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Effect of different doses of whey proteins on muscle strength, body composition and gene expression of mTOR and MuRF-1 in trained Wistar rats

Efecto de diferentes dosis de proteínas de suero sobre la fuerza muscular, composición corporal y expresión génica de mTOR y MuRF-1 en ratas Wistar entrenadas

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Abstract. Introduction: The combination of resistance training (RT) and whey protein supplementation (WPS) is widely practiced by both athletes and recreational exercisers to promote muscle growth and increase strength. Objective: This study examined the effect of different doses of whey proteins on muscle strength, body composition and gene expression of mTOR and MuRF-1 in trained Wistar rats. Methods: 80 male Wistar rats were divided into 8-groups (n=10): sedentary control (C), RT-control (TC), groups consuming whey protein at varying doses (W2, W4, and W6; respectively 2/4/6g/kg/day), and groups consuming whey protein at varying doses combined to RT (TW2, TW4, and TW6; respectively 2/4/6g/kg/day). The RT program was conducted for 12 weeks, three days a week, with the training intensity increasing from 50 to 100% of the rats' body weight. The rats receiving the whey supplement via the gavage method based on their body weight. Results: Muscle strength significantly increased in all trained groups ($p<0.0001$), with a more significant increase in the groups RT and WPS combined. In addition, the expression of mTOR was higher in the RT groups compared to the sedentary groups ($p<0.01$), but supplementation did not yield significant differences. WPS decreased MuRF-1 expression ($p<0.01$) independently of RT. Conclusion: In conclusion, RT combined with WPS for 12 weeks improved muscle strength. Furthermore, mTOR expression increased in trained rats, but not in sedentary rats who used different doses of WPS. However, WPS at any dose reduced MuRF-1 expression, independently of RT. Higher WPS doses did not enhance observed gains compared to a lower dose.
Keywords: whey protein, body composition, resistance training, mTOR, MuRF-1.

Resumen. Introducción: La combinación de entrenamiento de resistencia (RT) y suplementación de proteína de suero (WPS) es ampliamente practicada tanto por atletas como por deportistas recreativos para promover el crecimiento muscular y aumentar la fuerza. Objetivo: Este estudio examinó el efecto de diferentes dosis de proteínas de suero sobre la fuerza muscular, la composición corporal y la expresión génica de mTOR y MuRF-1 en ratas Wistar entrenadas. Métodos: 80 ratas Wistar macho se dividieron en 8 grupos (n = 10): control sedentario, control RT, suplementación de proteína de suero en diferentes dosis (WPS-2, WPS-4 y WPS-6 g/kg/día) y suplementación de proteína de suero en diferentes dosis combinadas con RT (RTWPS-2, RTWPS-4 y RTWPS-6 g/kg/día). El programa de RT se llevó a cabo durante 12 semanas, tres días a la semana, con la intensidad del entrenamiento aumentando del 50 al 100% del peso corporal de las ratas. Las ratas que recibieron el suplemento de suero a través del método de alimentación por sonda se basaron en su peso corporal. Resultados: La fuerza muscular aumentó significativamente en todos los grupos entrenados ($p<0,0001$), con un mayor aumento en los grupos RTWPS. Además, la expresión de mTOR fue mayor en los grupos RT en comparación con los grupos sedentarios ($p<0,01$), pero la suplementación no produjo diferencias significativas. La suplementación con WPS disminuyó la expresión de MuRF-1 ($p<0,01$) independientemente de RT. Conclusión: En conclusión, RT combinado con WPS durante 12 semanas mejoró la fuerza muscular. Además, la expresión de mTOR aumentó en ratas entrenadas, pero no en ratas sedentarias que usaron diferentes dosis de WPS. Sin embargo, WPS en cualquier dosis redujo la expresión de MuRF-1, independientemente de RT. Las dosis más altas de WPS no mejoraron las ganancias observadas en comparación con una dosis más baja.
Palabras clave: proteína de suero, composición corporal, entrenamiento de resistencia, mTOR, MuRF-1.

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Introduction

A little over a decade ago, protein supplements were predominantly used by bodybuilders or elite athletes. However, today, these supplements have gained widespread popularity among a diverse range of individuals, including those who may not participate in regular exercise (Samal & Samal, 2018)

Whey protein (WP) is especially sought after due to its high nutritional value and its association with muscle hypertrophy, even if indirectly. (Carrilho, 2013)

Whey protein (WP) is a milk-derived protein that contains branched-chain amino acids (BCAAs) such as leucine, isoleucine, and valine. These amino acids play a crucial role in various physiological and morphological processes, particularly in muscle protein synthesis. (Pal & Radavelli-Bagatini, 2013)

The optimal dosage of protein intake for enhancing muscle protein synthesis remains under active investigation, with recommendations varying based on factors such as age, training level, and training duration. In this sense, in a previous study performed by Naderi et al. (Naderi et al., 2016), it is suggested that young individuals consume 0.20 to 0.25 g/kg of body mass in frequent meals throughout the day or 20 to 25 g post-exercise. In this same study, the recommended dose for elderly individuals is 0.40 g/kg of body mass, and for adult athletes, 40 g/day post-exercise.

The American College of Sports Medicine ("Nutrition and Athletic Performance," 2016), in official positioning, recommends that the protein intake necessary to support

metabolic adaptation, repair, remodeling, and protein turnover ranges from 1.2 g/kg to 2.0 g/kg of body weight. (Phillips & van Loon, 2011) propose that sedentary individuals aim for 0.8 g/kg/day, while athletes may require between 1.2 and 2.0 g/kg/day. Therefore, protein dosage recommendations vary significantly depending on specific goals.

Resistance training (RT) combined with protein supplementation is a widely practiced strategy primarily aimed at increasing skeletal muscle mass. The mechanical stimulus provided by RT, combined with protein intake, activates important pathways that regulate muscle protein synthesis. (Cermak et al., 2012; Hornberger et al., 2006; Luciano et al., 2017)

Several studies involving both human and animal models have been conducted to explore the effects of resistance training on muscle hypertrophy signaling pathways. In this perspective, a previous study (Karagounis et al., 2010) used 36 rats to evaluate the impact of short-term resistance exercise on these pathways. Their findings indicated that animals across six different groups, whether engaged in resistance exercises with varying stimuli or not, exhibited significant influences of resistance training on multiple hypertrophic signaling pathways.

Similarly, Zanchi et al. (Zanchi et al., 2009) conducted a study with 20 sedentary female Wistar rats, utilizing a resistance training protocol performed on stairs with an intensity ranging from 80% to 95% of the maximum load voluntarily lifted during a strength test. The study demonstrated the effectiveness of this training method in enhancing muscle mass in the trained animals. This method was also employed by Padilha et al. (Padilha et al., 2019) who demonstrated that both intense and moderate training protocols effectively increased muscle mass in the trained rats.

Whey protein supplementation (WPS) supplementation in combination with RT has been extensively evaluated *in vivo* studies. Research performed by Avila et al. and Teixeira et al. (Avila et al., 2018; Teixeira et al., 2016) utilizing the water jumping method as a form of RT demonstrates that WP supplementation as nutritional support can enhance and positively influence muscle growth.

To monitor this positive influence, researchers have investigated intracellular biochemical mechanisms of gene expression, mainly focusing on pathways such as the *Mammalian target of rapamycin* (mTOR) and the E3 ligase *Muscle Ring Finger* (MuRF-1).

The mTOR protein plays a crucial role in regulating muscle mass in response to various stimuli, including mechanical stress, nutrient intake, growth factors, and hormonal signals (Ogasawara et al., 2014). The mechanical stimulus generated by RT activates the mTOR pathway through growth factors like insulin-like growth factor 1 (IGF-1), which stimulates mTORC1 (Guertin & Sabatini, 2007). RT has been shown to significantly elevate serum levels of IGF-1 following a period of consistent training (Galaviz Berelleza et al., 2020). IGF-1, a critical mediator of anabolic processes, plays a pivotal role in muscle hypertrophy and tissue repair by promoting protein synthesis and cellular growth.

Conversely, ubiquitin ligases, particularly MuRF-1, are known as E3 ligases within the ubiquitin-proteasome system and are associated with signaling pathways that lead to muscle protein degradation and autophagy (Murton et al., 2008). RT can also influence the ubiquitin-proteasome pathway through MuRF-1, which contains regulatory promoter regions that activate specific pathways involved in muscle catabolism (Murton et al., 2008).

It is also important to note that WPS has been linked to alterations in the gene expression of the anabolic pathways (Luo et al., 2013; Zanchi et al., 2009). Thus, the present study aimed to investigate the effects of varying doses of WPS on strength gains, body composition, and the expression of mTOR and MuRF1 in Wistar rats subjected to resistance ladder training.

Materials and methods

Ethical Considerations

All biological tests were conducted following the guidelines of the Brazilian Society for Laboratory Animal Science (SBCAL/COBEA, 2012). The research received approval from the Ethics and Research Committee on the Use of Animals at the Federal University of Maranhão (CEUA/UFMA) under registration No. 23115.011090/2016-48.

Animals and experimental protocols

Eighty healthy young male Wistar rats, aged eight weeks and weighing approximately 250-350 grams, were used in this study. The body weight was measured using an digital balance (AD-CAL, Mars Scientific, São Paulo, Brazil) with a precision of 0.01g. The animals were obtained from the vivarium of the Federal University of Maranhão (UFMA). The animals were housed under hygienic conditions in collective cages (dimensions 41x34x16 cm), with five rats per cage. The animals were housed in a room with a temperature range of 24ºC to 28ºC and a relative humidity of 50±5%, with a 12-hour lightdark cycle. These animals received a standard diet (CR-1) and water *ad libitum*.

Eighty male Wistar rats were divided into 8-groups (n=10): Sedentary control (C); WP supplementation 2g/kg/day (W2); WP supplementation 4g/kg/day (W4); WP supplementation 6g/kg/day (W6); RT control (TC); RT and WP supplementation 2g/kg/day (TW2); RT and WP supplementation 4g/kg/day (TW4); RT and WP supplementation 6g/kg/day (TW6); The RT protocol lasted 12 weeks. The supplementation of WP was performed daily and administered through the gavage method.

Gavage

The gavage procedure was performed daily for 12 weeks. Before the start of the experimental protocol, the animals underwent three adaptation sessions to the gavage process. In all supplemented groups, the daily WP dose was

administered in three gavages (5mL each), with a 60-minute interval between each gavage. The supplemented groups received their respective doses of WP, while the control training group received the same volume of filtered water. The body weight of the rats was monitored weekly to adjust the supplementation. WP supplementation was administered at a dosage of 2, 4, and 6 g/kg/day, dissolved in filtered water with a standardized concentration of 0.189 g/mL of the supplement (HI Whey: Essential Nutrition®), equivalent to 0.284 g/mL of WP. The WP was measured using an analytical balance (AD200, Mars Scientific, São Paulo, Brazil) with a precision of 0.001g to prepare the standard solution.

Resistance training

Resistance training (RT) was performed on a vertical ladder (110cm high, 18cm wide, grid with 2cm spacing between the steps and 80° inclination, with a 20x20x20cm box at the top of the stairs). RT was performed according to the animal model described by Horberger and Farrar (Hornberger Jr. & Farrar, 2004).

Adaptation to resistance training

One week before the start of the RT protocol, the rats were gradually adapted to climbing with the training apparatus attached to the tail without adding load. Four climbs were performed with 120 seconds of interval between climbs. This was performed for three days non-consecutive (48-hour interval between sessions).

Maximum Load-carrying Test

Two days after the familiarization procedure, the maximum load-carrying (MLC) test was performed. The test for load capacity was initiated with a climb carrying a load of 75% of the rat's body weight. Then, an incremental load of 30 grams was added to each successful climb. The animals received a resting period of 120 seconds between each climb. Were performed between 4 to 9 attempts until you get the MLC. Failure was defined as the rat's inability to continue climbing the ladder after three consecutive stimuli on the tail (using tweezers) (Hornberger Jr. & Farrar, 2004; Krug et al., 2016).

This procedure was applied every two weeks during the 12 weeks of training to determine the training intensity and to monitor the adaptations of the muscular strength.

Resistance Training protocol

After the maximum load-carrying test, the RT program was started, with a frequency of three sessions each week on alternate days (Mondays, Wednesdays, and Fridays) for 12 weeks. The RT sessions consisted of four climbs carrying a load of 50, 75, 90, and 100% of the previous maximum load. The exercise protocol was adapted from Hornberger and Farra (Hornberger Jr. & Farrar, 2004)

Euthanasia

Twenty-four hours after the last exercise training session, rats underwent a 12 h fast and were then anesthetized with intraperitoneal (ip) administration of xylazine (70 mg/kg) and ketamine (10 mg/kg), and subsequent decapitation.

Sample collection and gene expression analysis

The procedure for obtaining muscle tissue followed the recommendation of Xia et al. (Xia et al., 2016) and Hornberger and Farrar (Hornberger Jr. & Farrar, 2004). Thus, skeletal muscles (gastrocnemius, soleus, and flexor hallucis longus) were dissected and weighed. The superficial portions (white portions) of the gastrocnemius muscle of the right paw were snap-frozen in an RNA stabilizing solution (Invitrogen™; RNAlater™), then stored at -80°C for subsequent analysis in Real Time-PCR (RT-PCR).

Gene expression of mTOR and MuRF-1 (RT_PCR)

cDNA was synthesized with the SuperScript® IV First-Strand Synthesis System Kit (Thermofisher, USA) following the manufacturer's instructions. The RNA and cDNA were quantified spectrophotometrically using the NanoDrop-One (Thermofisher, USA). Subsequently, qRT-PCR was performed with the Power SYBR Green Master Mix Kit (Thermo Fisher Scientific©) on the StepOne Sequence Detection System. In addition, the GAPDH gene was used as an endogenous control for normalization and all samples were performed in duplicate. The primers used in this study are listed in Table 1.

Table 1.
Primers used for Real-Time PCR

Target	Forward sequence	Reverse sequence
mTOR rat	5'-CAGGACGAG CGAGTGAT -3'	5'- CGAGTTGGT GGACAGAGG -3'
MuRF-1 rat	5'-AGTCGCAGTT TCGAAGCAAT -3'	5'- AACGACCTCC AGACATGGAC -3'
GAPDH rat	5'-ACGGCAAGTTCA ACGGCACAGTCAA-3'	5'- GCTTTCCAGAGG GGCCATCCACA -3'

The primers were derived from *Rattus Novergicus* genes (National Center of Biotechnology Information GenBank), and similarities were evaluated using the Prime-BLAST program (http://ncbi.nlm.nih.gov/tools/primer-blast/).

Additionally, RT-PCR cycle threshold (C_t) values represent the amplification cycles required for the target gene to exceed a threshold level. In this sense, cycle threshold (C_t) values were calculated for each gene, and ΔC_t values (target gene C_t minus GAPDH C_t) were calculated for each group. Additionally, $\Delta\Delta C_t$ values (ΔC_t from the sample group minus ΔC_t from the control group) were calculated, mRNA amplification was determined as $2^{-\Delta\Delta CT}$, and the results were expressed as % of the control (Haraguchi et al., 2014).

Statistical analysis

Data were analyzed using the statistical software GraphPad Prism 8.0 (GraphPad, San Diego, California,

USA). The results are presented according to the descriptive statistics (mean and standard deviation). Data were assessed for deviations from a normal distribution using the Shapiro-Wilk test. For the comparison between baseline and post 12 weeks of RT was used the paired Student's t-test. Statistical significance was applied on a one-way or two-way analysis of variance (ANOVA), followed by Tukey's post hoc test for the eight groups evaluate. The significance level adopted was $p< 0.05$.

Results

Table 2 shows the body mass results (mean ± SD) before and after twelve weeks of RT and WP supplementation. The comparison between groups did not show statistically significant differences in the baseline moment and after the twelve weeks of intervention.

After twelve weeks of the study, the Control group showed a 59.9% increase in body weight ($p < 0.0001$). The W2 group experienced a 51.8% increase ($p < 0.0001$), W4 increased by 57.5% ($p < 0.0001$), and W6 by 61.6% ($p < 0.0001$). In the TC group, body weight increased by 57.8% ($p < 0.0001$), TW2 by 45.7% ($p < 0.0001$), TW4 by 48.5% ($p = 0.0004$), and TW6 by 47.6% ($p < 0.0001$).

Over 12 weeks, the body weight of rats increased in all groups. In addition, the RT associated with WP supplementation, independent of dose, promoted a lower increase in body weight in Wistar rats.

Table 2
Body weight (g) before and after 12 weeks of intervention (mean ± SD)

Group	Baseline (g)	After 12 wk.(g)	Increase (%)	p-value
C	290,6 ±16,39	464,8 ±34,51	59,9%	<0,0001
W2	299,6 ±21,13	455,0 ±29,64	51,8%	<0,0001
W4	267,5 ±26,12	421,5 ±42,46	57,5%	<0,0001
W6	254,8 ±20,41	411,9 ±46,08	61,6%	<0,0001
TC	287,1 ±9,79	453,1 ±25,28	57,8%	<0,0001
TW2	302,5 ±31,66	432,2 ±54,16	42,8%	<0,0001
TW4	280,4 ±23,43	416,5 ±21,91	48,5%	0,0004
TW6	297,6 ±16,28	430,9±37,08	44,8%	<0,0001

Legend: C: sedentary control; W2: WP supplementation 2g/kg/day; W4: WP supplementation 4g/kg/day; W6: WP supplementation 6g/kg/day; TC: RT control; TW2: RT and WP supplementation 2g/kg/day; TW4: RT and WP supplementation 4g/kg/day; TW6: RT and WP supplementation 6g/kg/day.

Table 3 and Figure 1 present the results of the MLC test before and after twelve weeks of intervention.

Table 3.
MLC (g) before and after 12 weeks of intervention (mean ± SD)

Group	Baseline (g)	After 12 wk.(g)	Increase (%)	p-value
TC	287,1 ±9,79	396,9 ±20,58	38,2%	<0,0001
TW2	278,9 ±27,20	540,9 ±28,82	93,9%	<0,0001
TW4	225,0 ±40,97	542,8 ±9,58	141,2%	<0,0001
TW6	267,7 ±48,28	530,8 ±46,28	98,2%	<0,0001

Legend: TC: RT control; TW2: RT and WP supplementation 2g/kg/day; TW4: RT and WP supplementation 4g/kg/day; TW6: RT and WP supplementation 6g/kg/day.

After 12 weeks of RT, the TC group showed a statistically significant increase of 38.2% in MLC ($p < 0.0001$), while the TW2 group exhibited a 93.9% increase in this parameter ($p < 0.0001$). In parallel, the TW4 group demonstrated a 141.2% improvement in strength ($p < 0.0001$). Finally, the TW6 group achieved a 98.2% increase compared to baseline ($p < 0.0001$).

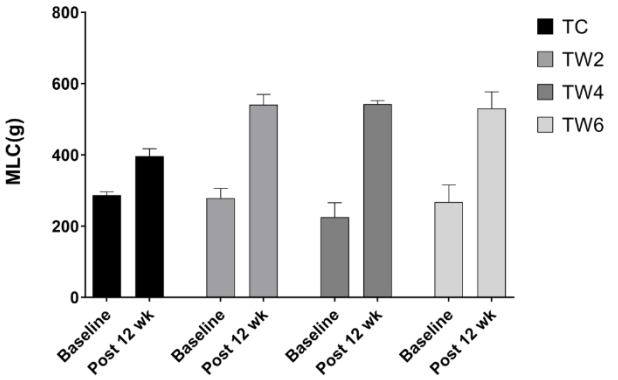

Figure 1: MLC (g) before and after 12 weeks of intervention (mean ± SD); Legend: TC: RT control; TW2: RT and WP supplementation 2g/kg/day; TW4: RT and WP supplementation 4g/kg/day; TW6: RT and WP supplementation 6g/kg/day. * p<0,0001 *vs.* Baseline.

All trained groups showed a statistically significant increase in maximum strength. However, regardless of the dose administered, WP supplementation appeared to result in a more substantial strength increase in these rats when compared those that underwent RT alone ($p < 0.0001$).

Figure 2 shows the mTOR gene expression in the gastrocnemius muscle. Thus, it was observed that mTOR was significantly higher in the TC group compared to the W2, W4, and W6 groups ($p = 0.0021$; $p = 0.0043$; $p = 0.0059$, respectively). However, no statistical difference was found between the sedentary control groups and all trained and supplemented groups. Interestingly, WP supplementation appeared to reduce mTOR gene expression in rats, while RT provided a protective effect, increasing mTOR expression in the trained groups.

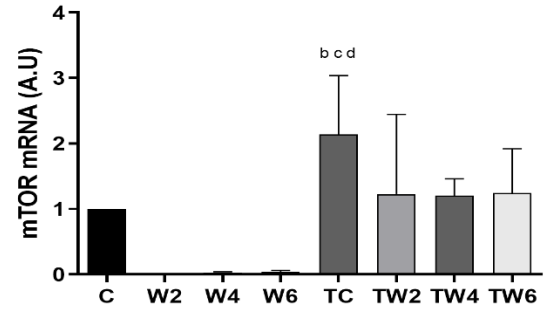

Figure 2. Expression of mTOR RNA after 12 weeks of intervention (mean ± SD). Legend: C: sedentary control; W2: WP supplementation 2g/kg/day; W4: WP supplementation 4g/kg/day; W6: WP supplementation 6g/kg/day; TC: RT control; TW2: RT and WP supplementation 2g/kg/day; TW4: RT and WP supplementation 4g/kg/day; TW6: RT and WP supplementation 6g/kg/day; (A.U.) arbitrary unity; [a] p<0,01 *vs.* C group. [b] p<0,01 *vs.* W2 group; [c] p<0,01 *vs.* W4 group; [d] p<0,01 *vs.* W6 group.

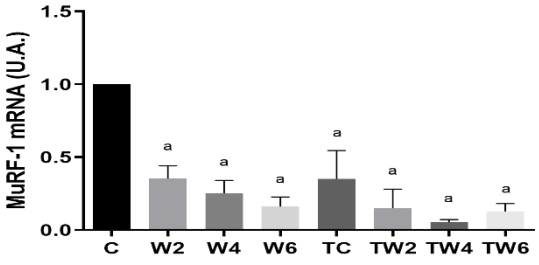

Figure 3. Expression of MuRF-1 RNA after 12 weeks of intervention (mean ± SD). Legend: C: sedentary control; W2: WP supplementation 2g/kg/day; W4: WP supplementation 4g/kg/day; W6: WP supplementation 6g/kg/day; TC: RT control; TW2: RT and WP supplementation 2g/kg/day; TW4: RT and WP supplementation 4g/kg/day; TW6: RT and WP supplementation 6g/kg/day; (A.U.) arbitrary unity; [a] $p<0,01$ *vs.* C group.

Discussion

Based on the results presented, resistance training combined with whey protein supplementation led to an increase in body mass, muscle strength, and mTOR RNA expression, while reducing MURF-1 RNA expression. Additionally, it was observed that increasing the dose of WP did not enhance these effects.

A previous study (Teixeira et al., 2016) reported increased body mass in rats subjected to RT and WP supplementation for eight weeks. In this study, all groups that received intervention presented higher weight when compared to the control. However, the trained groups, particularly the RT control group, exhibited fewer gains. These findings corroborate the present study, where the trained groups also showed reduced weight gain. In parallel, this study's results demonstrated that, regardless of the dose, rats supplemented with whey protein alongside RT presented the least body mass gain.

Furthermore, over the 12 weeks, all trained groups demonstrated improvement in muscle strength. In this sense, the TC, TW2, TW4, and TW6 groups showed increased strength in 28%, 93%, 141.2%, and 98.2%, respectively, compared to the baseline ($p<0.0001$). Similarly, Antonio-Santos et al. (Antonio-Santos et al., 2016) reported strength gains of approximately 33% in weight-bearing capacity in a RT model comparable to the current study.

An important finding of the present study was the greater MLC capacity in the groups supplemented with WP, regardless of the dose, compared to the control RT group ($p<0.0001$).

The increase in strength observed across all groups is attributed to the adaptations induced by RT. Additionally, the enhanced performance in the groups that combined RT with WP supplementation may be linked to increased muscle and liver glycogen stores, which are known to be boosted by WP supplementation, as previously reported in studies by Morifuji et al. (Morifuji et al., 2005)

In addition, concerning daily protein consumption, a dose of approximately 1.6 g/kg body weight is sufficient for potentiation in one repetition maximum (1RM) strength gains (Morton et al., 2018). The gene expression of mTOR was higher in the trained and supplemented groups, indicating a potential role of RT in stimulating protein synthesis pathways. Additionally, moderate-intensity RT (80% of body mass) increased muscle cross-sectional area. In this sense, Hellyer et al. reported that mTOR expression was similar between sedentary and trained groups of Sprague-Dawley rats after ten weeks of stair-climbing training (Hellyer et al., 2012)

Supporting these results, a study performed by Gil and collaborators evaluated the effect of RT combined with supplementation of leucine (10% or 50% of the recommended standard dose) on mTOR phosphorylation. In the study in question, higher mTOR phosphorylation was observed in the sedentary group supplemented with 50% leucine compared to the control group. However, it did not differ from the RT group. Moreover, trained groups supplemented with leucine (10% or 50%) showed higher mTOR phosphorylation levels than the control group ($p=0.01$). Additionally, the phosphorylation of mTOR in the trained group supplemented with 50% leucine was significantly greater than in the group that performed only RT ($p=0.03$) but not different from the group supplemented with 10% leucine (Gil & Kim, 2015). Although in Gil and Collaborators' study(Gil & Kim, 2015) the authors used leucine supplementation exclusively, the results related to the RT were similar to the findings of this study.

In another study, Haraguchi and Collaborators (Haraguchi et al., 2014) using male Fischer rats for eight weeks, evaluated the influence of RT promoted through the jumps inside a pool associated with WP consumption on the gene expression of mTOR. In this scenario, it was evidenced that mTOR gene expression was higher in the groups that consumed WP; in parallel, the control group who only performed resistance training showed a marked decrease ($p<0.05$) in mTOR gene expression compared to the other groups.

In previous study, an isolated WP dose did not seem to promote increased mTOR RNA expression. However, WP supplementation promoted increased myofibrillar synthesis rate and mTOR expression at doses 0.5g/kg and 2.0/g/kg (Nakayama et al., 2019)

WP supplementation associated with RT significantly reduced MuRF-1 gene expression. In addition, the groups that received only whey protein supplementation decreased MuRF-1 gene expression compared to the control group; however, there was no difference in MuRF gene expression when compared to different doses or the trained groups associated with supplementation. In another study, RT using the stair climbing model for 12 weeks reduced approximately 40% the MuRF-1 gene expression in rats. Additionally, it resulted in a significant increase of 12.0% ($p<0.01$) in the plantaris muscle mass in the resistance training group compared to the sedentary group. Interestingly, the plantaris muscle mass/body mass ratio also increased by 13.7% compared to the sedentary group (Zanchi et al., 2009). Furthermore, WP supplementation combined with RT reduced

MuRF-1 gene expression ($p<0.001$) in trained rats, regardless of dose (Haraguchi et al., 2014).

The results of this study indicated that although mTOR expression did not increase, there was a reduction in MuRF-1 expression, a gene implicated in muscle protein degradation. This effect was observed in rats supplemented with WP with or without RT. Therefore, the data suggest that whey protein supplementation effectively inhibits muscle protein degradation by negatively regulating proteins such as MuRF-1, contributing to maintaining muscle mass during RT. Furthermore, resistance training associated with whey protein supplementation increased muscle strength compared to the trained control group. Therefore, WP supplementation may be an effective strategy to improve strength performance in RT. Although the results indicated a significant improvement in muscle strength in the RT protocol combined with WP supplementation, it is essential to assess the extent of these results and compare it to a group that received only WP supplementation without RT. Therefore, including a control group in future studies would offer more precise information on the impact of WP supplementation on muscle strength improvement. Additionally, factors such as the duration of supplementation and the type of RT may influence the outcomes and should be considered in future research.

Conclusion

In conclusion, the findings of this study suggest that RT increased muscle strength after 12 weeks, and this effect was enhanced by WP supplementation, highlighting its benefits in this type of exercise. Additionally, mTOR expression did not increase in sedentary rats supplemented with 2, 4, or 6 g/kg/day of WP but increased in trained rats. Moreover, WP supplementation reduced MuRF-1 expression in trained or untrained groups. Interestingly, higher doses of whey protein (4 or 6 g/kg/day) did not amplify the gains observed with a lower dose (2 g/kg/day). Therefore, these results suggest that whey protein supplementation can effectively improve resistance training outcomes.

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Conflict of interest

The authors have no conflict of interest.

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Contributions

RFM, MRCM and FN conceived and designed the study. RFM, MRCM, AJSS and FCBV performed the experiment. RFM, CENA and ACN analyzed and interpreted all the data. All authors were involved in drafting and revising the manuscript.

Ethical approval

All animals were treated following the institutional guidelines for the care and use of animals, and this study was approved by the ethical committee on animal research.

Consent for publication

Not applicable.

Consent to participate

Not applicable.

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