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Moderate vs high-load resistance training on muscular adaptations in rats.

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Abstract

Aims: The main aim of this study was to investigate the moderate versus high-load resistance training on muscle strength, hypertrophy and protein synthesis signaling in rats. **Methods:** Twenty rats were randomly allocated into three groups as follow: control group (C, n = 6), high-load training (HL, n = 7) and moderate-load training (ML, n = 7). A latter climb exercise was used to mimic resistance exercise. ML resistance training consisted of a moderate load, allowing performance at higher volume of load inherent to higher number of repetitions (8-16 climbing). HL resistance training consisted of progressively increase training load, with low volume of load (4-8 climbing). C group remained with physical activity restricted to their cage space. This experiment was conducted over a six-weeks period. Forty-eight hours after the last resistance training session the animals were euthanized for tissue collection. **Results:** Both HL and ML regimens promoted similar increases in muscle strength, elevated protein synthesis signaling demonstrated by increased skeletal muscle total/phosphorylated P-70S6K ratio and similar increases in plantaris and FHL muscle hypertrophy, all compared to control. All these similarities were demonstrated even though testosterone/cortisol ratio was higher in HL group compared to ML and control. ML regimen caused higher total training volume and soleus muscle hypertrophy, which was not demonstrated in HL group. **Conclusion:** In conclusion, results suggest that both HL and ML induce muscle hypertrophy and increase on strength in a similar way. ML moreover seems to favor slow fiber hypertrophy due the higher training volume.

Keywords: Cortisol, muscle mass, protein synthesis, resistance exercise, strength, testosterone.

1. Introduction

Since Hornberger & Farrar [1] first proposed the ladder climb as a resistance training (RT) model in rats, several studies have used the ladder climb exercise to study the effects of RT in different heath and disease conditions in rodents [2-5]. Indeed, the ladder climbing can now be considered the most-used RT model in rodents. However, fifteen years later the proposal, little is known about the full range of possibilities to develop RT variances of this model in rodent studies. Most studies using the ladder climb as a RT model in rodents use high-load (HL) training, as first proposed by Hornberger & Farrar (2004) [1]. HL traditionally uses heavy loads combined with low number of repetition (low volume) [1, 6] to promote the recruitment of fast-twitch muscle fiber types and metabolic stimuli, consequently increasing strength and skeletal muscle hypertrophy [7, 8].

However, discussions have recently arisen about the idea that heavy loads are not necessary for optimizing the post-exercise muscular response for hypertrophy; studies have considering that low to moderate training loads combined with a higher number of repetitions (potentially to muscular failure) may induce similar adaptations when compared to a heavy-load RT regimen [9]. The moderate load (ML) regimen is based on the argument that simply performing an RT session to momentary muscular failure—regardless of load—could result in developing the full spectrum of available motor units, thus increasing the potential for hypertrophy [10, 11]. Indeed, some studies demonstrate that fatiguing contractions result in an increased contribution of higher threshold motor units engaged to maintain force output [7, 11]. Therefore, whether ML with more repetitions can produce similar results in muscle strength and hypertrophy is still up for debate. Whether RT regimens of different load-volume combinations can be adapted to a rat ladder climbing model is also unknown. Importantly, the impact of different loading regimens on rats' skeletal muscular

hypertrophy may improve our knowledge about the molecular bases of hypertrophy, because having the muscle tissue is the best way to measure hypertrophy signaling pathways in skeletal muscle.

Therefore, the aim of this study was to investigate the effect of two different RT load regimens—HL versus ML resistance training—on strength, skeletal muscle hypertrophy, and hormonal and protein synthesis signaling in rats. Our initial hypothesis was that the ML training regimen would promote similar levels of strength and muscular hypertrophy as the HL training regimen, even though ML generates a lower training load compared to the HL training regimen. Our study is relevant because RT has been studied as a health promotion tool in several disease models over the past few years, including in elderly and muscle-wasting diseases—conditions that are in several cases incompatible with heavy-load RT protocols.

2. Methods

2.1 Rats and experimental design

This experimental study was performed in accordance with Animals in Research: Reporting In Vivo Experiments (ARRIVE). All procedures were approved by the Ethics Committee for Animal Use at the State University of Londrina and were in accordance with the Guidelines of the Brazilian College of Experiments with Animals (COBEA). The sample size and power of analysis were calculated using G*Power 3.1 (total sample size = 6, effect size f = 0.8, and statistical power [1-β= 0.85]).

Twenty male Wistar rats, each weighing 210 ± 7.4 g, were obtained from the Biological Sciences Center at the State University of Londrina. The rats were housed in collective cages on an inverted 12-h-light/12-h-dark cycle at a mean temperature of 22ºC, with free access to food and water throughout the experimental period, a total of seven weeks. All groups were fed with the same standard commercial diet from Nuvilab® [Quintia, Curitiba, Brazil (carbohydrates 62.7%, protein 24.8%, and fat 12.4%)]. After one week of acclimatization, the rats were randomly allocated

using a random sequence generator (www.Random.org) into three groups, as follows: sedentary control group (C, n = 6), RT exercise group with a moderate-load regimen (ML, n = 7), and RT exercise group with a high-load regimen (HL, n = 7). The rats from the ML and HL groups were submitted to an RT routine as detailed below, while the rats from the control group had their physical activity restricted to their cage space throughout the experimental period. Forty-eight hours after the last RT session, the rats were euthanized for tissue collection.

2.2 Resistance training regimen

The RT exercise regimen consisted of climbing a ladder (1.1 x 0.18 m, 2-cm grid, 90° incline) 3 times a week for a total of six weeks, in a regimen adapted from Hornberger and Farrar (2004) [1]. The length of the ladder was determined so that the rats could complete 8-12 dynamic movements per climb. At the top of the ladder, a dark covered chamber had been constructed (20 x 20 x 20 cm) for interval resting between climbing bouts. One week before starting the HL and ML protocols, all rats were familiarized with the exercise apparatus. In the familiarization week, the rats were placed at the lower part of the ladder and stimulated to climb by being pushed to initiate movements. The pushing stimuli were performed until each rat was capable of climbing the entire ladder. At the top of the ladder, the rats could rest for two minutes. At the end of the familiarization period, all rats were able to voluntarily climb the ladder without stimulus. No attached load was used during this period.

After the familiarization week and before the HL and ML training regimens, all rats were evaluated with the maximal strength test (adapted from Hornberger and Farrar, 2004). Initially, all rats were made to climb the ladder with a load corresponding to 75% of their body weight, attached to their tail with adhesive tape. After successfully climbing with the initial load, an additional 30g was added to the load. This procedure was successively repeated until a load was reached with which the rat was unable to climb the complete ladder length for three consecutive attempts. In

those cases, the weight load of the most recent successful climbing attempt was defined as the rat's maximal strength. This test was repeated at weeks 2, 4, and 6 as a strength gain parameter.

Forty-eight hours after the maximal strength test, the ML and HL regimens were initiated. The HL training regimen consisted of four ladder climbs while carrying 50%, 75%, 90%, and 100% of their maximal carrying capacity, respectively. At fifth ladder climb, an additional 30g was added to the load. This procedure was successively repeated until a load was reached with which the rat was unable to climb the complete ladder length for three consecutive attempts, or a maximum of eight total successful climbs. The load pulled in the last successful climbing attempt was used as maximal strength and used to adjust load training for the next subsequent HL training session; thus, the HL training load was adjusted every training session. In this way, the HL regimen involved heavy loads (high-intensity) and low number (4-8) of climbs (low volume) training sessions.

The ML training regimen consisted of 8 to 16 ladder climbs while carrying 70% (in weeks 1-2), 80% (in weeks 3-4), and 85% (in weeks 5-6) of their maximal strength. The ML training load was adjusted only in weeks 2, 4, and 6, based on their maximal strength test. In this way, the ML regimen involved low/moderate-load (low/moderate intensity), high number (8-16) of climbs (high volume) training sessions.

2.3 Necropsy and tissue preparation

Forty-eight hours after the last training session, the rats were anesthetized with an intramuscular injection of a ketamine and xylazine mixture (65 mg/kg) between 8 AM and 12 PM. The rats were then euthanized by exsanguination. Blood was collected from the inferior cava vein and centrifuged at 3,000 rpm for 10 min at 4 ºC, and the serum was stored at -80ºC for later testosterone, cortisol, and creatine kinase (CK) analysis. Epididymal and retroperitoneal fat were identified, extracted, and weighed. The flexor hallucis longus (FHL), soleus, and plantaris muscles

were dissected, weighed, and half-sectioned for cross-sectional area (CSA) analysis. A half portion of FHL muscle was frozen at -80ºC for further analysis by Western blotting.

2.4 Muscle histological analysis

For optical microscopy analysis, three portions of muscles—one of the soleus, one of the plantaris, and one of the FHL—were fixed in 4% formaldehyde for 24 hours, dehydrated with graded ethanol, and embedded in paraffin blocks according to routine procedures. Semi-thin sections (5μm) were cut in a microtome, applied to silane-coated slides, and deparaffinated. Pieces were stained in hematoxylin and eosin; images were then captured on an optical microscope at a magnification of 100x, and the CSA muscle fibers were quantified (~880 fibers per group) using Image-J.

2.5 Testosterone, cortisol and CK assay

Serum concentrations of testosterone and cortisol were measured using ELISA kits (Abcam, Cambridge, UK catalogue# ab108666 and Mybiosource, San Diego, California Catalogue# MBS2883557, respectively). CK activity was measured using a commercially available kit (Labtest, Lagoa Santa, Brazil).

2.6 Western blotting analyses

Proteins from the FHL muscle were extracted using the extraction buffer 1:10 [50 mM HEPES, 40 mM NaCl, 2mM EDTA, 1,5 mM Na3VO4, 50 mM NaF, 0,1% sodium dodecyl sulfate (SDS), 0,1% Triton X-100, proteases and phosphatase's inhibitors cocktail (#5872 Cell Signaling Technology)]. The total protein was determined by the BCA method (QPRO-BCA protein assay, Cyanagen, Bologna, Italy). Equivalent amounts of 80 μg protein were electrophoresed on 10% SDS-PAGE in the running buffer [25 mM Tris-base, 1,92M glycine, pH 8.6 e 1% SDS], as

described by Laemmli (1970) [12]; gels were blotted into a polyvinylidene difluoride (PVDF) (Immun-Blot® PVDF Membrane Bio-Rad) in the transfer buffer [25 mM Tris, 192 mM glycine, pH 8.3 and 20% methanol]. Then, non-specific binding was blocked with 5% (w/v) dry non-fat milk in TBS-T [100 mM Tris, 1.5 mM NaCl, pH 8.0 and 0.5% Tween 20]. After that, membranes were incubated overnight with a primary antibody in 5% defatted bovine serum albumin in TBS-T (anti-p70S6K total 1:1000 Cell Signaling Technology, anti-p70S6K phosphorylated [Thr389] 1:1500 Cell Signaling Technology) at 4 ºC, then washed and incubated with secondary horseradish peroxidase-conjugated anti-Rabbit (anti-Rabbit IgG 1:4000 Bio-Rad). Immunoreactivity bands were detected by enhanced chemiluminescence (ECL) (GE Healthcare) according to the manufacturer's procedure; images were quantified using Image-J.

3. Statistical analysis

Normality was checked by the Shapiro–Wilk test. The data were expressed in mean and standard deviation (SD). One-way analysis of variance (ANOVA) was applied in order to compare groups. Two-way ANOVA for repeated measures was used for both within-group and between-group comparisons. When an F-ratio was significant, Tukey's HSD post hoc test was used to identify significant differences. In variables where sphericity was violated—as indicated by Mauchly's test—the analyses were adjusted with a Greenhouse–Geisser correction. The data were analyzed using SPSS software, version 24.0 for Mac (SPSS Inc., Chicago, IL, USA), and GraphPad prism version 7.0.

4. Results

A similar increase in body weight was seen in all groups during the six weeks of the experimental period (Figure 1A). The HL and ML groups progressively increased ($p < 0.01$) the maximal strength at a similar rate, compared to the control group (Figure 1B). The ML training

regimen generated significantly higher ($p < 0.01$) volume of load at week 6 of training (Figure 1C) and a significantly higher ($p < 0.01$) number of climbs over the six weeks of exercise, compared to the HL training regimen (Figure 1D).

Table 1 presents FHL, soleus, and plantaris muscle weights, as well as epididymal and retroperitoneal fat weights, after 6 weeks of exercise. The plantaris muscles were heavier in the HL group compared to the other two groups. No significant differences were found among the groups in soleus and FHL muscle weight. Therefore, epididymal fat in the HL group was significantly reduced compared to the ML and control groups.

*****Insert Figure 1 here*****

*****Insert Table 1 here*****

Both the HL and ML training regimens significantly ($p < 0.05$) increased the CSA of the plantaris and FHL muscles (Figure 2A and B) compared to the control group. The increased CSA was similar across the HL and ML groups. Moreover, the ML training regimen showed a significantly higher increase ($p < 0.01$) in the soleus CSA compared to the HL group and the control group (Figure 2C).

*****Insert Figure 2 here*****

The ML training regimen provided similar protein synthesis stimuli to HL. Both the HL and ML groups presented higher levels of phosphorylated P-70S6K protein expression ($p = 0.01$, $F = 6.82$) and total/phosphorylated P-70S6K ratio ($p = 0.048$, $F = 3.80$) compared to the control group (Figure 3). The higher rates of phosphorylated P-70S6K protein and of the total/phosphorylated P-70S6K ratio were also similar between the HL and ML groups.

Testosterone and cortisol analysis demonstrated that the HL training regimen caused a significant increase in the plasma levels of testosterone ($p = 0.0033$, $F = 8.15$) and the testosterone/cortisol ratio ($p = 0.023$, $F = 4.69$) compared to the ML and control groups (Figure 4A). No significant changes ($p = 0.292$, $F = 1.31$) were seen among the groups regarding cortisol

plasma levels (Figure 4B). In addition, no significant differences (p = 0.295, F = 1.32) in creatine kinase activity were demonstrated among the groups (Figure 4D).

*****Insert Figure 3 here*****

*****Insert Figure 4 here*****

Discussion

Our main new finding was that the ML training regimen proposed in this study was similarly effective in developing muscular hypertrophy and strength gains as the HL training regimen proposed by Hornberger & Farrar [1]; this is probably due to similar protein synthesis stimuli, demonstrated by similar increases in levels of phosphorylated-p70S6K protein expression, although an elevated testosterone/cortisol ratio favored the HL training regimen. Importantly, ML seems to favor slow-twitch muscle fiber hypertrophy, due the higher volume compared to the HL training regimen. These findings are in accordance with our initial hypothesis that the ML training regimen would develop a level of muscular hypertrophy and strength similar to HL, due to ML's ability to overload skeletal muscle with a combination of moderate intensity and higher volume compared to the HL training regimen. Our findings are significant because they demonstrate that the ladder climbing model designed for rats can mimic human RT systems, which adapt to different RT load regimens. Moreover, different ladder climbing RT regimens can be used to better understand the molecular bases of hypertrophy using rat studies, since it is easier to determine changes in skeletal muscle hypertrophy signaling in rats compared to humans. In addition, RT is now largely used as a preventive and treatment tool in several muscle disorders, including muscle wasting, cachexia, sarcopenia, and others [13-15]—disorders that are not compatible with heavy-load training regimens.

The past few years have seen a questioning of the assumption that heavy weights are necessary to optimize skeletal muscle hypertrophy generated by RT [16, 17]. Authors have demonstrated similar strength and hypertrophic gains when comparing low- *vs* high-load training

programs in humans [7, 18]. Schoenfeld et al. (2017) conducted a meta-analysis comparing low- *vs* high-load RT programs, and they concluded that while maximal strength benefits are obtained from the use of heavy loads, muscle hypertrophy can be equally achieved across a spectrum of loading ranges [19]. In terms of animal RT models, Tibana and colleagues (2017) were one of the few studies comparing different loading regimens using ladder climbing models. Comparing two RT regimens for rats, these authors demonstrated similar results to studies in humans comparing low- *vs* high-volume training regimens. They demonstrated that both low- and high-volume training generated similar disturbance to skeletal muscle proteins as well as gains in skeletal muscle hypertrophy [20]. These data agree with ours, which demonstrated similar strength and hypertrophic responses to ML and HL training regimens.

Although the ML training regimen cannot engage as many motor units as high-load RT (especially fast-twitch muscle fiber motor units) [21], low to moderate-load RT have demonstrated to develop similar protein synthetic responses as heavy-load training programs [9]. Burd et al. (2010) demonstrated in humans that training to failure at 30% of 1 maximal repetition produced a similar acute muscle protein synthetic response compared to training at 90% of 1 maximal repetition, 4h after exercise. Furthermore, phosphorylation of p70S6K was significantly increased after 4h, and myofibrillar muscle protein synthesis remained elevated at 24h only in the 30% 1 RM condition [22]. Our results also demonstrated comparable phosphorylation of p70S6K in the ML and HL regimens, even though the testosterone-cortisol ratio was only elevated in the HL group. The similar protein synthesis stimulation by the mTOR axis can explain the analogous gains in strength and hypertrophy in both the HL and ML groups in our study.

These findings suggest that low/moderate training load RT, when performed at higher volume, promotes similar adaptive responses as training with heavy loads. This occurs because mechanical load is probably the most important stimulus to hypertrophy. When mechanical load is absent or reduced, other stimuli have small effects on muscle size (e.g. metabolic stress, muscle damage)

[23]. This is accomplished through the phenomenon of mechanotransduction, whereby sarcolemma-bound mechanosensors (such as integrins and focal adhesions) convert mechanical loading-induced musculoskeletal stress into chemical signals that stimulate intracellular anabolic and catabolic pathways, a process that ultimately leads to myofiber enlargement [24]. Thus, heavy- and moderate-load RT seem to demonstrate similar mechanotransduction to the mTOR anabolic pathway, demonstrated in our study by an elevated level of protein expression of p70S6K, a key mTOR down-stream target related to skeletal muscle protein synthesis.

Notably, the ML regimen favors slow-twitch fiber hypertrophy, demonstrated by higher cross-sectional area in the soleus muscle compared to the soleus muscle of HL-trained rats. Evidence shows that the predominantly slow-twitch soleus muscle is much less responsive to high-load RT compared to the primarily fast-twitch muscle [25]. However, whether slow-twitch fibers are more responsible to low-load, high-volume exercise is not well known. A study by Netreba et al. (2007) is the only one to demonstrate that traditional HL strength training increased the cross-section area of fast-twitch fibers, while the low-intensity strength training without relaxation increased the slow-twitch fiber cross-section area of the quadriceps femoris. Despite the scant amount of evidence, this hypothesis—that low-load, high-volume/to failure RT may develop specific slow-twitch muscle fibers hypertrophy—is intriguing in theory and must be examined in future studies [26].

The present study has at least one limitation that should be considered. Different RT regimens must promote fiber type-specific hypertrophy and/or fiber type shifts, which were not measured in this study. Instead, we used skeletal muscles mostly composed of slow-twitch (soleus) and fast-twitch (plantaris) fibers to identify fiber type-specific hypertrophy induced by different RT regimens.

In conclusion, ML training is proposed to be equally effective as HL training in developing muscular hypertrophy and strength gains in rats. This is probably due to the similar protein

synthesis stimuli, demonstrated by analogous increases in the levels of phosphorylated-p70S6K protein expression. Markedly, moderate-load, high-volume training seems to favor slow-twitch fiber hypertrophy, which was not demonstrated in the HL training regimen. Therefore, low- to moderate-load training regimens adapted to rodents may be an important strategy to study animal models of elderly, myopathy, and muscle wasting diseases, as well as other disorders that may not tolerate resistance training at high load intensities.

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Author contributions: CSP, FAV and RD designed the study. CSP, FAV, PSC, MTJT, PCM, PBG and KCI participated in data collection, contributed to analysis and interpretation of data. CSP, ASR, FAV and RD assisted in the preparation of the manuscript. CSP, ASR, FAV and RD wrote the initial draft of the manuscript. ASR, FAV, PSC, MTJT, PCM and PBG critically reviewed the manuscript. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Figure Captions

Figure 1. Body weight gain (A), maximal carrying load (B), volume of load (C) and cumulative number of climbing (D) for groups C: control group, HL: high-load and ML: moderate-load regimen over six weeks of experiment. * indicates differences between groups in the same week; # indicate difference from the previous week ($P < 0.05$; two-way ANOVA repeated measures followed by Tukey post-hoc test).

Figure 2. Plantaris (A), FHL (B) and soleus (C) muscle cross sectional are (CSA) average and distribution for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA followed by Tukey post-hoc test).

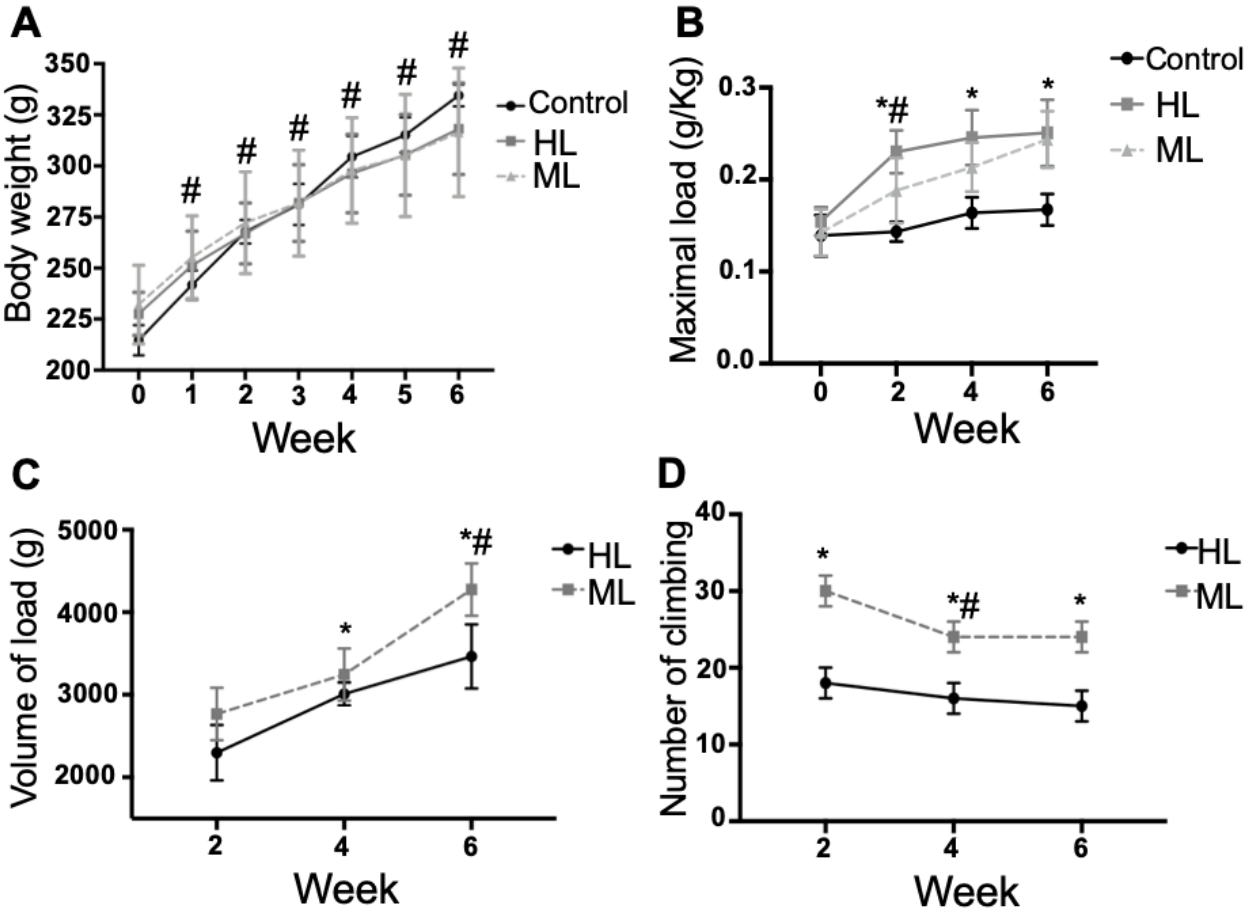
Figure 3. FHL muscle total (A), phosphorylated (B) and total/phosphorylated ratio (C) P70S6K protein expression for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA followed by Tukey post-hoc test).

Figure 4. Serum levels of testosterone (A) and cortisol (B), testosterone/cortisol ratio (C) and creatine kinase activity (D) for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA with post hoc test of Tukey).

Table 1– Final body and tissues weight of control (C), high-load (HL) and moderate-load training regimen (ML) groups.

	C (n = 6)	**HL (n = 7)**	**ML (n = 7)**
Final body weight (g)	334.5 ± 5.32	321.2 ± 22.8	323.3 ± 28
Soleus/body weight (%)	0.049 ± 0.004	0.045± 0.002	0.049 ± 0.007
FHL/body weight (%)	0.17 ± 0.006	0.18 ± 0.02	0.19 ± 0.01
Plantaris/body weight (%)	0.10 ± 0.007	0.11 ± 0.004*	0.10 ± 0.007#
Epididymal fat/body weight (%)	0.01 ± 0.001	0.001 ± 0.002*	0.001 ± 0.002#
Retroperitoneal fat/body weight (%)	0.01 ± 0.003	0.01 ± 0.004	0.01 ± 0.003

Data are presented as means ± SD. * indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA followed by Tukey post-hoc test).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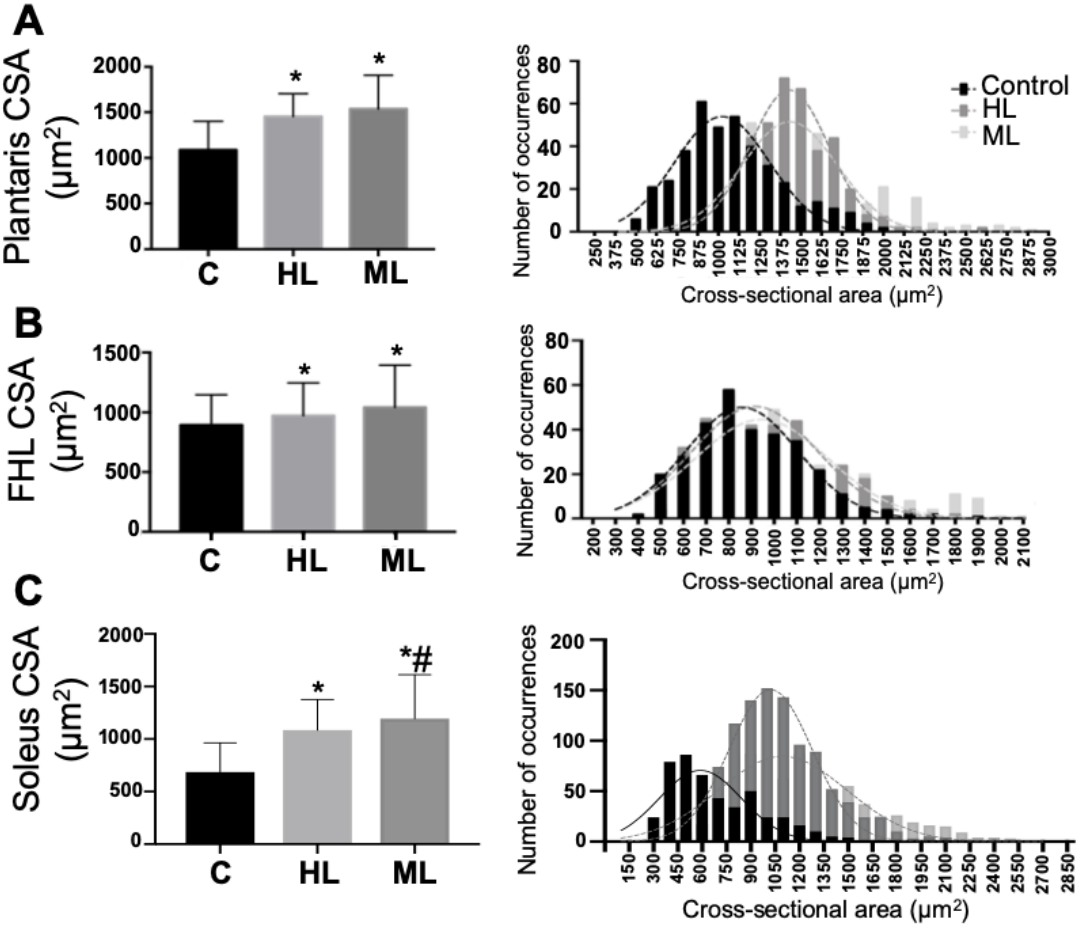
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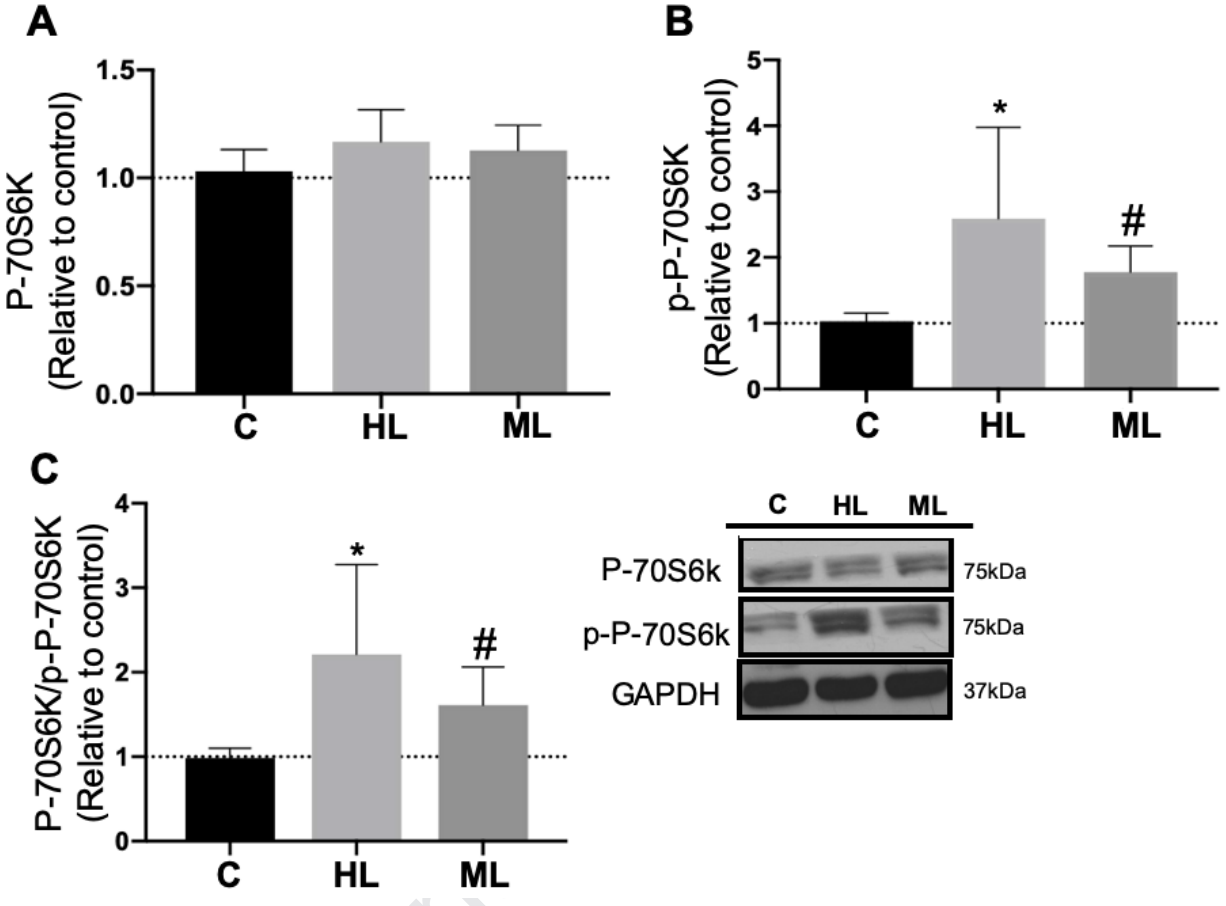
ML
250 375 500 625 750 875 1000 1125 1250 1375 1500 1625 1750 1875 2000 2125 2250 2375 2500 2625 2750 2875 3000
Cross-sectional area (μm²)
B
FHL CSA (μm²)
1500
1000
500
0
*
*
C
HL
ML
Number of occurrences
80
60
40
20
0
200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 1600 1700 1800 1900 2000 2100
Cross-sectional area (μm²)
C
Soleus CSA (μm²)
2000
1500
1000
500
0
*
*#
C
HL
ML
Number of occurrences
200
150
100
50
0
150 300 450 600 750 900 1050 1200 1350 1500 1650 1800 1950 2100 2250 2400 2550 2700 2850
Cross-sectional area (μm²)


A
P-70S6K
(Relative to control)
1.5
1.0
0.5
0.0
C
HL
ML
B
p-P-70S6K
(Relative to control)
5
4
3
2
1
0
*
#
C
HL
ML
C
P-70S6K/p-P-70S6K
(Relative to control)
4
3
2
1
0
*
#
C
HL
ML
C
HL
ML
P-70S6k
75kDa
p-P-70S6k
75kDa
GAPDH
37kDa

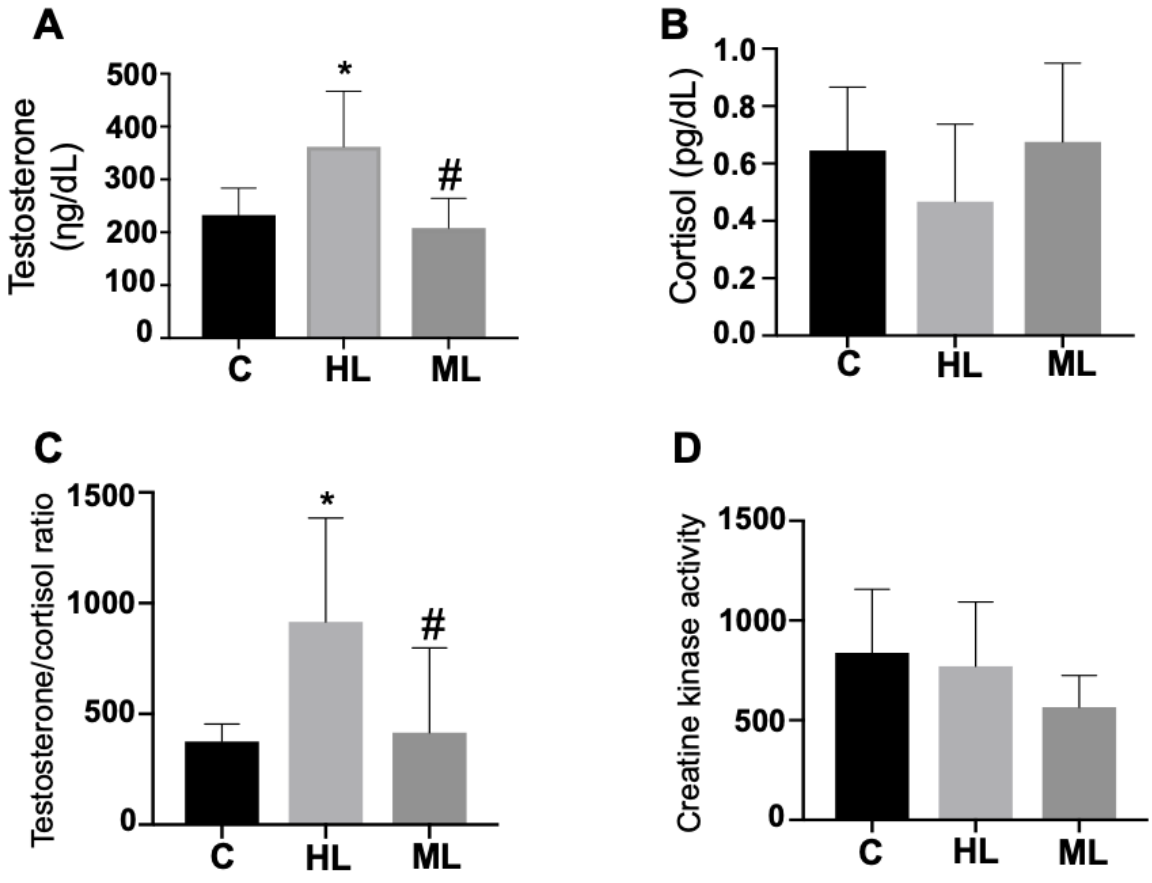

A
Testosterone (ng/dL)
500
400
300
200
100
0
*
#
C
HL
ML
B
Cortisol (pg/dL)
1.0
0.8
0.6
0.4
0.2
0.0
C
HL
ML
C
Testosterone/cortisol ratio
1500
1000
500
0
*
#
C
HL
ML
D
Creatine kinase activity
1500
1000
500
0
C
HL
ML

Figure Captions

Figure 1. Body weight gain (A), maximal carrying load (B), volume of load (C) and cumulative number of climbing (D) for groups C: control group, HL: high-load and ML: moderate-load regimen over six weeks of experiment. * indicates differences between groups in the same week; # indicate difference from the previous week ($P < 0.05$; two-way ANOVA repeated measures followed by Tukey post-hoc test).

Figure 2. Plantaris (A), FHL (B) and soleus (C) muscle cross sectional are (CSA) average and distribution for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA followed by Tukey post-hoc test).

Figure 3. FHL muscle total (A), phosphorylated (B) and total/phosphorylated ratio (C) P70S6K protein expression for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA followed by Tukey post-hoc test).

Figure 4. Serum levels of testosterone (A) and cortisol (B), testosterone/cortisol ratio (C) and creatine kinase activity (D) for groups C: control, HL: high-load and ML: moderate-load regimen. * Indicates difference from C group; # indicates difference from HL group ($P < 0.05$; one way ANOVA with post hoc test of Tukey).