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Original Article

Plasma Biomarkers of Poor Muscle Quality in Older Men and Women from the Baltimore Longitudinal Study of Aging

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Abstract

Aging is characterized by progressive decline in muscle mass, strength, and quality all of which contribute to functional impairment, falls, mobility disability, and frailty. Circulating factors may provide clues on the mechanisms for decline in muscle quality with aging. Characterizing the metabolic profile associated with reduced muscle quality in older persons could have important translational implications for the early identification of subjects at high risk of developing sarcopenia and the identification of targets for new preventive strategies and treatments. In a pilot cross-sectional, case–control study nested in the Baltimore Longitudinal Study on Aging, we compared circulating metabolites between 79 participants with low muscle quality ratio and 79 controls with high muscle quality, matched by age, sex, and height. The concentrations of 180 metabolites were determined by LC MS/MS, using the Biocrates p180 system, a targeted metabolomics approach. Participants with low muscle quality had significantly higher levels of leucine, isoleucine, tryptophan, serotonin, and methionine, while those with high muscle quality had significantly lower levels of putrescine and the selected phophatidylcholine (PCs) and lysoPCs. The results of this study open a new road for future investigations aimed at identifying new metabolic pathways involved in the decline of muscle quality with aging.

Keywords: Sarcopenia—Muscles—Metabolomics

Aging is characterized by a progressive and inexorable decline in muscle strength, resulting in mobility disability and higher risk of adverse health outcomes (1–6). However, a large interindividual variability exists and only in some individuals, but not others the decline in muscle strength crosses a critical threshold and results in functional consequences. The underlying pathophysiological mechanisms for heterogeneous rated of muscle strength decline are still not understood. Recent evidence suggested that the age-related decline in muscle strength is greater than what expected by the decline in muscle mass alone, with consequent decrease of the strength to mass ratio, which has been termed biomechanical muscle quality (7,8). Interestingly, decreased muscle strength is a much better predictor of functional limitation and poor health in older adults that muscle mass alone (9). Several hypotheses have been proposed for the decline in muscle quality with aging, including decrease in fiber size and number, intrinsic reduction of contractility in the intact fibers, fat micro and macro infiltrations, increase in collagen, alteration of the motor unit, mitochondrial dysfunction, and impaired neurological modulation of contraction (10). In spite of considerable research conducted in this field, the true reason for the decline in contractile capacity of skeletal muscle with aging remains unknown. For example, a previous study reported that multiple branched chain amino acids (BCAA) levels were associated with thigh muscle cross-sectional area (CSA) in older adults (11), but no analyses for predictor of muscle strength and quality were reported.

In recent years, the study of the biology of aging has been revolutionized by the introduction of –omics disciplines, including genomics, proteomics, and metabolomics. Metabolomics, unlike the other –omics, provides a direct signature of the metabolic state of the organism (12–14). Recent advances in mass spectrometry allow the simultaneous analysis of hundreds of metabolites from small amounts of biological material (15,16). It is assumed that the biological pathways leading to pathologic conditions causes altered concentrations of specific metabolites and that studying a subset of these metabolites may offer important clues on the pathophysiology of these conditions. Indeed, metabolomics has been recently used for the identification of novel

biomarkers and the elucidation of novel biological pathways for a variety of diseases (15,17). For example, Collino and co-workers studied the metabolic phenotype of longevity by comparing the metabolomics profile of centenarians, elderly, and young people (18,19). In this study, alterations of the glycerophoshpolipids and sphingolipids were observed in the centenarian population, helping elucidate mechanistic routes for the longevity phenotype and demonstrating the power of this method. As a result, characterizing the metabolic profile associated with reduced muscle quality and poor muscle strength in older persons could have important translational implications, such as early identification of subjects at high risk of developing sarcopenia, improvement of preventive strategies, and development of new treatments. However, very little research has focused on the identification of metabolic biomarkers for poor muscle mass and strength in older adults (11). Here we report the findings of a pilot cross-sectional, nested case–control study using a targeted metabolomics approach, where the concentrations of 180 metabolites belonging to six classes (amino acids, biogenic amines, amino acid metabolites, acyl carnitines, glycerolphospholipids, sphingomyelins, and glucose) were determined by LC MS/MS, using the Biocrates p180 system (15,16). In particular, we contrasted plasma metabolites concentrations between 79 participants with low muscle quality ratio versus 79 controls with high muscle quality who were matched by age, sex, and height.

Methods

Study, Setting, and Design

The design was a nested case–control study derived from a large cohort of men and women enrolled in the Baltimore Longitudinal Study of Aging (BLSA), which is a study of human aging established in 1958 and conducted by the National Institute on Aging Intramural Research Program (NIA-IRP). A general description of the sample and enrollment procedures and criteria has been previously reported (20,21). Briefly, the BLSA continuously enrolls healthy volunteers aged 20 and older who are followed for life with follow-up visits conducted at intervals of 1–4 years, with more frequent follow-up for older persons. Participants are assessed at the NIA Clinical Research Unit in Baltimore, Maryland, over 3 days of testing. Certified nurse practitioners and certified technicians administer all assessments following standardized protocols. All participants receive an extensive description of the study protocol, procedures, and risk associated with participation and consent to be part of the study at each single visit.

A total of 917 BLSA participants, aged 50 or older assessed between January 2006 and February 2014 had available a plasma sample, a CT-based measure of quadriceps mid-thigh cross-sectional area and knee extension isokinetic dynamometry. Cases and controls were defined as participants, respectively, in the lowest and in the highest (age and sex-specific) quartiles of age (decades) and sex-specific muscle quality (see below for the definition of muscle quality) distribution in the sample population (Figure 1). Of these, 79 pairs of cases and controls, matched by age (±2.5 years), sex, and height (±1.5 cm), were included in the analysis (Table 1).

Muscle Quality Assessment

Muscle quality (Nm/cm^2) was operationalized as the ratio of the maximum quadriceps strength, measured by isokinetic dynamometer, to the thigh CT-scan cross-sectional muscle area.

Maximum quadriceps strength was defined as the highest value of torque (peak torque) from either leg in up to three consecutive measures of concentric knee extensor strength (Newton per meters, Nm) using isokinetic dynamometer at an angular velocity of 0.52 rad/s (30°/s). The Kinetic Communicator (Kin-Com Model 125E, version 3.2; Chattanooga Group, Chattanooga, TN) dynamometer was used to assess strength in BLSA until 2010 (22). From 2010 up to now, muscle strength was tested by the Biodex Multi-Joint System-PRO (Biodex Medical System, Advantage Software V.4X, Inc., Shirley, NY) dynamometer. Validity and reliability of both dynamometers were

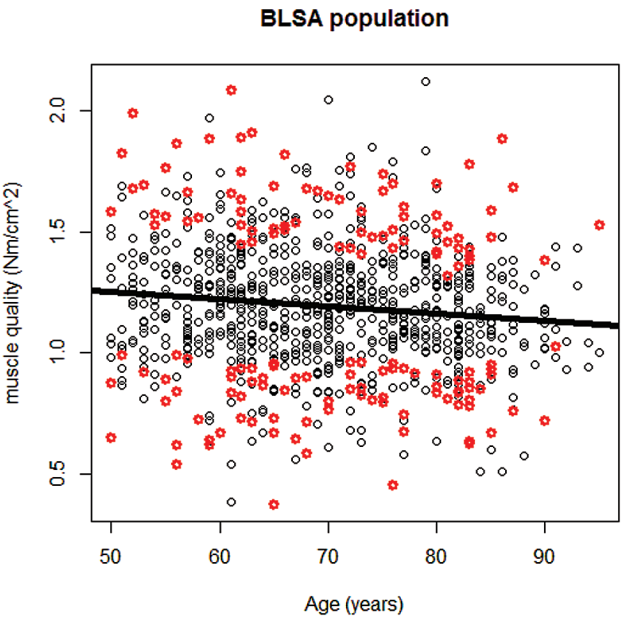

Figure 1. 79 pairs (⭕) of cases and controls, matched by age (±2.5 years), sex and height (±1.5 cm), respectively, in the lowest and in the highest (age and sex-specific) quartiles of age (decades) and sex-specific muscle quality sex-specific muscle quality (Nm/cm^2) in the BLSA population (○).

Table 1. Characteristics of the Population According to Cases (low muscle quality) and Controls (high muscle quality)

	Cases	Controls
All	*N* = 79	*N* = 79
Age (years)	70.62	70.32
Sex (men)	39	39
Height	168.5	168.5
Muscle quality (Nm/cm^2)	0.8	1.6
Age group: 50–59	*N* = 14	*N* = 14
Age (years)	55.1	54.5
Sex (men)	7	7
Height (cm)	170.3	170.6
Muscle quality (Nm/cm^2)	0.8	1.7
Age group: 60–69	*N* = 23	*N* = 23
Age (years)	64.1	64.0
Sex (men)	11	11
Height	171.0	171.1
Muscle quality (Nm/cm^2)	0.8	1.6
Age group: 70–79	*N* = 19	*N* = 19
Age (years)	74.26	74.01
Sex (men)	9	9
Height	166.5	166.7
Muscle quality	0.8	1.5
Age group: 80+	*N* = 23	*N* = 23
Age (years)	83.6	83.1
Sex (men)	12	12
Height	166.5	166.5
Muscle quality	0.8	5.0

reported in previous studies (23–25). The dynamometer's calibration settings were verified on a regular basis throughout the data collection period according to the manufacturers' guidelines. To address possible systematic differences in strength measures due to the change in instrument (dynamometer), we developed a conversion equation using data from 76 participants who underwent both Kin-com and Biodex concentric knee extensor strength tests at the same visit. A scatter plot was used to explore the relationship between strength data obtained with the two different technologies. [right leg: $r = 0.87$, $p < .001$; left leg: $r = 0.87$, $p < .001$]. A regression equation describing this correlation was then developed [right leg: Biodex = 0.9*Kin-Com + 15.6; left leg: Biodex = 0.74*Kin-Com + 19.2]. Equations developed separately in men and women were not statistically different and, therefore, the overall conversion equation was used in the analysis. After the conversion, sensitivity analyses excluded a residual effect of the type of instrument on strength values in the sample population. Cross-sectional muscle area (CMA) of the thigh was measured from 10 mm CT images captured at midfemur by a Somatom Sensation 10 CT scanner (Siemens, Malvern, PA) and quantified using Geanie software version 2.1 (BonAlyse, Jyvaskyla, Finland; http:// www.bonalyse.com) (8). Different tissues in the analysis were separated according to different density thresholds: a density value of 35 mg/mm was used to separate fat from muscle tissue, and 180 mg/mm to separate muscle from bone tissue. Analyzed images were visualized and checked for mistakes in the identification and contouring of different tissue. Macroscopically detectable intramuscular fat was not included in the calculation of muscle area.

Sampling

In BLSA participants, blood samples were collected in the morning after an overnight fast. Blood samples were centrifuged at 3,000 rpm for 30 min at 4°C. Plasma was carefully collected and was snap frozen on dry ice and subsequently stored at –80°C, until used for further analyses.

Plasma Metabolites

Metabolites were extracted and concentrations were assessed using the AbsoluteIDQ kit p180 (Biocrates Life Science AG, Austria) following the manufacturers protocol for the API5500 LC-MS/MS System (AB SCIEX) running with Analyst 1.5.2 software equipped with an electrospray ionization source, a Shimadzu CBM-20A command module, LC-20AB pump, and a Shimadzu SIL-20AC-HT autosampler and a CTO-10Ac column oven heater (15,16). Briefly, 10 µl of plasma-EDTA samples were pipetted onto a 96-well Biocrates kit. The samples were dried at room temperature (RT) for 30 min. About 50 µl of 5% PITC reagent was added and incubated for 20 min and the plate was dried under nitrogen for 1 h. 300µl of 5mM Ammonium acetate in methanol was added and incubated at RT on a shaker (450 rpm) for 30 min. The plate was centrifuged at 500× g for 2 min and labeled; 50 µl of each sample was transferred to a 96 deep well LC plate and 10 ul of each sample was transferred to the 96 deep well FIA plate. To the LC plate, 450 µl of 40% methanol (in HPLC grade water) was added. To the FIA plate, 490 µl of FIA running solvent was added. 10 µl was injected onto the Eclipse XDB C18, 3.5 µm, 3.0 × 100 mm with a Phenomenex C18 Security Guard Cartridge, 3.0 mm ID. The mobile phase consisted of solvent A (water containing 0.2% formic acid) and solvent B (acetonitrile containing 0.2% formic acid), with the following gradient: 0–0.5 min 0% B, 5.5 min: 95% B; 6.5min: 95% B; 7.0 min: 0% B; 9.5 min: 0% B for the LC plate Evaluation of the samples was carried out using the MetIDQ software. The FIA plate was run with 20 µl injection directly onto the MS at a flow of 30 µl/min with water/acetonitrile (1:1) containing 0.2% formic acid as the mobile phase, with the following flow rate program: 0–1.6 min: 30 µl/min; 2.4 min: 200 µl/min; 2.80 min: 200 µl/min and 3.00 min: 30 µl/min. Concentrations were calculated using the Analyst/MetIDQ software and reported in µM (Supplementary Table 1).

Statistical Methods

Characteristic of the population according to cases and controls are reported as mean ± standard deviations (SD) or percentage. Only metabolites detected in more than 80% of the sample population were included in the statistical analysis. Metabolites with a skewed distribution were log-transformed and after transformation they all approached normal distribution. Paired-*t* test was used to test the cross-sectional association between each metabolite and muscle quality in age, sex, and height-matched cases and controls.

Statistical analyses were performed using the SAS statistical package, version 9.3 (SAS institute Inc., Cary, NC) and R programming language version 3.1.3 (http://www.r-project.org).

Results

Characteristics of the Population

The study population included 158 participants in the BLSA, aged 50–95 (mean age ± SD: 70.5 ± 10.7) years old, of whom 80 were women (50.6 %). They were categorized in 79 pairs of age, sex, and height-matched cases (low muscle quality ratio) versus controls (high muscle quality ratio) (Figure 1). Main characteristics of the population according to cases and controls are presented in Table 1. Fasting insulin levels trended higher in cases with lower muscle quality versus control ($p = .079$), while the homeostatic model assessment, a model of insulin-resistance (HOMA index) was significantly higher in cases versus control ($p = .011$), which is consistent with previous reports where insulin resistance was associated with a reduction of muscle quality (26). In addition, a strong correlation was observed between insulin levels and body mass index (BMI); patients with lower muscle quality had higher BMI when compared with controls ($p < 0.001$). As the patients were matched by height, this finding is mostly due to different weight between cases and controls. Further, only putrescine ($p = .012$) was negatively associated with weight when adjusting for age, sex, and height.

Plasma Analysis

Of the 180 metabolites, 126 were reproducibly measured in plasma obtained from the 158 BLSA participants. Mean values of plasma concentration for the metabolites included in the present analysis are also provided in Table 2. While all the amino acids were measured, of the biogenic amines and amino acid metabolites, only asymmetrical dimethylarginine (ADMA), alpha-amino adipic acid (alpha-AAA), creatinine, kynurenine, histamine, symmetrical dimethylarginine (SDMA), spermidine, and putrescine, had concentration at or above the lower limit of quantitation (Table 2). Participants with low muscle quality ratio presented significantly higher concentrations of isoleucine, leucine, and tryptophan compared to controls ($p = .012$, $p = .033$, and $p = .045$, respectively, Table 2). A similar trend without reaching full significance was also found for methionine ($p = .069$) and serotonin ($p = .078$). In the case of the biogenic amines only putrescine was significantly lower in cases with low muscle quality ratio ($p = .018$) than in controls. The sum of the BCAAs was not significantly different between cases and controls, while the sum of aromatic amino acids was higher in cases versus controls, although the difference was not statistically significant ($p = .069$). Differences of amino acids and biogenic amine concentrations between cases and controls are depicted in Figure 2. While significantly lower concentrations of glycerophospholipids (PC diacyl (aa)

Table 2. Results from Paired *t* Tests Testing Differences in Plasma Concentrations of Amino Acids and Biogenic Amines Between Age, Sex, and Height-Matched Cases (low muscle quality) and Controls (high muscle quality)

Metabolite	Difference (case–control)	*p* value
Alanine	22.55	.284
Arginine	−0.65	.856
Asparagine	19.65	.248
Citrulline	10.05	.364
Glutamine	20.09	.354
Glutamate	−4.47	.645
Glycine	−2.68	.844
Histidine	2.04	.359
Isoleucine	5.76	.012[#]
Leucine	10.09	.033[#]
Lysine	0.74	.926
Methionine	1.43	.067[#]
Ornithine	−0.22	.962
Phenylalanine	2.55	.137
Proline	−10.75	.129
Serine	4.04	.326
Threonine	10.69	.189
Tryptophan	3.78	.045
Tryosine	3.75	.148
Valine	2.43	.725
Asymmetric dimethylarginine	0.02	.338
Symmetric dimethylarginine	0.02	.405
Total dimethylarginine	−0.01	.832
Creatinine	69.04	.311
Kynurenine	−0.06	.642
Putrescine	−0.025	.018
Serotonin	0.07	.078
Spermidine	0.012	.298
Taurine	4.35	.268

Note: [#]*p* values after log transformation.

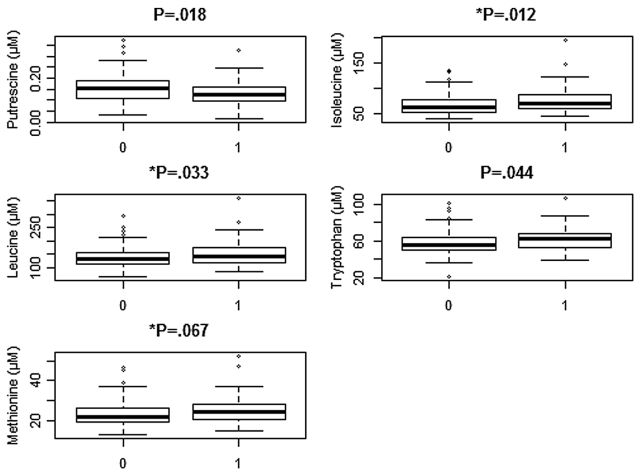

Figure 2. Differences observed between cases (1) and controls (0) for biogenic amine (putrescine) and amino acids (Isoleucine, leucine, tryptophan, and methionine) (*p* values from paired *t*-test (*log transformation)).

C32:2, C34:3, C34:4 and C42:4, PC acyl-alkyl (ae) C34:1, C38:1, C38:2 C38:3, C40:2, C40:3, C40:4, C40:5, C42:1, C42:3, C42:4, C42:5, C44:3, C44:4 and lysoPC acyl (a) C16:1, C18:1 and C18:2) (Table 3) were observed for participants with low muscle quality compared to controls, no difference was observed for sphingolipids or acylcarnitines. However, in the latter case, only the C0, C2, and C3 acyl carnitines were measurable and no difference was observed between cases and controls. In addition, to these metabolites, of the custom metabolite indicators studied, the only ratios reaching significance were putrescine/ornithine indicative of ornithine decarboxylase activity and kynurenine/tryptophan ratios indicative of indoleamine 2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) activity, which were lower in participants with low muscle quality compared to controls.

Discussion

Using a targeted metabolomic approach and comparing 79 pairs (158 BLSA participants) of age, sex, and height-matched cases (low muscle quality ratio) and controls (high muscle quality ratio), we explored the relationship between poor muscle quality and 126 plasma metabolites concentrations, including amino acids, biogenic amines, and lipids.

Amino Acids and Biogenic Amines

Participants with low muscle quality ratio presented significantly higher concentrations of isoleucine and leucine compared to controls. Branched chain amino acids (BCAA, leucine, isoleucine, and valine) account for 35% of the essential amino acids found in muscle proteins (27,28). While the degradation of most amino acids occurs in the liver, BCAA are mostly metabolized in muscle tissue by mitochondrial dehydrogenase and branch-chain ketoacid dehydrogenase to produce the branch-chain keto acids (29). The generated ketoacids are used by the muscle to generate ATP via the Kreb's cycle or transported to the liver for oxidation (27,28). Studies have suggested that muscle protein synthesis is upregulated by amino acids with leucine being the most potent (30,31). Interestingly, in supplementary analyses, leucine and isoleucine levels significantly correlated with BMI but not with insulin. The increased levels of fasting insulin levels in cases versus controls, would be expected to result in an increase in uptake of BCAAs into the skeletal muscle and conversely a decrease in circulating plasma levels. On the contrary, participants with low muscle quality had significantly higher levels of isoleucine and leucine, suggesting that low muscle quality is characterized by impaired transport of amino acids and/or a decreased mitochondrial dehydrogenase or branch-chain keto acid dehydrogenase activity, which would result in decreased uptake in skeletal muscle and increased levels in circulating plasma. This hypothesis should be tested in future studies where parallel metabolomics analyses are performed in muscle tissue and plasma. Interestingly, catabolism of isoleucine and leucine begins in the muscle and results in increased levels of NADH and FADH2 which is subsequently used for ATP generation.

Participants with low muscle quality ratio also presented with significantly higher concentrations of tryptophan compared to controls. A similar trend was also observed for methionine and serotonin although differences were not statistically significant, however no difference was observed for kynurenine between case and control. Tryptophan circulates in blood in a free and albumin bound form (32). Noteworthy in our study, we only measured total tryptophan and not free tryptophan. Normal fasting circulating concentrations ranges of tryptophan are between 55 and 65 μM (33), similar to what was observed in our study (48–62 μM). In order to determine which pathway was more likely responsible for the decrease in kynurenine pathway, the ratio of serine/tryptophan and kynurenine/tryptophan was determined. Physiologically, tryptophan is degraded by TDO (liver) or IDO1 (blood, placenta, lungs, and brain) or IDO2 into kynurenine (34,35). However, IDO2 has low enzymatic activity and its biological

Table 3. Results from Paired *t* Tests Testing Differences in Plasma Concentrations of Phosphatidylcholines Between Age, Sex, and Height-Matched Cases (low muscle quality) and Controls (high muscle quality)

Metabolite	Difference (case–control)	*p* Value
Lysophosphatidylcholine acyl C16:0	−4.59	.365
Lysophosphatidylcholine acyl C16:1	−0.33	.038
Lysophosphatidylcholine acyl C17:0	−0.18	.129
Lysophosphatidylcholine acyl C18:0	−1.79	.339
Lysophosphatidylcholine acyl C18:1	−2.92	.026[#]
Lysophosphatidylcholine acyl C18:2	−4.17	.075
Lysophosphatidylcholine acyl C20:3	−0.18	.262
Lysophosphatidylcholine acyl C20:4	−0.76	.182
Lysophosphatidylcholine acyl C28:1	−0.01	.335
Phosphatidylcholine diacyl C28:1	0.06	.629
Phosphatidylcholine diacyl C30:0	−0.32	.101
Phosphatidylcholine diacyl C30:2	−0.005	.898
Phosphatidylcholine diacyl C32:0	−0.17	.814
Phosphatidylcholine diacyl C32:1	−1.85	.134
Phosphatidylcholine diacyl C32:2	−0.70	.031
Phosphatidylcholine diacyl C32:3	−0.06	.137
Phosphatidylcholine diacyl C34:1	−8.60	.302
Phosphatidylcholine diacyl C34:2	0.49	.979
Phosphatidylcholine diacyl C34:3	−2.12	.025
Phosphatidylcholine diacyl C34:4	−0.26	.057
Phosphatidylcholine diacyl C36:0	0.05	.793
Phosphatidylcholine diacyl C36:1	−0.43	.800
Phosphatidylcholine diacyl C36:2	−1.01	.922
Phosphatidylcholine diacyl C36:3	−3.95	.505
Phosphatidylcholine diacyl C36:4	−6.42	.532
Phosphatidylcholine diacyl C36:5	−1.71	.540
Phosphatidylcholine diacyl C36:6	−0.09	.242
Phosphatidylcholine diacyl C38:0	0.11	.508
Phosphatidylcholine diacyl C38:1	−0.07	.532
Phosphatidylcholine diacyl C38:3	1.95	.262
Phosphatidylcholine diacyl C38:4	0.48	.942
Phosphatidylcholine diacyl C38:5	−1.62	.540
Phosphatidylcholine diacyl C38:6	3.89	.448
Phosphatidylcholine diacyl C40:2	−0.09	.101
Phosphatidylcholine diacyl C40:3	−0.08	.06
Phosphatidylcholine diacyl C40:4	0.01	.959
Phosphatidylcholine diacyl C40:5	0.34	.442
Phosphatidylcholine diacyl C40:6	2.47	.138
Phosphatidylcholine diacyl C42:0	−0.03	.275
Phosphatidylcholine diacyl C42:1	−0.005	.715
Phosphatidylcholine diacyl C42:2	−0.01	.403
Phosphatidylcholine diacyl C42:4	−0.03	.055
Phosphatidylcholine diacyl C42:5	−0.01	.614
Phosphatidylcholine diacyl C42:6	0.01	.637
Phosphatidylcholine acyl-alkyl C30:0	−0.02	.248
Phosphatidylcholine acyl-alkyl C30:2	−0.006	.330
Phosphatidylcholine acyl-alkyl C32:1	−0.05	.647
Phosphatidylcholine acyl-alkyl C32:2	−0.04	.282
Phosphatidylcholine acyl-alkyl C34:0	−0.07	.335
Phosphatidylcholine acyl-alkyl C34:1	−0.74	.052
Phosphatidylcholine acyl-alkyl C34:2	−1.00	.099
Phosphatidylcholine acyl-alkyl C34:3	−0.75	.157
Phosphatidylcholine acyl-alkyl C36:0	−0.01	.715
Phosphatidylcholine acyl-alkyl C36:1	−0.74	.064
Phosphatidylcholine acyl-alkyl C36:2	−1.34	.052
Phosphatidylcholine acyl-alkyl C36:3	−0.51	.175
Phosphatidylcholine acyl-alkyl C36:4	0.19	.834
Phosphatidylcholine acyl-alkyl C36:5	−0.01	.989
Phosphatidylcholine acyl-alkyl C38:0	−0.08	.581
Phosphatidylcholine acyl-alkyl C38:1	−0.45	.082
Phosphatidylcholine acyl-alkyl C38:2	−0.47	.064
Phosphatidylcholine acyl-alkyl C38:3	−0.75	.068
Phosphatidylcholine acyl-alkyl C38:4	−0.54	.369
Phosphatidylcholine acyl-alkyl C38:5	−0.22	.795
Phosphatidylcholine acyl-alkyl C38:6	0.18	.674
Phosphatidylcholine acyl-alkyl C40:1	−0.06	.361
Phosphatidylcholine acyl-alkyl C40:2	−0.25	.022
Phosphatidylcholine acyl-alkyl C40:3	−0.53	.053
Phosphatidylcholine acyl-alkyl C40:4	−0.36	.033
Phosphatidylcholine acyl-alkyl C40:5	−0.44	.046
Phosphatidylcholine acyl-alkyl C40:6	−0.04	.849
Phosphatidylcholine acyl-alkyl C42:1	−0.04	.074
Phosphatidylcholine acyl-alkyl C42:2	−0.04	.184
Phosphatidylcholine acyl-alkyl C42:3	−0.08	.024
Phosphatidylcholine acyl-alkyl C42:4	−0.09	.033
Phosphatidylcholine acyl-alkyl C42:5	−0.19	.054
Phosphatidylcholine acyl-alkyl C44:3	−0.01	.076
Phosphatidylcholine acyl-alkyl C44:4	−0.03	.010
Phosphatidylcholine acyl-alkyl C44:5	−0.07	.256
Phosphatidylcholine acyl-alkyl C44:6	−0.05	.385
Hydroxysphingomyelin C16:1	0.04	.840
Hydroxysphingomyelin C22:1	0.01	.974
Hydroxysphingomyelin C22:2	0.06	.855
Hydroxysphingomyelin C24:1	0.11	.680
Sphingomyelin C16:0	−0.87	.820
Sphingomyelin C16:1	0.06	.917
Sphingomyelin C18:0	0.83	.292
Sphingomyelin C18:1	0.19	.671
Sphingomyelin C20:2	−0.05	.241
Sphingomyelin C22:3	−0.46	.292
Sphingomyelin C24:0	0.57	.398
Sphingomyelin C24:1	−0.13	.920
Sphingomyelin C26:0	0.002	.721
Sphingomyelin C26:1	−0.01	.572

[#]*p* values after log transformation.

significance is unclear (36). The activity of TDO determines the amount of dietary tryptophan from the portal to the systemic circulation and is the primary enzyme responsible for tryptophan degradation (>95%), and has a Km an order of magnitude higher than IDO. While TDO is specific to l-tryptophan, IDO1 metabolizes serotonin to formyl-5-hydroxykynuramine (34), in addition to metabolizing tryptophan to kynurenine. The kynurenine/tryptophan are lower in participants with low muscle quality compared to controls, however, no difference was observed in the case of the serotonin/tryptophan levels, suggesting that a dysfunction of TDO is responsible for the increased levels of tryptophan and serotonin. Further, IDO1 unlike TDO, is activated by proinflammatory cytokines (IL-1β, IL-6, IFN-α, TNF-α) (34,37) and inhibited by anti-inflammatory cytokines (IL-4 and IL-10). However, in our study no correlation was seen with the decrease in tryptophan metabolism and the levels of IL-6, as a result, a dysfunction of TDO is most likely responsible for the increased levels of tryptophan and serotonin.

The polyamine metabolic pathway was also assessed by studying the concentration of putrescine, spermidine, and spermine. These biogenic amines are involved in many functions, including cell growth, cell survival, and proliferation. There has been increased

interest in recent years to elucidate the function of polyamines and their physiological effects. While, higher levels have been reported in cancer cells, more recently, it was demonstrated that polyamines may play a role in longevity (37–40). In fact, polyamine levels have been shown to decline with age in many organisms (41). Further, muscle mass was strongly associated with polyamine concentrations in rodents (42). The polyamine pathway has recently been implicated in the effects of growth hormone and the androgen receptor on skeletal muscle hypertrophy (42). The biosynthetic pathway for polyamines begins with the production of ornithine from arginine. In our study, no difference was observed between cases and controls for arginine or ornithine, however participants with lower muscle quality had significantly lower levels of putrescine. Putrescine is produced from the decarboxylation of ornithine via ornithine decarboxylase (ODC). Consequently, the activity of ODC was lower in participants with low muscle quality compared to controls, even though the difference between cases and controls did not reach statistical significance. This is consistent with previous studies demonstrating that higher levels of polyamines correlated with reduced inflammation and greater longevity (43,44). No other association between any of the amino acids and biogenic amines was found.

Lipids

Phosphatidyl cholines (PCs) are the major phospholipid in cell membranes and PCs containing the two fatty acid chains (C16-C20) are the major component of the lipoproteins. In our study, participants with low muscle quality presented significantly lower concentrations of PC diacyl (aa) C32:2, C34:3, C34:4 and C42:4, PC acyl-alkyl (ae) C34:1, C38:1, C38:2 C38:3, C40:2, C40:3, C40:4, C40:5, C42:1, C42:3, C42:4, C42:5, C44:3, C44:4 (Table 3). The decreased levels of PC in patients with lower muscle quality may result from the dysfunctional mitochondria that has been shown to play a role in muscle function decline (45). Mitsuhashi *et al.* (46), demonstrated that decreased mitochondrial function resulted in decreased PC levels in mice, and conversely, the muscle mitochondrial dysfunction in patients with lower muscle quality may be responsible for the observed decreases in PC, however, further studies are needed for confirmation. PCs are also converted to lysophosphatidylcholines via phospholipase A2. In this study, there was also a decrease in lysoPC acyl (a) C16:1, C18:1 and C18:2 in patients with lower muscle quality relative to control. While, typically lysoPCs can induce proinflammatory signaling it is currently believed that the resulting decrease in lysoPCs is a direct result of the decrease of PC levels.

Conclusions

In spite of substantial research in model organisms and humans, the mechanisms that lead to decline in muscle mass, strength, and quality with aging are still unknown. Searching for clues on putative pathways that could be targeted for prevention and cure of sarcopenia, we performed a metabolomics analysis on plasma samples collected in 79 pairs of BLSA participants with low muscle quality and high matched for age, sex, and height. The data from this study indicate that the concurrent increase in leucine, isoleucine, tryptophan, serotonin, and methionine with a concomitant decrease in putrescine and the selected PCs and lysoPCs may be indicative a decreasing muscle quality. If replicated in an independent population, these findings indicate where effort for future research in this field should be directed.

This study has both limitations and strengths. The most important limitation is the small sample size that did not allow the partition of the sample in a discovery set and a validation set. Another limitation is that no Bonferroni adjustment to the p-value was carried out, as this is a preliminary study. Therefore, our findings should be replicated in an independent sample and the hypothesis that metabolic signatures can be identified as risk factors for the development of age-related sarcopenia should be tested in a longitudinal design. Main strengths of this study are the high quality and standardization of the phenotype, the matched design and the nature of the results that harmonically point to specific pathways that have face validity as potential pathogenic mechanisms of sarcopenia.

Supplementary Material

Please visit the article online at http://gerontologist.oxfordjournals.org/ to view supplementary material.

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