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Sustained release of branched-chain amino acids from slow-digesting whey enhances endurance running performance and improves skeletal muscle protein synthesis in mice

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ABSTRACT

Post-exercise whey protein isolate (WPI) supplement is beneficial for skeletal muscle recovery due to the stimulation of branched chain amino acids (BCAAs). This implies us that intake slow digestion rate of protein to sustain BCAAs releasing rate may facilitate muscle protein synthesis. To examine this hypothesis, we conducted a series of protein supplements including modified slow-digesting whey (SDW), whey, hydrolyzed whey and casein, orally to mice undergoing endurance running. Our results showed that the SDW gavage constant supplied BCAAs in the serum of mice within 6 h and significantly enhanced ($P < 0.01$) endurance exercise capacity, compared to other groups. In addition, the SDW supplementation increased the cross-sectional area of mice gastrocnemius fibers, as well as their muscle and liver glycogen content. It also increased the testosterone/cortisol ratio in serum and interleukin-6 (IL-6) levels in muscle, while it decreased the tumor necrosis factor-alpha (TNF-α) levels and oxidative stress in muscle. Moreover, it may activate mechanistic target of rapamycin signaling by upregulating mRNA (*bcat-1* and *pgc-1α*) expression. Thus, our findings illustrate that prolonged BCAAs supply duration promotes mice endurance running capacity and skeletal muscle growth, contributing to the advancement of sports nutrition practices.

1. Introduction

Highly intense exercise induces muscle damage resulting in muscle degeneration *via* the degradation of structural proteins, such as myofibrils and cytoskeletal proteins[1]. The ultrastructural damage and muscle protein degradation can persistently exist during the first 12–72 h post-exercise, and these metabolic alterations increase the skeletal muscle cell's demand for dietary protein over a long period[2-3]. Branched chain amino acids (BCAAs) positively influence mammalian muscle protein synthesis (MPS) and muscle development[4]. Consequently, the consistent and prolonged release of BCAAs from protein supplements has promising prospects. Among various proteins, the intimate association between whey protein isolate (WPI) enriched with BCAAs and MPS has been established over last few decades[5]. And it can also mitigate muscle inflammation and expedite muscle recovery[6-7]. Owing to the rapid digestion rate, a single intake of WPI cannot maintain sustained sufficient serum BCAAs. Despite casein being generally recognized as a slow-digesting protein, it contains a lower concentration of BCAAs compared to whey, which results in limited effects on MPS[8-9]. Interestingly, other researches indicate that intravenous injection of BCAAs alone did not result in higher protein anabolism than catabolism[10]. Although, only intake of BCAAs stimulated the translation initiation pathway, it did not

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maximally stimulate postexercise myofibril MPS. In the presence of an adequate supply of leucine, the order of stimulation to MPS is complete protein > essential amino acids (EAAs) > BCAAs > leucine supply alone[11]. A possible explanation for the lower stimulation of MPS by ingestion of BCAAs alone than with whey protein is that the limited availability of EAAs can not provide sufficient substrates for MPS[12]. It is necessary to intake of protein enriched with BCAAs rather than BCAAs supplements alone. Thus, a slow digestive protein with sustained BCAAs release duration is needed. Transglutaminase (TG) and thermal treatment are mild, effective and convenient strategies to prepare WPI gel[13]. Compared with readily water-soluble WPI, the modified protein gel including protein aggregates and stable gel structure leads to less access to digestion enzyme. It (denoted as slow-digesting whey, SDW) may result in slow down the digestion rate of WPI and constant supply of BCAAs to improve post-exercise muscle recovery.

In addition to stimulating post-exercise muscle synthesis, BCAAs can also mitigate fatigue and enhance sports performance[14]. Endurance running exposes athletes to extreme physiological circumstances resulting in consuming glycogen storage, releasing hormone, increasing oxidative stress[15]. Supplementation with BCAAs has a dose dependent effect on promoting a higher hepatic and muscle glycogen content in trained animals, and branched chain α-keto acids are endogenous metabolites of BCAAs can alleviate oxidative stress[16-17]. Nevertheless, the impact of supplementing with different rates of BCAA release on endurance exercise performance remain controversial. Intake of rapidly digesting proteins, such as WPI and hydrolyzed WPI (HWPI), leads to an immediate peak of BCAAs in serum, while SDW ingestion results in a high level and prolonged BCAAs supply. Hence, to investigate the influence of sustained BCAAs supply and instantaneous BCAAs supply on sports performance, forced uphill endurance running is employed on mice subjected to different digestion rate of protein gavage.

Herein, we present the SDW produced through the combination of TG enzyme and thermal treatment, providing a continuous supply of BCAAs *in vivo*. Additionally, a forced endurance running mouse model was selected to verify the impact of varying BCAAs releasing rate (from ingested SDW, WPI, HWPI and casein) on improving post-exercise muscle recovery and endurance capability. Besides this, the glycogen storage, testosterone/cortisol ratio in serum, muscle oxidative stress, inflammatory factors and muscle mRNA relative expression were assessed. Our results may reveal the potential regulation of BCAAs releasing rate as a post-exercise supplement for promoting muscle growth, attenuating muscle inflammation and enhance endurance running performance.

2. Materials and methods

2.1 Sample preparation

WPI 292 (containing 90% protein) was purchased from Fonterra Co-operative Group Ltd. (Auckland, New Zealand). The WPI aqueous solution (10% (*m*/*V*) , pH 7, Fig. 1a) was heated at 80 °C for 30 min. After cooling to 50 °C, it was mix with TG (200 U/g protein) to produce SDW (Fig. 1b). HWPI (degree of hydrolysis 12%) was obtained from WPI 292 treated with Alcalase 2.4 L (Novozymes Biotechnology Co., Ltd., Beijing, China). Casein was purchased from Beijing InnoChem Science & Technology Co., Ltd. (Beijing, China).

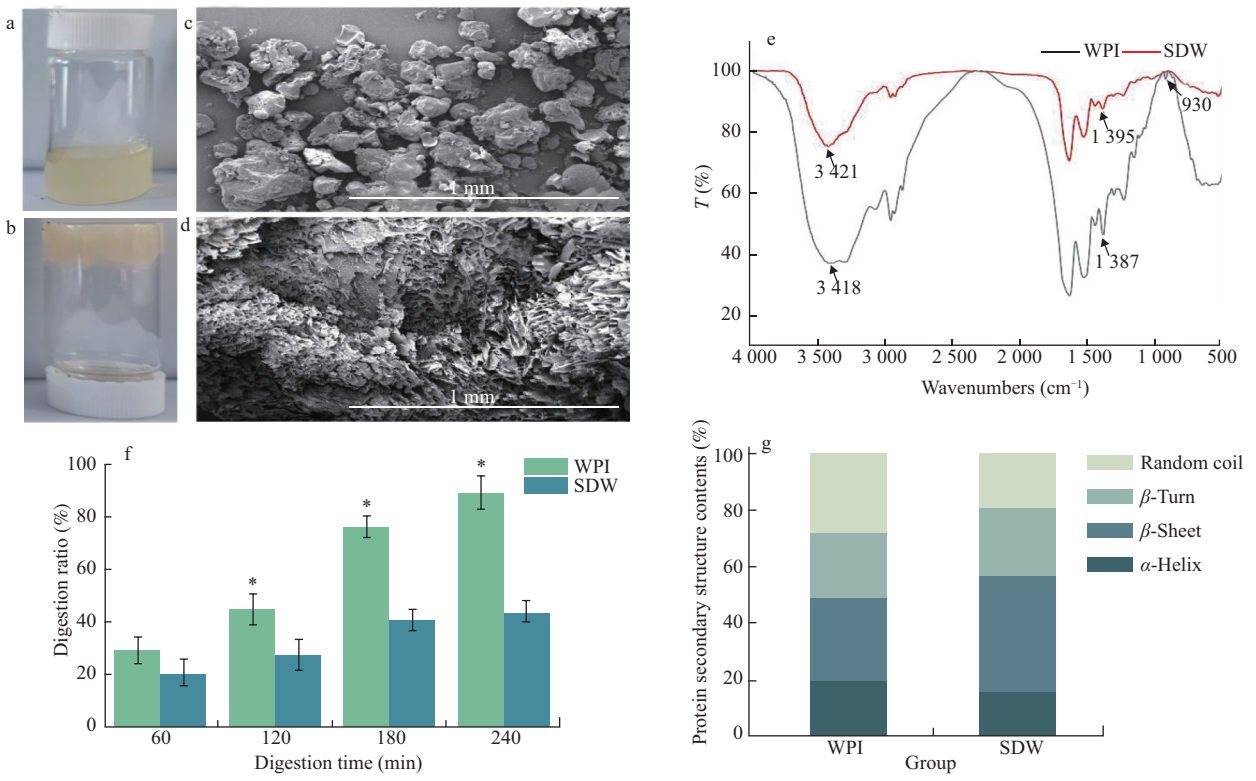

Fig. 1 Morphological appearance of (a) WPI and (b) SDW samples. SEM micrographs of (c) WPI and (d) SDW samples, and the magnification is shown in each photograph. (e) FTIR spectra of WPI and SDW. (f) The *in vitro* digestion ratio of WPI and SDW, * means significant difference ($P < 0.05$, compared with SDW group). (g) Secondary structure contents of WPI and SDW. The concentration of BCAAs in mice serum released from (h) WPI and (i) SDW.

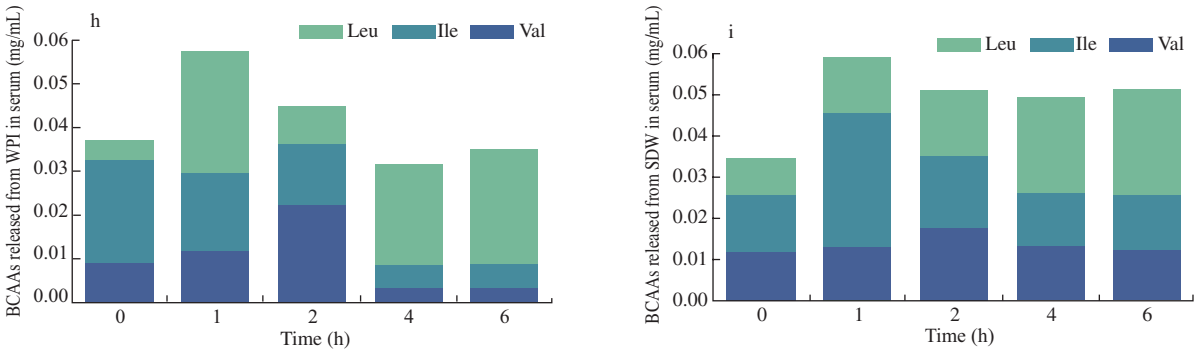

Fig. 1 (*Continued*)

2.2 Scanning electron microscope (SEM) analysis

After freeze-drying, the WPI sample was dispersed powder, while the SDW sample was a whole block due to its gel properties. The SDW gel sample was cut into cubes with a side length of 2–3 mm to expose the internal microstructure. The gold sputter-coating was conducted prior to SEM analysis. Subsequently, the images were observed using scanning electron microscopy (FEI, Eindhoven, the Netherlands) at 10 kV.

2.3 Fourier transform infrared (FTIR) spectra Analysis

Freeze-dried WPI and SDW (1 mg) were individually mixed with 200 mg KBr and laminated to fabricate transparent film. The FTIR spectra were acquired using the FT-NIR/IR spectrometer (IS10, Nicolet, USA) within the wavelength range of 4 000–500 cm^{-1} by the acquisition of 32 scans at a resolution of 4 cm^{-1}.

2.4 In vitro digestibility of WPI and SDW

The *in vitro* INFOGEST simulated digestion tests of WPI and SDW were conducted according to the methods propose by Yao et al.[18]. And the protein digestible rate was calculated according to our previous study[13].

2.5 Secondary structure characterization

The circular dichroism (CD) spectra of WPI and SDW were recorded with a spectropolarimeter (V100, Applied Photophysics Ltd., UK) in the far-UV region (190–260 nm) at 25 °C. The sample concentration was 1 mg/mL in a 0.1 mm quartz cell. Alterations in the content of protein secondary structures were calculated by CDPro software (https://www.bmb.colostate.edu/cdpro/).

2.6 Animals

Male young adult C57BL/6 mice (n = 98; Gempharmatech Co., Ltd., Jiangsu, China; 2 months) were used in this study. The trial was conducted in accordance with the Chinese guidelines for animal welfare and received approval from the Ethics Committee of the Jiangnan University of Science and Technology (JN.No20220315c0800610[017]). Animals were habituated to the animal facility for one week before experiments started and kept under a 12-h light/dark cycle, with a temperature of (22 ± 1) °C. Food and water were given ad libitum.

2.7 The analysis of BCAAs releasing rate in vivo

Two groups of mice (n = 25 per group) were conducted to evaluate the BCAAs releasing rate from WPI and SDW, separately. After 12 h of fast, mice were orally administrated with an equal amount of protein (200 μL 100 mg/mL WPI solution and SDW gel), individually. Blood was collected from the mice at 0, 1, 2, 4 and 6 h after oral gavage, and serum was separated by centrifugation (3 000 r/min, 10 min). Each group consisted of 5 mice at each timepoint. The BCAAs concentration in serum was measured by an automatic amino acid analyzer (Sykam GmbH, Germany).

2.8 Mice forced running experiments

Mice were randomly assigned to one of 6 treatments (n = 8) including one stationary group and five forced running groups, details provided in Fig. 1. In forced running groups, the mice underrunning controlment a forced uphill running task at a 15° incline, reaching a final velocity of 12 m/min for 30 min. This running training lasted for 8 weeks and was conducted 6 times per week. Post-running, mice were administered different samples *via* gavage, and the total protein intake is the equal among SDW, WPI, HWPI and casein treatment groups. After final behavior experiment, mice were euthanized to collect blood, liver and gastrocnemius muscles for further analysis as described in a previous study[19].

2.9 Endurance running test

The endurance capabilities of the mice were assessed on the final day of the 8-week exercise protocol. Following a 30-min forced run, a rotating rod fatigue mice assay was conducted according to the method described by Zhong et al.[20]. The rotating speed was set at 30 r/min, and the duration from when each mouse began running to when it left the rotating rod was recorded as the endurance running time.

2.10 Skeletal muscle and liver glycogen content measurements

Mice skeletal muscle and hepatic glycogen content was measured by using commercial kit purchased from Solarbio Science and Technology Co., Ltd. (Beijing, China).

2.11 Gastrocnemius muscles histology

The gastrocnemius muscles histology was examined using the following methods[21]. Briefly, the gastrocnemius muscles was excised and fixed in 4% paraformaldehyde solution for 24 h, and the samples were then dehydrated with increasing concentrations (50%, 70%, 85%, 95%) of ethanol and transparentized in xylene. The samples were embedded in molten paraffin, sectioned into 5 μm slices using a microtome, and placed on clean glass slides to dry at 37 °C overnight. The tissue sections were dewaxed in xylene for 5–10 min twice and dehydrated in different concentrations of ethanol for 2–5 min. These sections were subsequently stained with hematoxylin and eosin (H&E) for morphological and structural analysis of the muscles.

2.12 Quantification of muscle fiber cross-sectional area

Quantification of the muscle histology was performed over the entire gastrocnemius fiber cross section by using ImageJ software (http://rsbweb.nih.gov/ij).

2.13 Biochemical analysis in muscle tissue

Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) in gastrocnemius muscle was tested using commercial kits (Solarbio, China). Superoxide dismutase (SOD), nitric oxide (NO), catalase (CAT) and malondialdehyde (MDA) content in skeletal muscle was evaluated using commercial kits (Nanjing Jiancheng Bioengineering Insitate, Nanjing, China) according to the manufacturer's instruction.

2.14 Serum testosterone and cortisol evaluation

The testosterone and cortisol in mice serum was measured using ELISA kit (Shanghai Enzyme Linked Biotechnology Co., Ltd.).

2.15 RNA extraction and real-time quantitative polymerase chain reaction (PCR)

Total RNA was isolated from the skeletal muscle by using RNAiso Plus (Takara Biomedical Technology (Beijing) Co., Ltd.). Subsequently, the cDNA was then performed using the PrimeScript™ RT Master Mix (Takara). After cDNA synthesis, the LightCycler 480 II RT-PCR system (Roche Co., Ltd., USA) was employed to detect the mRNA expression level of branched-chain aminotransferase 1 (*bcat-1*) and peroxisome proliferator-activated receptor γ coactivator 1α (*pgc1-α*). The expression of glyceraldehyde-3-phosphate dehydrogenase (*gaphd*) was used as the reference gene. The sequences of the PCR primers were shown in Table S1.

2.16 Statistical analysis

All results are expressed as the mean ± standard deviation from a minimum of 3 independent experiments. One-way analysis of variance (ANOVA) was used for statistical significance analysis by using GraphPad Prism 8. A P-value less than 0.05 was deemed statistically significant. The significance levels are denoted as follows: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3. Results and discussion

3.1 The characterization of SDW and WPI

The visual appearance of WPI and SDW is shown in Figs. 1a-b. In comparison to the WPI aqueous solution, the SDW sample formed a stable gel that could remain at the bottom of an inverted bottle. This phenomenon illustrates that the combination of heat and TG enzyme (H-TG) cross-linking treatment results in the mutual interaction of denatured proteins, inducing gel formation. The dimensional properties and micromorphology of WPI and SDW can be intuitively observed by SEM. The depiction in Fig. 1c reveals that WPI exhibits a dispersed structure, leading to excellent water solubility and a rapid digestion rate[22]. Conversely, the SDW shows the distinct compacted sheet structures, a result of the H-TG treatment. Evidently, the thermal treatment unfolds the tertiary structure of SDW, exposing ε-amino on the lysine residues and enhancing the cross-linking efficiency of TG enzyme, resulting in a stable protein gel[23]. Conversely, the SDW (Fig. 1d) shows the distinct compacted sheet structures.

As presented in Fig. 1e, FTIR spectra of WPI and SDW were performed to provide better information about the effect of H-TG treatment on chemical bonds of protein. The Amide A band (3 750–3 000 cm^{-1}) corresponds to intermolecular hydrogen bonds involving N–H and O–H stretching vibrations[24]. The peak of WPI, initially located at 3 421 cm^{-1}, was slightly red-shifted to 3 418 cm^{-1}. The H-TG treatment generated covalent linkages and iso-peptide bonds, resulting in an increase in the amplitude of amide A due to the induction of numerous bonded N–H groups[25]. The peaks between 3 000 and 2 750 cm^{-1} belong to the Amide B band, relating to C–H stretching vibrations of $–CH_2$. The higher amplitudes of the peaks observed in SDW spectra could be attributed to interactions involving hydrogen bonds with water, protein, and TG enzyme[26]. In contrast, the spectrum of SDW showed a new characteristic absorption peak at 930 cm^{-1}, generated by C–O–C stretching vibrations.

The *in vitro* digestible ratio of SDW and WPI is depicted in Fig. 1f. WPI could be digested nearly 90% at 4 h, while SDW could be digested for approximately 40% after 4 h digestion, and the digestible ratio of SDW was significantly lower ($P < 0.05$) than that of WPI during 2–4 h. It may result from that the protein aggregates and gel structure reduced the SDW contact with digestive enzymes.

The secondary structure contents of WPI and SDW were calculated and shown in Fig. 1g. The H-TG treatment induced plenty of changes to SDW secondary structures. Specifically, the concentration of random coil decreased from 28.4% (WPI) to 19.2% (SDW), indicating that the H-TG treatment increased structural order. The concentration of α-helix showed a slight decline from 19.0% to 15.6%. This finding aligns with a previous

report on H-TG treatment affecting *Decapterus maruadsi* surimi, which was attributed to the rupture or weakening of hydrogen bonds to form stronger intermolecular hydrogen bonds[27-28]. However, the β-sheet contents markedly increased from 30.2% to 41.1%, suggesting that the protein structure was more extended prior to undergoing H-TG treatment[29].

The fluctuations of BCAAs concentration in mice serum during 6 h were shown in Figs. 1h-i. Initially, the BCAAs concentration was low due to overnight fasting. One hour post-gavage, the BCAAs concentration rose to 0.059 mg/mL (WPI) and 0.057 mg/mL (SDW). BCAAs in WPI group were sharply declined to the beginning concentration in 4 h. This illustrates that the WPI undergoes rapid digestion, releasing abundant BCAAs immediately, but it cannot sustainably supply BCAAs. BCAAs in SDW group maintained a slightly lower level than that of WPI in 1 h post-gavage, but it consistently remained higher than the WPI group during the subsequent 5 h. The slow BCAAs releasing rate of SDW is due to stable gel structure, reducing the contact with digestive enzymes, and it could also be attributed to the changes in the secondary structures, particularly the increase of β-sheet structures leading to a decrease in digestibility[13,30]. In a word, the SDW is slow digestive protein that constantly releases BCAAs into serum within 6 h.

3.2 SDW treatment enhances mice exercise endurance and improves glycogen storage in liver and skeletal muscle

Mice receiving different proteins orally manifested different endurance exercise durations, as presented in Fig. 2a. Given the constant running speed, the longer running endurance time indicates the stronger endurance exercise capacity. The SDW treatment group demonstrated the longest running time ((87.50 ± 28.05) s, $P < 0.01$), significantly longer than other groups. SDW, WPI and HWPI contain higher concentration of BCAAs compared with casein, while SDW can sustain 6 h high serum BCAAs concentration (Fig. 1h). Thus, the sustained BCAAs release enhances endurance running ability of mice due to the high serum BCAAs concentration, which competes with tryptophan, its precursor, for passage across the blood-brain barrier, thereby reducing cerebral serotonin synthesis, decreasing fatigue, and enhancing endurance performance in athletes[31-32]. Furthermore, glycogen, a branched polymer of glucose primarily stored in the liver and skeletal muscle, serves as a major energy source for endurance sports[33]. The relative concentration of muscle glycogen reserve is highly correlated with exercise performance[34]. The glycogen contents in mice skeletal muscle and liver were shown in Figs. 2b-c. The muscle glycogen of stationary control group ((0.62 ± 0.10) mg/mg prot) was the lowest, consistent with the notion that endurance sports training improves muscle glycogen accumulation[35]. Skeletal muscle glycogen of SDW treatment group was (1.24 ± 0.17) mg/mg prot, significantly higher than that of running control ((0.87 ± 0.07) mg/mg prot, $P < 0.05$) and WPI treatment ((0.86 ± 0.05) mg/mg prot, $P < 0.05$). Muscle glycogen synthesis following its depletion by endurance exercise is insulin dependent, and WPI enriched with BCAAs can stimulate the insulin secretion[36-37]. Besides this, mice receiving a SDW gavage maintained high BCAAs level in serum for long time can increase muscle and hepatic glycogen storage[16]. In addition, hepatic glycogen mobilizes in response to the incremental glucose demands from skeletal muscle, and is another key regulator of endurance capacity in mice[38]. Its content was highest in the SDW treatment group at (15.48 ± 0.57) mg/mg prot and significantly higher ($P < 0.05$) than that of stationary control groups ((10.60 ± 2.64) mg/mg prot). As noted above, mice in SDW treatment group with high level muscle and hepatic glycogen enhanced endurance running performance.

3.3 SDW treatment reduces mice gastrocnemius inflammation and increases muscles fiber aera

It is well-established that exhausted endurance or high-intensity sports can impair muscle fibers, induce muscle inflammation, and increase muscle soreness[39-41]. The HE-stained cross sections of gastrocnemius muscle fibers were shown in Figs. 3a-f. Several

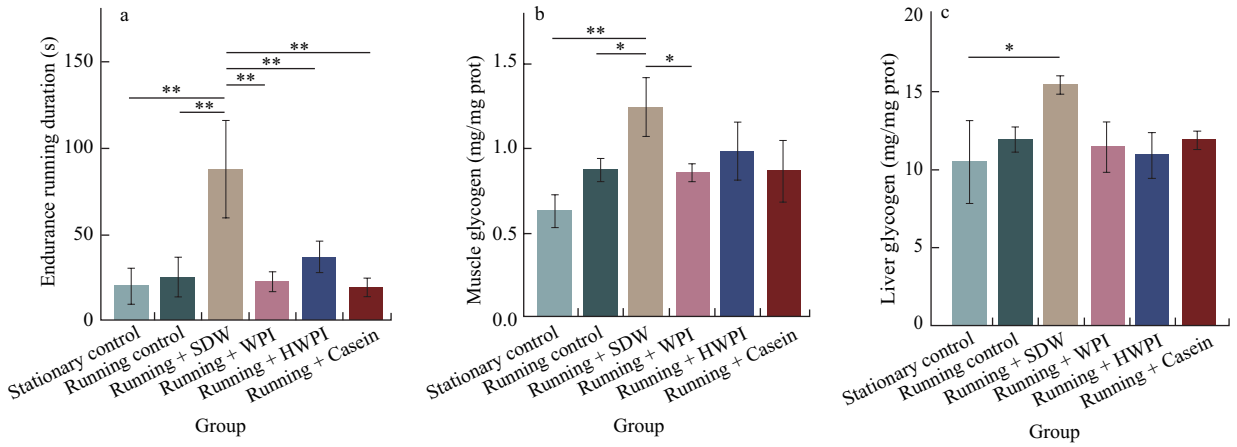

Fig. 2 (a) The mice endurance running time, (b) gastrocnemius muscle glycogen contents, and (c) liver glycogen contents. The error bar represents the standard deviation error. * $P < 0.05$ and ** $P < 0.01$, the same as below.

regions of inflammatory infiltrate were present in the four running groups (marked with red circle in Figs. 3b, d-f) except stationary control and SDW treatment group. Despite following the same running protocol, no obvious inflammatory area was observed in gastrocnemius of the SDW treatment group. Without suitable protein supplementation, unrecovered muscles are disruptive to locomotor performance, which were consistent with endurance running ability (Fig. 2a). The total protein intake among SDW, WPI, HWPI and casein treatments is equal, thus the BCAAs supply form is critical for alleviating post-exercise muscle damage. The quantification of mice average gastrocnemius fiber cross-sectional area was shown in Fig. 3g, and the muscle fiber size is positively correlated with strength and leg extension[42]. No significant distinctions in the size of muscle fibers were noticed between mice in stationary control and endurance running control groups, although Kazior et al.[43] suggested that human subjected to long-term endurance running training enlarge human muscle fibers. This could be attributable to murine having a lower proportion of slow twitch muscle fibers than human[44]. Mice in SDW treatment group had the biggest average gastrocnemius fiber size ((922.28 ± 161.30) μm^2, $P < 0.01$), significantly larger than other groups. It is more likely due to the fact that sustained BCAAs supply may lead to stimulation of muscle anabolism rather than inhibition of catabolic processes. The average gastrocnemius fiber cross-sectional area of HWPI treatment group ((673.17 ± 160.97) μm^2) was the second biggest and significantly bigger than that of WPI treatment group ((783.87 ± 169.29) μm^2, $P < 0.05$). The immediate BCAAs release is associated with positive post-exercise MPS effect, while it is still weaker than that of sustained BCAAs release.

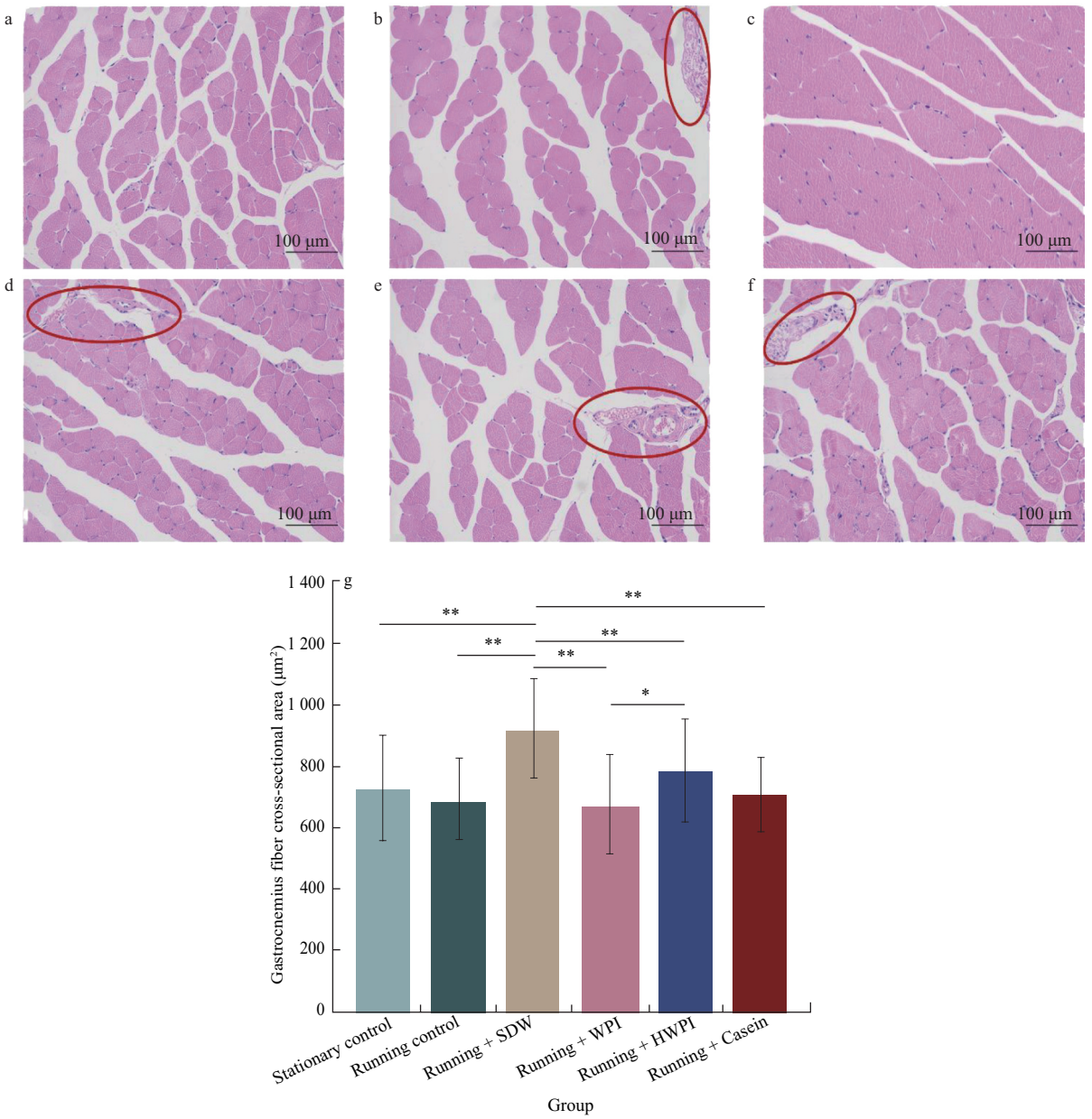

Fig. 3 The HE-stained paraffin sections of mice gastrocnemius in (a) Stationary control, (b) Running control, (c) Running + SDW, (d) Running + WPI, (e) Running + HWPI, (f) Running + Casein, (f) RCA, scale bar: 100 μm; inflammation area in muscle fibers marked with red circle. (g) Quantification of average gastrocnemius fiber cross-sectional area.

3.4 SDW treatment elevates testosterone/cortisol ratio in serum

Testosterone is a naturally secreted androgenic-anabolic hormone that promotes intramuscular amino acid uptake, leading to muscle growth[45]. Furthermore, it can protect against development of widespread, long-lasting activity-induced muscle pain[46]. No difference of the testosterone concentration in serum (Fig. 4) were seen among six groups, while mice receiving SDW ((1.95 ± 0.23) ng/mL) showed an upward trend, compared to control groups. In contrast to testosterone, cortisol negatively correlates with MPS, and reduces muscle strength and mass[47]. The concentration of cortisol (Fig. 4b) in SDW treatment group ((1.88 ± 0.37) ng/mL) showed a downward trend. Although, there were no significant difference in serum testosterone and cortisol content among all groups, the ratio of these 2 hormones is critical for post-exercise recovery of muscle function. Therefore, it is imperative to investigate whether the form of post-exercise BCAA supplementation can enhance testosterone levels and suppress cortisol levels, thereby positively impacting muscle growth. The testosterone/cortisol ratio (Fig. 4c) of mice in SDW treatment group (1.06 ± 0.24) was significantly higher than that of stationary control (0.56 ± 0.17, $P < 0.05$), running control (0.56 ± 0.10, $P < 0.05$) and HWPI treatment (0.54 ± 0.04, $P < 0.05$) groups. Mice administered sustained BCAAs supply demonstrated the strong effect on increasing testosterone and reducing cortisol in serum, facilitating MPS, and further validating the largest muscle fiber area observed in Fig. 3.

3.5 SDW treatment mitigates the oxidative stress of skeletal muscle

Endurance running and other exercise that increase oxygen utilization can raise the likelihood of free radical formation, which are scavenged by a crucial antioxidant SOD[48]. The SOD activities in mice skeletal muscle were measured and presented in Fig. 5a. The SDW treatment group showed significantly higher SOD activity ((480.62 ± 77.79) U/mg prot, $P < 0.05$) than stationary control ((330.13 ± 40.35) U/mg prot, $P < 0.05$), running control ((365.06 ± 48.27) mg/mg prot, $P < 0.05$) and casein treatment ((311.21 ± 33.85) U/mg prot, $P < 0.05$) groups. Low SOD activity in the muscles of the running control groups may be due to insufficient post-exercise BCAAs supplementation. Interestingly, post-exercise casein supplementation did not increase SOD activity, aligning with previous findings that rats fed a casein diet could not suppress the production of muscle peroxidative products *in vivo*[49]. The CAT is another enzyme to buffer bursts of intracellular reactive oxygen species (ROS) and peroxide production[50]. The CAT activity (Fig. 5b) in muscle of SDW treatment group ((199.28 ± 35.02) U/mg prot) was significantly higher than that of stationary control ((68.04 ± 38.15) U/mg prot, $P < 0.01$) and running control groups ((99.83 ± 19.19) U/mg prot, $P < 0.05$). And the CAT activity of WPI treatment was significantly ($P < 0.05$) higher than that of stationary control. Our results correspond with previous evidence that forced running exercise can increase CAT expression in muscle[51]. Besides this, compared to WPI supplementary, SDW gavage showed an improvement in CAT activities, demonstrating that the slow digestion modification of WPI resulting in sustained BCAAs supply has a stronger ability to adapt to oxidative stress. Without adequate SOD or CAT enzymes present in muscle, free radicals can peroxidize cell membrane lipids, generating MDA, which is particularly harmful to muscle tissue[52]. As shown in Fig. 5c, the MDA content of SDW treatment group ((0.23 ± 0.05) nmol/mg prot) was significantly lower than that of stationary control ((0.38 ± 0.07) nmol/mg prot, $P < 0.05$), running control ((0.55 ± 0.08) nmol/mg prot, $P < 0.001$), ((0.39 ± 0.02) nmol/mg prot, $P < 0.01$), HWPI treatment ((0.42 ± 0.11) nmol/mg prot, $P < 0.01$) and casein treatment ((0.52 ± 0.07) nmol/mg prot, $P < 0.001$), illustrating that the sustained BCAAs supply considerably suppresses the production of MDA. MDA content of running control group was the higher than that of stationary control group, indicating that the endurance running induces oxidative stress resulting to formation of MDA. And the it was significantly higher ($P < 0.01$) than that of WPI treatment group, which was consistent with that supplementation of whey protein decreased the levels of malondialdehyde in rat serum[53].

NO is an ergogenic aid that enhances vasodilation leading to increased blood flow, delivering more oxygen and nutrients to muscles, and inhibiting calpain to protect cells from ion-induced proteolysis[54]. Inhibition of NO can delay and restrict the extent of muscle repair[55]. As depicted in Fig. 5d, the NO content in muscle of SDW treatment group ((2.98 ± 0.30) μmol/mg prot) was significantly higher ($P < 0.05$) than that of running control group ((1.46 ± 0.13) μmol/mg prot). This indicates that mice with sustained BCAAs release elevated the NO content in muscle, which may stimulate the muscle growth.

Overall, the prolonged BCAAs release increases activities of SOD and CAT, which can high effectively scavenge free radicals and hydrogen peroxide, therefore protect the body against the destructive effects. On the other hand, it the decreases concentration of MDA in muscle implying strong antioxidant capacity. Moreover, it leads to high NO content promoting MPS.

3.6 Decrease of INF-α and increase of IL-6 in mice muscle by SDW administration

Overwhelming evidence suggests that muscle injury is related to TNF-α, which up-regulates IncRNAs expression, leading to myotube atrophy[56]. Inhibiting post-trauma TNF-α can enhance myofiber diameter, the ratio of muscle weight to body weight and the twitch muscle force[57]. As presented in Fig. 6a, TNF-α concentration in the muscle of SDW treatment group ((12.41 ± 10.86) pg/mg prot) was significantly lower than that of stationary control ((80.24 ± 45.87) pg/mg prot, $P < 0.05$), HWPI treatment ((70.73 ± 34.67) pg/mg prot, $P < 0.05$) and casein treatment ((88.76 ± 40.11) pg/mg prot, $P < 0.05$). This demonstrates that a sustained release of BCAAs has a more pronounced effect on reducing TNF-α in muscles compared to an instantaneous release of BCAAs, which may aid muscle recovery after endurance running.

Contrary to detrimental effect of TNF-α on muscles, IL-6 plays different role primarily in muscle growth[58]. Muscle-induced and transient expression of IL-6 is an important signaling molecule to promote muscle satellite cells activation, proliferation and terminal differentiation[59]. As shown in Fig. 6b, the IL-6 content of SDW treatment group ((58.44 ± 12.48) pg/mg prot, $P < 0.05$) is

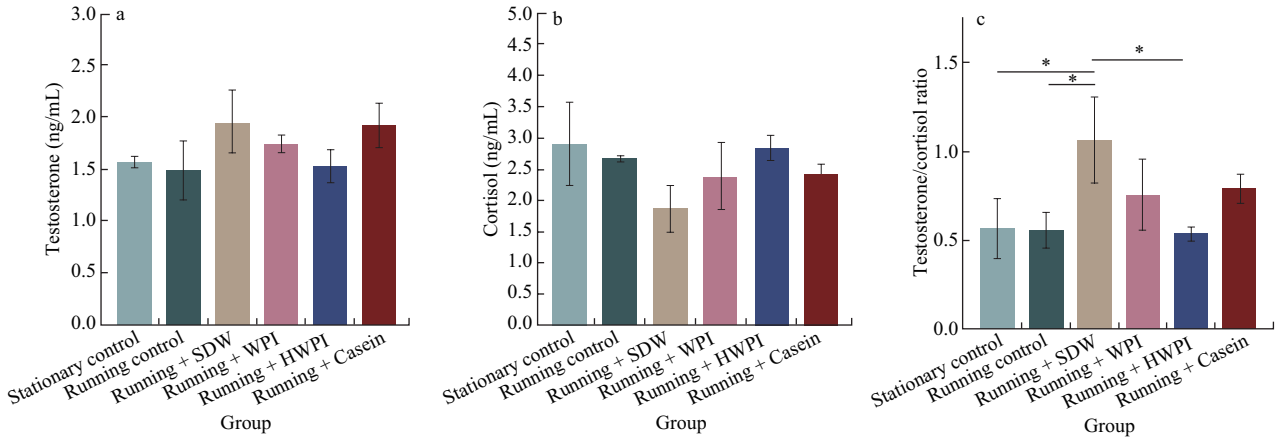

Fig. 4 (a) The testosterone content in serum: nanogram per milliliter, (b) cortisol content in serum: nanogram per milliliter, and (c) ratio of serum testosterone content/serum cortisol content.

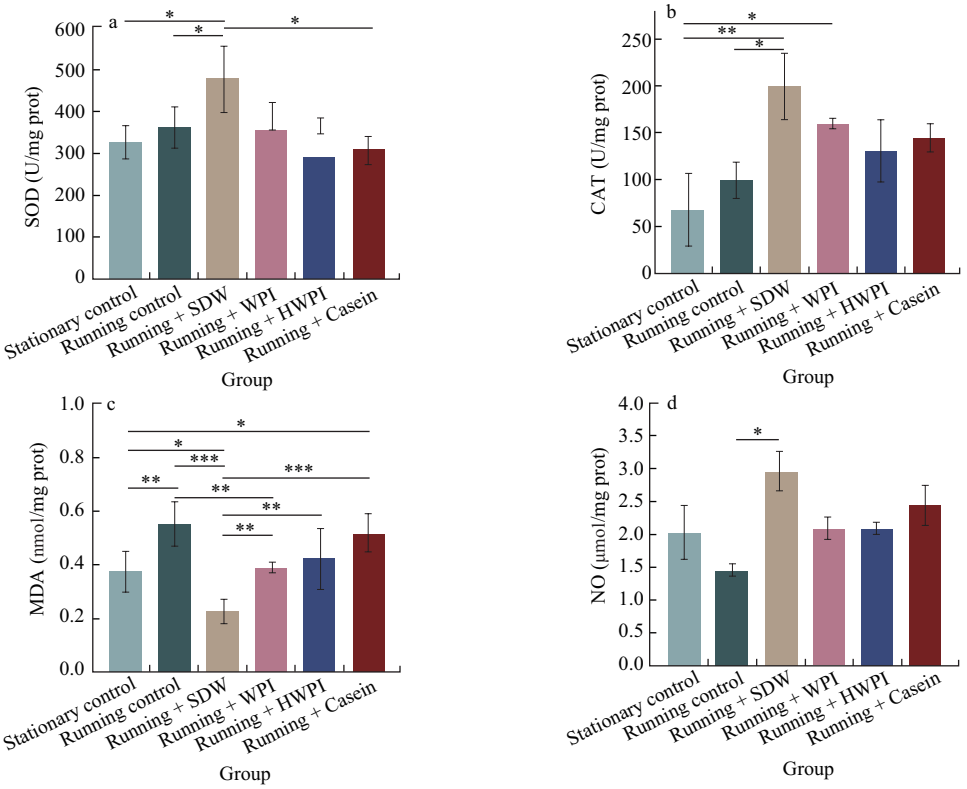

Fig. 5 Activity of (a) SOD and (b) CAT, concentration of (c) MDA and (d) NO in skeletal muscle.

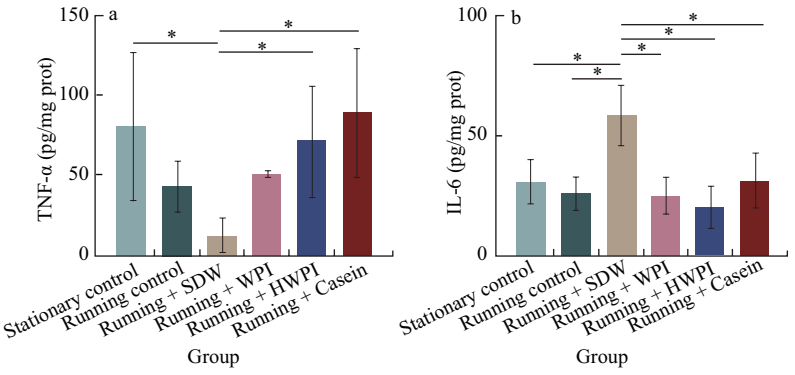

Fig. 6 The concentration of (a) TNF-α and (b) IL-6 in skeletal muscle.

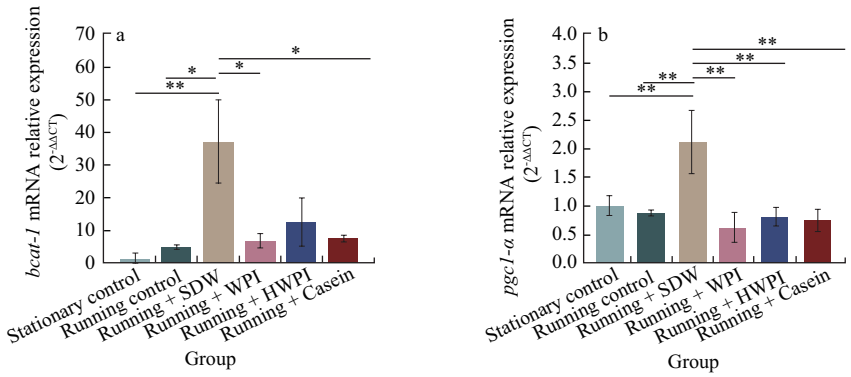

Fig. 7 Relative expression of (a) *bcat-1* and (b) *pgc1-α* mRNA in skeletal muscle.

significantly higher than other groups. Mice gavage with SDW have a long duration of high BCAAs contents in serum, which provides substrates for cytokine and immunoglobulin synthesis and may contribute to lymphocyte proliferation[60]. Thus, the high post-exercise IL-6 level in SDW treatment group can stimulate muscle repair and regeneration.

3.7 SDW upregulating bcat-1 and pgc-1α

The mechanistic target of rapamycin (mTOR) signaling is a critical factor in maintaining skeletal muscle mass[61]. It can trigger *bact-1* expression that facilitates the initial metabolic processing of BCAAs, and this process involves the transfer of the amine group to alpha-ketoglutarate, which results in the production of alpha keto acids and glutamate[62]. In SDW treatment group, bcat-1 expression (Fig. 7a) was upregulated, which was significantly higher than that of running control ($P < 0.01$), stationary control ($P < 0.05$), WPI treatment ($P < 0.05$) and casein treatment ($P < 0.05$) groups. The slow digestion of SDW constantly releasing BCAAs into the serum could potentially activate mTOR signaling, which could upregulate bact-1 expression, promoting growth rate of muscle cells and decreasing ROS levels[63-64]. And our findings, as illustrated in Fig. 3 and Fig. 5, support the notion that the SDW treatment group experienced an increase in muscle fiber area and improved adaptation to oxidative stress. Likewise, the enhancement of mTOR signaling induces the expression of *pgc1-α*, which is a transcriptional coactivator that activates BCAA metabolism[65]. Its expression is increased in the skeletal muscle during exercise and is used for energy in the tricarboxylic acid (TCA) cycle[66]. As shown in Fig. 7b, *pgc1-α* expression in the SDW treatment group was significantly upregulated ($P < 0.01$) compared to other groups. And the expression of *pgc1-α* in skeletal muscles could increase endurance running ability, which interpreted mice in SDW treatment group showing the longest endurance running duration[67]. Thus, *bcat-1* and *pgc1-α* were upregulated in muscle cell can elevate BCAAs metabolism and promote MPS.

4. Conclusion

The SDW is conveniently fabricated by the combination of TG enzyme and heat treatment, sustaining a constant supply of BCAAs *in vivo*. The mouse model demonstrates that SDW gavage effectively enhanced skeletal muscle mass and elevated endurance running capability. The underlying mechanism seems to involve a sustained release of BCAAs that increased the serum testosterone/cortisol ratio and IL-6 levels while concurrently inhibiting TNF-α and oxidative stress. In addition, the mRNA of *bcat-1* and *pgc-1α* expression were upregulated. Thus, our study confirms that the sustained BCAAs release is beneficial to skeletal muscle recovery, constituting a promising approach to widen our perspectives on post-exercise protein supplement and improve athlete' sports performance .

Conflict of interests

All the authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250333.

References

[1] J. Kim, C. Lee, J. Lee, Effect of timing of whey protein supplement on muscle damage markers after eccentric exercise, J. Exerc. Rehabil. 13 (2017) 436-440. https://doi.org/10.12965/jer.1735034.517.

[2] W.J. Evans, Muscle damage: nutritional considerations, Int. J. Sport Nutr. Exe. 1 (1991) 214-224. https://doi.org/10.1123/ijsn.1.3.214.

[3] I. Fatouros, A. Jamurtas, Insights into the molecular etiology of exercise-induced inflammation: opportunities for optimizing performance, J. Inflamm. Res. 9 (2016) 175-186. https://doi.org/10.2147/JIR.S114635.

[4] G. D'Hulst, E. Masschelein, K. de Bock, Resistance exercise enhances long-term mTORC1 sensitivity to leucine, Mol. Metab. 66 (2022). https://doi.org/10.1016/j.molmet.2022.101615.

[5] G.X. Ren, Y.I. Suqing, H. Zhang, et al., Ingestion of soy-whey blended protein augments sports performance and ameliorates exercise-induced fatigue in a rat exercise model, Food Funct. 8 (2017) 670. https://doi.org/10.1039/c6fo01692h.

[6] J.D. Philpott, C. Donnelly, I.H. Walshe, et al., Adding fish oil to whey protein, leucine and carbohydrate over a 6 week supplementation period attenuates muscle soreness following eccentric exercise in competitive soccer players, Int. J. Sport Nutr. Exerc. Metab. (2018) 1-28. https://doi.org/10.1123/ijsnem.2017-0161.

[7] L. Eddens, S. Browne, E.J. Stevenson, et al., The efficacy of protein supplementation during recovery from muscle-damaging concurrent exercise, Appl. Physiol. Nutr. Metab. 42 (2017) 716-724. https://doi.org/10.1139/apnm-2016-0626.

[8] A. Kanda, K. Nakayama, C. Sanbongi, et al., Effects of whey, caseinate, or milk protein ingestion on muscle protein synthesis after exercise, Nutrients 8 (2016) https://doi.org/10.3390/nu8060339.

[9] S. Reitelseder, Whey and casein labeled with *L*-[1-^{13}C] leucine and muscle protein synthesis: effect of resistance exercise and protein ingestion, Am. J. Physiol. Endocrinol. Metab. 300 (2011) 231-242. https://doi.org/10.1152/ajpendo.00513.2010.

[10] R.J. Louard, E.J. Barrett, R.A. Gelfand, Effect of infused branched-chain amino acids on muscle and whole-body amino acid metabolism in man, Clin. Sci. 79 (1990) 457-466. https://doi.org/10.1042/cs0790457.

[11] M. Moberg, W. Apró, B. Ekblom, et al., Activation of mtorc1 by leucine is potentiated by branched-chain amino acids and even more so by essential amino acids following resistance exercise, Am. J. Physiol. Cell Physiol. (2019), https://doi.org/10.1152/ajpcell.00374.2015.

[12] X. Lv, C. Zhou, Q. Yan, et al., Elucidating the underlying mechanism of amino acids to regulate muscle protein synthesis: effect on human health, Nutrition 103-104 (2022) 111797. https://doi.org/10.1016/j.nut.2022.111797.

[13] J. Leng, B. Wang, L. Li, et al., Modified whey protein isolate gel prepared by thermal aggregation combined with transglutaminase crosslinking achieves Casein-like slow digestion *in vitro* and *in vivo*, Food Res. Int. (2023). https://doi.org/10.1016/j.foodres.2023.112573.

[14] C.C. Yang, C.L. Wu, I.F. Chen, et al., Prevention of perceptual-motor decline by branched-chain amino acids, arginine, citrulline after tennis match, Scand. J. Med. Sci. Sports (2017). https://doi.org/10.1111/sms.12717.

[15] N. Mach, D. Fuster-Botella, Endurance exercise and gut microbiota: a review, J. Sport Health Sci. 6 (2017) 179-197. https://doi.org/10.1016/j.jshs.2016.05.001.

[16] J.A. de Araujo, G. Falavigna, M.M. Rogero, et al., Effect of chronic supplementation with branched-chain amino acids on the performance and hepatic and muscle glycogen content in trained rats, Life Sci. 79 (2006) 1343-1348. https://doi.org/10.1016/j.lfs.2006.03.045.

[17] W. Dong, M. Zhou, M. Dong, et al., Keto acid metabolites of branched-chain amino acids inhibit oxidative stress-induced necrosis and attenuate myocardial ischemia-reperfusion injury, J. Mol. Cell Cardiol. 101 (2016) 90-98. https://doi.org/10.1016/j.yjmcc.2016.11.002.

[18] Y. Yao, Y. Zheng, Y. Zhang, et al., Pressure-dominated steam explosion for modifying textured soy proteins: structure and *in vitro* digestion kinetics, Food Res. Int. 180 (2024) 114071. https://doi.org/10.1016/j.foodres.2024.114071.

[19] G. Kou, Z. Li, C. Wu, et al., Citrus tangeretin improves skeletal muscle mitochondrial biogenesis *via* activating the AMPK-PGC1-α pathway *in vitro* and *in vivo*: a possible mechanism for its beneficial effect on physical performance, J. Agric. Food Chem. (2018). https://doi.org/10.1021/acs.jafc.8b04124.

[20] S. Zhong, P. Xu, L. Xu, et al., Analysis of the molecular mechanism of punicalagin in the treatment of Alzheimer's disease by computer-aided drug research technology, ACS Omega 7 (2022) 6121-6132. https://doi.org/10.1021/acsomega.1c06565

[21] L. Wang, Q. Lin, T. Yang, et al., Oryzanol modifies high fat diet-induced obesity, liver gene expression profile, and inflammation response in mice, J. Agric. Food Chem. (2017). https://doi.org/10.1021/acs.jafc.7b03230.

[22] L. Cornacchia, C. Forquenot de la Fortelle, P. Venema, Heat-induced aggregation of whey proteins in aqueous solutions below their isoelectric point, J. Agric. Food Chem. 62 (2014) 733-741. https://doi.org/10.1021/jf404456q.

[23] Y. Chen, J. Ling, M. Li, et al., Understanding the impact of protein-excipient interactions on physical stability of spray-dried protein solids, Mol. Pharm. (2021). https://doi.org/10.1021/acs.molpharmaceut.1c00189.

[24] J. Gao, X. Li, S. Jia, et al., Structural characterization and antioxidant activity of a glycoprotein isolated from shiitake mushrooms, Food Biosci. 53 (2023) 102608. https://doi.org/10.1016/j.fbio.2023.102608.

[25] H. Staroszczyk, J. Pielichowska, K. Sztuka, et al., Molecular and structural characteristics of cod gelatin films modified with EDC and TGase, Food Chem. 130 (2012) 335-343. https://doi.org/10.1016/j.foodchem.2011.07.047.

[26] C. Paluszkiewicz, E. Stodolak, M. Hasik, et al., FT-IR study of montmorillonite-chitosan nanocomposite materials, Spectrochim. Acta A Mol. Biomol. Spectrosc. 79 (2011) 784-788. https://doi.org/10.1016/j.saa.2010.08.053.

[27] W. Ji, F. Yang, M. Yang, Effect of change in pH, heat and ultrasound pre-treatments on binding interactions between quercetin and whey protein concentrate, Food Chem. 384 (2022) 132508. https://doi.org/10.1016/j.foodchem.2022.132508.

[28] Q. Li, S. Yi, W. Wang, et al., Different thermal treatment methods and TGase addition affect gel quality and flavour characteristics of, Foods (Basel, Switzerland). 11 (2021). https://doi.org/10.3390/foods11010066.

[29] K. Yilmaz, S. Turhan, F.T. Saricaoglu, et al., Improvement of physicochemical, mechanical, thermal and surface properties of anchovy by-product protein films by addition of transglutaminase, and the correlation between secondary structure and mechanical properties, Food Packaging Shelf Life 24 (2020). https://doi.org/10.1016/j.fpsl.2020.100483.

[30] A. Martínez-Velasco, C. Lobato-Calleros, B.E. Hernández-Rodríguez, et al., High intensity ultrasound treatment of faba bean (*Vicia faba* L.) protein: effect on surface properties, foaming ability and structural changes, Ultrason Sonochem. 44 (2018) 97-105. https://doi.org/10.1016/j.ultsonch.2018.02.007.

[31] L. Schiphof-Godart, B. Roelands, F.J. Hettinga, Drive in sports: how mental fatigue affects endurance performance, Front. Psychol. 9 (2018) 1383. https://doi.org/10.3389/fpsyg.2018.01383.

[32] I.S. Cheng, Y.W. Wang, I.F. Chen, et al., The supplementation of branched-chain amino acids, arginine, and citrulline improves endurance exercise performance in two consecutive days, J. Sports Sci. Med. 15 (2016) 509-515.

[33] D.S. Allende, S. Gawrieh, O.W. Cummings, et al., Glycogenosis is common in nonalcoholic fatty liver disease and is independently associated with ballooning, but lower steatosis and lower fibrosis, Liver Int. 41 (2021). https://doi.org/10.1111/liv.14773

[34] J. Nielsen, H.C. Holmberg, H.D. Schrøder, et al., Human skeletal muscle glycogen utilization in exhaustive exercise: role of subcellular localization and fibre type, J. Physiol. 589 (2011) 2871-2885. https://doi.org/10.1113/jphysiol.2010.204487.

[35] J.S. Greiwe, R.C. Hickner, P.A. Hansen, et al., Effects of endurance exercise training on muscle glycogen accumulation in humans, J. Appl. Physiol. 87 (1999) 222-226. https://doi.org/10.1152/jappl.1999.87.1.222.

[36] Y. Takahashi, J. Sarkar, J. Yamada, et al., Enhanced skeletal muscle glycogen repletion after endurance exercise is associated with higher plasma insulin and skeletal muscle hexokinase 2 protein levels in mice: comparison of level running and downhill running model, J. Physiol. Biochem. 77 (2021) 469-480. https://doi.org/10.1007/s13105-021-00806-z.

[37] J.L. Ivy, C.H. Kuo, Regulation of GLUT4 protein and glycogen synthase during muscle glycogen synthesis after exercise, Acta Physiol. Scand. 162 (1998) 295-304. https://doi.org/10.1046/j.1365-201X.1998.0302e.x

[38] I. López-Soldado, J.J. Guinovart, J. Duran, Increased liver glycogen levels enhance exercise capacity in mice, J. Biol. Chem. 297 (2021) 100976. https://doi.org/10.1016/j.jbc.2021.100976.

[39] G. Boccia, D. Dardanello, C. Tarperi, et al., Decrease of muscle fiber conduction velocity correlates with strength loss after an endurance run, Physiol. Meas. 38 (2017) 233. https://doi.org/10.1088/1361-6579/aa5139.

[40] P. Rankin, E. Stevenson, E. Cockburn, The effect of milk on the attenuation of exercise-induced muscle damage in males and females, Eur. J. Appl. Physiol. 115 (2015) 1-17. https://doi.org/10.1007/s00421-015-3121-0.

[41] P. Baumert, M.J. Lake, C.E. Stewart, et al., Genetic variation and exercise-induced muscle damage: implications for athletic performance, Eur. J. Appl. Physiol. 116 (2016) 1595-1625. https://doi.org/10.1007/s00421-016-3411-1.

[42] G.G. Sleivert, R.D. Backus, H.A. Wenger, Neuromuscular differences between volleyball players, middle distance runners and untrained controls, Int. J. Sports Med. 16 (1995) 390-398. https://doi.org/10.1055/s-2007-973026.

[43] Z. Kazior, S.J. Willis, M. Moberg, et al., Endurance exercise enhances the effect of strength training on muscle fiber size and protein expression of Akt and mTOR, PLoS ONE 11 (2016). https://doi.org/10.1371/journal.pone.0149082.

[44] B. Darin, Q. Joe, B. Rebecca, Rapid determination of myosin heavy chain expression in rat, mouse, and human skeletal muscle using multicolor immunofluorescence analysis, PLoS ONE 7 (2012) e35273. https://doi.org/10.1371/journal.pone.0035273.

[45] J.R. Florini, Effects of testosterone on qualitative pattern of protein synthesis in skeletal muscle, Biochemistry 9 (1970) 909-912. https://doi.org/10.1021/bi00806a027.

[46] J.B. Lesnak, S. Inoue, L. Lima, et al., Testosterone protects against the development of widespread muscle pain in mice, Pain. Publish Ahead of Print (2020), https://doi.org/10.1097/j.pain.0000000000001985.

[47] K. Shunsuke, Y.U. Maki, U. Hironobu, et al., Impact of cortisol on reduction in muscle strength and mass: a mendelian randomization study, J. Clin. Endocrinol. Metab. (2021). https://doi.org/10.1210/clinem/dgab862.

[48] Z. Liu, C.Y. Qu, J.X. Li, et al., Hypoglycemic and hypolipidemic effects of malonyl ginsenosides from American ginseng (*Panax quinquefolius* L.) on type 2 diabetic mice, ACS Omega 6 (2021) 33652-33664. https://doi.org/10.1021/acsomega.1c04656.

[49] M. Morifuji, Y. Aoyama, Dietary orotic acid affects antioxidant enzyme mRNA levels and oxidative damage to lipids and proteins in rat liver, J. Nutr. Biochem. 13 (2002) 403-410. https://doi.org/10.1016/s0955-2863(02)00182-1.

[50] A. Zhuang, C. Yang, Y. Liu, et al., SOD2 in skeletal muscle: new insights from an inducible deletion model, Redox Biology 47 (2021) 102135. https://doi.org/10.1016/j.redox.2021.102135.

[51] C.A.O. Bartra, L.A. Jager, A.A.O. Alcarraz, et al., Antioxidant molecular brain changes parallel adaptive cardiovascular response to forced running in mice, Antioxidants 11 (2022). https://doi.org/10.3390/antiox11101891.

[52] S. Arslan, K. Erdem S Fau-Kilinç, A. Kilinç K Fau-Sivri, et al., Free radical changes in rat muscle tissue after exercise, Rheumatol Int. (2001). https://doi.org/10.1007/s002960000094.

[53] G. Ren, S. Yi, H. Zhang, et al., Ingestion of soy-whey blended protein augments sports performance and ameliorates exercise-induced fatigue in a rat exercise model, Food & Funct. 8 (2017) 670-679. https://doi.org/10.1039/C6FO01692H.

[54] M. McIntosh, R. Cochrum, R. Conners, et al., The effect of a nitric oxide supporting pre-workout supplement on muscular endurance: 866, Medicine & Science in Sports & Exercise 53 (2021) 288. https://doi.org/10.1249/01.mss.0000762456.97950.95.

[55] J.E. Anderson, A role for nitric oxide in muscle repair: nitric oxide-mediated activation of muscle satellite cells, Mol. Biol. Cell 11 (2000) 1859-1874. https://doi.org/10.1091/mbc.11.5.1859.

[56] B.J. Hardin, K.S. Campbell, J.D. Smith, et al., TNF-alpha acts *via* TNFR1 and muscle-derived oxidants to depress myofibrillar force in murine skeletal muscle, J. Appl. Physiol. 104 (2008) 694-699. https://doi.org/10.1152/japplphysiol.00898.2007.

[57] I. Stratos, A.K. Behrendt, C. Anselm, et al., Inhibition of TNF-alpha; Restores muscle force, inhibits inflammation, and reduces apoptosis of traumatized skeletal muscles, Cells 11 (2022) 2397. https://www.mdpi.com/2073-4409/11/15/2397.

[58] T. Kishimoto, Interleukin-6: from basic science to medicine—40 years in immunology, Annu. Rev. Immunol. 23 (2005) 1-21. https://doi.org/10.1146/annurev.immunol.23.021704.115806.

[59] L. Forcina, C. Miano, A. Musarò, The physiopathologic interplay between stem cells and tissue niche in muscle regeneration and the role of IL-6 on muscle homeostasis and diseases, Cytokine Growth Factor Rev. (2018) S1359610118300583. https://doi.org/10.1016/j.cytogfr.2018.05.001.

[60] P. Li, Y.L. Yin, D. Li, et al., Amino acids and immune function, Br. J. Nutr. 98 (2007) 237. https://doi.org/10.1017/S000711450769936X.

[61] M.S. Yoon, mTOR as a key regulator in maintaining skeletal muscle mass, Front Physiol. 8 (2017) 788. https://doi.org/10.3389/fphys.2017.00788.

[62] M.A.O. Go, E. Shin, S.Y. Jang, et al., BCAT1 promotes osteoclast maturation by regulating branched-chain amino acid metabolism, Exp. Mol. Med. 54 (2022) 825-833. https://doi.org/10.1038/s12276-022-00775-3.

[63] H. Ouyang, X. Gao, J. Zhang, Impaired expression of BCAT1 relates to muscle atrophy of mouse model of sarcopenia, BMC Musculoskeletal Disord. 23 (2022) 1-10. https://doi.org/10.1186/s12891-022-05332-7.

[64] T. Matsumura, Y. Morinaga, S. Fujitani, et al., Oral administration of branched-chain amino acids activates the mTOR signal in cirrhotic rat liver, Hepatology Res. 33 (2005) 27-32. https://doi.org/10.1016/j.hepres.2005.07.001.

[65] A. Rocchi, C. Milioto, S. Parodi, et al., Glycolytic-to-oxidative fiber-type switch and mTOR signaling activation are early-onset features of SBMA muscle modified by high-fat diet, Acta Neuropathol. 132 (2016) 127-144. https://doi.org/10.1007/s00401-016-1550-4.

[66] Y. Kamei, Y. Hatazawa, R. Uchitomi, et al., Regulation of skeletal muscle function by amino acids, Nutrients 12 (2020) 261. https://www.mdpi.com/2072-6643/12/1/261.

[67] T. Miki, M. Shinji, K. Yuko, et al., Skeletal muscle-specific expression of PGC-1α-b, an exercise-responsive isoform, increases exercise capacity and peak oxygen uptake, PLos ONE 6 (2011) e28290. https://doi.org/10.1371/journal.pone.0028290.