Serum Amino Acids Patterns and 4-Year Sarcopenia Risk in Community-Dwelling Chinese Older Adults

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Abstract

Introduction: Dietary protein intake and serum amino acids (AAs) are factors controlling the rate of muscle protein synthesis and catabolism. This study examined the association between serum AAs patterns and incident sarcopenia in community-dwelling older adults. Methods: Chinese older adults in Hong Kong aged ≥65 years attended a health check at baseline and 4-year follow-up. At baseline, fasting blood was collected to measure 17 serum AAs. Serum AAs patterns were identified using principal component analysis. Dietary protein intake was assessed using a validated food frequency questionnaire. A composite score was computed by summing the principal component score and sex-standardized protein intake. Six composite scores representing each AAs pattern were available for each participant. Sarcopenia was defined using the updated version of the Asian Working Group for Sarcopenia. Crude and adjusted multiple logistic regressions were performed to examine the associations between each of the 6 composite scores and sarcopenia over 4 years. Results are presented as odds ratio (OR) and 95% confidence interval (CI). To address multiple testing, a Bonferroni correction was applied using a corrected significance level of \( p < 0.008 \) (\( \alpha = 0.05/6 \) patterns). Results: Data of 2,610 participants (mean age 71.6 years, 45.4% men) were available. In men, serum AAs patterns characterized by high branched-chain AAs (BCAAs) (OR 0.77, 95% CI 0.69–0.87, \( p < 0.001 \)) and tyrosine, tryptophan, and phenylalanine (OR 0.79, 95% CI 0.71–0.89, \( p < 0.001 \)) were significantly associated with a lower risk of sarcopenia over 4-year follow-up. After adjusting for confounders, the associations were no longer significant. In women, serum AAs patterns characterized by glutamine, glutamic acid, and methionine (OR 1.28, 95% CI 1.11–1.47, \( p = 0.001 \)) and arginine, taurine, and serine (OR 1.20, 95% CI 1.06–1.35, \( p = 0.003 \)) were associated with a higher risk of sarcopenia. After adjusting for confounders, serum AAs pattern characterized by high BCAAs (adjusted OR 1.52, 95% CI 1.25–1.86, \( p < 0.001 \)) and arginine, taurine, and serine (adjusted OR 1.30, 95% CI 1.09–1.56, \( p = 0.004 \)) were significantly associated with a higher risk of sarcopenia. No association between other AAs patterns with incident sarcopenia was found. Conclusions: In community-dwelling Chinese older adults, serum AAs patterns characterized by high BCAAs and nonessential AAs (arginine, taurine, and serine) were associated with a higher risk of sarcopenia in women. Findings may allow identifying new targets for interventions.
Introduction

Sarcopenia is age-related low muscle mass and muscle strength [1] and is associated with a higher risk of adverse outcomes, including falls, fractures, functional decline, and mortality [2, 3]. The prevalence of sarcopenia was approximately 10% among healthy older adults [4] and up to 31% among older adults with chronic diseases [5]. Apart from dietary protein intake, circulating amino acids (AAs) are important factors in the control of the rate of muscle protein synthesis and catabolism [6, 7]. Disarrangement in protein-AA metabolism may become clinically evident with signs such as sarcopenia [8].

To unveil the pathophysiological mechanisms underlying sarcopenia, track its progression, and identify targets for interventions, a better understanding of the AA profiles could be useful [9, 10]. Several studies demonstrated the relationships of circulating AAs with sarcopenia in older adults. Higher circulating proline [11], glutamic acid, and taurine [12], lower circulating branched-chain AAs (BCAAs) [13, 14], and lower circulating methionine [12, 13] and phenylalanine [13] were independently associated with a higher risk of sarcopenia in older adults. However, these studies were of cross-sectional design and did not take dietary factors into consideration. Moreover, only one study attempted to identify a unique pattern of circulating AAs in older adults with sarcopenia [12]. Since circulating AAs are potentially highly correlated and protein synthesis is limited by the AA in the least amount [15], it is important to develop a comprehensive study of circulating AAs to identify specific AAs patterns that are associated with the risk of sarcopenia. To the best of our knowledge, there is no priori AAs pattern score/index available in the literature. For this, a posteriori approach, such as principal component analysis (PCA) to derive major patterns from the data can be applied.

Given the literature gap in these aspects, this study aims to examine the association between serum AAs patterns and incident sarcopenia risk in community-dwelling Chinese older adults. The findings may facilitate the early identification of individuals at high risk of sarcopenia and the design of the nutritional intervention.

Materials and Methods

Study Participants

Data were derived from Mr. Os and Ms. Os Hong Kong study, which is a prospective cohort study examining the risk factors of osteoporosis in Chinese older men and women [16]. 2,000 Chinese men and 2,000 Chinese women aged ≥65 years old living in the community in Hong Kong were invited to attend a health check between 2001 and 2003. Older adults who had a bilateral hip replacement or were unable to give informed consent were excluded. Included participants were able to walk or take public transport to the study site. A stratified sampling method was adopted to equally assign participants into each of these age-groups: 65–69, 70–74, and ≥75 years old. Participants were followed up by a visit to the study site at the 4th year. Participants who did not have serum AAs data, had sarcopenia at baseline, had incomplete or invalid dietary data (daily energy intake ≥5,000 and ≤500 kcal), and without follow-up assessment of sarcopenia at the 4th year were excluded from the analysis (shown in Fig. 1). The sample size for the final analysis was 2,610. Informed consent was obtained from all participants. The study was conducted in compliance with the Declaration for Helsinki and has been approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong.

Demographic Data

A standardized and structured interview was conducted to collect information on age, sex, body mass index (BMI), smoking habits, alcohol use, physical activity level, cognitive function level, number of diseases, and subjective social status. BMI was calculated as body weight (kg) divided by height in meters squared (m²). Participants were asked to wear light clothing, and the body weight was measured using the Physician Balance Scale (Healthometer; Illinois, USA). Body height was measured using the Holtain Harpenden Stadiometer (Holtain Ltd, Crosswell, UK). Physical activity level was assessed using the Physical Activity Scale for the Elderly (PASE) [17]. The PASE consists of 12 items assessing the average number of hours per day spent in leisure, household, and occupa-
tional physical activities over the last 7 days. Activity weights for each item were determined based on the amount of energy spent. Each item score was then computed by multiplying the activity weight with the frequency of daily activity. A composite PASE score was computed by summing each item score. A higher PASE score represents a higher physical activity level. Cognitive function was assessed using the cognitive part of the Community Screening Instrument for Dementia (CSID) [18]. The possible range of the CSID score is 0–33, with a higher score representing a better cognitive function. Participants were classified into normal, borderline, and probably dementia according to the CSID score of ≥29.5, 28.4–29.49, and <28.4, respectively. A pre-defined list was used to assess the number of diseases. Participants were asked if a doctor or other health-care professional has ever told that they had or have any of the diseases in the list, supplemented by the identification of medications brought to the interviewers. Subjective social status was assessed by asking participants to mark their self-perceived position on a picture of an upright ladder with 10 rungs. The lowest rung represents the most undesirable and the highest rung represents the most desirable state with respect to their standing in the community (community status ladder) and in Hong Kong (Hong Kong ladder) [19].

### AAs Assays

At the baseline visit, blood was taken after an overnight fast. Serum was separated within 3 h and stored at −80°C until assayed. Liquid chromatography-tandem mass spectrometry, using a modified version of a previously described method, was used to measure AAs in the stored serum [20, 21]. In brief, isotopically labeled internal standards were added to serum, followed by reduction of disulfides using dithioerythritol and then protein precipitation using 5-sulfosalicylic acid. Before analysis, an aqueous solution of formic acid (0.5%) and heptafluorobutyric acid (0.3%) was used to dilute the extracts. Liquid chromatography-tandem mass spectrometry was carried out using a Shimadzu LC-20ADXR Prominence LC system (Kyoto, Japan) coupled to a Sciex QTRAP5500 mass spectrometer with a Turbo V ion source and TurbolonSpray probe (Framingham, MA, USA). Chromatographic separation was achieved on a Phenomenex Kinetex Core-Shell C18 (100 × 4.6 mm, 2.6 μm) LC column (Torrance, CA, USA) with an aqueous solution of formic acid (0.5%) and heptafluorobutyric acid (0.3%) and acetonitrile gradient mobile phase. Detection was performed using positive mode multiple reaction monitoring. Quantification was conducted using linear calibration curves of the peak area ratios of the analyte and internal standard. The coefficient of variation for the analytes was 3–7%. Spiked serum QA samples from an external quality assurance scheme ERNDIM (www.erndim.org) was used to validate the method for 16 of the 17 analytes. Serum AAs measured in this study included BCAAs (isoleucine, valine, and leucine), essential AAs (EAAs) (methionine, phenylalanine, and tryptophan), and non-EAAs (glutamine, glutamic acid, taurine, serine, homocysteine, cystathionine, cysteine, arginine, proline, ornithine, and tyrosine).

### Serum AAs Patterns

PCA was applied using the 17 serum AAs to identify serum AAs patterns. One advantage of PCA is that potential unknown interactions between individual AAs could be uncovered because a priori biological assumptions are not required. The correlation matrix (>0.3), Kaiser-Meyer-Olkin measure of sampling adequacy (>0.6), and Bartlett’s test of sphericity (p < 0.05) were inspected to determine whether a meaningful PCA could be performed. All cri-

### Table 1. Component loadings for the 6 AAs patterns

| 17 AAs                | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Factor 6 |
|----------------------|----------|----------|----------|----------|----------|----------|
| Isoleucine           | 0.90     | 0.21     | 0.04     | 0.08     | 0.10     | 0.16     |
| Leucine              | 0.88     | 0.29     | 0.08     | 0.14     | 0.09     | 0.11     |
| Valine               | 0.88     | 0.26     | 0.04     | 0.03     | 0.09     | 0.11     |
| Tyrosine             | 0.21     | 0.82     | −0.06    | 0.05     | 0.06     | 0.16     |
| Tryptophan           | 0.33     | 0.72     | 0.14     | 0.03     | −0.03    | 0.00     |
| Phenylalanine        | 0.37     | 0.53     | −0.21    | 0.36     | 0.29     | 0.08     |
| Glutamine            | 0.11     | 0.10     | 0.94     | 0.06     | 0.13     | 0.10     |
| Glutamic acid        | 0.05     | 0.11     | −0.93    | 0.17     | −0.02    | 0.05     |
| Methionine           | 0.30     | 0.52     | 0.55     | 0.11     | 0.06     | 0.32     |
| Arginine             | 0.00     | 0.15     | −0.06    | 0.73     | 0.09     | −0.09    |
| Taurine              | 0.26     | −0.13    | 0.02     | 0.69     | 0.09     | 0.01     |
| Serine               | −0.08    | 0.26     | −0.04    | 0.62     | −0.08    | 0.45     |
| Homocysteine         | 0.13     | −0.14    | 0.15     | 0.03     | 0.81     | 0.22     |
| Cysteine             | 0.03     | 0.30     | 0.04     | 0.20     | 0.79     | −0.04    |
| Ornithine            | 0.03     | 0.18     | 0.05     | 0.02     | 0.10     | 0.72     |
| Proline              | 0.41     | −0.03    | 0.09     | 0.06     | 0.03     | 0.57     |
| Cystathionine        | 0.16     | −0.02    | −0.01    | −0.08    | 0.40     | 0.45     |

Component loadings with an absolute value of ≥0.2 are shown in bold. For AAs that load >1 AAs pattern, only the highest absolute value of loading is bolded. AAs, amino acids.
Dietary Assessment
Since diet may have an influence on serum AAs levels [23, 24] and it is known to be a lifestyle determinant of sarcopenia [25], a dietary assessment was conducted in order to obtain dietary data to address this potential confounder in the present investigation. Dietary intake was assessed at baseline using a validated food frequency questionnaire [26]. The food frequency questionnaire consisted of 280 food items. Participants were asked to complete the questionnaire using the past 12 months before the interview as a reference period. The portion size was explained to the participants using a catalog of pictures of individual food portions. The amount of cooking oil was estimated according to the usual cooking methods of preparing a standardized portion of different foods consumed by the participants. The mean nutrient quantification per day was calculated using food composition tables derived from McCance and Widdowson [27] and the Chinese Medical Sciences Institute [28]. The total protein intake in grams per day was calculated.

Definition of Sarcopenia
Sarcopenia was defined according to the updated version of the Asian Working Group for Sarcopenia (AWGS 2019) [29]. Participants who had low muscle mass and low muscle strength or low physical performance were defined as having sarcopenia. Muscle mass was measured using the dual-energy X-ray absorptiometry (Hologic QDR-4500W, software version 11.2; Hologic, Inc., Waltham, MA, USA). The total appendicular skeletal muscle mass was calculated by summing the lean mass of the 4 limbs, with the operator adjusting the cut lines of the limbs according to specific anatomical landmarks [30]. Low muscle mass was defined as appendicular skeletal muscle mass/height$^2$ <7.0 kg/m$^2$ in men and <5.4 kg/m$^2$ in women [29]. Handgrip strength was measured using a dynamometer (JAMAR hand dynamometer 5030JO; Sammons Preston, Bolingbrook, IL, USA). Two measurements were performed for each side and the best value of the right or left side was used for analysis. Low muscle strength was defined as <28 kg in men and <18 kg in women [29]. Gait speed was measured using a 6-m walking test at the usual pace. The best time to complete the walking test was used for analysis. Low physical performance was defined as gait speed <1.0 m/s for both genders [29].

Statistical Analysis
Statistical analyses were performed stratified by gender using the statistical package SPSS version 26.0 (IBM SPSS Statistics for Windows, Version 26.0.; IBM Corp., Armonk, NY, USA). Continuous data are presented as mean and standard deviation as they are normally distributed. Categorical variables are presented as numbers and percentages (%). The differences between sarcopenia and nonsarcopenia participants were tested using the independent t tests and the χ² tests as appropriate.

Total protein intake (g/day) was first standardized into sex-specific z-scores. A composite score was then computed by summing the principal component score and standardized protein intake. Since 6 AAs patterns were identified, 6 composite scores representing each AAs pattern were available for each participant. A higher composite score represents greater conformity with the derived AAs pattern and higher dietary protein intake. Binary logistic regressions were performed to estimate the associations between each of the 6 composite scores and the incidence of sarcopenia. Model 1 was unadjusted. Model 2 was adjusted for age, BMI, current smoker (yes/no), current alcohol use (yes/no), PASE score, cognitive function (normal/borderline/probable dementia), number of diseases (0–1/2+), subjective social status (community ladder and Hong Kong ladder), and daily energy intake. The odds ratios (ORs) and 95% confidence intervals (CIs) are presented. To address multiple testing, a Bonferroni correction was applied using a corrected significance level (individual AAs: 0.05/17, p < 0.003; AAs patterns: 0.05/6, p < 0.008).

Results
Baseline Characteristics of Participants
Table 2 shows the characteristics of our study population according to sarcopenia status at the 4-year follow-up. Of the 2,610 participants, 201 men (17.0%) and 125 women (8.8%) were newly identified as sarcopenia. Men with sarcopenia were significantly older, had lower BMI, more likely to be current smokers, had lower physical activity level, less likely to have a normal cognitive function, and had lower protein and energy intake than men without sarcopenia. Women with sarcopenia were significantly older, had lower BMI, lower physical activity level, and higher protein intake than women without sarcopenia. In both men and women, those with sarcopenia at the 4-year follow-up had significantly lower baseline muscle mass and handgrip strength than those without sarcopenia.

Table 3 shows the serum AAs levels and AAs patterns by 4-year sarcopenia status. Overall, the mean (standard deviation) serum AAs patterns scores were 0.03 (0.99),
Table 2. Characteristics of participants by 4-year sarcopenia status ($n = 2,610$)

|                        | All          |              | p value | All          |              | p value | All          |              | p value | All          |              | p value |
|------------------------|--------------|--------------|---------|--------------|--------------|---------|--------------|--------------|---------|--------------|--------------|---------|
|                        | nonsacopenic | sacopenic    |         | nonsacopenic | sacopenic    |         | nonsacopenic | sacopenic    |         | nonsacopenic | sacopenic    |         |
| Age, years             | 71.4 (4.6)   | 73.3 (5.2)   | <0.001  | 70.9 (4.1)   | 73.2 (5.1)   | <0.001  | 71.7 (4.9)   | 73.4 (5.2)   | <0.001  | 71.7 (4.9)   | 73.4 (5.2)   | 0.001  |
| BMI, kg/m$^2$          | 24.5 (3.0)   | 21.7 (2.5)   | <0.001  | 24.5 (2.8)   | 21.9 (2.5)   | <0.001  | 24.6 (3.2)   | 21.4 (2.5)   | <0.001  | 24.6 (3.2)   | 21.4 (2.5)   | 0.001  |
| Current smoker, %      | 4.7          | 8.9          | 0.003   | 9.0          | 13.9        | 0.038   | 1.5          | 0.8          | 1.000   | 1.5          | 0.8          | 1.000   |
| Current drinker, %     | 12.8         | 14.4         | 0.429   | 26.1         | 20.9        | 0.130   | 2.8          | 4.0          | 0.399   | 2.8          | 4.0          | 0.399   |
| PASE, score            | 95.2 (43.1)  | 89.4 (42.1)  | 0.024   | 103.8 (52.2) | 94.1 (47.6) | 0.016   | 88.7 (33.3) | 81.8 (30.0) | 0.027   | 88.7 (33.3) | 81.8 (30.0) | 0.027   |
| Cognitive function, %  |              |              |         |              |              |         |              |              |         |              |              |         |
| Normal                 | 74.1         | 78.8         |         | 91.8         | 86.6        |         | 60.8         | 66.4         |         | 60.8         | 66.4         |         |
| Borderline             | 11.6         | 12.0         | 0.044   | 6.1          | 9.0         | 0.045   | 15.8         | 16.8         | 0.237   | 15.8         | 16.8         | 0.237   |
| Probable dementia      | 14.3         | 9.2          |         | 2.1          | 4.5         |         | 23.5         | 16.8         |         | 23.5         | 16.8         |         |
| Diseases, %            |              |              |         |              |              |         |              |              |         |              |              |         |
| 0–1                    | 45.1         | 45.4         | 0.953   | 46.0         | 47.8        | 0.698   | 44.4         | 41.6         | 0.573   | 44.4         | 41.6         | 0.573   |
| 2+                     | 54.9         | 54.6         |         | 54.0         | 52.2        |         | 55.6         | 58.4         |         | 55.6         | 58.4         |         |
| Social status (HK), rung | 4.6 (1.8) | 4.4 (1.9) | 0.116 | 4.5 (1.8) | 4.5 (1.9) | 0.820 | 4.7 (1.9) | 4.4 (1.8) | 0.084 |
| Social status (community), rung | 7.0 (2.2) | 6.7 (2.2) | 0.044 | 6.4 (2.2) | 6.5 (2.2) | 0.952 | 7.4 (2.1) | 7.1 (2.2) | 0.167 |
| Protein intake, g      | 76.7 (33.4)  | 79.7 (33.2)  | 0.137   | 91.2 (35.5)  | 83.7 (32.8) | 0.006   | 65.8 (27.1) | 73.1 (32.9) | 0.005   |
| Energy intake, kcal    | 1,831 (588)  | 1,872 (541)  | 0.228   | 2,147 (594)  | 1,997 (538) | <0.001  | 1,592 (456) | 1,672 (482) | 0.063   |
| ASM, kg                | 16.9 (3.6)   | 15.8 (3.2)   | <0.001  | 20.3 (2.3)   | 17.9 (1.9)  | <0.001  | 14.3 (1.7)  | 12.3 (1.2)  | <0.001  |
| ASM/height$^2$, kg/m$^2$ | 6.8 (0.9) | 6.2 (0.8) | <0.001 | 7.6 (0.7) | 6.7 (0.6) | <0.001 | 6.3 (0.6) | 5.4 (0.4) | <0.001 |
| Handgrip strength, kg  | 28.8 (8.4)   | 28.5 (7.0)   | 0.537   | 36.3 (6.1)   | 32.7 (5.0)  | <0.001  | 23.0 (4.4)  | 21.7 (3.5)  | <0.001  |
| Gait speed, m/s        | 1.05 (0.23)  | 1.07 (0.20)  | 0.104   | 1.13 (0.22)  | 1.10 (0.19) | 0.057   | 0.99 (0.21) | 1.01 (0.20) | 0.210   |
| 4-year follow-up       |              |              |         |              |              |         |              |              |         |              |              |         |
| ASM, kg                | 16.6 (3.6)   | 15.0 (3.1)   | <0.001  | 20.1 (2.3)   | 17.2 (1.6)  | <0.001  | 14.1 (1.7)  | 11.6 (1.0)  | <0.001  |
| ASM/height$^2$, kg/m$^2$ | 6.8 (0.9) | 5.9 (0.8) | <0.001 | 7.5 (0.7) | 6.5 (0.5) | <0.001 | 6.2 (0.6) | 5.1 (0.3) | <0.001 |
| Handgrip strength, kg  | 26.5 (8.3)   | 24.9 (7.0)   | <0.001  | 33.8 (6.1)   | 28.6 (6.0)  | <0.001  | 20.8 (4.4)  | 18.8 (3.6)  | <0.001  |
| Gait speed, m/s        | 0.95 (0.23)  | 0.85 (0.18)  | <0.001  | 1.04 (0.22)  | 0.87 (0.17) | <0.001  | 0.88 (0.22) | 0.82 (0.19) | <0.001  |

Values are presented as mean (SD) or percentage. ASM, appendicular skeletal muscle mass; BMI, body mass index; HK, Hong Kong; PASE, Physical Activity Scale for the Elderly score; SD, standard deviation.
Table 3. Serum AAs level and AAs patterns of participants by 4-year sarcopenia status (n = 2,610)

| All (N = 2,610) | Men | Women |
|----------------|-----|-------|
| **total**      | **nonsarcopenic** | **sarcopenic** | **p value** | **nonsarcopenic** | **sarcopenic** | **p value** | **nonsarcopenic** | **sarcopenic** | **p value** |
| (n = 2,284)    | (n = 984) | (n = 201) |          | (n = 1,100) | (n = 125) |          | (n = 1,300) | (n = 125) |          |
| **Branched-chain AAs** |     |       |    |     |       |    |     |       |    |    |
| Isoleucine, µM | 80 (15) | 81 (15) | 81 (16) | 0.615 | 86 (15) | 83 (15) | 0.005 | 76 (14) | 75 (16) | 0.482 |
| Valine, µM    | 293 (46) | 294 (46) | 288 (45) | 0.047 | 310 (43) | 298 (43) | **<0.001** | 281 (44) | 273 (43) | 0.051 |
| Leucine, µM   | 149 (24) | 150 (24) | 148 (26) | 0.267 | 160 (23) | 154 (24) | **<0.001** | 141 (22) | 139 (25) | 0.207 |
| **EAAs**      |     |       |    |     |       |    |     |       |    |    |
| Methionine, µM| 27 (6) | 27 (6) | 28 (6) | 0.004 | 31 (5) | 31 (5) | 0.045 | 24 (4) | 24 (5) | 0.754 |
| Phenylalanine, µM | 98 (14) | 98 (14) | 96 (15) | 0.047 | 99 (13) | 97 (14) | 0.086 | 97 (14) | 94 (16) | 0.056 |
| Tryptophan, µM| 59 (11) | 59 (11) | 58 (11) | 0.138 | 62 (11) | 60 (11) | 0.004 | 57 (10) | 55 (11) | 0.188 |
| **Non-EAAs**  |     |       |    |     |       |    |     |       |    |    |
| Glutamine, µM | 455 (144) | 449 (144) | 499 (138) | **<0.001** | 568 (112) | 577 (98) | 0.279 | 359 (89) | 374 (94) | 0.066 |
| Glutamic acid, µM | 187 (85) | 190 (85) | 164 (79) | **<0.001** | 134 (64) | 125 (53) | 0.070 | 233 (74) | 227 (73) | 0.424 |
| Taurine, µM   | 171 (32) | 171 (32) | 172 (34) | 0.402 | 172 (32) | 173 (35) | 0.533 | 170 (32) | 171 (33) | 0.728 |
| Serine, µM    | 169 (23) | 168 (23) | 170 (24) | 0.160 | 168 (22) | 170 (25) | 0.351 | 168 (23) | 170 (23) | 0.300 |
| Homocysteine, µM | 14 (5) | 14 (5) | 15 (5) | 0.061 | 15 (5) | 16 (5) | 0.221 | 13 (4) | 13 (4) | 0.349 |
| Cystathionine, µM | 260 (199) | 257 (184) | 280 (278) | 0.143 | 281 (159) | 322 (336) | 0.094 | 239 (199) | 213 (119) | 0.165 |
| Cysteine, µM  | 356 (44) | 356 (43) | 358 (50) | 0.515 | 359 (43) | 364 (49) | 0.164 | 353 (43) | 347 (49) | 0.151 |
| Arginine, µM  | 139 (28) | 139 (28) | 140 (27) | 0.837 | 135 (29) | 138 (28) | 0.328 | 142 (26) | 143 (26) | 0.760 |
| Proline, µM   | 203 (58) | 203 (57) | 208 (60) | 0.137 | 216 (59) | 216 (60) | 0.885 | 192 (54) | 195 (59) | 0.602 |
| Ornithine, µM | 131 (40) | 131 (39) | 132 (48) | 0.770 | 140 (35) | 141 (35) | 0.703 | 125 (40) | 117 (29) | 0.038 |
| Tyrosine, µM  | 84 (15) | 84 (15) | 81 (14) | **0.001** | 86 (15) | 82 (13) | **<0.001** | 83 (14) | 80 (15) | 0.053 |
| **AAs patterns** |     |       |    |     |       |    |     |       |    |    |
| Factor 1      | 0.03 (0.99) | 0.04 (0.98) | −0.04 (1.02) | 0.167 | 0.37 (0.95) | 0.10 (1.02) | **<0.001** | −0.21 (0.93) | −0.28 (0.99) | 0.431 |
| Factor 2      | 0.03 (1.00) | 0.05 (1.00) | −0.12 (0.97) | **0.004** | 0.21 (1.02) | −0.05 (0.96) | **0.001** | −0.06 (0.96) | −0.23 (0.97) | 0.064 |
| Factor 3      | −0.04 (1.00) | −0.08 (1.00) | 0.27 (0.92) | **<0.001** | 0.70 (0.74) | 0.78 (0.64) | 0.208 | −0.68 (0.72) | −0.54 (0.70) | 0.035 |
| Factor 4      | −0.01 (1.00) | −0.02 (0.99) | 0.07 (1.04) | 0.104 | −0.11 (1.00) | 0.01 (1.08) | 0.125 | 0.04 (0.98) | 0.18 (0.95) | 0.149 |
| Factor 5      | −0.02 (0.98) | −0.03 (0.96) | 0.03 (1.09) | 0.324 | 0.03 (0.97) | 0.21 (1.11) | 0.024 | −0.07 (0.94) | −0.24 (1.00) | 0.058 |
| Factor 6      | −0.01 (0.99) | −0.03 (0.96) | 0.09 (1.14) | 0.041 | 0.14 (0.92) | 0.27 (1.24) | 0.150 | −0.15 (0.97) | −0.19 (0.88) | 0.663 |

Values are presented as mean (SD). Bold values represent significant p after Bonferroni correction was applied for multiple testing using a corrected significance level of p < 0.003 (α 0.05/17 AAs) and p < 0.008 (α 0.05/6 AAs patterns). Factor 1: isoleucine, leucine, and valine; Factor 2: tyrosine, tryptophan, and phenylalanine; Factor 3: glutamine, glutamic acid, and methionine; Factor 4: arginine, taurine, and serine; Factor 5: homocysteine and cysteine; Factor 6: ornithine, proline, and cystathionine. AAs, amino acids; SD, standard deviation.
0.03 (1.00), −0.04 (1.00), −0.01 (1.00), −0.02 (0.98), and −0.01 (0.99) for factor 1, factor 2, factor 3, factor 4, factor 5, and factor 6, respectively. Serum valine, leucine, and tyrosine levels were significantly lower in men with sarcopenia than men without sarcopenia ($p < 0.003$). “Factor 1” and “factor 2” AAs pattern scores were also significantly lower in men with sarcopenia than those without sarcopenia ($p < 0.008$). Serum AAs levels and AAs patterns were not significantly different by sarcopenia status in women.

**Associations between Serum AAs Patterns and Sarcopenia**

In men, “factor 1” serum AAs pattern characterized by high BCAAs (OR 0.77, 95% CI 0.69–0.87, $p < 0.001$) and “factor 2” AAs pattern characterized by tyrosine, tryptophan, and phenylalanine (OR 0.79, 95% CI 0.71–0.89, $p < 0.001$) were significantly associated with a lower risk of sarcopenia over 4-year follow-up (Table 4). After adjusting for confounders, the associations were no longer significant (“factor 1”: adjusted OR 1.11, 95% CI 0.95–1.31, $p = 0.198$; “factor 2”: adjusted OR 0.89, 95% CI 0.76–1.05, $p = 0.160$). In women, “factor 3” AAs pattern characterized by glutamine, glutamic acid, and methionine (OR 1.28, 95% CI 1.11–1.47, $p = 0.001$) and “factor 4” AAs pattern characterized by arginine, taurine, and serine (OR 1.20, 95% CI 1.06–1.35, $p = 0.003$) were associated with a higher risk of sarcopenia in the crude model. After adjusting for confounders, “factor 1” serum AAs pattern characterized by high BCAAs (adjusted OR 1.52, 95% CI 1.25–1.86, $p < 0.001$) and “factor 4” AAs pattern (adjusted OR 1.30, 95% CI 1.09–1.56, $p = 0.004$) were significantly associated with a higher risk of sarcopenia over a 4-year follow-up.

**Discussion**

This study identified 6 serum AAs patterns using PCA and suggested an association between AAs patterns and incident sarcopenia in community-dwelling Chinese older adults. However, such association was only observed in women, in that serum AAs patterns characterized by high BCAAs and non-EAAs (arginine, taurine, and serine)
were associated with a higher risk of sarcopenia over a 4-year follow-up. No association between other AAs patterns with incident sarcopenia was found in community-dwelling Chinese older adults.

To our knowledge, there is no published article examining the potential relationship between serum AAs patterns and incident sarcopenia in older adults. The gender difference in the results deserves further investigation. We found that the associations were observed in women only. A possible explanation is that women have lower muscle mass and muscle strength, and therefore the associated consequences are higher than men, resulting in better response to serum AAs levels. Of note, the gender-specific differences in mechanisms underlying age-related sarcopenia have been suggested [31, 32]. The absence of association suggested that there may be other stronger risk factors for sarcopenia in men. Our prospective analysis suggested that higher age, lower BMI, lower PASE, and lower energy intake were significantly associated with incident sarcopenia in men other than serum AAs patterns (details not shown).

Cross-sectional studies investigating individual circulating AAs showed that high non-EAs (proline, glutamic acid, and taurine) [11, 12] and low EAAs (methionine and phenylalanine) [12, 13] were associated with a higher risk of sarcopenia in older adults. Our study found that serum AAs pattern characterized by high arginine, taurine, and serine was associated with a higher risk of sarcopenia. Oxidative stress and chronic inflammation play an important role in age-related sarcopenia [33]. It has been suggested that taurine are released from cells following oxidative stress and chronic inflammatory stimulation [34], whether this explains the association of serum AAs pattern characterized by high taurine level and sarcopenia remains to be explored. Contrast to some studies which found that higher circulating BCAAs was associated with a lower risk of sarcopenia [13, 14], our study found that a serum AAs pattern characterized by high BCAAs (isoleucine, valine, and leucine) was associated with a higher risk of sarcopenia in women. Conversely, one study showed no significant differences in the serum BCAAs between sarcopenic older adults and nonsarcopenic older adults [12]. The different findings may be explained by not accounting dietary factors and physical activity level in the analysis [11–14]. These lifestyle factors may have an influence on AA profiles and sarcopenia. Other reasons could be the collection of circulating AAs from nonfasting participants [14], and the cross-sectional nature of the studies [11–14].

BCAAs are important for maintaining and increasing muscle mass as they are involved in muscle protein synthesis, activation of satellite cells, and inhibitory effect on proteolysis [35]. It should be noted that the degradation of most AAs occurs in the liver, while BCAAs are mostly metabolized in muscle tissue by mitochondrial dehydrogenase and branch-chain ketoacid dehydrogenase [36]. It has been suggested that decreased uptake in skeletal muscle and increased levels in circulating BCAAs may reflect low muscle quality which is characterized by impaired transport of AAs and/or a decreased mitochondrial dehydrogenase or branch-chain ketoacid dehydrogenase activity [37]. Whether this explains our findings of the association between serum BCAAs pattern and a higher risk of sarcopenia in women remains to be elucidated. Of note, a 12-week intervention among community-dwelling Chinese sarcopenic older adults showed that β-hydroxy β-methylbutyrate (HMB) supplementation increased muscle mass only during the intervention period, but had no additional effect on the exercise-induced improvement of muscle strength and physical performance measures [38]. A systematic review found that leucine or HMB supplementation alone increased muscle mass but not for muscle strength or physical performance [39]. Existing studies are limited by factors, such as a small number of participants, lack of evidence on sarcopenia as an outcome measure, and issues on the interaction of BCAAs or HMB with other nutrients and with exercise programs [39, 40]. Whether BCAAs or HMB supplementation alone is effective in the management of sarcopenia will require further studies to confirm.

The present study has several strengths. This is the first prospective study examining serum AAs patterns and how they are related to the risk of sarcopenia in older adults. Due to the use of PCA, the issue of multiple comparisons between a wide range of AAs and sarcopenia was minimized. Furthermore, a wide range of lifestyle variables was considered in the analysis. However, several limitations of this study should be acknowledged. Only a subgroup of participants from our study had complete serum AAs data and was used for the analysis. Moreover, the study sample was comprised of Chinese older adults and the AAs patterns generated from our study may limit its generalizability to other ethnic groups with different cultures. Finally, although a large number of serum AAs were assayed in this study, it cannot be ruled out that other AAs that were not available in our dataset may be involved in the pathophysiology of sarcopenia.

To conclude, in community-dwelling Chinese older adults, 6 serum AAs patterns were identified. Serum AAs
patterns characterized by high BCAAs and high non-EAAs (arginine, taurine, and serine) at baseline were associated with a higher risk of sarcopenia over a 4-year follow-up in women. None of the other AAs patterns were associated with incident sarcopenia. Exploring the unique pattern of serum AAs that is associated with sarcopenia provides new insights into the role of specific serum AAs in the prevention and treatment of sarcopenia in older adults.

Statement of Ethics

The subjects have given their written informed consent and that the study protocol was approved by the Clinical Research Ethics Committee of the Chinese University of Hong Kong (CRE 2003.102).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Authors contributed to this work as follows: S.S.Y.Y. performed the statistical analysis, interpreted the results, and wrote the manuscript; Z.L.Y.Z. performed the statistical analysis, reviewed, and edited the final manuscript; T.K. designed and conducted the research, supervised the data collection, contributed to discussions about the results, reviewed, and edited the final manuscript; J.W. contributed to discussions about the results, critically revised, and edited the final manuscript. All the authors read and approved the final version submitted.

Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.
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