they also remove and degrade less insulin, due to the lower expression of the insulin-degrading enzyme in the liver, possibly with long-term consequences. Thus, the reduced insulin clearance to control hypoinsulinaemia in malnourished mice might lead to hyperinsulinaemia, when they are exposed to a normal and/or a high-nutrient diet (Rezende et al. 2014). The higher HOMA-IR index in R rats compared with $LPHC_{45}$ rats (but not with C_{45} rats), did not seem to have significance *in vivo*, since

it was not accompanied by alterations in the area under curve in the OGTT used to evaluate glucose tolerance.

Thus, the data suggest that the different response in $LPHC_{45}$ as compared with C_{45} rats (increased glucose tolerance, hepatic glycogen and lower fatty acid) and in R group as compared with $LPHC_{45}$ (increased total cholesterol, insulin resistance and fed insulin), can be related to different levels of adiponectin among groups.

AUTHOR CONTRIBUTIONS

Researchers P.C. and S.A.F. carried out biochemical analyzed and carcass composition. M.F. and M.P.S. carried out hormonal analyses. M.P.P. and T.S.Q. carried out lipid and glycogen determinations and F.H.S.S. carried out oral glucose tolerance test. Researchers P.C.L., C.M.B.A. and A.M.B. read the manuscript and contributed to the discussion. N.H.K. designed the experiment, helped analyze the data, wrote the manuscript, and supervised the project. All authors read and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

Figure S1.