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High-Protein Diet Associated with Resistance Training Improves Performance and Decreases Adipose Index in Rats

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ABSTRACT

International Journal of Exercise Science 13(2): 1366-1381, 2020. The study tested the hypothesis that a high protein diet based on isolated whey protein (IWP) associated with strength training improves performance and reduces body fat without promoting health damage. Male Wistar rats, 45 days old, were divided into four groups (n = 8 / group): normoprotein sedentary (IWP 14%; NS); hyperprotein sedentary (IWP 35%; HS); normoprotein trained (IWP 14%; NT) and hyperprotein trained (IWP 35%; HT). All groups performed the maximum load test at the beginning and after the vertical ladder training protocol for 6 weeks (3x/week). The performance improved in HT when compared to other groups. There was no difference in the plasma levels of testosterone, IGF-1 and the hematological parameters remained normal. The relative weights of the kidneys were higher in the groups fed with high protein; the liver was higher in HT compared to NS and NT, and the heart was higher in HS compared to NS and NT. Concerning relative muscle weight, quadriceps, and gastrocnemius, HT showed higher value compared to NT. Diet containing 35% isolate whey protein associated with resistance training improved performance as well as increased muscles and organs weight of the animals, without damaging the tissues related to protein metabolism (confirmed by unchanged hematological parameters), which may minimize the risk of developing cardiometabolic disorders.

KEY WORDS: Isolated whey protein, strength training, adipose tissue

INTRODUCTION

A balanced diet is one of the main factors regulating growth and development, and its important components (macronutrients [protein, carbohydrate, and lipids], and micronutrients [minerals

and vitamins]) promote cellular multiplication and energy storage (48). Protein is a macronutrient that is primarily responsible for positively modifying the resting energy consumption in both active and sedentary individuals, providing substrates for tissue regeneration, myofibrillar increase and, especially, for improving body composition (11, 22, 23).

An increase in dietary protein intake may stimulate a rise in total energy expenditure since it promotes higher thermogenesis (both mandatory and optional), resulting in elevated resting energy expenditure (8). Compared to other macronutrients, protein requires approximately 20-30% of usable energy to be metabolized and/or stored, whereas carbohydrates require 5-10%, and lipids about 3% (28). Isolated whey proteins (IWP) contain a proportion of essential amino acids near 43%, of which 26% are branched-chain (leucine, isoleucine, and valine). Because the amino acid composition has great similarity to skeletal muscle, it is believed that supplementing with IWP provides all amino acids necessary for the metabolism of this tissue (20, 21).

The effects of different types of dietary protein administration, applying or not the resistance training (RT), on the morphology and function of skeletal muscle require further investigation. Several studies have demonstrated that RT can prevent lean mass loss, in addition to generating positive effects on cardiometabolic function (10). Furthermore, skeletal muscle is the tissue that presents the greatest energy consumption in the body (45), and it is responsible for the oxidation and/or storage of a great amount of glucose (glycogen) and plasma lipids (45, 64). Besides, exercise and nutrient availability are the main determinants for muscle protein synthesis in adult individuals (50).

As it occurs with the adaptive mechanisms for the maintenance of the homeostasis after physical exercise, a high-protein diet (HD) seems to promote similar alterations, such as hyperaminoacidemia, increased plasma colloid osmotic pressure (i.e., increased water attraction, with consequent euvolemia elevation) and hyperammonemia. In theory, according to the quality of the protein, (i.e. the amino acid composition ratio) along with its digestibility and bioavailability, IWP would contribute to different responses including the ability to assist in the control of glycemic levels, stimulate protein synthesis and tissue repair, and improve body composition (26, 36, 54).

Protein-rich diets are used for maintenance and/or to improve body weight, muscle hypertrophy, and post-exercise recovery (12). There is controversy surrounding the intake of dietary protein type (e.g., whey, casein, meat, soy, rice) with inconclusive literature on the clinical and metabolic effects of protein intake and the real needs of the human body (12, 33). Nutritional recommendations proposed by the Institute of Medicine preconizes that protein values vary from 10% to 35% of total energy intake (46, 62). The positive and / or negative effects of consuming high-protein diets on organic functionality in sedentary individuals and/or subjected to resistance training are not fully understood (33, 52). HD was associated with several alterations, among them those that affect the kidneys (2, 13), and possible development of type 2 diabetes mellitus (18, 59). Protein dietary are important modulators in glucose metabolism (32). Cohort studies have indicated that the type of protein in the diet may influence the type 2 diabetes mellitus development (red meat and processed meat), but can also have a protective

effect, for instance, soy, dairy and dairy products (56, 60). Thus, experimental studies have shown that RT has demonstrated a protective effect on possible dietary damage (3, 13).

Proper and regular exercise practice increases lipid oxidation as well as decreases the body fat percentage, which is positively associated with improved physical performance and overall health (26). In this regard, it is of great scientific interest to verify the association of HD containing isolated whey protein and RT administration as prevention of obesity and cardiovascular and metabolic diseases (13).

The present study aimed to verify the effects of HD administration containing 35% IWP on performance, biochemical, hormonal and tissue parameters in rats that completed a vertical ladder RT protocol for 6 weeks performed 3 times per week. Additionally, we intended to contribute to the clarification of the real effects of excessive consumption of protein on an organism biologically similar to humans. In this sense, we hypothesize that HD containing IWP associated with proper and regular RT prevents metabolic damage modulated by body composition improvement as well as a reduced visceral and subcutaneous fat stores, and contributes directly to increased physical performance and overall health.

METHODS

Participants

Thirty-two male Wistar albino rats (Rattus norvegicus) were obtained from the facilities of Central Animal House of the Federal University of Mato Grosso (UFMT), Cuiabá, Mato Grosso, Brazil. As soon as the animals were 45 days old and an approximate weight of 70.0±2.0 g, they were transferred to the Laboratory of Animal Experimentation and Exercise of NAFIMES/FEF/UFMT. Animals were housed collectively in polyethylene cages (37.0 × 31.0 × 16.0 cm), maintained at 25±1 °C, relative humidity of 45-55% and artificial lighting with a photoperiod of 12/12 as follows: light cycle (06h00 p.m. to 06h00 a.m.) and dark cycle (06h00 a.m. to 06h00 p.m.). This study was approved by the Ethics Committee for the Use of Animals (CEUA) at UFMT (Protocol number: 23108.208999/2017-97). The study was conducted in accordance with the ethical principles of animal experimentation according to the Brazilian College on Animal Experimentation (COBEA) (Law no. 6638, of May 8, 1979, and Decree no. 26645 and 10 July 1934). This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (42).

The animals were kept under observation for 7 days (room and time zone adaptation) with food and water provided ad libitum (Purina® commercial chow and tap water). At the beginning of the second week, all animals were weighed and randomly assigned to groups according to the type of diet and resistance training received as described below:

- Normal protein diet Sedentary (NS; n = 8): sedentary animals fed with a purified diet containing 14% of the total energetic value composed of IWP;
- High-protein diet Sedentary (HS; n = 8): sedentary rats fed with a purified diet containing 35% of the total energetic value composed of IWP;

• Normal protein diet Trained (NT; *n* = 8): rats submitted to resistance training throughout the experiment and fed with a diet containing 14% of the total energetic value composed of IWP.
• High-protein diet Trained (HT; *n* = 8): rats submitted to resistance training throughout the experiment and fed with a diet containing 35% of the total energetic value composed of IWP.

Protocol

The semi-purified diets were prepared according to the guidelines of the American Institute of Nutrition model AIN-93M (47), to stay isocaloric (≅ 3.8 kcal/g). The diets were modified only concerning protein (Whey Protein Isolate ISOFORT/VITAFOR; percentage of purity = 85.4% determined by a specialized analytical laboratory) and carbohydrate (cornstarch) ratios; The lipid content (soy oil) as well as other dietary ingredients (i.e. dextrin, sucrose, fiber, salt mix, vitamin mix, L-cystine and choline bitartrate) of both diets were kept unchanged. The proportion of macronutrients in the Normal protein diet was 75.8% carbohydrate, 14.7% protein, and 9.5% lipid; while the High-protein diet was 55.5% carbohydrate, 35% protein, and 9.5% lipids (Tab.1 – supplementary). Both diets were offered in the form of pellets.

Resistance training adaptation occurred during the second and third weeks, with all animals stimulated to climb a vertical ladder apparatus (1.1 x 0.18m, 2 cm between the steps and 80o inclination) (24). The ladder had the necessary length for animals to perform 8-12 climbing ascent cycles. Conical flasks (Falcon®) were attached to the proximal portion of the tail, with the aid of a band of adherent tissue, for future overload training. With the apparatus attached to the tail, the animals performed climbing movement through contact stimuli (without pain and with minimal stress). Upon reaching the top of the ladder (Housing chamber; 20 cm x 20 cm x 20 cm) the animals remained at rest for 2 minutes. This procedure was repeated three consecutive times, five days per week to familiarize the type of training and minimize the possible stress caused by physical exercise.

All the animals completed a maximum load test (MLT) on two occasions: a) before the first session of the resistance training of ladder climbs (RTLC) (24); and b) at the end of the sixth week of training. The first climb was performed with an overload equivalent to 75% of the body mass. When reaching the housing chamber, the animal rested for 2 minutes. From the second climb onwards, the rest period remained the same, however, 30g of lead was added progressively per climb until the animal failed to complete the ascent. Ascent failure was defined as the absence of movement with two successive slight touch stimuli applied to the rear portion of the animal's body. The maximum load (ML) was determined to be the largest successive load carried the entire length of the ladder was considered as the maximum capacity.

After the familiarization period, the animals belonging to the NT and HT groups were submitted to RTLC for a total duration of 6 weeks. Training sessions were conducted 3x per week (Monday, Wednesday and Friday), and always at the same time of day (~2:00 p.m.). The RTLC sessions consisted of 4 sets of 2 ladder climbs with 2-minute rest intervals between each set. The overload

periodization was as follows: Weeks 1 and 2- 75% of ML, weeks 3 and 4- 80% of ML, and 5 and 6- 85% of ML (27).

Body weight, as well as food and water intake, were determinate 3x/week throughout the experiment. Body weight and food intake were measured using a digital scale (Denver Instruments®-P8001 model with 0.1 g graduation). Body weight gain during the experiment was determined by the difference of final weight from the initial weight. Water intake was determined with the aid of a 1000 mL polypropylene beaker with a minimum graduation of 5 mL.

At the end of the 6 weeks of RTLC, the animals remained without RT for 48 h with minimum levels of manipulation, and food was removed at least 12 h before euthanasia. Animals were anesthetized by inhalation of excess CO2 followed by exsanguination via decapitation using a guillotine (Insight, Stream Nearby, São Paulo, Brazil). Blood samples were immediately centrifuged at 3000 rpm for 15 min to obtain serum that was aliquoted into polypropylene microtubes (1.5 mL) (Eppendorf) in appropriate volumes to avoid degradation of analytes due to repetitive thawing and then stored at a temperature of −86°C (COLDLAB mark Ultra Freezer/CL58086V model) for further biochemical analysis.

Immediately after euthanasia and blood collection, the quadriceps, tibialis anterior, gastrocnemius, soleus, and long finger extensor (LFE) muscles were carefully extracted (from origin to insertion). Subcutaneous inguinal, epididymal, retroperitoneal, and omental adipose tissues were also extracted. The visceral adipose index post-mortem (VAI) was determined by using the formula: VAI = [Visceral adipose tissue (VAT) / BM] x 100. VAT (g) was determined by summing the following constituents of adipose tissue: epididymal + retroperitoneal + omental + perirenal (denotes the total storage body fat) (31). Finally, the liver, kidneys, and heart were removed and weighed for comparison among the groups.

The analyses were conducted in triplicate using standard commercial reagents (Bioclin® Quibasa Chemistry Ltda - Belo Horizonte, MG, Brazil) with the aid of a spectrophotometer (UV-mini 1240, SHIMADZU) or microplate reader in the case of ELISA assay (EspectraMax® 190 UV–Vis, Molecular Devises). For the determination of hormonal plasma concentrations, the samples were analyzed at the CEDIVET laboratory (Veterinary Diagnostic Center; CNPJ: 10.338.354/0001-02 – Cuiabá, Mato Grosso, Brazil).

Statistical analysis

Data were analyzed for normality (Shapiro-Wilk test) and homogeneity (Levene test). Firstly, the data were evaluated by analysis of variance (ANOVA), differences among groups were considered significant at $p < 0.05$ and, when observed, Tukey post hoc was used. Followed, the main effects of diet (D) and training (T), including their interactions (i.e. D x T) were analyzed, with diet and training maintained as fixed factors. The data were analyzed by factorial ANOVA (2x2) (Diet: normal protein, high-protein; Exercise: sedentary, trained). Data were expressed as mean ± standard deviation (SD). The analysis was performed using Statistical Package for the Social Sciences software (SPSS version 22; IBM®, Armonk, NY, USA).

RESULTS

Results of initial body weight, final body weight, body weight gain and food and water intakes are shown in table 1. There was no statistical difference in initial body weight (g) (p = 1.00), final body weight (p = 0.44) and body weight gain (p = 0.61) among groups. Food intake (g/day) was similar among groups (p = 0.90). On the other hand, water intake (mL) was higher in the HS and HT groups when compared to NS and NT (p < 0.01); there was the main effect for diet (p < 0.01), as well as interaction D x T (p = 0.04).

Table 1. Initial and final body weight and food and water intakes.

Values	Groups				p-value	Main Effects		Interaction
	NS	NT	HS	HT		D	T	DxT
Initial body weight (g)	186.5±26.3	186.7±26.7	187.4±25.6	187.4±26.2	1.00	-	-	-
Final body weight (g)	458.4±28.6	476.8±48.2	447.7±32.1	456.1±32.9	0.44	-	-	-
Body weight gain (g)	271.9±44.0	290.1±51.2	260.4±55.2	268.7±21.4	0.61	-	-	-
Food intake (g/day)	171.2±17.5	173.9±24.8	170.3±22.4	166.1±24.5	0.90	-	-	-
Water intake (mL/day)	185.5±17.1[a]	212.3±32.4[a]	368.9±84.9[b]	318.2±61.8[b]	<0.01	<0.01	0.52	0.04

Results expressed as Mean±SD and significance level p < 0.05. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted lettersab. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

Results regarding the evaluation of ML performance are described in table 2. The initial maximum load was not different among groups (p = 0.16). However, after 6 weeks of exercise training, the HT group carried a higher load when compared to HS, NT, and NS (p < 0.01). There were main effects of diet (p < 0.01) and training (p < 0.01) were observed concerning the final maximum load but without any interaction between groups.

Table 2. Initial and final maximum load tests.

Values	Groups				p-value	Main Effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Initial MLT (g)	265.7±18.9	255.1±30.6	258.0±23.9	234.2±35.5	0.16	-	-	-
Final MLT (g)	323.0±63.7[a]	472.6±72.7[b]	730.6±89.7[c]	964.8±117.6[d]	<0.01	<0.01	<0.01	0.19

MLT: maximum load test. Results expressed as Mean±SD and significance level p < 0.05. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted letters abcd. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

Table 3 displays the values for general biochemical parameters. Blood glucose levels were lower in HT when compared to NT, and similar to NS and HS, respectively (p < 0.05). There was a diet effect (p = 0.01) on glycemic levels. There was no difference in the activity of alanine

aminotransferase (ALT) (p = 0.83), aspartate aminotransferase (AST) (p = 0.77), total bilirubin levels (p = 0.22), creatinine (p = 0.25) and β-hydroxybutyrate (β-OH) (p = 0.45) among the groups.

Table 3. Biochemical parameters at the end of the 6-week experimental period.

Biochemical parameters	Groups				p-value	Main effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Glycemia (mg/dL)	165±14ab	176±22a	155±16ab	151±22b	<0.05	0.01	0.62	0.25
AST (U/L)	135±41	141±27	134±20	123±41	0.77	-	-	-
ALT (U/L)	32±8	35±6	33±2	33±4	0.83	-	-	-
Total bilirubin (mg/dL)	0.4±0.1	0.5±0.2	0.5±0.2	0.7±0.5	0.22	-	-	-
Creatinine (mg/dL)	0.7±0.1	0.7±0.1	0.6±0.1	0.7±0.2	0.25	-	-	-
β-OH (mmol/dL)	0.9±0.2	0.9±0.2	1.0±0.2	0.8±0.1	0.45	-	-	-

AST: aspartate aminotransferase; ALT: alanine aminotransferase; β-OH: β-hydroxybutyrate. Results expressed as Mean±SD and significance level $p < 0.05$. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted letters abc. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

The relative weight of the kidneys, liver, and heart are described in table 4. There were significant differences between the weight of the kidneys ($p < 0.01$), liver ($p < 0.01$) and heart ($p < 0.01$). The weight of the kidneys was higher in HS and HT compared to NS and NT ($p < 0.01$); there was a main effect for diet on kidney weight ($p < 0.01$). Liver weight was higher in the HT group compared to NS and NT ($p < 0.01$), but there was no difference for HS; there was a main effect for diet on liver weight ($p < 0.01$). Heart weight was higher in the HS group compared to NS and NT, and similar to the HT group ($p < 0.01$); the main effect for diet on heart weight was observed (p = 0.01).

Table 4. Relative weight of organs at the end of the experiment.

Organs	Groups				p-value	Main effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Σ kidneys (%)	0.58±0.04^{a}	0.59±0.02^{a}	0.72±0.05^{b}	0.70±0.04^{b}	<0.01	<0.01	0.80	0.37
Liver (%)	2.62±0.19^{ab}	2.60±0.19^{a}	2.88±0.22^{bc}	2.93±0.21^{c}	<0.01	<0.01	0.88	0.63
Heart (%)	0.29±0.02^{a}	0.27±0.01^{a}	0.32±0.02^{b}	0.30±0.03^{ab}	<0.01	0.01	0.09	0.88

Results expressed as Mean±SD and significance level $p < 0.05$. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted lettersabc. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

The relative weight of the gastrocnemius, soleus, tibialis anterior, LFE and quadriceps muscles are described in table 5. No differences were identified in the weight of the soleus (p = 0.25), tibialis anterior (p = 0.39) and LFE (p = 0.15). There was a significant difference in the weight of

the gastrocnemius (p = 0.05) and quadriceps (p = 0.02), with HT greater than NT. There was a main effect only for diet on gastrocnemius (p = 0.02) and quadriceps (p < 0.01) weight.

Table 5. Relative weight of the skeletal muscles at the end of the experiment.

Muscle	Groups				p-value	Main effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Gastrocnemius (%)	1.08±0.05^{ab}	1.05±0.09^{a}	1.11±0.10^{ab}	1.16±0.09^{b}	0.05	0.02	0.66	0.16
Soleus (%)	0.11±0.01	0.10±0.01	0.11±0.01	0.11±0.01	0.25	-	-	-
Tibialis anterior (%)	0.32±0.02	0.32±0.02	0.34±0.03	0.34±0.03	0.39	-	-	-
LFE (%)	0.06±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.15	-	-	-
Quadriceps (%)	1.56±0.08^{ab}	1.53±0.09^{a}	1.63±0.11^{ab}	1.67±0.09^{b}	0.02	<0.01	0.91	0.33

LFE: Long Finger Extension. Results expressed as Mean±SD and significance level p < 0.05. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted lettersab. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

Table 6 presents the results of the relative weight of the subcutaneous (inguinal), visceral (retroperitoneal, perirenal, omental and epididymal) tissues as well as the adiposity index. No differences were observed between groups in inguinal (p = 0.26), retroperitoneal (p = 0.12), perirenal (p = 0.20) and omental (p = 0.05) adipose tissue weights. There was a difference in the epididymal adipose tissue weight (p = 0.01) among the groups, with HT great than NT. The adiposity index was lower (p = 0.01) in HT compared to NT. Diet had a main effect on epididymal adipose tissue weight (p < 0.01) and adiposity index (p = 0.01).

Table 6. Relative weight of adipose tissues and adiposity index at the end of the experiment.

Adipose tissues	Groups				p-value	Main Effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Inguinal (%)	1.34±0.27	1.33±0.30	1.21±0.37	1.07±0.26	0.26	-	-	-
Retroperitoneal (%)	1.55±0.22	1.63±0.40	1.36±0.61	1.14±0.38	0.12	-	-	-
Perirenal (%)	0.50±0.14	0.54±0.14	0.47±0.15	0.39±0.14	0.20	-	-	-
Omental (%)	0.18±0.06	0.17±0.05	0.12±0.05	0.11±0.03	0.05	-	-	-
Epididymal (%)	2.21±0.25^{ab}	2.31±0.45^{a}	1.90±0.57^{ab}	1.71±0.11^{b}	0.01	<0.01	0.76	0.31
Adiposity index (%)	4.44±0.48^{ab}	4.65±0.79^{a}	3.85±1.18^{ab}	3.36±0.50^{b}	0.01	0.01	0.63	0.22

Results expressed as Mean±SD and significance level p < 0.05. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted lettersab. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

Table 7 shows that there were no significant differences in serum concentrations of testosterone (p = 0.09) and IGF-1 (p = 0.91) among the groups.

Table 7. Serum concentrations of testosterone and IGF-1 at the end of the experiment.

Hormones	Groups				p-value	Main effects		Interaction
	NS	NT	HS	HT		D	T	D x T
Testosterone (ng/dL)	27.2±11.9	75.3±25.3	67.4±53.4	67.7±44.0	0.09	-	-	-
IGF-1 (ng/mL)	26.3±6.0	28.8±8.3	28.1±5.5	28.1±8.3	0.91	-	-	-

IGF-1: Insulin-like growth factor type 1. Results expressed as Mean±SD and significance level $p < 0.05$. Analysis of variance (ANOVA), followed by Tukey HSD post hoc when differences were found are marked with superscripted lettersab. Main effects Diet (D) and Training (T), including interactions between diet x training (D x T) were analyzed.

DISCUSSION

The most relevant findings of the present study provide evidence that diet containing 35% IWP, whether associated with RTLC or not, improved performance, even though there was no change in final body weight or weight differences between groups. Also, there were decreases in the relative weight of the epididymal adipose tissue and the adiposity index. On the other hand, HD promoted greater water intake, increased relative weights of the liver, kidneys, and heart as well as the quadriceps and gastrocnemius muscles. Although HD altered the relative weights of the abovementioned organs/tissues, there was no significant alteration in the hematological parameters of the animals (Tab. 2 supplementary).

HD in combination with RT has well-known effects including a decrease in both visceral and subcutaneous fat, increased muscular mass, and improvement in performance variables (5, 7, 55, 57, 58). To explain the effect of diet on epididymal fat and adiposity index, it is suggested that the thermal effect of protein increases after HD intake when compared to other diets (28, 48). The variability of the thermal effect can be attributed to differences in molecular structure that significantly alter metabolism (4). A study showed that adult male rats fed HD (32% protein) for 9 weeks, reduced food intake and weight of epididymal and subcutaneous adipose tissues, as well as increasing fat-free mass (7). Although the present study did not observe a reduction in food intake, there was a reduction in the weight of epididymal adipose tissue and the adiposity index in the HS and HT groups; and we suggest that the thermal effect of HD accounts for this finding.

The volume of muscle mass employed together with the ability of the neuromuscular system to activate motor units results in muscle strength, power, and endurance, favoring a greater musculoskeletal performance (35, 51). Observing our results concerning the maximum load (higher loads in the trained groups), we believe that HD stimulated muscle protein synthesis and favored muscle hypertrophy since the HS group supported high final maximal loads. In both groups fed HD an increase in maximum load was observed, along with a greater weight of the quadriceps and gastrocnemius muscles. However, further investigation on whether HD increases muscle hypertrophy via increased protein synthesis is needed to confirm this hypothesis.

There are several contradictions about the effects of testosterone on adaptive responses triggered by RT. Evidence reports that RT, when performed at a relatively high intensity ranging around 70 and 85% of maximum load capacity, can increase muscle mass by stimulating protein synthesis rate (37). Despite this, studies emphasize that RT may increase testosterone secretion, thus promoting muscle hypertrophy, especially by inducing replication of satellite cells present in the extracellular matrix (51, 53). However, it should be clarified that muscle anabolic action is mediated by intracellular androgen receptors (AR), which seems to be more important for adaptive physiological responses than elevated blood testosterone levels (41). Previous research performed by Mitchell et al. (37) indicated a significant positive correlation between RT and AR gene expression, as well as between muscle volume and AR protein synthesis. Although we did not show an increase in blood testosterone levels and taking into consideration that the strength was increased in the HT group, is plausible to hypothetically suggest that RT associated with HD containing IWP may have induced positive adaptations by stimulating AR synthesis.

Similarly testosterone, IGF-1 plays a prominent role in modulating cell growth and repairing muscle tissue, suggesting that the expression and/or availability of IGF-1 plays a key role in muscle hypertrophy induced by training as it is synthesized and secreted during contractile activity (14, 25). In the present study, there was no difference in IGF-1 and testosterone levels even after 6 weeks of RTLC. It is hypothesized that these results may be due to the short training frequency performed (only 3 times per week).

Protein intake above the recommended amount can generate functional and morphological damage in tissues involved in metabolism, including the excretion of final products (1, 13, 15, 22, 48). Considering the liver and kidneys as the fundamental organs for homeostatic control, protein metabolism, and residual excretion, HD can cause different types of pathologies (15, 34).

The liver plays a crucial role in the upregulation of genes involved in amino acid metabolism. HD intake increases the hepatic metabolism of amino acids, as it promotes the cellular import of amino acids, deamination as well as an increase in the urea cycle (15, 39). In the present study, liver weight was higher in the high protein groups, as was the concentration of urea. These results suggest a greater load on the liver due to increased metabolic demand, but without altering ALT and AST enzymes or bilirubin total (Tab. 3 supplementary). Besides, ketogenic indicators were not altered, in particular, β-OH, which is synthesized mainly in the liver from lipid oxidation, and occurs during fasting, prolonged exercise and hypoglycemic diets (ex., HD) (44).

In a study conducted by our research group (13), 32 male rats completed training through aquatic jumps and fed with HD containing 35% casein. After 8 weeks of training (5x per week), the animals did not present with any hepatic damage. This result was confirmed by histological analysis and the absence of alterations in the activity of the ALT and AST enzymes; which are consistent with the results of the current investigation.

Although the actual impact of HD on renal function has not been fully elucidated, it is believed that the potentially harmful effect of excessive protein intake is due to the high demand for

protein from this organ's work (2), since the compounds produced by protein metabolism are eliminated in the urine. Thus, increased protein intake may increase both the intraglomerular pressure and the glomerular filtration rate, which may cause morphological and functional damage to the kidneys (1, 13, 34, 61). Furthermore, creatinine is a non-protein nitrogen metabolite, which acts as an important marker of muscle metabolic rate, protein intake and renal function (30). In the present study, creatinine values were not altered, which denotes an adaptive physiological effect for the high levels of dietary protein as well as for the training adopted. Thus, this outcome suggests, at least in part, that HD containing 35% IWP and RTLC did not promote renal overload.

In our study, the HD groups had higher values of water intake and relative kidney weight (indicative of greater renal work). It is known that the excess in the intake of ketogenic amino acids (isoleucine, leucine, lysine, threonine, and valine) (63) increases plasma osmolarity, which in turn, promotes a greater thirst sensation through hypothalamic activation, resulting in greater water intake (49).

Regular physical exercise promotes positive cardiovascular adaptations, resulting in improvements in performance at submaximal and maximal exercise intensities (43). Physiological cardiac hypertrophy is characterized by maintaining normal structure and improving cardiac function (9). In a recent study carried out by our research group, higher heart weight was observed in male rats fed with a high protein diet, indicating that both HD and RT through aquatic jumps can promote cardiovascular alterations (6). On the other hand, in the present study, there was an increase in the relative weight of the heart only in the HD-fed sedentary animals, suggesting that the vertical ladder RT promoted cardiac remodeling when compared to the aquatic jumps RT.

The molecular signaling pathway responsible for glucose uptake occurs because of increased expression and/or activity of the key proteins involved in glycemic regulation (40). IWP ingestion can stimulate GLUT-4 translocation to the plasma membrane, favoring glucose uptake without altering insulin levels (38). The participation of leucine in the uptake of glucose could explain the results obtained in the present study since the groups fed with HD presented lower glycemia when compared with the normoproteic groups. Moreover, it is possible to say that RTLC potentiated the uptake of glucose by the skeletal muscle via GLUT-4 (38), since glycemia was lower in HT.

Although the benefits of strength training are multifactorial, including improvement in hemostatic balance as well an increase in blood fibrinolytic potential (17), response to platelet counts in exercise (increase or decrease) is influenced by the intensity, duration, and physical state of the individual (16, 19). In our study, RTLC and HD did not change platelet counts, maintaining similar values among groups. Furthermore, it is important to note that the diet and the exercise protocol used may be considered adequate for the present study concerning the health of the animals since no alteration was observed in hematological parameters (29).

This study is not without limitations, for example, a) we did not use individual and metabolic cages, which could provide more in-depth and reliable information from the analysis of urine and feces; b) protein expression of GLUT-4 in skeletal muscle, and a glucose tolerance test to better explain our findings regarding glycemia, were not performed; c) histomorphological analysis of the kidney, liver, and heart were not performed, which could confirm our hypothesis related to the adaptive process of these organs to HD; d) cross-sectional area of the quadriceps and gastrocnemius muscles was not determined, as well as molecular analysis (investigation of specific signaling pathways) to confirm the hypothesis related to the occurrence of hypertrophy in these tissues.

From the results obtained in the present study, it is possible to conclude that the RTLC, HD containing 35% IWP, as well as the combination of RTLC and HD, promotes an improvement in the performance of healthy and eutrophic rats. However, only HD increased the relative weight of muscles (quadriceps and gastrocnemius), liver, kidneys, and heart. Moreover, RT and the administration of HD, alone or in combination, did not promote significant changes in blood, hormonal and biochemical parameters.

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