Metabolism Clinical and Experimental 94 (2019) 18–27

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Effects of isolated soy protein and strength exercise training on exercise performance and biochemical profile in postpartum mice

Li Wei [a,b,1], Ya-Ting Wen [c,1], Mon-Chien Lee [d], Hua-Ming Ho [d], Chi-Chang Huang [d,*], Yi-Ju Hsu [d,*]

[a] Graduate Institute of Injury Prevention and Control, College of Public Health, Taipei Medical University, Taipei 11031, Taiwan
[b] Division of Neurosurgery, Department of Surgery, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei City 23142, Taiwan
[c] Department of Neurosurgery, Taipei Medical University-Wan Fang Hospital, Taipei 11696, Taiwan
[d] Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan 33301, Taiwan

ARTICLE INFO

Article history:
Received 28 November 2018
Accepted 23 January 2019

Keywords:
Isolated soy protein
Strength exercise training
Postpartum
Fitness

ABSTRACT

Objective: Postpartum women are at an increased risk for obesity and metabolic diseases because of excessive weight gain during pregnancy and weight retention after delivery. Maintenance of good nutrition and regular physical activity is used as a therapeutic approach for promotion of health and well-being in postpartum women. The aim of this study is to assess the independent and additive effects of isolated soy protein (ISP) and strength exercise training (ET) on weight management, exercise performance and health maintenance in postpartum mice.
Design and methods: Thirty-two postpartum mice (ICR, 14-weeks old) were divided into four groups ($n = 8$ per group): Group 1 mice were the sedentary control with vehicle (SC), Group 2 mice were the sedentary control with ISP supplementation (8.95 $g \cdot kg^{-1}$, SC + ISP), Group 3 mice received vehicle with exercise training (ET) and Group 4 mice received isolated soy protein with exercise training (ISP + ET). Animals in the ET and ISP + ET groups underwent strength exercise training for 6 weeks, 5 days a week. 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: Combined intervention of ISP and ET increased lean muscle mass and prevented body weight and fat elevation. The grip strength and exhaustive swimming time of the ISP + ET group were significantly higher than the other groups. The ISP + ET group showed significantly decreased serum levels of lactate, ammonia and creatinine phosphate kinase (CPK), and increased glucose level after the 15-min swimming test. The serum levels of aspartate transaminase (AST), triglyceride (TG) and creatinine after sacrifice were significantly decreased in the ET + ISP group. ISP combined with ET promoted fat oxidation in brown adipose tissue (BAT) as evidenced from the increased utilization of plasma and BAT tissue triglyceride.
Conclusions: We suggest that long-term supplementation with ISP can have a wide spectrum of bioactivities on health promotion, performance improvement and fitness. ISP with ET conferred better energy utilization, improved biochemical profiles and may be an effective ergogenic aid in strength training.

1. Introduction

The retention of weight in women after postpartum is an important issue. Research has suggested that excessive weight gain is common during pregnancy and women who retain those extra weight also face an increased risk of long term overweight problem [1,2]. Study shows that parous women who gain excessive weight have a steeper weight trajectory, gaining more from 1 y follow-up to 15 y follow-up [1]. Studies indicate that excessive weight gain in postpartum women is associated with obesity-related diseases including gestational diabetes, high blood pressure and cardiovascular (CVD). Gestational diabetes should be an element of care planning during and after the postpartum period because of the high risk of developing Type 2 diabetes mellitus [2–5]. A few studies recommend regular physical activity as a way to maintain healthy weight during the pregnancy and postpartum period [2]. Suitable exercise training combined with diet modification could produce sustained improvements in glucose metabolism and weight loss among overweight and obese lactating women. It also decreases the risk factors for cardiovascular diseases and type 2 diabetes [6,7]. Physicians, nurses or dieticians who care for women after childbirth may

Abbreviations: ISP, isolate soy protein; ET, exercise training; SC, sedentary control; CPK, creatinine phosphate kinase; AST, aspartate aminotransferase; ALT, alanine transaminase; TG, triglyceride; LDH, lactate dehydrogenase; UA, uric acid; ALB, albumin; TC, total cholesterol; TP, total protein; UFP, uterine fat pads; BAT, brown adipose tissue.

* Corresponding authors at: Graduate Institute of Sports Science, National Taiwan Sport University, No. 250, Wenhua 1st Rd., Guishan Township, Taoyuan County 33301, Taiwan.
E-mail addresses: weili@tmu.edu.tw (L. Wei), 98142@w.tmu.edu.tw (Y.-T. Wen), 1061304@ntsu.edu.tw (M.-C. Lee), 1050214@ntsu.edu.tw (H.-M. Ho), john5523@ntsu.edu.tw (C.-C. Huang), 1041302@ntsu.edu.tw (Y.-J. Hsu).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.metabol.2019.01.012
0026-0495/

assess an individual's fitness goals and ability to perform strength physical training and support them with suitable nutritional advice.

Dietary soy protein has been investigated for its nutritional properties and multiple effects in physiology [8]. Soy protein is associated with clinically significant weight loss and improvement in several parameters of cardiometabolic risk [9]. In research, animal models of obesity demonstrate that soy consumption during a hypocaloric diet, promotes greater weight and fat loss [10–12]. Furthermore, soy protein isolates β-conglycinin and glycinin, also have a strong effect in lowering cholesterol [13]. Several reviews have pointed out that soy isoflavones from soybeans are useful for the prevention or maintenance of bone loss, because isoflavones are associated with estrogen synthesis, which is protective of bone turnover and bone mineral density [14,15].

Studies in postpartum mice that focus on the relationship between weight control and health after exercise training have mostly been epidemiological review. In this study we aim to investigate the beneficial effects of isolated soy protein and exercise training on body composition, biochemical profiles and physical performance in postpartum mice. ISP supplement may be helpful for women in weight management and health maintenance after pregnancy.

2. Materials and methods

2.1. Material animals and treatment design

Isolated soy protein was purchased from Best Jet Biotechnology/Gogodone Co. Ltd. (New Taipei City, Taiwan). The nutrients and amino acids present in the ISP product were analyzed by SGS Taiwan, Ltd. (New Taipei City, Taiwan) (Table 1). Thirty-two 14-week-old female Institute of Cancer Research (ICR) mice raised under specific pathogen-free (SPF) conditions were used in this study. All animals were given standard laboratory chow diet (No. 5001, PMI Nutrition International, Brentwood, MO) and distilled water ad libitum. The mice were housed at the National Taiwan Sport University's animal facility with a 12-h light-dark cycle, 25 ± 1 °C room temperature and 50%–60% humidity. Eighteen days after postpartum, mice were acclimatized for 3 days to the environment and diet. Experiments commenced at 21 days postpartum. 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-10528 approved by the IACUC ethics committee. All procedure adhered to the American College of Sports Medicine animal care standards.

Table 1
Nutrient composition of ISP.

Amino acids	g/40 g	%
Protein	23.2	64.44
Fat	0.8	5.00
Carbohydrate	11	30.56
Total calories	144.2 kcal	
Alanine	0.88	3.93
Arginine	1.89	8.43
Aspartic acid	2.69	12.00
Cysteine	0.26	1.16
Glutamic acid	4.81	21.46
Glycine	0.94	4.19
Histidine*	0.63	2.81
Isoleucine*#	1.2	5.35
Leucine*#	1.08	4.82
Lysine*	1.50	6.69
Methionine*	0.28	1.25
Phenylalanine*	1.26	5.62
Proline	1.18	5.27
Serine	1.12	5.00
Threonine*	0.49	2.19
Tryptophan*	0.27	1.20
Tyrosine	0.79	3.53
Valine*#	1.14	5.09
Total AA	22.41	100
Total EAA*	7.81	36.19
Total BCAA#	3.42	15.26
Isoflavones	mg/40 g	
Daidzin	1.5	
Daidzein	2.0	
Genistin	4.5	
Genistein	4.0	

Essential amino acids (EAA) are denoted by *.
Branched chain acids (BCAA) are denoted by #.

Animals were randomly divided into four groups (8 mice per group) for ISP supplementation and/or ET as follows: 1) sedentary control with vehicle (SC), 2) sedentary control with ISP supplementation (SC + ISP), 3) exercise training with vehicle (ET) and 4) exercise training with ISP supplementation (ET + ISP). Water consumption, food intake and animal weights were recorded twice a week.

2.2. ISP supplementation

Mice in the SC + ISP and ET + ISP groups were given ISP by oral feeding twice per day (before and 30 min after exercise training). The recommended use of ISP for human is about 40 g per day with normal diet and physical activity. The mouse ISP dose (8.95 g/kg) 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 (availablefromhttp://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm078932.pdf). Assuming a human weight of 60 kg, the human equivalent dose for $43.8\ g \times 60 \cdot kg^{-1}$ (0.73 g/kg) = 0.73, 0.73 × 12.3 = a mouse dose of $8.95\ g \cdot kg^{-1}$; the conversion coefficient 12.3 was used to account for differences in body surface area between a mouse and a human.

2.3. ET protocol

Animals in the ET and ET + ISP groups underwent exercise power training following the training protocol in Fig. 1A. They were placed in a plastic container (65 cm high, 20 cm in diameter) with tap water 15–16 cm deep maintained at 30 ± 1 °C. The loading weight was gradually increased from 3% to 35% of the animal's body weight.

2.4. Exhaustive swimming exercise

The endurance performance was evaluated by an exhaustive swimming test. After 6 weeks of ISP supplementation and ET training, a mouse was selected from each group and a lead sheet (5% of mouse

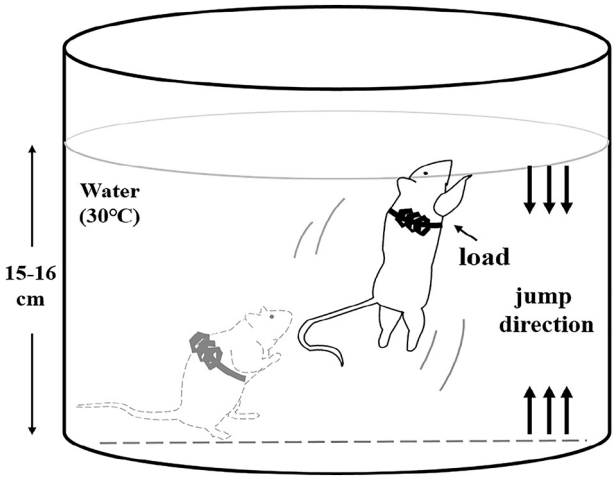

Fig. 1. Diagram of the strength exercise training set-up. Mice were placed in a plastic container (65 cm high, 20 cm in diameter) with tap water 15–16 cm deep maintained at 30 ± 1 °C.

body weight) attached to its tail. Each mouse was evaluated in a columnar swimming pool (65 cm high, 20 cm diameter, 40 cm deep) maintained at 28 ± 1 °C. The endurance performance 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 [16].

2.5. 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 [16]. The test of forelimb grip strength was performed after administration of the indicated ET protocol and ISP supplementation for 6 weeks. The maximal force (grams) recorded was used as an indicator of absolute grip strength.

2.6. Fatigue-associated biochemical variables

The effect on fatigue-associated biochemical indexes, based on our previous reports [16]. The blood sampled immediately after 15 min of acute exercise was tested to measure glucose, lactate, creatine phosphate kinase (CPK) and ammonia levels. The blood samples were centrifuged at 1000 ×*g* and 4 °C for 15 min after serum separation and analyzed using an autoanalyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

2.7. Tissue sample preparation

After the exercise training finished, the mice were sacrificed. The target organs and tissues collected include the heart, liver, lungs, kidneys, muscle tissue, uterine fat pads (UFP) and brown adipose tissue (BAT). Organs and tissues were carefully excised, rinsed in saline solution and blotted dry. The whole weight and the specific tissue weight (%) relative to individual body weight were recorded and calculated.

2.8. Blood biochemical assessments

After the experiments, blood samples were immediately collected from the submandibular duct of each mouse from the treated groups and centrifuged at 1500 ×*g*, 4 °C for 10 min for serum preparation. Clinical biochemical assessment of AST, ALT, albumin, total protein (TP), blood urea nitrogen (BUN), creatinine, CPK, UA, TC, TG and glucose levels were analyzed using an autoanalyzer (Hitachi7060, Hitachi, Tokyo, Japan).

2.9. Glycogen content analysis

Since liver and skeletal muscles are the two major tissues for glycogen deposition, we investigated whether glycogen contents of these two target tissues could be elevated. Liver and muscle tissues were excised and stored in −80 °C for glycogen content analysis as described previously [16].

2.10. Histological staining of tissues

Collected liver, muscles, lungs, kidney, and heart were fixed in 10% formalin for 24 h, cut transversely or longitudinally to obtain ventricular sections or longitudinally to obtain ventricular sections or four-chamber cross-sections. Tissues were embedded in paraffin and cut into 4-μm-thick slices for morphological and pathological evaluation. Sections were stained with hematoxylin and eosin (H&E) and examined using a light microscope equipped with a CCD camera (BX-51, Olympus, Tokyo, Japan). The area of UFP tissue was traced manually and intracellular lipid vacuoles in the BAT on the H&*E*-stained slides calculated using the ImageJ software (National Institutes of Health, Bethesda, MD).

2.11. Statistical analysis

Data are expressed as mean ± SEM. Two-way ANOVA was used to assess the effect of the ET and ISP 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 and statistical analyses was carried out using SAS v9.0 (SAS, Cary, NC, USA).

3. Results

3.1. Effect of ISP supplementation and ET on body and organ weight

The initial body weights for SC, SC + ISP, ET and ET + ISP groups were 39.4 ± 0.8 g, 39.8 ± 0.8 g, 39.9 ± 1.0 g, and 39.3 ± 0.9 g, respectively, with no difference between groups (Fig. 2B). After a 2-wk ET and ISP supplementation, the body weight was 6.1% lower in the ET + ISP than SC group ($P = 0.0254$). The 2-wk body weight was lower with ISP supplementation than ET alone ($P = 0.0225$). The body weights were lower by 8.6% ($P = 0.0164$), 6.5% ($P = 0.1008$) and 9.4% ($P = 0.0079$) in the SC + ISP, ET and ET + ISP groups respectively, than with SC group. However, there was no significant difference in the 2-wk and final body weights among the SC, SC + ISP and ET groups.

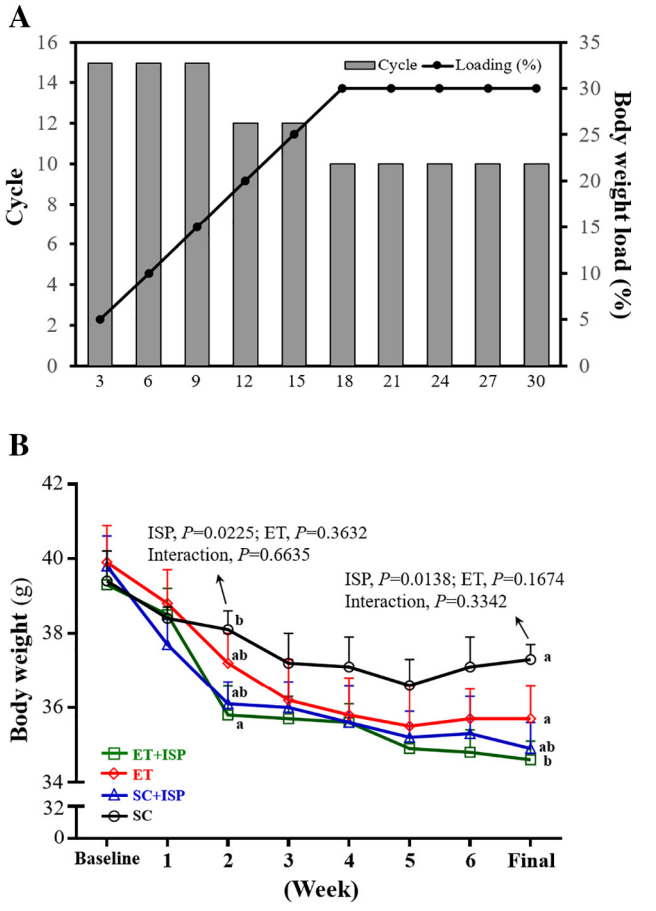

Fig. 2. (A) The training program of 6-wk ET. (B) Effect of ISP supplementation and 6-wk ET on body weight. Data are expressed as mean ± SEM for $n = 8$ mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P* values and interaction between ISP and ET by two-way ANOVA are indicated.

Table 2
General characteristics of the experimental groups.

Characteristic	SC	SC + ISP	ET	ET + ISP	*P* Values		
					Main effect of ISP	Main effect of ET	Interaction (ISP× ET)
Food intake (g/day)	5.56 ± 0.16[a]	5.46 ± 0.20[a]	5.57 ± 0.33[a]	5.51 ± 0.29[a]	0.2562	0.7399	0.7706
Water intake (mL/day)	7.72 ± 0.16[a]	7.03 ± 0.20[a]	10.02 ± 0.33[c]	9.17 ± 0.29[b]	**0.0032**	**<0.001**	0.7556
Liver (g)	2.10 ± 0.04[a]	1.97 ± 0.06[a]	1.98 ± 0.08[a]	1.95 ± 0.04[a]	0.172	0.2014	0.3463
Muscle (g)	0.33 ± 0.006[a]	0.33 ± 0.006[ab]	0.34 ± 0.009[ab]	0.35 ± 0.011[b]	0.1309	0.0745	0.5103
Kidney (g)	0.46 ± 0.010[b]	0.45 ± 0.015 [ab]	0.45 ± 0.11[ab]	0.42 ± 0.010[a]	0.1738	0.1441	0.2922
Heart (g)	0.21 ± 0.007[a]	0.21 ± 0.006[a]	0.21 ± 0.006[a]	0.20 ± 0.006[a]	0.4924	0.9216	0.207
Lung (g)	0.23 ± 0.007[a]	0.24 ± 0.008[a]	0.24 ± 0.006[a]	0.23 ± 0.003[a]	0.6858	0.8395	0.5446
UFP (g)	0.65 ± 0.083[b]	0.35 ± 0.029[a]	0.33 ± 0.046[a]	0.33 ± 0.027[a]	**0.0055**	**0.0032**	**0.0079**
BAT (g)	0.08 ± 0.006[a]	0.08 ± 0.006[a]	0.08 ± 0.009[a]	0.08 ± 0.011[a]	1.0000	0.5071	1.0000
Relative liver weight (%)	5.5 ± 0.138[a]	5.4 ± 0.097[a]	5.5 ± 0.075[a]	5.4 ± 0.086[a]	0.6111	0.771	0.9419
Relative muscle weight (%)	0.86 ± 0.020[a]	0.91 ± 0.020[a]	0.92 ± 0.020[a]	0.99 ± 0.017[b]	**0.003**	**0.0018**	0.4143
Relative kidney weight (%)	1.22 ± 0.027[a]	1.23 ± 0.050[a]	1.23 ± 0.017[a]	1.14 ± 0.030[a]	0.2671	0.2099	0.1158
Relative heart weight (%)	0.51 ± 0.024[a]	0.58 ± 0.018[b]	0.56 ± 0.021[ab]	0.52 ± 0.016[ab]	0.6266	0.8075	0.0133
Relative lung weight (%)	0.60 ± 0.016[a]	0.67 ± 0.027[b]	0.66 ± 0.020[ab]	0.66 ± 0.017[ab]	0.0973	0.2749	0.136
Relative UFP weight (%)	1.69 ± 0.194[a]	1.15 ± 0.056[b]	1.07 ± 0.162[b]	0.94 ± 0.083[b]	**0.0204**	**0.0048**	0.1305
Relative BAT weight (%)	0.22 ± 0.020[a]	0.22 ± 0.020[a]	0.24 ± 0.020[a]	0.23 ± 0.017[a]	0.8422	0.1706	0.5516

Data are expressed as mean ± SEM for $n = 8$ mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P* values and interaction between ISP and ET by two-way ANOVA are indicated. Muscle mass include both gastrocnemius and soleus muscles in the back part of the lower legs. BW: body weight; UFP: uterine fat pads; BAT: brown adipose tissue. Data in bold indicate significant *P* value.

The final body weight was lower with ISP supplementation than ET alone ($P = 0.0138$).

The food consumption, water intake and body compositions are summarized in Table 2. The food intake was not significantly different among the four groups. The water intake of the ET and ET+ ISP groups were significantly higher by 23.0% ($P < 0.0001$) and 29.8% ($P = 0.0001$) respectively, as compared with vehicle group. There was no significant difference among the SC and ISP groups. The main effect of ISP supplementation was a difference in water intake ($P = 0.0032$). ET led to an increase in water intake ($P < 0.0001$). The muscle weight of the ET + ISP group was significantly higher (8.1%, $P = 0.0228$) than the SC group. The relative muscle weight (%) was greater in the ET + ISP group than the SC, SC + ISP and ET groups by 13.1% ($P < 0.0001$), 8.6% ($P = 0.0053$) and 8.0% ($P = 0.0075$), respectively. Kidney weight was lower by 7.4% ($P = 0.0499$) for the ET + ISP than SC group, with no difference in relative kidney weights (%) among groups. Weight of uterine fat pads (UFP) was significantly lower in the SC + ISP, ET, and ET + ISP groups than SC group by 46.6% ($P = 0.0003$), 48.4% ($P = 0.0002$) and 49.5% ($P = 0.0001$) respectively, with a slight interaction between ISP supplementation and ET ($P = 0.0079$). The relative UFP weight (%) was lower in the SC + ISP, ET, and ET + ISP groups than SC group by 32.3% ($P = 0.0057$), 37.1% ($P = 0.0020$) and 44.3% ($P = 0.0004$), respectively. Overall, the main effect of ISP supplementation was decreased UFP weight ($P = 0.0055$) and relative UFP weight ($P = 0.0204$), and increased relative muscle weight ($P = 0.0030$). The main effects of ET were decreased UFP weight ($P = 0.0032$), decreased relative UFP weight ($P = 0.0048$) and increased relative muscle weight (%) ($P = 0.0018$).

The area of UFP tissue was calculated (Fig. 3B) and observed UFP morphology shown in Fig. 3A. The UFP areas for SC, SC + ISP, ET and ET + ISP groups were 267.3 ± 24.78 mm^2, 200.9 ± 10.01 mm^2, 173.1 ± 14.18 mm^2 and 170.2 ± 11.71 mm^2, respectively. Compared with the SC group, SC + ISP, ET and ET + ISP groups showed decreased UFP area by 24.8% ($P = 0.0003$), 35.2% ($P = 0.0073$) and 36.3% ($P = 0.0002$), respectively. ISP supplementation could significantly decrease UFP area ($P = 0.0417$) via the main effect of ISP supplementation. The main effect of ET alone was decreased UFP area ($P = 0.0006$).

3.2. Effect of ET and ISP supplementation on physical performance

The two physical performance tests included forelimb grip strength and exhaustive swimming exercise. As shown in Fig. 4A, the forelimb grip in the SC, SC + ISP, ET, and ET + ISP groups were 124 g, 137 g, 128 g and 147 g, respectively. Absolute and relative grip strength were greater by 1.19- ($P = 0.0004$) and 1.15-fold ($P = 0.0029$), and were greater by 1.18- ($P = 0.0021$) and 1.13-fold ($P = 0.0149$), respectively, in the ET + ISP group than the SC and ET group (Fig. 4). ISP

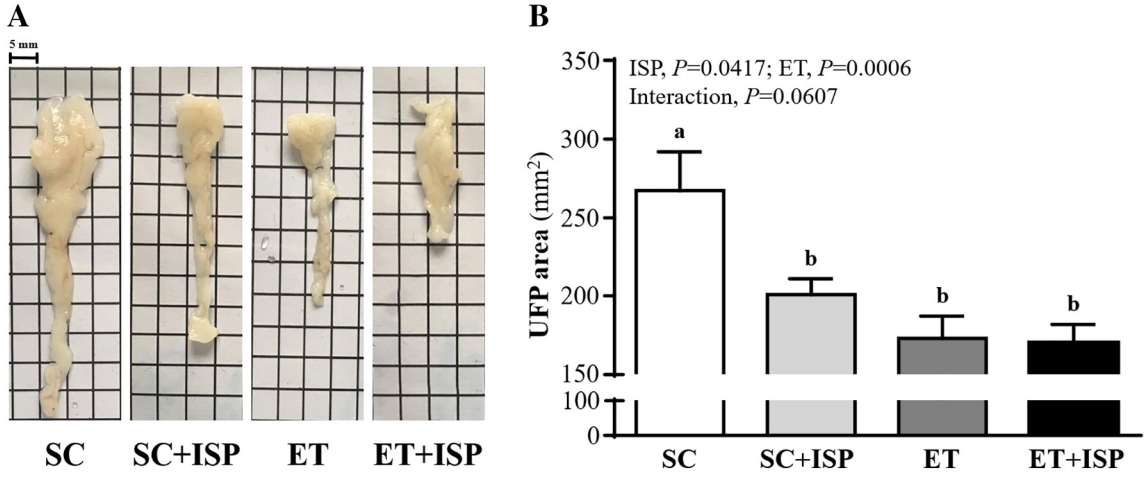

Fig. 3. Effect of ISP supplementation and 6-wk ET on (A) uterine fat pads (UFP) area (mm^2). (B) View of UFP. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P*-values and interaction between ISP and ET by two-way ANOVA are indicated.

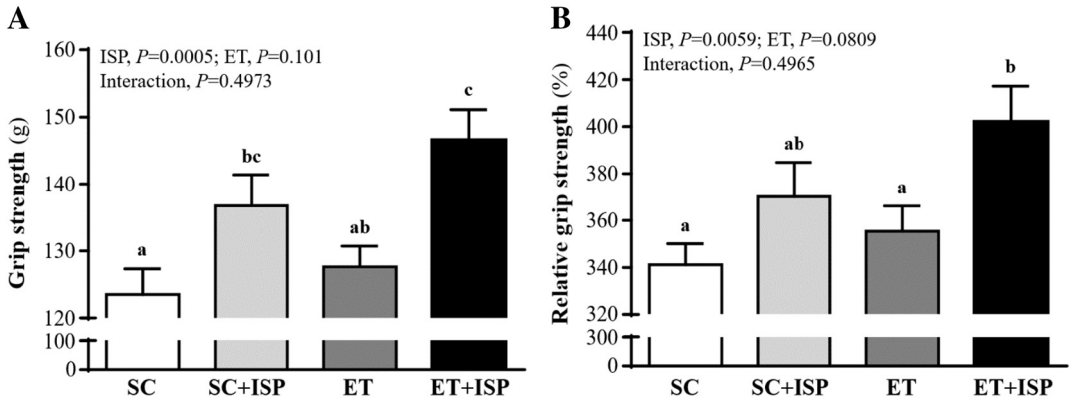

Fig. 4. Effect of ISP supplementation and 6-wk ET on (A) forelimb grip strength and (B) relative forelimb grip strength. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b and c) indicate significant difference at $P < 0.05$. Main-effect P values and interaction between ISP and ET by two-way ANOVA are indicated.

supplementation could significantly increase absolute ($P = 0.0005$) and relative grip strength ($P = 0.0059$) via the main effect of ISP supplementation. In the swimming test, the exercise endurance levels of the SC, SC + ISP, ET, and ET + ISP mice were 7.76 min, 9.53 min, 10.36 min, and 12.73 min, respectively (Fig. 5). The swimming time of the ET + ISP group was significantly higher (1.64-fold, $P = 0.0094$) than the SC group. The main effect of ET improved swimming time ($P = 0.0292$). However, there was no significant difference in swimming times among the SC, SC + ISP and ET groups.

3.3. Effect of ISP supplementation and ET on exercise fatigue-related indicators after acute exercise

The lactate, ammonia, glucose and CPK levels in the blood of the mice groups were measured. The measurements were conducted after swimming with a constant workload and the results shown in Fig. 6. Serum lactate concentrations were significantly lower by 35.2% and 33.0% in the ET and ET + ISP groups than the SC group (Fig. 6A). As shown in Fig. 6B, serum ammonia levels of SC + ISP, ET and ET + ISP groups were significantly lower by 16.5% ($P = 0.0048$), 17.8% ($P = 0.0027$) and 20.6% ($P = 0.0007$), respectively, as compared with the SC group. Fig. 6C shows that levels of serum glucose with the ET and ET + ISP groups respectively were significantly higher by 17.5% and 19.5% ($P = 0.0003$, $P < 0.0001$) than the SC group. CPK level with SC, SC + ISP and ET groups respectively was significantly higher by 56.1% ($P = 0.002$), 43.8% ($P = 0.047$) and 52.5% ($P = 0.0064$) than the ET + ISP group. ISP supplementation could significantly reduce serum levels of ammonia ($P = 0.0169$) and CPK ($P = 0.0052$) via the main effect of ISP supplementation. The main effect of ET alone was decreased serum levels of lactate ($P < 0.0001$) and ammonia ($P = 0.0078$) and increased glucose levels ($P < 0.0001$).

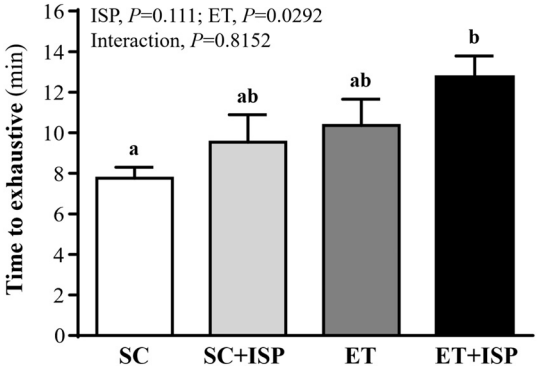

Fig. 5. Effect of ISP supplementation and 6-wk ET on exhaustive swimming test. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect P-values and interaction between ISP and ET by two-way ANOVA are indicated.

3.4. Effect of ISP 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. Levels of biochemical indices including ALT, Albumin, TP, CPK, UA, TC, and glucose, did not differ among groups ($P > 0.05$, Table 3). The AST level was significantly lower by 17.8% ($P = 0.043$) in the ET than SC group, with no significant difference between SC + ISP and ET alone groups. Serum BUN levels of SC + ISP and ET + ISP groups were significantly higher by 20.5% ($P = 0.0143$) and 19.1% ($P = 0.024$) respectively, as compared with the SC group. Level of creatinine in the SC + ISP, and ET + ISP groups were 23.2% ($P = 0.0078$) and 26.2% ($P = 0.0031$) respectively, significantly lower than that of the SC group. Serum levels of TG in the SC + ISP, ET and ET + ISP groups were 18.0% ($P = 0.0142$), 17.8% ($P = 0.013$) and 17.2% ($P = 0.0175$), significantly lower than that of the SC group. Overall, the main effect of ISP supplementation decreased serum levels of AST ($P < 0.001$) and creatinine ($P = 0.0026$) and increased level of BUN ($P < 0.001$). We found significant interaction between ISP supplementation and ET for levels of AST ($P = 0.0405$).

3.5. Effect of ET and ISP Supplementation on Tissue Glycogen Determination

Glycogen content in the liver and skeletal muscles of mice are shown in Fig. 7. Liver glycogen level did not differ among the four groups as seen in Fig. 7A. Fig. 7B shows that muscle glycogen of the ET + ISP group were 1.88-, 1.80- and 2.21-fold ($P < 0.0001$) higher than those of the SC, SC + ISP and ET groups. There was no significant difference between the SC, SC + ISP and ET groups. The muscle glycogen index demonstrated significant ISP supplementation main effect ($P < 0.0001$), ET main effect ($P = 0.0048$) and interaction effect ($P = 0.0001$).

3.6. Effect of ET and ISP supplementation on histology

As seen in Fig. 8, the four groups did not differ in the histological observations of the liver, kidney, heart, lung, muscle and UFP. The arrangement of sinusoid and hepatic cords in liver showed no changes with ISP or exercise training intervention (Fig. 8A). The structure of renal tubules and glomerulus did not differ among treatments (Fig. 8B). Hypertrophy and hyperplasia were not observed in heart cardiomyocytes (Fig. 8C) or rhabdomyocytes of gastrocnemius muscle (Fig. 8E). All animal showed typical tissue architectures of lung alveoli by H&E staining (Fig. 8D). In addition, adipose tissue morphology and fat cell size did not differ between groups (Fig. 8F). Histological examination of organs showed no apparent damage in any mice.

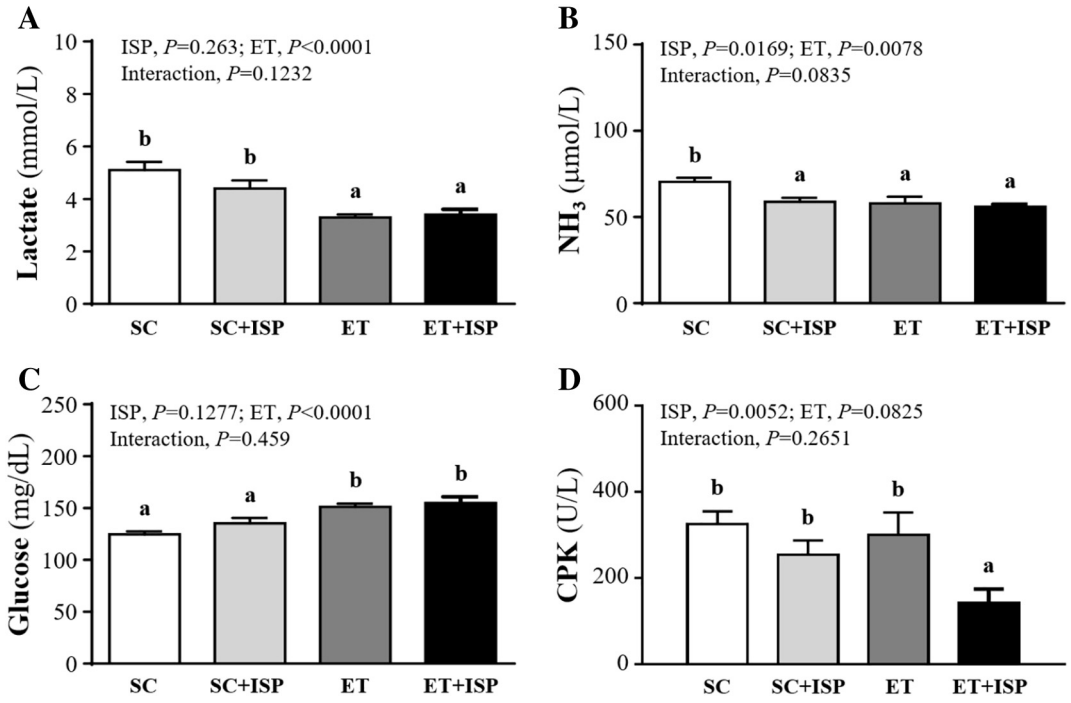

Fig. 6. Effect of ISP supplementation and 6-wk ET on serum (A) lactate; (B) ammonia; (C) glucose; and (D) creatine kinase (CPK) levels after acute exercise challenge. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P*-values and interaction between ISP and ET by two-way ANOVA are indicated.

Table 3
Biochemical analysis at the end of treatment.

Characteristic	SC	SC + ISP	ET	ET + ISP	*P* Values		
					Main effect of ISP	Main effect of ET	Interaction (ISP× ET)
AST (U/L)	111.4 ± 7.26[b]	94.9 ± 8.43[ab]	98.9 ± 6.59[ab]	91.5 ± 2.92[a]	<**0.001**	0.072	**0.0405**
ALT (U/L)	57.3 ± 3.15[a]	52.8 ± 4.95[a]	53.5 ± 4.11[a]	52.0 ± 4.03[a]	0.4713	0.5883	0.7178
Albumin (g/dL)	2.85 ± 0.02[a]	2.81 ± 0.03[a]	2.84 ± 0.06[a]	2.86 ± 0.05[a]	0.8783	0.6464	0.4461
TP (g/dL)	5.04 ± 0.08[a]	5.00 ± 0.05[a]	5.03 ± 0.08[a]	5.00 ± 0.46[a]	0.6459	0.9266	0.9266
BUN (mg/dL)	22.3 ± 1.79[a]	28.1 ± 1.41[b]	18.8 ± 1.26[a]	27.6 ± 1.72[b]	<**0.001**	0.2074	0.3404
Creatinine (mg/dL)	0.33 ± 0.03[b]	0.26 ± 0.01[a]	0.30 ± 0.01[ab]	0.25 ± 0.02[a]	**0.0026**	0.2125	0.4581
CPK (U/L)	326 ± 44.0[a]	228 ± 21.0[a]	234 ± 40.9[a]	266 ± 44.4[a]	0.3928	0.4933	0.1054
UA (mg/dL)	0.54 ± 0.08[a]	0.45 ± 0.02[a]	0.45 ± 0.02[a]	0.45 ± 0.02[a]	0.3314	0.3314	0.3314
TC (mg/dL)	127 ± 3.27[a]	130 ± 5.7[a]	140 ± 5.96[a]	129 ± 7.00[a]	0.5347	0.2749	0.2567
TG (mg/dL)	243 ± 11.82[a]	199 ± 10.55[b]	200 ± 13.53[b]	202 ± 10.62[b]	0.0807	0.0895	0.0624
Glucose (mg/dL)	144 ± 3.48[a]	144 ± 4.65[a]	145 ± 2.58[a]	142 ± 2.70[a]	0.6545	0.9001	0.6805

Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P* values and interaction between ISP and ET by two-way ANOVA are indicated. Muscle mass include both gastrocnemius and soleus muscles in the back part of the lower legs. AST, aspartate aminotransferase; ALT, alanine aminotransferase; TP, total protein; BUN, blood urea nitrogen; CK, creatine phosphate kinase; UA, uric acid; TC, total cholesterol; TG, triacylglycerol.
The statistically significant ($P < 0.05$) for the bold in this table.

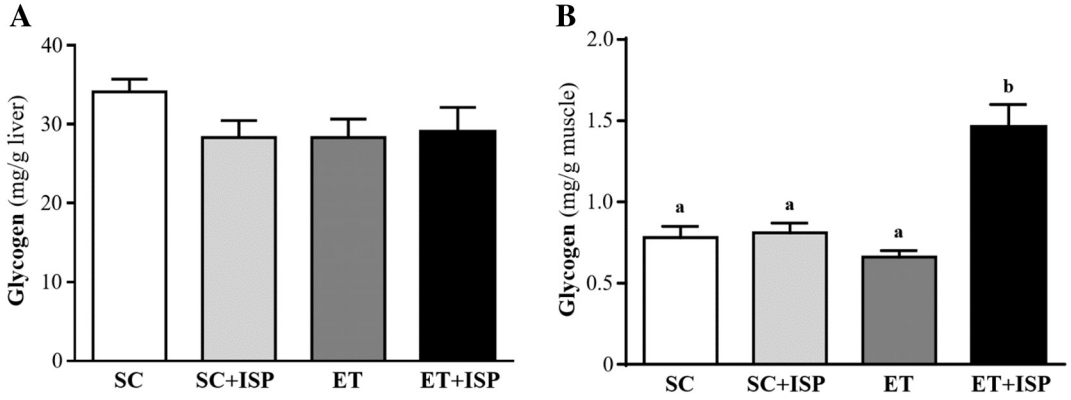

Fig. 7. Effect of ISP supplementation and 6-wk ET on glycogen content in (A) liver and (B) muscle. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P*-values and interaction between ISP and ET by two-way ANOVA are indicated.

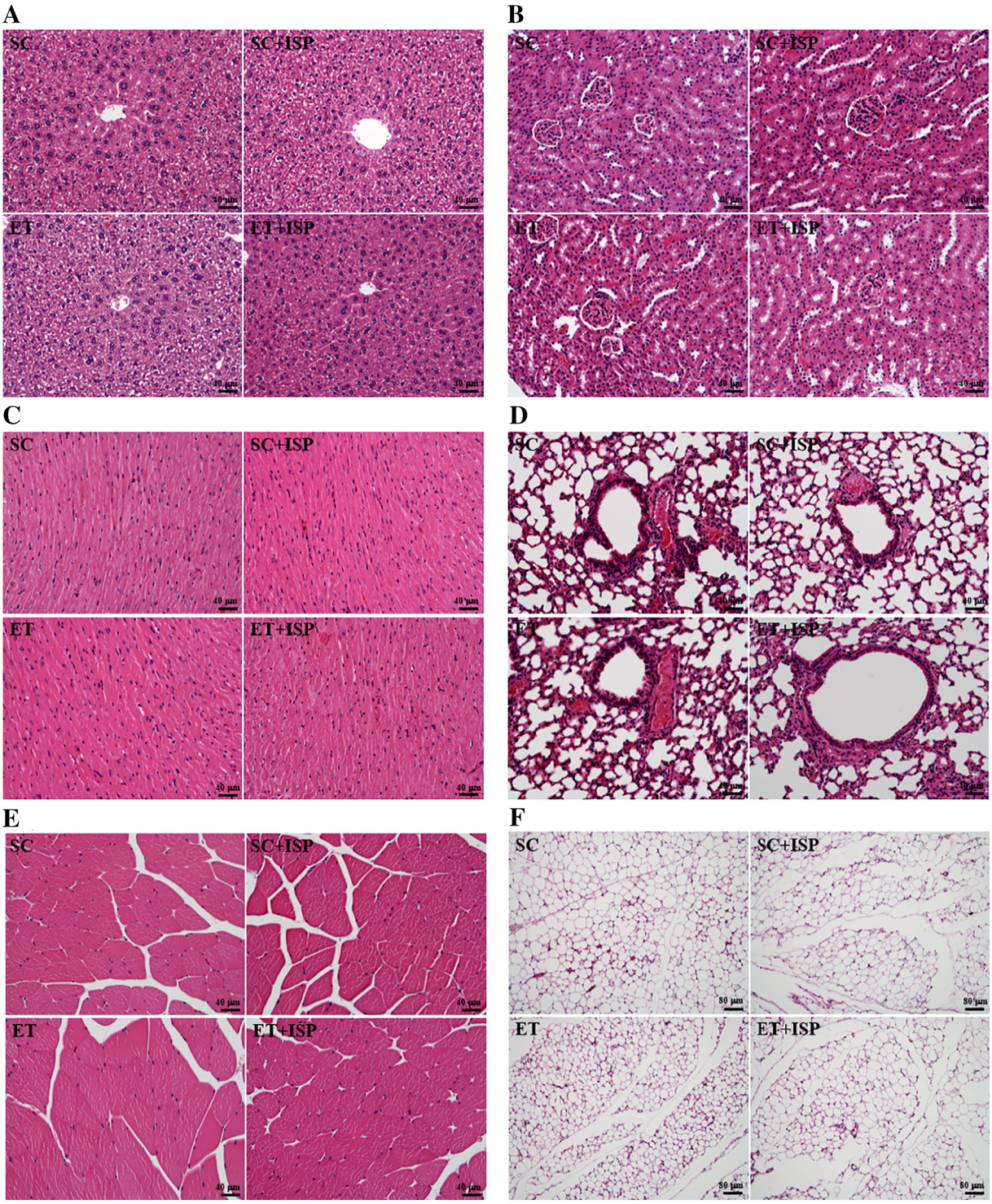

Fig. 8. Effect of ISP supplementation and 6-wk ET on morphology of (A) liver (B) kidney (C) heart (D) lung (E) skeletal muscle and (G) UFP in mice. Specimens were photographed using light microscopy. H&E stain, magnification: 100× or 200×; scale bar, 40 or 80 μm.

3.7. Effect of ET and ISP supplementation on BAT metabolism

Fig. 9 shows the pathological changes by ISP and exercise training intervention in BAT. The SC group had larger and more numerous lipid droplets in BAT than the other three groups. The lipid areas of BAT for SC, SC + ISP, ET and ET + ISP groups were 43.6 ± 0.4%, 21.3 ± 1.07%, 22.6 ± 1.26% and 21.9 ± 1.61%, respectively (Fig. 9B). Compared with the SC, SC + ISP, ET and ET + ISP groups showed decrease in lipid areas of BAT by 51.2%, 48.2%, and 49.8%, respectively. Measurement of the triglyceride content in BAT showed a significant reduction in the SC + ISP, ET and ET + ISP groups compared to the SC group by 52.4% ($P = 0.0001$), 51.2% ($P < 0.0001$) and 48.6% ($P = 0.0002$), respectively. For the lipid area and triglyceride content in BAT, significantly ISP supplementation main effect ($P = 0.0417$, $P = 0.0049$), ET main effect ($P = 0.0007$, $P = 0.007$) and interaction effect ($P = 0.0005$, $P = 0.0021$) were observed.

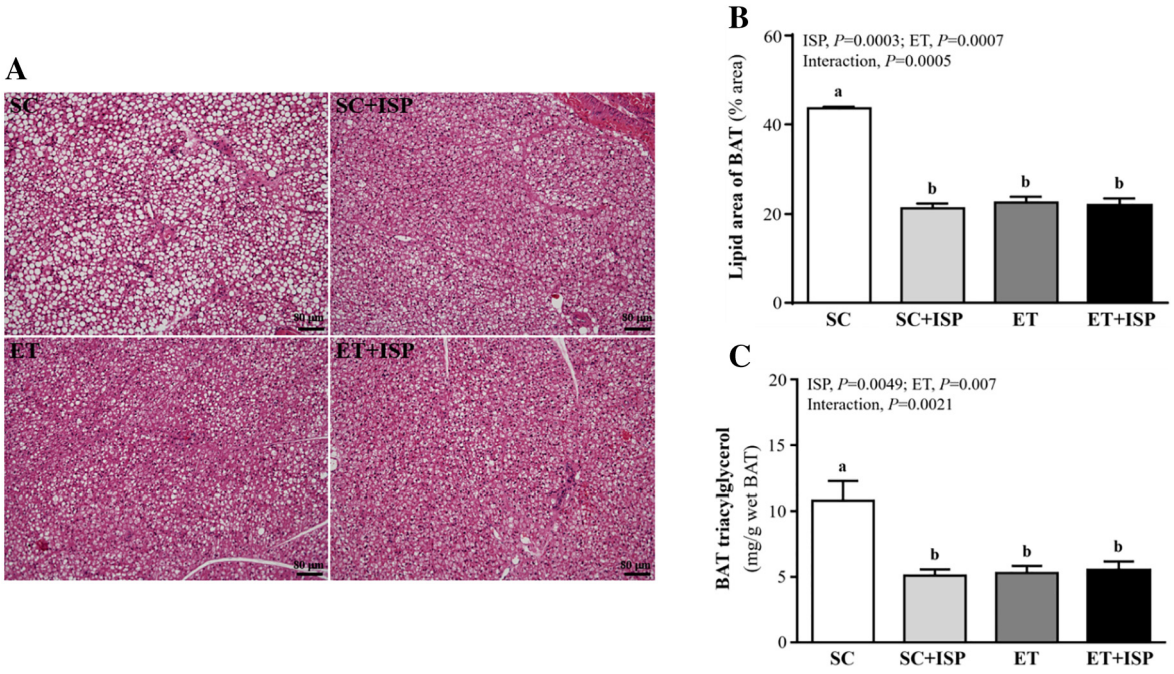

Fig. 9. Effect of ISP supplementation and 6-wk ET on the (A) morphology in BAT (B) Lipid droplet-positive area of BAT (C) BAT triglycerides (TG) content. Data are expressed as mean ± SEM for n = 8 mice in each group. Different letters (a, b) indicate significant difference at $P < 0.05$. Main-effect *P*-values and interaction between ISP and ET by two-way ANOVA are indicated. Specimens were photographed using light microscopy. H&E stain, magnification: 100×; scale bar, 80 μm.

4. Discussion

We found that after 6 weeks of supplementation, the mice in all the groups showed a decrease in body weight compared to baseline (Fig. 2B). Even though the mice in the SC group were inactive and sedentary, they still had a slight decrease in body weight over the 6 weeks. This is in agreement with previous studies showing that pregnant mice gain an average of 3–4 g in body weight, and the weight is lost postpartum over time [17]. The early weight loss occurs in the first few weeks and seems to be related to the loss of the placenta and amniotic fluid, as well as contraction of maternal blood volume and other body components [18]. We observed that 6-wk ET and ISP supplementation was able to significantly lower the body weight of the mice, compared with the sedentary (SC) mice (Fig. 2B). ET in combination with ISP appears to accelerate the speed of weight loss.

High-protein meals (30% of energy from protein) with soy-based protein sources showed increased appetite control and weight loss among obese men in a randomized crossover trial [19]. In this study, we did not find a difference in food intake between the 4 groups, showing that the appetite was not affected. A previous study reported that higher protein intake could improve postpartum body weight loss and reduce body fat percentage [20]. In our study, the attenuated body weight in the mice may be due to a decrease in fatty tissue (Table 2) and uterine fat area (Fig. 3). Physical activity should be maintained during pregnancy and postpartum in order to achieve a healthy weight and prevent negative health outcomes [21]. Another study suggests that although physical activity interventions promote postpartum weight loss, the most effective intervention strategies encompass both physical exercise and dietary interventions [22]. It is well accepted that intake of protein and exercise training are key to building muscle mass [23]. Compared to many other protein sources, ISP is a low-fat source of high-quality protein that can help build lean muscle mass. Intake of adequate protein quality is a necessary anabolic driver to maintain muscle mass in the absence of high volumes of training [24]. In this study, a combination of daily supplementation with ISP and strength ET is effective in promoting muscle mass growth and reducing body fat and weight.

Forelimb grip strength is a routine physical examination test. Our previous study found that muscle strength positively correlated with forelimb grip strength [25]. In this study, we observe greater grip strength with the ET + ISP group than other groups. Consistent with our previous study, whey protein and exercise training could improve muscle strength [26]. Lower physical activity levels in postpartum women may contribute to lower muscle strength, weakness and fatigue. On the other hand, stronger women are less fatigued and demonstrate better functional mobility [27]. Soy protein is a high-quality, plant-based protein providing indispensable amino acids necessary for protein synthesis in the body. Ingestion of soy protein after resistance exercise is an important strategy to prevent muscle loss or improve muscle mass and strength. Our experimental results indicate that combining ISP supplementation with strength training could increase muscle mass (Table 2) and benefit grip strength (Fig. 4) during postpartum.

The loaded-swimming model is a reliable measure of anti-fatigue effects used in the field of sports nutrition research [28]. After 6 weeks of exercise and ISP intervention, the swimming capacity of the mice showed varying amounts of improvement. The improvement was significantly greater for the ET + ISP group than SC group (Fig. 5). Interestingly, aerobic capacity and strength is known to decrease physiologically in the early postpartum period [29]. Evidence suggests that the $VO_{2\ max}$ increases significantly in postpartum women participating in exercise programs, with concomitant improvement in cardio-respiratory function [30]. Several studies have indicated that soy protein antioxidants could curtail exercise training induced free radical damage to the muscle, either by restricting antioxidant depletion or by enhancing antioxidant capacity [31,32]. ISP supplementation after ET can enhance the recovery process during long-term exercise training. Additionally, ISP might have an anti-fatigue effect and could improve physical exercise capacity.

Several biochemical parameters have been used to evaluate the extent of exercise-induced fatigue and injury after exercise, such as lactate, ammonia, glucose and CPK [33]. During exercise, energy demand expedites muscular glucose utilization and blood glucose, which are important fuels for increased ATP production within contracting skeletal muscle during exercise. When the ATP supply fails

to meet the consumption of ATP during exercise, the generation of ATP shifts from aerobic processes to anaerobic glycolysis or glycogenolysis, resulting in fatigue [34].

Under the conditions of physical training, serum lactate is a biomarker of muscle fatigue [35]. Serum ammonia closely follows the lactate response during exercise [36]. During exercise, ammonia is produced and accumulates in skeletal muscle when AMP is deaminated to IMP by AMP deaminase (AMPD) during resynthesis of ATP. Ammonia is very toxic and has harmful influences on the body, including activation of phosphofructokinase (PFK), the rate-limiting enzyme in glycolysis, and inhibition of pyruvate oxidation to acetyl-CoA [37]. There is one report showing that sufu, a traditional Chinese fermented soybean food, could also modulate serum lactic acid levels after 10 min of swimming. The enhancement of endurance capacity is attributed to the activity of isoflavones in the sufu [38]. In this study, strength ET in combination with daily ISP supplementation for 6 weeks, could significantly modulate the lactate and ammonia levels after the exercise test.

During exercise, energy demand expedites muscular glucose utilization and consequently increases blood glucose disposal [39]. The ET and ET + ISP groups exhibited significantly higher levels of glucose than the SC + ISP group. We postulate that long-term exercise training increases the glucose level, thereby increasing energy utilization. The CPK activity in serum is routinely examined after exercise in sports medicine as a biomarker of muscle damage [40]. Our study showed that the ET + ISP group exhibited lower CPK level than the other three groups. Another study also demonstrated that long-term ingestion of soy protein may modulate CPK activity after exhaustive swimming, helping exhausted rats to rapidly recover athletic ability [41]. The combination of ISP supplementation and ET, however, did not show an additive effect on the CPK levels.

In this study, we showed that the ALT and TG levels in the ISP group were lower than the other groups. In an experimental study with obese rats, a diet with soy protein isolate favored a lower AST/ALT ratio. The authors suggested that soy protein enriched with isoflavones had a favorable effect on the inflammatory status of obese mice [42]. Previous studies using fructose-induced insulin resistance rat suggest that soy isoflavones, particularly genistein, are responsible for reducing serum liver enzymes [43]. As shown in Table 1, the ISP supplement contains isoflavones, especially genistein. Our results suggest that the effect on liver enzymes could be due to genistein. Dietary soy protein has been shown to reduce TG levels and excess body fat, playing important roles in ameliorating metabolic diseases [44]. This is in agreement with our results, that long-term training combined with ISP supplementation could reduce TG levels and control postpartum obesity.

Liver and skeletal muscle glycogen are important sources of energy storage and supply for key metabolic regulators of exercise [45]. Glycogen is the predominant source of glycolysis for ATP production. The concentration of glycogen is strongly associated with the degree of fatigue development during endurance exercise [46]. We found the highest muscle glycogen in the ET + ISP group. Our results suggest that soy protein combined with exercise training could serve as an energy source to postpartum mice in preventing the depletion of energy during extensive exercise.

Histological data related to the pathological effects of long-term intake of ISP is quite limited, especially with regards to bioactive doses. We found no changes in arrangement of sinusoid and hepatic cords with ISP treatment in mice and no hypertrophy or hyperplasia in heart cardiomyocytes and rhabdomyocytes of gastrocnemius muscles. We also found no difference in structure of renal tubules and glomerulus with ISP treatment or alterations in the alveolar, bronchial and interstitial space. The adipose tissue morphology and fat cell size did not differ between groups. Histological examination of organs showed no apparent damage in any mice.

To evaluate the degree of lipolysis, we investigated the microscopic appearance of BAT. Exercise training has been shown to affect the composition and concentration of phospholipids and triglycerides in the lipidome of rodent BAT [47]. In addition, ISP also modestly promotes the oxidation of TG, likely by enhancing TG uptake by BAT activation. Skeletal muscle and BAT are functionally linked, with the interferon regulatory factor 4 (IRF4) activation in BAT able to inhibit serum myostatin and increase exercise capacity in muscle [48]. Increasing BAT thermogenesis may serve as a novel approach to modulate energy balance, which is primarily dependent on the uncoupling protein 1 (UCP1). Regulation of UCP1 activation could promote energy balance and prevent obesity [49]. Interestingly, ET and ISP treatment have similar effects in BAT. Our results are in agreement with another study where isoflavones from soy, especially daidzein, was able to induce expression of UCP1 in BAT of rats with concomitant decrease in body weight and fat mass [50,51]. We suggest that ISP treatment could be a new effective way to control body weight and visceral fat, leading to improved metabolic phenotype and energy balance. To our knowledge, there is no evidence of ISP supplementation increasing brown adipose tissue metabolic rate or energy expenditure. This mechanism in postpartum obese mice warrants additional studies and further investigation.

5. Conclusions

In conclusion, our results suggest that ISP treatment can significantly reduce body weight and regulate lipid metabolism during the postpartum period. ISP affected biochemical assessments with long-term aerobic swimming (considered an intensive training exercise) and enhanced exercise performance. We also provide safety evidence from pathological observations and assessments that consumption ISP is safe during the postpartum period. It is possible that the addition of ISP to the diet may provide a viable strategy for improving the health of postpartum women, especially in combination with physical activity. Additional studies on ISP supplementation and ET for older postpartum women are recommended in the future.

Author contributions

Yi-Ju Hsu, Ya-Ting Wen, and Chi-Chang Huang designed the experiments. Yi-Ju Hsu, Mon-Chien Lee and Hua-Ming Ho carried out the laboratory experiments. Ya-Ting Wen and Chi-Chang Huang contributed reagents, materials and analysis platforms. Yi-Ju Hsu, Mon-Chien Lee and Li Wei analyzed the data. Yi-Ju Hsu and Chi-Chang Huang interpreted the results, prepared the figures, wrote and revised the manuscript.

Disclosure summary

The authors have no conflict of interest to declare.

Acknowledgments

This work was supported by a grant from Taipei Medical University-Wan Fang Hospital (106-eva-15) and financial support from the Ministry of Science and Technology (MOST) of Taiwan (grant No. MOST 107-2410-H-179-006-MY3). The authors are grateful to graduate students at the Sport Nutrition Laboratory, National Taiwan Sport University, for their technical assistance in conducting the animal experiments.

References

[1] Linne Y, Dye L, Barkeling B, Rössner S. Weight development over time in parous women- the SPAWN study-15 years follow-up. Int J Obes Relat Metab Disord 2003;27:1516–22.
[2] Choi J, Fukuoka Y, Lee JH. The effects of physical activity and physical activity plus diet interventions on body weight in overweight or obese women who are pregnant or in ostpartum: a systematic review and meta-analysis of randomized controlled trials. Prev Med 2013;56:351–64.
[3] Goueslard K, Cottenet J, Marie AS, Giroud M, Cottin Y, Peti JM, et al. Early cardiovascular events in women with a history of gestational diabetes mellitus. Cardiovasc Diabetol 2016;15:1.

[4] Amorim Adegboye AR, Linne YM. Diet or exercise, or both, for weight reduction in women after childbirth. Cochrane Libr 2013;7:CD005627.
[5] Wasalathanthri S. Attenuating type 2 diabetes with postpartum interventions following gestational diabetes mellitus. World J Diabetes 2015;6:648.
[6] Ehrlich SF, Hedderson MM, Quesenberry CP, Feng J, Brown SD, Crites Y, et al. Postpartum weight loss and glucose metabolism in women with gestational diabetes: the DEBI Study. Diabet Med 2014;31:862–7.
[7] Brekke HK, Bertz F, Rasmussen KM, Bosaeus I, Ellegård L, Winkvist A. Diet and exercise interventions among overweight and obese lactating women: randomized trial of effects on cardiovascular risk factors. PLoS One 2014;9:e88250.
[8] Sugano M. Soy in health and disease prevention. CRC Press; 2005.
[9] Beavers KM, Gordon MM, Easter L, Beavers DP, Hairston KG, Nicklas BJ, et al. Effect of protein source during weight loss on body composition, cardiometabolic risk and physical performance in abdominally obese, older adults: a pilot feeding study. J Nutr Health Aging 2015;19:87–95.
[10] Aoyama T, Fukui K, Takamatsu K, Hashimoto Y, Yamamoto T. Soy protein isolate and its hydrolysate reduce body fat of dietary obese rats and genetically obese mice (yellow KK). Nutrition 2000;16:349–54.
[11] Nagasawa A, Fukui K, Funahashi T, Maeda N, Shimomura I, Kihara S, et al. Effects of soy protein diet on the expression of adipose genes and plasma adiponectin. Horm Metab Res 2002;34:635–9.
[12] Naaz A, Yellayi S, Zakroczymski MA, Bunick D, Doerge DR, Lubahn DB, et al. The soy isoflavone genistein decreases adipose deposition in mice. Endocrinology 2003;144:3315–20.
[13] Moriyama T, Kishimoto K, Nagai K, Urade R, Ogawa T, Utsumi S, et al. Soybean β-conglycinin diet suppresses serum triglyceride levels in normal and genetically obese mice by induction of β-oxidation, downregulation of fatty acid synthase, and inhibition of triglyceride absorption. Biosci Biotechnol Biochem 2004;68:352–9.
[14] Lanou AJ. Soy foods: are they useful for optimal bone health? Ther Adv Musculoskelet Dis 2011;3:293–300.
[15] Messina M. Soy foods, isoflavones, and the health of postmenopausal women. Am J Clin Nutr 2014;100(Supplement 1):423S–30S.
[16] Hsu YJ, Huang WC, Chiu CC, Liu YL, Chiu WC, Chiu CH, et al. Capsaicin supplementation reduces physical fatigue and improves exercise performance in mice. Nutrients 2016;8:648.
[17] Heyne GW, Plisch EH, Melberg CG, Sandgren EP, Peter JA, Lipinski RJ. A simple and reliable method for early pregnancy detection in inbred mice. J Am Assoc Lab Anim Sci 2015;54:368–71.
[18] Gunderson EP, Abrams B, Selvin S. Does the pattern of postpartum weight change differ according to pregravid body size? Int J Obes Relat Metab Disord 2001;25:853–62.
[19] Neacsu M, Fyfe C, Horgan G, Johnstone AM. Appetite control and biomarkers of satiety with vegetarian (soy) and meat-based high-protein diets for weight loss in obese men: a randomized crossover trial. Am J Clin Nutr 2014;100:548–58.
[20] de Castro MBT, Kac G, de Leon AP, Sichieri R. High-protein diet promotes a moderate postpartum weight loss in a prospective cohort of Brazilian women. Nutrition 2009;25:1120–8.
[21] World Health Organization, WHO. Obesity and overweight; 2016.
[22] Nascimento SL, Pudwell J, Surita FG, Adamo KB, Smith GN. The effect of physical exercise strategies on weight loss in postpartum women: a systematic review and meta-analysis. Int J Obes 2014;38:626.
[23] Markofski MM, Jennings K, Timmerman KL, Dickinson JM, Fry CS, Borack MS, et al. Effect of aerobic exercise training and essential amino acid supplementation for 24 weeks on physical function, body composition and muscle metabolism in healthy, independent older adults: a randomized clinical trial. J Gerontol A Biol Sci Med Sci 2018 (In press).
[24] Pasiakos SM, McLellan TM, Lieberman HR. The effects of protein supplements on muscle mass, strength, and aerobic and anaerobic power in healthy adults: a systematic review. Sports Med 2015;45:111–31.
[25] Kan NW, Ho CS, Chiu YS, Huang WC, Chen PY, Tung YT, et al. Effects of resveratrol supplementation and exercise training on exercise performance in middle-aged mice. Molecules 2016;21:661.
[26] Chen WC, Huang WC, Chiu CC, Chang YK, Huang CC. Whey protein improves exercise performance and biochemical profiles in trained mice. Med Sci Sports Exerc 2014;46:1517.
[27] Deering RE, Cruz M, Senefeld JW, Pashibin T, Eickmeyer S, Hunter SK. Impaired trunk flexor strength, fatigability, and steadiness in postpartum women. Med Sci Sports Exerc 2018 (In press).
[28] Xu C, Lv J, You S, Zhao Q, Chen X, Hu X. Supplementation with oat protein ameliorates exercise-induced fatigue in mice. Food Funct 2013;4:303–9.
[29] Treuth MS, Butte NF, Puyau M. Pregnancy-related changes in physical activity, fitness, and strength. Med Sci Sports Exerc 2005;37:832–7.
[30] Zourladani A, Zafrakas M, Chatzigiannis B, Papasozomenou P, Vavilis D, Matziari C. The effect of physical exercise on postpartum fitness, hormone and lipid levels: a randomized controlled trial in primiparous, lactating women. Arch Gynecol Obstet 2015;291:525–30.
[31] Brown EC, DiSilvestro RA, Babaknia A, Devo ST. Soy versus whey protein bars: effects on exercise training impact on lean body mass and antioxidant status. Nutr J 2004;3:22.
[32] Shenoy S, Dhawan M, Singh Sandhu J. Four weeks of supplementation with isolated soy protein attenuates exercise-induced muscle damage and enhances muscle recovery in well trained athletes: a randomized trial. Asian J Sports Med 2016;7:e33528.
[33] Hsu YJ, Huang WC, Lin JS, Chen YM, Ho ST, Huang CC, et al. Kefir supplementation modifies gut microbiota composition, reduces physical fatigue, and improves exercise performance in mice. Nutrients 2018;10:862.
[34] Hargreaves M. Exercise, muscle, and CHO metabolism. Scand J Med Sci Sports 2015;25:29–33.
[35] Wan JJ, Qin Z, Wang PY, Sun Y, Liu X. Muscle fatigue: general understanding and treatment. Exp Mol Med 2017;49:e384.
[36] Gosker HR, Schols AM. Fatigued muscles in COPD but no finishing line in sight. Eur Respir J 2008;31:693–4.
[37] Verlander JW, Chu D, Lee HW, Handlogten ME, Weiner ID. Expression of glutamine synthetase in the mouse kidney: localization in multiple epithelial cell types and differential regulation by hypokalemia. Am J Physiol Renal Physiol 2013;305:F701–13.
[38] Liu Y, Zhou Y, Nirasawa S, Tatsumi E, Cheng Y, Li L. In vivo anti-fatigue activity of sufu with fortification of isoflavones. Pharmacogn Mag 2014;10:367.
[39] Emhoff CAW, Messonnier LA, Horning MA, Fattor JA, Carlson TJ, Brooks GA. Gluconeogenesis and hepatic glycogenolysis during exercise at the lactate threshold. J Appl Physiol 2012;114:297–306.
[40] Baird MF, Graham SM, Baker JS, Bickerstaf GF. Creatine-kinase-and exercise-related muscle damage implications for muscle performance and recovery. J Nutr Metab 2012:960363.
[41] Ren G, Yi S, Zhang H, Wang J. Ingestion of soy–whey blended protein augments sports performance and ameliorates exercise-induced fatigue in a rat exercise model. Food Funct 2017;8:670–9.
[42] Hakkak R, Gauss CH, Bell A, Korourian S. Short-term soy protein isolate feeding prevents liver steatosis and reduces serum ALT and AST levels in obese female Zucker rats. Biomedicine 2018;6:55.
[43] Incir S, Bolayirli IM, Inan O, Aydın MS, Bilgin IA, Sayan I, et al. The effects of genistein supplementation on fructose induced insulin resistance, oxidative stress and inflammation. Life Sci 2016;158:57–62.
[44] Tovar AR, Torres N. The role of dietary protein on lipotoxicity. Biochim Biophys Acta 1801;2010:367–71.
[45] Adeva-Andany MM, González-Lucán M, Donapetry-García C, Fernández-Fernández C, Ameneiros-Rodríguez E. Glycogen metabolism in humans. BBA Clin 2016:85–100.
[46] Knuiman P, Hopman MT, Mensink M. Glycogen availability and skeletal muscle adaptations with endurance and resistance exercise. Nutr Metab 2015;12:59.
[47] May FJ, Baer LA, Lehnig AC, So K, Chen EY, Gao F, et al. Lipidomic adaptations in white and brown adipose tissue in response to exercise demonstrate molecular species-specific remodeling. Cell Rep 2017;18:1558–72.
[48] Kong X, Yao T, Zhou P, Kazak L, Tenen D, Lyubetskaya A, et al. Brown adipose tissue controls skeletal muscle function via the secretion of myostatin. Cell Metab 2018;28:631–43.
[49] Cannon B, Nedergaard J. Nonshivering thermogenesis and its adequate measurement in metabolic studies. J Exp Biol 2011;214:242–53.
[50] Takahashi Y, Ide T. (2008). Effects of soy protein and isoflavone on hepatic fatty acid synthesis and oxidation and mRNA expression of uncoupling proteins and peroxisome proliferator-activated receptor γ in adipose tissues of rats. J Nutr Biochem 2008;19:682–93.
[51] Crespillo A, Alonso M, Vida M, Pavón FJ, Serrano A, Rivera P, et al. Reduction of body weight, liver steatosis and expression of stearoyl-CoA desaturase 1 by the isoflavone daidzein in diet-induced obesity. Br J Pharmacol 2011;164:1899–915.