The effects of a high-protein diet and resistance training on organ mass and metabolic profile in rats

Os efeitos de uma dieta rica em proteínas e treinamento de resistência sobre a massa dos órgãos e o perfil metabólico em ratos

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ABSTRACT

A high-protein diet associated or not with strength exercise impacts satiety, fat accumulation, mass gain, changes biochemical and morphological. The study evaluated the association between adipose tissue mass and organs, in addition to the blood biochemical profile of rats fed a high-protein diet (HD) submitted to strength training (RT). Adult male Wistar rats were divided into groups (n=7/each): sedentary-normoprotein (SN-14%), sedentary-hyperprotein (SH-35%), trained-normoprotein (TN-14%), and trained-hyperprotein (TH-35%). RT consisted of 4 sets of 10 water jumps/8 weeks. HD and RT reduced the adiposity index (p<0.001). Regardless of HD, RT increased the mass of the gastrocnemius (p<0.001) and soleus (p=0.01). Heart mass was inversely correlated (p<0.01) with retroperitoneal fat. There was an inverse dependence between the mass of the gastrocnemius and retroperitoneal (p<0.01), omental (p<0.05), subcutaneous inguinal (p<0.01), and visceral adiposity (p<0.05). There was a positive dependence between kidney mass and serum creatinine levels (p<0.001). Liver mass showed a positive dependence (p<0.01) on total cholesterol, HDL-c (p<0.01), and triglycerides (p<0.05). The results showed that isolated HD and associated with RT reduced the visceral adiposity, but did not increase the gastrocnemius and soleus mass. The participation of DH and TR stands out as measures of behavior tendency among the studied variables.

Keywords: hyperprotein-diet, organ mass, visceral fat, skeletal muscle, resistance training.

RESUMO

Uma dieta rica em proteínas associada ou não ao exercício de força afeta a saciedade, acúmulo de gordura, ganho de massa, mudanças bioquímicas e morfológicas. O estudo avaliou a associação entre massa tecidual adiposa e órgãos, além do perfil bioquímico sanguíneo de ratos alimentados com uma dieta rica em proteína (HD) submetida a treinamento de força (RT). Os ratos Wistar machos adultos foram divididos em grupos (n=7/cada): sedentário-normoproteína (SN-14%), sedentário-hiperproteína (SH-35%), treinado-normoproteína (TN-14%) e treinado-hiperproteína (TH-35%). RT consistiu em 4 conjuntos de 10 saltos de água/8 semanas. HD e RT reduziram o índice de adiposidade (p<0,001). Independentemente do HD, o RT aumentou a massa do gastrocnêmio (p<0,001) e do soléu (p=0,01). A massa cardíaca foi inversamente correlacionada (p<0,01) com a gordura retroperitoneal. Houve uma dependência inversa entre a massa do gastrocnêmio e a retroperitoneal (p<0,01), omental (p<0,05), inguinal subcutânea (p<0,01) e adiposidade visceral (p<0,05). Havia uma dependência positiva entre a massa renal e os níveis de creatinina sérica (p<0,001). A massa hepática mostrou uma dependência positiva (p<0,01) do colesterol total, HDL-c (p<0,01), e triglicérides (p<0,05). Os resultados mostraram que a HD isolada e associada à RT reduziu a adiposidade visceral, mas não aumentou a massa gastrocnêmica e a massa do linguado. A participação de DH e TR se destaca como medidas de tendência comportamental entre as variáveis estudadas.

Palavras-chave: hiperproteína-dieta, massa de órgãos, gordura visceral, músculo esquelético, treinamento de resistência.

1 INTRODUCTION

Diets containing high protein content and low-carbohydrate represent an attractive strategy to individuals that aim for mass loss, decrease fat stores, and increase muscle mass associated with the low percentage of subcutaneous fat [1-3]. Although these dietary changes are popular and thought to be effective for long-term weight loss, the reduction of carbohydrates combined with high levels of fat and protein still needs to be investigated further, particularly in terms of the functional overload of organs like the kidneys, liver, and heart [4-6].

Is well established in the literature the main body regions responsible for storing energy in lipids form, among them visceral and subcutaneous adipose tissue, besides the regions referred to as ectopic, for example, liver and skeletal muscle [7]. While fat accumulation may occur in these tissues, visceral fat (intra-abdominal) and ectopic intrahepatic fat (which characterizes the diagnosis of hepatic steatosis) are the most important for the development of obesity-related diseases [7, 8].

The stimulation of pro-inflammatory active peptides such as monocyte chemoattractant protein-1 (MCP1), tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), plasminogen activator inhibitor-1 (PAI-1), and resistin, among others, is one of the important aspects of an excess of triacylglycerols in adipose tissue, especially in the intra-abdominal region; this can lead to metabolic and functional damage to cardiac tissues (such as cardiomyocytes), as well as an increased risk of obesity-related morbidities such atherosclerosis, dyslipidemias, acute myocardial infarction, and diabetes mellitus [9, 10].

The effects of a high-protein diet (HD) on humans [11-14] and non-human animal models [4, 15-17] show that this nutritional strategy leads to hunger control, body mass increase, and the prevention of overweight and obesity, as well as associated comorbidities. The foundation presented in most studies is based on the metabolic cost of protein compared to other energy nutrients (carbohydrate and fat), which would result in a decrease in appetite, lower food and total energy intake, and, as a result, reductions in body mass gain, body circumferences, and, most importantly, visceral fat deposits, promoting the improvement of the lipid profile associated with the preservation of lean mass [2, 18-21].

In the context of an isocaloric diet, a higher protein intake and lower carbohydrate intake lead to an increase in thermogenesis, both obligatory and facultative, and hence an increase in basal or resting metabolism. This effect can be explained by the fact that dietary protein has a higher energy demand to be used as an energy source, implying approximate values of 20-30% of its usable energy, whereas carbohydrates only require roughly 5-10% and fat only 3%, according to the literature [20].

Regular physical activity raises energy demand and accelerates nutrient oxidation (including fat oxidation) and, when it produces a continuous negative energy balance, the result is an improvement in body composition, which is linked to increased physical performance and overall health [22, 23]. In this aspect, there is great scientific interest in investigating whether the administration of HD associated with physical exercise acts in the prevention of obesity, cardiovascular and metabolic diseases.

The goal of this study was to see how HD and resistance training (RT-water jumping protocol) affected the relative mass of body fat tissues, organs, and blood biochemical parameters in healthy rats. parameters of healthy rats.

2 MATERIALS AND METHODS

2.1 ANIMALS

The Central Animal Facility of the Federal University of Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil, provided 28 male Wistar rats (Rattus norvegicus) with 45 days of age and a body mass of around 70.02.0 g. The animals were kept in collective polyethylene cages (37.0 31.0 16.0 cm) in the NAFIMES/FEF/UFMT Laboratory of Animal Experimentation and Exercise, under a controlled temperature of 25°C, humidity relative to air between 45 and 55 percent, and a light/dark cycle of 12/12h: light cycle (06:00h to 18:00h) and dark cycle (18:00h to 06:00h). The experiment was carried out following the ethical principles of animal experimentation in accordance with the Brazilian College of Animal Experimentation (COBEA) (Law nº 6638, May 8, 1979; and Decree nº 26,645, of July 10, 1934) and was approved by the Ethics Committee for the Use of Animals in Research of the Federal University of Mato Grosso, Brazil (CEUA/UFMT) under protocol number 23108.002839/14-2. The experiment was carried out in accordance with the ethical principles of animal experimentation established by the Brazilian College of Animal Experimentation (COBEA) (Law no 6638, May 8, 1979; and

Decree no 26,645, July 10, 1934) and approved by the Ethics Committee for the Use of Animals in Research of the Federal University of Mato Grosso, Brazil (CEUA/UFMT) under protocol number 23108.002839/14-2.

2.2 PREPARATION AND COMPOSITION OF THE DIET

The animals were initially kept for a week under observation, fed commercial chow (Purina®), and kept hydrated with free access to filtered water (ad libitum). From the second week onwards, all animals were weighed and randomly assigned to experimental groups based on the type of diet they were given and whether or not they were given resistance training through water jumps in a pool designed specifically for rats, as stated in the protocol below:

- Sedentary normal protein diet (SN, n=7): sedentary rats fed with a purified diet containing 14% of the total energy value composed of casein protein;
- Sedentary high-protein diet, (SH, n=7): sedentary rats fed with a purified diet containing 35% of the total energetic value composed of casein protein;
- Trained normal protein diet, (TN, n=7): rats submitted to resistance training throughout the experiment and were fed with a diet containing 14% of the total energetic value of casein protein;
- Trained high-protein diet, (TH, n=7): rats submitted to resistance training throughout the experiment and were fed a diet comprising 35% of the total energetic value of casein protein.

The manipulation of the experimental diets was carried out following the guidelines of the American Institute of Nutrition [24]. All the experimental diets were kept isocaloric (3.8 kcal/g), differing only in the proportion of protein (casein) and carbohydrates (cornstarch), considering the following macronutrient distributions: normal protein diet: 75.8% carbohydrates, 14.7% casein protein and 9.5% lipids; high protein diet: 55.5% carbohydrates, 35% casein protein and 9.5% lipids (Table 1). Both diets were offered in the form of pellets to respect the natural characteristics of the animals, that is, the rodents.

Table 1 Composition of purified isocaloric diets (normal and high proteins)

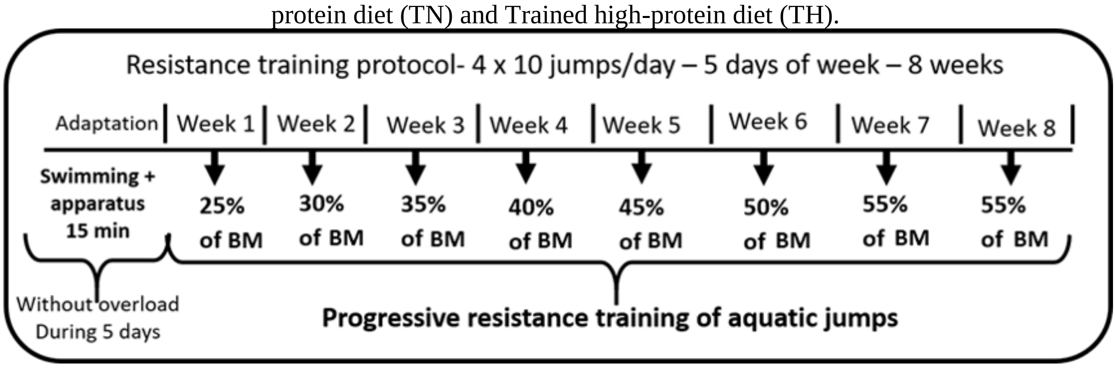
Diet components (g/kg)	Normal protein (g)	kcal	High protein (g)	kcal
Protein content (Casein>85%) [1]	140.0	560.0	332.7	1331.0
Cornstarch [1]	465.7	1862.8	272.9	1091.8
Dextrin [1]	155.0	620.0	155.0	620.0
Sucrose [1]	100.0	400.0	100.0	400.0
L- Cystina [1]	1.8	-	1.8	-
Soy oil [1]	40.0	360.0	40.0	360.0
Salt mixture (AIN – 93M MX) [1, 2]	35.0	-	35.0	-
Vitamin mixture (AIN-93-VX) [1, 3]	10.0	-	10.0	-
Fiber [1]	50.0	-	50.0	-
Choline chloride [1]	2.5	-	2.5	-
Total	1000.0	3802.8	1000.0	3802.8

[1] According to the *American Institute of Nutrition* (*AIN*-93M) [24]
[2] Salt Mixture according to *AIN* - 93M - MX [24]
[3] Vitamin Mixture according to *AIN* – 93M - VX [24]

2.3 ADAPTATION AND RESISTANCE TRAINING OF AQUATIC JUMPS

Adaptation and RT protocol were performed as established and performed by [16]. Following the water adaption protocol, the RT (aquatic jumps protocol) began the following week. The animals in the TN and TH groups were trained for eight weeks at a frequency of five times per week (Monday through Friday), always at the same hour (2:00 p.m.). The aquatic jump protocol required the animals to leap into a swimming pool that was specific for rats supporting overloads tied to the aminal's thorax with the aid of elastic at a depth of 150% of the animal's naso-anal length. The calculation to determine the overload was performed, together in two ways: diary, according to animal's body mass gain and, weekly, employing correction of the animal's body mass (BM) multiplied by the percentage of the previous week overload, plus 5% (Charge = BM * overload from previous week + 5%). The physical training schedule can be visualized in figure 1.

Figure 1 Adaptation and resistance physical training schedules applied to the groups: Trained normal protein diet (TN) and Trained high-protein diet (TH).



The RT session consisted of 4 sets of 10 underwater jumps separated by 1 minute. The animal's jump performance was only calculated after full flexion of the hind legs was achieved utilizing the swimming pool's base, followed by extension to exit and breathe.

2.4 EUTHANASIA AND BLOOD COLLECTION

At the end of the 8 weeks of RT, the animals remained at rest for 48 h. Animals in all groups have fasted for 12 h before euthanasia, which was performed by inhalation of excess CO2, followed by exsanguination by decapitation in a specific guillotine for this purpose (Insight, Córrego Near, São Paulo, Brazil). The blood samples, after coagulation, were centrifuged at 3000 rpm for 15 min to obtain the serum. The serum obtained was then aliquoted into polypropylene microtubes (1.5 mL) in volumes appropriate to the assays of interest in order to avoid degradation of the analytes due to repetitive thawing, and then stored at a temperature of -86°C (model COLDLAB brand Ultra Freezer/CL58086V).

2.5 EXTRACTION OF SKELETAL MUSCLE AND ORGANS

The gastrocnemius and soleus muscles were excised (gives rise to insertion) and weighed on a precision scale. The kidneys (right and left), liver and heart were extracted after a midline laparotomy procedure, then weighed and immediately frozen at -86°C in an Ultra Freezer (COLDLAB – model CL58086V).

2.6 BIOCHEMICAL DETERMINATIONS

The biochemical assays were carried out at the Laboratory of Experimental Biochemistry (LABE), of the Institute of Exact and Earth Sciences, UFMT, Cuiabá, Mato Grosso, Brazil. Serum samples were tested in triplicate using commercial reagents (Bioclin® Quibasa Química Ltda.—Belo Horizonte, MG, Brazil) with the aid of a spectrophotometer (UV-mini 1240, SHI-MATZU) or microplate reader in the case ELISA Assay (SpectraMax® 190 UV–Vis, Molecular Devices).

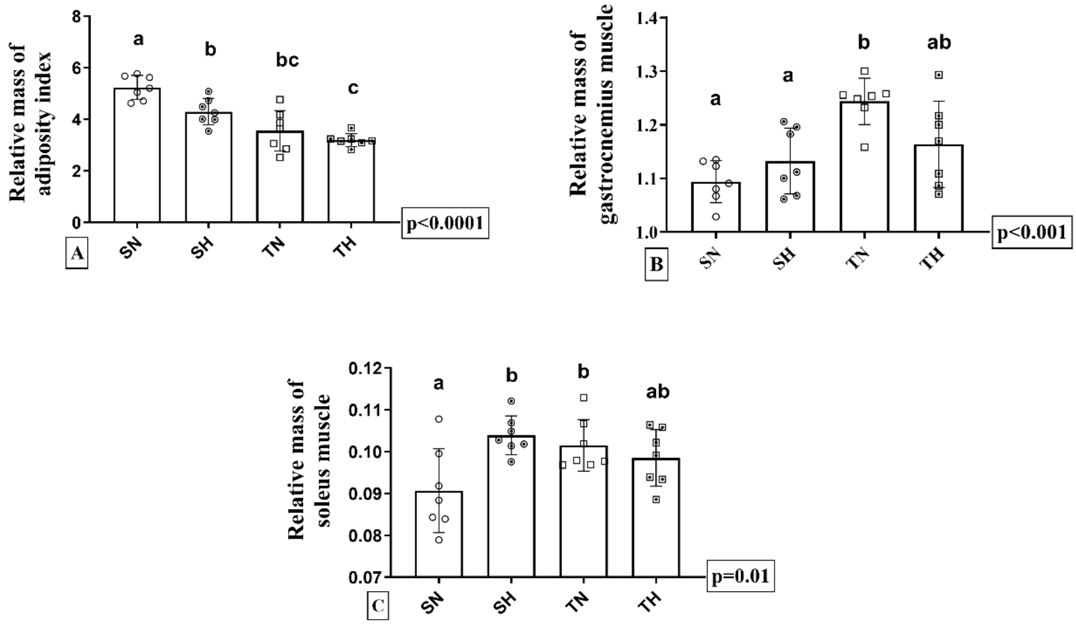
2.7 STATISTICAL ANALYSIS

Simple variance analysis tests (*one-way* ANOVA) followed by *post hoc* Tukey were used. The global average of the interest variables was weighted as the degree of linear dependence and direction performance by Pearson correlation coefficient (r). The determination of the relationship between variables was performed by linear regression (r^2). Level of significance adopted by $p< 0.05$.

3 RESULTS

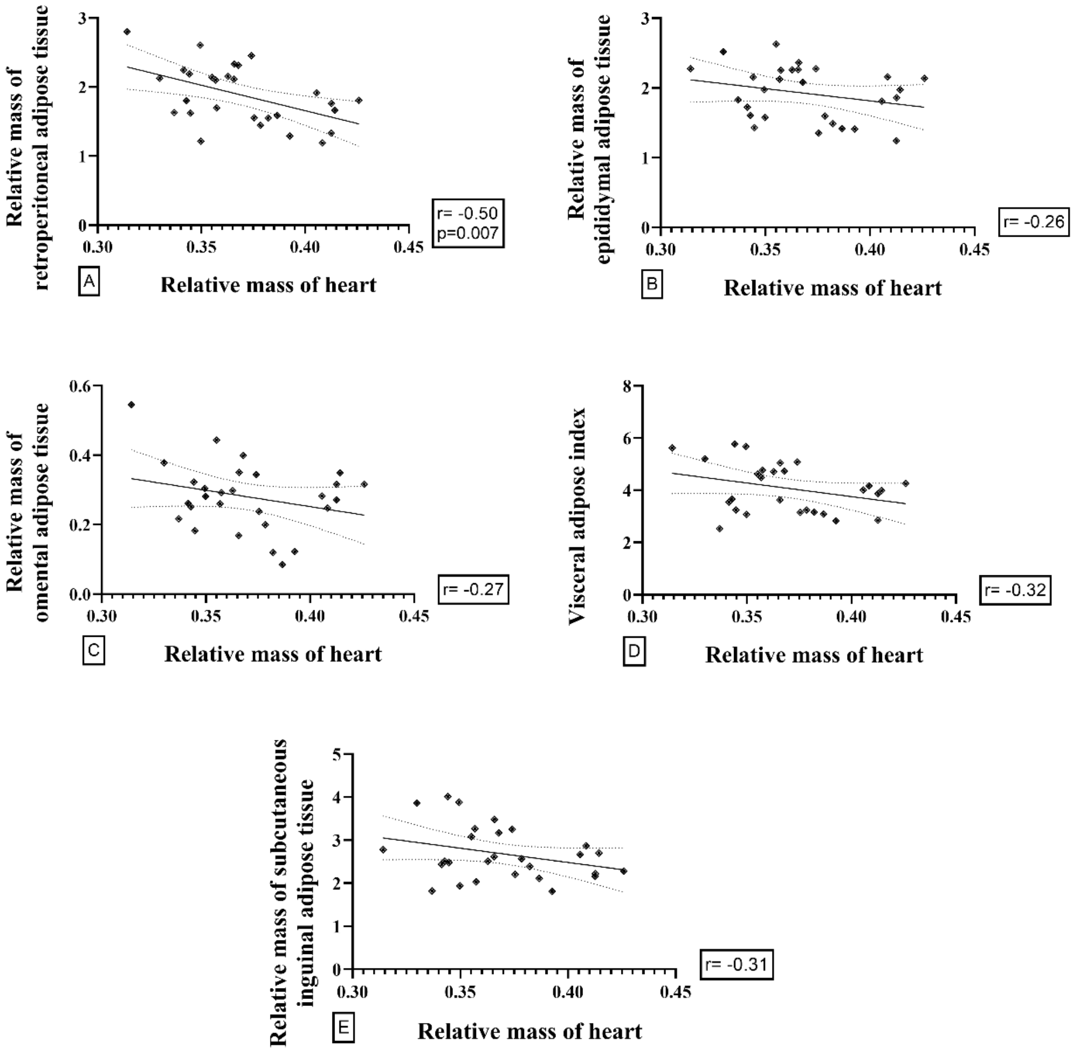
Figure 2A shows that TH (3.2±0.2), TN (3,5±0,8), and SH (4,3±0,5) groups had a lower relative visceral fat mass (g) ($p< 0.0001$) compared with SN (5,2±0,5) group. The relative gastrocnemius mass (g) is shown in Figure 2B, and TN (1.24±0.04) group showed higher values ($p< 0.001$) compared to SN e SH (1.09±0.04; 1.13±0.06) groups, and similar TH (1.16±0.08). The relative soleus muscle mass (g) was lower ($p< 0.05$) in SN (0.09±0.01) group compared to SH (0.10±0.004) e TN (0.10±0.006) groups, and similar to TH (0.09±0.007) (figure 2C).

Figure 2 Relative mass of adiposity index (A), gastrocnemius muscle (B), and soleus muscle (C); Sedentary normal protein diet (SN); Trained normal protein diet (TN); Sedentary high-protein diet (SH); Trained high-protein diet (TH). One-way ANOVA ($p<0.05$), followed by post hoc Tukey HSD [abc] when differences were found, marked by superscript letters. Results expressed as mean±SD (n= 7 animals per group).



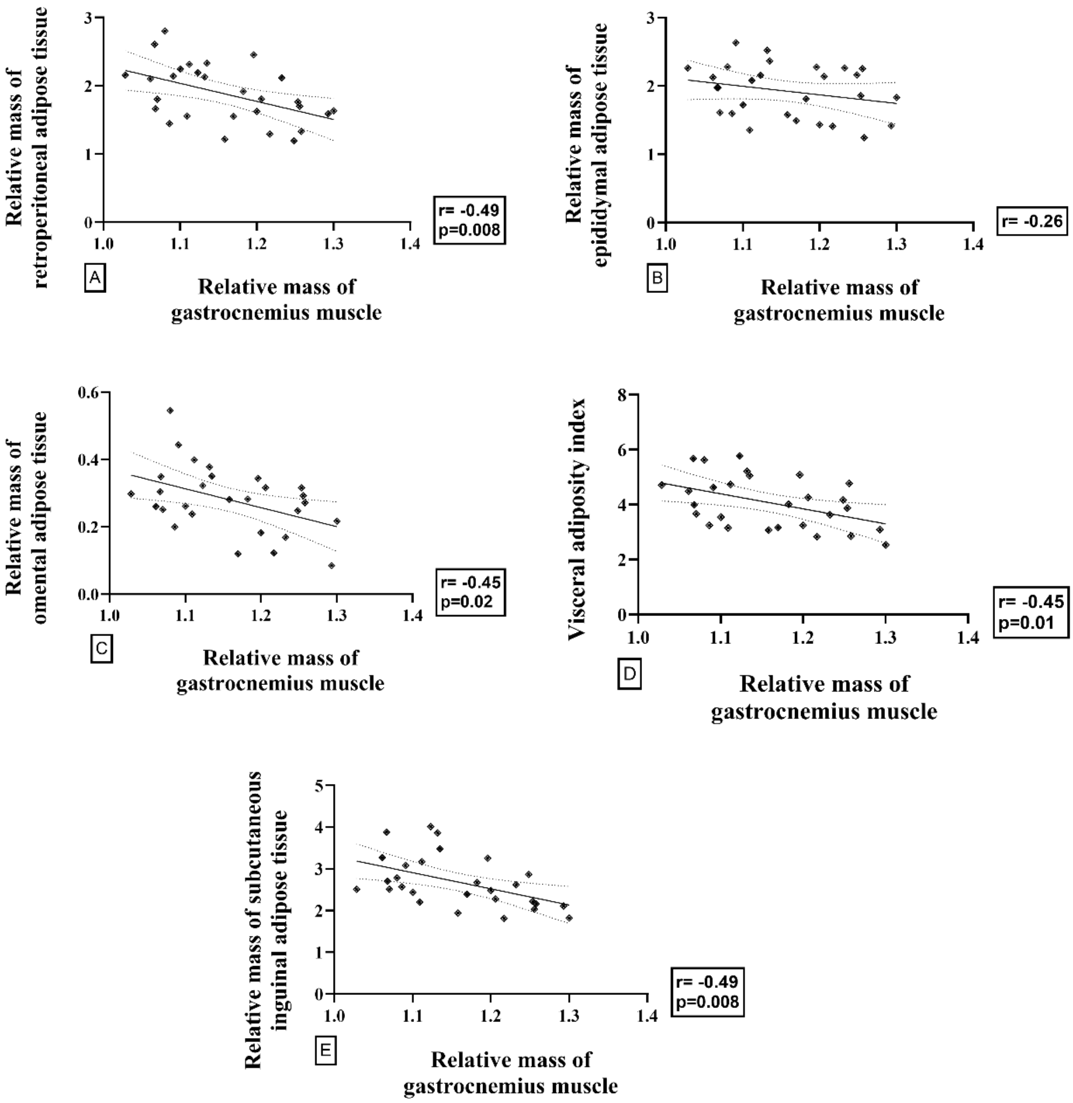
Correlation of relative heart mass with relative mass of retroperitoneal, epididimal, omental, visceral adiposity index, and inguinal subcutaneous adipose tissues are shown in the Figure 3. Relative heart mass showed moderate and inverse correlation ($r= -0.5$; $p< 0.01$) with relative retroperitoneal fat mass (figure 3A). There were no dependencies between relative heart mass and epididymal fat ($r= -0.26$) (figure 3B), omental fat (r= -0.27) (figure 3C), visceral adiposity index (r= -0.32) (figure 3D) and inguinal subcutaneous adipose (r= -0.31) (figure 3E).

Figure 3 Analysis Pearson correlation coefficient (r) between relative heart mass with relative mass retroperitoneal (A), epididymal (B), omental (C), adiposity index (D), and inguinal subcutaneous adipose tissues. Level of significance adopted by p< 0.05.



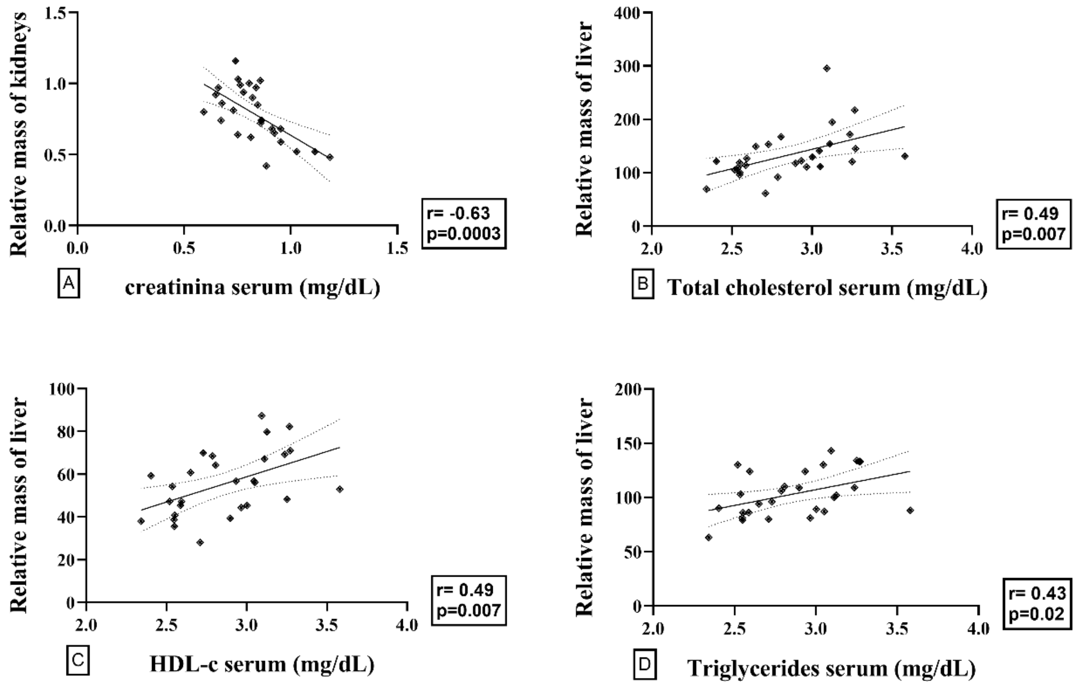
The variables relative gastrocnemius muscle mass relative retroperitoneal adipose tissue mass showed moderate and inverse dependency (r= -0.48; p< 0.01) (figure 4A). There was no dependency between relative mass of gastrocnemius muscle and epididymal adipose tissue (r= -0.25) (figure 4B). Figures 4C and 4D showed moderate and inverse dependency (r= -0.44; p< 0.05) between relative mass of gastrocnemius muscle and omental adipose tissue, as well as with the adiposity index (r= -0.45; p< 0.05) and the inguinal subcutaneous adipose tissue (r= -0.49; p< 0.01) (figure 4E).

Figure 4 Analysis of the Pearson correlation coefficient (r) between relative gastrocnemius muscle mass and relative mass of retroperitoneal adipose tissue (A), epididymal (B), omental (C) adiposity index (D), and inguinal subcutaneous adipose tissues (E). Level of significance adopted by p< 0.05.



Correlation between relative kidneys mass and creatinine levels, is shown in figure 5A, and there was moderate and inverse dependency (r= -0.63; p< 0.001). The relative liver mass showed moderate positive dependency (r= 0.50; p< 0.01) with cholesterol total (figure 5B), HDL-c (r= 0.50; p< 0.01) (figure 5C) and triglycerides (r= 0.43; p< 0.05) (figure 5D) serum levels.

Figure 5 Analysis of the Pearson correlation coefficient (r) between relative kidneys (right + left) mass and creatinine serum levels (A); Relative liver mass and total cholesterol (B), HDL- cholesterol (C), and triglycerides serum levels (D). Level of significance adopted by $p < 0.05$.



4 DISCUSSION

The present study showed that the HD, independent of resistance training (RT), reduced visceral fat (intra-abdominal), without promoting muscle mass increase. On the other hand, RT alone decreased visceral fat and increased the relative mass of the gastrocnemius and soleus skeletal muscles. In addition, was identified inverse dependence among relative heart mass and retroperitoneal adipose tissue; the same occurred among gastrocnemius muscle and retroperitoneal, omental, and inguinal subcutaneous adipose tissues. Interestingly, the relative kidneys mass presented inverse dependence with creatinine, whereas the liver showed positive dependence with cholesterol total, HDL-c, and triglycerides.

The results presented are consistent with previous studies, which showed that low-carb diets and high protein content (different types and percentages of protein), associated or not with physical training, control satiety, as well as decrease body mass and deposits of visceral and subcutaneous fat [11, 15, 17]. It is important to emphasize that the greater energy demand promoted by resistance training is associated with increased food intake and also with an increase in the mass of organs, which in turn is attributed to the adaptive response to increased food and protein intake, as well as processes of metabolism performed by the body [22].

Scientific evidence suggests that the thermic effect of food (TEF) is higher after protein intake than carbohydrates and fats [2, 12, 13, 19, 22, 25, 26]. Although, the effects of HD are not fully elucidated, is supposed that TEF contributes greatly to increased satiety and thermogenesis, these changes could explain the reduction of body mass gain, adiposity, and positives changes in the lipid profile of the animals in the present study.

To enable this hypothesis, [25], evaluated the energy expenditure after three different isoenergetic meals containing high levels of protein, carbohydrate, and lipid and found the relationship between TEF, the absorption of energy after these meals, as well as satiety feeling; the HD presented a greater thermogenic effect, being attributed to greater thermogenesis obligatory (greater sense of satiation up to 7 hours after the meal).

Based on the important study mentioned above, it is possible to suggest that the reduction in visceral adipose tissue mass verified in HD groups is associated with high energy expenditure by protein metabolism from digestion to the excretion of residues [12, 15, 17, 27]. It also should be highlighted that the protein type represents a determinant factor in the reduction of adiposity and favors greater satiety, besides positively modulating the blood lipid levels [15, 18]. In this case, the milk proteins, in particular those derived from milk serum (whey), have a great ability to reduce body mass gain, the adiposity index and, when carbohydrate intake is reduced, change the blood levels of lipid positively [17, 28].

In addition, the reduction in mass of the visceral fat tissues generated by RT was expected, since the physical training protocol applied (aquatic jumps, i.e RT) was high intensity, which has been reported to be beneficial to health due to its preventive action on metabolic risk factors and chronic diseases, especially to those related to excessive visceral adiposity.

Although, dietary protein intake, especially those of high biological value, associated with the potent anabolic of RT, represents a key link for muscle protein synthesis [29-31], was not observed increase in mass of gastrocnemius and soleus muscles in the present study.

Most of the energy used by cardiomyocytes is due to the catabolism of long-chain fatty acids (LCFA) for example the palmitic and the oleic, which are come, largely, from the circulation in the form of free fatty acids, where they are transported into the cells and, afterward, of mitochondria through cytosolic proteins and also to those bound to the mitochondrial membranes, especially by enzyme activity carnitine palmitoyltransferase I [32].

It is not fully understood in the literature what is the real effect of HD associated with RT on the oxidation of LCFA by the myocardium, nor the modulation of this association on the complex biochemical mechanism in other tissues. The fact that the present study verified an inverse association between the relative mass of the heart and visceral epididymal adipose tissue can be assigned, hypothetically, to the occurrence of a heart adaptation mechanism in response to the metabolic stress generated by both the energy inefficiency of HD and the high energy expenditure from RT. Future studies, based on specific analyzes (biochemical and molecular) in these two tissues, are needed to confirm this hypothesis since it is based only on the mass of the same is scientifically shallow.

The intake of HD (above the reference recommendations) is considered one of the main reasons for the increase in mass and function of the kidneys since promotes an increase in the rate of glomerular filtration with consequent renal hyperfiltration (RHF), which may lead to an increased risk of chronic kidney disease (CKD) [5, 26, 33]. In this regard, we believe that the inverse dependence of the kidneys on creatinine is related to RHF positively caused by HD administration. Given the limitation of the present study and despite the renal function of the animals has not been evaluated directly, it is worth raising a hypothesis about the protective effect of RT on the damage of HD to the renal system since great questions emerge from this subject.

Different nutritional strategies have been applied as an auxiliary measure in the prevention and control of dyslipidemias or risk factors for cardiovascular and hepatic diseases. In this context, it is important to point out that some dietary measures have specialized in modifying the paradigm of preventive recommendations described in *Guidelines* [34]. The nutritional strategies that consider the moderate replacement of carbohydrates by protein in a diet containing normal content of fat (saturated, mono, and poly saturated) result in a significant improvement in lipemic profiles and, consequently, reduce the usage of medication, such as statins. Da Rosa et al., [16], found that rats fed 35% protein (casein), submitted or not to the RT of aquatic jumps, exhibited increase serum levels of HDL cholesterol when compared to the control group. Although there were increased serum levels of glucose and triglycerides, there was no damage to the liver of animals, being confirmed by thorough histological analysis.

The most important limitations of this study should be highlighted, 1) the number of animals was an obstacle so that the investigation could show, more effectively, the link between the parameters investigated; 2) the general marginal mean of each variable to verify the dependence between them, i.e., were not considered the groups as isolated entities, this control does not always occur in correlation analyzes. It is essential to point out that the present study adopted as a premise the physiological similarities between rodents and humans. However, there is an extensive non-literature discussion that cast doubt on the relationship of phylogenetic responses among these species; therefore, it is essential to be cautious in extrapolating the results found.

In conclusion, our results delay the effective participation of HD independent of RT on lipid metabolism; this was not observed concerning its effects on organs, mainly the heart, liver, and kidneys. The association HD+RT seems to be positive since it was minimized the possible adverse effects of HD in isolation. It is worth noticing, that we verified the degree of association between heart mass and skeletal muscle with the visceral and subcutaneous adipose tissues, as well as the mass of the kidneys and liver for the biochemical parameters of the blood. Which denotes the participation of both HD and RT as measures of trend regarding the behavior of these variables.

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