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Original Research

^{1}H NMR-based metabolomics approach for exploring the effect of astaxanthin supplementation on plasma metabolites after high-intensity physical exercise

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Abstract

Purpose: In this study, proton nuclear magnetic resonance (^{1}H NMR) spectroscopy was used to evaluate the effect of astaxanthin (ASTA) supplementation on changes in human plasma elicited by high-intensity exercise.

Methods: Sixteen adult males were randomly divided into 2 groups (n = 8 per group), namely the control group C (placebo for 28 d, 4 weeks) and experimental group M (supplement medium dose ASTA: 12 mg/d for 28 d, 4 weeks). At 08:00 on the 29th day, fasting blood sampling was carried out on all the participants, and the samples were tested in the laboratory for the first time. Later, the participants performed acute exercise on a pedal-powered bicycle with full strength for 30 s $\times$ 3/3 min intervals (loading a weight of 0.075 kg/kg). Blood sampling was then respectively performed immediately, 1 h after the acute exercise, and 1 d after the acute exercise.

Results: (1) The metabolites of the subjects of the two groups were found to be diverse at different time points, and 34 types of metabolites were screened from the two groups. (2) The metabolites with differences between the two groups 1 h after exercise were β-hydroxybutyrate, creatine, and glycerol. The levels of β-hydroxybutyric acid and glycerol in group M were significantly lower than those in group C, while the level of creatine was significantly higher. Compared with the resting state 1 h after exercise, the metabolites in common between the two groups were leucine (Leu), valine (Val), and citric acid (CA), and their levels were significantly decreased. (3) During the period between 1 h and 1 d after exercise, the different metabolites between the two groups were methionine (Met) and glycerol. The glycerol levels of group M were significantly lower than those of group C, while the levels of Met were significantly higher. The co-metabolites of the subjects in groups C and M 1 d after exercise were creatine, glucose, and glycerol, the levels of which were all significantly increased.

Conclusions: (1) One hour after exercise, the consumption of creatine, amino acids, fatty acids, and CA was found to be obvious, and ASTA intake was conducive to their recovery. (2) After high-intensity exercise, changes occurred in the body's energy metabolism that involved the metabolism of glucose, lipids, and proteins, and basic recovery was found 1 d after exercise. The findings of this study suggest that ASTA intake can accelerate metabolic recovery induced by physical exercise.

Keywords

Astaxanthin; High-intensity exercise; Human plasma; Metabolomics

1. Introduction

Cellular metabolism in organisms is accompanied by the generation of unstable free radicals, such as reactive oxygen species, which easily cause oxidative damage to amino acids, proteins, DNA, etc. [1, 2]. Under normal conditions, the body's own redox reaction is in a state of equilibrium, but, during high-intensity exercise, the production of free radicals increases significantly [3]; this causes a series of adverse effects on the body and is the main cause of the occurrence of sports injuries and sports fatigue [4].

Astaxanthin (ASTA) is a lipid-soluble antioxidant carotenoid available for supplementation via the intake of *Haematococcus pluvialis*-derived antioxidant products. In 1987, ASTA was approved by the United States Food and Drug Administration (FDA) as a daily dietary supplement (health product). Recent research has shown that ASTA has strong anti-oxidation properties, and can alleviate chronic inflammatory diseases, diabetes, and cancer [5, 6]. In the field of sports science, studies have shown that ASTA has the effects of scavenging free radicals, relieving exercise damage to skeletal muscle, maintaining the normal contraction of skeletal muscle, synthesizing ATP, and delaying motor fatigue [2, 7–9]. The authors' previous research [10] has also shown that ASTA intake can eliminate free radicals produced in the body, thereby significantly reducing the increases in blood lactic acid and blood uric acid after high-intensity exercise.

Metabolomics was originally derived from the metabolic profiling proposed by Devaus *et al.* in the 1970s [11]. It is the "end product" of the genome, transcriptome, and proteome, which includes the phenotypic, direct, and comprehensive "biomarker" information that can be used to achieve a global observation of metabolites in the body at a certain moment. It has been widely used in the physiology, toxicology, disease diagnosis, drug efficacy, and drug mechanism fields, which have yielded numerous research results [12]. However, in the field of human kinesiology, research on the effects of antioxides on body functions via proton nuclear magnetic resonance (^{1}H NMR) spectroscopy has rarely been reported. The field of metabolomics can offer a significantly higher level of blood biomarkers for sports nutrition and performance monitoring. The present work attempts to elucidate the action mechanism of the anti-oxidation ability of ASTA and its effects on motor function from a metabolic perspective. We hypothesized that with the same workload situation, ASTA supplementation have a positive effect for movement body that can accelerate plasma metabolic recovery induced by physical exercise, compared to the non-ASTA.

2. Materials and methods

2.1 Experimental object and grouping

According to their height, weight, and sports level, sixteen male students from the 2018 physical education institution of Shanxi University were selected as the research sample (age: 20.3 $\pm$ 2.42, height: 180 $\pm$ 3.24, BMI: 17 $\pm$ 5.31). The participants were randomly divided into two groups (n = 8 per group), namely the control group C (placebo) and group M (supplement medium dose ASTA: 12 mg/d). All subjects were asked about their medical history and underwent a routine physical examination to confirm their health and that they did not suffer from any major diseases, upper respiratory tract infection, or cardiovascular problems. They were instructed to not take supplements that act as free-radical scavengers (vitamin E, vitamin C, lycopene) or traditional Chinese medicines during the experimental period. This clinical trial followed the Declaration of Helsinki, and informed consent was obtained from the subjects before the experiment.

This experiment was approved by the ethics committee of Shanxi University (No. SXULL2020064).

2.2 Experimental design and exercise protocol

The experiment was conducted over 29 days (Taking ASTA continuously for about 4 to 8 weeks will be effective [10]). The experimental group M took ASTA (12 mg/d, after dinner) at 21:00 7 d/week for 4 weeks. The control group took a placebo (the main component of which was soybean oil) with a similar appearance as ASTA for 4 weeks. On the morning of the 29th day at 08:00, all the participants underwent fasting blood sampling, and the samples were tested in the laboratory for the first time. Later, participants performed acute exercise on a pedal-powered bicycle with all their strength for 30 s $\times$ 3/3 min intervals (loading a weight of 0.075 kg/kg) [13]. Blood sampling was then respectively performed immediately, 1 h after the acute exercise, and 1 d after the acute exercise. According to the time of sampling, all blood samples were respectively marked as T1C, T2C, T3C, and T4C (control group), and T1M, T2M, T3M, and T4M (experimental group).

2.3 Experimental instruments and reagents

The experimental instruments included a Bruker AVANCE III 600 MHz NMR spectrometer (600.13 MHz proton frequency; Bruker, Germany), a TGL-16 vacuum centrifuge (Cence Company, Hunan, China), a Neofuge 13R high-speed refrigerated centrifuge (MiaoShen Science and Technology, Shanghai, China), a 101-3AB electrothermal blast dryer, and a QL-901 vortex mixer (Jingke Company, Shanghai, China). Moreover, ASTA soft capsules (containing ASTA $\geq$ 4 mg per capsule, Yaskoda Biotech Co., Ltd., Wuhan, China), D2O (Norell, USA), and phosphate buffer (0.01% TSP, 0.2 mol/L Na_2HPO_4/NaH_2PO_4, pH 7.4) were used in the experiment.

2.4 Sample collection, storage, and handling

Blood samples were collected in anticoagulant tubes after exercise. First, 1 mL of the supernatant obtained via the centrifugation of the blood sample (4 °C, 3000 r/min for 30 min) was placed in a 2-mL EP tube for labeling and stored in a -80 °C refrigerator for testing.

Samples were then processed for ^{1}H NMR spectroscopy. First, the blood samples were thawed at 4 °C, and 450 μL

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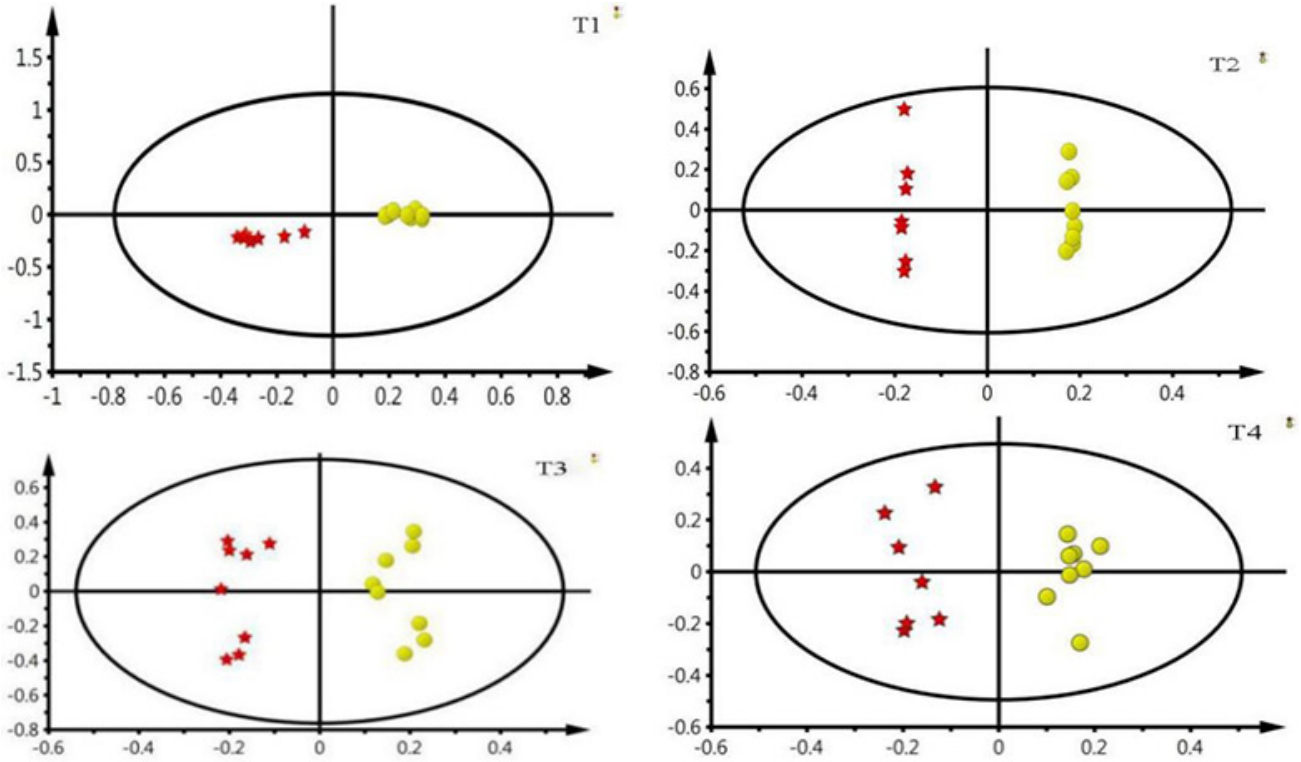

FIG. 1. Score maps of the OPLS-DA models (group C: five-pointed stars; group M: circles).

of the samples was placed in a 2-mL EP tube. Thereafter, 900 μL of methanol (to precipitate the protein) was added, and the samples were vortexed for 120 s and then placed in a refrigerated centrifuge at 4 °C and 13000 r/min for 0.5 h. Then, 900 μL of the supernatant was placed into a 5-mL EP tube. Finally, the samples were blow-dried using a vacuum centrifuge with 600 μL of phosphate buffer (0.01% TSP, 0.2 mol/L Na_2HPO_4/NaH_2PO_4, pH = 7.4). After centrifugation at 4 °C and 13000 rpm for 10 min, 550 μL of the supernatant was placed in a 5-mm NMR tube for NMR spectroscopy analysis.

2.5 NMR data acquisition and processing

The samples processed via ^{1}H NMR spectroscopy were detected using a Bruker AVANCE III 600 MHz NMR spectrometer, on which scanning could be carried out up to 64-fold with the Bruker 5-mm BBO probe at a 600.13-MHz proton frequency, a 298 K acquisition temperature, a Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, a spin relaxation of delay of 320 ms, and a spectral width of 8 kHz with 64 k data points of free induction decay.

Blood plasma maps were processed using MestreNova software (version 6.1.0-6224 Mestrelab Research, Santiago de Compostela, Spain). The TSP chemical shift was calibrated as δ0.00, the baseline of all maps was adjusted, and the phase was shifted. After manually removing the water peak (δ4.5-5.19), the range of the maps was limited to 0-9 ppm, and the integral width was 0.01 ppm; thereafter, the data were normalized and saved in MS Excel.

2.6 Multivariate statistical analysis

SIMCA-P (version 13.0, Umetrics, Sweden) was used to centralize and normalize the integration values. Thereafter, PLS-DA and OPLS-DA analyses were performed, and differential metabolites were screened by combining the *P*-value ($P \leq 0.01$ or $P \leq 0.05$) and VIP value (VIP > 1).

Student's *t*-test and one-way ANOVA ($P \leq 0.01$ or $P \leq 0.05$) were performed using SPSS (version 21.0) to compare the differential metabolites.

3. Results

3.1 OPLS-DA analysis

Via orthogonal partial least squares discriminant analysis (OPLS-DA), the irrelevant information on the independent variable matrix and the dependent variable was removed, and a more simplified explanatory model rendering was obtained, as shown in Figs. 1, 2.

Each distribution point in Fig. 1 represents one subject. Fig. 1 presents the respective score maps of the OPLS-DA models of groups C and M at the four sample collection times. It can be seen from the score image that the individuals in each group exhibited an aggregation tendency; the two groups are obviously separated, which indicates that the ASTA supplement had an obvious effect on the body.

Each point in the S-plot corresponds to the distribution of a metabolite based on the VIP value. The greater the distance from the origin, the higher the contributions of the rate and reliability, and the greater the VIP value. As shown in Fig. 2, the points that are separated from most other points and far

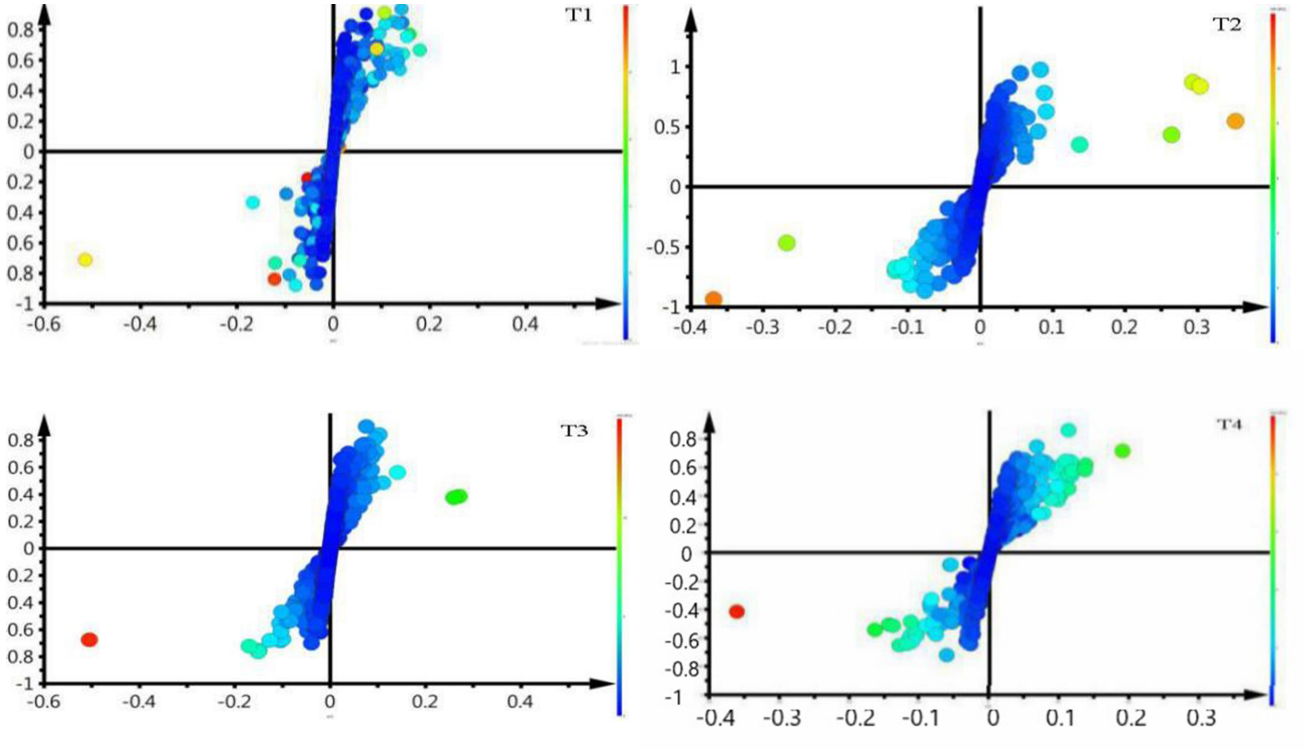

FIG. 2. S-plots of the OPLS-DA model at T1-T4.

from the origin are significant differential metabolites.

3.2 Metabolite identification of the ^{1}H NMR fingerprint

Using the internationally recognized Human Metabolome Database (www.hmdb.ca) and related metabolomics studies, 34 metabolites were identified via ^{1}H NMR spectroscopy. The main metabolic pathways of these substances were then determined via analysis (Fig. 3).

3.3 Screening of differential serum metabolites in groups C and M under quiet conditions

In the quiet state before exercise (after taking ASTA for 28 d), the levels of lipids ($P < 0.05$), glycerol ($P < 0.05$), phenylalanine (Phe; $P < 0.05$), lactic acid ($P < 0.05$), betaine (Bet; $P < 0.01$), and histidine (His; $P < 0.01$) were significantly higher in group C than in group M. In contrast, the levels of valine (Val), β-hydroxybutyric acid, glutamic acid (Glu), glutamine (Gln), trimethylamine oxide (TMAO), acetoacetate, glycine (Gly), glucose, tyrosine (Tyr), alanine (Ala), and creatine ($P < 0.05$) were significantly lower (Table 1).

3.4 Screening of differential serum metabolites in groups C and M 1 h after exercise

The levels of lipids ($P < 0.01$), leucine (Leu; $P < 0.01$), Val ($P < 0.01$), and CA ($P < 0.05$) in group C (T3C vs. T1C) were significantly lower 1 h after exercise than those in the quiet state. The levels of β-hydroxybutyrate ($P < 0.01$), CA ($P < 0.01$), Leu ($P < 0.05$), Val ($P < 0.05$), glycerol ($P < 0.05$), and acetoacetic acid ($P < 0.05$) were significantly decreased in group M (T3M vs. T1M), but the lactic acid level ($P < 0.01$) was significantly increased. At this time, the common metabolites, namely Leu, Val, and CA, were significantly reduced (Table 2).

In group C (T3C vs. T2C), excluding lactic acid ($P < 0.01$), the levels of methionine (Met), CA, creatine, acetoacetic acid, and glycerol were significantly increased ($P < 0.01$) 1 h after exercise as compared with those immediately after exercise. In group M (T3M vs. T2M), excluding lactic acid ($P < 0.01$), the levels of the other metabolites, namely lipids, Ala, creatine, Bet, Gly, and glycerol, were significantly increased ($P < 0.01$; Table 2).

One hour after exercise, there were three metabolic differences between the two groups; group M exhibited significant decreases in β-hydroxybutyrate and glycerol, and the creatine level was significantly higher than that in group C (Table 2).

3.5 Screening of differential serum metabolites in groups C and M 1 d after exercise

Compared with the quiet state, 1 d after exercise, the levels of creatine, glycerol, and glucose of group C (T4C vs. T1C) were significantly increased ($P < 0.05$). The concentrations of Phe ($P < 0.01$), Tyr ($P < 0.05$), and His ($P < 0.05$) of group M (T4M vs. T1M) were significantly decreased, but the concentrations of Bet ($P < 0.01$), glycerol, ($P < 0.01$), creatine ($P < 0.05$), and glucose ($P < 0.05$) were significantly increased. At this time, the common metabolites between the two groups, namely creatine, glucose, and glycerol, were significantly elevated (Table 3).

The concentrations of Leu, creatine, glycerol, and glucose in the serum of group C (T4C vs. T3C) were significantly

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TABLE 1. The variation trend of metabolites in group C and M ($\bar{x} \pm SD$, n = 8)

δ (ppm/peak/coupling constant)	Metabolite	Group C ($\bar{x} \pm SD$)	Group M ($\bar{x} \pm SD$)	C vs. M
0.86 (m)	Lipid	2.37 ± 0.89	1.46 ± 0.89	↓*
1.05 (d, 7.0)	Val	3.16 ± 0.65	3.97 ± 0.66	↑*
1.19 (d, 7.1)	β-hydroxybutyric acid	4.57 ± 1.31	6.37 ± 1.31	↑*
1.93 (s)	Acetic acid	4.01 ± 0.02	7.55 ± 0.23	↑*
2.07 (m)	Glu	0.98 ± 0.52	1.40 ± 0.05	↑*
2.45 (m)	Gln	3.34 ± 1.15	4.44 ± 1.17	↑*
3.26 (m)	trimethylamine oxide	16.11 ± 2.75	20.51 ± 2.70	↑*
3.27 (m)	Bet	8.79 ± 1.83	5.90 ± 1.87	↓*
3.48 (s)	Acetoacetate	15.73 ± 5.50	17.32 ± 5.27	↑*
3.57 (s)	Gly	8.48 ± 1.59	6.31 ± 1.59	↓*
3.67 (m)	Glycerol	12.00 ± 2.43	11.07 ± 2.53	↓*
5.24 (d, 3.7)	Glucose	4.59 ± 2.68	5.61 ± 2.68	↑*
7.33 (m)	Phe	0.30 ± 0.01	0.21 ± 0.01	↓*
7.19 (m)	Tyr	1.09 ± 0.001	1.42 ± 0.001	↑*
7.09 (s)	His	0.3 ± 0.1	0.01 ± 0.1	↓**
4.12 (d, 6.9)	Lactic acid	2.63 ± 0.59	1.60 ± 0.59	↓*
3.77 (d, 7.3)	Ala	17.05 ± 2.45	20.17 ± 2.75	↑*
3.05 (s)	Creatine	1.77 ± 0.53	2.68 ± 0.51	↑*

Notes: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

*: $P < 0.05$; **: $P < 0.01$.

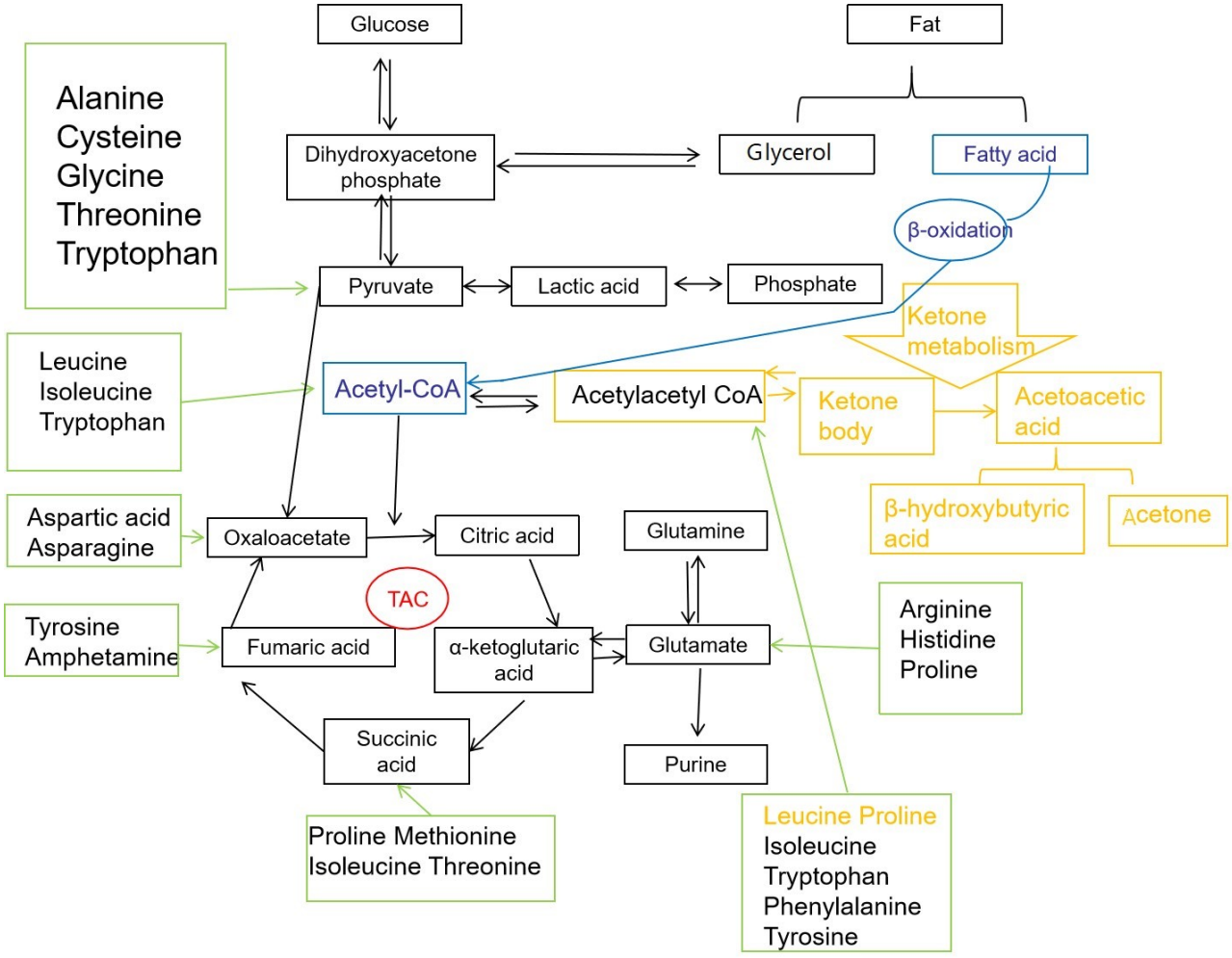

FIG. 3. Path maps involved in this study. Note: red indicates TAC circulation, blue indicates the fatty acid beta-oxidation pathway, and orange indicates the ketone metabolism pathway.

TABLE 2. The variation trend of metabolites in group C and M at 1 h after exercise ($\bar{x} \pm SD$, n = 8)

δ (ppm/peak/coupling constant)	Metabolite	T3C vs. T1C	T3C vs. T2C	T3M vs. T1M	T3M vs. T2M
0.86 (m)	Lipid	↓**			↑**
0.96 (d)	Leu	↓**		↓*	
1.05 (d, 7.0)	Val	↓**		↓*	
1.19 (d, 7.1)	β-hydroxybutyric acid			↓**	
4.12 (d, 6.9)	Lactic acid		↓**	↑**	↓**
1.48 (d, 7.3)	Ala				↑**
2.14 (s)	Met		↑**		
2.65 (s)	CA	↓*	↑**	↓**	
3.05 (s)	Creatine		↑**		↑**
3.27 (m)	Bet				↑**
3.48 (s)	Acetoacetic acid		↑**	↓*	
3.57 (s)	Gly				↑**
3.67 (m)	Glycerol		↑**	↓*	↑**

Notes: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.
*: $P < 0.05$; **: $P < 0.01$.

TABLE 3. The variation trend of metabolites in group C and M at 1d after exercise ($\bar{x} \pm SD$, n = 8)

δ (ppm/peak/coupling constant)	Metabolite	T4C vs. T1C	T4C vs. T3C	T4M vs. T1M	T4M vs. C T3M
0.96 (d)	Leu		↑*		↑**
1.05 (d, 7.0)	Val				↑*
4.12 (d, 6.9)	Lactic acid		↓**		↓**
3.05 (s)	Creatine	↑*	↑*	↑*	↑**
3.27 (m)	Bet			↑**	
3.48 (s)	Acetoacetic acid				↑**
3.67 (m)	Glycerol	↑*	↑*	↑**	↑**
5.24 (d, 3.7)	Glucose	↑*	↑*	↑*	
7.33 (m)	Phe			↓**	
7.19 (m)	Tyr			↓*	
7.09 (s)	His			↓*	

Notes: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.
*: $P < 0.05$; **: $P < 0.01$.

increased ($P < 0.05$) 1 d after exercise as compared with 1 h after exercise, but the concentration of lactic acid was significantly decreased ($P < 0.01$). In group M (T4M vs. T3M), the concentration of lactic acid was significantly decreased ($P < 0.01$), but the concentrations of Leu ($P < 0.01$), creatine ($P < 0.01$), acetoacetic acid ($P < 0.01$), glycerol ($P < 0.01$), and Val ($P < 0.05$) were significantly increased (Table 3).

One day after exercise, there were two metabolic differences between the two groups; the level of glycerol in group M was significantly lower than that in group C, while the level of Met was significantly higher.

4. Discussion

4.1 Effect of ASTA supplementation on immediate recovery 1 h after high-intensity exercise in humans

In this study, the levels of Leu and Val in groups C and M were found to be lower than those in the resting state 1 h after exercise, and the decreases in group C were more pronounced. It is speculated that the body has insufficient muscle glycogen content during exercise. Portions of Leu and Val are used as an energy supply to improve the body's ability to move, while the intake of ASTA can reduce the use of BCAA [14]; thus, the decline in group C was more obvious. Both Leu and Val are branched-chain amino acids (BCAAs), which are the main amino acids involved in energy metabolism in the body. In humans, BCAAs cannot be synthesized or converted from other substances, and can only be acquired through food. After a series of catabolism, Leu is finally degraded into acetoacetate and acetyl CoA; Val is decomposed into succinyl-CoA, which is involved in ketogenesis, and the sugar reaction enters the tricarboxylic acid (TCA) cycle [15]. In muscle tissue, in addition to being used as an energy matrix during exercise, BCAAs are involved in protein synthesis and serve as a precursor for the synthesis of other amino acids [16, 17]. Moreover, Leu can also enhance muscle protein anabolism and participate in skeletal muscle repair by promoting the release of insulin and growth hormones [18, 19], which may be mediated through signaling pathways that control protein synthesis; this is related to breastfeeding of the phosphorylation of the rapamycin target (mTOR) and the activation of the 70-kD ribosomal S6 protein kinase (p70 S6 kinase) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1). mTOR plays a central role in the muscle protein synthesis signaling pathway [20], and also

acts as motor and nutrient receptors [21, 22]. p70 S6 kinase is activated by the phosphorylation of Ser/Thr residues (including serine 424/threonine 421, Ser424/Thr421) [23]. Studies have shown that after supplementation with BCAAs or Leu for 2 or 6 h, the phosphorylation of p70 S6 kinase and 4E-BP1 in skeletal muscle increases, which in turn leads to the increased phosphorylation of mTOR, thereby promoting skeletal muscle repair [24].

The levels of CA significantly decreased in group C and group M. Studies have shown that CA has an antioxidant effect, which can reduce brain lipid peroxidation, inflammation, liver damage, and DNA fragmentation [25]. In addition, CA is a hydroxytricarboxylic acid compound, which is a good H^+ donor and can efficiently chelate metal ions such as Fe^{2+}, Mn^{2+}, Cu^{2+}, etc.; moreover, as the most common metal chelating agent, it can resist lipid peroxidation induced by metal ions [26]. CA acts as a starting material for acetyl CoA entering the Krebs cycle, and its concentration reflects the level of cyclic metabolism of the Krebs cycle. Studies have shown that the CA concentration is significantly lower 1 h after acute resistance exercise than at the quiet state, and that CA is not fully recovered 1 h after exercise [20], which is consistent with the results of the present study. It is speculated that during high-intensity exercise, the metabolism of TCA is intense and accompanied by the formation of a large number of free radicals. Large amounts of CA are consumed, and it cannot be completely recovered 1 h after exercise.

In group M, excluding β-hydroxybutyrate, the levels of glycerol and creatine were found to be significantly increased 1 h after exercise as compared with the levels immediately after exercise. β-hydroxybutyric acid and glycerol are the products of fat catabolism. When creatine is phosphorylated in skeletal muscle, phosphocreatine reserve energy is produced. Ca, β-hydroxybutyric acid, and glycerol are the three metabolites mainly involved in energy metabolism in the body. One possible factor that affects fat utilization is the co-localization of carnitine palmitoyltransferase I (CPT I) with fatty acid transposase (FAT/CD36) [27]. CPT I is located on the mitochondrial membrane and is the rate-limiting factor for the entry of fatty acyl-CoA into muscle mitochondria [28]. ASTA is an antioxidant in the mitochondrial membrane, and mitochondria are considered to be the main targets of ASTA [29]. Aoi found that ASTA facilitates the mutual promotion of CPT I and FAT/CD36 in exercise muscles. Changes in CPT I activity can affect the co-localization of CPT I with FAT/CD36, which regulates changes in lipid metabolism during exercise [30]. Moreover, Ikeuchi *et al.* found that the dietary supplementation of ASTA inhibited CPT I activity and promoted lipid metabolism in exercised rats as compared to mice with a normal diet [31]. It was found that the activity of CPT I was negatively correlated with lipid metabolism in the rats during exercise, and that ASTA enhances fatty acid metabolism by inhibiting CPT I activity by acting on mitochondrial targets. In addition, the ratio of acetoacetate to β-hydroxybutyrate was found to be proportional to the extent to which fatty acids are involved in energy metabolism [32]. In the present experiment, 1 h after exercise, the β-hydroxybutyrate level was significantly lower in group M than in group C, the concentration of acetoacetic acid did not change significantly, and the ratio of acetoacetic acid to β-hydroxybutyric acid increased. Fatty acids are involved in the enhancement of energy metabolism. The supplementation of ASTA may promote fatty acid catabolism and enhance the body's antioxidant capacity via the previously discussed pathways. Moreover, because the glycerol metabolic pathway is similar to the saccharolytic pathway of glucose, the findings of this study suggest that the significant decrease in the glycerol levels in group M may be related to the enhancement of aerobic metabolism. In addition, groups C and M exhibited increased creatine levels after 1 h after exercise compared that with immediately after exercise, but the changes of creatine ($P < 0.01$) in group M were more significant, indicating that ASTA supplementation increased creatine reserves.

4.2 Effect of ASTA supplementation on the recovery process from 1 h to 1 d after high-intensity exercise

The results of this experiment demonstrated that 1 d after high-intensity exercise, the serum creatine and glucose levels were increased significantly as compared with the quiet state, suggesting that the body had undergone excessive recovery, and the levels of creatine, BCAAs, and acetoacetic acid of group M were significantly higher than those of group C 1 h after exercise. This confirms that ASTA supplementation had a positive effect on the recovery of the body during this period. Creatine is a quinone compound synthesized from arginine (Arg) and Gly in the liver and kidney. Plasma creatine is transported into tissues through a special creatine transporter, and more than 90% of creatine is utilized by skeletal muscle [33]. As an energy source for extreme exercise, the energy supply of the phosphoric acid system can only last for 6-8 s [34]. The recovery process after creatine consumption is very short; it can be fully recovered in about 3 min [35]. As found in the present study, after 3 intermittent exhaustive cycling exercises, the muscle glycogen recovered about 44% of the total consumption within 30 min, it was recovered completely 24 h after exercise, and the recovery of glycogen during this period was basically free of ordinary diet and the effects of high sugar diets [36]. In the early stage of over-recovery, the reserves of energy substances are increased. First, the levels of glycogen and creatine phosphate increase, after which the levels of energy substances, such as proteins and phospholipids, gradually increase [37].

There were significant differences between the metabolites in groups C and M. The number of metabolites selected in group M was significantly higher than that in group C. In addition to glucose and creatine, the concentration of Bet was also significantly increased in group M, while the concentrations of CA, Tyr, His, and Phe were significantly decreased. This indicates that the supplementation of ASTA had a great influence on the metabolism of the body during the recovery period from 1 h after exercise to 1 d after exercise. The concentration of glycerol in the serum of group M

was significantly higher than that in the quiet state, but it was significantly lower than that in the serum of group C at this point, as was the concentration of lipids. This suggests that ASTA can still promote the metabolic energy supply of body lipids from 1 h after exercise to 1 d after exercise. The serum glycerol concentrations in groups C and M 1 d after exercise were significantly higher than those in the quiet state, which may be related to excessive recovery after the body's energy consumption.

The serum Bet concentration was increased significantly as compared with that in the quiet state. Studies have found that the consumption of Bet during exercise is related to the consumption of carnitine and choline during exercise [38]. During the recovery period after exercise, the body's consumed materials recover gradually, and over-recovery occurs. In the early stage of excessive recovery, the occurrence of excessive lipid recovery occurs relatively late as compared to the recovery of sugar and amino acid energy substances [39]. The supplementation of ASTA can promote the body's recovery, which may cause the excessive recovery of phospholipids, etc., to occur in advance. Additionally, the level of choline, a substrate for the synthesis of phospholipids, will also increase accordingly, which may be one of the metabolic mechanisms that increased the concentration of Bet in the serum of group M. In addition, the increase in Bet concentration may also be associated with the significant increase in Met in the serum of group M, as it is involved in the methyl transfer of synthetic Bet. These two factors caused a significant increase in the serum Bet concentration of group M as compared with that in the quiet state, whereas there was no significant change in group C.

ASTA has the effect of scavenging free radicals and increasing glutathione (GSH) activity. ASTA can also maintain or increase the serum levels of Bet, choline, carnitine, and creatine, thereby reducing the consumption of Met. The Met serum concentrations were therefore significantly increased in group M 1 d after exercise. Met is a glucogenic amino acid that can be converted into succinyl-CoA for energy in the TCA cycle [40]. Met is also an important precursor for the synthesis of S-adenosyl Met (SAM), which is a significant donor for the methyl group in multiple methyltransferase reactions; it is therefore involved in the metabolic pathway of methyl transport [24, 41]. Met can be involved in the methylation of phospholipids in cell membranes, in the synthesis of phospholipids to maintain the integrity of biofilms, and in the synthesis of creatine, carnitine, choline, etc. The body can improve its exercise capacity by supplementing food containing Met-chelated selenium to accelerate the elimination of free radicals produced by exercise, alleviate the oxidative damage to the body, and strengthen the expression of the antioxidant enzyme activity of the body [42].

In this study, in the serum of group M, the levels of Phe, Tyr, and His were significantly lower than those in the quiet state (T4M vs. T1M), but there was no significant change in the serum of group C as compared with the quiet state (T4C vs. T1C), suggesting that ASTA supplementation may play a role in regulating the metabolism of hormones in the exercising organism. The body's demand for thyroxine, dopamine, and carnosine increases during exercise, and studies have found that Tyr levels are significantly reduced after exercise [42]. Tyr and His are involved in the synthesis of thyroxine, dopamine, and carnosine [43], while Phe can be catalyzed by Phe hydroxylase to synthesize Tyr; its metabolic pathway in vivo is basically the same as that of Tyr. Research has shown that high-intensity exercise with 3 × 80 m intermittent 10 s or 1 min results in a decrease in urinary Phe levels after exercise [44]. In the recovery period, the substances consumed during exercise are gradually recovered. The excessive recovery of protein and amino acids occurs after the sugar, and the lipids are the latest, while the recovery of enzymes such as enzymes and hormones restored occurs in the late stage of the over-recovery period [37]. During this period the rapid synthesis of regulated metabolites may be responsible for the decrease in the levels of Phe, Tyr, and His.

5. Conclusions

(1) One hour after high-intensity exercise, the body's consumption of creatine, amino acids, fatty acids, CA, and other metabolites is obvious, and ASTA supplementation is beneficial to their recovery.

(2) High-intensity exercise changes the body's levels of sugars, fats, amino acid metabolism, etc., during basic recovery one day after exercise. The supplementation of ASTA accelerates the recovery process of the body's metabolic changes induced by exercise.

Author contributions

L-LW: First author, Revising the manuscript and confirmation of final version to be published and Submitted manuscript. ZS: Corresponding author, Substantial contributions to conception and design, performed sample collection and the data analysis, revising the manuscript and confirmation of final version to be published. A-PC: Corresponding author, Revising the manuscript. Providing the funding to this research. L-JW: Substantial contributions to conception and design.

Ethics approval and consent to participate

This experiment was approved by the ethics committee of Shanxi University (No. SXULL2020064).

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Conflict of interest

The authors declare no conflict of interest.

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