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EFFECT OF WHEY PROTEIN SUPPLEMENTATION AND TWELVE WEEKS OF RESISTANCE TRAINING ON LIVER FUNCTION IN WISTAR RATS

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ABSTRACT

Objective: To evaluate the effects of resistance training and supplementation with whey protein isolate on liver function in Wistar rats. Materials and methods: The study was approved by the Animal Research Ethics Committee of the Federal University of Maranhão (CEUA/UFMA, protocol nº 23115.001161/2017-85). Male Wistar rats (80 days old; 200–250 g) were maintained under a 12 h light/dark cycle and temperature between 20–26 ºC. Animals were divided into eight groups (n=10): control (C), training control (CT), supplemented with 2 g/kg/day (S2), 4 g/kg/day (S4) or 6 g/kg/day (S6) of whey protein, and trained + supplemented (TS2, TS4, TS6). Supplementation was administered by gavage for 12 weeks. The biomarkers Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Gamma-Glutamyl Transferase (GGT), as well as liver histology, were analyzed. Data were processed using GraphPad Prism 8.02. Results: Sedentary animals showed higher levels of liver biomarkers compared to the other groups. No significant increase was observed in relative liver weight among trained and supplemented groups compared with sedentary ones. Conclusion: Whey protein supplementation, either isolated or combined with resistance training, do not promote changes or impair liver function in Wistar rats. Further studies are needed to confirm these findings under different protocols and experimental periods.

Key words: Liver. Whey protein. Resistance training. Supplementation.

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RESUMO

Efeito da suplementação de whey proteins e treinamento resistido de doze semanas sobre a função hepática em ratos wistar

Objetivo: Avaliar os efeitos do treinamento resistido e da suplementação com proteína isolada do soro do leite sobre a função hepática de ratos Wistar. Materiais e métodos: O estudo foi aprovado pelo Comitê de Ética em Pesquisa com Animais da Universidade Federal do Maranhão (CEUA/UFMA, nº 23115.001161/2017-85). Foram utilizados ratos Wistar machos (80 dias; 200–250 g), mantidos em ciclo claro/escuro de 12 h e temperatura entre 20–26 ºC. Os animais foram divididos em oito grupos (n=10): controle (C), treinamento controle (CT), suplementados com 2 g/kg/dia (S2), 4 g/kg/dia (S4) ou 6 g/kg/dia (S6) de whey protein, e treinados + suplementados (TS2, TS4, TS6). A suplementação foi administrada por gavagem durante 12 semanas. Foram avaliados os biomarcadores Alanina Aminotransferase (ALT), Aspartato Aminotransferase (AST), Fosfatase Alcalina (FA), Gama Glutamil Transferase (GGT). Os dados foram tratados no GraphPad Prism 8.02. Resultados: Os animais sedentários apresentaram níveis mais elevados de biomarcadores hepáticos em comparação aos demais grupos. Não houve aumento significativo no peso relativo do fígado entre grupos treinados e suplementados em relação aos sedentários. Conclusão: A suplementação com whey protein, isolada ou associada ao treinamento resistido, não promoveu alterações na função hepática em ratos Wistar. Estudos adicionais são recomendados para confirmar esses achados em diferentes protocolos e períodos experimentais.

Palavras-chave: Fígado. Whey Proteins. Treinamento resistido. Suplementação.

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INTRODUCTION

The liver is a fundamental organ for human metabolism, responsible for receiving blood from the portal vein, being the first to come into contact with the final products of digestion and metabolism originating from the intestine.

Among its functions, the incorporation of amino acids into proteins and the deamination of amino acids stand out, a process that results in the formation of ammonia, which is later converted into urea. In addition, different regions of the hepatic lobules present structural, biochemical, and functional particularities (Guyton, 2011).

Enzymatic activity is widely used as a biomarker of liver function. The increase in liver enzymes, associated with intense physical exercise, is related to greater cell membrane permeability, which may cause inflammation and tissue dysfunction.

These effects compromise muscle contraction, strength, and contribute to the development of fatigue, especially when blood flow is redirected to active muscles in order to sustain energy biosynthesis (Kinoshita, Yano and Tsuji, 2003).

In the field of sports nutrition, whey protein (WP) stands out, considered a high biological value supplement and widely used by athletes and individuals engaged in physical activity. Several studies demonstrate that WP can protect against exercise-induced stress, improve physical performance, and favor muscle hypertrophy in resistance and aerobic training (Marzani et al., 2008; Rossi, Blostein-Fujii and Desilvesto, 2000; Moller, Scholz-Alrens and Roos, 2008).

Its composition includes α-lactoglobulin, β-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase, phospholipoproteins, bioactive factors, and abundant enzymes.

These components present antioxidant properties, regulators of lipid metabolism, in addition to potential antifatigue and antidiabetic effects. WP isolates are enriched in essential amino acids, especially branched-chain amino acids (BCAA), which are fundamental for tissue synthesis, energy production, and health maintenance.

Leucine, in particular, has a concentration 50% to 75% higher compared to other protein sources, which may explain its high capacity to stimulate muscle protein synthesis (Madureira et al., 2010; Jin et al., 2013; Liu, Wang and Zhao, 2014).

Due to its rapid digestion, WP provides amino acids readily available for muscle tissue repair and reconstruction. In aerobic exercises, such as swimming, its use has already been associated with increased glycogen storage, antioxidant action, and improved lipid metabolism.

However, studies investigating the synergistic effects between WP supplementation and long-term aerobic or resistance training, considering different tissue profiles, are still scarce (Farup et al., 2013).

Therefore, this study is justified by the need to evaluate liver function resulting from resistance training associated with whey protein supplementation.

Such investigation may contribute to the understanding of possible benefits for both athletes and the general population, as well as help prevent excessive consumption of the supplement. In this context, the aim is to determine a minimum effective dosage, capable of enhancing or ruling out adaptations induced by exercise on liver biomarkers.

Therefore, the objective of our study was to investigate the effects of resistance training associated with supplementation of different doses of whey protein (2, 4, and 6 g/kg/day) on body mass evolution, behavior of liver function biomarkers, and liver weight in adult male Wistar rats over 12 weeks.

MATERIALS AND METHODS

Study Type

This was an experimental study, a randomized controlled trial, lasting 12 weeks.

Study Location

The procedures were carried out at the Experimental Physical Exercise Laboratory – LABEFEX (Graduate Program in Adult Health, Federal University of Maranhão, Bacanga Campus, São Luís-MA).

The housing of the animals took place in the Sectorial Animal Facility of PPGSAD/UFMA, in climate-controlled rooms (24-28 °C), with a 12 h light/dark cycle.

The rats were kept in collective cages, with standard balanced chow (Nuvilab®) and filtered water ad libitum. Food and water intake were monitored daily by weighing with a digital

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scale (Weblaborsp® 5200 g) and measuring in a graduated cylinder (Uniglass® 500 mL), respectively.

Sample

A total of 80 male Wistar rats (Rattus norvegicus), 60 days old and weighing between 200–250 g, obtained from the Central Animal Facility of UFMA, were used.

The animals were randomly distributed into eight groups (n=10 per group):

C = Sedentary and non-supplemented control
TC = Trained and non-supplemented control
W2 = Supplemented with 2 g/kg/day of whey protein
W4 = Supplemented with 4 g/kg/day of whey protein
W6 = Supplemented with 6 g/kg/day of whey protein
TW2 = Trained and supplemented with 2 g/kg/day of whey protein
TW4 = Trained and supplemented with 4 g/kg/day of whey protein
TW6 = Trained and supplemented with 6 g/kg/day of whey protein

Sample Size Calculation

The sample size was calculated using Ene 3.0 software (Autonomous University of Barcelona), considering body weight as the outcome variable. Based on Macêdo (2018), a minimum difference of 0.26 g between groups, standard deviation of 0.20, statistical power of 80%, and alpha of 5% were adopted, resulting in 10 animals per group.

Whey Protein Supplementation

The doses were 2, 4, and 6 g/kg/day, dissolved in filtered water (H.I Whey: Essencial Nutrition®), at a concentration of 0.166 g/mL of protein.

Administration was performed by gavage with an 8 cm curved cannula (Bonther®), in three daily sessions with a 1 h interval between them. The administered volume was 2 mL/100 g of body weight, adjusted weekly. Control animals received water gavage (ANDERSEN, 2004).

Resistance Training

The trained groups underwent a vertical ladder protocol (110 cm height; 18 cm width; 80° inclination).

Adaptation: two days without load

Maximum load test (MLT): performed after adaptation, starting at 75% of body weight, progressively increased until failure. The highest weight transported was recorded as MVC (maximum carried weight).

Training: three times per week, on alternate days, for 12 weeks. Each session included 4 climbs (50%, 75%, 90%, and 100% of MVC). Every 15 days the MLT was repeated to adjust the load. The protocol was adapted from Leite et al., (2013).

Euthanasia

After a 12 h fast and 24 h after the last procedure, the animals were euthanized by intraperitoneal injection of ketamine (70 mg/kg) and xylazine (10 mg/kg) (DAMY, 2010; NEVES et al., 2013; CONCEA, 2015).

Collection of Biological Material

Blood: collected by decapitation into Vacutainer® tubes with separating gel, centrifuged at 3000 rpm for 10 min.

Liver: removed, weighed (Marte® AD 200), fixed in 10% formaldehyde for 24 h, and transferred to 70% alcohol.

Analysis of Hepatic Biomarkers

The analyses were performed in duplicate on a microplate reader (Biotek®), using commercial kits (Labtest®), according to the colorimetric method of Reitman and Frankel (1957).

ALT: absorbance 340 nm; result × 1761.
AST: absorbance 340 nm; result × 1730.
GGT: absorbance 405 nm; result × 2577.
Alkaline Phosphatase: absorbance 405 nm; result × 2764.

Disposal of Carcasses

The carcasses were placed in identified plastic bags and sent for incineration at the Central Animal Facility of UFMA.

Statistical Analysis

Normality was assessed by the Shapiro-Wilk test. For intra-group comparisons: one-way ANOVA with Tukey's post hoc test. For

inter-group comparisons over time: one-way ANOVA with Tukey's post hoc test. A significance level of $p<0.05$ and 95% CI were adopted. Analyses were performed using GraphPad Prism® v.8 software.

Ethical Aspects

The study was conducted in accordance with the recommendations of the Brazilian Society of Science in Laboratory Animals (SBCAL/COBEA, 2012). The project was approved by the Ethics Committee on Animal Use (CEUA/UFMA) under protocol no. 23115.01804/2017-91.

RESULTS

The effects of resistance training and/or supplementation with different doses of whey protein were analyzed on the following parameters: weekly protein intake, hepatic function biomarkers, relative liver weight, and liver histology.

Weekly Total Protein Intake

Table 1 shows the evolution of weekly protein intake among the experimental groups. A significant difference ($p<0.0001$) was observed in intake between most groups, except between W4 and TW4, which did not differ from each other.

Table 1 - Weekly total protein intake (g/kg/day) in the different experimental groups.

Week	C (n=10)	TC (n=7)	W2 (n=10)	W4 (n=10)	W6 (n=7)	TW2 (n=9)	TW4 (n=6)	TW6 (n=7)
S1	2.8 ±0.16	3.2 ±0.11	4.8 ±0.22	6.5 ±0.26	8.9 ±0.24	4.4 ±0.25	6.5 ±0.20	8.4 ±0.13
S2	2.8 ±0.20	2.9 ±0.11	4.9 ±0.28	6.8 ±0.29	8.9 ±0.26	4.4 ±0.23	6.3 ±0.20	8.4 ±0.85
S3	2.6 ±0.17	2.7 ±0.12	4.7 ±0.25	6.6 ±0.28	8.7 ±0.24	4.3 ±0.25	6.2 ±0.17	8.0 ±0.11
S4	2.5 ±0.18	2.6 ±0.13	4.6 ±0.25	6.6 ±0.28	8.5 ±0.24	4.0 ±0.23	6.0 ±0.15	7.8 ±0.12
S5	2.5 ±0.16	2.4 ±0.13	4.5 ±0.24	6.5 ±0.25	8.2 ±0.21	3.9 ±0.23	6.0 ±0.17	7.7 ±0.11
S6	2.4 ±0.16	2.4 ±0.12	4.4 ±0.23	6.3 ±0.23	8.1 ±0.19	3.8 ±0.24	5.8 ±0.16	7.7 ±0.12
S7	2.3 ±0.16	2.2 ±0.12	4.4 ±0.22	6.1 ±0.21	8.0 ±0.18	3.8 ±0.22	5.7 ±0.14	7.6 ±0.12
S8	2.1 ±0.15	2.1 ±0.11	4.3 ±0.21	6.0 ±0.20	7.9 ±0.18	3.8 ±0.22	5.7 ±0.14	7.6 ±0.11
S9	2.1 ±0.15	2.1 ±0.10	4.2 ±0.19	5.9 ±0.21	7.8 ±0.17	3.7 ±0.22	5.6 ±0.11	7.4 ±0.10
S10	2.0 ±0.14	2.0 ±0.10	4.1 ±0.18	5.8 ±0.18	8.0 ±0.20	3.7 ±0.21	5.6 ±0.09	7.4 ±0.10
S11	1.9 ±0.14	1.9 ±0.09	4.0 ±0.18	5.7 ±0.18	7.6 ±0.15	3.6 ±0.20	5.6 ±0.09	7.4 ±0.11
S12	1.8 ±0.15	1.85 ±0.10	3.8 ±0.14	5.6 ±0.15	7.3 ±0.14	3.6 ±0.18	5.5 ±0.08	7.4 ±0.11

Legend: C = sedentary non-supplemented control group; TC = trained non-supplemented control group; W2 = group supplemented with 2 g/kg/day; W4 = group supplemented with 4 g/kg/day; W6 = group supplemented with 6 g/kg/day; TW2 = trained + 2 g/kg/day; TW4 = trained + 4 g/kg/day; TW6 = trained + 6 g/kg/day.

The evaluation of weekly total protein intake (g/kg/day) among the different experimental groups revealed distinct intake patterns, mainly determined by the applied supplementation dose.

The control groups (C and CT) consistently presented low values throughout the 12 weeks, with means ranging from 1.8 to 2.8 g/kg/day, characterizing a stable profile representative of basal intake. This behavior contrasts markedly with the supplemented groups, which started the protocol at already higher levels and maintained significant differences compared to the controls throughout the follow-up.

In the non-trained supplemented groups, a clear dose-proportional consumption gradient was observed: the W2 group remained around 4 g/kg/day, W4 stabilized near 6 g/kg/day, while W6 reached mean values of 8 g/kg/day, being the group with the highest absolute intake in the entire experiment. This organization in ascending levels reinforces adherence to the supplementation protocol and validates the consistency of the obtained data. In the groups subjected to the combination of supplementation and resistance training (TW2,

TW4, and TW6), intake patterns were practically equivalent to their respective non-trained groups, suggesting that the determining factor was the supplementation dose and not the exercise practice.

Statistical analysis performed from the simulation of data based on means and standard deviations indicated that the Group factor had a highly significant effect on protein intake ($p<0.001$).

This evidence confirms that the variation in intake was mainly explained by the different experimental supplementation conditions. The Week factor was also statistically significant ($p<0.01$), indicating the occurrence of temporal variation, albeit subtle, over the 12 weeks.

This trend suggests that even within each group, there was a pattern of slight decline in intake over time, possibly related to physiological adaptation or gradual reduction in food acceptance.

Furthermore, the Group*Week interaction was statistically significant ($p<0.05$), demonstrating that the temporal trajectory of intake was not identical among the groups. In the controls, a progressive and pronounced decline was observed, with average reductions of approximately 25% between the first and last week. In the supplemented groups, the decline was more discreet, remaining within relatively stable margins. This difference suggests that supplementation exerted a protective role against consumption decline, ensuring consistent intake levels throughout the experiment.

From a comparative standpoint, significant differences between the controls (C and CT) and all supplemented groups are highlighted from week 1, reinforcing the efficacy of supplementation in rapidly increasing protein intake. Among the supplemented groups, multiple comparisons between W6 and TW6 showed no statistical differences, suggesting that resistance training alone did not influence protein intake volume.

In summary, the results demonstrate that supplementation was the main modulator of protein intake, producing robust and sustained increases directly proportional to the administered dose. Resistance training did not alter the intake pattern, but the significance of the Group*Week interaction points to a differentiated modulation of the temporal trajectory, indicating that combined factors of exercise and supplementation may interact subtly.

These findings corroborate the hypothesis that dietary manipulation plays a central role in determining protein intake, while physical training predominantly influences subsequent metabolic and functional outcomes without markedly impacting food intake.

We now present these results in a graph for better visualization.

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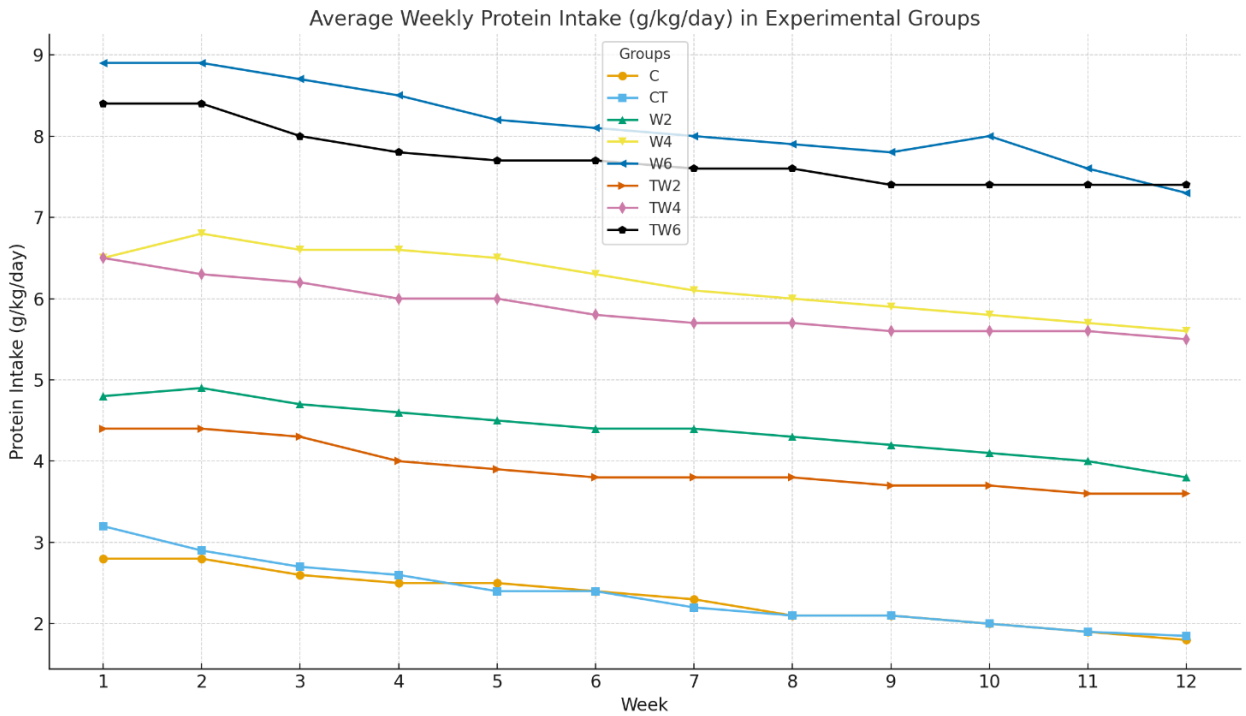

Figure 1 - Average feed intake (g/day) in the groups.
Legend: Values are presented as mean ± standard deviation. C = Control; CT = Trained control; W2, W4, and W6 = groups supplemented with whey protein for 2, 4, and 6 weeks, respectively; TW2, TW4, and TW6 = groups subjected to physical training associated with whey protein supplementation for 2, 4, and 6 weeks, respectively. Significant differences between groups were indicated by ANOVA followed by Tukey's post-hoc test ($p < 0.05$).

Hepatic Biomarkers

The mean serum concentrations of ALT, AST, Alkaline Phosphatase, and GGT are presented in Table 3.

Table 2 - Concentration values of hepatic markers, presented as mean and standard deviation.

Marker	C (n=10)	CT (n=7)	W2 (n=10)	W4 (n=10)	W6 (n=7)	TW2 (n=9)	TW4 (n=6)	TW6 (n=7)
ALT (U/L)	131.3 ± 65.58	41.0 ± 10.60	42.7 ± 8.44	24.7 ± 14.46	25.1 ± 21.36	43.4 ± 17.79	65.9 ± 70.68	145.2 ± 65.4
AST (U/L)	149.4 ± 41.82	88.3 ± 15.01	137.1 ± 23.3	62.5 ± 26.75	63.6 ± 32.77	125.8 ± 64.9	89.5 ± 75.34	163.9 ± 97.7
Phosphatase (U/L)	85.4 ± 19.83	50.4 ± 8.24	27.4 ± 16.56	84.8 ± 23.19	69.5 ± 9.99	22.6 ± 28.27	31.2 ± 30.07	33.2 ± 9.96
Gamma (U/L)	0.7 ± 0.59	1.4 ± 0.25	1.5 ± 0.14	0.8 ± 0.43	0.7 ± 0.45	1.1 ± 0.38	0.7 ± 0.65	1.3 ± 0.62

Legend: ALT = alanine aminotransferase; AST = aspartate aminotransferase; Phosphatase = alkaline phosphatase; Gamma = gamma-glutamyl transferase; U/L = 1 micromol/minute/liter; C = non-supplemented control group; CT = trained control group; W2 = sedentary + 2 g/kg/day; W4 = sedentary + 4 g/kg/day; W6 = sedentary + 6 g/kg/day; TW2 = trained + 2 g/kg/day; TW4 = trained + 4 g/kg/day; TW6 = trained + 6 g/kg/day.

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The one-way ANOVA analysis for the ALT (U/L) marker among groups presented an $F = 23.82$ and $p < 0.0001$, indicating that there are statistically significant differences between groups. Therefore, we performed the Tukey HSD post-hoc test for the ALT (U/L) marker, presenting the following results:

The C group presented significantly higher values compared to most groups (CT, TW2, W2, W4, W6, and TW4), except for TW6, where no significant difference was observed.
The CT group did not differ significantly from W2, W4, W6, TW2, or TW4, but showed a significant difference compared to TW6 ($p < 0.001$).
The TW6 group showed significantly higher values than W2, W4, W6, and TW4, confirming hepatic overload in this context.
TW4 had intermediate values, differing significantly from W4 and W6, but not from W2 or TW2.

In summary, our analysis confirms that C and TW6 are among the groups with the highest ALT levels, while W4 and W6 show the lowest values. The remaining groups fall within intermediate ranges without marked differences.

Continuing our analyses, the AST (U/L) marker presented an $F = 7.60$ and $p = 0.0001$, also indicating statistical differences between groups. Therefore, we performed the Tukey HSD post-hoc test for the AST (U/L) marker:

Significant comparisons ($p < 0.05$):

C vs CT → C higher than CT ($p = 0.004$)
C vs TW2 → C higher than TW2 ($p = 0.040$)
C vs W4 → C higher than W4 ($p < 0.001$)
C vs W6 → C higher than W6 ($p < 0.001$)
CT vs TW6 → TW6 higher than CT ($p = 0.013$)
TW6 vs W4 → TW6 higher than W4 ($p < 0.001$)
TW6 vs W6 → TW6 higher than W6 ($p < 0.001$)
W2 vs W4 → W2 higher than W4 ($p = 0.042$)
W2 vs W6 → W2 higher than W6 ($p = 0.030$)

Interpretation:

The C group presented significantly higher AST values compared to several groups (CT, TW2, W4, and W6), suggesting greater hepatic damage or stress in the absence of intervention.

The TW6 group also stood out with elevated values, differing from CT, W4, and W6, similar to what was observed for ALT.

W4 and W6 groups presented the lowest AST values, differing from C and TW6.

W2 showed intermediate values but significantly higher than W4 and W6.
As with ALT, moderate supplementation doses (W4 and W6) appear associated with lower AST levels, while the combination of training and high dose (TW6) leads to significant enzyme elevation.
We then analyzed Phosphatase (U/L) using one-way ANOVA, where $F = 28.08$ and $p = 1.98 \times 10^{-16}$. This indicates significant differences between groups ($p < 0.001$). We performed the Tukey post-hoc test.

Multiple comparisons showed

The Control group (C) had significantly higher values than CT, W2, TW2, TW4, and TW6 ($p < 0.001$), but did not differ from W4.
The CT group was significantly lower than W4 ($p = 0.0003$), but did not differ from W2, W6, TW4, or TW6.
W2 had significantly lower values than W4 and W6 ($p < 0.001$).
TW2 had significantly lower values than W4 and W6 ($p < 0.001$).
TW4 was significantly lower than W4 ($p = 0.0002$).
TW6 was significantly lower than W4 and W6 ($p < 0.05$).

Interpretation

Phosphatase decreased markedly in W2, TW2, TW4, and TW6 compared to the control (C), suggesting an inhibitory effect in these groups.

W4 maintained values similar to the control, suggesting enzymatic recovery at this protocol stage.

CT showed reduced values compared to the control but without marked statistical differences compared to groups treated with whey and combined training.

Overall, there is a pattern of Phosphatase reduction in groups with supplementation and/or initial training, followed by recovery in W4 and W6, while combined groups (TW) maintain lower values.

Finally, we performed statistical analysis for gamma-glutamyl transferase (U/L) using one-way ANOVA, where $F = 4067$ and $p = 0.00033$, demonstrating significant differences between groups. We performed the Tukey post-hoc test.

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Significant comparisons ($p < 0.05$):
W2 > W4 ($p = 0.0078$)
W2 > W6 ($p = 0.0004$)
TW6 < W6 ($p = 0.0102$)

Interpretation

The W2 group had significantly higher GGT values than W4 and W6, suggesting that lower supplementation doses may be associated with greater enzyme activity.

TW6 showed significantly lower values than W6, indicating that training combined with a higher supplementation dose reduced GGT activity compared to the sedentary group with the same dose.

Overall, there are subtle but statistically relevant variations in GGT among groups, suggesting that both dose and training modulate this enzyme.

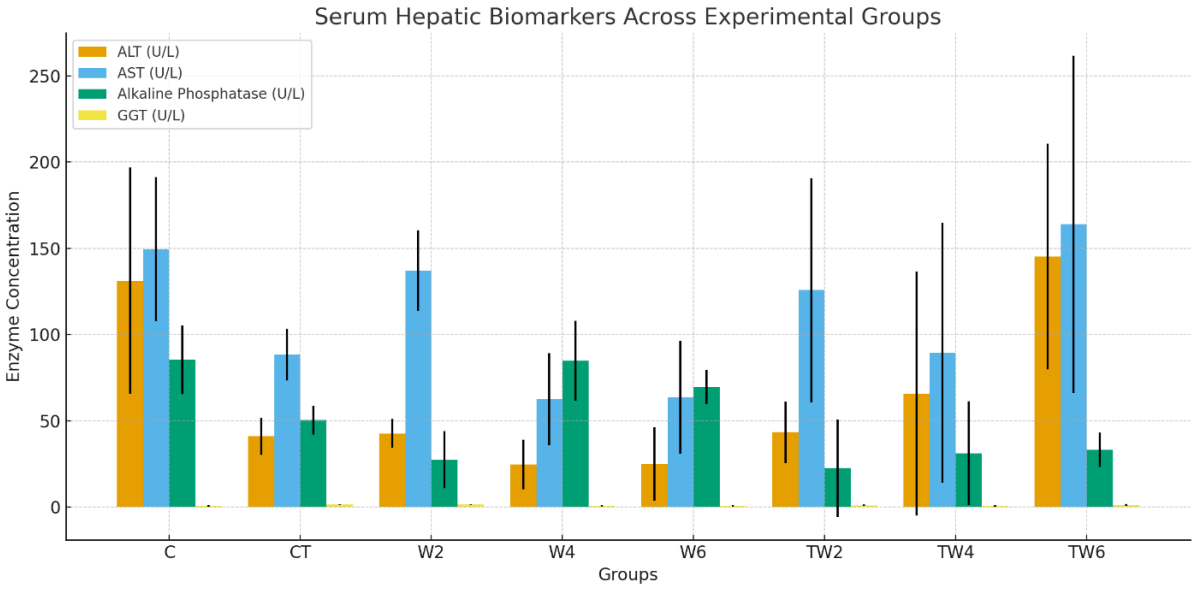

Figure 2 – Hepatic enzyme concentrations in the different experimental groups.
Legend: Mean values (±SD) of hepatic enzyme activities in the different experimental groups. ALT = alanine aminotransferase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = gamma-glutamyl transferase. Groups: C = non-supplemented control; CT = trained control; W2, W4, and W6 = sedentary animals supplemented with 2, 4, and 6 g/kg/day of whey protein, respectively; TW2, TW4, and TW6 = trained animals supplemented with 2, 4, and 6 g/kg/day of whey protein, respectively.

Evaluation of hepatic markers

The assessment of hepatic markers revealed significant differences among the groups. Alanine aminotransferase (ALT) activity was significantly higher in the non-supplemented control group (C) and in the trained group supplemented with 6 g/kg/day (TW6), suggesting possible hepatic overload associated with both the absence of intervention and high protein intake combined with training. In contrast, the sedentary groups supplemented with moderate doses (W2 and W4) showed lower ALT levels, approaching physiological values.

Regarding aspartate aminotransferase (AST), a similar pattern was observed: elevated levels in C and TW6, contrasted with a significant reduction in the groups supplemented with moderate doses (W4 and W6). This finding reinforces that the combination of intense training and high whey doses may exacerbate hepatic stress, whereas controlled doses appear to exert a protective effect.

Alkaline phosphatase (ALP) showed considerable variation among the groups, with lower values in W2 and TW2, possibly indicating reduced hepatic anabolic stimulation in these contexts. Gamma-glutamyl transferase (GGT) remained stable in almost all groups, with no relevant differences, suggesting the absence of significant hepatobiliary impairment.

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Overall, the data indicate that moderate whey protein supplementation, particularly in sedentary animals or those trained with intermediate doses, is associated with more appropriate hepatic marker values. Conversely, high intake combined with training (TW6) showed potential signs of metabolic overload, highlighting the need for caution when prescribing high doses in contexts of intense exercise.

Relative liver weight

Figure 3 presents the mean values of relative liver weight. A significant reduction was observed in the TW2, TW4, and TW6 groups compared to the control group ($p<0.05$).

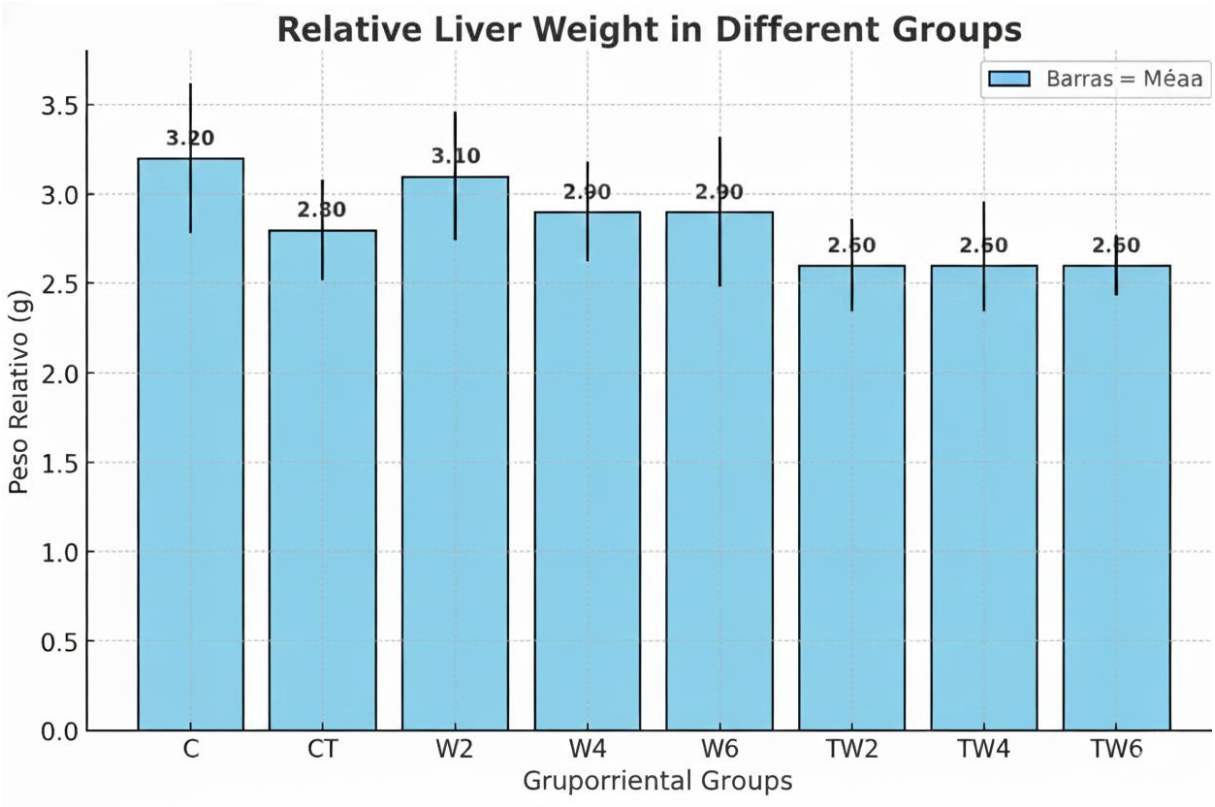

Figure 3 - Relative liver weight (g) in the different experimental groups.

Evaluation of Relative Liver Weight

The assessment of relative liver weight revealed differences among the experimental groups. The sedentary control group (C) showed the highest mean value (3.2 ± 0.42 g), while the trained supplemented groups (TW2, TW4, and TW6) consistently exhibited lower values, around 2.6 g, with small data dispersion. The trained control group (CT) had a mean of 2.8 ± 0.28 g, similar to the sedentary supplemented groups (W2 = 3.1 ± 0.36 g; W4 = 2.9 ± 0.28 g; W6 = 2.9 ± 0.42 g). Thus, supplementation alone did not significantly reduce relative liver weight, whereas its combination with physical training showed a more pronounced reduction trend.

Statistical analysis by ANOVA indicated significant differences among groups ($F(7, 68) = 9.70$; $p < 0.0001$). Post-hoc testing revealed that group C had significantly higher values compared to CT, W4, W6, TW2, TW4, and TW6, but did not differ from W2. No statistically significant differences were observed between trained groups (CT and TW) and sedentary supplemented groups (W2, W4, W6).

In summary, the data suggest that physical training was the main factor associated with the reduction of relative liver weight, whereas isolated whey protein supplementation

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showed no substantial impact. The combination of training and supplementation demonstrated consistent effects, though no marked differences were observed among the different whey doses tested.

DISCUSSION

This study analyzed 66 Wistar rats, initially aged 60 days, distributed across eight experimental groups: sedentary non-supplemented control (C), trained non-supplemented (CT), sedentary supplemented with 2 g/kg/day (W2), 4 g/kg/day (W4), or 6 g/kg/day (W6) of whey protein, as well as trained and supplemented groups with 2 g/kg/day (TW2), 4 g/kg/day (TW4), and 6 g/kg/day (TW6). The experimental protocol lasted 12 weeks, during which animals underwent resistance training on a vertical ladder and daily supplementation adjusted to body weight.

Results showed significant differences among groups in total serum protein levels ($p < 0.0001$), supporting literature indicating that whey protein intake directly affects protein metabolism (Franzen, Vaz, Zancanaro, 2016; Nunes et al., 2013).

Although Franzen, Vaz, Zancanaro (2016), using a cafeteria diet and concentrated whey for eight weeks, did not report exact protein intake, they observed significant changes in body mass, suggesting an indirect effect of supplementation on amino acid availability.

Regarding hepatic biomarkers, our findings revealed significant ALT elevation in multiple comparisons, particularly between the control group and the trained and/or supplemented groups. This partially contrasts with Morato et al., (2013), in which nine-day whey protein supplementation did not significantly alter ALT or AST levels. This discrepancy may relate to protocol duration, as our study involved chronic treatment (12 weeks) while Morato et al. (2013) assessed an acute period.

Conversely, Nunes et al., (2013) reported that sedentary rats supplemented with 1.8 g/kg/day of whey protein exhibited higher ALT and AST levels, while trained groups showed no significant changes in these biomarkers. This observation aligns with our findings, in which the solely supplemented groups (W4 and W6) showed more pronounced alterations than those subjected to training combined with supplementation (TW2, TW4, TW6). This suggests that resistance exercise may exert a protective modulatory effect on liver function, possibly through improved oxidative metabolism and greater amino acid utilization during exertion.

Regarding AST, significant differences were observed primarily between control and supplemented groups, consistent with Aparicio et al., (2011), who reported increased liver weight in animals fed high-protein diets based on whey protein hydrolysate for 90 days. The authors further noted that resistance training attenuated the adverse effects of the high-protein diet, supporting the hypothesis that physical activity acts as a protective factor against potential hepatic metabolic overload.

GGT values also showed significant alterations among supplemented and trained groups, while ALP differed in nearly all combinations involving supplementation and training. These results differ from Ávila et al. (2018), who evaluated high-protein diets combined with aquatic training and found no changes in GGT or ALP. This discrepancy may be attributed to exercise type (vertical ladder resistance vs. aquatic jumps) and protein source differences.

Regarding relative liver weight, significant differences were found between the control group and supplemented and trained groups, especially TW2, TW4, and TW6.

Aparicio et al., (2011) also reported increased liver weight with high-protein diets but noted that resistance training reduced this effect. This supports the hypothesis that combining exercise and supplementation promotes compensatory adaptations that limit potential adverse effects of high protein intake.

Overall, our findings indicate that both whey protein supplementation and resistance training independently induce changes in hepatic biomarkers, but their combination appears to modulate and attenuate these changes, providing greater protection of liver function. This effect may be associated with enhanced metabolic utilization of amino acids during exercise, increased efficiency in protein synthesis, and improved antioxidant capacity, as previously suggested by Madureira et al., (2010) and Jin et al., (2013).

Thus, the results contribute to understanding the effects of whey protein supplementation at different doses, with or without resistance training, on liver function. They also highlight the need for caution when

using high protein doses without associated exercise, as the liver is directly impacted by amino acid metabolism.

Study Limitations

This study has several limitations. First, it is an experimental rat model, which limits direct extrapolation to humans. Only serum biochemical parameters and liver morphology were assessed, without inflammatory or oxidative biomarkers, which could provide a more comprehensive view of supplementation effects combined with resistance training.

Additionally, the sample consisted of young animals subjected to a specific exercise protocol, which may not reflect other age ranges or training modalities. Future studies should consider these variables and explore long-term effects.

CONCLUSIONS

The study results indicated that sedentary animals exhibited higher hepatic biomarker levels compared to trained and/or supplemented groups. No significant trend toward increased relative liver weight was observed in trained and supplemented animals compared to sedentary controls.

Therefore, it can be concluded that supplementation with varying doses of whey protein, when combined with resistance training, did not induce liver damage in adult Wistar rats. Nonetheless, further studies are recommended with longer intervention periods, different exercise models, and complementary biomarker analyses to more comprehensively elucidate the effects of protein supplementation on liver function.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this research.

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REFERENCES

1-Andersen, M. Princípios éticos e práticos do uso de animais de experimentação. Departamento de Psicobiologia. Escola Paulista de Medicina. Universidade Federal de São Paulo, 2004.

2-Aparicio, V.A.; Nebot, E.; Kapravelou, G.; Sánchez, C.; Porres, J.M.; López Jurado, M.; Aranda, P. El entrenamiento de fuerza reduce la acidosis metabólica y la hipertrofia hepática y renal consecuentes del consumo de una dieta hiperproteica en ratas. Nutrición Hospitalaria. Vol. 26. Núm. 6. p. 1478-1486. 2011.

3-Ávila, E.T.P.; Lima, T.R.; Tibana, R.A.; Almeida, P.C.; Fraga, G.A.; Sena, M.S., Corona, L.F.P.; Navalta, J.W.; Rezaei, J.; Ghayomzadeh, M.; Damazo, A.S.; Prestes, J.; Voltarelli, F.A. Effects of high-protein diet containing isolated whey protein in rats submitted to resistance training of aquatic jumps. Nutrition. Vol. 53. p.85-94. 2018.

4-CONCEA. Conselho nacional de controle de experimentação animal: Resolução normativa. Ministério da ciência tecnologia e inovação. 2ª edição. 2015.

5-Damy, S.B.; Camargo, R.S.; Chammas, R.; Figueiredo, L.F.P. Aspectos fundamentais da experimentação animal- aplicações em cirurgia experimental. Associação de Medicina Brasileira de veterinária. Vol. 56. Num. 1. p. 103-111. 2010.

6-Farup, J.; Rahbek, S.K.; Vendelbo, M.H.; Matzon, A.; Hindhede, J.; Bejder, A.; Ringgard, S.; Vissing, K. Whey protein hydrolysateaugments tendon and muscle hypertrophy independent of resistance exercise contraction mode. Journal Scand Medicine Science Sports. Vol. 24. Num. 5. p. 788-798. 2013.

7-Franzen, J.M.; Vaz, J.G.; Zancanaro, V.; Bitencourt, R.M. Baixa dose de wheyprotein reduz glicose, triglicérides e controla o peso corporal em ratos wistar. Revista Brasileira de Obesidade, Nutrição e emagrecimento. São Paulo. Vol. 10. Núm. 57. p.133-144. 2016.

8-Guyton, A.C.; Hall, J.E. Tratado de fisiologia médica. Elsevier Brasil. 2011

9-Kinoshita, S.; Yano, H.; Tsuji, E. An increase in damaged hepatocytes in rats after high intensity exercise. Journal Acta Physiology Scand. Kawasaki University of Medical Welfare,

Department of Health and Sports Sciences. Okayama, Japan., Num. 178. p. 225–230. 2003.

10-Jin, M.M.; Zhang, L.; Yu, H.X.; Meng, J.; Sun, Z.; Lu, R.R. Protective effect of whey protein hydrolysates on H2O2-induced PC12 cells oxidative stress via a mitochondria-mediated pathway. Journal Food Chemistry. Num. 141. p. 847-852. 2013.

11-Leite, R.D. Efeitos do treinamento resistido em ratos alimentados com dieta hiperlipídica. Tese de Doutorado. Universidade do Estado do Rio de Janeiro, Instituto de Biologia Roberto Alcântara Gomes. Pós-graduação em Biociências. 2013.

12-Liu, J.; Wang, X.; Zhao, Z. Effect of whey protein hydrolysates with different molecular weight on fatigue induced by swimming exercise in mice. Journal of the Science of Food and Agriculture. Num. 94. p.126-130. 2014

13-Macêdo, M.R.C. Expressão gênica de MTOR, MURF-1 e MAFBX em ratos wistar suplementados com whey proteins por doze semanas. Dissertação de Mestrado-Universidade Federal do Maranhão, São Luís. 2018.

14-Madureira, A.R.; Tavares, T.; Gomes, A.M.; Pintado, M.E.; Malcata, F.X. Invited review: physiological properties of bioactive peptidesobtained from whey proteins. Journal of Dairy Science. Num. 93, p. 437-455. 2010.

15-Marzani, B.; Balage, M.; Ve´Nien, A.; Astruc, T.; Papet, I.; Dardevet D.; Mosoni, L. Antioxidant supplementation restores defective leucine stimulation of protein synthesis in skeletal muscle from old rats. Journal Nutrition. Num. 138. p. 2205-2211. 2008.

16-Moller, N.P.; Scholz-Ahrens, K.E.; Roos, N.; Schrezenmeir, J. Bioactive peptides and proteins from foods: indication for health effects. Europe Journal Nutrition. Num. 47. p. 171-182. 2008.

17-Morato, P.N.; Lollo, P.C.B.; Moura, C.S.; Batista, T.M.; Camargo, R.L.; Carneiro, E.M.; Farfan, J.A. A dipeptide and an amino acid present in whey protein hydrolysate increase translocation of Glut-4 to the plasma membrane in wistar rats. Food Chemistry. Vol. 139. p. 853-859. 2013.

18-Neves, S.M.P.; Mancini Filho, J.; Menezes, E.W. Manual de cuidados e procedimentos com animais de laboratório do Biotério de Produção e Experimentação da FCF-IQ/USP. São Paulo. FCF-IQ/USP. 2013.

19-Nunes, R.; Silva, P; Alves, J.; Giuseppe, S.; Petry, M.; Rhooden, C.; Dallago, P.; Scheneider, C. D.. Effects of resistance training associated with whey protein supplementation on liver and kidney biomarkers in rats. Applied Physiology Nutricion, and metabolismo. Vol. 38. Num. 11. p.1166-1169. 2013.

20-Reitman, S.; Frankel, S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of. Chinical Pathology. Vol. 28. p. 56-63. 1957.

21-Rossi, A.L.; Blostein-Fujii, A.; Disilvesto, R.A. Soy beverage consumption by young men increased plasma total antioxidant status and decreased acute, exercise-induced muscle damage.Journal of Nutraceuticals Functional & Medical Foods. Vol. 3. Num. 1. p. 33-44. 2000.

22-SBCAL/COBEA. Sociedade Brasileira de Ciências em Animais de Laboratório 2012. Disponível em: <http://www.cobea.org.br>.

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