Possible-sarcopenic Screening with Disturbed Plasma Amino Acid Profile in the Elderly

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Research Article

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Abstract

Background

The mass and strength of skeletal muscle decline with age, leading to its progressive dysfunction. High-throughput metabolite profiling provides the opportunity to reveal metabolic mechanisms and the identification of biomarkers. However, the role of amino acid metabolism in possible sarcopenia remains unclear.

Objectives

The aim of this study included exploring changes in plasma amino acid concentrations in elderly individuals who may have possible sarcopenia and attempting to characterize a distinctive plasma amino acid profile through targeted metabolomics.

Methods

A cross-sectional, correlational research design was used for this study. Thirty possible-sarcopenic elderly participants were recruited (n = 30), as determined by the Asian Working Group for Sarcopenia (AWGS). Meanwhile, a reference group of non-sarcopenic (sex-, age-, and Appendicular Skeletal muscle Mass Index (ASMI)-matched non-sarcopenic controls, n = 36) individuals was included in their comparisons to reflect potential differences in the metabolic fingerprint of the plasma amino acids associated with sarcopenia. Both groups were conducted the body composition analysis, physical function examination, and plasma amino acid-targeted metabolomics. The amino acids in plasma were measured using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS). Also, orthogonal partial least-squares-discriminant analysis (OPLS-DA) was applied to characterize the plasma amino acid profile.

Results

With respect to Handgrip Strength (HGS), the Five-Repetition Chair Stand Test (CS-5), the Six-Minute Walking Test (6MWT), the arm curl, the 30s-Chair Stand Test (CST), the 2-Minute Step Test (2MST), the 8-Feet Timed Up-and-Go Test (TUGT), there was a decline in skeletal muscle function in the possible-sarcopenic group compared to the non-sarcopenic group. The mean plasma concentrations of arginine, asparagine, phenylalanine, serine, lysine, glutamine, and threonine were significantly lower in the possible sarcopenia group, whereas cirulline, proline, serine, and glutamic acid concentrations were higher. According to the multi-analysis, glutamine, serine, lysine, threonine, and proline were the potential markers that could have indicated possible sarcopenia.

Conclusions

The findings characterize the significantly altered plasma amino acid metabolisms in the elderly with possible sarcopenia, which aids to screening people who are at a high risk of developing condition, allowing for the design of new preventive measures and therapeutic options.

1 Introduction

A progressive decline in muscle strength and an increase in muscle fatigability translate into a generalized deterioration of physiological function, an increase in the rate of disability and dependency[1]. Sarcopenia, the term for the aging-related loss of skeletal muscle, has serious physiological and clinical repercussions[2–5]. Recent researches have indicated that the age-related decline in muscle strength is greater than what would be expected by the decline in muscle mass alone, with consequent decrease in the strength to mass ratio, also known as biomechanical muscle quality[6, 7]. Although the age-related decline in muscle strength is associated with the loss in muscle size[8], longitudinal studies discovered a 1.5 to 5 times greater decline in muscle strength compared with muscle size[9, 10]. In addition, there was a stronger correlation between muscle strength and disability compared to that between muscle mass and strength[11]. Evidence suggests that physical performance is influenced by muscular strength, and that this relationship is curvilinear in older individuals[12–14]. Interestingly, decreasing muscle strength rather than muscle mass alone is a far stronger predictor of functional limitation and poor health in older adults[15]. Further strategies for early detection of those suffering from, or at risk of developing sarcopenia, are advised by the Asian Working Group for Sarcopenia (AWGS) 2019 to enable necessary interventions in contexts lacking advanced diagnostic equipment[16]. In particular, AWGS 2019 introduces “possible sarcopenia”, defined as poor muscle strength with or without reduced physical performance, which is recommended for use in primary healthcare and preventive services[16]. The etiology of skeletal muscle mass and function loss may include satellite cell senescence, death of motor neurons, decreased activity of neuromuscular junctions, hormonal state, pro-inflammatory cytokines, impaired mitochondrial function, abnormal myokine synthesis, and weight loss accompanied by decreased appetite[3–5]. The actual cause of the reduction in skeletal muscle's ability to contract with age, despite extensive research in this area, is still a mystery.

All organ systems in the human body are connected by plasma free amino acids (PFAA), which are abundantly circulated and whose profiles have been shown to be influenced by metabolic variations in specific organ systems induced by certain diseases[17]. Recent research from the Baltimore Longitudinal Study of Aging found that distinct amino acid signatures were associated with muscle mass in elder adults with functional limitations[18] and poor muscle quality[19]. Low plasma levels of essential amino acids (EAAs) characterized the amino acid profile of severely frail Japanese older people compared to their non-frail peers[20]. High levels of serum branched-chain amino acids (BCAA) are related with a higher fat-free mass in older adults[21] and better skeletal muscle in older adults, respectively. Abnormal

PFAA profiles in age-related diseases[18, 22, 23], indicating that skeletal muscular decline may be accelerated by defective PFAA metabolism. Furthermore, leucine-rich EAA supplementation plus physical exercise can increase the amount and the strength of skeletal muscle in sarcopenic older adults[24]. Therefore, the existence of an amino acid signature in advanced aged individuals with weak skeletal muscle raises the possibility that specific metabolic alterations might be involved in the pathogenesis of this condition. The discovery of biochemical indicators for diminished muscle power in older persons has, however, received very little study attention.

Although the pathophysiology of aging is complicated and multifaceted, the major role given to muscle recession suggests that biomarkers related to it should be utilized to unveil its underlying mechanisms and identify worthwhile targets for interventions. As a result, amino acid profiling may be served as a potent analytical approach to explore the potential function of protein-amino acid networks in possible sarcopenia, especially when coupled with multivariate statistical analysis. Therefore, the objectives of the current study were to characterize the plasma amino acids print and develop a metabolite predictor from possible sarcopenia, thus to provide an analytical explanation of complicated metabolic processes.

2 Experimental Procedures

2.1 Study design

The cross-sectional study was based on baseline data from RCT study of the National Key Research and Development Program of China (RCTs registered at the Chinese Clinical Trial Registry on Oct. 19, 2022; ChiCTR2200064801) conducting in October, 2022. Volunteers between the ages of 81 and 90 years were recruited from the Hua-Du Aged-care Center (Shandong Province, China). After participants received a detailed explanation of procedures, risks of the investigation, and provided with a written consent, the survey was launched. Sixty-six senior adults were enrolled after written informed consent was obtained. According to the AWGS 2019 recommendation, the possible sarcopenia was defined as low muscle strength with or without reduced physical performance: low muscle strength diagnostic cutoffs were handgrip < 28.0 kg for men and < 18.0 kg or women. Hence, participants were divided into two groups with possible sarcopenia and non-sarcopenia.

A questionnaire was used to collect demographic information (gender, age, illnesses, present medical issues, and lifestyle choices) and estimate dietary consumption, which has a great impact on metabolite. We asked about the intake frequency of eggs, red meat, poultry, freshwater fishes, seafood, soybean products, milk. Meanwhile, blood specimens specimens were collected from the study subjects and muscle mass, physical function examination was also performed. Basic information, physical function indexes and plasma amino acids metabolites were compared between possible sarcopenic and non-sarcopenic control groups. The data used in this study were anonymized and masked for analysis.

Inclusion criteria: $81 \leq$ age ≤ 90; ability to communicate with others; ability to perform physical function test; no disease that might impair exercise performance

Exclusion criteria: To prevent issues that could jeopardize each subject's involvement in the study, each participant was thoroughly screened. Any of the following conditions exclude a participant from fulfilling the following experiments: uncontrolled hypertension; acute musculoskeletal injuries, joint contractures or internal metal implants such as total joint arthroplasty; cardiovascular, pulmonary disorders or serious sequelae that could prevent them from engaging in exercise; neurological impairment.

Ethical Procedure

All experiments were approved by the Ethics Committees of Beijing Sport Universityfor Sports Science Experimental (trial number 2020082H). The norms of the China of Health were met, according to Ethical review measures for biomedical research involving human beings of Health, Science and Education [2007]17. All methods were performed in accordance with the relevant guidelines and regulations. The procedures and risks of the investigation were thoroughly explained to participants and written informed consent was obtained prior to participation.

2.2 Assessments and procedures for data collection

2.2.1 Measurements

InBody S10 (InBody Co. Ltd, Seoul,Korea) was used to estimate muscle mass (kg/m^2) in the standing position. After height and weight were measured, four electrodes were attached to both upper and lower extremities to obtain appendicular and trunk muscle mass. ASMI was calculated by dividing the upper and lower extremitiesthe muscle mass by height squared in meters[25].

According to the literatures, physical function used a wide range of physical performance tests, including Handgrip Strength (HGS)[26], the Five-Repetition Chair Stand Test (CS-5)[27], the Six-Minute Walking Test (6MWT)[28], the arm curl, the 30s-Chair Stand Test (CST)[28], the 2-Minute Step Test (2MST), the 8-Feet Timed Up-and-Go Test (TUGT)[28].

2.2.2 Blood Sample Collection and plasma amino acids quantification

For measures of plasma amino acids metabolites, blood samples were collected from the forearm vein into vacutainers containing lithium-heparin after an overnight fast at the same day with physical function examination. The plasma-fraction was then obtained by centrifugation at 3,000for 30at 4°C. The samples were immediately stored at -80 C for further analysis[30].

The amino acids in plasma were measured using ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS-MS). Samples were analyzed on a QTRAP 5500 LC-MS/MS system (SCIEX, Framingham, MA) equipped with a Waters UPLC (Waters, USA)[31].

For the quantification of amino acids, 40μL plasma was mixed with 20 stable-isotope-labeled internal standard (IS) in sulfosalicylic acid to precipitate proteins, and this process was then vortexed and centrifuged (4000 rmp, 4°C, 20). Chromatographic separation was achieved on an HSS T3 column (100×2.1 mm, 1.8 μm, Waters, USA), and the column temperature was maintained at 40°C. The UPLC system employed a gradient elution program consisting of water with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). The linear gradient was optimized and was as follows: 0.3-6 min, 2–40% B; 6–9 min, 40% B, 9-9.5 min for 40–90% B, with a 0.5 ml/min flow rate. Calibration was achieved by spiking plasma with various concentrations of amino acid standards. Data were further analyzed using MultiQuant software (SCIEX, USA)[31].

3 Statistical Methods

3.1 Descriptive Statistics

Mann-Whitney U-test was implemented to evaluate the demographic information, physical function and plasma amino acid concentration between the possible-sarcopenic and non-sarcopenic group with IBM SPSS software 23.0. Data are presented as mean ± standard deviation (SD), with statistical significance value $p< 0.05$.

3.2 The plasma amino acid analysis

The data generated by the plasma amino acid metabolomics were then imported separately into the SIMCA-P + 14.1 software package. We used principle component analysis (PCA) and orthogonal partial least-squares-discriminant analysis (OPLS-DA) to identify differences of plasma amino acids. The contribution of each metabolite ion to the discrimination of each group is reflected in the variable importance in projection (*vip*) obtained in OPLS-DA processing. Variables with a vip > 1 and $p < 0.05$ were different, and the metabolites with statistical significance in multivariate and univariate analyses (vip >1.0 and $p< 0.05$) were able to distinguish the possible sarcopenia group from the control group.

4 Result

4.1 The characteristics of participants information and skeletal muscle function

Information such as ages, food frequency questionnaire results and skeletal muscle function characteristics for the entialed participants between possible sarcopenia and non-sarcopenia were listed in Table 1. There was no discernable difference in ages, food frequency and Appendicular Skeletal muscle Mass Index (ASMI) between two groups. However, physical function was poorer in participants who have possible sarcopenia than in controls, as expected ($p < 0.01$).

Table 1
The main details characteristics and skeletal muscle function values between two groups

Physical Performance	possible sarcopenia (n = 30)	Non-sarcopenia (n = 36)	*p-value*
Age (y)	85.77 ± 3.53	84.69 ± 2.73	0.169
coarse cereals	20.00 ± 4.75	19.67 ± 4.60	0.78
eggs	17.66 ± 6.19	16.67 ± 2.24	0.238
red meat	20.24 ± 17.57	21.22 ± 4.44	0.476
poultry	20.59 ± 18.17	21.28 ± 5.37	0.063
freshwater fishes	23.17 ± 8.51	24.56 ± 4.77	0.741
seafood	25.38 ± 8.33	25.61 ± 3.24	0.151
soybean products	23.59 ± 7.06	24.22 ± 4.16	0.811
milk	18.34 ± 7.07	17.61 ± 4.63	0.824
ASMI	6.13 ± 0.97	6.19 ± 1.42	0.835
HGS	15.60 ± 5.24	25.21 ± 6.95	0.00
CS-5	15.94 ± 2.24	9.16 ± 1.70	0.00
6MWT	278.15 ± 78.52	416.36 ± 99.51	0.00
Arm curl	13.03 ± 4.75	17.86 ± 7.29	0.003
CST	8.20 ± 2.72	16.19 ± 2.65	0.00
2-MST	45.50 ± 17.29	89.08 ± 16.27	0.00
TUGT	17.85 ± 6.34	9.78 ± 3.14	0.00

Note:Data are expressed as mean ±SD (standard deviation).Variables were analyzed by Mann-Whitney U tests,significantly (p<0.05)different between two groups.The food frequency questionnaire results are listed in times per week. ASMI (Appendicular Skeletal muscle Mass Index), HGS (handgrip strength,kg), CS-5 (Five-Repetition Chair Stand Tests,s), 6MWT (the six-minute walking test,m), Arm Curl (repetitions), CST (the 30s-chair stand test, repetitions), 2MST, (2-minute step test repetitions), TUGT (8-feet timed up-and-go test, seconds). Gestational age of subjects represents the time at which plasma samples were collected.

4.2 Plasma amino acids concentration

Nine plasma amino acids showed the substantial variations between the two groups. The general pattern of citrulline, glutamic acid, and proline plasma concentrations generally increased in possible-sarcopenic group. The concentrations of seven out of twenty amino acids decreased with possible sarcopenia, namely arginine, serine, asparagine, glutamine, threonine, phenylalanine and lysine (Table 2).

Table 2
Plasma amino acids concentration between two groups

Plasma amino acid	possible sarcopenia (n = 30)	Non-sarcopenia (n = 36)	*p-value*
Alanine	430.24 ± 144.98	422.26 ± 186.74	0.849
Arginine	68.5 ± 31.34	83.43 ± 27.60	0.044
Asparagine	29.97 ± 8.74	39.16 ± 8.75	0.000
Aspartic acid	7.03 ± 5.87	5.66 ± 4.47	0.285
Citrulline	45.61 ± 17.28	37.02 ± 16.09	0.041
Glutamine	329.39 ± 81.31	397.80 ± 113.72	0.008
Glutamic acid	81.51 ± 41.75	63.57 ± 34.31	0.001
Histidine	59.90 ± 18.78	54.70 ± 12.31	0.199
Isoleucine	74.72 ± 26.59	75.40 ± 24.73	0.914
Leucine	126.54 ± 43.96	119.95 ± 34.45	0.507
Lysine	204.04 ± 67.04	228.28 ± 56.25	0.022
Methionine	24.05 ± 8.12	25.09 ± 5.94	0.551
Ornithine	53.85 ± 25.33	54.38 ± 24.33	0.931
Phenylalanine	61.93 ± 20.52	69.21 ± 14.20	0.012
Proline	358.79 ± 244.53	228.22 ± 111.63	0.010
Serine	67.90 ± 30.67	86.63 ± 37.80	0.030
Threonine	99.77 ± 30.24	119.08 ± 37.38	0.026
Tryptophan	45.67 ± 12.05	41.68 ± 10.60	0.157
Tyrosine	70.82 ± 21.10	69.11 ± 17.50	0.719
Valine	215.83 ± 67.12	222.37 ± 58.40	0.673

Note:Data are expressed as mean ±SD (standard deviation). Variables were analyzed by Mann-Whitney U tests, significantly ($p<0.05$)different between two groups.

4.3 Classification of plasma amino acid based on OPLS-DA

In order to verify the existence of distinct patterns of amino acids in participants with possible sarcopenia, a OPLS-DA classification model was constructed and validated. Age, gender and ASMI did not significantly differ between the two groups in this study. Aside from age and gender, diversity in

exercise, nutrition, and living conditions has some effects on metabolomics results. In order to reduce the impact of interference factors on the experimental results, OPLS-DA, which combines partial least squares discriminant analysis and orthogonal signal correction, can screen the differential variables by removing individual differences. The PCA score plot (Fig. 1A) which used two components (R2Xcum = 0.621, Q2cum = 0.485), showed separate trends when comparing maternal plasma from the two groups. These results indicate that plasma contains a particular pattern of amino acids. The twenty amino acids in plasma samples were subjected to OPLS-DA analysis, with the model parameters being R2X(cum) = 0.787, R2Y(cum) = 0.727, Q2(cum) = 0.582. OPLS-DA model has great explanatory and prediction power, as evidenced by the considerable differences in plasma amino acid profile in possible sarcopenia, which were completely distinguishable in the score (Fig. 1B and 1C). In addition, permutation testing proves that the model is stable and reliable without over-fitting issues (Fig. 1D).

In order to identify the metabolites that were mostly responsible for discriminating between two groups, the values of the VIP indices were examined. The variables with VIP greater than one were listed in Table 3. The amino acid-targeted metabolomic profiling identified five amino acids altered in possible sarcopenia, of which four were markedly down-regulated, including glutamine, threonine, serine, lysine and threonine, whereas proline were noticeably up-regulated.

Table 3
Plasma amino acids signatures

Metabolites	*vip value*	possible sarcopenia	Non-sarcopenia	*p-value*
Glutamine	1.81	329.39 ± 81.31	397.80 ± 113.72	0.008
Proline	2.68	358.79 ± 244.53	228.22 ± 111.63	0.010
Serine	1.11	67.90 ± 30.67	86.63 ± 37.80	0.030
Lysine	1.03	204.04 ± 67.04	228.28 ± 56.25	0.022
Threonine	1.01	99.77 ± 30.24	119.08 ± 37.38	0.026

Note: Plasma concentrations of discriminant analytes, variable importance in projection (*vip*) values between possible sarcopenia and non-sarcopenia. Plasma concentrations are shown as mean ±SD (standard deviation).

5 Discussion

Impaired mobility and functional decline[32] are two of the most prominent physiological changes associated with aging. While there are many factors that contribute to aged-related physical limits, one of the most noticeable factors is that skeletal muscle performance progressive declines and deteriorates over time[33]. Skeletal muscle strength, power, endurance, and contractile function gradually reduce with aging[11,34–37]. Similar to this, we have also observed that skeletal muscle function gradually declines as the likelihood of sarcopenia increases. Examples include tests for HGS, CS-5, 6MWT, arm curls, CST, 2MST, TUGT.

Age-related acceleration of these declines in skeletal muscle physiological function begins between the ages of 30 and 40 years[38]. Protein metabolism throughout aging is responsible for a number of physiological and functional abnormalities in skeletal muscle, which are aggravated by decreased dietary protein intake and slowed protein synthesis responses to stimuli. Additionally, amino acids serve as both nutrients and regulators for the production of various macromolecules, including proteins, which are essential to maintain both physical and mental functions. There have been reports on the relationships between circulatory amino acid concentrations and muscle mass and strength[21, 39, 40], which are consistent with studies demonstrating that BCAA are a marker of muscle mass/strength in healthy individuals[39, 40]. Our research's findings revealed that the pattern of plasma amino acid concentrations had changed, and that possible sarcopenia had been associated with the presence of some distinctive metabolites. Plasma levels of free amino acids are in a dynamic equilibrium that is altered by the daily protein intake[41]. The variations in plasma amino acid profile would be attributed to possible sarcopenia according to the food frequency questionnaire. To put it another way, systemic amino acid metabolism is different in older adults compared to older persons with reasonably normal skeletal muscle strength and function. This is the first study that, to our knowledge, has linked possible sarcopenia with the plasma amino acid metabolomics profiles.

Studies have shown that perturbations of amino acid metabolism are widespread in elderly with skeletal muscle hypofunction[42]. In line with these observations, we discovered that possible sarcopenia was associated with lower circulating levels of the EAA including serine, lysine and threonine. In addition, there was a decreasing trend of valine for possible sarcopenia, yet this difference was not statistically significant. Both sexes were found to have lower serum levels of a number of EAAs as they aged[20]. Thus, amino acid metabolic disorders may also be risk factors for the development of possible sarcopenia. When there is a lack of amino acids, skeletal muscle proteins are degraded together with amino acids, which incurs severe problems of skeletal muscle health degradation speed is fast. According to reports, EAA is primarily responsible for the anabolic effect of amino acids on muscle proteins[43]. By activating the mammalian target of rapamycin complex, EAAs, particularly branched-chain amino acids, influence protein synthesis and nutritional status[44, 45]. Meanwhile, we found significantly decreased glutamine levels and markedly increased glutamic acid levels in possible sarcopenia. Glutamine has been shown to regulate mTORC1 by affecting autophagy, transamination and other specific functions, which is considered an essential tissue size and mass regulator, either in healthy or ill patients[46, 47]. The mTORC1 pathway is highlighted as a key therapeutic target to prevent sarcopenia by the age-related alterations in skeletal muscle[48–51]. In fact, in skeletal muscle, mTORC1 activation regulates protein synthesis and regulates skeletal muscle mass[52]. Moreover, the activity of amino acids, particularly leucine, as anabolic inducers in muscle cells is hampered by mTOR when glutamine is not available[53]. Another reason for the formation and progression of sarcopenia is protein anabolic resistance brought on by insulin resistance[54, 55]. Cheng et al. reported that plasma glutamine, glutamic acid, and the glutamine/glutamic acid ratio were highly correlated with insulin resistance traits in two different cohorts, the Framingham Heart Study and the Malmö Diet and Cancer Study[56]. The

findings suggest the possibility that skeletal muscle mass and function may be affected by the decreased glutamine concentrations in the elderly. In order to delay the deterioration of possible sarcopenia with aging, glutamine depletion may have a role in both prevention and treatment. Besides, our multivariable analysis revealed that a higher plasma concentration of proline was the variable associated with possible sarcopenia. The results corroborate those that were found in earlier research on sarcopenia in the elderly[57]. Ilaiwy et al. also observed that muscle cell atrophy was related to the elevated proline concentrations in culture media in vitro, again agreeing with our findings[58]. Likewise, a high concentration of proline has also been confirmed to induce oxidative damage to protein, lipids, and DNA in rats[59].

In summary, a low-quality protein diet or malabsorption may have reduced the amount of EAAs, which limits protein synthesis and may have therapeutic effects on the quality of skeletal muscle in the elderly with possible sarcopenia. The profile appears to indicate metabolic abnormalities that are subsequent to potential sarcopenia, which is another more likely explanation. The anaplerotic pathway of the tricarboxylic acid cycle receives amino acids as a source of energy for metabolism. When the elderly with possible sarcopenia are malnourished, amino acids, especially proline, are produced by proteolysis in skeletal muscle and used for energy metabolism. By contrast, in healthy elderly individuals with sufficient carbohydrate intake, amino acids are not metabolized via the anaplerotic pathway, resulting in the higher concentrations of those amino acids. However, further research is required to clarify the relationship between skeletal muscle quality and circulating amino acids in the context of possible sarcopenia to provide new preventive strategies and treatments.

There were some limitations to this study. First, selection bias cannot be ruled out. Given that the participants were volunteers from two regional healthcare center and not chosen at random, some findings may have biases. Second, since this was a cross-sectional study, we were unable to relate our findings to the likelihood of possible sarcopenia in the elderly. Finally, there was no nutrition restriction before the study visits. Dietary variations among participants, particularly differences in protein intake, may have increased variability to the results of the amino acids or affected them in ways that were not measured.

Declarations

Ethics approval and consent to participate This research was approved by the Sports Science Experiment Ethics Committee of Beijing Sport University, registration number 2020082H. The norms of the China of Health were met, according to Ethical review measures for biomedical research involving human beings of Health, Science and Education [2007]17. We confirm that all methods were performed in accordance with the relevant guidelines and regulations. All subjects of human experimentation gave informed consent before participating in the study. All procedures and risks of the investigation were thoroughly explained to participants and written informed consent was obtained prior to participation.

Consent for publication: Not Applicable

Availability of data and materials: The dataset supporting the conclusions of this article is included within the article. All data generated and analyzed during this study are included in this published article.

Competing interests: There are none conflict of interests.

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Figures

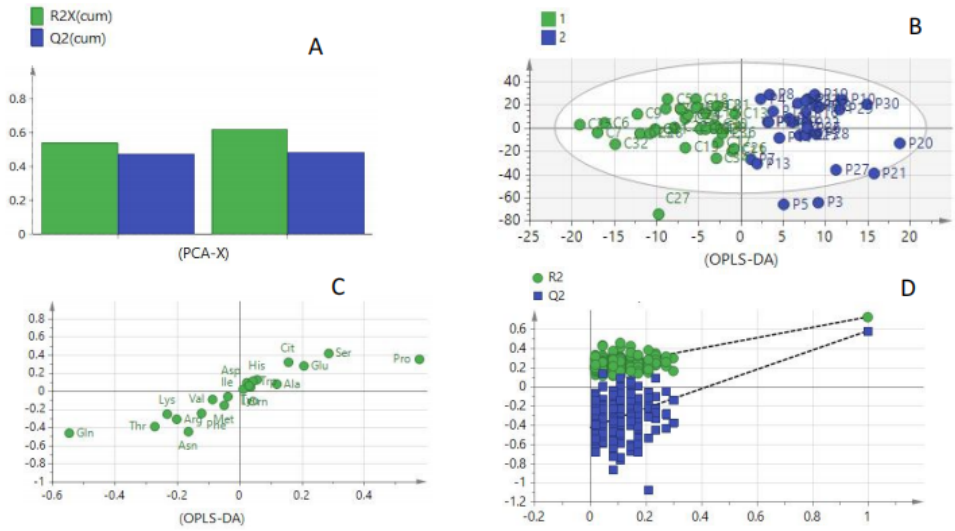

Figure 1. A=PCA (principal component analysis) Scores plot between two groups; B=Scores plot showing the separation of participants according to the plasma concentrations of amino acids and derivatives in the space spanned by the two latent variables (R2X and R2Y), as determined by orthogonal partial ;least-squares-discriminant analysis (OPLS-DA). 1=non-sarcopenia, 2=possible sarcopenia; C=loadings plot； D=Permutation testing

Figure 1

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