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ORIGINAL CONTRIBUTION

Whey protein precludes lipid and protein oxidation and improves body weight gain in resistance-exercised rats

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Abstract
Background Resistance exercise such as weight-lifting (WL) increases oxidation products in plasma, but less is known regarding the effect of WL on oxidative damage to tissues. Dietary compounds are known to improve antioxidant defences. Whey protein (WP) is a source of protein in a variety of sport supplements and can enhance physical performance.
Aim To evaluate the effect of WL on biomarkers of lipid and protein oxidation, on liver antioxidants and on muscle growth in the absence or presence of WP in rats.
Methods Thirty-two male Fisher rats were randomly assigned to sedentary or exercise-trained groups and were fed with control or WP diets. The WL programme consisted of inducing the animals to perform sets of jumps with weights attached to the chest. After 8 weeks, arteriovenous blood samples, abdominal fat, liver and gastrocnemius muscle were collected for analysis.
Results WP precludes WL-mediated increases in muscle protein carbonyl content and maintains low levels of TBARS in exercised and sedentary animals. WL reduced liver CAT activity, whereas WP increased hepatic glutathione content. In addition, WL plus WP generated higher body and muscle weight than exercise without WP.
Conclusions These data suggest that WP improves antioxidant defences, which contribute to the reduction of lipid and protein oxidation as well as body and muscle weight gain in resistance-exercised rats.

Keywords Whey protein · Weight-lifting · Lipid oxidation · Protein oxidation · Antioxidants

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Introduction

Physical exercise is known to increase the generation of reactive oxygen species (ROS) in response to increased oxygen utilisation [1, 2]. However, it has been proposed that different types and intensities of physical exercise could differentially affect the antioxidant system [3, 4]. A moderate intensity of regular physical exercise produces a mild amount of ROS, which, in turn, causes an adaptation of antioxidant and repair systems, being considered itself as an antioxidant [5]. On the other hand, acute, exhaustive exercise leads to a burst of ROS generation, causing a disturbance in the pro-oxidant/antioxidant balance in favour of the former, which results in oxidative stress. ROS induced by aerobic endurance exercise of different intensities has also been reported to promote muscle fatigue [6, 7] and DNA damage [8]. Regarding the effect of resistance exercise, such as weight-lifting and oxidative stress, oxidised macromolecules have been reported to

increase following exercise, with differences in the magnitude and time course of elevation [9–11]. Plasma is still the biological sample most commonly used to measure oxidation products and antioxidants markers, induced by weight-lifting exercise in human studies [9–12]. Muscle samples are often used [13, 14], whereas liver biopsy is impractical due to the invasive nature of tissue biopsy in human studies and due to the difficulty of imposing weight-lifting exercise on laboratory animals. Uchiyama et al. [15] reported that a weight-lifting exercise model in rats resulted in muscle fibre damage, as estimated by serum creatine kinase (CK) activity and indirect markers such as serial changes in total muscle superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) activity.

Oxidative damage induced by exercise could induce inflammation and tissue dysfunction, affecting muscle contraction, strength and the development of muscle fatigue [7, 16]. Although the effect of oxidative stress on muscle protein synthesis has not been well defined, it is possible that ROS could be involved in the impairment of the anabolic effect of nutrients [17, 18]. Recently, Marzani et al. [17] reported that antioxidant supplementation reversed impairment in the ability of leucine to stimulate in vitro muscle protein synthesis from old rats.

Antioxidants are generally believed to protect against exercise-induced oxidative stress and enhance exercise performance [17, 19]. Dietary compounds have been thought to improve antioxidant defences [20, 21]. Among these compounds, whey protein (WP) deserves special attention because it is a popular source of protein in a variety of supplements in sports nutrition. Designed as a high-biological-value protein supplement, studies have reported its benefits in the field of physical performance [22] as well as its ability to improve the antioxidant system during endurance and aerobic training [22, 23]. In addition, WP has successfully been used in the treatment of human immunodeficiency virus [24], liver diseases [25], cancer [26] and bone formation [27]. However, the effects of WP on the antioxidant system and biomarkers of oxidation products in tissues induced by weight-lifting exercise are less clear. In addition, it is unknown whether WP could be associated with muscle growth that is mediated by a reduction of protein and lipid oxidation induced by resistance exercise.

Therefore, the aim of this study was to evaluate the effect of a weight-lifting exercise, a form of resistance exercise, on products of protein and lipid oxidation in the muscle and liver, on liver antioxidants and on muscle growth in rats. In addition, we evaluated the ability of WP to enhance or preclude these resistance exercise-induced adaptations.

Materials and methods

Animals

Thirty-two male 60-day-old Fisher rats weighing approximately 110 g were used. Four groups of eight animals were housed individually in galvanised wire cages in a room with controlled temperature (23 ± 1 °C) and a 12-h light/dark cycle. The animals were maintained and used in accordance with the guidelines of the Canadian Council on Animal Care (CCAC). The research was approved by the Ethical Committee of Ouro Preto University—protocol # 036/2008.

Diets

Rats were randomly assigned to four groups: a control sedentary (CS) and a control exercised group (CE) fed with a standard diet—AIN-93 M [28]; a WP sedentary (WS) and a WP exercised group (WE) received the AIN-93 M modified diet with WP instead of casein. The diet composition is presented in Table 1, and the amino acid composition of the proteins (casein as control and WP) utilised is presented in Table 2. Food and water were provided ad libitum. Body weight and food consumption were measured every week. Food consumption was corrected for spillage.

Exercise training programme and experimental procedure

The aim of the resistance exercise programme was to mimic a weight-lifting training model for hindlimb muscles

Table 1 Composition (g/1,000 g) of control sedentary (*CS*), control exercised (*CE*), whey protein-sedentary (*WS*) and whey protein-exercised (*WE*) diets

Ingredients	CS/CE	WS/WE
Casein[a]	140	–
Whey protein[b]	–	150
Mineral mixture[c]	35	35
Vitamin mixture[d]	10	10
Soybean oil	40	40
Choline	2.5	2.5
Cellulose	50	50
Sucrose	100	100
Cornstarch	622.5	612.5
Total	**1,000**	**1,000**

[a] Isofar® (Rio de Janeiro, Brazil), containing 85% protein as determined by the Kjeldahl method [29]

[b] Isoprowhey® (Probiótica, Brazil), containing 80% protein as determined by the Kjeldahl method [29]

[c] Mineral mixture for the AIN-93 M diet [28]

[d] Vitamin mixture for the AIN-93 M diet [28]

Table 2 Amino acid composition of control (casein) and whey (*WP*) proteins as well as minimum requirements for rodent diets (g/100 g protein)

Amino acid	Casein	WP	Minimum requirements[a]
Threonine	3.9	8.0	3.7
Valine	6.4	6.3	5.6
Isoleucine	5.2	7.2	4.7
Leucine	8.9	11.2	8.7
Lysine	7.6	8.8	7.3
Methionine	2.8	2.2	2.6
Phenylalanine	4.9	3.1	4.9
Tryptophan	1.2	1.9	1.3
Tyrosine	5.3	3.2	–
Cysteine	0.5	2.7	–
Aspartic acid[b]	6.6	11.9	–
Arginine	3.5	2.2	–
Serine	4.9	5.3	–
Histidine	2.9	2.1	–
Glutamic acid[c]	20.1	18.9	–
Glycine	1.7	1.7	–
Alanine	2.9	5.3	–
Proline	10.6	7.8	–

[a] Estimated minimal nutrient composition of AIN-93 M rodent diets [28]

[b] Aspartic acid: aspartic acid + asparagine

[c] Glutamic acid: glutamic acid + glutamine

and consisted of two steps. In the first one, an adaptation period, rats of each exercised group (CE and WE) were subjected to swimming for 15 min in a 40-cm-deep swimming pool, with water temperature set to 32 ± 1 °C and without weights, for 1 week. After the adaptation period, the exercise training consisted of inducing the animals to perform jumps in a circular plastic container at a water level corresponding to 150% of their body length. Weights were attached to the animal's chest to promote its submersion. When the rats touched the bottom of the container, they jumped to emerge and breathe. The water level in the container and the overload attached to the animal's chest generated the resistance to the exercise. The animals performed four sets of 10 jumps/day, five times per week, for 8 weeks. Each set of jumps was interrupted by a 1-min resting interval. The exercise intensity was increased weekly through the attached weights, according to the animal's body weight (25% of BW in week 1, 30% in week 2, 35% in week 3, 40% in week 5, 50% in week 6, and 55% in weeks 7 and 8). Animals of the CS and WS groups were kept in a 5-cm-deep swimming pool for a period of 15 min to generate the same amount of stress as experienced by the animals that performed exercise. At the end of the 8 week of training, all rats were fasted for 12 h. Arteriovenous blood samples were collected under anaesthesia from all animals and centrifuged at 10,000 rpm for 15 min at 4 °C. After blood collection, the abdominal cavity was opened, and the abdominal fat was excised and weighed. Liver and gastrocnemius muscle were also quickly excised, washed, weighed and stored at −80 °C for further analysis.

Gastrocnemius muscle glycogen content

Muscle glycogen concentration was determined by a method previously described by Varnier et al. [30]. Briefly, 30–40 mg muscle tissue cleaned of connective tissue, visible fat and blood were homogenised in 250 μL distilled water using a homogeniser on ice. The homogenate was transferred to screw-cap tubes containing 50 μL of 6 mol/L hydrochloric acid and incubated in a boiling water bath for 2 h. After cooling, the mixture was neutralised with 150 μL of 2 mol/L potassium hydroxide. Samples were centrifuged at 9,000 rpm for 10 min at 4 °C, and muscle glycogen concentrations were determined using glucose residues (Labtest Diagnóstica, Lagoa Santa, Brazil).

Tissue preparation

Liver and/or gastrocnemius muscle samples were homogenised using a Potter–Elvehjem glass at 4 °C with specific solutions (10% w/v): sulfosalicylic acid (5%) for total liver glutathione, potassium phosphate buffer (pH 7.2) for liver catalase activity, Tris–HCl for liver and muscle TBARS, and (20% w/v) of potassium phosphate buffer (pH 6.7) with EDTA to assay liver and muscle protein carbonyls. After homogenisation, samples were centrifuged for 10 min at 10,000 rpm at 4 °C and the supernatant was used for analysis.

Thiobarbituric acid-reactive substances (TBARS)

The formation of TBARS is used as an indicator of peroxy lipid content. Malondialdehyde (MDA), a product of lipid peroxidation, reacts with thiobarbituric acid (TBA) at low pH and high temperature to form a pink-coloured complex. Liver and muscle TBARS were determined according to Beuge & Aust [31]. Briefly, 250 μL of 28% TCA were added to 500 μL of sample supernatant, 250 μL of TBA (1% in acetic acid 1:1) and 125 μL of BHT (5 mM in ethanol). Samples were then incubated at 95 °C for 15 min. The mixture was subsequently centrifuged at 10,000 rpm for 15 min. Supernatant absorbance was measured at 535 nm using a spectrophotometer. The level of peroxy lipid was calculated using the molar extinction coefficient of the TBA-MDA complex (1.56×10^5 mol^{-1} cm^{-1}). Values were expressed in nmoles per mg of protein.

Protein carbonyls

The introduction of protein carbonyl (PC) groups into proteins by oxidative mechanisms was assayed by reaction of carbonyl groups with primary amines to form semi-stable Schiff bases through reaction with 2,4-dinitrophenylhydrazine (DNPH), as previously described [32]. In brief, proteins were precipitated using TCA (10%) and incubated with DNPH and HCl at room temperature for 30 min. TCA (10%) was added to the precipitate and centrifuged at 5,000 rpm for 5 min at 4 °C. After discarding the supernatant, the precipitate was washed twice with ethanol/ethyl acetate (1:1), dissolved in 6% SDS solution and centrifuged at 10,000 rpm for 10 min at 4 °C. Supernatant absorbance was measured at 370 nm using a spectrophotometer. Carbonyl content was calculated using the DNPH molar extinction coefficient (21×10^3 mol^{-1} cm^{-1}), and the results were expressed as nmol of carbonyl groups per mg of protein.

Total liver glutathione content

Total liver glutathione (GSSG + GSH) content was determined using Sigma kit #CS0260 (Saint Louis, USA). This assay utilises a kinetic method to measure total glutathione content based on the reduction of DTNB (5,5′-dithio-bis-(2-nitrobenzoic) acid) to TNB, which can be spectrophotometrically measured at 412 nm. The sensitivity of the assay was up to 2 nmol/mL. A solution of reduced glutathione (G4251-Sigma) was used to determine the standard curve. Total glutathione was expressed in nmoles per ml of sample.

Catalase activity

Catalase (CAT) activity was assessed by quantification of the H_2O_2 degradation rate, measured spectrophotometrically at 240 nm at 25 °C [33]. Briefly, 10 µL of sample supernatant was mixed with 50 µL of K_2HPO_4, 40 µL of milli-Q water (Millipore, Bedford, MA, USA) and 900 µL of 2.5 mmol/L H_2O_2. Concentrations of H_2O_2 and samples were chosen such that the degradation rate was linearly proportional at 30 s, 1 min, 2 min and 3 min. The rate of decomposition of H_2O_2 was calculated using the molar extinction coefficient, 0.071 M^{-1} cm^{-1}. One U of catalase is equivalent to the hydrolysis of 1 µmol of H_2O_2 per min.

Protein assay

Total protein concentrations were determined using the method described by Lowry et al. [34] with bovine serum albumin as the standard.

The precision of CAT enzyme activity (intra-assay = 12%; inter-assay = 13%); total glutathione content (intra-assay = 7%; inter-assay = 10%); TBARS (intra-assay = 12%; inter-assay = 11%) and protein carbonyl (intra-assay = 15%; inter-assay = 14%) levels were calculated using the variation coefficient (in per cent) of repeated measurements of control samples.

Statistics

All variables were tested for normal distribution using the Kolmogorov–Smirnov test ($p > 0.05$). Data were analysed by two-way analysis of variance; the classification factors were diet (CS + CE × WS + WE) and exercise (CS + WS × CE + WE) as well as the interaction between diet and exercise (CS × CE × WS × WE). Tukey's post-hoc test was used to determine the differences among the four groups when a statistical diet-by-exercise interaction was observed. Sigmaplot version 11.0 was used.

Results

Food intake, body weight gain, muscle weight, muscle glycogen and abdominal fat

Data related to food intake, body weight gain, abdominal fat weight and gastrocnemius muscle weight and glycogen content are presented in Table 3. Throughout the experimental period, food intake was slightly lower in exercised when compared to sedentary rats and was not modified by WP treatment. Body weight gain and gastrocnemius muscle weight were similar in all groups except for control exercised (CE) animals, which yielded the lowest values. While body and muscle weight in the WE group were comparable with those of the CS and WS groups, these values were higher than those obtained for the CE group. In addition, rats in the WE group had higher levels of muscle glycogen than did rats in the other groups. Exercised rats also had less abdominal fat than the sedentary ones.

Lipid and protein oxidation products

The effects of a resistance-exercise model, in which rats were stimulated to perform a set of jumps with attached overload mimicking weight-lifting training, on biomarkers of muscle and liver protein and lipid oxidation in the absence or presence of WP are presented in Figs. 1–2. Protein carbonyl (PC) groups were affected differentially by resistance exercise, depending on tissue type. The weight-lifting training increased muscle PC content but reduced PC levels in the liver (Fig. 1). Interestingly, muscle PC level in the WE group was lower than that in the

Table 3 Food intake, body weight gain, gastrocnemius muscle weight, muscle glycogen and abdominal fat in the studied groups

Variables	Experimental groups				Two-way ANOVA (P)		
	CS	CE	WS	WE	D	E	D × E
Initial body weight (g)	113.96 ± 15.72	113.55 ± 8.65	113.65 ± 7.98	113.46 ± 8.85	0.957	0.933	0.975
Food intake (g/day)	13.18 ± 0.66	11.64 ± 0.73	13.00 ± 1.28	12.35 ± 0.81	0.382	0.002	0.209
Body weight gain (g/day)	3.14 ± 0.17[a]	2.59 ± 0.29[b]	3.42 ± 0.34[a]	3.51 ± 0.57[a]	<0.001	0.032	0.032
Muscle weight (mg)	1,708 ± 131[a]	1,487 ± 88[b]	1,679 ± 169[a]	1,716 ± 96[a]	0.032	0.046	0.007
Muscle glycogen (mg/100 mg tissue)	0.08 ± 0.03[b]	0.09 ± 0.04[b]	0.11 ± 0.04[b]	0.21 ± 0.09[a]	<0.001	0.004	0.043
Abdominal fat (g)	5.31 ± 0.81	4.06 ± 0.69	5.99 ± 0.35	4.31 ± 1.03	0.172	<0.001	0.430

Values were expressed as the mean ± SD. $n = 6$–8 animals per group. D and E correspond to diet and exercise, respectively. D × E is the interaction between the corresponding parameters. Within a row, statistically different values are marked with different superscript letters when a significant interaction was observed ($p < 0.05$)

ANOVA analysis of variance, *CS* control sedentary group, *CE* control exercised group, *WS* whey protein-sedentary group and *WE* whey protein-exercised group

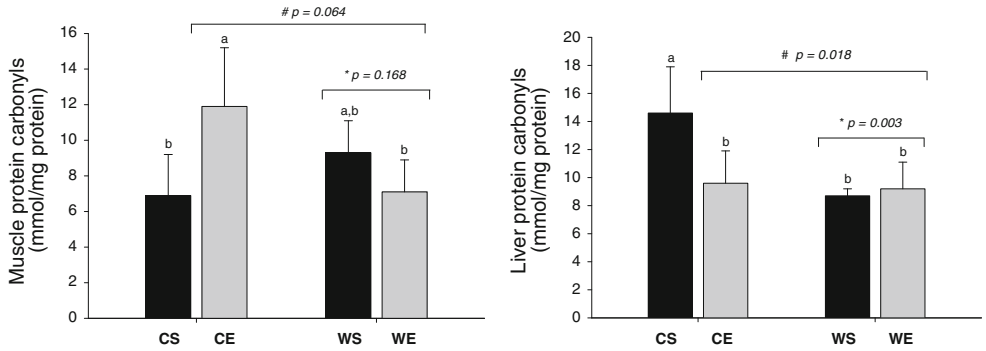

Fig. 1 Muscle and liver protein carbonyl levels of sedentary (*black bars*) or resistance-trained (*grey bars*) rats fed with control (CS and CE) or WP (WS and WE) diets. Results are expressed as means ± SD; $n = 6$–8 per group. *Diet effect; #Exercise effect; statistically different values are marked with superscript letters when a significant interaction was observed. Diet × Exercise ($p = 0.001$ and $p < 0.001$ for muscle and liver protein carbonyl levels, respectively)

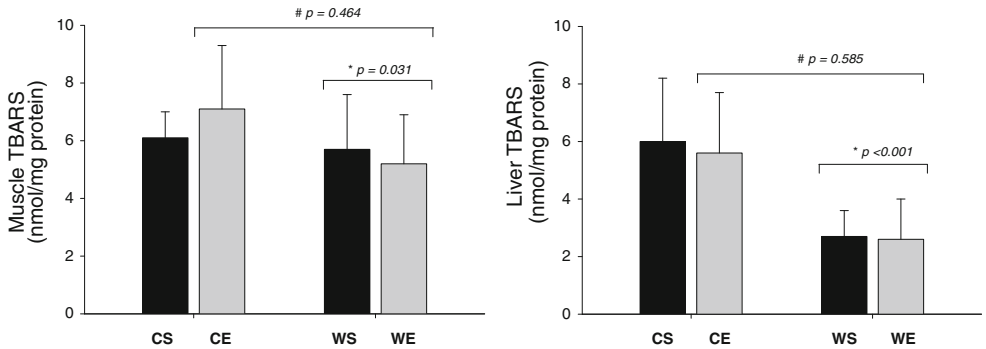

Fig. 2 Muscle and liver TBARS levels of sedentary (*black bars*) or resistance-trained (*grey bars*) rats fed with control (CS and CE) or WP (WS and WE) diets. Results are expressed as means ± SD; $n = 6$–8 per group. *Diet effect; #Exercise effect; Interaction between Diet × Exercise ($p = 0.420$ and $p = 0.202$ for muscle and liver TBARS, respectively)

CE group and comparable to levels in the CS and WS groups, whereas the liver PC content of WP rats was comparable with that in CE rats. These findings demonstrate the ability of WP to prevent the increase in muscle PC induced by resistance exercise and prevent the increase of liver PC content in exercised as well as sedentary rats. Fig. 2 shows the end products of lipid peroxidation in gastrocnemius muscle and liver, as represented by levels of TBARS, a well-known biomarker of lipid peroxidation [35]. Although the exercise did not significantly affect

muscle and liver levels of TBARS in either the CE or the WE group, WP treatment was able to significantly reduce TBARS levels in both tissues with no significant interaction observed ($p = 0.420$ and $p = 0.202$ for muscle and liver, respectively).

Liver catalase activity and total liver glutathione content

Data for total liver glutathione content and liver catalase (CAT) activity of sedentary or exercised rats in the presence or absence of WP are presented in Fig. 3. Resistance exercise did not improve the antioxidant parameters in the liver, as measured by total glutathione content and CAT activity. Although WP treatment increased total glutathione content ($p = 0.025$), there were no statistical differences among groups. Both exercise and WP treatment significantly reduced liver CAT activity, although CAT activity did not differ significantly between the WE group and the WS group. Rats in the CS group exhibited higher levels of CAT activity than did rats in any of the other groups.

Discussion

The aim of this study was to evaluate the effect of a weight-lifting exercise model for rats on biomarkers of protein and lipid oxidation in the muscle and liver, liver antioxidants and muscle weight. In addition, we evaluated the ability of WP to enhance or preclude the adaptations induced by resistance exercise. It was demonstrated that WP treatment was able to preclude muscle protein oxidation induced by resistance training through the reduction of PC levels. Notably, weight-lifting is known to promote plasma protein oxidation [9–12]. Hudson et al. [12] found increases in plasma PC immediately after and 60 min after acute hypertrophy-resistance exercise in trained subjects. Bloomer et al. [36] also found elevated plasma PC after a single bout of weight-lifting in trained men. However, these studies did not measure muscle PC levels in situ, where PC levels have been associated with fatigue and impaired contractility [7, 16]. Although mechanical stress such as forced stretching of muscle fibres is considered a possible cause of weight-lifting-induced muscle damage, oxidative stress could also induce muscle damage [37] and could affect muscle protein metabolism in response to anabolic stimuli [17, 18]. However, in contrast to observations in muscle in situ, exercise reduced liver PC in control rats, and WP was able to maintain similarly low PC levels in exercised and sedentary rats (Fig. 2). These results suggest that WP may attenuate oxidative damage in muscle and liver, regardless of whether the oxidative damage present resulted from resistance exercise. Similarly, levels of TBARS, a well-known biomarker of lipid peroxidation [35], are known to increase in plasma after weight-lifting training [10]. In this study, resistance exercise did not increase TBARS levels in CE and/or WE rats, while levels were reduced by WP treatment, suggesting WP-mediated protection of muscle and liver tissues against lipid peroxidation that is independent of exercise.

Resistance exercise did not improve antioxidant parameters in the liver, as measured by CAT and total glutathione content. Resistance exercise reduced CAT activity in both exercised groups. Previous studies have shown that exercise training can increase or reduce CAT activity, depending on the type and intensity of exercise [38, 39]. Recently, da Silva et al. [3] observed that continuous running and downhill running (45 min daily, 5 day/week for 8 week) reduced liver CAT activity in trained mice. However, intermittent running (3 × 15 min daily, 5 day/week for 8 week) did not induce the same adaptations. Taysi et al. [40] reported that treadmill running (2.1 km/h, 1.5 h/day, 5 day/week for 8 week) also reduced liver CAT

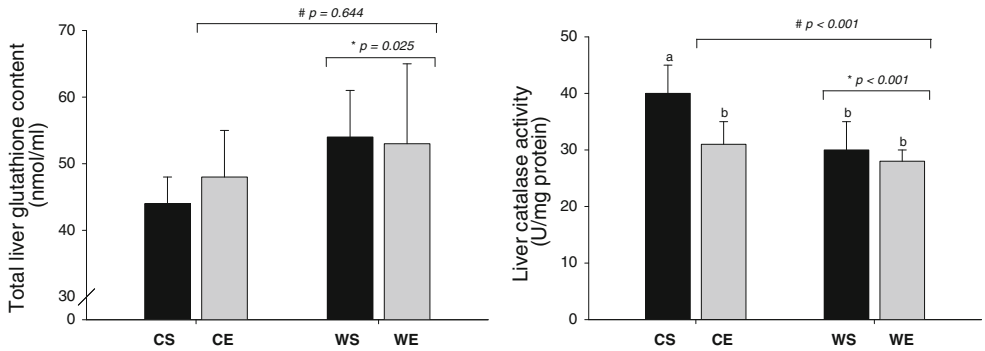

Fig. 3 Total liver glutathione content and liver catalase (CAT) activity of sedentary (*black bars*) or resistance-trained (*grey bars*) rats fed with control (CS and CE) or WP (WS and WE) diets. Results are expressed as means ± SD; $n = 6$–8 per group. *Diet effect; #Exercise effect; statistically different values are marked with different superscript letters when a significant interaction was observed; Interaction between Diet × Exercise ($p = 0.353$ and $p = 0.018$ for total glutathione and CAT activity, respectively)

activity, but Kakarla et al. [41] reported that lower-intensity treadmill running (1.4 km/h, 0.5 h/day, 5 day/week for 12 week) increased CAT activity. In the present study, high-intensity exercise, in which rats were stimulated to perform a set of jumps with attached overload reaching 55% of body weight, reduced liver CAT activity in both exercised groups. Taken together, these findings suggest that liver CAT activity is inversely correlated with exercise intensity: high-intensity exercises appear to reduce CAT activity, whereas low-intensity exercises appear to elevate CAT activity. In addition, exercise did not significantly affect total liver glutathione content, a key component of antioxidant defence [42]. On the other hand, it was observed that WP treatment increased total liver glutathione levels ($p = 0.025$). Tissue glutathione synthesis is dependent on the dietary supply of amino acid, mainly cysteine, the rate-limiting substrate for intracellular glutathione synthesis [42]. WP is an important source of glutamylcysteine groups, which are rare in other food proteins [43], and has a cysteine content up to five-fold higher than that of casein (Table 2). Thus, consistent with previous findings [22, 43], data from the present study indicate that WP represents an effective substrate source for glutathione replenishment in WP animals, suggesting that WP should ameliorate the oxidative equilibrium, which could also contribute to less ROS-induced oxidative tissue damage, as evidenced by PC and TBARS levels in rats fed with WP. In addition, an increase in total glutathione concentration augments potentially the availability of reducing equivalents used by the enzyme GPx to detoxify hydrogen peroxide [44], what could improve the oxidative balance and partially reduce the activity of liver CAT.

Resistance-training programmes with laboratory animals represent an alternative to study muscle hypertrophy [45]. The results of the present study show differences between whole body and muscle tissue in CE and WE groups. Rats in the CE group show reduced body and muscle weight, as is typically observed in resistance-trained animals [46, 47]. Interestingly, WP counteracts the effects promoted by resistance exercise, resulting in rats with similar body and muscle weight to those observed in the CS and WS groups, but higher than observed for CE rats. Differences in the amino acid composition of both proteins (casein and WP) cannot entirely explain the difference in weight gain between CE and WE groups because casein provides the minimum requirements for the rodent diet (Table 2), and no significant differences in body and muscle weight gain were observed between CS and WS groups. In fact, casein is a standard for biological evaluation of proteins and allows differences in protein nutritional quality to be readily demonstrated [48]. Although WP exhibits deficient levels of phenylalanine and methionine, it has been demonstrated that commercial WP fulfils more biological requirements than casein [49]. In addition to its greater digestibility, protein efficiency ratio and net protein ratio, bioactive peptides present in WP can positively affect the utilisation of protein for growth [49], which could partially explain differences in body and muscle weight gain. However, significant differences were observed only in exercised rats. Studies have reported that BCAA, particularly leucine, is an established modulator of muscle protein metabolism. BCAAs have also been identified as key regulators in the initiation of muscle protein synthesis [50]. In comparison with those of casein, WP digestion/absorption kinetics appear to promote a blood leucine peak [51] and the stimulation of protein synthesis after resistance exercise [52]. Therefore, WP is currently described to have the potential to affect muscle and strength development during resistance exercise [53, 54] and could partially explain differences in body and muscle weight between rats fed with casein or WP.

Alternatively, oxidative stress induced by resistance exercise can generate alterations in DNA, lipids and proteins [7, 10, 15], resulting in decreased biological function and potentially mediating the anabolic effect of leucine [17, 18]. Marzani et al. [17] observed that antioxidant supplementation with rutin, zinc, selenium and vitamins E and A improved the response of protein synthesis to leucine in muscles from old rats. It has been reported in in vitro studies that oxidative stress impairs insulin signalling [55], leading to protein synthesis modulation. However, the exact molecular mechanisms responsible for the ROS-induced impairment of protein synthesis remain unknown. Marzani et al. [17] failed to demonstrate that the decrease in leucine's effect on muscle protein synthesis in old rats was associated with changes in oxidative damage to the S6K protein. The authors did not observe a clear effect of antioxidants on muscle mass, although they did not evaluate these parameters in association with exercise. In the present study, it was observed that exercised rats fed with WP show higher body and muscle weight than CE rats, concomitant with a strong reduction in oxidative products, as evidenced by PC and TBARS levels. These results suggest that differences in muscle and body weight may have been mediated through a reduction of local (in situ muscle) and liver oxidative damage. The findings also suggest that a synergistic effect between the antioxidant properties [22, 23] and that the leucine content of WP may improve muscle protein anabolism, although the exact mechanisms involved remain to be determined. Lastly, it was also observed that muscle glycogen content was higher in exercise-trained rats that were fed with WP (Table 3). Morifuji et al. [46] observed a similar effect, with a significant positive correlation ($r = 0.86$; $p < 0.001$) with skeletal muscle glycogen synthase activity, suggesting that WP increased skeletal muscle glycogen content through

this pathway. In addition, WP increases activity and mRNA expression of fatty acid synthase in skeletal muscle, stimulating skeletal muscle to enhance intramuscular triacylglycerol accumulation [56], which is an important source of energy during exercise and could spare muscle glycogen stores [57].

In conclusion, the results of the present study reveal that weight-lifting exercise increased protein oxidation in muscle but reduced protein oxidation in liver. In contrast, peroxy lipid content in both tissues was not affected by exercise. It was clearly demonstrated that WP inhibits the oxidation of muscle proteins induced by exercise, maintaining low levels of peroxy lipids in exercised and even sedentary animals. Although the resistance exercise did not enhance liver antioxidant parameters, the WP diet significantly increased hepatic glutathione content, which could also reduce ROS-induced protein and lipid oxidation. Concomitant with a reduction in protein and lipid oxidation, weight-lifting training plus WP treatment results in rats with higher body and muscle weight than in rats subjected to exercise without WP. These results suggest that these differences in muscle and body weight are partially mediated by WP's antioxidant properties.

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Conflict of interests None.

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