Research Article

Metabolites Associated with Walking Ability Among the Oldest Old from the CHS All Stars Study

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Abstract

Background: Low walking ability is highly prevalent with advancing age and predicts major health outcomes. Metabolomics may help to better characterize differences in walking ability among older adults, providing insight into potentially altered molecular processes underlying age-related decline in functioning. We sought to identify metabolites and metabolic pathways associated with high versus low walking ability among 120 participants ages 79–95 from the CHS All Stars study.

Methods: Using a nested case–control design, 60 randomly selected participants with low walking ability were matched one-to-one on age, gender, race, and fasting time with 60 participants with high walking ability. High versus low walking ability was defined as being in the best versus worst tertiles for both gait speed (≥ 0.9 vs < 0.7 m/s) and the Walking Ability Index (7–9 vs 0–1). Using liquid chromatography-mass spectrometry, 569 metabolites were identified in overnight-fasting plasma.

Results: Ninety-six metabolites were associated with walking ability, where 24% were triacylglycerols. Triacylglycerols that were higher among those with high walking ability consisted mostly of polyunsaturated fatty acids, whereas triacylglycerols that were lower among those with high walking ability consisted mostly of saturated or monounsaturated fatty acids. Body composition partly explained associations between some metabolites and walking ability. Proline and arginine metabolism was a top pathway associated with walking ability.

Conclusion: These results may partly reflect pathways of modifiable risk factors, including excess dietary lipids and lack of physical activity, contributing to obesity and further alterations in metabolic pathways that lead to age-related decline in walking ability in this older adult cohort.

Keywords: Metabolomics, Gait speed, Lipids, Triacylglycerols

Currently, almost all ($\approx 92\%$) U.S. older adults ages ≥ 65 have a gait speed < 1.0 m/s (1), a clinically relevant threshold for detecting older adults at risk for multiple major health outcomes (2). Gait speed is a marker of overall health and well-being, where a decline may be a manifestation of accumulating chronic conditions and adverse age-related changes (2). Metabolomics, the large-scale study of endogenous and exogenous metabolites (3), may help to further our

understanding of the biology underlying aging-related variation in walking ability. Identifying metabolites associated with walking ability among older adults may facilitate the identification of potentially altered metabolic pathways to intervene on at earlier points in pathogenesis to prevent disability and promote healthy aging.

Few studies have examined metabolomics of gait speed, one identifying mostly glycerophospholipids and phosphosphingolipids

(4) and another identifying salicylurate and 2-hydroxyglutarate (5) as correlated with gait speed. In this report, we sampled older adults from the Cardiovascular Health Study (CHS) All Stars study who were in the extremes of walking ability to provide more power to detect metabolic differences by physical functioning. The CHS All Stars study was a well-characterized cohort of older adults (median age: 85 years) alive at year 18 of the original CHS (6). Examining metabolomics of walking ability in such a cohort of long-lived participants may indicate metabolic pathways involved in healthy aging and longevity. Thus, the aims of this report were to identify metabolites and metabolic pathways associated with walking ability extremes using a nested case-control study of 120 CHS All Stars ages 79–95 years with high versus low walking ability, matched one-to-one on age, gender, race, and fasting time. We hypothesized a metabolomic signature more reflective of healthy aging would be associated with high versus low walking ability.

Methods

Cohort

The CHS All Stars study was an ancillary study of 1,862 participants alive at year 18 of the CHS (6). The CHS was a multicenter prospective longitudinal cohort of 5,201 mostly white participants recruited during 1988–1989 and 687 mostly black participants recruited during 1992–1993 (7). Participants had annual examinations until 1999 and semiannual telephone interviews until 2016. The CHS All Stars study held an additional in-person clinic or home examination for surviving CHS participants during 2005–2006 and was designed to examine healthy aging and longevity. Both the CHS and the CHS All Stars study were approved by the Human Research Protection Office at each participating university and all participants provided informed consent.

For this report, we designed a nested case-control study of 120 CHS All Stars. Figure 1 includes the breakdown of eligible participants. Sixty participants with low walking ability were randomly selected and matched one-to-one on age (± 1 year), gender, race, and fasting time (± 1 hour) to a participant with high walking ability. During the CHS All Stars examination, plasma samples were collected after an overnight fast of ≥ 8 hours and were stored at -80°C from the time of collection (2005–2006) until 2018 when metabolites were measured. There were no differences in time of day plasma

was collected or time between sample storage and metabolomics by matched pairs.

Metabolites

Plasma aliquots were organized in a random, specified order prior to assay with a sample from a participant with high walking ability and their low walking ability pair in every other position to limit confounding by batch effects. Four complimentary liquid chromatography-mass spectrometry (LC-MS) methods were used to measure: (i) amines and polar metabolites (eg, amino acids), (ii) central carbon metabolites and polar metabolites (eg, purine and pyrimidines), (iii) lipids (eg, triglycerides), and (iv) metabolites of intermediate polarity (eg, fatty acids). Metabolite values were LC-MS peak areas (8). More detail is available in the [Supplementary Material](#).

Among the 605 known metabolites identified, 497 (82%) were detected in all participants and 72 (12%) contained missing values, but were detected in at least 80% of participants. Missing values were assumed to be due to true values being below detectable limits and for analytic purposes, were replaced with half the minimum recorded value for the respective metabolite (9). Thirty-six (6%) metabolites were excluded because they were measured in less than 80% of the participants (10).

Walking Ability

Walking ability was defined using tertiles of gait speed and the Walking Ability Index (11), which is the sum of self-reported level of difficulty or ease walking $\frac{1}{2}$ mile and walking one mile ([Supplementary Table S1](#)). Walking Ability Index scores were ranked into tertiles based on all participants with an in-person visit ($n = 1,077$); the worst, middle, and best tertile ranged from 0 to 1, 2 to 6, and 7 to 9, respectively. Fifteen feet gait speed was ranked into tertiles using information from all participants ($n = 981$). The slowest, middle, and fastest tertiles were <0.7 , ≥ 0.7 to <0.9 , and $\geq 0.9\text{ m/s}$, respectively.

High walking ability was defined as gait speed in the fastest tertile ($\geq 0.9\text{ m/s}$) and a Walking Ability Index score in the best tertile (scores 7–9). Similarly, low walking ability was defined as gait speed in the slowest tertile ($<0.7\text{ m/s}$) and a Walking Ability Index score in the worst tertile (scores 0–1). Gait speed provided an objective measure of walking ability across 15 feet and the Walking Ability Index provided self-reported information on more strenuous activities: walking $\frac{1}{2}$ mile and walking one mile. Tertiles of gait speed were used instead of established thresholds because the majority (64%) of participants (ages 77–102) walked $<0.8\text{ m/s}$.

Examination

Participants self-reported demographics, education, smoking status, alcohol consumption, and difficulty with activities of daily living. History or presence of heart disease (myocardial infarction or congestive heart failure), stroke (including transient ischemic attack), cancer (excluding non-melanoma skin cancer), arthritis (of the back, hip, or knee), asthma, chronic bronchitis, and emphysema was determined by self-reported physician diagnosis. Hypertension was defined as systolic blood pressure $\geq 140\text{ mmHg}$, diastolic blood pressure $\geq 90\text{ mmHg}$, or taking antihypertensive medication. Diabetes was defined as self-reported physician's diagnosis confirmed by medication use or fasting glucose $\geq 126\text{ mg/dL}$. Participants brought in all medications used in the past 2 weeks for a medication inventory. Waist circumference and body mass index were measured.

Figure 1. Flow chart of CHS All Stars eligible for our nested case-control study examining metabolites associated with walking ability extremes.

Participants were instructed to fast for ≥ 8 hours and self-reported fasting time. Plasma cholesterol and triglycerides and serum glucose, interleukin-6, and creatinine were measured by the CHS central laboratory. Dietary information was assessed at year 8 (1995–1996) using an interviewer-administered food frequency questionnaire developed by the Willet Group at the Harvard School of Medicine.

Statistical Analysis

Mean (standard deviation) or frequency (percent) described differences by walking ability and were tested using paired *t* tests for continuous measures and McNemar's tests for categorical measures. Metabolite peak areas were log-transformed and standardized. Paired *t*-tests were used to adjust for the matched design and determine metabolites associated with high versus low walking ability ($p < .05$). To account for multiple comparisons, a Benjamini-Hochberg correction was used (12) with 30% false discovery rate since this was a hypothesis-generating report (10).

Metabolites associated with walking ability were examined in a pathway analysis using MetaboAnalyst (13), which compared the metabolites associated with walking ability against the metabolites involved in metabolic pathways. Fisher's Exact Tests determined whether the number of metabolites associated with walking ability and involved in a pathway was more than expected by chance. Pathway impact scores range from 0 to 1 and indicate how centrally located walking ability-associated metabolites are on a pathway; scores of zero versus one indicate metabolites associated with the phenotype account for none versus all of the pathway importance, respectively.

We hypothesized more commonly measured risk factors, eg body mass index, may be upstream factors causing differences in metabolites that then contribute to aging-related walking ability differences. Though, it should be noted that this is a cross-sectional study, so mediation and temporality cannot be determined. We informally examined whether the association between body mass index and walking ability was attenuated after adjusting for a metabolite using the following three steps: (i) determined whether body mass index was associated with walking ability ($p < .05$) using conditional logistic regression; (ii) determined which of the walking ability-associated metabolites were also associated with body mass index ($p < .05$) using random intercept models; and (iii) calculated percent attenuation in the association between body mass index and walking ability after adjusting for a metabolite using conditional logistic regression. Percent attenuation was calculated as: $100 \times (b_1 - b_2) / b_1$, where b_1 was the coefficient of interest from an unadjusted model and b_2 was the same coefficient after further adjustment. We also examined the extent to which body mass index attenuated the association between a metabolite and walking ability. Percent attenuations in associations between metabolites and walking ability after adjusting for body mass index (*y*-axis) was plotted against percent attenuations in the association between body mass index and walking ability after adjusting for the respective metabolite (*x*-axis), which allows one to visualize which attenuation is greater.

Last, we examined the amount of attenuation in the association between body mass index and walking ability extremes after adjusting for multiple metabolites. The metabolites included in the multivariable model were determined by the following steps. Among the set of metabolites that were associated with both body mass index and walking ability extremes, we first identified highly related

metabolites ($|\text{Spearman correlation}| \geq 0.70$) and among those highly related metabolites, we only included the metabolite that was most strongly associated with walking ability. Next, we applied a stepwise selection approach using a conditional logistic regression model of walking ability extremes on the remaining metabolites, we used *p*-values of .10 as the criteria for metabolites to enter the model and to remain in the model. Once we obtained a final set of metabolites, we then added body mass index to the model to determine whether adjusting for multiple metabolites attenuated its association with walking ability. The same steps were repeated for the following variables that differed significantly by walking ability extremes and were thought to be upstream factors of metabolite differences by walking ability extremes: waist circumference, arthritis, number of prescription medications, and proinflammatory cytokine, interleukin-6.

Results

Participants were 85 years old, on average, 40% were men, and 10% were black. Consistent with the matched design, we found no differences in age, gender, race, or fasting time by walking ability (Table 1). Participants with high versus low walking ability were more likely to have more than a high school education; had lower average body mass index, waist circumference, fasting glucose, and interleukin-6 levels; and were less likely to have had a stroke, asthma, arthritis, and difficulty with ≥ 1 activity of daily living.

Among the 569 metabolites, 96 were associated with walking ability (Supplementary Table S2). Forty-five metabolites were lower and 51 were higher, on average, among those with high walking ability (Figure 2). Supplementary Table S3 includes the Human Metabolome Database taxonomy classifications of the 96 metabolites; slightly more than half were lipids and lipid-like molecules (eg, glycerolipids and glycerophospholipids), specifically 23 metabolites were triacylglycerols. There were more triacylglycerols associated with walking ability than expected by chance (Fisher's exact $p = .001$). Remaining metabolites associated with walking ability were mostly organic acids and derivatives (mostly amino acids) and organoheterocyclic compounds (mostly purines and purine derivatives).

Among the 96 metabolites associated with walking ability, 88 had an identification number in the Human Metabolome Database version 4.0 (13) and were included in a pathway analysis. Table 2 includes the top ten pathways among 28 that involved at least one metabolite associated with walking ability. Most significant pathways with the largest impact scores were caffeine metabolism and arginine and proline metabolism. The match status for caffeine metabolism was 4/21; meaning 21 known metabolites are involved in caffeine metabolism, of which four were associated with walking ability (caffeine, 3-methylxanthine, 7-methylxanthine, and theophylline; Supplementary Table S4). The match status was 6/77 for arginine and proline metabolism (ornithine, L-arginine, L-proline, N-acetylputrescine, 4-acetamidobutanoic acid, and sarcosine; Supplementary Table S4).

Body mass index, waist circumference, arthritis, interleukin-6, and number of prescription medications were significantly associated with walking ability (Table 1). Among the 96 metabolites associated with walking ability, 32, 40, 14, 9, and 32 were also associated with body mass index, waist circumference, arthritis, number of prescription medications, and interleukin-6, respectively (Supplementary Table S5).

Table 1. Characteristics of 120 Randomly Selected CHS All Stars by Walking Ability, Matched One-to-One on Age, Gender, Race, and Fasting Time

Mean (SD) or Frequency (percent)	High Walking Ability <i>n</i> = 60	Low Walking Ability <i>n</i> = 60	Paired Test <i>p</i> value
Matching variables:			
Age	85 (2.9)	85 (2.9)	.27
Men	24 (40%)	24 (40%)	.99
Black race	6 (10%)	6 (10%)	.99
Number of hours since last meal	14 (1.6)	14 (1.7)	.17
Personal history:			
Clinic site:			
Wake Forest University School of Medicine	19 (32%)	7 (12%)	.02
University of California, Davis	21 (35%)	30 (50%)	
Johns Hopkins University	3 (5%)	10 (17%)	
University of Pittsburgh	17 (28%)	13 (22%)	
More than a high school education	32 (54%)	17 (28%)	.01
Never smoked cigarettes	43 (72%)	34 (57%)	.10
Weekly alcohol consumption	1.8 (3.6)	0.7 (2.4)	.03
Body mass index (kg/m ²)	25 (3.2)	28 (5.6)	<.0001
Waist circumference (cm)	92 (11)	103 (15)	<.0001
Chronic conditions:			
Heart disease	10 (17%)	18 (30%)	.08
Stroke	5 (8%)	14 (23%)	.04
Hypertension	48 (80%)	51 (85%)	.49
Diabetes	5 (8%)	12 (20%)	.07
Cancer	17 (28%)	16 (27%)	.82
Asthma	3 (5%)	14 (23%)	.01
Emphysema or chronic bronchitis	6 (10%)	13 (22%)	.10
Arthritis	17 (28%)	40 (67%)	.0005
Total number of prescription medications	7.7 (3.3)	10.1 (5.4)	.01
Difficulty with ≥1 Activities of Daily Living	2 (4%)	21 (40%)	.005
Blood-based markers:			
Total cholesterol (mg/dL)	182 (36)	186 (40)	.57
High-density lipoprotein cholesterol (mg/dL)	58 (16)	56 (16)	.50
Low-density lipoprotein cholesterol (mg/dL)	99.9 (30)	103 (31)	.69
Triglycerides (mg/dL)	120 (65)	131 (75)	.39
Fasting glucose (mg/dL)	96 (17)	106 (37)	.04
Interleukin-6 (pg/mL)	3.2 (2.2)	5.0 (2.9)	.0003
Creatinine (mg/dL)	1.1 (0.4)	1.2 (1.0)	.72
Dietary intake per day at year 8			
Calories (kcal)	1909 (604)	2103 (834)	.15
Calories from fat (kcal)	1898 (605)	2092 (835)	.15
Protein (g)	80 (28)	83 (30)	.66
Caffeine (mg)	140 (159)	214 (225)	.09

Body Mass Index

One standard deviation (=4.8 kg/m²) higher body mass index was associated with 65% lower odds of high walking ability (95% confidence interval [CI]: 0.19, 0.67). When adjusting for one of the 32 metabolites (Supplementary Table S6), the association between body mass index and walking ability was attenuated by ≤24% (Figure 3a). Whereas associations between metabolites and walking ability after adjusting for body mass index were attenuated by ≥40% for 18 of the 32 metabolites. Adjusting for multiple select metabolites in a single model (proline, cinnamoylglycine, imidazole propionate, 1-methylguanine, and cys-gly-oxidized) attenuated the association between body mass index and walking ability by 46% (Supplementary Table S6).

Waist Circumference

One standard deviation (=14.3 cm) higher waist circumference was associated with 66% lower odds of high walking ability

(95% CI: 0.18, 0.64). When adjusting for one of the 40 metabolites (Supplementary Table S7), the association between waist circumference and walking ability was attenuated by ≤26%, whereas attenuations in associations between metabolites and walking ability after adjusting for waist circumference were attenuated by ≥40% for 18 metabolites (Figure 3b). Adjusting for multiple select metabolites in a single model (proline, 1-methylguanine, 3-methylxanthine, dimethylurate, and serotonin) did not attenuate the association between waist circumference and walking ability (Supplementary Table S7).

Arthritis

Participants with arthritis had 77% lower odds of high walking ability (95% CI: 0.10, 0.53). When adjusting for one of the 14 metabolites (Supplementary Table S8), the association between arthritis and walking ability was attenuated by ≤13%, whereas attenuations in associations between metabolites and walking ability after

adjusting for arthritis were $\geq 25\%$ for seven metabolites (Figure 3c). Adjusting for multiple select metabolites in a single model (bilirubin, imidazole propionate, ornithine, and C36:3 PS plasmalogen) attenuated the association between arthritis and walking ability by 38% (Supplementary Table S8).

Medications

One standard deviation higher (≈ 4.6) number of prescription medications was associated with 37% lower odds of high walking ability (95% CI: 0.42, 0.93). Adjusting for one of the 9 metabolites (Supplementary Table S9) resulted in attenuations ranging from 10%–21% of the association between number of medications and walking ability (Figure 3d). Adjusting for one metabolite, glycerate, resulted in a reverse attenuation of 66%. Adjusting for total number of medications resulted in similar attenuations in

associations between metabolites and walking ability, ranging from 6%–29%, with reverse attenuation of 75% for glycerate. Adjusting for multiple select metabolites in a single model (3-methylxanthine, C34:5 PC plasmalogen, and glycerate) did not attenuate the association between total number of medications and walking ability (Supplementary Table S9).

Interleukin-6

One standard deviation higher log-transformed interleukin-6 was associated with 57% lower odds of high walking ability (95% CI: 0.24, 0.75). Adjusting for one of the 32 metabolites (Supplementary Table S10) attenuated the association between interleukin-6 and walking ability by $\leq 26\%$, whereas adjusting for interleukin-6 attenuated associations between metabolites and walking ability by $\geq 40\%$ for 10 metabolites (Figure 3e). Adjusting for multiple select metabolites in a single model (C58:11 triacylglycerol, proline, and 1-methylguanine) attenuated the association between interleukin-6 and walking ability by 41% (Supplementary Table S10).

Discussion

Differences in patterns of 96 plasma metabolites were observed by walking ability among 120 CHS All Stars ages 79–95 using a nested case–control design, matched on age, gender, race, and fasting time. Arginine and proline metabolism was a top pathway associated with walking ability. Body mass index, waist circumference, arthritis, and interleukin-6 only partly explained associations between a subset of metabolites and walking ability; though associations between 35 of the metabolites and walking ability extremes were not attenuated by these more commonly measured variables (attenuations $< 10\%$).

There was little overlap in metabolites associated with walking ability when compared to previous publications. Among adults ages ≥ 50 from the Baltimore Longitudinal Study of Aging, only eight metabolites, out of 148, were associated with gait speed (false discovery rate < 0.05) adjusting for age and gender (4). All eight were lipids and lipid-like molecules, mostly sphingolipids and glycerophospholipids, of which three sphingolipids were also associated with walking ability in this report (C18:0, C18:1, and C16:0). Among black men from the Health, Aging, and Body Composition (Health ABC) study (median age: 74), seven metabolites, out of 350, were associated with gait speed ($p \leq .01$), adjusting for age, site, smoking, and weight change (5), of which none were associated with walking ability among the CHS All Stars. Minimal overlap in metabolites associated with walking ability could be due to differences in LC-MS methodology with fewer metabolites measured in past reports and due to cohort differences in functional status and age. Average gait speed was 0.5 versus 1.0 m/s

Figure 2. Volcano plot of significance versus mean standardized paired difference in the association between 96 metabolites and walking ability among 120 CHS All Stars. Full color version is available within the online issue.

Table 2. Top Pathways Involving ≥ 1 Metabolite(s) Associated with Walking Ability Among 120 CHS All Stars

Pathway name	Match Status	p Value	False Discovery Rate	Impact
Caffeine metabolism	4/21	.0003	0.02	0.38
Arginine and proline metabolism	6/77	.001	0.05	0.37
D-Arginine and D-ornithine metabolism	2/8	.007	0.18	0
Glycerophospholipid metabolism	3/39	.02	0.48	0.23
Glycerolipid metabolism	2/32	.09	1.00	0.07
Nicotinate and nicotinamide metabolism	2/44	.16	1.00	0.02
Phenylalanine metabolism	2/45	.16	1.00	0.04
Glycine, serine and threonine metabolism	2/48	.18	1.00	0.05
Glycosylphosphatidylinositol (GPI)-anchor biosynthesis	1/14	.20	1.00	0.04
Linoleic acid metabolism	1/15	.22	1.00	0

Figure 3. Percent attenuations in associations between select metabolites and walking ability after adjusting for a more commonly measured variable versus percent attenuations in association between the more commonly measured variable and walking ability after adjusting for the respective metabolite, organized by taxonomy class among 120 CHS All Stars. Full color version is available within the online issue.

for those with low versus high walking ability, respectively among the CHS All Stars, that is, even those with “high” walking ability did not actually walk “fast,” whereas previous reports were in healthier and younger older adult cohorts with faster average gait speeds (4,5), and were restricted by race and/or gender. We were also more likely to find significant associations since extremes of walking ability were sampled, providing more power to detect differences.

Half of the metabolites associated with walking ability were lipids and lipid-like molecules. Specifically, 23 were triacylglycerols, that is, glycerols bonded to three fatty acids. Interestingly, all 12 triacylglycerols that were higher among those with high walking ability consisted mostly of polyunsaturated fatty acids (eg, linoleic acid, alpha-linolenic acid, arachidonic acid, and docosahexaenoic acid), whereas all 11 triacylglycerols that were lower among those with high walking ability consisted mostly of saturated or monounsaturated fatty acids (eg, palmitic acid or oleic acid). Triacylglycerols containing polyunsaturated fatty acids were reported as inversely correlated with insulin resistance, waist circumference, and diabetes (14,15). Some polyunsaturated fatty acids have anti-inflammatory effects. For example, omega-3 fatty acids have reduced inflammation in animal models resulting in improved insulin sensitivity (16). The CHS All Stars with high walking ability also had higher levels of docosahexaenoate, an omega-3 fatty acid most often found in fish oil.

Differences in the direction of associations between triacylglycerols and walking ability could reflect underlying differences in diet and energy expenditure, though we cannot rule out differences being due to metabolism, including absorption, clearance, and endogenous production. Triacylglycerols composed primarily of polyunsaturated fatty acids may be a protective set of lipids against adverse aging-related metabolic changes, especially when paired with low levels of triacylglycerols containing mostly saturated or monounsaturated fatty acids.

Among the 23 triacylglycerols associated with walking ability, seven were positively associated with body mass index or waist circumference, of which all but one was also associated with body mass index among the Health ABC black men (17). Higher levels of all seven triacylglycerols were associated with low walking ability, where adjusting for body size attenuated the associations by $\geq 40\%$. Among the other 29 lipid/lipid-like molecules associated with walking ability, 12 were associated with body mass index or waist circumference in our cohort, where associations between those metabolites and walking ability were attenuated by 30%–60% after adjusting for body size. Thus, associations between select lipids and lipid-like molecules and walking ability were partly explained by differences in body composition. Though there remained 16 triacylglycerols and 19 other lipids and lipid-like molecules associated with walking ability, but not correlated with body mass index or waist circumference, suggesting a profile of lipids and lipid-like molecules related to walking ability independent of body size.

Triacylglycerols are used mainly to store fats, making them a major energy reservoir (18). In a healthy individual, dietary triacylglycerols are roughly equal to the amount used for energy (19). However, when there are more triacylglycerols than needed for energy expenditure, adipose tissue expands to store excess, contributing to obesity over time if the ratio is not altered (16). Triacylglycerol overload can cause adipocytes to secrete monocyte chemoattractant protein-1, which attracts macrophages that promote tumor necrosis factor- α , a protein involved in systemic inflammation (19). As a result, stored lipids in adipose tissue begin to break down and are released into circulation at an increased rate (16). Chronic high levels of circulating free fatty acids can cause storage elsewhere, for example, in myocytes contributing to insulin resistance and in the liver causing fatty liver disease. The triacylglycerols associated with low walking ability, which were partly explained by higher body mass index in this report, may be markers of adverse aging-related molecular changes due to body composition.

Obesity contributes to decline in walking ability through multiple pathways that can be broadly classified into two main mechanisms: (i) biomechanical burden of excess weight on lower extremities and (ii) biochemical differences (eg, higher levels of circulating proinflammatory cytokines and free fatty acids) that have adverse effects on metabolism (20). The latter mechanism involving a potential causal pathway of worse body composition contributing to altered metabolites and pathways that contribute to lower walking ability. Here, we informally examined whether a metabolite was a mediator of the relationship between body size and walking ability, but found the association between body size and walking ability was minimally attenuated by any single metabolite (attenuations $\leq 26\%$). This is likely because body mass index and waist circumference are more global measures impacted by multiple factors when compared to a single metabolite. Since body composition likely impacts multiple metabolites simultaneously with differential effects depending on amount of lean versus fat mass, longitudinal studies with detailed

body composition measures need to assess the true mediation of multiple metabolites, simultaneously, by having enough power to include all relevant metabolites in the same model or by computing metabolite composite scores to determine the direct and indirect effects between body composition and walking ability.

Proline, an amino acid that can be synthesized from glutamate, was the most strongly associated metabolite with walking ability. One standard deviation higher proline level was associated with 3.5 times the odds of low walking ability. Higher proline levels have been associated with abnormal fasting glucose among adults ages 50+ from the Baltimore Longitudinal Study of Aging (21), reported among patients with Alzheimer's disease when compared to controls (22), and with sarcopenia among an older Japanese cohort (23). Similar to our report, arginine and proline metabolism was also a top pathway that included more of the metabolites associated with muscle mass and strength than expected by chance (driven by glutamate and aspartate) among a younger cohort of White women (24). Other reports have also identified amino acids as top metabolites associated with sarcopenia or frailty-related phenotypes, though these other reports did not observe a significant association with proline specifically (9,25,26). Here, we observed six metabolites associated with walking ability and involved in arginine and proline metabolism, where all, but one (arginine), were higher in those with low walking ability. Conditions causing high circulating lactate levels have been suggested as contributing to high proline levels since lactate inhibits the breakdown of proline (27). Consistent with this, higher levels of both proline and lactate were associated with low walking ability in our study.

A limitation of this report was the unit-less LC-MS peak areas for metabolite values. Concentrations of metabolites may have made it possible to assess whether values were outside a healthy range, though a healthy range for many metabolites is unknown. Another limitation was lack of dietary information at the time metabolites were measured. It should also be noted that 65% of participants in the "high" walking ability group had a gait speed below 1.0 m/s, a clinically relevant threshold for predicting major health outcomes (1,2). Thus, in this report of community-dwelling adults ages 79–95 years, we are comparing those who walk slowly versus those who walk extremely slow. Strengths included our well-characterized cohort of community-dwelling older adults, carefully collected and stored plasma samples that had never been thawed, the use of four LC-MS profiling platforms to cover a wide range of metabolites, and sampling extremes allowing for more variability and power to detect differences, while matching on important confounders.

Several metabolites, particularly lipids, were associated with high versus low walking ability using a nested case-control study of 120 CHS All Stars matched on age, gender, race, and fasting time. Associations between a subset of lipids and walking ability were partly explained by differences in body composition. Triacylglycerols consisting of mostly polyunsaturated fatty acids were positively associated with walking ability and appeared to be a protective set of lipids. The reproducibility and generalizability of these results need to be determined to understand whether differences in these metabolites truly characterize differences in walking ability among older adults. Understanding these molecular differences may provide insight into biologic mechanisms that possibly become altered with aging and disease that contribute to a decline in walking ability which can then be targeted in interventions to promote independence throughout life.

Supplementary Material

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

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Conflict of Interest

None reported.

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