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GENE EXPRESSION OF MTOR, MURF-1 AND MAFBX IN WISTAR RATS SUPPLEMENTED WITH WHEY PROTEINS

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ABSTRACT

To verify the effect of supplementation of different doses of Whey Proteins for 12 weeks on the gene expression of MTOR, MURF-1, MAFBX and on the alteration of the cellular diameter of the gastrocnemius muscle. Sample: 38 Wistar rats and supplemented for 12 weeks. The white portion of the right gastrocnemius muscle was embedded in Rna Latter solution and stored at -80 ° C and subjected to real-time polymerase chain reaction analysis. The left gastrocnemius muscle was intended for histological analysis. Gene expression of MTOR mRNA had lower statistical significance in the groups of Whey 2, Whey 4, Whey 6 than in the control group, in the order of 100%, 97%, 96%, respectively. Likewise, the gene expression of MURF-1 mRNA had lower statistical significance in the groups of Whey 2, Whey 4, Whey 6 than the control group, in the order of 65%, 75%, 84%, respectively. In line, the gene expression MAFBX mRNA had lower statistical significance in the groups Whey 2, Whey 4, Whey 6 in relation to the control group, in the order of 99%, 97%, 99%, respectively. There was no statistical difference in the cellular area of the gastrocnemius muscle between the groups. Therefore, supplementation of Whey Proteins at doses of 2, 4, 6 g / kg / day for 12 weeks in sedentary animals was not able to increase the gene expression of MTOR mRNA, however, it was able to reduce the gene expression of MURF -1 mRNA, MAFBX mRNA.

Key words: Whey Proteins. MTOR. MUFR-1. MAFBX. Gastrocnemius.

RESUMO

Expressão genica de MTOR, MURF-1 e MAFBX em ratos Wistar suplementados com Whey Proteins

Verificar o efeito da suplementação de diferentes doses de Whey Proteins por 12 semanas na expressão gênica de MTOR, MURF-1, MAFBX e na alteração do diâmetro celular do músculo gastrocnêmio. Amostra: 38 ratos Wistar e suplementados por 12 semanas. A porção branca do músculo gastrocnêmio direito foi embebida em solução de Rna Latter e armazenada a -80°C e submetida à análise de reação em cadeia da polimerase em tempo real. O músculo gastrocnêmio esquerdo foi destinado à análise histológica. A expressão gênica do mRNA MTOR teve menor significância estatística nos grupos Whey 2, Whey 4, Whey 6 do que no grupo controle, na ordem de 100%, 97%, 96%, respectivamente. Da mesma forma, a expressão gênica do mRNA de MURF-1 teve menor significância estatística nos grupos Whey 2, Whey 4, Whey 6 do que no grupo controle, na ordem de 65%, 75%, 84%, respectivamente. Em linha, a expressão gênica MAFBX mRNA teve menor significância estatística nos grupos Whey 2, Whey 4, Whey 6 em relação ao grupo controle, na ordem de 99%, 97%, 99%, respectivamente. Não houve diferença estatística na área celular do músculo gastrocnêmio entre os grupos. Portanto, a suplementação de Whey Proteins nas doses de 2, 4, 6 g/kg/dia por 12 semanas em animais sedentários não foi capaz de aumentar a expressão gênica de MTOR mRNA, porém, foi capaz de reduzir a expressão gênica de MURF - 1 mRNA, mRNA MAFBX.

Palavras-chave: Whey Proteins, MTOR, MUFR-1, MAFBX, Gastrocnêmio.

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INTRODUCTION

According to the WHO (2003) the recommendations for total protein intake ranges from 10 to 15% of the total energy value. In this sense, according to the Dietary Reference Intake, the intake of proteins for adult men and women ranges from 0.8 to 1.0 g/kg/day of high biological value. In face of this, when the intake of proteins occurs between 1.8 to 3.3 g/kg/day a hyper protein diet is considered (Lancha Junior colaboradores, 2009; IOM, 2006).

In this sense, the whey proteins, in special, has received great attention because of its benefits on skeletal muscle hypertrophy, reduction of body fat, performance, appetite regulation, immune system regulation, lipid profile regulation and decrease of the metabolic stress (Krissansen, 2007).

Due to this, the national industry of nutritional supplements reached a growth of 10% in its production in 2016, with a profit around R$ 1.49 billion (Brasnutri, 2016).

With Regard to protein supplements, it is understood that the proteins are a vital element to a well-succeed promotion in the process of muscle hypertrophy and reduction of body fat, especially in consequence of exercise with overloads.

Being this importance often overestimated, leading athletes and individuals who practice physical activities to consume very high doses of protein, being able to reach values ranging from 4.0 to 6.2 g/kg/day (Bacurau, 2009).

This study is justified by the need of a determination of gene expression of proteins related to the protein synthesis and degradation in function of supraphysiologic doses of Whey Proteins.

In face of this, the hypothesis of this present study is, an alternative hypothesis, where the whey proteins are able to promote change in the gene expression of MTOR, MURF-1 and MAFBX or the not changing of the gene expression (null hypothesis) of those proteins related to the synthesis and to the protein degradation.

The aim of this experiment was verify the effect of the supplementation of different doses of Whey Protein for 12 weeks in the gene expression of MTOR, MURF-1 and MAFBX and in the changing in the diameter of the cell of the gastrocnemius muscle.

MATERIALS AND METHODS

The biological essays were based in the (7) and the experimental protocol received an initial consolidated opinion with approval and protocol number 23115.014424/2015-54 in the Ethic Commission in Use of Animals of the Federal University of Maranhão-UFMA.

An experimental research design was adopted with randomized groups, divided according to the received supplementation: (C) Not supplemented (control); (W2) supplemented with 2g/kg/day of whey proteins; (W4) supplemented with 4g/kg/day of whey proteins; and (W6) supplemented with 6g/kg/day of whey proteins.

This present study was performed with 38 males Rattus novergicus albinus of the Wistar linage with an initial age of 60 days and an initial body mass between 218 and 323 grams, from the Animal Facility of the Federal University of Maranhão-UFMA.

The rats stood in hygienic conditions in collective cages, kept in a climatized laboratory with control of temperature between 24°C and 28°C under the cycle light/dark of 12 hours. They were fed with a standard balanced ration for rodents (Labina®) and water ad libitum.

The supplementation with Whey Proteins followed the dosages 2 g/kg/day, 4 g/kg/day and 6 g/kg/day dissolved in water, having a common concentration of 0.189 g/mL of the supplement (H.I Whey: Essencial Nutrition®), which corresponds to 0.166 g/mL of whey proteins.

The supplementation was performed by a gavage needle during 12 weeks, daily, three times daily, in doses of 5 mL, with 1 hour-interval between each gavage.

The Control Group received filtered water in the same frequency and volume of the interventions group.

The chart 1 and 2 present the nutritional content and the aminogram of the supplement used in the experiment.

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Chart 1 - Nutrition Facts of whey proteins H.I Whey Protein.

Nutrition Facts		
Amount per serving (25 grams)		
Nutrient	Amount per serving (g)	Daily Value (%)
Carbohydrates	0 grams	0%
Proteins	22 grams	29%
Total fat	0 grams	0%
Dietary Fiber	0 grams	0%
Sodium	79 milligrams	3%
Calcium	118 milligrams	12%
Phosphorus	63 milligrams	9%
Magnesium	22 milligrams	8%

Chart 2 - Aminogram of whey proteins H.I Whey Protein.

Aminogram	
Amount per Serving (25 grams)	
Amino acids	Amount per serving
Aspartic acid	2.6 grams
Glutamic acid	3.7 grams
Alanine	1.2 grams
Arginine	0.5 grams
Cystine	0.6 grams
Phenylalanine	0.7 grams
Glycine	0.4 grams
Histidine	0.3 grams
Isoleucine	1.5 grams
Leucine	2.3 grams
Lysine	2.2 grams
Methionine	0.5 grams
Proline	1.4 grams
Serine	1.1 grams
Tyrosine	0.6 grams
Threonine	1.7 grams
Tryptophan	0.3 grams
Valine	1.3 grams

The body mass of the rats was evaluated weekly for readjustment of dosage of the Whey Proteins Supplements using a Weblasborsp® precision scale 5.200 grams, with 0.1 gram of precision.

After 24 hours of final experimental procedures and with 12 hours of food restriction, the rats were euthanized by ketamine/xylazine overload (75:10 mg/kg) (Neves, 2013; Leary e colaboradores, 2013).

After that moment, the obtaining of the muscle tissue followed the recommendations of (Xia, 2016).

The superficial portion (white) of the gastrocnemius muscle of the right leg was separated, weighted in a Marte digital scale model AD-200, with precision of 0.001 gram and immediately conserved in Latter RNA solution, then the sample of the muscle was stored in a temperature of -80°C for further analysis of real-time polymerase chain reaction. The gastrocnemius muscle of the left leg was intended for histological analysis.

For the isolation of the total Ribonucleic Acid molecule, the Promega® RNA Tissue Miniprep System ReliaPrep™ Kit was used. Then, for the reverse transcriptase reaction, the SuperScript® IV First-Strand Synthesis System Kit from Thermo Fisher Scientific® was used. Then, for the real-time Polymerase Chain Reaction was used the Thermo Fisher Scientific® Power SYBR Green Master Mix Kit.

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The Polymerase Chain Reaction in real time was performed at the StepOne Sequency Detection System for the following primers:
- MTOR rato forward, 5'-CAGGACGAGCGAGTGAT -3', and reverse, 5'-CGAGTTGGTGGACAGAGG -3';
- MURF-1 rato forward, 5'-AGTCGCAGTTTCGAAGCAAT -3', and reverse, 5'- AACGACCTCCAGACATGGAC -3';
- MAFBX rato forward, 5'-CTACGATGTTGCAGCCAAGA -3',and reverse, 5'- GGCAGTCGAGAAGTCCAGTC -3';
- GAPDH rato forward, 5'-ACGGCAAGTTCAACGGCACAGTCAA -3', and reverse, 5'-GCTTTCCAGAGGGGCCATCCACA -3'.

GAPDH was used as endogen control.

The measurement of muscle cells was performed as recommended by (Bodine, 2012), where 150 cells were analyzed and measured for each sample, using a trinocular biological optical microscope of the brand ANATOMIC, in the 40-fold increase, obtaining the cell area in square micrometers (μm^2). The images were analyzed using the program AxioVision, version 4.9.1.

The variables described were tested for normal distribution using the Shapiro-Wilk test ($p > 0.05$). The quantitative data of the feed intake were analyzed by the two-way ANOVA; the classification variable was the interaction between diet and supplementation (C, W2, W4, W6).

The Tukey post-test was used to determine the statistical differences between groups (W, W2, W4, W6). The software used for the analyzes was GraphPad Prism 7.

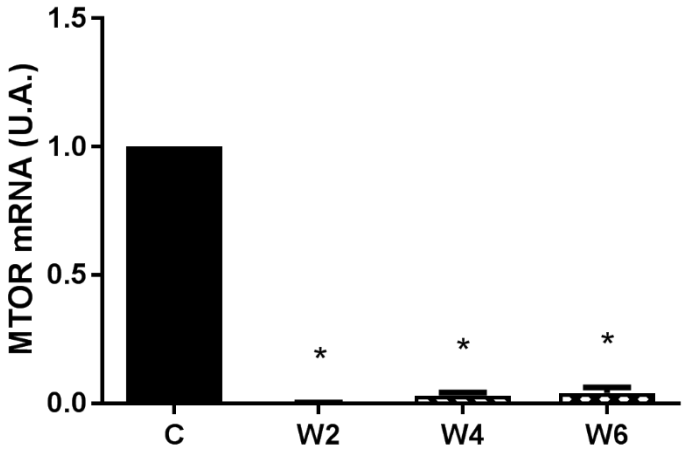
RESULTS

As observed in the chart 3 and illustrated in the figure 1, the gene expression of MTOR mRNA was significant statically in the Whey 2 ($p<0.0001$), Whey 4 ($p<0.0001$) and Whey 6 ($p=0.0001$) groups in relation to the control group, in range of 100%, 97% and 96%, respectively. There was no statistical difference between the supplemented groups.

Chart 3 - Comparison of gene expression of MTOR mRNA between groups (n=36).

	Control (n=8)	W2 (n=11)	W4 (n=10)	W6 (n=7)
Control (n=8)	-	<0.0001	<0.0001	<0.0001
W2 (n=11)	-	-	0.2206	0.0700
W4 (n=10)	-	-	-	0.9142
W6 (n=7)	-	-	-	-

Legend: W2 = Group supplemented with 2g/kg/day Whey Proteins; W4= Group supplemented with 4g/kg/day Whey Proteins; W6= Group supplemented with 6g/kg/day Whey Proteins; ANOVA One-way ($p=0.0306$); Tukey's Post-hoc.



Legend: *p<0.05 compared to the control group.
Figure 1 - Alteration of gene expression of MTOR mRNA.

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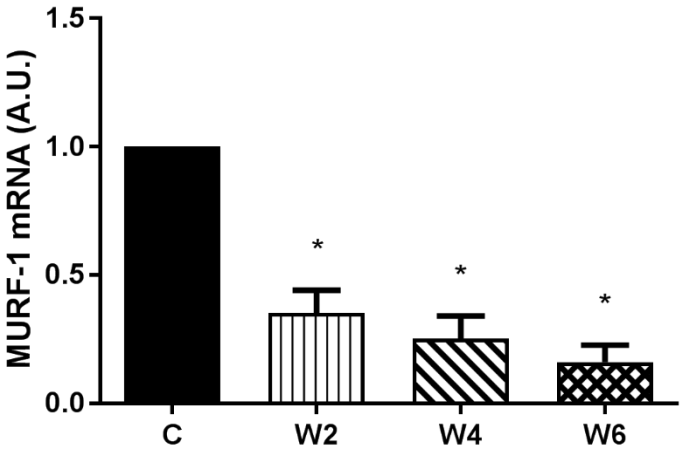
As observed in the chart 4 and illustrated in the figure 2, the gene expression of MURF-1 mRNA was significant statistically in Whey 2 (p<0.0001), Whey 4 (p<0.0001) and Whey 6 (p<0.0001) groups in relation to the control group, in range of 99%, 97% and 99%, respectively. There was no statistical difference between the supplemented groups.

Chart 4 - Comparison of gene expression of MURF-1 mRNA between the groups (n=36)

	Control (n=8)	W2 (n=11)	W4 (n=10)	W6 (n=7)
Control (n=8)	-	<0.0001	<0.0001	<0.0001
W2 (n=11)	-	-	0.7607	0.2865
W4 (n=10)	-	-	-	0.8451
W6 (n=7)	-	-	-	-

Legend: W2 = Group supplemented with 2g/kg/day Whey Proteins; W4= Group supplemented with 4g/kg/day Whey Proteins; W6= Group supplemented with 6g/kg/day Whey Proteins; ANOVA One-way (p<0.0001); Tukey's Post-hoc.



Legend: *p<0.05 compared to the control group.
Figure 2 - Alteration of gene expression of MURF-1 mRNA.

As observed in the chart 5 and illustrated in the figure 3, the gene expression of MAFBX mRNA was significant statistically in Whey 2 (p<0.0001), Whey 4 (p<0.0001) and Whey 6 (p<0.0001) in relation to the control group, in range of 99%, 97% and 99% respectively. There was no statistical difference between the supplemented groups.

Chart 5 - Comparison of gene expression of MAFBX mRNA between groups (n=36).

	Control (n=8)	W2 (n=11)	W4 (n=10)	W6 (n=7)
Control (n=8)	-	<0.0001	<0.0001	<0.0001
W2 (n=11)	-	-	0,6606	>0.9999
W4 (n=10)	-	-	-	0.7569
W6 (n=7)	-	-	-	-

Legend: W2 = Group supplemented with 2g/kg/day Whey Proteins; W4= Group supplemented with 4g/kg/day Whey Proteins; W6= Group supplemented with 6g/kg/day Whey Proteins; ANOVA One-way (p<0.0001); Tukey's Post-hoc.

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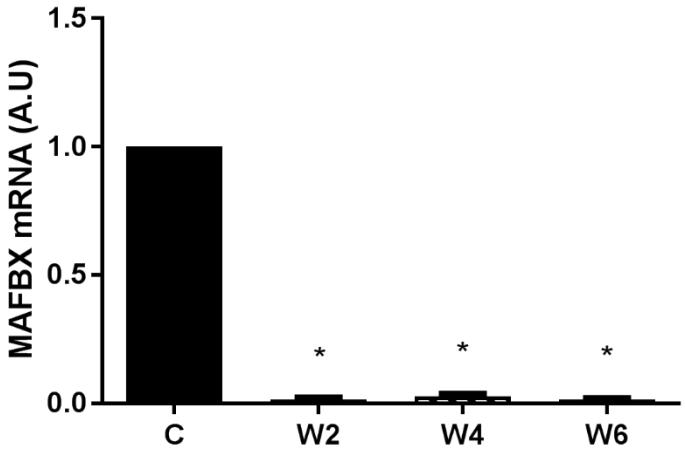

Legend: *p<0.05 compared to the control group.
Figure 3 - Alteration of gene expression of MAFBX mRNA.

As observed in the charts 6 and 7 and illustrated in the figure 6, there was no significant statistical difference (p>0.05) in the area of the gastrocnemius muscle cells in the comparisons between groups.

Chart 6 - Area of the gastrocnemius muscle cells of the groups.

Area (µm²)	C (n=8)	W2g (n=11)	W4g (n=10)	W6 (n=7)
Gastrocnemius muscle	2648.18 ±832.15	2462.56 ±825.70	2369.25 ±696.25	2383.56 ±631.19

Legend: W2 = Group supplemented with 2g/kg/day Whey Proteins; W4= Group supplemented with 4g/kg/day Whey Proteins; W6= Group supplemented with 6g/kg/day Whey Proteins; ANOVA One-way (p=0.2245); Tukey's Post-hoc. The values are expressed in square micrometers (µm²). * p<0.05 compared to the control group.

Chart 7 - Comparison of the cell area of the gastrocnemius muscle between the groups (n=36).

	Control (n=8)	W2 (n=11)	W4 (n=10)	W6 (n=7)
Control (n=8)	-	0.5426	0.2168	0.3302
W2 (n=11)	-	-	0.8885	0.9459
W4 (n=10)	-	-	-	0.9996
W6 (n=7)	-	-	-	-

Legend: W2 = Group supplemented with 2g/kg/day Whey Proteins; W4= Group supplemented with 4g/kg/day Whey Proteins; W6= Group supplemented with 6g/kg/day Whey Proteins; ANOVA One-way (p=0.000000); Tukey's Post-hoc.

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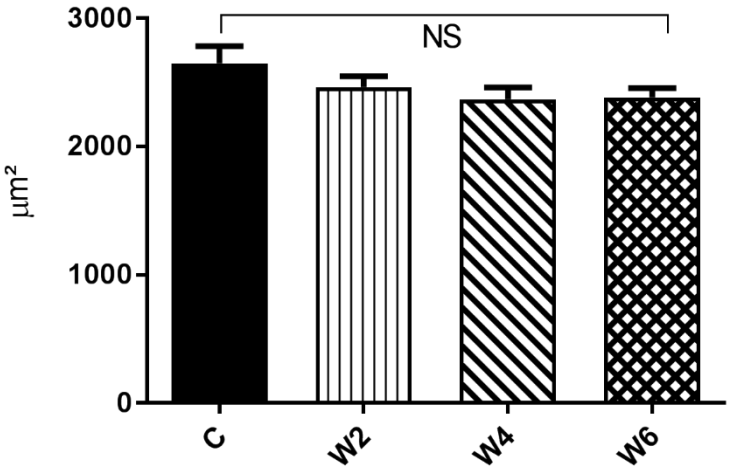

Legend: NS p>0.05 in comparison.
Figure 4 - Alteration of the area of the gastrocnemius muscle cells.

DISCUSSION

According to the results presented in the chart 3, 4, and 5 and illustrated in the figures 1, 2 and 3, the gene expression of MTOR mRNA had a significant statistic in Whey 2 (p<0.0001), Whey 4 (p<0.0001) and Whey 6 (p=0.0001) groups in relation to the control group, in range of 100%, 97% and 96%, respectively.

In the same way, the gene expression of MURF-1 mRNA had a significant statistic in Whey 2 (p<0.0001), Whey 4 (p<0.0001) and Whey 6 (p<0.0001) in relation to the control group, in range of 99%, 97% and 99%, respectively.

These findings corroborate those of (Lomonosova, 2014), that underwent 48 hours of experimentation, with 4 experimental groups containing 14 male Wistar rats each, aged 90 days, with body mass between 254 and 276 grams, organized into control group (maintained as the control conditions); exercise group (submitted to the eccentric declined aerobic training); the exercise group 90 (submitted to the eccentric declined aerobic training and arginine supplementation at 90 mg/kg/day); and exercise group 500 (Submitted to the eccentric declined aerobic training and arginine supplementation at 500 mg/kg/day).

By analyzing only, the sedentary groups, the gene expression of MAFBX was reported and MURF-1 was excluded because of arginine supplementation (although the author did not present the "p" value found in the statistical test).

It is important to emphasize that the authors did not evaluate the gene expression of MTOR mRNA and the variable training was not evaluated in the present study, however, the amino acids and Whey Proteins supplementations look signalize for a reduction of gene expression of pathways related to protein degradation.

In contrast, (Lomonosova, 2011) in 14 days of research, with 3 experimental groups, containing 14 male Wistar rats each, aged 10 weeks, with body mass approximately 220 grams, distributed in control group (maintained as the control conditions); suspended group (hindlegs suspended); and supplemented suspended group (hindlegs suspended and administration of L-arginine 500 mg/kg with water). It was reported an increase in the gene expression (although the author does not present the “p” value found in the statistical test). In contrast, there was no difference in the gene expression between the supplemented suspended group and control group.

In this case, the authors did not evaluate the gene expression of MTOR mRNA and they used the suspension of the hindlegs for stimulate the muscle disuse, what was not evaluated in the present study, in addition, the supplementation of the arginine amino acid was not able to reduce the gene expression of MURF-1 and MAFBX, whereas in the present study the gene expression of both was reduced in all experimental dosage, suggesting that the

consumption and/or supplementation of isolated amino acids may not change the gene expression of these proteins.

In the findings of (Mobley e colaboradores, 2015), with 180-minutes duration, with 4 experimental groups containing 8 to 13 males Wistar rats, (the author did not inform the age), with body mass of 250 grams, organized into control group (treated with 1 ml of water); phosphatidic acid group (treated with 0.029 grams of phosphatidic acid); Whey group (treated with 0.193 grams of concentered Whey Proteins); and Whey + phosphatidic acid group (treated with 0.193 grams of concentered Whey Proteins and 0.029 grams of phosphatidic acid).

It's was reported that there was an increase of 2.3 times in the gene expression of MURF-1 in Whey + phosphatidic acid group in comparison to the control (although the author does not present the "p" value found in the statistic test), however, the gene expression of MAFBX was similar in both groups.

In this case, in addition to the temporal difference and not evaluating the gene expression of MTOR mRNA, supplementation with Whey Proteins occurred at lower doses when compared to the present study (2, 4 or 6 g/kg/day), signaling with the increase of gene expression of MAFBX and the unaltered of the gene expression of MAFBX between groups, whereas in the present study, with 12-week duration, both expressions were reduced in the supplemented groups, thus suggesting that the chronic consumption of supraphysiological doses of Whey Proteins are capable of reducing the gene expression of these proteins.

On the other hand, in the experiment performed by (Haraguchi, 2014), with 8-weeks duration, performed with 4 experimental groups containing 8 male Fischer rats each, aged 60 days, body mass of approximately 110 grams, divided as follows form: sedentary control group (standard ration based on casein); control exercise group (standard ration based on casein); sedentary Whey Proteins group (ration of whey protein in substitution for casein); and group Whey Proteins exercise (ration with Whey Proteins in substitution for casein). It was observed that, in relation to the gene expression of MTOR, that the Whey Proteins group presented values similar to the sedentary control groups and Whey Proteins sedentary, however presented significant difference in relation to the exercise control (p=0.005).

In relation to the gene expression of MURF-1, there was a significant decrease in groups of exercise in relation to the sedentary groups ($p<0.001$), with regard to gene expression of MAFBX, there was a significant decrease in Whey Protein groups, in the presence or absence of resistance exercise (p=0.008).

In the study of (Haraguchi, 2014), an offer of ration enriched with Whey Proteins occurred, added to the physical exercise, which was not evaluated in the present study.

With regard to the gene expression of MTOR, the values were similar between the sedentary control group and the Sedentary Whey Proteins group, which disagrees to the findings in the present study, in which the gene expression of MTOR was lower in the Whey Proteins groups in comparison to the sedentary control group.

The disparity in the results probably occurred due the difference in the doses of Whey Proteins, thus being a modifying factor of the gene expression.

In relation to the gene expression of MAFBX, there was a reduction in the groups that consumed the ration containing Whey Proteins, corroborating with the findings in the present study, in which there was also a reduction in the gene expression of this proteins in the groups supplemented with Whey Proteins.

In an addiction, (Mobley e colaboradores, 2015) in a research with 180-minutes duration, performed with 5 experimental groups containing 6 to 14 male rats, aged 8 to 9 weeks, with body mass of 250 grams, organized in the following way: Control group (maintained the control conditions); WPC group (supplemented with 0.19g of concentered whey protein); 70W/30E group (supplemented with 0.19g of protein, being 30% concentered whey protein and 70% albumin). It was reported that, in gene expression of MAFBX there was not alteration at the first 90 minutes in all groups, but it increased in the 70W/30E group in comparison to the control (p=0.049) 180 minutes after the supplementation.

In relation to the gene expression of MURF-1. It was reported that there was not alteration in the first 90 minutes in all groups but increased in the WPC and 70W/30E groups in comparison to the control (p=0.020 and p=0.032, respectively) 180 minutes after the supplementation.

In the study of (Mobley e colaboradores, 2015) in which the authors did not evaluated the gene expression of MTOR

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rRNA and of lower duration to the present study, it was supplemented Whey Proteins and/or albumin, different from the present study, where it was supplemented only whey protein. With regard to the gene expression of MRUF-1 and MAFBX the result disagrees the findings in the present study, which there was a large reduction in the gene expression of these proteins. This difference can be explained by the total content of protein supplemented and by the duration of the study.

The same way, in the research made by (Batistela e colaboradores, 2014), with 15-days duration, performed with 2 experimental groups containing each 7 male rats, aged approximately 30 days, with body mass of 95 grams, organized in the following way: Control group (it received a diet consisting of 63% of carbohydrates and 17% of proteins) and the LPHC group (it received a diet consisting of 74% of carbohydrates and 6% of proteins). It was reported that the gene expression of MURF-1 and MAFBX was reduced in 57% and 34%. Respectively, in the rats of the LPHC group (although the author does not present the "p" value found in the statistical test)

In this study, the authors did not evaluate the gene expression of MTOR mRNA and modulated the macronutrients of the diet in order to watch the alteration of the gene expression, not being the focus of the present study.

Regarding to the gene expression of MURF-1 and MAFBX, the results disagree the founds in the present study, since the content of Whey Proteins supplemented in the present study promoted the reduction in the gene expression of these proteins. It can be explained by the low protein content offered and the time of experimentation, which may have promoted a reduction of the protein turnover, so the gene expression of these proteins.

In the investigation of (Luo e colaboradores, 2010) with 14-days duration, 3 experimental groups, each group with 10 males Prague-Dawley rats, body mass of 62.55 grams (the author did not inform the age), organized in casein group (submitted to the controlled consumption of ration based in casein); soy group (submitted to the controlled consumption of ration based in isolated soy protein); and the zein group (submitted to the controlled consumption of ration based in protein of corn). It was reported that the gene expression of MTOR increase in the zein group (although the author does not present the "p" value found in the statistical test).

In relation to the gene expression of MURF-1, there was a significant increase of approximately 8 times in the zein group in comparison to the casein group and the soy group (p=0.003)

In line, the gene expression of MAFBX was 4 times bigger in the zein group in comparison to the casein group and the soy group (although the author does not present the "p" value found in the statistical test).

In the study of (Luo e colaboradores, 2010), 3 protein sources were used to evaluate the modification of the gene expression of synthetic pathway and protein degradation, differing in the present study in the protein source and time of exposition to the protein intake. In this sense the results found in the increase of the gene expression of MTOR, MURF-1 and MAFBX in the group supplemented with corn protein are opposite to the present study, witch there was a reduction in the expression of these same proteins in the 3 groups supplemented.

The author discusses that the increase in the gene expression can be related to the increase in the amino acids pool caused by the exposition to the ratio enriched with corn protein, not promoting this way the protein degradation, since that occurred an increase in the synthetic pathway and degradation.

In this same sense, the reduction of the gene expression of these 3 proteins in the present study can't be directly related to the protein degradation, since that there was a decrease in the synthetic pathway and in the degradation pathway, suggesting a lower utilization of amino acids from Whey Protein supplementation and the ration consumption, suggesting a stabilization between the protein synthesis and degradation. And this inference is possible due the results be supported by the equivalence of the cellular area of the gastrocnemius muscle, as demonstrated in the chart 6 and 7 and figure 6.

Therefore, the supplementation of whey protein at doses of 2 g/kg/day, 4 g/kg/day and 6 g/kg/day for 12 weeks in sedentary rats was not able to generate positive effects on the muscle hypertrophy.

Although have occurred alterations in the gene expression of MTOR mRNA, MURF-1 mRNA and MAFBX mRNA.

CONCLUSION

The founds in the present study support the alternative hypothesis, confirming that the supplementation with whey protein at doses at 2, 4 and 6 g/kg/day is able to alter the gene expression of MTOR mRNA, MURF-1 mRNA and MAFBX mRNA.

In this sense, the Whey Protein supplementation at doses 2, 4 and 6 g/kg/day was not able to increase the gene expression of MTOR mRNA and MAFBX mRNA, however, it was able to reduce the gene expression of MURF-1 mRNA and MAFBX mRNA.

The sum of these effects not necessarily is bind to the increase of protein synthesis, thus providing a physiological stabilization within the parameters evaluated.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest on any aspect.

ACKNOWLEDGMENT

*The scholars thank the Coordination of Higher Education Personnel (CAPES) for the support to make the study feasible.

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Recebido para publicação em 29/07/2022
Aceito em 04/09/2022