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Fatigue Exercise Alters the Profiles of Physiological Biomarkers in Association with Four Metabolic Pathways: A Metabolomic Study

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Abstract

Background: This study aimed to investigate the effect of fatigue exercise on metabolic markers in skeletal muscles through proton-nuclear magnetic resonance (1H-NMR) spectrometric analysis. METHODS: Twenty 7-week-old, male SD (Sprague Dawley) rats were randomly divided into control (C; n=10) and exercise (E; n=10) groups. Skeletal muscle tissue was harvested 21 d after exercise intervention; they were then prepared for 1H-NMR spectroscopic analysis. Spectral analysis was performed using MestReNova, SIMCA-P, and SPSS, and differential metabolites were screened.

Results: (1) Consequently, 18 different metabolites were screened in the two groups via 1H-NMR spectroscopy. Leucine (Leu), lactic acid, trimethylamine oxide (TMAO), hypoxanthine (Hx), creatine, phosphorylcholine, acetic acid ($p<0.01$), citric acid (CA), glycerol, and choline ($p<0.05$) levels were significantly higher in group E than in group C. Alanine (Ala), carnitine, α-glucose, succinic acid ($p < 0.01$), glutamine (Gln), betaine (Bet), tyrosine, and cysteine ($p<0.05$) levels were significantly lower in group E than in group C.

(2) On Metabolomic Pathway Analysis (MetPA), differences were observed among 18 metabolites in 30 metabolic pathways. Four metabolic pathways were potentially involved in fatigue metabolism: alanine, aspartate, and glutamate metabolism, the TCA cycle, pyruvate metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis.

(3) Alanine, Glutamine, succinic acid, Citric acid, lactic acid, acetic acid, and tyrosine were potential markers of fatigue metabolism herein.

Conclusions: The present results indicate that fatigue exercise potentially reduces physiological functions associated with changes key metabolites in alanine, aspartate, and glutamate metabolism, the TCA cycle, pyruvate metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis.

Keywords: Fatigue Exercise; Proton-Nuclear Magnetic Resonance Spectrometric Analysis; Metabolomic Pathway Analysis; Rat; Skeletal Muscle.

Introduction

As a stressor, exercise markedly influences physiological functions. High-to-moderate-intensity exercise promotes aerobic metabolism, enhances cardiopulmonary function, and increases immunity. Acute high-intensity or prolonged and heavy exercise leads to a redox imbalance, resulting in oxidative damage and muscle fatigue [1-2], even resulting in various conditions including neurological diseases, immune and inflammatory injury, and joint and muscle damage [3-5].

Metabolomics is a rapidly developing biological tool since the Human Genome Project and Human Proteome Project in the late 1990s and depends on high-throughput detection and data processing as means, considering information modeling and system conformity as the goal and group index analysis as the basis [6]. All metabolites can be scanned in a "panoramic" manner without pre-setting specific analytical indicators; hence, metabolomics reflects the effects of external factors including drugs, environmental pollution, and exercise on metabolism [7-9]. Metabolomics is currently widely used in studies on physiology, functional genomics, toxicology, disease diagnosis, and the efficacy and mechanism of action of pharmacotherapeutic agents, [10]. However, certain studies in sports physiology have used a metabolomics approach. Based on the marked physiological effects of fatigue exercise, this study aimed to investigate the metabolic status of skeletal muscle metabolites upon fatigue exercise in rats through 1H-NMR spectroscopy. Furthermore, this study attempted to elucidate the pathway and mechanism underlying the effect of fatigue exercise from the metabolic viewpoint.

Materials and Methods

Grouping of experimental animals

Twenty-four 7-week-old, male SD male rats weighing 160~180 g were obtained from Beijing Vital River Laboratory Animal Technology Co., Ltd. (animal license number: SCXK [Beijing, China] 2012-0001). All rats were adaptively fed for 1 week and then randomly divided into a control (C) group and an exercise (E) group (n=10 rats per group). Excluding three inactive mice and one that ran away. Furthermore, rats were placed in four cages, each containing five rats, at 25–28 °C and a relative humidity of 40~60%. Thereafter, rats were subjected to physical exercise by swimming in water and maintained at 30±2 °C (approximately 40-cm depth).

This experimental has been approved by the committee of Scientific research in Shanxi University (No. SXULL2020064) (Taiyuan, China).

Establishment and validation of a rat model of muscle fatigue

The exercise sequence comprised fatigue swimming that referenced the exercise model of Kalantari and Hou [10-11]. Before initiation of the formal experiment, rats in group E were accustomed to swimming for 3 d. Formal exercise was carried out in three stages, each lasting 7 d and 21 d in total. In stage 1, rats swam for 100 min at 2:30 pm; stage 2, rats swam for 3 h at 2:30 pm; stage 3, rats swam for 3 h each at 8:00 am and 2:30 pm. Furthermore, during the swimming period, the rats were subjected to continuous exercise, and the floating rats were driven with wooden sticks.

Fatigue was defined as uncoordinated movement of rat limbs during swimming and the failure to rise to the surface for spontaneous breathing within 10 s [10-12].

Experimental analysis

Laboratory instruments and reagents

Brucker600-MHz AVACNCE III NMR spectrometer (600.13 MHz proton frequency,) （Bruker，Germany）; TGL-16 Vacuum centrifuge (Cence company, China, HuNan); Adjustable high-speed homogenate machine FSH-2A (YouYi Instrument research institute, China, Changzhou); High-speed refrigerated centrifuge Neofuge13R (MiaoShen Science and Technology, China,

Shanghai); Vortex mixer QL-901(Jingke company, China,Shanghai); Methanol solution (purity≥99.5%); Phosphate buffer (0.01% TSP, 0.2 mol/L Na2HPO4/NaH2PO4, pH 7.4).

Tissue preparations

Skeletal muscle samples were harvested after training and washed with physiological saline, dried with filter paper, wrapped with aluminum foil, placed in a sealed bag, and stored in -80 °C for analysis.

Samples were then processed for proton-nuclear magnetic resonance (1H-NMR) spectrometry. First, skeletal muscle samples were thawed at 4 °C and 0.2 g of these samples were placed in a 5-ml EP tube. Thereafter, 600 ml methanol and 300 ml distilled water were added to the tube and homogenized on ice. Thereafter, 500 ml of the supernatant obtained through centrifugation (4 °C, 13000 rpm for 15 min) of the skeletal muscle homogenate was placed in a 2-ml EP tube. Samples were blow-dried using a vacuum centrifuge with 600 ul phosphate buffer (0.01% TSP, 0.2 mol/L Na2HPO4/NaH2PO4, pH=7.4). After centrifugation at 4 °C and 13000 rpm for 10 min, 550 ul of the supernatant was placed in a 5-mm NMR tube for NMR spectrometry.

1H-NMR spectrometry

The 1H-NMR spectrometer. Samples processed through 1H-NMR spectrometry were detected using a Brucker600-MHz AVACNCE III NMR spectrometer. Scanning could be carried out up to 64-fold with the Bruker 5-mm BBO probe, at a 600.13-MHz proton frequency, 298 K acquisition temperature, Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, spin relaxation of delay of 320 ms, 64 k data points of free induction decay, and a spectral width of 8 kHz.

Statistical analyses

Skeletal muscle maps were processed using the MestReNova software (version 6.1.0-6224 Mesterlab Research, Santiago de Compostela, Spain). The TSP chemical shift was calibrated as δ0.00, and the baseline of all maps was adjusted and the phase was shifted. After manually removing the water peak (δ4.60–5.20), the range of 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.

SIMCA-P (version 13.0 Umetric, Sweden) was used to centralize and normalize the integration values. Thereafter, PLS-DA and OPLS-DA analysis 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.

Results

Statistical analysis of 1H-NMR spectroscopic data

Partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were performed to analyze the 1H-NMR spectrometric data. The results of model verification presented adequate predictive potential and was reliable (Figure 1) and helped ascertain statistical significance to the results of PLS-DA and OPLS-DA (Figure a). The model validation plot shows the skeletal muscle scores of group C and E via PLS-DA respectively and facilitates supervised pattern recognition. Model validity was evaluated on the basis of a 200-permutation test (R2X=0.721, R2Y=0.991, Q2=0.961). The closer the parameter to 1, the higher the value of the reliability of the model. Figure a shows that the rightmost points of R2 and Q2 are significantly higher than those at other points, and the trend of R2 is higher that of than Q2 and can be well separated. Figure c shows that the spatial independent areas of each group are completely separated, indicating a significant difference between groups C and E.

Score and s-plots of group C and E. Figure b shows differences between groups C and E, indicating that the intervention of exercise fatigue induced metabolic changes in rat skeletal muscle. Figure d shows that the VIP value of single variables was larger when it was further from the starting point and significantly different from other metabolic markers, indicating its potential as a biomarker.

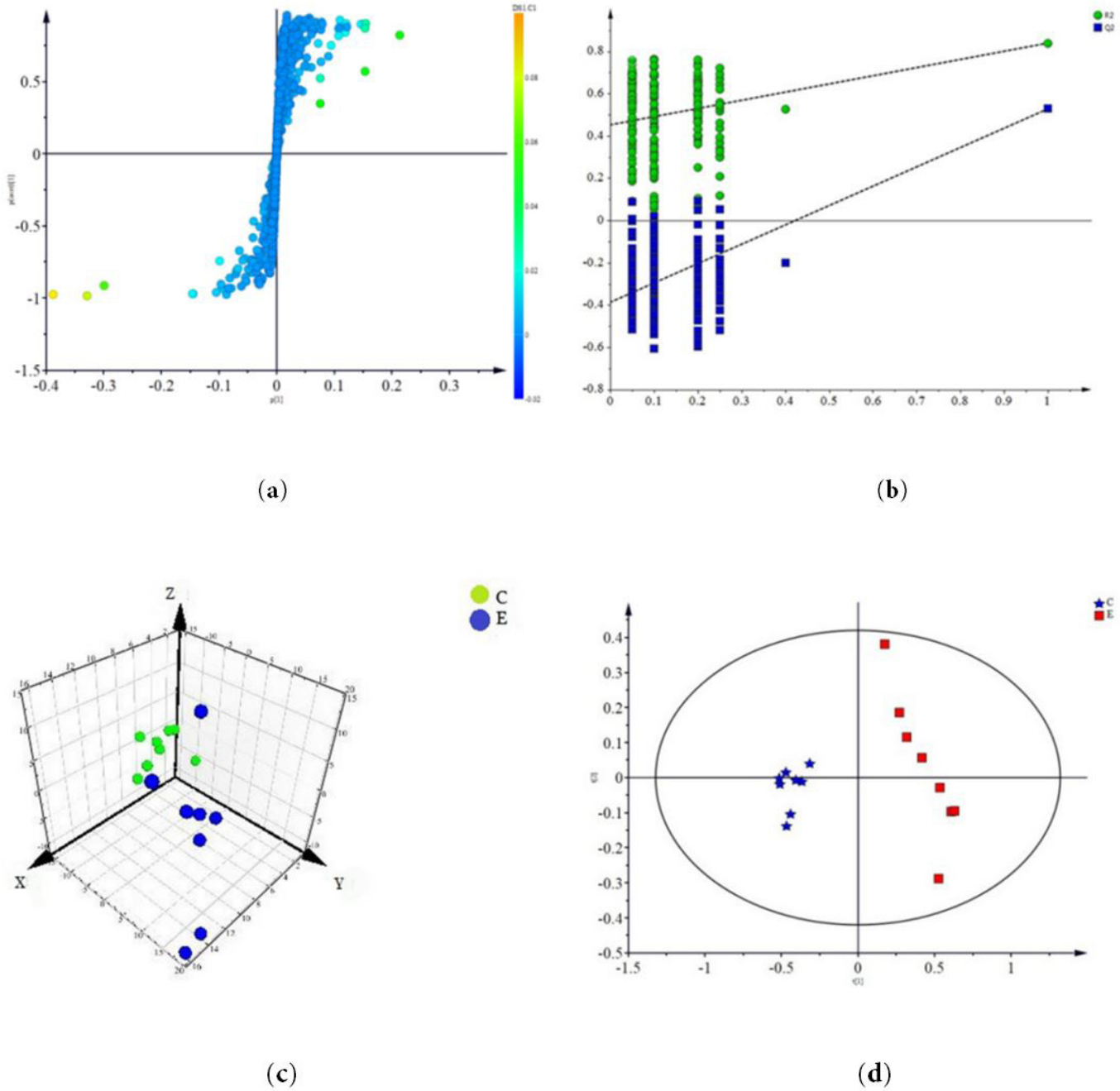

Figure 1: PLA-DA permutation test (a), OPLS-DA scores (b), OPLS-DA 3D (c), S-Plots (d)

Using the Human Metabolome Database (WWW.hmdb.ca), an internationally recognized database, and related metabolomics studies, 18 metabolites were identified via 1H-NMR spectrometry (Table 1). The metabolomic spectrum is shown in Figure 2.

NO.	δ(ppm/peak/coupling constant)	Metabolite	VIP	E vs C
1	1.0(d)	Leucine	1.92	↑**
2	1.34(d), 4.12(q)	Lactic acid	1.05	↑**
3	1.48(d,7.3)	Alanine	1.41	↓**
4	2.15, 2.45 (m)	Glutamine	1.42	↓*
5	2.68(d)	Citric acid	1.18	↑*
6	3.20(s)	Carnitine	4.29	↓**
7	3.26(m)	Trimethylamine N-oxide	1.55	↑**
8	3.43(t), 4.45(d)	Glucose	2.97	↓**
9	3.78(m)	Glycerol	2.52	↑*
10	3.95(s)	Betaine	1.50	↓*
11	8.17, 8.25(s)	Hypoxanthine	2.74	↑**
12	3.04(s)/3.93(s)	Creatine	5.50	↑**
13	3.21(s)	Phosphorylcholine	1.90	↑**
14	7.06(d)	Tyrosine	1.88	↓*
15	3.24(s)	Choline	2.53	↑*
16	2.41(s)	Succinic acid	2.05	↓**
17	4.03(dd)	Cysteine	1.42	↓*
18	1.92(s)	Acetic acid	1.40	↑**

Notes: s：singlet；d: doublet；t: triplet；q：quartet；m：multiplet；dd：double doublet；*: p <0.05; **: p<0.01

Table 1: The variation trend of metabolites in group E and C (X±SD, n=8)

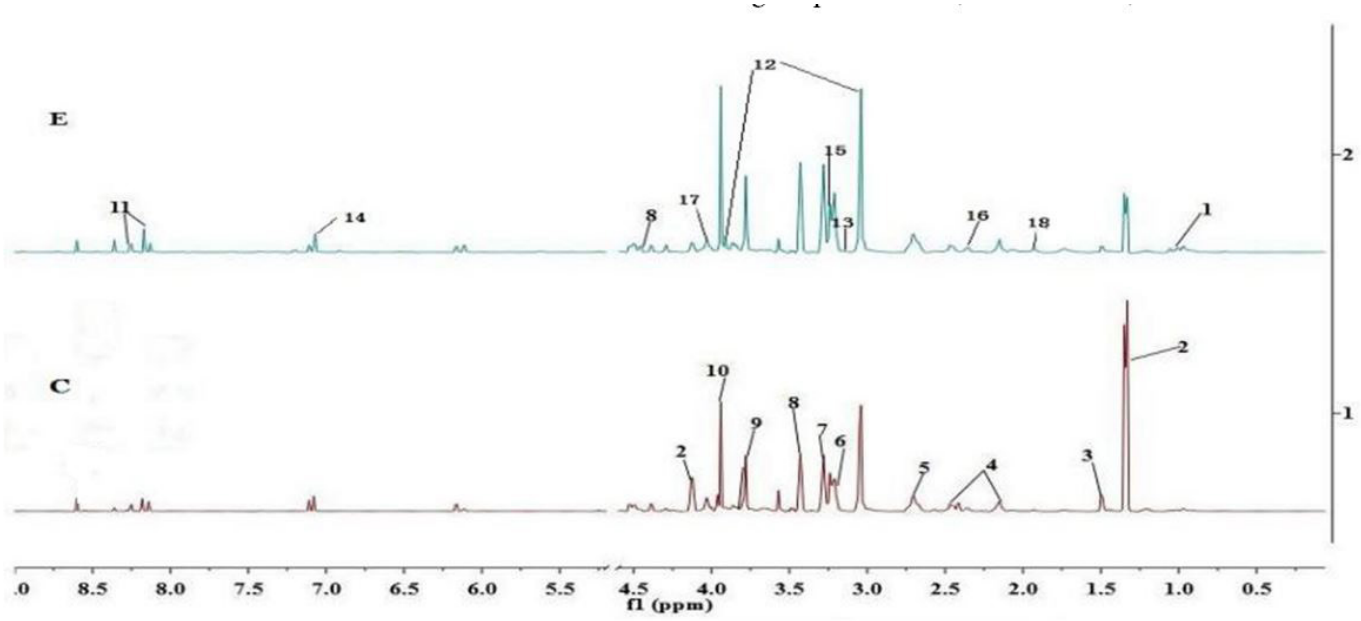

Figure 2: 1H-NMR metabolism map of rat skeletal muscle

Variation trend of metabolites in rat skeletal muscles in groups E and C

On fatigue exercise, 18 different metabolites were present in rat skeletal muscle. Leucine (Leu), lactic acid, Trimethylamine N-oxide (TMAO), hypoxanthine (Hx), creatine, choline phosphate ($p<0.01$), acetic acid, citric acid (CA), glycerol, and choline ($p<0.05$) levels were significantly higher in group E than in group C, while alanine (Ala), carnitine, alpha-glucose, succinic acid ($p<0.01$), glutamine (Gln), betaine (Bet), tyrosine (Tyr), and cysteine ($p<0.05$) levels were significantly lower.

Metabolic pathways of differential metabolites

To identify metabolic pathways associated with exercise fatigue, KEGG (http://www.genome.jp) and HMDB (http://www.hmdb.ca/) were first adopted to quantitatively analyze metabolites, relevant transporters, and the number of pathways (Table 2). MetaboAnalyst 4.0 (http://www. metaboanalyst.ca) was used to analyze the differential metabolites between groups C and E. Eighteen differential metabolites were imported into pathway analysis, revealing 30 metabolic pathways in total (Table 3). Figure 3 shows the pathway impact in the abscissa obtained through topological analysis and represents the importance of metabolic pathways, and –log P in the ordinate represents the significance level of metabolic pathway enrichment analysis. The higher the value of the pathway impact and –log P, the higher the correlation of differential metabolites between groups E and C and larger the circle. Based on the high pathway impact value and P value ($P<0.05$), four metabolic pathways significantly correlated with fatigue exercise were selected as potential targets. The following four metabolic pathways were involved: alanine, aspartic acid, and glutamate metabolism, the TCA cycle, pyruvate metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis (Figure 4).

Query	HMDB ID	KEGG ID	Pub Chem	metabolic pathways	relevant enzymes
Leucine	HMDB0000687	C00123	6106	15	9
Lactic acid	HMDB0000190	C00186	107689	13	10
Alanine	HMDB0000161	C00041	5950	18	37
Glutamine	HMDB0000641	C00064	5961	20	37
Citric acid	HMDB0000094	C00158	311	23	10
Carnitine	HMDB0000062	C00318	2724480	2	7
Trimethylamine N-oxide	HMDB0000925	C01104	1145	3	3
Glucose	HMDB0000122	C00031	5793	32	84
Glycerol	HMDB0000131	C00116	753	8	20
Betaine	HMDB0000043	C00719	247	3	7
Hypoxanthine	HMDB0000157	C00262	790	1	13
Creatine	HMDB0000064	C00300	586	3	5
Phosphorylcholine	HMDB0001565	C00588	1014	3	8
Tyrosine	HMDB0000158	C00082	6057	33	33
Choline	HMDB0000097	C00114	305	2	19
Succinic acid	HMDB0000254	C00042	1110	32	86
Cysteine	HMDB0000574	C00097	5862	22	39
Acetic acid	HMDB0000042	C00033	176	21	99

Table 2: Fatigue exercise differential metabolites basic comments

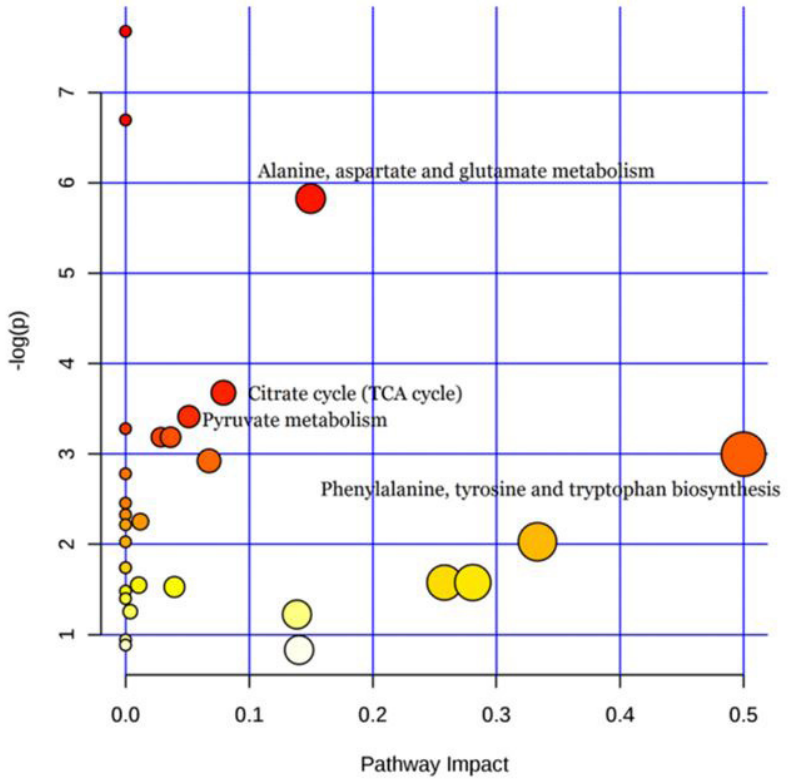

Figure 3: Metabolic pathways of rat skeletal muscle

	Total Cmod	Ex-pected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Glycine, serine and threonine metabolism	31	0.39	4	4.64E-04	7.68E+00	3.80E-02	3.80E-02	0.00
Aminoacyl-tRNA biosynthesis	69	0.88	5	1.24E-03	6.70E+00	1.00E-01	5.07E-02	0.00
Alanine, aspartate and glutamate Metabolism	24	0.30	3	2.95E-03	5.83E+00	2.36E-01	8.07E-02	0.15
Citrate cycle (TCA cycle)	20	0.25	2	2.53E-02	3.68E+00	1.00E+00	4.24E-01	0.08
Pyruvate metabolism	23	0.29	2	3.29E-02	3.41E+00	1.00E+00	4.24E-01	0.05
Ubiquinone and other terpenoid-quinone biosynthesis	3	0.04	1	3.77E-02	3.28E+00	1.00E+00	4.24E-01	0.00
Glycolysis or Gluconeogenesis	26	0.33	2	4.14E-02	3.19E+00	1.00E+00	4.24E-01	0.03
Galactose metabolism	26	0.33	2	4.14E-02	3.19E-02	1.00E+00	4.24E-01	0.04
Phenylalanine, tyrosine and tryptophan biosynthesis	4	0.05	1	4.99E-02	3.00E+00	1.00E+00	4.41E-01	0.50
Glycerophospholipid metabolism	30	0.38	2	5.37E-02	2.92E+00	1.00E+00	4.41E-01	0.07
D-Glutamine and D-glutamate metabolism	5	0.06	1	6.20E-02	2.78E+00	1.00E+00	4.62E-01	0.00

	Total Cmod	Ex-pected	Hits	Raw p	-log(p)	Holm adjust	FDR	Impact
Thiamine metabolism	7	0.09	1	8.58E-02	2.46E+00	1.00E+00	5.86E-01	0.00
Taurine and hypotaurine metabolism	8	0.10	1	9.74E-01	2.33E+00	1.00E+00	5.96E-01	0.00
Arginine and proline metabolism	44	0.56	2	1.05E-01	2.25E+00	1.00E+00	5.96E-01	0.01
Nitrogen metabolism	9	0.11	1	1.09E-01	2.22E+00	1.00E+00	5.96E-01	0.00
Phenylalanine metabolism	11	0.14	1	1.32E-01	2.03E+00	1.00E+00	6.35E-01	0.00
Valine, leucine and isoleucine biosynthesis	11	0.14	1	1.32E-01	2.03E+00	1.00E+00	6.35E-01	0.33
Selenoamino acid metabolism	15	0.19	1	1.75E-01	1.74E+00	1.00E+00	7.57E-01	0.00
Pantothenate and CoA biosynthesis	15	0.19	1	1.75E-01	1.74E+00	1.00E+00	7.57E-01	0.00
Glyoxylatc and dicarboxylate metabolism	18	0.23	1	2.07E-01	1.58E+00	1.00E+00	7.73E-01	0.26
Glycerolipid metabolism	18	0.23	1	2.07E-01	1.58E+00	1.00E+00	7.73E-01	0.28
Purine metabolism	68	0.86	2	2.13E-01	1.55E+00	1.00E+00	7.73E-01	0.01
Starch and sucrose metabolism	19	0.24	1	2.17E-01	1.53E+00	1.00E+00	7.73E-01	0.04
Propanoate metabolism	20	0.25	1	2.27E-01	1.48E+00	1.00E+00	7.75E-01	0.00
Butanoate metabolism	22	0.28	1	2.47E-01	1.40E+00	1.00E+00	8.09E-01	0.00
Glutathione metabolism	26	0.33	1	2.85E-01	1.26E+00	1.00E+00	8.93E-01	0.00
Cysteine and methionine metabolism	27	0.34	1	2.94E-01	1.22E+00	1.00E+00	8.93E-01	0.14
Valine, leucine and isoleucine degradation	38	0.48	1	3.89E-01	9.45E+00	1.00E+00	1.00E-01	0.00
Pyrimidine metabolism	41	0.52	1	4.12E-01	8.86E-01	1.00E+00	1.00E+00	0.00
Tyrosine metabolism	44	0.56	1	4.35E-01	8.32E-01	1.00E+00	1.00E+00	0.14

Total: total number of compounds; Hits: the number of actual matches with the metabolome database; Raw p: original p value obtained by pathway analysis; FDR: false defect rate; Impact: the impact value obtained by topological analysis

Table 3: FFatigue exercise differential metabolites pathway analysis results by MetPA

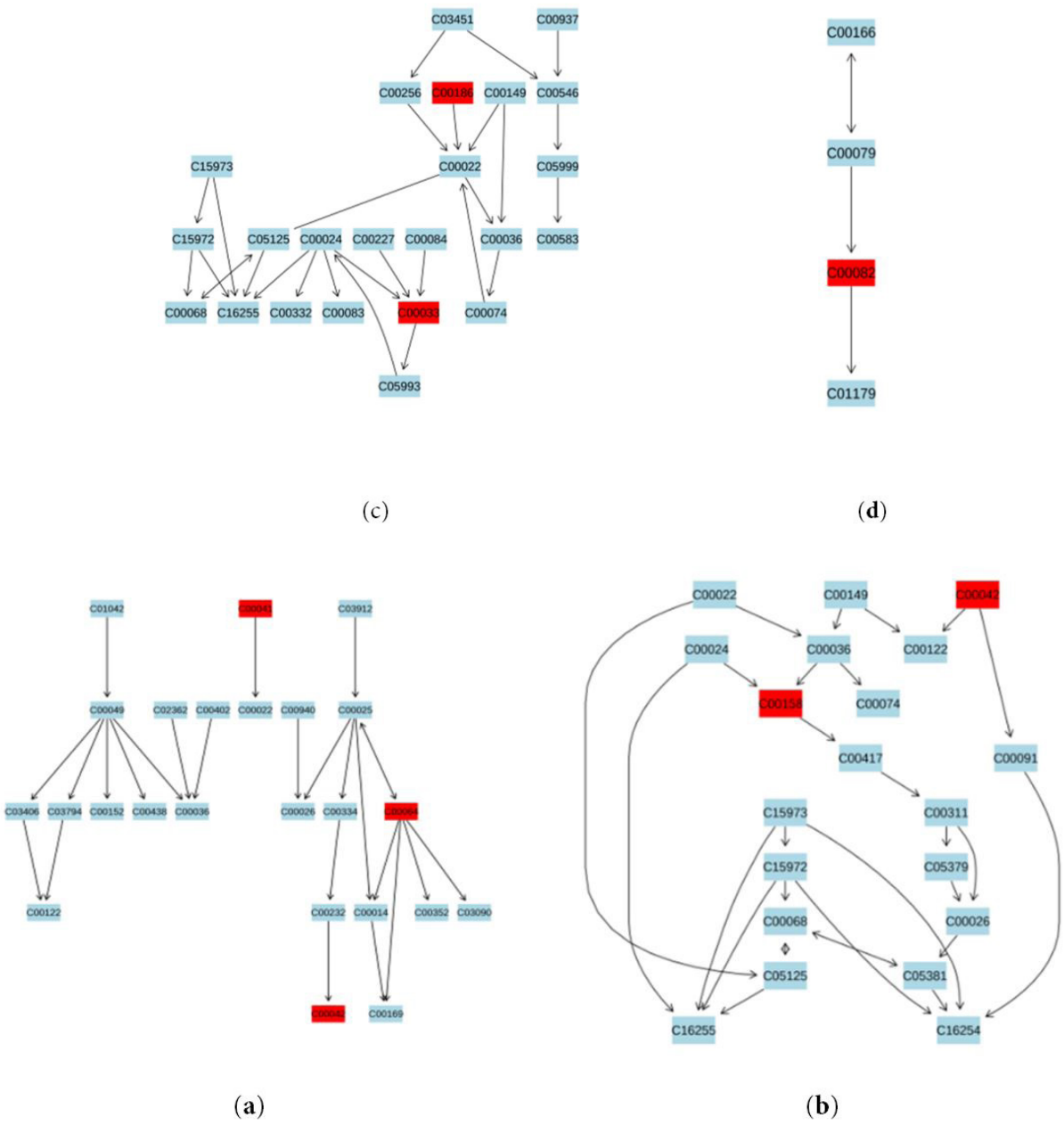

Figure 4: Metabolic pathways associated with exercise fatigue: Alanine, aspartate and glutamate Metabolism (a), TCA cycle (b), Pyruvate metabolism (c), Phenylalanine, tyrosine and tryptophan biosynthesis (d)

Effect of fatigue exercise on metabolic biomarkers in rat skeletal muscle

This study evaluated the diagnostic potential of differential metabolites in fatigue exercise through receiver operating characteristic (ROC) curve analysis. The area under the ROC curve (AUC) represents the diagnostic markers. The closer the value of the AUC to 1, the better the diagnostic performance of the test. If AUC > 0.9, the accuracy is high; when the AUC value is 0.7~0.9, it has a relative accuracy; when the AUC value is 0.5~0.7, it has a low accuracy; when AUC ≤ 0.5, it does not have a diagnostic value. Herein, 17 differential metabolites were selected on the basis of the AUC and the P-value (P <0.05), including leucine, lactic acid, alanine, glutamine, citric acid, carnitine, trimethylamine N-oxide, glucose, glycerin, hypoxanthine, creatine, choline phosphate, tyrosine, choline, succinic acid, acetic acid, and cysteine. Alanine (AUC=1.0), glutamine (AUC= 0.86), succinic acid (AUC=1.0), citric acid (AUC= 0.83), lactic acid (AUC=1.0), acetic acid (AUC= 0.94), and tyrosine (AUC= 0.89) were involved in four fatigue-associated metabolic pathways. These metabolites were considered potential markers of fatigue metabolism herein.

Discussion

To Metabolomics is an essential tool in biological studies, facilitating the assessment of metabolic mechanisms from the overall metabolite profile and combines modern molecular biology techniques with computer information technology. Using a metabolomics approach, this study shows that fatigue exercise can cause differential changes in skeletal muscle metabolism in rats. Herein, 18 differential metabolites were involved in 30 metabolic pathways. MetPA analysis revealed that fatigue exercise altered skeletal muscle metabolism in rats, which is potentially associated with alterations in 4 metabolic pathways including alanine, aspartic acid, and glutamate metabolism, the TCA cycle, pyruvate metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis.

Herein, alanine (c00041), glutamine (c00064), and succinic acid (c00042) were involved in alanine, aspartate, and glutamate metabolism (Figure 4a). In the skeletal muscle, alanine aminotransferase transfers an alpha-amino group from glutamate to pyruvate to form alanine through the "glucose-alanine cycle." Alanine released from skeletal muscle tissue is used as a substrate for hepatic gluconeogenesis [13]. Alanine intake during exercise training reportedly improves exercise performance, attenuates neuromuscular fatigue, and prolongs the duration of exercise [14]. Alanine levels are significantly reduced 30 min after increasing the exercise load [15], concurrent with our previous findings. Alanine levels were significantly decreased in group E ($P < 0.01$), suggesting that fatigue exercise may result in alanine consumption as a substrate for gluconeogenesis in rat skeletal muscle, thus altering alanine, aspartate, and glutamate metabolism. Glutamine (Gln) is the most abundant amino acid in the body and can be converted to upstream glutamic acid (Glu) in the metabolic pathway, which is one of the pathways for ammonia metabolism. Simultaneously, Gln is involved in energy metabolism, regulating blood ammonia and lactate levels, leading to glutathione (GSH) biosynthesis, and protecting against oxidative stress and inflammation [16, 17] reported that plasma and tissue Gln levels in rats, especially in the skeletal muscle, were sharply decreased during resistance training and endurance exercise. If Gln levels are insufficient, muscles decompose to fulfil the physiological demand of Gln, thus potentially affecting muscle volume. Furthermore, thus decreases immunity owing to the release of glutamine from the immune system, which was primarily derived from skeletal muscles [18, 19, 20] reported that dietary Gln supplementation potentially reverses impaired macrophage function resulting from overload training. Herein, Gln levels were significantly decreased after fatigue exercise in rats. These potentially constitute reasons underlying the reduction in immunity owing to fatigue exercise.

The TCA cycle is a fundamental energy-generating aerobic metabolic pathway in humans and is the intersection of glucose, lipid, and amino acid catabolism. TCA cycle intermediates serve as the starting material for various biosynthetic pathways. This study shows that succinic acid (c00042) and citric acid (CA) (c00158) are involved in the TCA cycle (Figure 4b). CA was converted to α-ketoglutarate in the TCA cycle, and the initial phase of CA accumulation did not inhibit the TCA cycle. Reductions in fumaric acid and isocitrate levels and an increase in oxaloacetate, pyruvate, and CA levels occurred during the induction of CA accumulation. A reduction in α-ketoglutarate dehydrogenase potentially decreases succinic acid and fumaric acid levels [21]. The concentration of CA, the starting material for acetyl-CoA entry into the TCA cycle, reflects the extent of TCA metabolism. [22] reported an increase in CA levels in the skeletal muscle in rats after 5 h of aerobic exercise, concurrent with alterations in CA levels herein. The TCA cycle is a comprehensive reflection of energy metabolism [23]. These intermediate metabolite alterations potentially result in TCA cycle dysfunction. Herein, CA levels were significantly increased; however, succinic acid levels significantly decreased in group E, thus resulting in disorders of aerobic metabolism and fatigue. MetPA revealed that succinic acid is not only associated with the TCA cycle but also with alanine, aspartic acid, and glutamate metabolism.

The metabolic pathway involving lactic acid (c00186) and acetic acid (c00033) is pyruvate metabolism (Figure 4c). L-lactic acid (c00186), D-lactic acid (c00256) and L-malic acid (c00149) upstream of the metabolic pathway produce pyruvic acid (c00022). In the secondary metabolic pathway, acetic acid is metabolized by upstream acetyl-coenzyme A (c00024), acetyl phosphate (c00227), and acetaldehyde (c00084). Under adequate oxygenation, tissues such as skeletal muscle and the myocardium directly convert lactic acid into pyruvic acid via lactate dehydrogenase, thus contributing to energy generation. Under normoxic conditions, glycolysis is enhanced, and pyruvic acid is converted into lactic acid by lactate dehydrogenase. The accumulation of lactic acid during exercise can decrease the body pH, reduce neural regulation, deter ATP synthesis, lead to weakened nerve excitability, decrease muscle working capacity, and ultimately lead to exercise fatigue [24]. Lactic acid levels significantly increased after an

acute bout of submaximal endurance exercise [25]. Furthermore, lactic acid activates hypoxia-inducible factor-1 (HIF-1), thus inducing the expression of carbonic anhydrase (CAIX) in tumor cells, promoting the maintenance of cancer cell invasion under normoxic conditions [26]. Herein, lactic acid and acetic acid levels significantly increased after exercise, indicating that the rate of formation of lactic acid and other acidic substances was higher than their elimination rate, and pyruvate metabolism was disrupted.

Tyrosine (TYR) is the precursor for catecholamine (CA) neurotransmitters, dopamine (DA), and norepinephrine (NE). The metabolic pathways involving tyrosine (c00082) are phenylalanine, tyrosine, and tryptophan metabolism (Figure 4d). When CA neurons fire frequently, they become depleted of Tyr neurotransmitter reserves [27]. Exogenous elevation of plasma Tyr plasma levels may prevent the depletion of catecholamines in the central nervous system, and under conditions of acute stress, Tyr administration can significantly improve various physiological and behavioral parameters [28]. Furthermore, brain serotonin (5-HT) and dopamine (DA) neurotransmitters are associated with fatigue, indicating that increased serotoninergic activity reduces performance, while increased dopaminergic activity is associated with increased performance [29, 30] reported that DA levels were significantly decreased during skeletal muscle fatigue. [31] reported that increased brain DA levels inhibit serotonin synthesis, suggesting that a high brain dopamine-serotonin ratio (DA/5-HT ratio) helps prolong exercise and a low ratio induces lethargy and reduced motivation. Brain DA has several roles including movement initiation, motor control, increased arousal, and endurance, resulting in neural excitation. Changes in body Tyr levels are consistent with those of DA levels [32]. Herein, Tyr levels in the skeletal muscle of rats were significantly decreased after exercise, indicating that fatigue exercise can reduce precursor substances in synthetic DA, finally leading to fatigue.

Conclusions

Eighteen differential metabolites and 4 metabolic pathways were screened through 1H-NMR spectrometry and MetPA in comparison with the control group. Fatigue exercise causes differential changes in metabolites potentially associated with alterations in key metabolites in four metabolic pathways including alanine, aspartate, and glutamate Metabolism, the TCA cycle, pyruvate metabolism, and phenylalanine, tyrosine, and tryptophan biosynthesis.

Author Contributions

Zhuo Sun.:First author. Performed sample collection and the data analysis, substantial contributions to conception and design, drafting the manuscript, revising the manuscript and confirmation of the final version for publication and submission. Shui Hu (Lecturer): Substantial contributions to conceptualization and design, acquisition of the financial support for the project leading to this publication. Lijun WU Prof.: Substantial contributions to conceptualization and design.Lu-lu Wei (Lecturer): The data analysis, sample collection.Hui Wang (Phd student):The data analysis, sample collection.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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