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Whey Protein Improves Exercise Performance and Biochemical Profiles in Trained Mice

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ABSTRACT

CHEN, W.-C., W.-C. HUANG, C.-C. CHIU, Y.-K. CHANG, and C.-C. HUANG. Whey Protein Improves Exercise Performance and Biochemical Profiles in Trained Mice. *Med. Sci. Sports Exerc.*, Vol. 46, No. 8, pp. 1517–1524, 2014. **Purpose**: The objective of this study is to verify the beneficial effects of whey protein (WP) supplementation on health promotion and enhance exercise performance in an aerobic-exercise training protocol. **Methods**: In total, 40 male Institute of Cancer Research mice (4 wk old) were divided into four groups (n = 10 per group): sedentary control with vehicle (SC) or WP supplementation (4.1 $g \cdot kg^{-1}$, SC + WP), and exercise training with vehicle (ET) or WP supplementation (4.1 $g \cdot kg^{-1}$, ET + WP). Animals in the ET and ET + WP groups underwent swimming endurance training for 6 wk, 5 $d \cdot wk^{-1}$. Exercise performance was evaluated by forelimb grip strength and exhaustive swimming time as well as by changes in body composition and biochemical parameters at the end of the experiment. **Results**: ET significantly decreased final body and muscle weight and levels of albumin, total protein, blood urea nitrogen, creatinine, total cholesterol, and triacylglycerol. ET significantly increased grip strength; relative weight (%) of liver, heart, and brown adipose tissue (BAT); and levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatine kinase, and total bilirubin. WP supplementation significantly decreased final body, muscle, liver, BAT, and kidney weight and relative weight (%) of muscle, liver, and BAT as well as levels of aspartate aminotransferase, lactate dehydrogenase, creatine kinase, and uric acid. In addition, WP supplementation slightly increased endurance time and significantly increased grip strength and levels of albumin and total protein. **Conclusion**: WP supplementation improved exercise performance, body composition, and biochemical assessments in mice and may be an effective ergogenic aid in aerobic exercise training.
Key Words: WHEY PROTEIN, EXERCISE TRAINING, EXERCISE PERFORMANCE, ERGOGENIC AID

BASIC SCIENCES

Milk protein is mostly composed of whey protein (WP) and casein, about 20% and 80%, respectively (23). During cheese manufacturing, WP is generated as a by-product of casein precipitation. WP is the most popular protein supplement sold in powder format. It contains valuable food ingredients because of its nutritional value and functional bioactivity. WP contains β-lactoglobulin, α-lactalbumin, immunoglobulins, bovine serum albumin, lactoferrin, lactoperoxidase, phospholipoprotein, bioactive factors, and enzymes in order of abundance (26). The biological components of WP and its isolates have been reported to benefit antioxidation (21) and regulation of lipid metabolism (27) and have antifatigue (25) and antidiabetic properties (20).

WP isolates contain enriched essential amino acids, including branched chain amino acids, which the body needs for tissue synthesis, energy, and health. The high leucine content (50%–75% more than other protein sources), one of the branched chain amino acids, in WP could explain its ability to stimulate muscle protein synthesis (14) and upregulate mammalian target of rapamycin signaling in high concentration (3). With WP supplementation, resistance exercises can result in muscle adaption and hypertrophy, regardless of the contraction mode (13). WP is marketed as a dietary supplement and as an aid for muscle development with resistance training. Because of its rapid rate of digestion, WP provides a rapid source of amino acids that can be taken up by the muscles to repair and rebuild muscular tissue. The use of WP to enhance aerobic exercises and swimming training has only been reported in terms of glycogen storage (28), antioxidation (12), and lipid metabolism. Few reports have shown the beneficial synergistic effects of WP and long-term aerobic exercise training (ET) on biochemical profiles in specific tissues.

Moderate exercise or individual training is beneficial to heart and lung functions, improving exercise performance,

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Submitted for publication October 2013.
Accepted for publication December 2013.

0195-9131/14/4608-1517/0
MEDICINE & SCIENCE IN SPORTS & EXERCISE®

DOI: 10.1249/MSS.0000000000000272

and as preventive medicine helpful in reducing the incidence of chronic disease (34). High-intensity workouts, ET, and athletic competition affect the body's hemostasis, with resulting pathological syndromes. Physiological functions such as oxidative systems and important tissues are affected by long-term, high-intensity exercise that exceeds the body's endurance (18,19). Several clinical biochemistry parameters considered as biomarkers in evaluating physiological functions or status after exercise or training include aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK) (5). In a previous report, WP supplementation was found to reduce weight increase and alleviate glucose intolerance, improve insulin sensitivity, and reduce plasma cholesterol in an animal model of high-fat-diet–induced obesity (36). A combination of resistant exercise and WP benefitted the lipid profile, especially plasma triglycerides and cholesterol (2).

In this study, we aimed to investigate the beneficial synergistic effects of WP supplementation and swimming ET on exercise performance, biochemical profiles, and pathological responses after long-term supplementation. WP supplementation may be helpful to athletes focusing on resistance training for maximal strength performance related to muscle hypertrophy or performing aerobic exercise such as marathons, long-distance cycling, and swimming as well as for overall physiologic protective effects.

METHODS

Animals and treatment design. Specific pathogen-free male Institute of Cancer Research mice (4 wk old) were purchased from BioLASCO (Yi-Lan, Taiwan). All animals were given distilled water *ad libitum* and a standard laboratory chow diet (No. 5001; PMI Nutrition International, Brentwood, MO) and appropriately housed in the animal facility at National Taiwan Sport University at a 12-h light–dark cycle and 25°C ± 1°C and 50%–60% humidity. Before the experiments, the mice were acclimatized for 1 wk to the environment and diet. The Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University approved all animal experimental protocols, and the study conformed to the guidelines of protocol IACUC-10111 approved by the IACUC ethics committee; all procedures adhered to the American College of Sports Medicine animal care standards.

All animals were randomly divided into four groups (10 mice per group) for WP supplementation and/or ET, as follows: 1) sedentary control with vehicle (SC) or 2) WP supplementation (SC + WP) and 3) ET with vehicle or 4) WP supplementation (ET + WP). Food intake and water consumption were recorded daily, and all animals were weighed weekly.

WP supplementation. Mice in the SC + WP and ET + WP groups were given WP by oral feeding within 30 min after the ET. The WP (EAS 100% WP, vanilla) was purchased from a local wholesaler (Costco, Taoyuan, Taiwan) and prepared and dissolved in distilled water. The recommended use of WP for humans is about 20 g per one intake with a normal diet and exercise program. The mouse WP dose (4.1 $g \cdot kg^{-1}$) used in this study was converted from a human equivalent dose on the basis of body surface area by the following formula from the US Food and Drug Administration (available from http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf): assuming a human weight of 60 kg, the human equivalent dose for 20 g × 60 kg^{-1} (0.333 $g \cdot kg^{-1}$) = 0.333 × 12.3 = a mouse dose of 4.1 $g \cdot kg^{-1}$; the conversion coefficient 12.3 was used to account for differences in body surface area between a mouse and a human.

ET protocol. Animals in the ET and ET + WP groups underwent swimming endurance training, following the training protocol shown in Figure 1A. Animals underwent an aerobic swimming training program adapted from other studies (12,31). They were placed in a plastic container (65 cm high, 20 cm in diameter) with tap water 40 cm deep maintained at 34°C ± 1°C. They trained for 30 min on the first day, 45 min on the second day, and then 60 $min \cdot d^{-1}$, 5 $d \cdot wk^{-1}$. The swimming training was maintained for 1 h from weeks 2 to 6. After the first week, the swimming training consisted of five weekly sessions of 60 min of forced swimming with a 1%

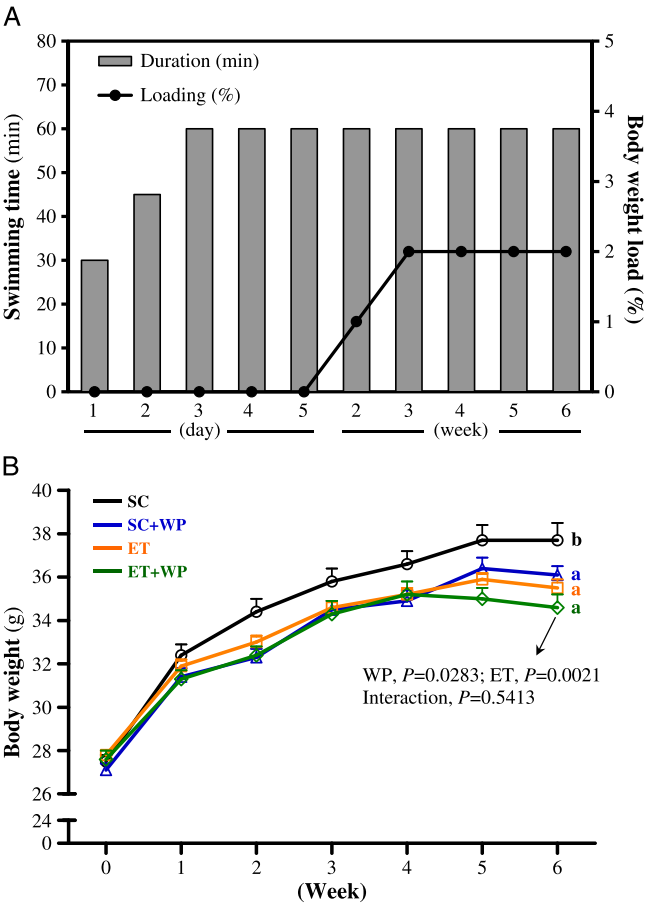

FIGURE 1—A, Protocol for 6-wk swimming ET. B, Effect of WP supplementation and 6-wk ET on body weight. Data are mean ± SEM. Different letters (a, b, and c) indicate significant difference at $P < 0.05$. Main-effect P values and interaction between WP and ET by two-way ANOVA are indicated.

loading of body weight at week 2. From weeks 3 to 6, animals underwent a 2% loading of body weight training protocol, which consisted of five weekly swimming sessions for 60 min each. Body weight was measured weekly, and the load was estimated and increased accordingly. A load of <3% of the body weight was defined as aerobic exercise, a frequency of three weekly sections was considered a moderate training protocol, and a five times a week exercise protocol was considered heavy training (31).

Exhaustive swimming exercise. The exercise performance was evaluated by an exhaustive swimming test as we have previously described (39). After the 6-wk WP supplementation and ET regimen, a mouse was selected from each group and a lead sheet (5% of mouse body weight) was attached to the tail. Each mouse was evaluated in a columnar swimming pool (65 cm high, 20 cm diameter) with water 40 cm deep maintained at 27°C ± 1°C. The endurance for each mouse was measured as the swimming time, recorded from the beginning to exhaustion, which was determined by the observation of uncoordinated movements and failure to swim to the surface within 7 s.

Forelimb grip strength. A low-force testing system (Model-RX-5; Aikoh Engineering, Nagoya, Japan) was used to measure forelimb grip strength of mice undergoing the indicated treatments. The amount of tensile force was measured by a force transducer equipped with a metal bar (2 mm in diameter, 7.5 cm long) for each mouse. The detailed procedure was described in our previous report (39). The test of forelimb grip strength was performed after administration of the indicated ET protocol and WP supplementation for 6 wk. The maximal force (grams) recorded was used as an indicator of absolute grip strength.

Tissue sample preparation. After the ET was finished, the mice were killed. The target organs and tissues collected included the heart, liver, lungs, kidneys, muscle tissue, epididymal fat pad (EFP), and brown adipose tissue (BAT). Organs and tissues were carefully excised and rinsed in saline solution, and then blotted dry with a KimWipe. The whole weight and the specific tissue weight (%) relative to individual body weight were recorded and calculated.

Histological staining of tissues. Collected liver, muscles, lungs, kidneys, and heart were fixed in 10% formalin for 24 h, and then cut transversely or longitudinally to obtain ventricular sections or four-chamber cross-sections, respectively. Tissues were embedded in paraffin and cut into 4-μm-thick slices for morphological and pathological evaluation, and then stained with hematoxylin and eosin (H&E) and examined by use of a light microscope equipped with a CCD camera (BX-51; Olympus, Tokyo, Japan).

Blood biochemical assessments. After the 6-wk experiments, blood samples were immediately collected from the submandibular duct of each mouse from the treated groups and centrifuged at 1500*g* and 4°C for 10 min for serum preparation. Clinical biochemical assessment of levels of AST, ALT, alkaline phosphatase (ALP), LDH, CK, total bilirubin (TBIL), total protein (TP), blood urea nitrogen (BUN), uric acid, total cholesterol (TC), and triacylglycerol (TG) involved use of an autoanalyzer (Hitachi 7060; Hitachi, Tokyo, Japan).

Statistical analysis. Data are expressed as mean (SE). Two-way ANOVA was used to assess the effects of the ET and WP supplementation on general mouse characteristics, including body weight, organ weight, biochemical values, swimming exhaustion times, and grip strength. Tukey (HSD) test was used to compare individual means among treatment groups. $P < 0.05$ was considered statistically significant. Statistical analyses involved use of SAS v9.0 (SAS, Cary, NC).

RESULTS

Effect of WP supplementation and ET on body and organ weight. The initial body weight for SC, SC + WP, ET, and ET + WP groups was 27.5 ± 0.3, 27.1 ± 0.3, 27.8 ± 0.2, and 27.6 ± 0.4, respectively, with no differences between groups (Fig. 1B). After 6-wk ET and WP supplementation, the body weight was lower, by 4.2% ($P = 0.0475$), 5.8% ($P = 0.0083$), and 8.2% ($P = 0.0003$), with SC + WP, ET, and ET + WP, respectively, than SC alone. The final body weight was lower with WP supplementation than ET alone ($P = 0.0283$). As expected, the final body weight was lower for trained than nontrained mice ($P = 0.0021$).

Food intake and water consumption did not differ among the treatment groups (Table 1). Organ weights for intervention and control animals can provide information about the health status of test mice. Liver weight was lower with SC + WP and ET + WP than SC and ET alone, by 8.8% ($P = 0.0063$) and 7.5% ($P = 0.0160$), respectively (Table 1). The relative liver weight (%) was lower with SC + WP and ET + WP than SC and ET alone, by 4.8% ($P = 0.0007$) and 3.0% ($P = 0.0150$), respectively.

Muscle mass was lower, but not significantly, with SC + WP and ET than SC alone, by 5.4% ($P = 0.0639$) and 5.7% ($P = 0.0523$), respectively. Muscle mass was lower with ET + WP than SC alone, by 12.7% ($P < 0.0001$), with a significant interaction between WP supplementation and ET ($P = 0.0038$). In addition, relative muscle weight (%) was lower with ET + WP than SC and ET alone, by 2.9% ($P = 0.0253$) and 3.0% ($P = 0.0208$), respectively. Heart weight did not change among the groups. However, relative heart weight (%) was greater with SC + WP and ET + WP than SC alone, by 1.18- ($P = 0.0004$) and 1.14-fold ($P = 0.0040$), respectively. Kidney weight was lower with ET + WP than SC and SC + WP, by 12.3% ($P = 0.0362$) and 12.8% ($P = 0.0281$), respectively, with no differences in relative kidney weight (%) among groups. BAT weight was lower with SC + WP and ET + WP than SC and ET alone, by 19.3% ($P = 0.0487$) and 25.9% ($P < 0.0001$), respectively, with a slight interaction between WP supplementation and ET ($P = 0.0983$). Also, relative BAT weight (%) was lower with SC + WP and ET + WP than SC and ET alone, by 15.7% ($P = 0.0438$) and 22.6%

TABLE 1. General characteristics of the experimental groups.

Characteristic	SC	SC + WP	ET	ET + WP	*P* Values		
					Main effect of WP	Main effect of ET	Interaction (WP × ET)
Food intake ($g \cdot d^{-1}$)	6.4 ± 0.2^{a}	6.3 ± 0.3^{a}	6.6 ± 0.2^{a}	6.4 ± 0.2^{a}	0.5809	0.5045	0.6626
Water intake ($mL \cdot d^{-1}$)	9.0 ± 0.6^{a}	9.3 ± 0.4^{a}	9.3 ± 0.6^{a}	9.3 ± 0.7^{a}	0.7964	0.8634	0.7472
Weight (g)							
Liver	1.57 ± 0.04^{bc}	1.43 ± 0.03^{a}	1.61 ± 0.03^{c}	1.49 ± 0.03^{ab}	**0.0005**	0.2120	0.7918
Muscle	0.37 ± 0.01^{b}	0.35 ± 0.01^{b}	0.35 ± 0.01^{b}	0.32 ± 0.01^{a}	**0.0037**	**0.0025**	**0.0038**
Heart	0.23 ± 0.01^{a}	0.24 ± 0.01^{a}	0.26 ± 0.01^{a}	0.24 ± 0.01^{a}	0.4562	0.1957	0.2660
Kidney	0.64 ± 0.03^{b}	0.64 ± 0.03^{b}	0.63 ± 0.02^{ab}	0.56 ± 0.02^{a}	**0.0279**	0.0810	0.1584
EFP	0.61 ± 0.03^{b}	0.47 ± 0.06^{a}	0.46 ± 0.03^{a}	0.51 ± 0.03^{ab}	0.2313	0.1797	**0.0162**
BAT	0.09 ± 0.01^{b}	0.07 ± 0.01^{a}	0.14 ± 0.01^{d}	0.11 ± 0.01^{c}	<**0.0001**	<**0.0001**	0.0983
Relative weight (%)							
Liver	4.17 ± 0.03^{b}	3.97 ± 0.04^{a}	4.52 ± 0.05^{d}	4.38 ± 0.03^{c}	<**0.0001**	<**0.0001**	0.4157
Muscle	0.98 ± 0.01^{b}	0.97 ± 0.01^{ab}	0.98 ± 0.01^{b}	0.95 ± 0.01^{a}	**0.0239**	0.3519	0.2957
Heart	0.62 ± 0.02^{a}	0.65 ± 0.02^{ab}	0.73 ± 0.02^{c}	0.70 ± 0.02^{bc}	0.7160	**0.0004**	0.1209
Kidney	1.68 ± 0.05^{a}	1.77 ± 0.06^{a}	1.76 ± 0.05^{a}	1.64 ± 0.03^{a}	0.7636	0.6522	**0.0321**
EFP	1.62 ± 0.07^{b}	1.27 ± 0.14^{a}	1.29 ± 0.06^{a}	1.50 ± 0.08^{ab}	0.4755	0.5589	**0.0044**
BAT	0.23 ± 0.01^{b}	0.20 ± 0.01^{a}	0.40 ± 0.01^{d}	0.31 ± 0.02^{c}	<**0.0001**	<**0.0001**	0.0983

Data are mean ± SEM for $n = 10$ mice in each group. Data in the same line followed by different letters (a, b, and c) differ significantly at $P < 0.05$ by two-way ANOVA. Muscle mass includes both gastrocnemius and soleus muscles in the back part of the lower legs. Data in bold indicate significant *P* values that will make it easy for readers to understand the differences in each parameter.

($P < 0.0001$), respectively, with a slight interaction between WP supplementation and ET ($P = 0.0983$). Weight of EFP, an important indicator of white adipose tissue in the body, was lower with SC + WP and ET + WP than SC alone, by 24.3% ($P = 0.0120$) and 25.2% ($P = 0.0092$), respectively, with a significant interaction between WP supplementation and ET ($P = 0.0162$). Relative EFP weight (%) was lower with SC + WP and ET than SC alone, by 21.4% ($P = 0.0117$) and 20.6% ($P = 0.0146$), respectively, with a significant interaction between WP supplementation and ET ($P = 0.0044$).

Overall, the main effect of ET was decreased muscle weight ($P = 0.0025$) and increased relative liver weight (%) ($P < 0.0001$) and relative heart weight (%) ($P = 0.0004$) as well as BAT weight ($P < 0.0001$) and relative BAT weight (%) ($P < 0.0001$). The main effect of WP supplementation was decreased muscle weight ($P = 0.0037$) and relative muscle weight (%) ($P = 0.0239$), liver weight ($P = 0.0005$) and relative liver weight (%) ($P < 0.0001$), BAT weight ($P < 0.0001$) and relative BAT weight (%) ($P < 0.0001$), and kidney weight ($P = 0.0279$).

Effect of WP supplementation and ET on biochemical assessments. Biochemical results at the end of the experiment could provide clinical information about the health status of test animals. Serum levels of AST, ALT, ALP, LDH, CK, and TBIL were higher with ET than those with SC, by 2.40- ($P < 0.001$), 2.26- ($P = 0.0342$), 1.19- ($P = 0.0089$), 2.01- ($P < 0.0001$), 22.74- ($P < 0.0001$) and 2.13-fold ($P < 0.0001$), respectively (Table 2), whereas levels of albumin, TP, creatinine, TC, and TG were lower with ET than those with SC, by 10.2% ($P < 0.0001$), 8.7% ($P < 0.0001$), 11.4% ($P = 0.0039$), 17.1% ($P = 0.0018$), and 60.6% ($P < 0.0001$), respectively. Serum levels of AST, LDH, and CK were lower with ET + WP than ET alone, by 59.4% ($P < 0.0001$), 32.7% ($P = 0.0043$), and 84.2% ($P < 0.0001$), respectively, but levels of albumin and TP were higher with ET + WP than ET alone, by 1.10- and 1.08-fold ($P < 0.0001$), respectively. We found significant interactions between WP supplementation and ET for levels of AST ($P = 0.0035$), CK ($P = 0.0006$), albumin ($P = 0.0019$), TP ($P = 0.0021$), and creatinine ($P = 0.0269$).

Overall, the main effect of ET was decreased levels of albumin ($P < 0.0001$), TP ($P < 0.0001$), BUN ($P = 0.0015$), creatinine ($P = 0.0468$), TC ($P = 0.0045$), and TG ($P < 0.0001$) and increased levels of AST ($P < 0.0001$), ALT ($P = 0.0077$), ALP ($P < 0.0001$), LDH ($P < 0.0001$), CK ($P < 0.0001$), and TBIL ($P < 0.0001$). The main effect of WP supplementation was decreased levels of AST ($P < 0.0001$), LDH ($P = 0.0055$), CK ($P = 0.0003$), and uric acid ($P = 0.0462$) and increased levels of albumin ($P = 0.0035$) and TP ($P = 0.0002$).

TABLE 2. Effect of WP supplementation and 6-wk swimming ET on biochemical serum levels at the end of the experiment.

Parameter	SC	SC + WP	ET	ET + WP	*P* Values		
					Main effect of WP	Main effect of ET	Interaction (WP × ET)
AST ($U \cdot L^{-1}$)	97 ± 12^{a}	63 ± 5^{a}	234 ± 30^{b}	95 ± 6^{a}	<**0.0001**	<**0.0001**	**0.0035**
ALT ($U \cdot L^{-1}$)	38 ± 5^{a}	33 ± 4^{a}	86 ± 13^{b}	72 ± 27^{ab}	0.5489	**0.0077**	0.7750
ALP ($U \cdot L^{-1}$)	169 ± 7^{a}	159 ± 9^{a}	201 ± 8^{b}	202 ± 9^{b}	0.6298	<**0.0001**	0.5325
LDH ($U \cdot L^{-1}$)	435 ± 16^{ab}	329 ± 25^{a}	874 ± 97^{c}	588 ± 86^{b}	**0.0055**	<**0.0001**	0.1834
CK ($U \cdot L^{-1}$)	88 ± 9^{a}	29 ± 6^{a}	1995 ± 427^{b}	316 ± 74^{a}	**0.0003**	<**0.0001**	**0.0006**
Albumin ($g \cdot dL^{-1}$)	3.3 ± 0.0^{b}	3.3 ± 0.0^{b}	3.0 ± 0.1^{a}	3.3 ± 0.0^{b}	**0.0035**	<**0.0001**	**0.0019**
TBIL ($\mu g \cdot dL^{-1}$)	70 ± 3^{a}	88 ± 3^{b}	149 ± 7^{c}	145 ± 7^{c}	0.1722	<**0.0001**	0.0550
TP ($g \cdot dL^{-1}$)	5.7 ± 0.1^{b}	5.8 ± 0.1^{b}	5.2 ± 0.0^{a}	5.7 ± 0.0^{b}	**0.0002**	<**0.0001**	**0.0021**
BUN ($mg \cdot dL^{-1}$)	27.3 ± 1.0^{ab}	29.3 ± 1.4^{b}	24.2 ± 0.9^{a}	25.0 ± 0.9^{a}	0.2026	**0.0015**	0.5477
Creatinine ($mg \cdot dL^{-1}$)	0.31 ± 0.01^{b}	0.29 ± 0.01^{ab}	0.27 ± 0.01^{a}	0.29 ± 0.01^{ab}	0.8526	**0.0468**	**0.0269**
UA ($mg \cdot dL^{-1}$)	1.33 ± 0.12^{b}	1.17 ± 0.06^{ab}	1.26 ± 0.06^{ab}	1.07 ± 0.09^{a}	**0.0462**	0.3277	0.8605
TC ($mg \cdot dL^{-1}$)	161 ± 6^{b}	154 ± 6^{b}	134 ± 4^{a}	146 ± 7^{ab}	0.6466	**0.0045**	0.0880
TG ($mg \cdot dL^{-1}$)	82 ± 4^{b}	74 ± 10^{b}	32 ± 3^{a}	37 ± 3^{a}	0.7818	<**0.0001**	0.2858

Data are mean ± SEM for $n = 10$ mice in each group. Data in the same line with different letters (a, b, and c) differ significantly, $P < 0.05$ by two-way ANOVA.
UA, uric acid.

Effect of ET and WP supplementation on physical performance. The two physical performance tests included forelimb grip strength and exhaustive swimming exercise. ET (ET and ET + WP groups) increased absolute and relative grip strength ($P = 0.0005$ and $P < 0.0001$, respectively) as compared with no training (SC and SC + WP groups) (Fig. 2A). As expected, ET could significantly increase absolute and relative grip strength, by 1.19- ($P = 0.0023$) and 1.26-fold ($P < 0.0001$), as compared with SC alone. WP supplementation could significantly increase absolute and relative grip strength via the main effect of WP supplementation ($P < 0.0001$). In addition, absolute and relative grip strength were greater by 1.26- and 1.32-fold, respectively, in SC + WP than SC alone ($P < 0.0001$) and were greater by 1.10- ($P = 0.0419$) and 1.15-fold ($P < 0.0001$), respectively, in ET + WP than SC + WP.

In the exhaustive swimming test (Fig. 2B), ET or WP supplementation did not have any effect as compared with the main effect of ET or WP supplementation ($P = 0.1381$ and $P = 0.0735$, respectively). The SC, SC + WP, and ET groups did not differ in test results ($P > 0.05$), but the results were higher for the ET + WP than SC group ($P = 0.0229$).

Effect of ET and WP supplementation on histology. The four groups did not differ in gross observations of liver, kidneys, heart, lungs, muscles, and other organs.

The four groups did not significantly differ in visual observation of the muscle morphology (Fig. 3A) or histology of muscle tissues (Fig. 3B).

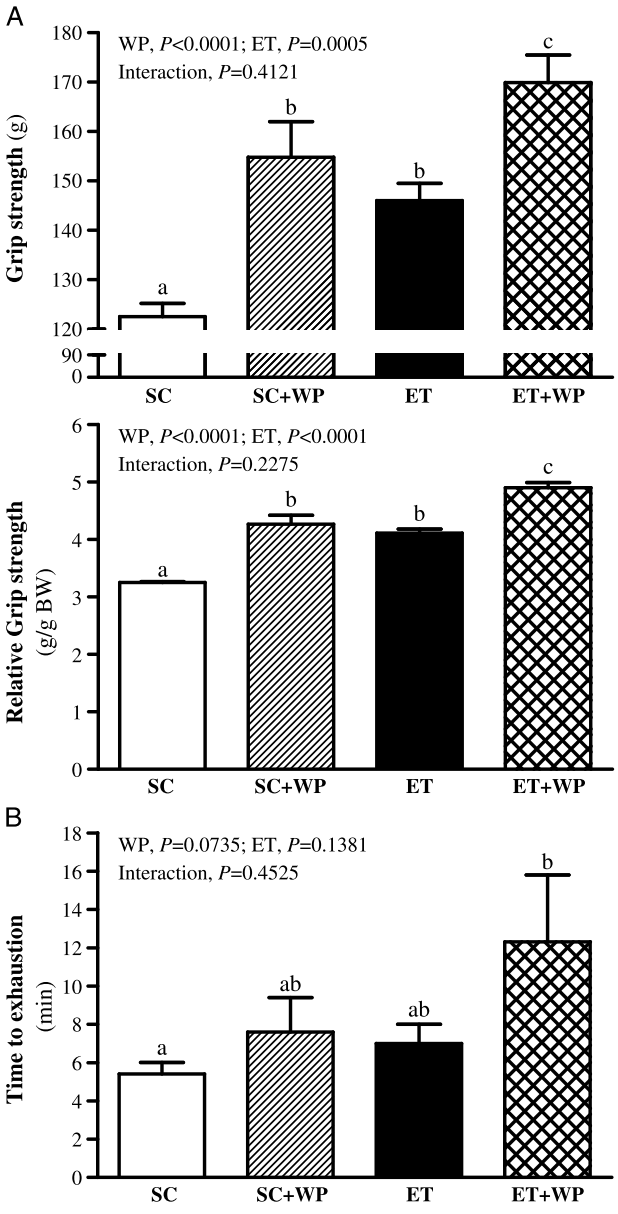

FIGURE 2—Effect of WP supplementation and 6-wk ET on physical performance, including forelimb grip strength (A) and exhaustive swimming time (B). Data are mean ± SEM. Different letters (a, b, and c) indicate significant difference at $P < 0.05$. Main-effect P values and an interaction between WP and ET by two-way ANOVA are indicated.

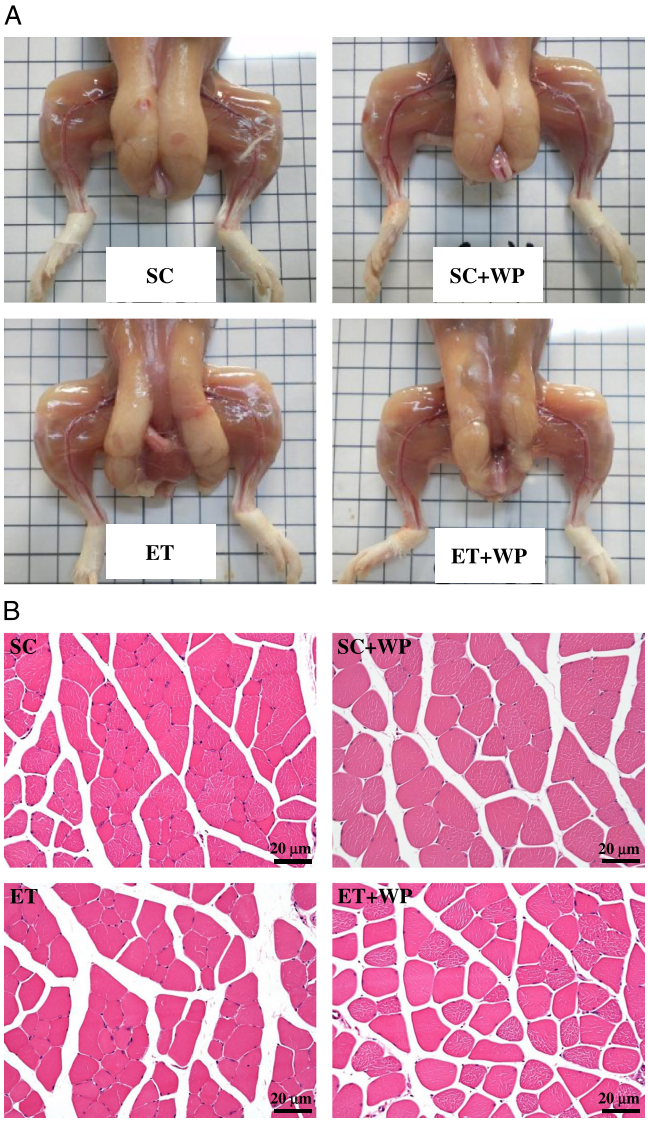

FIGURE 3—Effect of WP supplementation and 6-wk ET on skeletal muscles: ventral view of animals (A) and histology of muscle tissues (B). Specimens were photographed by light microscopy (H&E staining, magnification: ×200; scale bar, 20 μm).

In liver sections (Fig. 4A), normal liver cells were arranged in proper order, with no necrotic cells in lobes. There was no vacuolization accumulated with lipids or glycogens, fibrosis, chronic lobular hepatitis, or chronic hepatitis infiltrated by inflammatory cells with treatment. Therefore, livers with different treatments showed no lesions. Kidneys showed no glomerular atrophy, tubular atrophy or expansion, glomerular fibrosis or compensatory hypertrophy, or destruction of the junction of the renal medullary unit (Fig. 4B). They showed no infiltration of inflammatory cells, fibrosis or lesions, for no lesions with treatment. Heart (Fig. 4C) and lung (Fig. 4D) tissue showed no pathological effects with treatment.

DISCUSSION

We found that 6-wk ET and WP supplementation could significantly lower the body weight of mice as compared with sedentary mice (Fig. 1B). A high-protein diet could play an important role in regulating energy expenditure or central appetite (15). A previous study showed that WP administration strongly suppressed hunger and decreased food intake as compared with casein or soy and egg albumin (37). However, in our study, food intake did not differ between SC + WP and SC groups. In addition, mean energy intake was significantly lower with WP supplementation as compared with other protein sources such as tuna, eggs, and turkey in healthy subjects (32). Therefore, energy usage could be regulated by WP supplementation in this study.

Forelimb grip strength is a routine physical examination test. Our previous study had found that muscle strength was positively correlated with forelimb grip strength (39). In this study, we found greater grip strength with SC + WP, ET, and ET + WP than with SC alone. These data agreed with previous results finding that WP could improve muscle strength because of its amino acid composition (i.e., branched chain amino acids) (6). In addition, a recent study demonstrated that branched chain amino acids, especially leucine, play an important role in protein synthesis and enhanced glycogen storage in skeletal muscles (40).

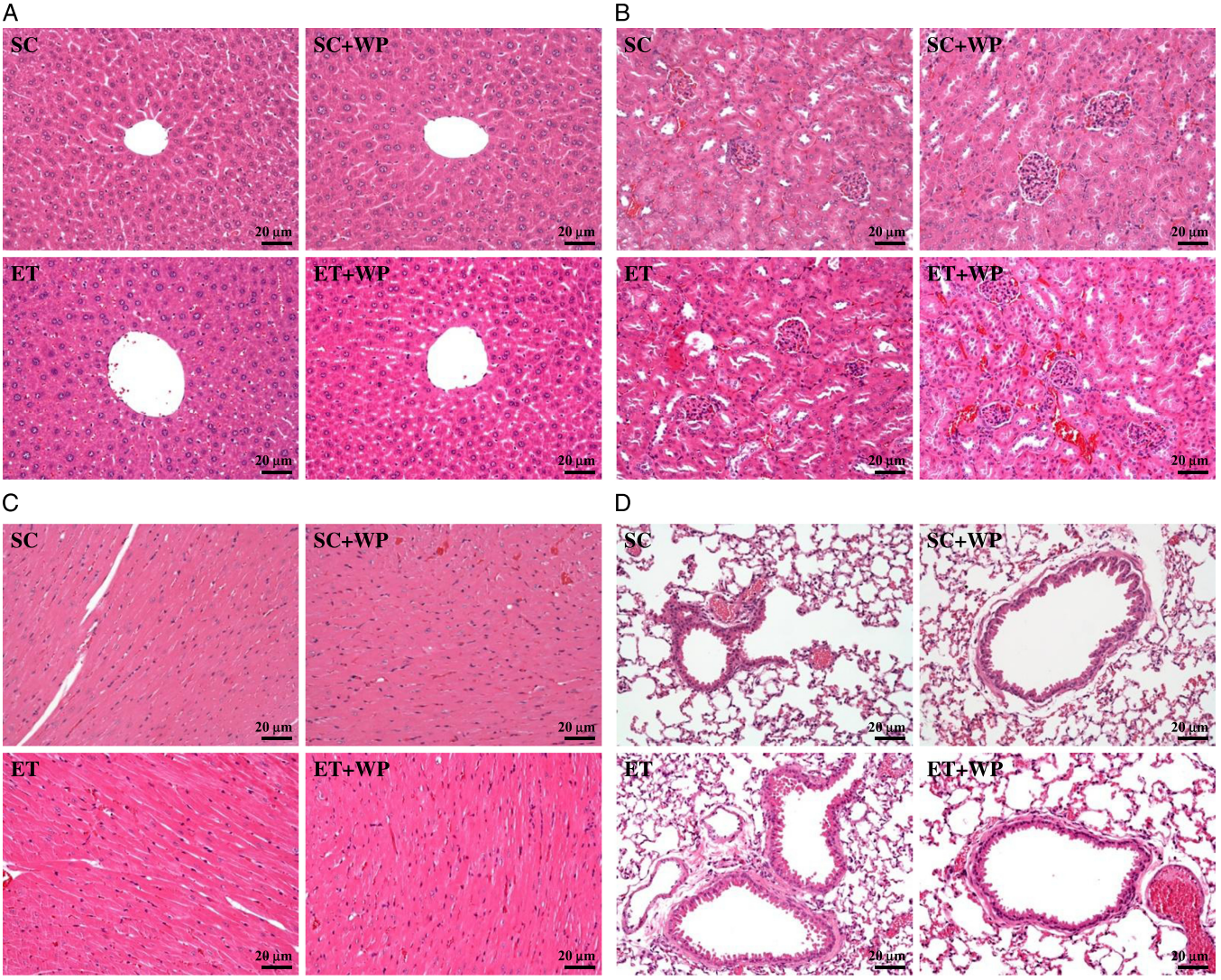

FIGURE 4—Effect of WP supplementation and 6-wk ET on morphology of liver (A), kidney (B), heart (C), and lung (D) tissues. Specimens were photographed by light microscopy (H&E staining, magnification: ×200; scale bar, 20 μm).

To examine the effectiveness of ET and/or WP supplementation on improving exercise endurance capacity, all animals underwent a swim-to-exhaustion exercise test. As compared with ET alone, WP supplementation could significantly prolong the swimming time to exhaustion, so WP could significantly improve the exercise endurance of test animals after 6-wk ET. Simultaneously, WP supplementation could increase the serum albumin levels and protect against long-term ET-induced acute phase response (Table 2). Previous reports have demonstrated that long-term ET can imbalance antioxidant status and result in acute tissue injury or muscle fatigue (10). In addition, oxidative stress can induce muscle damage and affect protein metabolism in the muscle (4). WP supplementation could thus inhibit the oxidation of muscle proteins induced by ET (16). WP supplementation after ET may reduce the resulting long-term ET physiologic fatigue, thereby contributing to improved exercise performance.

Concerning lean body mass, many studies reported that protein synthesis could be upregulated by the branched chain amino acids of WP, especially leucine (3). The combination of daily supplementation with WP and resistance ET was effective in promoting muscle hypertrophy (13). However, our 6-wk aerobic swimming ET did not increase muscle weight. The type of ET could be an important factor in stimulating muscle reconditioning when combined with WP supplementation.

Previous studies reported that moderate- or high-intensity aerobic exercise had the highest potential to reduce visceral adipose tissue in overweight subjects (38). The observed browning of the visceral fat, by a supposed white-to-brown transdifferentiation phenomenon, suggested that exercise could be a new physiological stimulus in counteracting obesity by providing an adrenergic-regulated recruitment of brown adipocytes (8). In the present study, we found that 6-wk swimming exercise could significantly decrease white adipose tissue, the EFP, and increase BAT, which was consistent with previous data. A WP isolate diet was demonstrated to regulate muscle lipid and fatty acid metabolism by decreasing the mRNA levels of Aldh1a7, Fasn, leptin, Nr4a3, and Scd1 (35). The high fat diet reduced the adipose tissue mRNA expression of GLUT4 and insulin receptor that can result in the fat accumulation. In contrast, WP supplementation was also found to reduce fat mass by preventing the reduction in the adipose tissue mRNA levels of insulin receptor and GLUT4 and reduce susceptibility to weight gain (27). However, the effect of WP supplementation on both adipose tissue types could be an interesting issue for further investigation of physiological functions and metabolic activation.

During intensive exercise or long-term training, biochemical variables could be significantly altered. Acute aerobic physical exercise, such as a exhaustive swimming exercise, might significantly elevate the activity of traditional biomarkers such as LDH, AST, CK, and bilirubin (39). These postexercise biomarkers of cardiac and skeletal muscle damage remain elevated at 24 h postworkout (29). Many kinds of nutrient supplements, within different experimental models, have been found effective for their protective effects on these biomarkers, but few reports have shown the long-term effects of WP supplementation, when combined with ET, on these physiological markers. In a previous study, WP supplementation could attenuate strength decline and decrease plasma LDH index after eccentrically induced muscle damage in healthy subjects (7). In our study, ET could significantly increase LDH, AST, and CK levels, which is consistent with previous results. We also provide evidence of the possible protective effects for a significant decrease in these biomarkers with long-term training combined with WP supplementation.

ET is an effective approach to increase lean body mass and reduce fat mass (22) as well as improve the lipid–lipoprotein profile (9), insulin sensitivity (24), and blood pressure (33). Our data showed that ET could significantly decrease TC and TG levels, between 16.7% and 60%, respectively, as compared with SC alone, which is consistent with the effects of ET; however, WP supplementation did not have significant main effects ($P = 0.646$ and $P = 0.7818$, respectively). Previous reports showed that long-term WP supplementation, after 14 wk, could significantly decrease animal plasma cholesterol as compared with the control (30) and as confirmed by the acute effects of WP on postprandial TG levels in obese nondiabetic subjects (17). The duration of WP supplementation may affect lipid metabolism.

Safety is a primary concern when considering the use of specifically processed foods, such as nutrient supplements, as medicinal or healthcare products. Some of these products have been widely used by athletes to enhance the benefits of regular training. In previous studies targeting subchronic toxicity, WP consumption at intake levels up to 3 $g \cdot kg^{-1} \cdot d^{-1}$ had a no-observed-adverse-effect level (11), and the hydrolysate of WP at 2 $g \cdot kg^{-1}$ as a food additive resulted in no adverse effects or mortality (1). In this study, the WP dose was 4.1 $g \cdot kg^{-1}$, which is equivalent to 20 g of WP per 60 kg body weight for humans. Observation of different tissues in SC and SC + WP groups did not reveal any adverse effects. However, long-term ET also did not cause any tissue-related lesions. Therefore, certain biochemical variables could truly reflect the physiological effects caused by WP supplementation.

In conclusion, we provide evidence that WP affected biochemical assessments with long-term aerobic swimming, considered an intensive training exercise, and enhanced exercise performance without muscle hypertrophy. For future investigations, WP could be used in humans who focus on aerobic endurance training for protective and health purposes. We also provide the basic safety evidence from pathological observations and assessments. This study suggests alternative uses of WP as a nutrient supplement worthy of good health considerations.

We appreciate the efforts of the anonymous referees and their useful comments and suggestions for improving the manuscript. We also thank Laura Smales for her careful reading of the manuscript. The corresponding author acknowledge the National Science Council of Taiwan (grant no. NSC102-2628-H179-001-MY2) for the financial support on this study.

All authors declare that they have no conflicts of interest concerning the contents of this article. Furthermore, publication of these results of the present study does not constitute an endorsement by the American College of Sports Medicine.

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