Original article (short paper)

Metabolic profile and spontaneous physical activity modulation under short-term food restriction in young rats

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Abstract — Aims: The aim of this study was to investigate the effects of short-term food restriction (6-weeks) on metabolic profile and spontaneous physical activity (SPA) of young male Wistar rats. Methods: Thirty rats had their baseline SPA measured at 21 days-old and were separated into two groups at 28 days-old: Control (CG) and 50% of food restriction (FR). The food restriction protocol lasted six weeks, being the SPA measured weekly by a gravimetric apparatus. At the end of the experiment, biochemical analyses were performed in serum and tissue samples with statistical significance set at 5%. Results: FR showed less SPA than CG, as occurred for body mass, water intake, adipose tissue and liver, heart and soleus glycogen, serum glucose, total protein, triglycerides and total cholesterol ($P<0.05$). Conclusion: Data set demonstrates that low substrate stores signaled to decrease spontaneous physical activity to save energy.

Keywords: spontaneous activity, food restriction, gravimetric apparatus, rats.

Introduction

Spontaneous physical activity (SPA) comprises all movements performed without consideration of the volitional or forced physical exercise [1], which represents an important component of the total daily energy expenditure [2] and consequently to the control of energy balance and fat mass storage [3]. Therefore, efforts in order to identify the mechanisms involved in SPA regulation have been made [4] and despite the recognition of some peptides, hormones, their targets and communication [4], the complex control of SPA currently remains under investigation [5, 6, 7].

Santos-Pinto [8] consistently postulated that the total daily energy expenditure, which is given by the sum of energy spent through thermic effect of food, resting metabolic rate and physical activity [9], is modulated in relation to food intake [8]. It is well established that disproportional energy intake in relation to energy spent towards positive energy balance chronically leads to catastrophic consequences, firstly characterized by obesity and then ectopic fat deposition in many tissues, such as liver, kidney and heart, promoting insulin resistance and metabolic syndrome. The alarming growth of this scenario around the world called for studies employing protocols to reduce the energy intake, to increase the energy expenditure, or both simultaneously. Food restriction (FR) protocols have been extensively employed in this context.

Martin [2] postulated that it is unclear if the effect of calorie restriction in SPA is its reduction. Indeed, some authors confirm this statement [10, 11, 12, 13] and others found the inverse outcome [14, 15, 16, 17]. Such different statements are certainly also due to the employment of distinct protocols for calorie restriction with different durations. It has also been described that an acute increase of SPA during FR in rats is due to the increased activity hypothalamic neuropeptides, which is a transient behavior motivated by food seeking [18] and literature suggests that if FR is chronically maintained it leads to a decrease in SPA [18]. In this regard, an interesting idea of a mechanism to save energy in times of FR was proposed [8], being recently improved by involving neuronal, endocrine and metabolic elements [4].

Whereas it is obvious that efficiency in fuel utilization of mammals is higher in long-term food restricted animals [19], it is not clear whether differences regarding SPA also exist in short periods of FR. Furthermore, it is not known to date how FR could affect metabolic responses in young rats. This is a critical issue because eating disorders, e.g. anorexia nervosa and bulimia nervosa, and depression are more prevalent in young populations, which intentionally restrict caloric intake to substantially reduce body weight [20].

Based on such background, the aim of this study was to investigate the effects of short-term food restriction (6-weeks) on metabolic profile and SPA of young male Wistar rats.

Material and Methods

Animals

We assessed thirty 21-day old male Wistar rats obtained from the institutional facility. The animals were housed (five per cage of 41 x 34 x 16 cm) at 21±1°C, 45-55% of air relative humidity and light/dark cycle of 12/12h with lights turn on at 06:00h. The experiment was conducted after approval of the institutional ethics commission on the use of animals to research under protocol 4100-1.

Experimental design

The first procedure employed was the measurement of total daily amount of food consumed during a week by 21-day old rats. Then, the animals were randomly separated into two identical groups at 28-days old. The control group (CG; n=15) was fed with balanced standard rodent chow (Nuvilab®, Brazil) ad libitum, while the food restriction group (FR; n=15) received 50% of the CG food, relativized by body mass. Body mass, food and water intake and spontaneous physical activity were measured once a week at each cage, during six weeks, being food, water intake and spontaneous physical activity correspondent to 24h-period.

Spontaneous physical activity measurement

The spontaneous physical activity (SPA) was measured weekly using a gravimetric method that allows the assessment at a normal cage and absolutely no perception by the rats, according to Beck [21]. SPA of rats was measured for 24 h for each group. Animal`s cages were placed on two iron platforms where a load cell was fixed between them (PLA30Kgf, Lider Balanças ®). The signal was amplified (MKTC5-10®, MK control and instrumentation™) and then processed through analog/digital conditioning module (USB-6008®). Signals were collected at a frequency of 30 Hz using LabView Signal Express® software (National Instruments™). The signal acquisition system was calibrated by applying known mass. Regression equations (R^2= 0.99) were then computed enabling conversions of milivolts (mv) signals to kilograms (kg) units.

Obtaining and storage of biological material

At the end of the experiment 71-day old animals were anesthetized with sodium thiopental (30-40mg/Kg, intraperitoneal) and after confirming the absence of caudal and pupillary reflex were euthanized by thoracotomy followed by diaphragm rupture. Blood samples were collected through cardiac puncture, centrifuged at 3000RPM for 15 minutes and the serum was stored at -80°C in aliquots to avoid future thaw cycles for biochemical analysis. The animals remained in the regular food regime before euthanasia according to each group, in order to analyze the six-week effects on blood and tissues. Moreover, the euthanasia followed similar time of day to avoid any chronobiologic interference.

After blood collection, we extracted tissue samples from the liver, heart, white gastrocnemius, soleus, interscapular brown adipose tissue and visceral white adipose tissue (summation of epididymal and retroperitoneal white adipose tissue). The procedure was conducted in less than 10 minutes by two experienced researchers, being the tissue samples immediately stored in liquid nitrogen.

Biochemical analysis

Serum glucose, triglycerides, total cholesterol and total protein were analyzed using colorimetric kit (*In vitro Diagnóstica Ltda*®, Itabira, MG, Brazil). All of the analyses were performed in a microplate reader (ASYS Expert Plus UV, Biochrom, Cambs, CB4 0FJ, UK) and the measured absorbance were normalized against a calibration curve based on the manufacturer's instructions.

Free fatty acids levels were determined using the Regouw`s method [22]. 0.2 ml of serum was added to 7 ml of the solvent mixture containing chloroform, heptane and methanol at a ratio of 28: 21: 1, followed by vigorous shaking and centrifugation. The precipitate at the bottom of the tubes was aspirated and transported to other tubes, added to a solution containing copper (II) nitrate [0.05 M], triethanolamine [0,10], sodium hydroxide [0,035 N] and sodium chloride [35%] at pH 8.1, followed by further shaking and centrifugation. Finally, 3.0 ml of the precipitate was aspirated and added to 0.5 ml of sodium diethyldithiocarbamate solution. The FFA concentration was 435 nm against palmitic acid calibration curve.

The liver (500 mg), heart, soleus and gastrocnemius muscles (200–250 mg) were digested in potassium hydroxide. Sodium sulfate was added and the precipitated glycogen was purified using ethanol [70%]. The glycogen concentration was analyzed according to a colorimetric method using 10 μL of phenol and 2.0 mL of sulfuric acid with absorbance at 490 nm, according to Dubois [23].

Statistical analysis

Data were described in means and standard error of the means. Data normality was tested using Kolmogorov-Smirnov test. Independent *t* tests were used to compare the differences between groups (control and food restriction) for the variables measured after six weeks of experiment. Measurements taken over multiple weeks, such as SPA, body mass and food and water intake were analyzed by factorial two-way ANOVA to determine the main effects (time and diet effects) and interaction. Newman-Keuls post hoc test was used when appropriate. Statistical significance was set at 5%.

Results

Figure 1 shows data obtained during six weeks of experiment. Although significant differences were not found in the same week in the measurement between groups ($P>0.05$), food restriction group (FR) showed less SPA than control group (CG), demonstrating the diet effect on SPA outcomes ($F=27.95$; $P<0.01$; Figure 1A). Analyzing all the animals, there was also significant decrease in SPA during the experiment (six weeks) ($F=11.39$; $P<0.01$), leading to no significant interaction between diet and time for this parameter ($F=0.45$; $P=0.81$).

Body mass was significantly higher in CG than FR from the second week onwards ($P<0.01$), showing significant diet effect on body mass ($F=1041.6$; $P<0.01$; Figure 1B). Body mass increased through time ($F=118.36$; $P<0.01$), however, the FR group did not change the body mass when we compared all the weeks ($P>0.05$), whereas CG increased body weight every week, statistically from the second week onwards ($P<0.01$). These results lead to a significant interaction between diet and time for body mass ($F=102.28$; $P<0.01$).

The relative food intake was significantly smaller in FR than CG from the second week onwards ($P<0.01$) and a significant diet effect was found ($F=192.94$; $P<0.01$; Figure 1C). It caused a

significant decrease in relative food intake throughout the experiment (F=115.29; P<0.01). We found significant interaction between diet and time effects for relative food intake (F=6.16; <0.01).

The FR group also shows a decrease in relative water intake for FR in relation to CG during the experiment (F=4.99; P=0.03; Figure 1D). We also found a decrease through time (F=26.18; P<0.01) without interaction between effects.

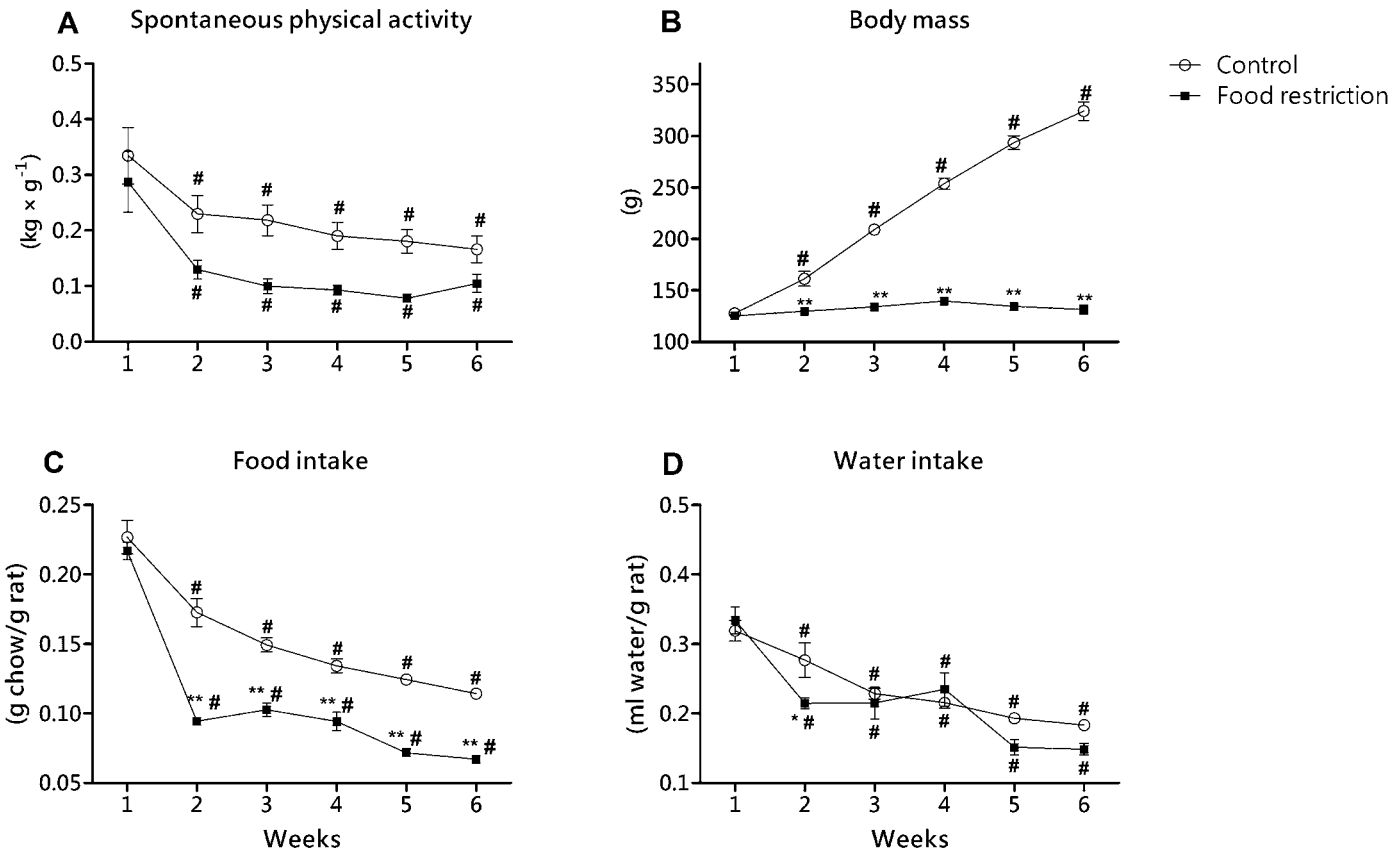

Figure 1. Data obtained during the six-week experiment for the control and food restriction groups; * P<0.05 in relation to control group to the same week; ** P<0.01 in relation to control group to the same week. # P<0.05 in relation to the first week for the same group.

Figure 2 illustrates the results from adipose tissue extracted at the end of the six-week experiment. The epididymal and retroperitoneal summation, relative to body mass, was higher for CG than FR (Figure 2A), whereas the relative brown adipose tissue showed no differences between groups (Figure 2B).

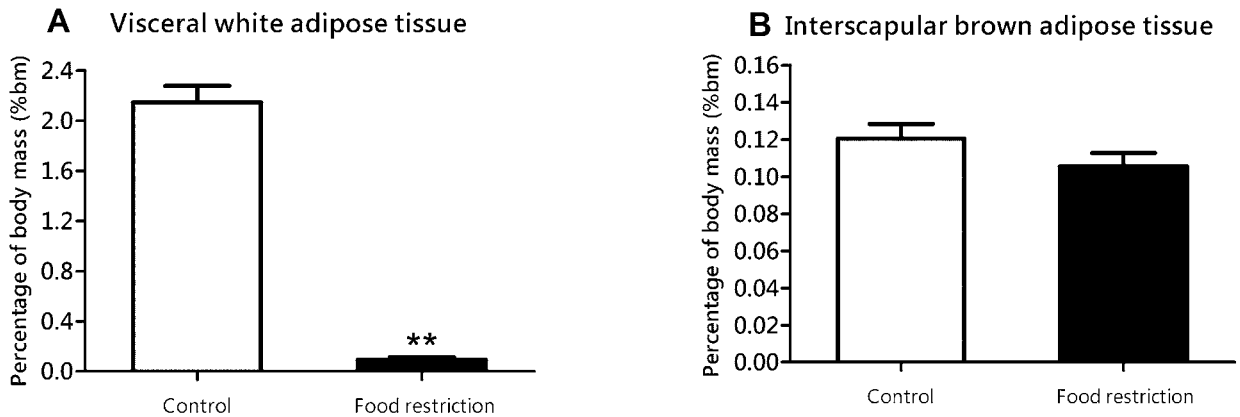

Figure 2. Visceral white adipose tissue (Figure 2A) and interscapular brown adipose tissue (Figure 2B) of the control and food restriction animals at the end of the six-week experiment; ** P<0.01 in relation to control group.

Except for the white gastrocnemius, the glycogen of all other tissues showed significant differences between groups in the end of the experiment (Figure 3).

The serum parameters were significantly modified by the diet employed, as showed in figure 4.

Discussion

The main findings of the present study were that the short-term food restriction adopted leads to a significant decrease in SPA and significant modulations for the metabolic profile of the rats through time.

Regarding the spontaneous physical activity, our results were in agreement with some authors [10, 11, 12, 13] but in discordance with others [14, 15, 16, 17]. The classic behavior towards acute increase of SPA under food restriction [18] was not observed in our experiment, and indeed, SPA significantly decreased through time. The lack of interaction between diet and time was found probably due to the apparent higher decrease in SPA for FR through time, when compared to smaller decrease in SPA for CG. In fact, behavior observed in our experiment follows the hypothesis of Santos-Pinto [8], who attribute the lower oxygen consumption with saving energy for rats under food restriction protocol, a behavior probably triggered by low energy stores in communication with brain neuropeptides responsible for SPA modulation. It is interesting to note that the important SPA differences between groups through time occurred independent of brown adipose tissue (Figure 2), which is associated with thermogenesis [24] and likely modulations of locomotion.

Relative food and water intake were lower for the food restriction group, as well as absolute body mass. However, when considering all of the animals of the experiment we found an increase in body mass through time. It occurred at a diet-dependent manner since when comparing the same group through time we found statistical increase in such parameter only for the control group. The decrease in food intake was dependent of the diet employed, as expected. Probably the decrease in relative food intake occurred also due to the disproportional increase in body mass in relation to food intake of the CG. It is interesting to note that the relative food intake behavior was similar to the spontaneous physical activity, in which the FR group showed apparent higher decrease than CG (Figure 1). Other interesting result was that the decrease in relative water intake through time was not influenced by the diet.

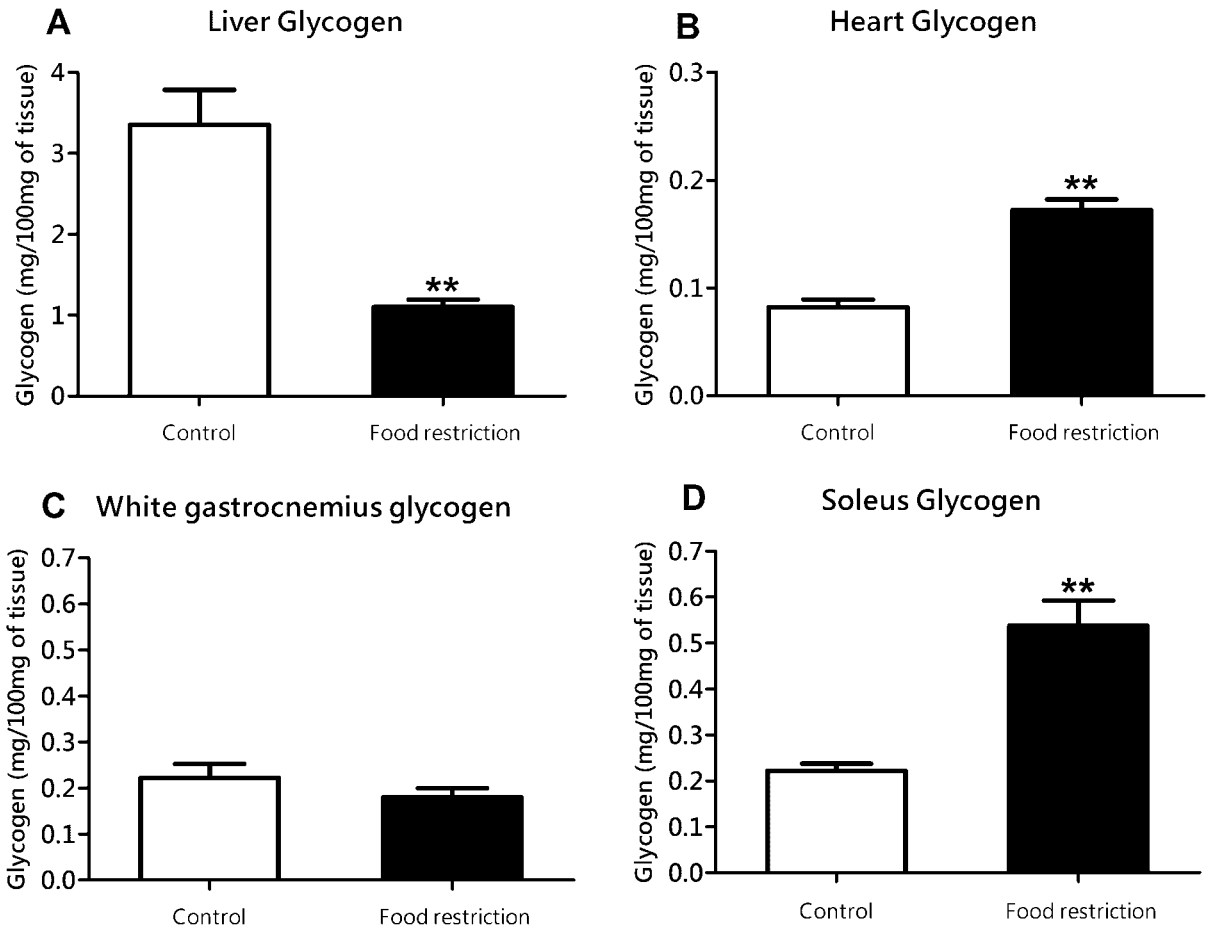

Figure 3. Glycogen measurements in the liver (Figure 3A), heart (Figure 3B), white gastrocnemius (Figure 3C) and red soleus (Figure 3D) for control and food restriction groups in the end of the experiment. ** P<0.01 in relation to control group.

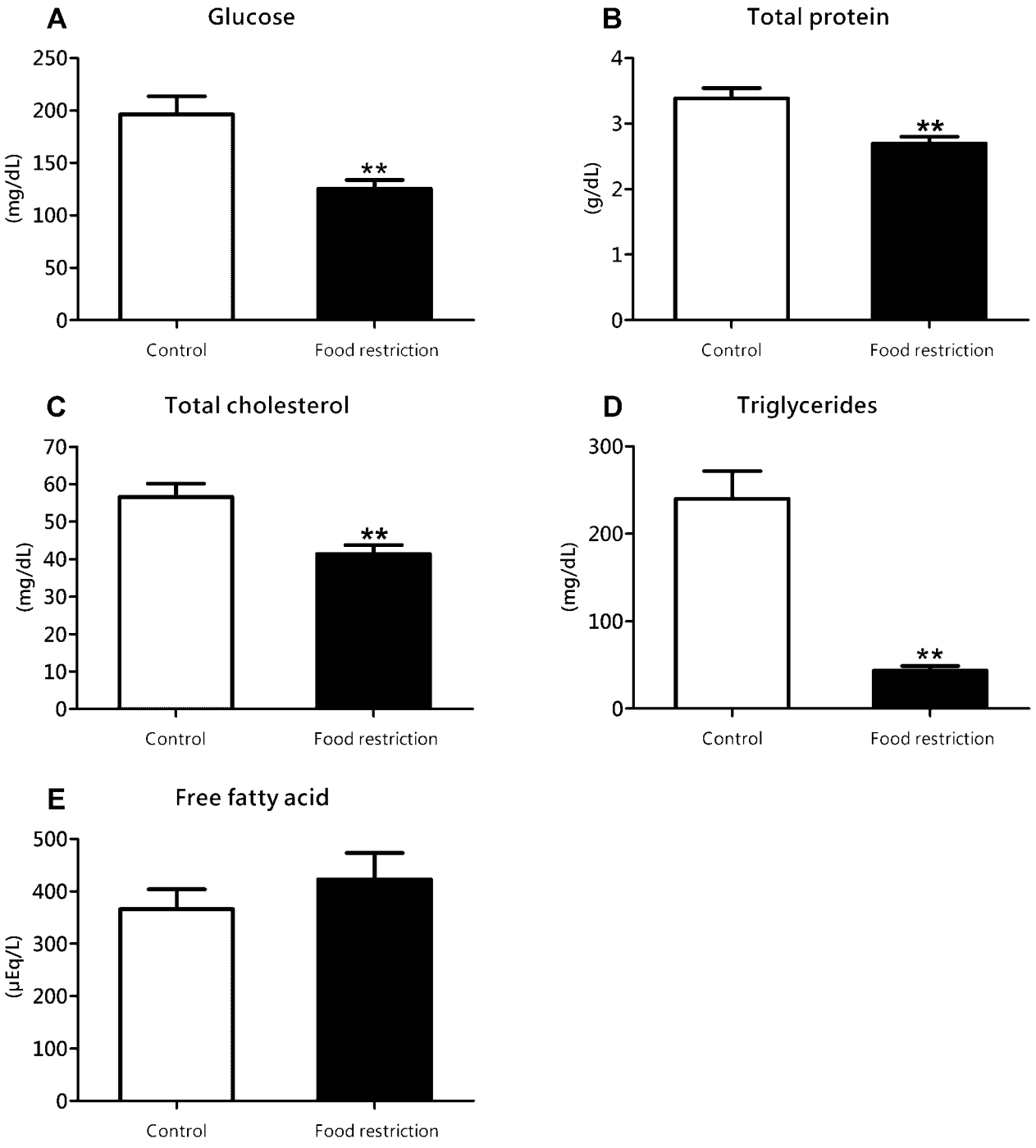

Figure 4. Serum concentration of glucose (Figure 4A), total protein (Figure 4B), total cholesterol (Figure 4C), triglycerides (Figure 4D) and free fatty acid (Figure 4E) determined at the end of the experiment for control and food restriction groups; ** P<0.01 in relation to control group.

The lower food availability leads to lower white adipose tissue for FR group, probably due to the use of this energy for replacement of poor food intake, as occurred for the liver glycogen and serum glucose, triacylglycerol and cholesterol. In periods of food restriction it was described a change from glycolytic to oxidative muscles [19] and it is known that the glucose uptake by the oxidative muscles increases during food restriction [25], so, it is expected a decrease in serum glucose followed by decrease in the liver glycogen, as classically described to glycidic liver metabolism [26]. That glucose uptake by the oxidative muscles also leads to an increase in glycogen stores for heart and soleus, as found in our experiment. Therefore, observing the big picture of the metabolic status, FR forced the organism balancing and reallocating substrates to survive, showing a high efficiency on energy usage, corroborating with some authors statements[4, 19].

Allthose modulations of metabolic and locomotor activity discussed above have been associated with neural communication, being the neuropeptide Y (NPY) and agouti-related peptide (AgRP) signals the most involved [27, 28]. In situations of negative energy balance NPY and AgRP activity increase [6], however,

during food restriction there is no possibility of feeding and it is possible that those signals persist. If correct, the consequences of chronically higher activity of NPY and AgRP in this case, with no food available, can be the inhibition of thermogenesis [24] and SPA [18] when trying to promote positive (or less negative) energy balance and maybe influencing the efficiency of energy usage [4, 19].

In summary, our experiment showed that even short-term food restriction of 50% imposes significant modulations on the metabolic profile towards the use of energy stores preferably in oxidative tissues of young rats. This condition led to lower energy stores, which literature postulated that increases signaling for NPY and AgRP activity in the arcuate nucleus of hypothalamus, promotes a downstream signaling that consequently decrease the spontaneous physical activity. Such scenario occurs in the effort to establish a positive energy balance, which was not possible due to the diet regime employed.

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