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ARTICLE

Ingestion of soy-whey blended protein augments sport performance and ameliorates exercise-induced fatigue in a rat exercise model

Guangxu Ren^{a*}, Suqing Yi^a, Hongru Zhang^a, and Jing Wang^{a*}

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This study sought to determine the effects of soy-whey blended protein supplementation on sport performance and related biochemical parameters after long-term training. After a week of adaptation, eighteen 6-week-old male Wistar rats were randomly assigned to 3 groups: the standard chow diet plus whey protein (Whey) group, the standard chow diet plus soy-whey blended protein (BP) group and the standard chow diet only (control) group. Each group included 6 rats for the seven-week experiment. Before the experiment, baseline values of body weight, grasping force and time to exhaustion due to the loaded-swimming test were recorded for each group. During the experimental period, all rats performed the loaded-swimming test until exhaustion five days each week. The results showed that the mean maximum grasping force of the BP group was significantly increased between the 5th and 7th weeks ($p < 0.05$) compared with the other groups. The ingestion of blended protein for 7 weeks significantly increased the mean time to exhaustion due to swimming by 1.5-fold and 1.2-fold compared with the control and Whey groups, respectively. Plasma levels of leucine, isoleucine and valine were significantly higher at 60 min after the blended protein intervention compared with the Whey and control interventions ($p < 0.05$). Furthermore, the ingestion of soy-whey blended protein enhanced the activities of lactate dehydrogenase and superoxide dismutase and decreased the levels of malondialdehyde in serum. These results collectively suggest that soy-whey blended protein ingestion following resistance exercise can improve sport performance and ameliorate exercise-induced fatigue in rats.

1 Introduction

Muscle strength is widely accepted as an important determining factor in sport performance¹. Thus, improving muscle mass is a successful strategy to enhance sporting abilities. Resistance exercise can lead to substantial gains in strength and muscle hypertrophy². However, previous investigations have indicated that resistance exercise is a double-edged sword: it can stimulate muscle protein synthesis (MPS)³ but has adverse effects on muscle mass, such as increasing the rate of muscle protein breakdown⁴⁻⁶ and producing fatigue due to free radicals⁷. A net muscle protein balance exists in the synthesis and breakdown that follows resistance exercise. Although muscle protein is constantly and simultaneously synthesized and degraded *in vivo*³, the process of muscle protein turnover induced by resistance exercise is relatively slow^{8, 9}. Studies have found that although MPS is markedly elevated after resistance exercise, the net protein balance remains negative until essential amino acids are

provided⁴⁻⁶. Evidence indicates that physical exercise alone can restrain net muscle protein catabolism but does not directly promote net protein deposition during the recovery phase of exercise. In addition, intense exercise can generate free radicals and result in oxidative stress¹⁰. Oxidative stress contributes to muscle damage and fatigue⁷. Given the above findings, resistance exercise must be incorporated with other factors such as essential amino acid (EAA) consumption, antioxidant substance use, or both to improve sport performance and reduce the disadvantages caused by exercise.

Several studies have indicated that supplements containing high-quality protein combined with resistance exercise can synergistically elevate MPS rates¹¹⁻¹³ during the recovery phase. Interestingly, dietary proteins from different sources possess different capacities to stimulate MPS both at rest¹⁴⁻¹⁶ and following resistance exercise^{15, 16}. The possible mechanisms responsible for these differences might be related to crucial differences in the amino acid profile and the digestion/absorption kinetics of dietary proteins. Whey is an acid-soluble protein with higher branched-chain amino acid (BCAA) content (primarily leucine) compared with other high-quality proteins¹⁷. BCAAs play a crucial role in MPS. Thus, whey protein is commonly used to build muscle or maintain sport performance among people who exercise. However, whey protein has two major drawbacks with regard to improving

^a Institute of Food and Nutrition Development, Ministry of Agriculture, Beijing 100081, China

*Corresponding author:

wangjing07@caas.cn, Tel: +86-10-82107740;
renguangxu@caas.cn, Tel: +86-10-82105482; Fax: +86-10-82105184

muscle mass. One drawback is that whey protein rapidly degrades after ingestion. Whey protein remains soluble in the stomach. Thus, it is emptied rapidly¹⁸. BCAAs are released into the blood and cause a transient hyperaminoacidemia following the ingestion of whey protein¹⁹. Previous studies have suggested that muscle protein accretion occurs during the recovery phase after exercise rather than during the actual exercise period^{20, 21}. Therefore, if only whey proteins are ingested after exercise, then the supply of BCAAs cannot meet the demands for MPS during the recovery phase. The other drawback is that because whey protein is deficient in antioxidants, oxidative stress during exercise cannot be eliminated effectively via oral supplementation with whey protein, thereby leading to muscle damage and fatigue^{7, 22}. However, soy protein, which is a high-quality plant protein, can remove the drawbacks of whey protein. Soy protein contains all of the EAAs and has the advantage of stimulating MPS¹⁷. In addition, soy protein, which has a relatively slower degradation rate compared with whey protein, contains a mixture of antioxidants, including isoflavones, saponins, and copper, a component in numerous antioxidant enzymes⁷.

These findings raise the question of whether soy-whey blended protein has more advantages than the single whey protein in improving sport performance. To address this issue, we conducted a 7-week resistance exercise experiment using a Wistar rat loaded-swimming model to compare the effects of soy-whey blended protein and whey protein alone on sport performance. We assessed changes in the mean time to exhaustion, grip strength, biochemical parameters such as blood EAAs, tissue-damage-related indicators, energy-related indicators and antioxidant capacity.

2 Materials and methods

2.1 Animals

Eighteen 6-week-old specific pathogen-free (SPF) Wistar male rats, each weighing 109-120 g, were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). All of the animals were housed in individually ventilated cages (IVC, 3 rats/cage) under standard laboratory

Fig. 1 Illustration of the experimental procedure. (A) Schematic illustration of experimental design; (B) Baseline and final detection procedures; (C) Illustration of the loaded-swimming model; (D) Daily operating procedure.

conditions (12-h light/dark cycle at room temperature [$23\pm 1^\circ\text{C}$] with standard rodent maintenance feed and water *ad libitum*) at the Institute of the Laboratory Animal Sciences, CAMS & PUMC. This project was approved by and performed in strict accordance with the guidelines of the Institutional Animal Care and Use Committee, Institute of Food and Nutrition Development, Ministry of Agriculture. All operations were made to minimize suffering.

2.2 Experimental design

The uniform weight of the SPF-grade Wistar male rats were housed in IVC cages for 1 week to allow them to adapt to the feeding environment and the swimming and grasping exercises. After this adaptation period, 18 rats were randomly divided into 3 equal-sized groups: a standard chow diet feeding group (control group), a standard chow diet plus whey protein feeding group (Whey group) or a standard chow diet plus soy-whey blended protein feeding group (BP group). First, we recorded baseline characteristics regarding weight, grasping force and time to exhaustion due to loaded-swimming training for the three groups on day 8 (Fig. 1A). After an overnight fast (~10 h) and daily swimming training, the dynamic profiles of the EAA release from the whey protein and blended protein were measured via tail-vein blood at 0 (after exhaustive swimming), 30, 60, 90 and 150 min after the gavage administration of target protein supplements (Fig. 1B). In order to minimize the gastric damage caused by the gavage, we only performed the gavage administration for 5 days a week. We conducted the standard daily operation including the quota of standard diet, free drinking, loaded-swimming training and dietary protein intervention for 5 days (Monday to Friday) per week from day 9 to day 56 (Fig. 1A and D). Finally, after the 7-week experimental period, the body weight,

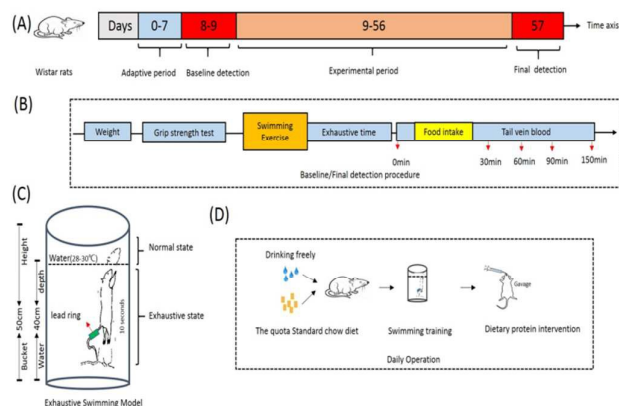


Table 1. The diet table of the three groups

Diets	Dietary patterns	The basic daily chow diet	Nutritional intervention (via gavage)		Contents
		GMCF ^①	Whey protein isolate ^②	Soy protein isolate ^③	
Groups	Protein Source	Soy protein	Whey protein	Soy protein	^① : GMCF: crude protein is composed of soy protein (20% of GMCF, Table 2); ^② : Whey protein concentration: 94.6% (Table 3); ^③ : Soy protein concentration: 92.5%(Table 3) ;
	Company	Yuwang	Hilmar	Yuwang	
	Control	+	-	-	
	Whey	+	+	-	GMCF (30g/day/rat)+Gavage (218.4 mg whey protein dissolved in 2ml of sterile water/day/rat)
	BP	+	+	+	GMCF (30g/day/rat)+Gavage (109.2 mg whey protein and 90.8 mg soy protein dissolved in 2ml of sterile water/day/rat)
	Notes	Providing daily nutrition	Protein nutritional intervention ^④		GMCF: Every day; Gavage: from Monday to Friday

^④: Nitrogen equilibrium: Whey protein (in Whey group) =Soy-whey blended protein (in BP group); Whey protein (in BP group) = Soy protein (in BP group).

grasping force and mean time to exhaustion for each rat were recorded before sacrifice.

2.3 Diet and protein supplements

The standard SPF-grade growth maintenance chow feeds (GMCF; GB14924.3-2010, China National Standard) were purchased from Beijing Keao Xieli feed Limited Company (SPF-GMCF, Beijing, China). The crude protein source for GMCF is soy protein which was purchased from Shandong Yuwang Industrial Co. Ltd. (Shandong, China). For daily feeding, all rats were maintained with standard chow feed (Table 1, 30 g/day/rat) and water intake *ad libitum*. According to the results of our preliminary experiment, the average daily intake of 6 week old rats was 30 grams. Fresh feed and water were provided at 8:00 AM every day. Due to the limitation of the rat's gastric volume, the maximum volume of the stomach perfusion was 2 ml. Thus, we tested the maximum solubility of the blended proteins (whey: Soy=1:1) in the 2ml sterilized water. Finally, we determined the optimal amount for BP protein in 2ml sterilized water was 200 mg (whey protein=100mg; Soy protein=100mg). Then, we used Kieldahl Azotometer (KDY-9820; Scipin, Beijing, China) to match the protein content of the Whey and BP groups (nitrogen equilibrium: 218.4 mg of Whey =200 mg of Blended protein). Briefly, the rats were given 218.4 or 200 mg total protein

dissolved in 2ml of sterilized water via gavage for the whey or blended proteins, respectively (Table 1).Whey protein isolate was purchased from Hilmar food international, Inc. (Hilmar9410, Livingston, CA, USA). Soy protein isolate was purchased from Shandong Yuwang Industrial Co. Ltd. (YP928H, Shangdong, China).

2.4 Loaded-swimming test

The loaded-swimming test was used as described previously with modifications²³. This test was conducted by forcing rats to swim until exhaustion. In our preliminary test, we examined the swimming time period and the appropriate load weight of swimming rats. To avoid floating, the rats were loaded with lead rings that weighed 10% of their body weight on their tails and were then placed in the swimming tank (50-cm high) filled to a depth of 40 cm with fresh water, and maintained at 28-30°C (Fig.1C). The endurance for each rat was measured as the time to exhaustion when swimming, recorded from the beginning to exhaustion. Exhaustion was defined as the loss of coordinated movements and a failure to return to the surface within 10 s. Rats were removed at exhaustion before drowning. In this test, the rats were trained and measured in a blinded fashion by the independent investigator who didn't know the details. All rats were numbered randomly. The relationship between the random number and the experimental ID for each

Table 2 The nutrients of GMCF^①

Nutrients	Value	Unit
Dry matter	89	%
Crude Protein ^②	20	%
Crude fat	4.5	%
Crude fiber content	3.7	%
Crude Ash	6.53	%
Calcium	1.19	%
Phosphorus	0.77	%
Lysine	0.93	%
Methionine+Cystine	0.63	%
Arginine	1.02	%
Histidine	0.5	%
Tryptophan	0.22	%
Phenylalanine + Tyrosin	1.5	%
Threonine	0.78	%
Leucine	1.59	%
Isoleucine	0.76	%
Valine	0.9	%
Vitamin+A	10.7	KIU/kg
Vitamin+D	1.5	KIU/kg
Vitamin+E	103.05	IU/kg
Vitamin+K	6.14	Mg/kg
Vitamin+B1	16	Mg/kg
Vitamin+B2	16.03	Mg/kg
Vitamin+B6	10.43	Mg/kg
Nicotinic acid	7.45	Mg/kg
Magnesium	0.26	%
Kalium	0.64	%
Sodium	0.32	%
Ferrum	180	Mg/kg
Zinc	56.2	Mg/kg
Selenium	0.16	Mg/kg

^①: Nutrient composition per kilogram of feed

^②: Crude protein is composed of soy protein.

rat was recorded to the cross-references. For the daily training, the independent investigator forced rats to conduct the loaded-swimming for 45 s per day from Monday to Friday during the whole experimental period.

Table 3 The ingredients of whey and soy protein isolate

Ingredients	Whey protein isolate	Soy protein isolate
Ash	2.5%	3.3%
Fat	1.4%	1.0%
Lactose content	0.2%	<0.2%
Protein concentration	94.6%	92.5%
Bacillus cereus presumptive	<10 CFU/g	<10 CFU/g
Yeast	<10 CFU/g	<10 CFU/g
Staph	<10 CFU/g	<10 CFU/g
MPN Ecoli	<3.0 MPN/g	<3.0 MPN/g
Lead	<0.005 ppm	<0.005 ppm

2.5 Grasping test

The grip strength of the rats in this study was quantified using a dynamometer (YLS-13A, Jinan Yinan Yiyuan Technology and Development Co., Ltd., Jinan, China). The grasping test was conducted as previously described²⁴. In brief, rats were gently placed on the grid of the dynamometer and pulled by their tails in the opposite direction. The maximum grip strength exerted by the rat before losing grip was recorded. The rate of "pulling" the rats by their tails was 2 minute intervals of each test. We calculated the mean of three grasping attempts.

2.6 Plasma EAA kinetics

The EAA concentrations in the plasma were quantified using high performance liquid chromatography (HPLC, Waters 2695, MA, USA). Sample extracts were chromatographed on a column that was kept at -80°C and monitored via fluorescence-detection. H₂O was used in the mobile phase at a flow-rate of 1.0 ml/min. Then, 20 µl plasma was mixed uniformly with 100 µl of derivative reagent after thawing, and 20 µl of mixed liquid was injected into the HPLC pump to measure the plasma concentrations of amino acids. The measurements for all samples were repeated in triplicate²⁵.

2.7 Biochemical parameter measurements

The level of malondialdehyde(MDA) in serum was measured by using a commercial reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Superoxide dismutase (SOD) activity was tested using the SOD Assay Kit (Institute of Biological Engineering of Nanjing Jianchen, Nanjing, China). Lactic dehydrogenase (LDH) was determined by using a commercial diagnostic kit (Biosino Bio-technology and Science Inc., Beijing, China). Serum glucose was measured by the oxygen rate method using the Beckman Synchron LX System (Beckman Coulter, Fullerton, CA, USA). Serum insulin was detected by using a radioimmunoassay commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum creatine kinase (CK) was measured by a simple colorimetric method using a commercial creatine kinase assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum ALT and AST levels were measured using a standard clinical automatic analyzer (Roche Modular P800 Automatic Chemistry Analyzer).

2.8 Statistical analysis

The data are presented as means±standard errors (SEs). Two-way analyses of variance (ANOVAs), repeated-measures ANOVAs and Bonferroni post hoc tests were performed using GraphPad Prism 4.0 software (GraphPad Inc., San Diego, CA, USA). *p*-values <0.05 were considered as significant.

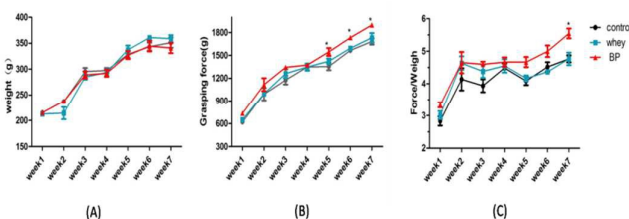


Fig. 2 Dynamics of body weight and grip strength. The values of body weight (A) and grip strength (B) were continuously recorded across the experimental period. The grip strength to body weight ratios were calculated and presented in (C). The results are expressed as the means \pm SEs of the 6 rats per group. (*) denotes significantly greater than the Whey and control groups, $p < 0.05$.

3 Results

3.1 Changes in body weight and grip strength in different groups

Body weight-matched rats were selected at the start (control: 214.8 ± 6.1 g, Whey: 212 ± 6.2 g, BP: 215.0 ± 4.6 g) of this study. Three groups of rats consumed 30 g of standard chow feed daily. The rats in the Whey and BP groups were administered 218.4 and 200 mg intervention protein, respectively, each day for 7 weeks. Body weight increased progressively, and no significant differences were observed among the three groups at each monitoring time point (Fig.2A). The results of the grasping test are shown in Figure 2B. At weeks 5, 6 and 7, the mean maximum grip strength of the BP group was significantly increased ($p < 0.05$) compared with the other groups. In addition, the ratio of grip strength to body weight was used to standardize grip strength (Fig.2C). No significant differences were observed in the ratios among the groups between the 1st and 2nd weeks. However, the ratio of the BP group showed an increasing trend beginning in the 3rd week. This trend produced a significant difference in grip strength compared with the other groups ($p < 0.05$) beginning in the 7th week.

3.2 Loaded-swimming test performance

At the beginning of this study, we used mean time to exhaustion to evaluate the basic exercise performance of the rats from the three groups. As Figure 3 illustrates, no significant differences were observed in time to exhaustion among the groups at week 1. After seven weeks of swimming tests, the exercise capacity of the rats from the control group had dramatically increased compared with the first week. Interestingly, the seven-week blended protein intervention significantly improved the mean time to exhaustion due to swimming by 1.5 fold and 1.2 fold compared with the control and whey interventions, respectively (Fig.3). Within-group

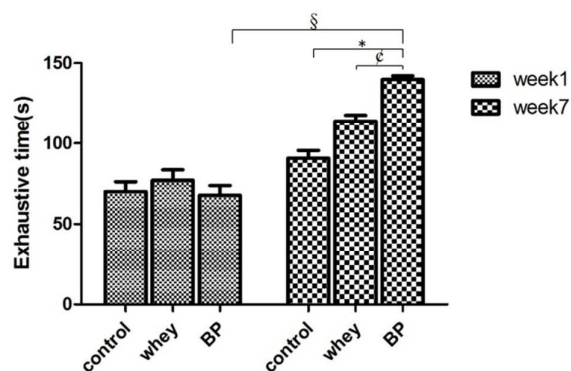


Fig. 3 Time to exhaustion based on the loaded-swimming test. Mean time to exhaustion due to the loaded-swimming test was recorded at weeks 1 and 7 during the experimental period. Data are shown as the means \pm SEs of 6 rats per group. (*) denotes significantly greater than the control group at week 7, $p < 0.05$. (§) denotes significantly greater at week 7 than week 1 in the BP group, $p < 0.05$. (c) denotes significantly greater than the Whey group at week 7, $p < 0.05$.

comparisons revealed the mean time to exhaustion increased 1.2 fold, 1.4 fold and 2.0 fold after the 7-week exercise and protein interventions for the control, Whey and BP groups, respectively.

3.3 Plasma EAA levels

The plasma levels of leucine, isoleucine and valine were significantly higher at 60 min after the blended protein intervention compared with the Whey and control groups

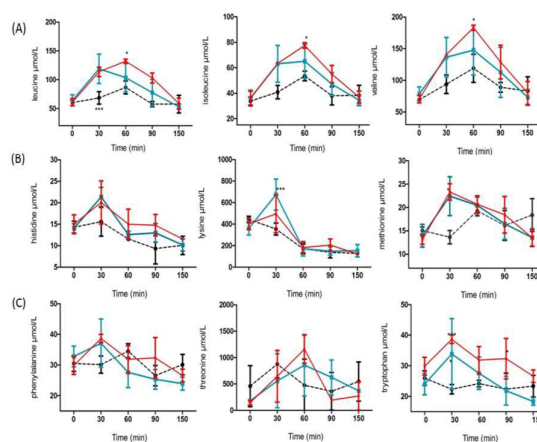


Fig. 4 EAA dynamics in plasma. Dynamic changes in EAAs were measured at five time points after the loaded-swimming test. (A) BCAAs including leucine, isoleucine and valine. (B) Non-BCAAs including histidine, lysine and methionine. (C) Non-BCAAs including phenylalanine, threonine and tryptophan. Data are shown as the means \pm SEs of 6 rats per group. (*) denotes significantly greater than the control group, $p < 0.05$.

($p < 0.05$, Fig.4A). This increased trend in the BP group was maintained through 90 min after protein intervention compared with the other groups. Moreover, the concentration of tryptophan was significantly increased in the BP group compared with the Whey and control groups between 30 and 150 min ($p < 0.05$, Fig.4C). However, no significant difference was observed between the Whey and BP groups with regard to other essential non-BCAAs at any monitoring point (Fig.4 B and C).

3.4 Levels of tissue-damage-related indicators

Acute exercise can induce tissue or cellular membrane damage in both human and non-human animals^{26, 27} and can lead to the release of cellular contents into the plasma. These phenomena can be identified by some indicators such as creatine kinase (CK), lactate dehydrogenase (LDH), alanine transaminase (ALT), and aspartate transaminase (AST). We collected serum samples at 0 (after exhaustive swimming), 30, 60, 90 and 150 min after the gavage administration of target protein supplements at the 7th week to monitor dynamic changes in the activities of LDH and CK (Fig.5). Significant increase in the activities of LDH and CK were observed within 30 minutes post-ingestion (Fig.5, $p < 0.05$). The highest activities of LDH and CK at 30 min (4985 ± 705 U/L, $p < 0.05$) were observed for the BP group and were increased by 36% and 22%, respectively, relative to those of the control group ($p < 0.05$). Although LDH activity in the BP group decreased after 30 min, it was consistently higher than the levels of the control and Whey groups at all time points (Fig.5A). In contrast, CK activity in the BP group was much lower than the activities in the control and Whey groups beyond 30 min (Fig.5B). The ALT and AST enzymes are produced in the liver and can be used as markers of hepatic damage²⁸. As shown in Table 4, although serum ALT and AST levels did not significantly differ among the three groups after exhaustive swimming (Table 4), there was a trend of higher ALT levels in the Whey group than in the control ($p = 0.1$) and BP groups ($p = 0.08$).

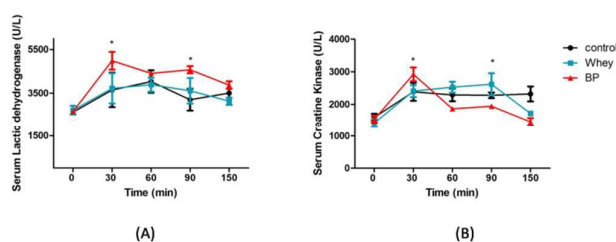


Fig. 5 Serum LDH and CK levels. Dynamic changes in LDH (A) and CK (B) levels were measured at five time points after the loaded-swimming test. Data are shown as the means \pm SEs of 6 rats per group. * $p < 0.05$ the BP value V.S. the control value (A); * $p < 0.05$ the BP value V.S. the whey value (B);

Table 4 The ingredients of whey and soy protein isolate

Serum levels (U.L ⁻¹)	Control (N=6)	Whey (N=6)	BP (N=6)	<i>p</i> -value
ALT	61.5 \pm 9.4	80.6 \pm 14.4	59.3 \pm 14.7	<i>n.s</i>
AST	215.3 \pm 33.1	204.1 \pm 12.4	216.6 \pm 30.0	<i>n.s</i>

Data are shown as the means \pm SEs of 6 rats per group.

n.s: $p > 0.05$.

ALT, alanine aminotransferase;

AST, aspartate transaminase

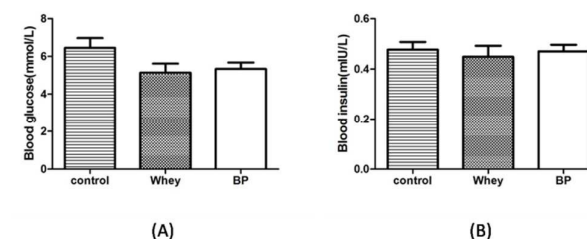


Fig. 6 Blood glucose and insulin levels. Blood glucose (A) and insulin (B) levels were measured at 0 min after exhaustive swimming. Data are shown as the means \pm SEs of 6 rats per group.

3.5. Levels of energy-related indicators

Fatigue during prolonged exercise often coincides with low glycogen content²⁹, and endurance performance can be improved by increasing blood glucose levels. Thus, we investigated whether differences in energy-related indicators (blood glucose and insulin) were apparent among the three groups at the resting phase before the loaded-swimming test at week 7. As shown in Figure 6A, the blood glucose levels of the blended protein group were not significantly higher than that of the other two groups ($p > 0.05$). Similarly, serum insulin concentrations did not differ among the groups (Fig.6B). Therefore, the observed differences in sport performance among the three groups cannot be explained by differences in energy.

3.6 Antioxidant capacity evaluation

Serum samples were collected to measure antioxidant capacity of the three groups at -0 min (before exhaustive swimming) and 0 min (after exhaustive swimming) at the 7th week. Given that superoxide dismutase (SOD) and malondialdehyde (MDA) are widely used in evaluating the role of in vivo antioxidants in disease-associated conditions, we used SOD and MDA as in vivo biomarkers of antioxidant capacity. For the baseline detection, we did not observe an obvious difference in the serum

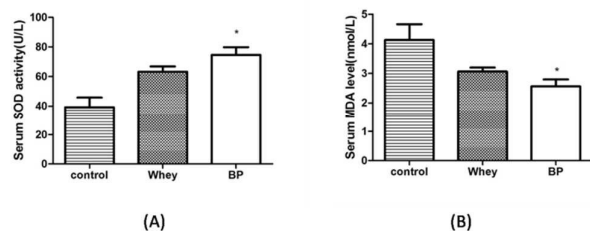


Fig. 7 Serum SOD activity and MDA level. Serum SOD activity (A) and MDA (B) levels were measured at 0 min after exhaustive swimming. Data are shown as the means \pm SEs of 6 rats per group. * $p < 0.05$ the BP value V.S. the control value (A and B);

antioxidant capacity between groups before exhaustive swimming (data not shown). Interestingly, as Figure 7A illustrates, serum SOD activity was higher in the Whey group than in the control group. The average serum SOD activity of rats in the BP group was 78 U/L, representing a 95% increase relative to the control group ($p < 0.05$). The serum MDA levels were significantly lower in rats of the BP group than those of the control group ($p < 0.05$, Fig. 7B).

4 Discussion

Because muscle strength and power are important determinants of sporting ability¹, improving muscle mass can be a successful strategy to enhance performance. Previous studies have focused on whey protein and its effects on lean mass gain due to the proposed superiority of this protein over other isolated protein sources³⁰. Whey protein has been referred to as a “fast” protein due to its rapid degradation rate³¹, which may affect its ability to increase lean body mass since the rate of protein digestion is an independent factor regulating protein retention³². In addition, whey protein is deficient in antioxidants such that oxidative stress during exercise cannot be effectively eliminated via oral supplementation with whey protein, leading to muscle damage and fatigue^{7, 22}. Soy protein has been described as an “intermediate” protein since the modest increases in plasma amino acid concentrations occur more gradually and are prolonged relative to those of whey protein³³. Furthermore, soy protein contains high-quality (in composition and concentration) EAAs^{17, 33} and natural bioactive substances that enhance plasma antioxidant activity³³. Therefore, we investigated whether blending whey and soy protein might be more beneficial than single protein sources for sport performance due to a potentially wider range of benefits (e.g., a balanced BCAA profile, antioxidant capacity, and contributions to muscle strength). To test the hypothesis that blended protein is superior, a rat loaded-swimming model was applied. As we assumed, the major result of this study showed that the ingestion of whey and soy proteins after resistance exercise for 7 weeks resulted in a prolonged mean time to exhaustion based on the loaded-swimming test in rats.

The loaded-swimming model is commonly used in the field of sports nutrition research³⁴. Compared with other models, such as the treadmill training, the interference of the rat is relatively small and easy to maintain exercise intensity at a relatively high level. In addition, as the rats are naturally good at swimming, they will not produce strong resistance during the training. With regard to the long-term swimming training, we employed multiple ways to maximally ensure the accuracy of the data in this study. First, the experimental animals were similar. All of the rats were selected based on the same criteria for gender and body weight at baseline. Sex differences in muscle fatigue have been reported frequently, with females generally exhibiting a greater relative fatigue resistance than males³⁵⁻³⁷. For this reason, male rats were chosen to evaluate the effects of soy-whey blended protein on sport performance. Second, the animals’ basic diets were controlled. According to our preliminary experiment, 30 g of standard chow diet provide enough energy to meet the per-day growth needs of a rat (Table 1 and Table 2). Third, the loads were similar. To avoid long-term rat floating and increase the intensity of exercise during the loaded-swimming test, lead rings were attached to their tails³⁸. Our preliminary data showed that rats with the tail loads weighing 10% of their body weight were able to achieve the best effect during this test. Fourth, the exhaustion criterion was well defined. Exhaustion was characterized as the moment when the rats were no longer able to maintain themselves on the water surface and remained submerged for 10 s³⁹. To maximize the effects of training, all rats were forced to perform a loaded-swimming exercise until exhaustion for five days every week. In addition, previous studies have shown that muscle protein accretion can occur during the recovery phase after exercise rather than during the exercise period itself^{20, 40}. Thus, in this study, we conducted protein interventions following resistance exercise.

No significant differences in weight were observed among the control and protein intervention groups during the 7-week training period. This result may be due to two possible factors. One potential factor is our dietary restriction of the rats each day, which may have limited potential weight increases. The other factor is the swimming training, which may have stabilized the body weight of the rats^{41, 42}. Interestingly, the rats administered blended protein showed more grip strength than the control and Whey groups later in training at weeks 5-6. In particular, the force/weight value was significantly higher for the BP group than the other groups (Fig.2). We did not observe marked differences in swimming ability among the three groups at baseline, at the beginning of the experiment. The increases in grip strength improved the rats’ capacity for loaded swimming. After 7 weeks of exercise and protein intervention, the swimming capacity of the rats improved by different degrees. The degree of improvement was significantly higher in the BP group than the Whey group (Fig. 3). We also performed a correlation analysis between the exhaustive time (s) and weeks of gavage. However, we could not find a significant correlation during these seven weeks. The reason may be that the improvement of sport ability is a

process of accumulation. It is not reflected in the initial stage of nutrition intervention.

The intake of BCAAs (leucine, isoleucine, and valine) can enhance muscle anabolic signaling⁴³. Previous studies have confirmed that BCAAs (especially leucine) can stimulate an increase in MPS through the activation of the mTOR-P70s6K pathway in animals^{44, 45}. However, BCAAs are commonly oxidized by muscle during exercise to provide energy once short-term glycogen stores are expended^{46, 47}. In this study, we found that the Whey and BP groups shared a similar non-BCAA spectrum after protein ingestion. However, the rats administered the blended protein supplement demonstrated a prolonged aminoacidemia with regard to leucine, isoleucine and valine (Fig.4). Therefore, a potential advantage of soy-whey blended protein is the provision of sufficient BCAAs to stimulate skeletal muscle protein synthesis without causing high levels of amino acid oxidation.

It is been demonstrated that resistance exercise causes micro muscle damage along with associated changes in the levels of serum muscular enzymes (e.g., LDH, CK)^{48, 49}. LDH is an enzyme in the glycolytic pathway, which is released from the cell into peripheral blood when the cell membrane is ruptured. It is dependent on NAD⁺ for the interconversion of pyruvate and lactate⁵⁰. In the present study, the soy-whey blended protein intervention yielded increased LDH activity at all time points (Fig.5A); such activity can eliminate lactic acid and prevent fatigue³⁴. Strenuous exercise for long periods of time may cause more muscle damage of the rats. It may explain why the LDH activity was elevated in the rats from the BP group. In addition, the CK activity in serum is routinely examined after exercise in sports medicine. CK is used as a biomarker of muscle damage⁴⁸. The serum CK activity of all of the rats reached maximum levels within 30 min post-ingestion, with the highest values observed in the BP group (Fig.5). Interestingly, resistance exercise caused a rapid elevation in CK activity in the rats fed soy-whey blended protein from 1546±185 to 2924±363 U/L; subsequently, CK activity rapidly decreased to basal levels within 150 min. In sports medicine, CK concentrations could potentially be used to monitor the return to activity of athletes with muscular injury⁵¹. Our results indicate that the long-term ingestion of soy-whey blended protein may help exhausted rats to rapidly recover athletic ability. To exclude the effect of liver damage caused by the loaded-swimming test on sport performance, we evaluated two major biomarkers (ALT and AST) of liver injury. No difference was observed among three groups (Table 4), indicating that the loaded-swimming test did not cause liver damage.

Coyle et al. showed that ingestion of a glucose polymer (1.8 g min⁻¹) could increase exercise time from 192 to 252 min during cycling⁵². In light of this finding, we investigated whether the improvement in the mean time to exhaustion of rats in the blended protein group was due to differences from the other treatment groups in serum glucose and insulin. We measured the concentrations of serum glucose and insulin in all of the rats. As shown in Figure 6A, rats treated with whey and blended protein had lower blood glucose levels than those

of the control group, although this difference was not significant ($p>0.05$). Similarly, serum insulin concentrations did not differ among the groups (Fig.6B). Therefore, we conclude that the observed differences in sport performance among the three groups cannot be explained by group differences in energy.

Growing evidence indicates that exercise-induced protein oxidation can generate reactive oxygen species (ROS), resulting in muscle fatigue⁵³. Accordingly, cells have evolved a number of enzymatic and non-enzymatic antioxidant defenses that function to alleviate oxidative-stress-mediated body fatigue⁵⁴. SOD is the first line of antioxidant defense against oxidative stress. SOD acts by converting superoxide to hydrogen peroxide, which can subsequently be converted to water by catalase or peroxiredoxin. Since antioxidant capacity is weakened in the process of fatigue, the enhancement of antioxidant enzyme activities can help prevent fatigue⁵⁵. As mentioned above, serum SOD activity was significantly higher in the BP group than in the control and Whey groups. The results clearly show the positive effect of soy-whey blended protein on anti-fatigue ability. MDA is one of the final-stage byproducts of lipid peroxidation, and it is an indicator of oxidative stress in cells and tissues⁵⁶. A lower MDA level indicates weaker oxidant stress and less lipid peroxidation⁵⁷. Our study revealed that soy-whey blended protein significantly reduced serum MAD levels compared to the levels observed in the control and Whey groups.

5 Conclusions

The findings obtained from the current animal model strongly support the idea that the ingestion of a soy-whey blended protein augments sport performance in rats after 7 weeks of training. Moreover, our results suggest that ingesting a soy-whey blended protein prolongs aminoacidemia, especially that of BCAAs during post-exercise recovery; enhances the activities of lactidrogenase and superoxide dismutase; and decreases the levels of malondialdehyde in serum. Thus, our data show that supplementation with a soy-whey blended protein ameliorates exercise-induced fatigue in a rat exercise model.

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Author contributions

GR designed the study and performed the animal studies. SY and HZ participated in the sample measurement. GR drafted the manuscript. JW participated in study coordination.

The conflicts of Interest

The authors declare that they have no competing interests.

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