Moderate, Rather Than Vigorous Exercise Benefits Plasma Amino Acids Profile in Patients With Nonalcoholic Fatty Liver Disease

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Research

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Abstract

Backgrounds: Exercise benefits nonalcoholic fatty liver disease (NAFLD), and amino acids (AAs) are close associated with the development and progression of NAFLD. However, it is unclear whether AAs profile changes following long term exercise training.

Methods: NAFLD participants (n=220) were recruited and randomly assigned to control group, moderate exercise group, and vigorous exercise group with a 6-month followed-up. Clinical characteristics were carefully calculated and plasma AAs concentrations were determined using a validated ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method.

Results: At baseline, AAs concentrations were close associated with clinical characteristics in NAFLD, particularly, the sum of branched chain amino acids (BCAAs) were positively associated with intrahepatic triglyceride (IHTG) content (r=0.18, P=0.007). After 6 month exercise intervention, IHTG content was reduced by both exercise intensities without a significant difference (P=0.45). Compared to control, several AAs concentrations altered, and different exercise intensity showed inversely impact on histidine, serine, glutamine, valine, tyrosine and tryptophan concentrations changes, particularly, moderate exercise was much more efficient on BCAAs decreasing than vigorous exercise with a significant difference (P=0.0008). Conclusions: Several AAs were close associated with IHTG content in NAFLD patients, and 6 month moderate exercise was more efficient on AAs concentrations alteration, especially BCAAs decreasing than vigorous exercise.

Trial registration number: NCT01418027

Introduction

Nonalcoholic fatty liver disease (NAFLD) has become increasingly a pandemic disease as the improvement of people's life, which is the hepatic manifestation of the metabolic syndrome and insulin resistance [1]. Many studies have demonstrated that exercise was a major recommended treatment due to the effect on decreasing of visceral adipose tissue, liver fat, body fat, weight, and the improvement of cardiovascular risk factors [2–4].

Recently, Targeted and non-targeted metabolomics studies have discovered that altered circulating levels of amino acids (AAs) were typical features in NAFLD subjects, metabolic homeostasis of which plays an important role in the development and progression of NAFLD [5–8]. In particular, branched-chain amino acids (BCAAs, leucine, isoleucine, and valine) have been proved to be elevated in NAFLD patients [9], and strongly associated with increased risk of NAFLD [10], insulin resistance, obesity [11, 12] and future clinical decompensation in NAFLD [13]. It was also revealed that the changes in hepatic BCAA composition were strongly associated with transcriptomic metabolism profiles during the progression of NAFLD [14]. Various exercise intensities impacted plasma metabolic profile differently [15–17], for now, there were fewer studies that examined the circulating AAs alteration after exercise training [18–22], and the relationship between AAs metabolism and exercise intensity was little known. Our previous clinic trial
demonstrated that intrahepatic triglyceride (IHTG) content in NAFLD patients were decreased significantly by both 6 month vigorous and moderate exercise training, and high intensity exercise offers no additional benefit to moderate intensity exercise in reducing liver fat [4, 23], although vigorous exercise was more effective on weight, waist circumference, body fat and visceral fat. However, the metabolic profile of plasma AAs in these NAFLD subjects were unknown, and whether AAs concentrations altered after different exercise intensity training were still unclear [24].

Thus, we performed a targeted metabolomics study on eighteen plasma AAs concentrations analysis in NAFLD subjects using a validated ultrahigh performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) method, and analyzed the correlation between AAs concentrations and clinical characteristics, especially IHTG content, and also assessed the effect on AAs concentrations alteration after long term vigorous and moderate exercise training.

Methods

Study participants

This study protocol, informed consent, and all steps from blood extraction to AAs concentrations analysis were approved by a steering committee, institutional review boards of Xiamen University and the First Affiliated Hospital of Xiamen University in China. All methods were carried out in accordance with the relevant guidelines and regulations.

Study participant and protocol were described in our previous study [4], briefly, a total of 220 individuals with NAFLD were selected and randomly assigned to control group (n = 74), moderate exercise group (n = 73, brisk walking 150 minutes per week at 45%-55% of maximum heart rate), and vigorous-moderate exercise group (n = 73, jogging 150 minutes per week at 65%-80% of maximum heart rate for 6 months and brisk walking 150 minutes per week at 45%-55% of maximum heart rate for another 6 months). Blood samples were collected in the early morning after an overnight fast, and then the plasma samples were stored at -80 °C until analysis. In this present study, clinical characteristics and plasma AAs concentrations of all study participants at baseline and 6-month exercise intervention were studied.

Determination Of Plasma AAs

Plasma AAs concentrations were determined accurately using Agilent 6460 triple stage quadrupole mass spectrometer equipped with an Agilent 1290 HPLC system (Agilent Technologies, Santa Clara, CA, USA), after sample preparation, chromatographic conditions and mass spectrometer parameters for AAs analysis were carefully optimized, and linearity, sensitivity, intra-day and inter-day precision, accuracy, and matrix effects were strictly performed for method validation (data were provided in supplementary material).
20 µL of plasma was added 1 µL L-phenyl-d5-alanine (10 µg/mL) and 80 µL acetonitrile, the mixture was vortex-mixed for 30 s and centrifuged at 19,000 × g for 10 min at 4 °C, 40 µL of the supernatant was then mixed with 120 µL water, then the aliquot of which (5 µL) was injected into the UHPLC-MS/MS system for analysis (LC-MS condition for determination of eighteen AAs were provided in supplementary material). Data of AAs concentrations were acquired and processed using Agilent Mass Hunter Workstation Data Acquisition and Quantitative Analysis B.07.00 (Agilent Technologies, Santa Clara, CA, USA).

Using this approach, we were able to quantify eighteen AAs concentrations in human plasma: taurine (Tau), methionine (Met), glutamine (Gln), histidine (His), lysine (Lys), leucine (Leu), isoleucine (Ile), valine (Val), arginine (Arg), phenylalanine (Phe), tryptophan (Trp), proline (Pro), threonine (Thr), alanine (Ala), serine (Ser), glutamine (Gln), glycine (Gly), and tyrosine (Tyr).

**Statistical analysis**

Data were summarized using frequencies and counts for categorical variables and means and standard deviations for continuous variables. Data were analyzed according to participants’ randomization assignments, regardless of their subsequent status. The general linear models were performed to assess the effects of exercise programs on the change in plasma AAs levels, with adjustment for age, sex, BMI at baseline, value for the respective outcome traits at baseline. Pearson’s correlation coefficient (for normally distributed variables) and Spearman’s rank correlation (for non-normally distributed variables) were used for correlation analysis of plasma AAs levels and clinical characteristics at baseline. Heatmap analysis was performed using GraphPad Prism 7.0. SAS statistical software, version 9.4 (SAS Institute Inc), was used to obtain point estimates and SEs of the treatment effects and to test for differences between treatments. \( P < 0.008 \) (0.05/6 comparisons) was considered statistically significant.

**Results**

**Clinical characteristics of study participants**

A total of 220 eligible trial participants were recruited and followed up for 12 months [4], in the present study, only 6 month intervention were concerned (74 participants completed in the 6 month control group, 69 participants completed the 6 month moderate exercise and 68 participants completed the 6 month vigorous exercise, respectively).

Baseline clinical characteristics were balanced among the three groups (Supplement Table 4), including the general information and clinical parameters. After 6 month exercise training, IHTG content was reduced by both exercise intensities (5% by vigorous exercise, \( P < 0.001 \); 4.2% by moderate exercise, \( P < 0.001 \)) with an equal effect \( (P = 0.45) \), while weight, waist circumference, body fat, visceral fat and blood pressure were only decreased significantly after vigorous exercise (Supplement Table 5).
Association Between Aas Concentrations And Clinical Characteristics At Baseline

Baseline plasma AAs concentrations of NAFLD subjects in control, moderate exercise and vigorous exercise groups were summarized in Table 1. At baseline, there were no significant difference between these three groups on seventeen AAs concentrations, while plasma Phe concentrations were much higher in moderate exercise group than control ($P = 0.0012$) and vigorous exercise group ($P = 0.0684$), respectively.

| AAs | Control (n = 74) | Moderate exercise (n = 73) | Vigorous exercise (n = 73) | $P$ values |
|-----|------------------|---------------------------|---------------------------|------------|
| Orn | 6.1 (2.5)        | 5.7 (1.9)                 | 6.3 (3.1)                 | 0.7183     |
| Lys | 7.8 (2.1)        | 8.0 (1.8)                 | 8.7 (2.6)                 | 0.0460     |
| Arg | 5.8 (2.3)        | 6.3 (1.8)                 | 6.0 (1.5)                 | 0.3621     |
| His | 10.6 (1.5)       | 10.6 (1.4)                | 11.4 (4.0)                | 0.1095     |
| Gly | 16.0 (3.5)       | 16.3 (4.0)                | 16.3 (6.9)                | 0.7110     |
| Ser | 11.9 (1.8)       | 12.1 (2.1)                | 12.7 (2.9)                | 0.6992     |
| Gln | 72.7 (11.1)      | 72.1 (10.4)               | 76.4 (7.9)                | 0.0149     |
| Ala | 35.3 (6.3)       | 36.2 (6.2)                | 35.6 (5.7)                | 0.7080     |
| Tau | 16.9 (3.4)       | 16.2 (3.7)                | 16.3 (4.0)                | 0.4731     |
| Thr | 13.1 (1.9)       | 12.8 (2.1)                | 13.0 (2.9)                | 0.6840     |
| Pro | 17.7 (4.6)       | 18.6 (4.9)                | 18.9 (4.8)                | 0.2776     |
| Val | 22.4 (4.0)       | 23.3 (4.1)                | 22.7 (4.5)                | 0.4155     |
| Met | 2.8 (0.6)        | 2.8 (0.4)                 | 2.7 (0.5)                 | 0.9176     |
| Ile | 7.0 (1.7)        | 7.2 (1.5)                 | 7.2 (1.8)                 | 0.6029     |
| Tyr | 9.0 (2.3)        | 9.2 (1.7)                 | 9.1 (2.3)                 | 0.8630     |
| Leu | 15.1 (3.0)       | 15.7 (2.9)                | 15.2 (3.1)                | 0.3535     |
| Phe | 11.8 (2.0)       | 12.4 (1.6)                | 11.4 (2.0)                | 0.0050     |
| Trp | 12.2 (2.0)       | 12.5 (2.1)                | 11.7 (2.1)                | 0.9077     |

Data were presented as mean (standard deviation).

$P < 0.008$ (0.05/6 comparisons) was considered statistically significant.
We assessed which kind of AAs was most close associated with the main clinical characteristics in NAFLD subject using bivariable correlations. Most of AAs had strong relationship with clinical parameters at baseline Fig. 1; in particular, all kinds of BCAAs Ile, Val and Leu were positively associated with IHTG content, liver enzymes (alanine transaminase and glutamyl transpeptidase), weight, BMI (body mass index), waist circumference and visceral fat, and inversely associated with body fat and HDL-C, respectively. Furthermore, the correlation analysis between the sum of BCAAs and IHTG content were performed (r = 0.18, P = 0.007, Fig. 2). Similarly, both aromatic AAs Phe and Tyr were positively correlated with weight, BMI and waist circumference.

Changes of plasma AAs concentrations response to 6 month exercise training

As showed in Table 2, concentrations of Orn, Lys, Arg, Thr and Pro did not change following the 6 month exercise training program. Compared to control, concentrations of His, Gly, Ser, Ala, Tau, Val, Ile, Tyr, Leu, Phe and Trp were decreased and concentrations of Gln was increased significantly after 6 month moderate exercise; concentrations of Gln was decreased and concentrations of His, Ser, Val, Met, Tyr and Trp was increased significantly after 6 month vigorous exercise, respectively. In addition, there were significant differences in the changes of Gly, Gln, Tau, Met, Ile, Leu and Phe between moderate and vigorous exercise intervention. Furthermore, the changes of the sum BCAAs concentrations after 6 month intervention (Fig. 3) were calculated, it was found that compared to vigorous exercise, moderate exercise (P = 0.006) was much more efficient on decreasing BCAAs concentrations with a significant difference between these two exercise intensity (P = 0.0008).
Table 2
Changes of plasma AAs concentrations after 6 month exercise intervention.

| AAs | Changes (95% CI) | P values |
|-----|------------------|----------|
|     | Control          | Moderate exercise | Vigorous exercise | Moderate vs Control | Vigorous vs Control | Vigorous vs Moderate |
| Orn | 0.6 (-0.6 to 1.8) | -0.1 (-1.0 to 1.6) | 0.6 (-0.2 to 2.1) | 0.9063 | 0.9247 | 0.9824 |
| Lys | 0.9 (-0.8 to 2.1) | 0.7 (-0.7 to 2.0) | 0.5 (-0.6 to 1.58) | 0.7241 | 0.4779 | 0.2946 |
| Arg | 0.5 (-1.2 to 1.2) | 0.7 (-0.7 to 1.9) | 0.4 (-0.40 to -0.8) | 0.0233 | 0.9253 | 0.0203 |
| His | 0.5 (-0.1 to 1.1) | 0.05 (-1.1 to 0.7) | 0.7 (0.03 to 1.8) | <.0001 | 0.0002 | 0.1835 |
| Gly | 1.3 (-0.7 to 3.0) | -0.3 (-1.4 to 1.4) | 3.3 (1.5 to 5.1) | 0.0008 | 0.9771 | 0.0012 |
| Ser | 1.3 (0.4 to 2.7) | 0.3 (-0.8 to 1.1) | 2.2 (1.4 to 3.7) | <.0001 | 0.0001 | 0.1633 |
| Gln | -4.8 (-9.9 to 2.1) | 0.5 (-4.4 to 7.4) | -5.0 (-8.0 to -2.0) | 0.0037 | 0.0006 | <.0001 |
| Ala | 2.2 (-2.2 to 6.2) | -2.7 (-7.1 to 0.1) | 2.9 (-1.6 to 6.7) | <.0001 | 0.0204 | 0.0481 |
| Tau | 0.7 (-0.8 to 2.5) | -1.2 (-3.8 to 2.0) | 2.1 (0.03 to 4.7) | <.0001 | 0.6612 | <.0001 |
| Thr | 0.1 (-1.2 to 1.4) | 0.3 (-1.0 to 1.7) | 1.5 (-0.6 to 2.7) | 0.3926 | 0.0921 | 0.4038 |
| Pro | 0.3 (-1.4 to 3.1) | -0.6 (-2.6 to 1.5) | -0.01 (-1.9 to 2.0) | 0.0777 | 0.2217 | 0.6023 |
| Val | 0.8 (-1.9 to 3.1) | -0.7 (-2.9 to 1.2) | 1.0 (-0.2 to 2.9) | <.0001 | 0.0007 | 0.0185 |
| Met | 0.04 (-0.2 to 0.4) | -0.1 (-0.3 to 0.3) | 0.2 (-0.2 to 0.4) | 0.4806 | 0.0003 | 0.0040 |
| Ile | 0.4 (-0.6 to 1.1) | -0.1 (-0.9 to 0.6) | 0.3 (-0.1 to 1.0) | <.0001 | 0.1139 | 0.0006 |
| Tyr | 0.4 (-0.4 to 1.3) | -0.4 (-1.3 to 0.4) | 0.7 (-0.2 to 1.1) | <.0001 | 0.0002 | 0.0090 |
| Leu | 0.7 (-0.6 to 2.2) | -0.2 (-1.6 to 0.9) | 1.3 (0.4 to 2.3) | <.0001 | 0.8250 | <.0001 |

*P* < 0.008 (0.05/6 comparisons) was considered statistically significant.
| AAs     | Changes (95% CI) | Pvalues |
|---------|------------------|---------|
|         | Control          | Moderate exercise | Vigorous exercise | Moderate vs Control | Vigorous vs Control | Vigorous vs Moderate |
| Phe     | 0.7 (-0.1 to 1.5) | -0.8 (-1.5 to 0.1) | 1.3 (0.7 to 2.0) | < .0001 | 0.0994 | < .0001 |
| Trp     | -0.5 (-2.2 to 1.2) | -1.0 (-2.1 to 0.4) | -0.2 (-2.0 to 1.3) | 0.0003 | 0.0062 | < .0001 |

*P < 0.008 (0.05/6 comparisons) was considered statistically significant.*

**Discussions**

A simple UHPLC-MS/MS method with a small volume plasma sample for determination of eighteen AAs in human plasma was developed and validated in our study, and then we analyzed the association between AAs concentrations and clinical characteristics, and assessed AAs changes after long term exercise training in NAFLD subjects. To our best knowledge, this was the first study that reported the relationship between plasma AAs concentrations and clinical characteristics in NAFLD patients, and compared the effect on AAs alteration following different long term exercise intensities.

Our study observed that AAs concentrations were close associated with clinical characteristics in NAFLD, Gly and Ser were inversely correlated with IHTG content, Ala and BCAAs (Val, Ile, Leu) were positively correlated with IHTG content, respectively. Thus, these AAs might be another potential indicators of NAFLD, since IHTC were diagnostic criteria for NAFLD in clinic (IHTG content > 5% was considered as NAFLD). Prior studies have shown a close association between BCAAs and HOMA-IR, liver fat 9, our work indicated a statistically significant association between BCAAs and IHTG content (r = 0.18, \( P = 0.007 \)), which verified the vitally important role of BCAAs in NAFLD progression.

After 6 moderate exercise training, BCAAs concentrations was decreased, the linking mechanisms remain uncertain. NAFLD was associated with down regulation of BCAA catabolic enzymes, which contribute to accumulation of BCAA in plasma [25], might be partially reversed in response to exercise, leading to a decrease of BCAAs in blood. In another hand, exercise training improved the oxidative potential of muscle mitochondria [26], where BCAAs catabolism occurred [27], thus improvement of mitochondrial oxidative capacity would likely impact the processes for consuming excess plasma BCAAs.

Our study found that plasma AAs concentrations changed differently following different exercise intensities. Compared to control, moderate exercise and vigorous exercise showed inversely impact on His, Ser, Gln, Val, Tyr and Trp concentrations changes, which might because muscle metabolic variables were different during various exercise condition: a clinical trial found that in overweight human, some AAs levels were did not change on average with month training program [18–20], in healthy adult males, plasma BCAAs concentrations were decreased by an acute exercise bout [21] or increased after a ten-
week standardized exercise program [22]. We hypothesized at first that high intensity exercise could largely change AAs concentrations, since a recent reported monitored that the strongest response of serum metabolic fingerprint was seen after resistance exercise, followed by high-intensity interval exercise, while effect of continuous moderate-intensity exercise was weak in men with metabolic syndrome [28]. However, the results in our work indicated that 6 moth moderate exercise seemed more efficient on AAs metabolism improvement in a conflict with prior study, especially on BCAAs decreasing. For now, the inner mechanism of amino acids changes following various intensity exercises were limited, we considered that exercise intensity was an important modulator of plasma AAs profile, and moderate exercise benefits AAs metabolism better than vigorous exercise due to the better impact on the enzymatic and molecular processes regulating AAs delivery and oxidation in liver and/or skeletal muscle, according to the finding that low-intensity exercise favors a fat oxidation rate than in the high intensity exercise group with a greater decrease in body mass and fat mass [29], which needed much more further investigation to confirm these hypothesis.

The major strength of our study was a moderately large number of participants treated with long term exercise training in different exercise intensities, and a combination with a novel targeted metabolomics study for AAs profile analysis. Nonetheless, we acknowledge several limitations of our study. No healthy people were participated in the clinic trial, thus we did not know whether AAs concentrations in NAFLD subjects after long term exercise training remained greater or returned to normal levels. Moderate exercise was more efficient on AAs changes than vigorous exercise with a lack of inner mechanism, and impact on BCAAs concentrations after exercise in tissues where BCAAs metabolized (muscle, adipose and liver) were unknown, thus, a corresponding animal model was necessary to verify the clinical finding.

In conclusion, several plasma AAs were close associated with main clinical characteristics in NAFLD patients, and altered after 6 month exercise training. Moderate exercise was more efficient on AAs changes than vigorous exercise, especially on BCAAs decreasing. These results add to the ongoing research efforts to map the metabolic profile responses to different exercise intensities and optimize exercise guidelines for individuals with NAFLD.

**Abbreviations**

NAFLD
nonalcoholic fatty liver diseas
AAs
amino acids
UHPLC-MS/MS
ultrahigh performance liquid chromatography-tandem mass spectrometry
BCAAs
branched chain amino acids
IHTG
intrahepatic triglyceride
Declarations
Ethics approval and consent to participate:
This study protocol, informed consent, and all steps from blood extraction to AAs concentrations analysis were approved by a steering committee, institutional review boards of Xiamen University and the First Affiliated Hospital of Xiamen University in China. All methods were carried out in accordance with the relevant guidelines and regulations.

Consent for publication:
not applicable

Availability of data and materials:
Data sharing is not applicable to this article as no datasets were generated

Competing interests:
no financial/commercial conflicts of interest.

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Authors’ contributions:
Jia Li, Xuejun Li and Zhong Chen contributed the study conception and design, Jia Li wrote the manuscript, Xiulin Shi was responsible for data analysis, Yinxiang Huang, Yan Zhao, Zheng Chen, were participated in clinical trial study, clinical characteristics detection, data analysis and blood sample collection. Caoxin Huang and Xuejun Li provided the funding.

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Figures
Figure 1

Heatmap analysis of correlation between AAs concentrations and clinical characteristics in NAFLD participants at baseline (n=220). *P<0.05, **P<0.01, ***P<0.001.
Figure 2

Correlation between the sum of BCAAs concentrations and IHTG content in NAFLD participants at baseline (n=220).
Figure 3

The sum of BCAAs concentrations changes after 6 month moderate and vigorous exercise training.

Supplementary Files

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