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Research Article

# Systemic and Metabolic Signature of Sarcopenia in Community-Dwelling Older Adults

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## Abstract

**Background:** Evidence suggests the pivotal contribution of nutrition as a modifiable risk factor for sarcopenia. The present cross-sectional study characterized the nutritional and metabolic profile of sarcopenia through an extensive exploration of a wide array of blood biomarkers related to muscle protein metabolism and transcriptomic signatures in community-dwelling elderly adults.

**Methods:** Among 189 older individuals with a mean age of 73.2 years, sarcopenia was diagnosed according to the Asian Working Group for Sarcopenia criteria based on appendicular lean mass measured by dual-energy X-ray absorptiometry scan, muscle strength, and gait speed. Nutritional status was evaluated using the mini-nutritional assessment (MNA). In addition, we assessed specific blood biomarkers of nutritional status (plasma essential amino acids [EAAs], vitamins), nicotine-derived metabolites, and an extensive microarray analysis from peripheral blood mononuclear cells.

**Results:** Malnutrition defined by low MNA score was independently associated with sarcopenia ($p < .001$). Sarcopenic elderly showed lower body mass index and leptin and higher adiponectin and high-density lipoproteins. Levels of EAAs including lysine, methionine, phenylalanine, threonine, as well as branched-chain AAs and choline, were inversely associated with sarcopenia. Furthermore, nicotine metabolites (cotinine and trans-3′-hydroxycotine) and vitamin B6 status were linked to one or more clinical and functional measures of sarcopenia. Differentially expressed genes and ingenuity pathway analysis supported the association of nutrition with sarcopenia.

**Conclusions:** Herein, the characterization of a nutritional and metabolic signature of sarcopenia provides a firm basis and potential identification of specific targets and directions for the nutritional approach to the prevention and treatment of sarcopenia in aging populations.

**Keywords:** Sarcopenia, Nutrition, Metabolites, Ingenuity pathway analysis, Modifiable risk factors

Sarcopenia is a central feature of the clinical frailty phenotype presenting as age-associated progressive loss of skeletal muscle mass, coupled with decreases in muscle strength and function, which in turn can significantly contribute to the progression of disability, impairment of quality of life, and mortality (1,2). It is estimated that sarcopenia will affect the health of 500 million elderly adults by 2050 (3). The cause of sarcopenia is complex and multifaceted and involves the imbalance of anabolic and catabolic factors which may be aggravated further by nutritional deficiency, insufficient physical activity, insulin resistance, atherosclerosis, smoking, and alterations of inflammatory status and endocrine function (4–6). Recent clinical and epidemiological research suggests a major impact of nutrition that contributes to the variation in the risk and rate of sarcopenia during aging (7).

Specifically, both human and animal studies investigating the association between malnutrition and muscle mass loss or physical performance decline with age have provided evidence for the roles of protein intake inadequacy, deficiencies in antioxidants, vitamin D, and n-3 class of polyunsaturated fatty acids in the elderly adults (8–10). However, few studies have been conducted to examine the role of those nutritional factors in elderly participants with a well-defined clinical phenotype of sarcopenia and conflicting results were generated. Alemán-Mateo et al. (11) provided protein-rich food on top of a habitual diet (additional 15.7 g protein/d) to sarcopenic elderly adults aged above 60 years and did not observe any change in appendicular skeletal muscle mass compared with the sarcopenic controls who maintained their habitual diet ($n$ = 40). On the other hand, supplementation with 8 g/d essential amino acid (EAA) mixture increased whole-body lean mass compared with placebo in a study of 41 older adults with sarcopenia (12). Beyond protein and amino acid intake and their role in lean tissue accrual (13), receptors of other nutrients found in human muscle cells such as 25-hydroxy vitamin D [25(OH)D] may also be involved in protein homeostasis and muscle physiology (14). In the KNHANES IV study of older Koreans who were 50 years and older, serum 25(OH)D levels positively correlated with lean muscle mass in participants classified as sarcopenic only based on muscle mass loss (15), but further gender-specific analysis found this relationship in females only (16). In addition, a higher proportion of sarcopenic elderly adults had poor micronutrient intakes based on estimates from a 3-days food record below the recommended dietary allowances (RDAs) compared with their counterparts without sarcopenia (17). These studies highlight the importance of specific dietary components such as proteins with specific amino acids, and vitamins as potential modulators of sarcopenia.

In this context, the present study aimed to (a) identify specific nutritional and metabolic biomarkers of sarcopenia through extensive blood measurements, including EAAs, vitamins, and nicotine-derived metabolites [as smoking is a risk factor for muscle wasting (18)]; (b) investigate the gene expression profile of interest in sarcopenic versus nonsarcopenic older individuals.

## Subjects and Methods

### Study Design and Participants

In this cross-sectional study, participants were recruited from the baseline measurements of the ongoing Singapore Longitudinal Ageing Study Wave 2 [SLAS-2 (19)] in 2014–2015. Individuals aged 65–90 years ($n$ = 189) of Chinese ethnicity with data available for sarcopenia identification participated. Additionally to sarcopenia, participants were assessed for functional health status [frailty status (20), physical activity, functional dependency, health-related quality of life, number of comorbidities], cognitive and psychological function (Mini-Mental State Examination, Geriatric Anxiety Inventory, Geriatric Depression Scale, Satisfaction With Life Scale). The demographic variables we measured included age, gender, body mass index (BMI), smoking status, the frequency of alcohol drinking, marital status, and housing type as an indicator of socioeconomic status. Fasting blood samples were collected and plasma and peripheral blood mononuclear cells (PBMCs) were isolated and stored at −80°C and liquid nitrogen, respectively until further analysis. The study was approved by the National Health Group (NHG) Domain Specific Review Board (DSRB) of Singapore, and all participants provided written informed consent.

### Identification of Sarcopenia

The sarcopenic status of each participant was established by dual-energy X-ray absorptiometry (DXA) scan, lower limb strength measurements, and 6-m gait speed test to measure appendicular lean mass, muscle strength, and function. Total and regional lean body mass was measured by DXA scan with the use of Hologic densitometer. After having voided bowel and bladder, the participant laid in a supine position on the DXA table with limbs close to the body. Scans were performed in accordance with the manufacturer's protocol in the Department of Diagnostic Radiology, National University Hospital (NUH) of Singapore. Lower limb strength was assessed using Lord's strap and strain gauge assembly component of the Physiological Profile Assessment (PPA) (21), and the gender and BMI standardized average value from three trials was calculated. Cutoff values for low lower limb strength were ≤18 kg for men and ≤16 kg for women. Participants were required to complete the 6-m fast gait speed test as described by Nelson and colleagues (22) and low gait speed was defined as the average speed of two trials ≤0.8 m/s. Figure 1 summarizes the process utilized for diagnosis of sarcopenia in accordance to the Asian Working Group for Sarcopenia (AWGS) criteria released in 2014 (7).

### Nutritional and Metabolic Biomarkers

The short-form mini-nutritional assessment (MNA) (23), an established nutritional screening tool for the elderly adults, was used to assess the nutritional status of the participants. Participants with a score ≤11 were considered at risk of malnutrition. The levels of fasting insulin and leptin were measured using MILLIPLEX MAP Human Bone Magnetic Bead Panel (Millipore Corp., Billerica, MA) and analyzed using Bioplex Manager 6.0 software (Bio-Rad Laboratories, Hercules, CA). The level of adiponectin was determined using commercially available enzyme-linked immunosorbent assay (ELISA) kit from Cusabio Biotech (Wuhan, China). The concentration of fasting glucose, high-density lipoproteins, low-density lipoproteins, and total cholesterol was measured using standard laboratory methods at the Department of Laboratory Medicine, NUH of Singapore. Plasma profiling of EAAs (histidine, lysine, methionine, phenylalanine, threonine, tryptophan, isoleucine, leucine, and valine), vitamins and associated metabolites or vitamers (all-trans retinol, 25-hydroxy vitamin D3, α-tocopherol, total B6, functional B6, total B12, choline, and 5-methyl-tetrahydrofolate), and nicotine-derived metabolites (cotinine and trans-3′-hydroxycotine) was performed blindly by Bevital Laboratory (Norway) with previously validated and reported analytical methods (http://www.bevital.no/).

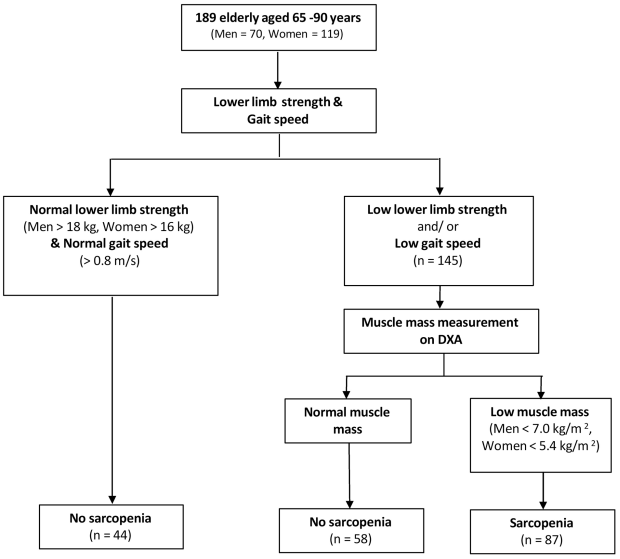


**Figure 1.** Flow chart of sarcopenia identification according to the Asian Working Group for Sarcopenia (AWGS) criteria. Muscle mass and function were firstly assessed by lower limb strength and gait speed. Participants with normal lower limb strength (men > 18 kg, women > 16 kg) and gait speed (>0.8 m/s) were classified as no sarcopenia ($n$ = 44), and the rest ($n$ = 145) were further screened for muscle mass measured by dual-energy X-ray absorptiometry (DXA) scan. Those who also had low muscle mass (men < 7.0 kg/m$^2$, women < 5.4 kg/m$^2$) were defined as having sarcopenia ($n$ = 87). Participants with low lower limb strength and/or low gait speed but normal muscle mass ($n$ = 58) were classified as no sarcopenia as well.

### Gene Expression Analysis

Total ribonucleic acid (RNA) was isolated from PBMCs using the mirVana miRNA isolation kit (Thermo Fisher Scientific, San Jose, CA). Complementary DNA (cDNA) was synthesized (Reverse Transcription Master Mix and Second Strand Master Mix, Thermo Fisher Scientific) and purified. Gene expression was analyzed by microarray using the Illumina human HT-12 V4.0 bead chip platform. The raw gene expression data output from *Illumina Genome Studio* was exported in batches of 96 samples. Quality control (QC) and preprocessing of data were done using the Bioconductor packages of R software and Array Studio.

## Statistical Analysis

Data analysis was performed using IBM SPSS 21 software (IBM), Bioconductor packages of R software version 3.4.1, and ingenuity pathway analysis (IPA) software. Independent $t$-tests and chi-squared tests were used for the comparison between sarcopenia and nonsarcopenia subjects as appropriate. Logistic regressions were performed to estimate the association of nutritional and metabolic biomarkers with the presence of sarcopenia, with the adjustment for potential confounding by age and gender (and additionally smoking status, BMI, physical activity time [percentage of activity time in activity plus sedentary time], the frequency of alcohol drinking, housing type as an indicator of socioeconomic status, and number of comorbidities). The odds ratio (OR) and its 95% confidence interval (CI), and $p$ values computed from the multiple regression models were presented. In linear regression models, the relations of identified nutritional and metabolic markers and components of sarcopenia (ASMI, lower limb strength, and gait speed) were investigated while controlling for confounders. Differentially expressed genes (DEGs) were identified using generalized linear model (GLM) adjusting for batches of microarray experiments, age, and gender of subjects. Bioconductor lima algorithm outlier detection was performed based on the distance from the sample to the center. We performed variance stabilizing transformation (vs. lumi), $\log_2$ transformation, quantile normalization (global method), and robust spline normalization (RSN, specific Illumina bead chip microarray method). Volcano plot was generated using RStudio version 1.0.136. Pathway and network analysis of DEGs was performed in sarcopenia versus nonsarcopenia using IPA software.

## Results

### Sarcopenia Characterization According to the Criteria Suggested by the AWGS

Participants in the present study ($n$ = 189) were all Chinese and had a mean age of 73.2 years ($SD$ = 5.3). There were 119 (63.0%) individuals who are females and 70 (37.0%) individuals who were males. As shown in Figure 1, sarcopenia was defined according to an algorithm suggested by the AWGS in 2014. The proportion of elderly adults with sarcopenia in this study (46%) is consistent with the prevalence of sarcopenia of above 40% in older adults over the age of 70 years worldwide (24,25).

Table 1 shows the sociodemographic and functioning characteristics of participants with and without sarcopenia. Sarcopenic elderly adults were more likely to be smokers ($p$ = .001), frailer ($p$ = .001), and had more depressive symptoms as assessed by GDS ($p$ = .042), consistent with the poor health, physical and mental function status found in sarcopenia (26,27). The sarcopenic group tended to be older than the nonsarcopenic group (73.9 years vs 72.5 years, $p$ = .070). No difference was found between the two groups for other demographics, physical and functional status, cognitive and psychological function, and clinical laboratory markers.

Sarcopenia and its components among men and women are presented in Supplementary Table S1. In all, 106 (56.1%) had low muscle mass, 142 (75.1%) had low lower limb strength, 26 (13.8%) had low gait speed, and 87 (46.0%) were sarcopenic, consisting of 32 (45.7%) men and 55 (46.2%) women ($p$ = .946 for gender difference). However, there were gender differences in components of sarcopenia. Given that men had higher absolute values of ASMI, lower limb strength, and gait speed than women, the application of gender-specific cutoffs of ASMI and lower limb strength revealed that there was a higher proportion of women who had low lower limb strength ($p$ = .001), whereas no gender difference was observed in terms of the proportions of low muscle mass or gait speed.

### Identification of Nutritional and Metabolic Markers Associated With Sarcopenia and Its Components

Logistic regressions were performed to investigate the association of nutritional and metabolic markers with the presence of sarcopenia after adjusting for age and gender. As given in Table 2, the presence of sarcopenia was significantly associated with lower MNA score as a continuous variable (OR = 0.453, $p$ < .001) and risk of malnutrition analyzed as a categorical variable (MNA < 11) (OR = 9.877, $p$ < .001). BMI (OR = 0.577, $p$ < .001) and leptin (OR = 0.941, $p$ < .001) were negatively associated whereas adiponectin (OR = 1.014, $p$ = .046) and high-density lipoproteins (OR = 3.266, $p$ = .004) were positively associated with sarcopenia. There were significant negative associations between sarcopenia and levels of lysine,

**Table 1.** Sociodemographic and Functional Characteristics of Study Participants With and Without Sarcopenia (Singapore Longitudinal Aging Study Wave 2, SLAS-2)

| | Sarcopenia | | | |
|---|---|---|---|---|
| | Yes ($n$ = 87) | No ($n$ = 102) | $t/\chi^2$ | $p$ |
| *Demographics* | | | | |
| Age (years) | 73.92 ± 5.27 | 72.52 ± 5.25 | 1.825 | .070 |
| Gender, $n$ (%) | | | | |
| Male | 32 (36.8) | 38 (37.3) | 0.005 | .946 |
| Female | 55 (63.2) | 64 (62.7) | | |
| Smoking status, $n$ (%) | | | | |
| Past or present smoker | 11 (12.8) | 1 (1.0) | 10.530 | .001 |
| Nonsmoker | 75 (87.2) | 98 (99.0) | | |
| Frequency of alcohol drinking | | | | |
| Never or rarely | 85 (97.7) | 94 (93.1) | 2.200 | .138 |
| More than one drink/mo | 2 (2.3) | 7 (6.9) | | |
| Marital status, $n$ (%) | | | | |
| Married | 52 (59.8) | 68 (66.7) | 0.963 | .326 |
| Not married | 35 (40.2) | 34 (33.3) | | |
| Housing type (indicator of socioeconomic status) | | | | |
| 1–3 room HDB | 38 (50.7) | 37 (40.7) | 1.662 | .197 |
| 4–5 room HDB or private housing | 37 (49.3) | 54 (59.3) | | |
| *Physical and functional status* | | | | |
| Frailty status, $n$ (%) | | | | |
| Robust | 21 (24.4) | 35 (35.0) | 8.704 | .013 |
| Pre-frail | 31 (36.0) | 45 (45.0) | | |
| Frail | 34 (39.5) | 20 (20.0) | | |
| Physical activity time (%) | | | | |
| Light activity | 36.01 ± 13.79 | 36.78 ± 10.33 | −0.406 | .685 |
| Moderate activity | 3.06 ± 2.62 | 3.58 ± 3.15 | −1.173 | .165 |
| Vigorous activity | 0.17 ± 0.89 | 0.32 ± 1.50 | −0.755 | .451 |
| Sedentary | 60.76 ± 15.62 | 59.32 ± 11.55 | 0.674 | .501 |
| BADL | 17.82 ± 0.95 | 17.83 ± 0.57 | −0.139 | .890 |
| EQ-5D index | 0.22 ± 0.64 | 0.22 ± 0.54 | 0.061 | .951 |
| Number of comorbidities | 1.98 ± 1.49 | 2.36 ± 1.51 | −1.760 | .080 |
| *Cognitive and psychological function* | | | | |
| MMSE | 27.47 ± 2.72 | 27.66 ± 2.03 | −0.536 | .593 |
| GAI | 0.49 ± 1.70 | 0.54 ± 1.90 | −0.170 | .865 |
| GDS | 0.83 ± 1.42 | 0.47 ± 0.88 | 2.048 | .042 |
| Life satisfaction | 1.89 ± 1.57 | 2.01 ± 1.35 | −0.580 | .562 |
| *Clinical laboratory tests* | | | | |
| White blood cell count (thousand/μL) | 5.57 ± 1.76 | 5.74 ± 1.60 | −0.693 | .489 |
| Red blood cell count (thousand/μL) | 4.65 ± 1.02 | 4.52 ± 1.01 | 0.859 | .393 |
| Platelets (thousand/μL) | 125.25 ± 57.59 | 121.97 ± 64.30 | 0.364 | .716 |
| Lymphocytes (%) | 35.06 ± 8.67 | 35.69 ± 8.67 | −0.493 | .623 |
| Monocytes (%) | 5.13 ± 1.90 | 5.49 ± 1.89 | −1.269 | .206 |
| Granulocytes (%) | 59.45 ± 9.43 | 57.95 ± 9.52 | 1.085 | .279 |
| Anemia | | | | |
| Yes | 43 (49.4) | 58 (56.9) | 1.044 | .307 |
| No | 44 (50.6) | 44 (43.1) | | |
| CRP (ng/mL) | 1.86 ± 2.53 | 1.91 ± 2.24 | −0.146 | .884 |

Notes: Data are presented as $n$ (%) or mean ± $SD$. Sarcopenia was defined according to the AWGS criteria as the presence of low muscle mass with low muscle strength or low physical performance. BADL = basic activities of daily living; CRP = C-reactive protein; EQ-5D = Euro Quality of life five dimensions questionnaire; GAI = Geriatric Anxiety Inventory; GDS = Geriatric Depression Scale; HDB = housing development board; MMSE = Mini-Mental State Examination.

methionine, phenylalanine, threonine, BCAAs, and choline which are known to be important for muscle protein turnover and overall metabolism. Furthermore, cotinine, trans-3′-hydroxycotine, and functional biomarker of vitamin B6 status, which are respectively markers of nicotine metabolism (ie, smoking indicator) and the 3-hydroxykynurenine:xanthurenic acid ratio (where higher HK/XA ratio represents low vitamin B6 level), was associated with the presence of sarcopenia.

Further sensitivity analysis was performed after adjusting for age, gender, and additionally smoking status, BMI, physical activity time (percentage of activity time in activity plus sedentary time), the frequency of alcohol drinking, housing type as an indicator of socioeconomic status, and number of comorbidities. As shown in Supplementary Table S2, BMI, methionine, threonine, cotinine, and trans-3′-hydroxycotine remained significantly associated with sarcopenia after adjustment for potential confounders. Histidine was

**Table 2.** Identification of Nutritional and Metabolic Markers Associated With Sarcopenia

| | Sarcopenia | | | | |
|---|---|---|---|---|---|
| | Yes ($n$ = 87) | No ($n$ = 102) | OR | 95% CI | $p$ |
| *Nutrition score and general metabolic markers* | | | | | |
| MNA score | 12.24 ± 0.14 | 13.01 ± 0.13 | 0.453 | 0.315–0.651 | <.001 |
| Risk of malnutrition, $n$ (%) | 24(32.4) | 4 (4.5) | 9.877 | 3.210–30.390 | <.001 |
| BMI (kg/m$^2$) | 21.66 ± 0.33 | 25.37 ± 0.30 | 0.577 | 0.488–0.683 | <.001 |
| Insulin (pg/mL) | 286.68 ± 46.87 | 365.35 ± 42.76 | 0.999 | 0.998–1.000 | .213 |
| Leptin (ng/mL) | 10.17 ± 1.61 | 17.16 ± 1.47 | 0.941 | 0.910–0.973 | <.001 |
| Adiponectin (mg/L) | 22.53 ± 2.57 | 15.54 ± 2.35 | 1.014 | 1.000–1.028 | .046 |
| Fasting glucose (mmol/L) | 6.28 ± 0.18 | 6.17 ± 0.16 | 1.018 | 0.844–1.227 | .853 |
| High-density lipoproteins (mmol/L) | 1.54 ± 0.04 | 1.40 ± 0.04 | 3.266 | 1.457–7.321 | .004 |
| Low-density lipoproteins (mmol/L) | 3.35 ± 0.13 | 3.25 ± 0.12 | 1.142 | 0.882–1.479 | .314 |
| Cholesterol (mmol/L) | 5.59 ± 0.15 | 5.35 ± 0.14 | 1.191 | 0.959–1.480 | .114 |
| *Essential amino acids* | | | | | |
| Histidine (μmol/L) | 62.25 ± 0.79 | 64.06 ± 0.72 | 0.969 | 0.929–1.011 | .147 |
| Lysine (μmol/L) | 158.48 ± 2.83 | 164.57 ± 2.58 | 0.988 | 0.976–0.999 | .040 |
| Methionine (μmol/L) | 22.50 ± 0.36 | 24.12 ± 0.33 | 0.853 | 0.773–0.941 | .001 |
| Phenylalanine (μmol/L) | 52.23 ± 0.68 | 54.54 ± 0.62 | 0.936 | 0.891–0.985 | .010 |
| Threonine (μmol/L) | 100.84 ± 2.23 | 108.30 ± 2.03 | 0.975 | 0.960–0.991 | .002 |
| Tryptophan (μmol/L) | 49.90 ± 0.76 | 50.98 ± 0.69 | 0.976 | 0.934–1.019 | .269 |
| Isoleucine (μmol/L) | 52.48 ± 1.22 | 56.91 ± 1.11 | 0.965 | 0.938–0.993 | .015 |
| Leucine (μmol/L) | 100.80 ± 1.93 | 108.15 ± 1.76 | 0.977 | 0.960–0.994 | .009 |
| Valine (μmol/L) | 206.26 ± 3.80 | 223.18 ± 3.46 | 0.985 | 0.975–0.994 | .001 |
| Total BCAA (μmol/L) | 359.54 ± 6.68 | 388.23 ± 6.09 | 0.992 | 0.987–0.997 | .002 |
| *Vitamin metabolism* | | | | | |
| All-trans retinol (μmol/L) | 1.51 ± 0.05 | 1.60 ± 0.04 | 0.599 | 0.293–1.223 | .159 |
| 25-Hydroxy vitamin D3 (nmol/L) | 56.96 ± 1.61 | 58.10 ± 1.47 | 0.992 | 0.972–1.012 | .425 |
| α-tocopherol (μmol/L) | 30.30 ± 0.75 | 28.75 ± 0.68 | 1.040 | 0.995–1.087 | .086 |
| Total B6 (nmol/L) | 97.12 ± 20.45 | 118.24 ± 18.66 | 0.999 | 0.998–1.001 | .490 |
| Functional B6 | 4.27 ± 0.21 | 3.69 ± 0.19 | 1.198 | 1.010–1.420 | .038 |
| Total B12 (pmol/L) | 78.65 ± 3.35 | 84.65 ± 3.04 | 0.991 | 0.980–1.002 | .095 |
| Choline (μmol/L) | 9.64 ± 0.26 | 10.23 ± 0.24 | 0.877 | 0.769–1.000 | .049 |
| 5-Methyl-tetrahydrofolate (nmol/L) | 26.41 ± 1.93 | 22.68 ± 1.76 | 1.017 | 0.999–1.035 | .065 |
| *Nicotine metabolism* | | | | | |
| Cotinine (nmol/L) | 148.81 ± 21.75 | 37.73 ± 19.84 | 1.002 | 1.001–1.004 | .008 |
| Trans-3′-hydroxycotine (nmol/L) | 61.59 ± 11.64 | 15.18 ± 10.62 | 1.004 | 1.000–1.008 | .031 |

Notes: All data are adjusted for age and gender. BCAA = branched-chain amino acid; BMI = body mass index; MNA = mini-nutritional assessment.

associated with the reduced possibility of sarcopenia (OR = 0.934). The effects of other parameters on sarcopenia reduced to or remained nonsignificant after further adjustment.

In linear regression models, the independent associations of identified nutritional and metabolic markers with the three components of sarcopenia were examined (Supplementary Table S3) with adjustment for age, gender, and total body fat (%). MNA score (β = 0.436, $p$ < .001) was positively and risk of malnutrition (β = –0.367, $p$ < .001) was negatively associated with ASMI. BMI was positively associated with ASMI (β = 0.973, $p$ < .001) and lower limb strength (β = 0.192, $p$ = .038). There was a positive association between leptin and a negative association between high-density lipoproteins and ASMI. The effect of adiponectin on the components of sarcopenia was not observed except a borderline negative association with gait speed. Regarding EAAs, the levels of methionine were positively associated with lower limb strength and gait speed, and borderline with ASMI. Total BCAA and leucine were associated with the increase of ASMI and gait speed; valine, phenylalanine, and threonine were associated with increased ASMI but not gait speed or lower limb strength; isoleucine was positively associated with gait speed. Choline was positively associated, whereas cotinine and trans-3′-hydroxycotinine were negatively associated with ASMI. Results after adjustment of additional potential confounders are shown in Supplementary Table S4. The associations of identified nutritional and metabolic markers with ASMI and lower limb strength remained significant or reduced. Cotinine and BMI were found significantly associated with lower limb strength and gait speed, respectively.

## DEGs and Pathway and Network Analysis in Sarcopenia Versus Nonsarcopenia

After data QC and preprocessing, the final pipeline detected a total of 27,867 statistically significant genes. The gene expression profile of the biological process of Gene Ontology (GO) molecular function terms EAA, vitamin, and nicotine metabolism (available in the GO database) were assessed using GLM adjusting for batches of microarray experiments, and age and gender of subjects. As shown in Supplementary Table S5, 196 DEGs were identified out of the 1,937 genes of interest. The most significant DEGs ($p$ < .01) were plotted in Figure 2. Data in the figure were presented as $\log_2$ (fold change) and $-\log_{10}$ ($p$ value) for the sarcopenia group versus the nonsarcopenia group. Labels were attached to genes falling above the line of $-\log_{10}$ (0.01). Dots in red and green indicate, respectively, upregulated and downregulated genes. The most upregulated genes in sarcopenia by fold change were FOLR3, AVPI1, PFKFB3, NAMPT, TNFAIP3,

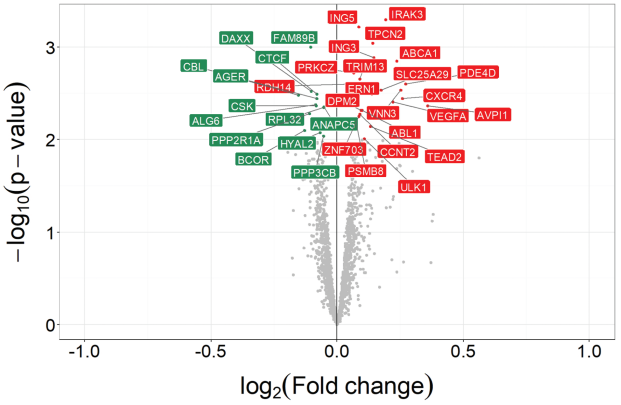


**Figure 2.** Volcano plot of differentially expressed genes (DEGs) in subjects with versus without sarcopenia.The gene expression profile of Gene Ontology (GO) molecular function terms essential amino acid, vitamin, and nicotine metabolism (available in the GO database) were assessed using generalized linear model (GLM) adjusting for batches of microarray experiments, and age and gender of subjects. Among the 1,937 genes we focus, 196 DEGs were identified. Data were presented as $\log_2$(fold change) and $-\log_{10}$(*p* value) for the sarcopenia group versus the nonsarcopenia group. Labels were attached to genes falling above the line of $-\log_{10}(0.01)$. Dots in red and green indicate, respectively, upregulated and downregulated genes.

SLC7A5, PDE4D, CXCR4, VNN3, and PDE4B. On the other hand, the most downregulated genes by fold change were GATA2, PIM1, CBL, CEBPA, DGKQ, BCOR, PTPN6, HSPA1A/HSPA1B, KIDINS220, and PSMD1.

Figure 3 depicts pathway and network analysis of DEGs in sarcopenia versus nonsarcopenia using IPA software. There was significant overlap of 221 canonical pathways ($p < .05$). Figure 3A shows top canonical pathways [$-\log_{10}(p \text{ value}) > 5.0$] which were connected with protein and lipid metabolism, diabetes mellitus signaling, cancer, immune cell response, and growth factor signaling. IPA upstream functional analysis was performed to explore the upstream transcriptional regulators from DEGs in sarcopenia. The network of top upstream regulators and genes regulated by them are shown in Figure 3B. The most significant upstream regulator was enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2) which is involved in epigenetic regulation of muscle development and differentiation pathways. EZH2 had seven main interactors in our data set: BCL2 (apoptosis), CDKN1A (p21, cell cycle inhibition), VEGFA (vascular endothelial growth factor A), CDKN1B (p27, cell cycle inhibition), FLT3 (FMS-like tyrosine kinase-3), KCNK1 (Potassium channel subfamily K member 1), and CDKN2A (p16, cell cycle inhibition). The DEGs were compared with disease gene sets to evaluate their association with diseases and disorders. The top disease and disorder identified by IPA are connective tissue disorders, inflammatory disease, organismal injury and abnormalities, and skeletal and muscular disorders as shown in Figure 3C.

## Discussion

Malnutrition is widely reported during aging, affecting as much as 50% of the elderly population (28). This incidence of malnutrition may in part be due to both inadequate nutritional intake and impaired digestive function (28), which together may result in reduced nutrient uptake and utilization by peripheral tissues (29). In the present study, we report that nutritional status is associated with sarcopenia and its components, and we further identified risk and protective nutritional and metabolic biomarkers of sarcopenia via an extensive exploration of global nutritional markers, EAAs, vitamins or vitamers, and nicotine metabolites in fasting plasma. Specifically, our results showed that the MNA score (OR = 0.453, 95% CI = 0.315–0.651, $p < .001$) and risk of malnutrition (OR = 9.877, 95% CI = 3.210–30.390, $p < .001$) were independently associated with the presence of sarcopenia after adjusting for age and gender. Inspecting the components of sarcopenia, nutritional status measured by the MNA was mainly associated with the preservation of muscle mass and to a lesser extent muscle strength, consistent with recent work (30). Furthermore, the present work proposes additional molecular insights which we identified through plasma metabolic and transcriptomic profiling.

Among the general nutritional and metabolic markers, BMI and leptin were found to be protective, while adiponectin and HDL were identified as risk factors for sarcopenia. In agreement with other studies, our results support the strong negative association of BMI with sarcopenia and positive association with muscle mass (31,32) and muscle strength, although majority of the participants in this study presented with normal BMI ranges (underweight [<18.5 kg/m$^2$]: 13 [6.9%]; normal [18.5–23 kg/m$^2$]: 78 [41.3%]; overweight [above 23 kg/m$^2$ and below 27.5 kg/m$^2$]: 70 [37.0%]) and only 28 (14.8%) subjects had a BMI of 27.5 kg/m$^2$ or above.

Adiponectin and leptin are adipokines which modulate glucose and lipid metabolism, inflammatory status, muscle metabolism, and insulin sensitivity (33). Bucci and colleagues (34) reported that circulating adiponectin has a negative correlation with quadriceps torque and handgrip strength in older individuals. They did not observe any correlation between leptin and muscle strength but found that leptin/adiponectin ratio was positively associated with quadriceps torque and handgrip strength. Our findings of the association of adiponectin with sarcopenia and decreased muscle functioning support the role of adiponectin on muscle strength (34,35). The related mechanisms involving AMP-activated protein kinase (AMPK) signaling and insulin sensitivity in sarcopenia warrants further work. We also observed a link between leptin and its protective role and sarcopenia and particularly muscle mass, possibly due to the regulation of leptin in energy balance and glucose homeostasis. The changes of these general nutritional and metabolic markers at a systemic level may reflect an inappropriate or altered activation of important signaling pathways in sarcopenia.

In healthy humans, skeletal muscle accounts for 40%–45% of total body mass and contains approximately 7 kg of protein primarily in the form of the contractile proteins (36). Out of all the EAAs, leucine has been shown to be a potent stimulator of skeletal muscle protein synthesis (MPS) via the activation of the mTOR complex-1 (mTORC1) signaling pathway (13). However, there have been only few human studies which investigated the role of AAs in properly diagnosed sarcopenia. In one study conducted by Aleman-Mateo and colleagues (11), 40 older individuals with sarcopenia were administered protein-rich food at a dose of 15.7 g protein per day. No change was observed in terms of appendicular skeletal muscle mass at the end of 3 months. In the other study by Solerte and colleagues (12), 41 older sarcopenic individuals were assigned to consume either an EAA mixture supplementation (8 g/d) or a placebo for 18 months and those who took EAA mixture had increased whole-body lean mass compared with individuals who took the placebo. We found that seven (methionine, lysine, phenylalanine, threonine, and the three BCAAs) out of the nine EAAs were negatively associated with sarcopenia. Furthermore, these amino acids had a positive association with sarcopenia components: muscle mass; methionine,

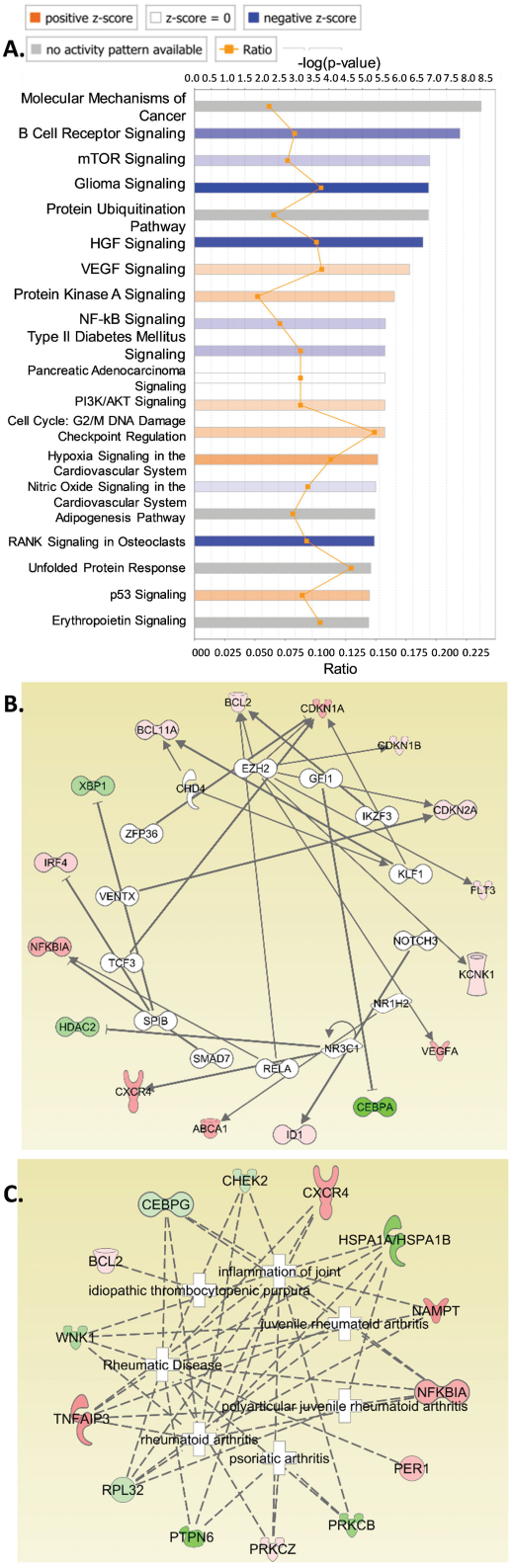


**Figure 3.** Pathway and network analysis of DEGs in sarcopenia versus non-sarcopenia using Ingenuity pathway analysis (IPA) software. (**A**)The top canonical pathways were ranked by $-\log_{10}(p \text{ value})$. (**B**) Network of top upstream regulators and genes regulated by them. (**C**) The top disease and disorder identified by IPA is connective tissue disorders, inflammatory disease, organismal injury and abnormalities, and skeletal and muscular disorders. The color of the bar reflects the direction of change for the function based on the *Z*-score, with orange indicating positive *Z*-score and blue indicating negative *Z*-score.

isoleucine, leucine, and total BCAA were positively associated with skeletal muscle function; and methionine was also associated with muscle strength. However, as the subjects were in a postabsorptive state, it is not possible to ascertain whether or not the circulating amino acids were of an endogenous or exogenous source.

Nine out of the 10 most downregulated genes were annotated to threonine and/or lysine functions. A top canonical pathway shown in IPA is downregulated mammalian target of ramapycin (mTOR) signaling which is an atypical threonine/lysine kinase. Other identified top canonical pathways such as protein ubiquitination pathway and protein kinase A signaling involve multiple amino acids and are related to connective tissue development and function (37). These findings are consistent with earlier genetic studies suggesting the involvement of protein catabolism pathway in sarcopenia in human (38) and nonhuman (39,40) samples. Taken together, our results suggest a close association between protein metabolism and sarcopenia. The anabolic action of AAs on MPS is is mainly due to the EAA content of a meal. Furthermore, the dose required to elicit such a response may be higher in elderly adults as compared with healthy younger individuals. Protein quality (AA composition and availability) has to be carefully evaluated before any intervention. We suggest that the elderly adults meet at least the current recommended intake of dietary protein by consuming varied protein sources (such as meat, fish, poultry, eggs, legumes, and soy) which provide diversity and complementarity of EAAs, to sustain lean tissue mass. Considering that the functional efficiency of the digestive system is more impaired during ageing, EAAs served as pharmaceutical supplements may be more effective for optimum MPS stimulation in the elderly adults.

The relationship between vitamins and sarcopenia is rarely reported except for vitamin D. We did not find any difference in the levels of 25-hydroxy vitamin D3 (25OHD3, the major circulating form of vitamin D) between sarcopenic and nonsarcopenic elderly adults. However, in our study, PIM1 (proto-oncogene serine/threonine-protein kinase) gene was among the most downregulated genes in sarcopenia versus nonsarcopenia. PIM1 kinase interacts with the DNA binding domain of the vitamin D receptor and is implicated in 25OHD3 signaling pathway, suggesting an alteration in vitamin D downstream signaling in the mechanism of sarcopenia independently of intake. Vitamin B6 was assessed with direct marker of total B6 status (plasma pyridoxal phosphate and pyridoxal [PLP + PL]) and functional B6 status as the ratio of 3-hydroxykynurenine and xanthurenic acid (HK/XA). Although the difference of total B6 between those with and without sarcopenia did not reach statistical significance, sarcopenic elderly adults had higher HK/XA ratio which suggests a deficiency of vitamin B6 and associated metabolic functions, for example, AA metabolism through specific vitamin B6 dependent enzymes. Choline, a water-soluble vitamin-like essential nutrient, was reduced in sarcopenia. Choline is the precursor for the neurotransmitter acetylcholine, involved in functions such as muscle control and a source for methyl groups via its metabolite—trimethylglycine (betaine), which participates in the biosynthesis of s-adenosylmethionine (SAM) and methionine metabolism. Taking together, our work underscores the role of B vitamins and choline in sarcopenia which can be explained by their modulation of homocysteine level and the methylation process to produce methionine, thus promoting muscle mass maintenance and strength. These findings also suggest that the so-called one-carbon (1C) metabolism is altered in sarcopenia. Further clinical trials are required to determine whether sarcopenic patients could benefit from drugs or supplementation strategy targeting 1C metabolism. Additionally, we found a significantly higher number

of smokers among the sarcopenic elderly adults in comparison with their nonsarcopenic counterparts. Nicotine metabolites consisting of cotinine and trans-3′-hydroxycotinine were observed to be strongly associated with sarcopenia and especially muscle mass loss. Genes annotated to nicotine response (ABAT and KCNK1) were downregulated in sarcopenia versus nonsarcopenic individuals. Nicotine metabolites may enter the bloodstream and reach the skeletal muscles or indirectly alter B vitamins status which is instrumental in the overall 1C metabolism, thus accelerating muscle wasting in smokers (18). Taken together, our results suggest that nutrition and smoking as lifestyle-related factors contribute to the risk of sarcopenia.

The present study has several strengths. We clearly defined sarcopenia based on appendicular lean mass measured by DXA scan, muscle strength assessed by lower limb strength, and muscle function shown as gait speed, in accordance to the standardized criteria recommended by the AWGS. We identified the nutritional and metabolic signature of sarcopenia through blood measurements of an extensive array of general nutritional markers, EAAs, vitamins, and nicotine-derived metabolites. Furthermore, we investigated the independent association of nutrition with the presence of sarcopenia as well as its components in an Asian population. However, the findings of this study do not unequivocally infer a causal relationship which should be established in a longitudinal study design. Another limitation is the relatively small sample size ($n$ = 189) that requires to be confirmed in a larger group of older individuals.

## Conclusions

In summary, the present study characterized the nutritional profile of sarcopenia using specific nutritional and metabolic biomarkers of EAAs, vitamins, and nicotine metabolism. DEGs and IPA indicate top canonical pathways, such as mTOC signaling and protein ubiquitination. The findings from this study provide a firm basis and potential specific targets and directions for the nutritional approach to the prevention and treatment of sarcopenia in aging populations.

## Supplementary Material

Supplementary data are available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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## Conflict of interest statement

None declared.

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