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Original Article

Metabolites Associated With Lean Mass and Adiposity in Older Black Men

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Abstract

To identify biomarkers of body mass index, body fat, trunk fat, and appendicular lean mass, nontargeted metabolomics was performed in plasma from 319 black men in the Health, Aging and Body Composition study (median age 72 years, median body mass index 26.8 kg/m^2). Body mass index was calculated from measured height and weight; percent fat, percent trunk fat, and appendicular lean mass were measured with dual-energy x-ray absorptiometry. Pearson partial correlations between body composition measures and metabolites were adjusted for age, study site, and smoking. Out of 350 metabolites, body mass index, percent fat, percent trunk fat, and appendicular lean mass were significantly correlated with 92, 48, 96, and 43 metabolites at p less than .0014. Metabolites most strongly correlated with body composition included carnitine, a marker of fatty acid oxidation (positively correlated), triacylglycerols (positively correlated), and amino acids including branched-chain amino acids (positively correlated except for acetylglycine and serine). Gaussian Graphical Models of metabolites found that 25 lipid metabolites clustered into a single network. Groups of five amino acids, three plasmalogens, and two carnitines were also observed. Findings confirm prior reports of associations between amino acids, lean mass, and fat mass in addition to associations not previously reported. Future studies should consider whether these metabolites are relevant for metabolic disease processes.

Keywords: Body composition—Aging—Metabolomics—Obesity

Aging is generally accompanied by loss of lean mass with stable or increasing body fat (1,2). Substantive evidence has associated these changes in body composition with increased risk of mortality (3), disability (4,5), and numerous chronic diseases including diabetes (6) and cancer (7). Genetic, metabolic, neuroendocrine, and behavioral factors explain some but not all of the association between body composition and these health outcomes. Discovery of additional metabolic pathways related to body composition may provide better insight on underlying and potentially modifiable mechanisms.

Metabolomics is the study of small endogenous and exogenous molecules involved in cellular metabolism. Advances in metabolic profiling technologies have made it possible to simultaneously assay

hundreds of metabolites. Several studies have identified metabolites associated with body mass index (BMI) (8,9), lean mass (10–12), and fat mass (13), which has led to the discovery of amino acids as potentially important mediators of lean and fat mass as well as insulin resistance (9,13) and diabetes development (14,15). However, with few exceptions (10), these studies used targeted metabolomics which limits the number of metabolites examined (9,16), have had samples fewer than 100 individuals (11,12), and have predominately assessed white populations (14).

Metabolic profiles associated with body composition may be particularly important to study in at-risk populations. Black individuals have the highest prevalence of obesity of any ethnic group in the United States and a high burden of obesity-related chronic disease (17). With respect to muscle, black individuals have approximately 5%–8% more muscle than white individuals (18). This is reflected in estimates of sarcopenia, which are generally lower in older black individuals despite having a higher prevalence of functional limitations than white individuals (19). Examining metabolic biomarkers of body composition may provide insight into these seemingly contradictory observations. Black men are also a largely understudied population with respect to metabolomics.

This study explores metabolic correlates of lean mass and adiposity in older black men. We conducted a hypothesis-generating exploration of metabolites associated with BMI, percent fat, percent trunk fat, and appendicular lean mass (ALM) to provide insights on the influence of lean mass and adiposity on human metabolism. The overall goal is to identify perturbations of metabolism that may impact diseases and to guide future targeted studies of metabolic pathways.

Materials and Methods

Population

The Health, Aging and Body Composition (Health ABC) study is a prospective, longitudinal cohort study of 3,075 community-dwelling, initially well-functioning, black and white men and women aged 70–79 years. Participants were recruited from a random sample of white Medicare beneficiaries in selected zip codes and all black Medicare-eligible residents in the Memphis, Tennessee, and Pittsburgh, Pennsylvania areas. Eligibility criteria included: (i) no reported difficulty walking one quarter of a mile, climbing 10 steps without resting, or performing activities of daily living, (ii) no active treatment for cancer in the prior 3 years, (iii) intention to stay in the study area for at least 3 years, and (iv) no active participation in a lifestyle intervention trial.

Body Composition

Body weight was measured to the nearest 0.1 kg with a standard balance beam scale. Body height was measured to the nearest 0.1 cm using a wall-mounted stadiometer at the baseline study visit. Total body and regional measures (trunk, arms, and legs) of fat mass, bone-free lean mass, and bone mineral content were acquired using fan-beam dual-energy x-ray absorptiometry (Hologic QDR 4500A; Hologic, Bedford, MA). Trunk fat was determined using the standard Hologic procedure (20) with the bottom of the trunk region defined as the region on top of the iliac crests. ALM was calculated as the sum of bone-free lean mass in the arms and legs. To account for variation in overall body size, variables are expressed relative to total body mass as percent fat, percent trunk fat, and kg/m^2 for ALM as is standard practice (21–23).

The validity and reproducibility of the dual-energy x-ray absorptiometry scanner for assessing fat-free mass have been reported previously (24,25). The scanner in Pittsburgh overestimated total mass by approximately 2% compared with scale weight. The Hologic 4500A overestimated fat-free mass by approximately 5% compared with criterion methods (26). Data were corrected accordingly.

For this analysis, a random sample of 319 black men was selected from the Health ABC population of black men with stored plasma ethylenediaminetetraacetic acid at the 12-month study visit. The 12-month study visit was chosen as this coincided with dual-energy x-ray absorptiometry. Due to the modest size of this pilot study, men were selected as the population of interest to minimize heterogeneity since body composition is known to vary by gender. Body composition measures were missing for $N = 7$ (ALM), $N = 13$ (percent fat), and $N = 18$ (trunk fat), resulting in differing numbers of participants by measure. Ethylenediaminetetraacetic acid plasma samples were taken after an overnight fast (mean fast = 14 hours). Samples had not been previously thawed and were stored at −80°C until metabolite profiling.

Metabolite Profiling

A total of 350 metabolites were measured using the metabolite profiling platform of the Broad Institute of the Massachusetts Institute of Technology. Methodologies were similar to those described in Townsend and colleagues (27). In brief, three liquid chromatography–mass spectroscopy (LC-MS) methods were used to measure polar metabolites and lipids in plasma extracts. Lipids were analyzed using a Nexera X2 U-HPLC (Shimadzu, Marlborough, MA) coupled to an Exactive Plus orbitrap mass spectrometer (Thermo Fisher Scientific, Waltham, MA). Plasma extracts (2 µL) were injected onto a ACQUITY UPLC BEH C8 column (1.7 µm, 2.1 × 100 mm; Waters, Milford, MA). The column was initially eluted isocratically at a flow rate of 450 µL/min with 80% mobile phase A (95:5:0.1 vol/vol/vol 10 mM ammonium acetate/methanol/formic acid) for 1 minute followed by a linear gradient to 80% mobile phase B (99.9:0.1 vol/vol methanol/formic acid) over 2 minutes, a linear gradient to 100% mobile phase B over 7 minutes, then 3 minutes at 100% mobile phase B. MS analyses were carried out using electrospray ionization in the positive ion mode (source voltage was 3 kV, source temperature was 300°C, sheath gas was 50.0, auxiliary gas was 15) using full scan analysis over m/z 200–1,100 and at 70,000 resolution. Hydrophilic interaction liquid chromatography analyses of water-soluble metabolites were conducted in the positive ion mode using a Nexera X2 U-HPLC (Shimadzu)-Q Exactive orbitrap (Thermo Fisher Scientific) LC-MS instrument. Plasma samples (10 µL) were extracted using 90 µL of 74.9:24.9:0.2 vol/vol/vol acetonitrile/methanol/formic acid containing stable isotope-labeled internal standards (valine-d8, Isotec; and phenylalanine-d8, Cambridge Isotope Laboratories, Andover, MA). The samples were centrifuged (10 minutes, 9,000*g*, 4°C) and the supernatants were injected directly onto a 150 × 2 mm Atlantis hydrophilic interaction liquid chromatography column (Waters). The column was eluted isocratically at a flow rate of 250 µL/min with 5% mobile phase A (10 mM ammonium formate and 0.1% formic acid in water) for 1 minute followed by a linear gradient to 40% mobile phase B (acetonitrile with 0.1% formic acid) over 10 minutes. The electrospray ionization voltage was 3.5 kV and data were acquired using full scan analysis over m/z 70–800 at 70,000 resolution. Hydrophilic interaction liquid chromatography analyses of water-soluble metabolites in the negative ion mode were acquired using an ACQUITY UPLC (Waters) coupled with a 5500 QTRAP triple quadrupole MS (AB SCIEX) as described previously (27).

Progenesis CoMet (Nonlinear Dynamics) and TraceFinder 3.1 (ThermoFisher Scientific) software were used to integrate LC-MS peaks in data generated using orbitrap MS systems while MultiQuant 2.1 software (SCIEX) was used to automatically integrate 55000 QTRAP data and for manual review of data quality (28). Reference standards for each metabolite were used to determine chromatographic retention times. Metabolite peaks were compared against known standards to confirm identity. Metabolite signals were measured as LC-MS peak areas proportional to metabolite concentrations. Intraclass correlation coefficients were determined from 16-blinded duplicates (5% of 319). The median (interquartile range) intraclass correlation coefficient across 350 metabolites was 0.92 (0.81–0.97), indicating a high level of reliability.

Covariates

Covariates from the 12-month visit related to adiposity were selected a priori including age, education, study site, smoking status, prevalent disease (cancer, coronary heart disease, and type 2 diabetes), and self-reported physical activity. Prevalent disease was determined from a combination of self-reported physician diagnosis, medication use, and physiological measures. Serum cystatin C, a biomarker of kidney function that has been found to be a better predictor of glomerular filtration rate than creatinine (29), was included despite its measurement at baseline as data were not available at the 12-month visit. A BNII nephelometer (Dade Behring, Deerfield, IL) was used to measure cystatin C with a particle-enhanced immunonephelometric assay (N Latex Cystatin) (30).

Statistical Analysis

Metabolite values were skewed and thus were log transformed to approximate a normal distribution. Metabolites were grand mean centered to a mean of 0 and variance of 1: $\log(Xi) - \text{mean}(\log(Xi))/SD(\log(Xi))$ (31). Missing metabolites were assumed to be below the limit of detection and were imputed with half of the minimum value of the nonmissing metabolite. The median level of missingness before imputation across the 350 metabolites was less than 1%.

As this was a hypothesis-generating analysis, partial Pearson correlations between metabolites and body composition measures were minimally adjusted for study site, age, and smoking status (Model 1). To determine the robustness of correlations, additional adjustment for prevalent disease and cystatin C was performed (Model 2). Significance was determined at a Bonferroni adjusted alpha of p less than .00014 (.05/350 statistical tests).

To evaluate the potential independence of metabolic associations, we estimated the pairwise Pearson correlations between all metabolites significantly associated with each body composition measure. For the top-ranked metabolites—that is, those metabolites ranked in the top 25 for correlations with either ALM or trunk fat—we additionally evaluated pairwise correlations after conditioning on other top-ranked metabolites. These conditional correlations were then mapped using a Gaussian Graphical Model (GGM) approach (32). The GGM was visualized as nodes (metabolites) connected by edges representing direct conditional correlations of 0.3 or above. This GGM approach eliminates many indirect pairwise relationships from the model, allowing a parsimonious depiction of metabolite interrelationships. Prior tests of GGMs against known metabolic pathways have found that GGMs more accurately identify these pathways than do alternative approaches (32). To illustrate how distinct metabolic clusters relate to adiposity and lean phenotypes, we overlaid correlation results from the primary analysis for the top 10 ranked metabolites for each of ALM and trunk fat, in order.

Partial correlation analyses were performed with STATA version 14.0 (StataCorp, College Station, TX), and GGM analyses were conducted in R (R Project, version 3.1.2, Vienna, Austria) with GGM visualization using Cytoscape (33).

Results

Characteristics for the sample of 319 participants are shown in Table 1. The mean (*SD*) age of the sample was 74 (2.82) years. Participants predominantly had less than a high school education, were likely to have reported current or former smoking, had prevalent disease, and low levels of physical activity. The median BMI was overweight with the interquartile range spanning normal weight to obese BMI. The median percent fat was 28% and median percent trunk fat was 14%.

Absolute correlations between all 350 measured metabolites and (i) BMI, (ii) percent fat, (iii) percent trunk fat, and (iv) ALM are shown in age-, smoking-, and site-adjusted models (Model 1; Figure 1). Lipid and organic acid metabolites were the top correlated metabolites for each body composition measure. Correlations for metabolites statistically significantly associated with body composition measures are shown in Supplementary Tables S2–S5 and ranged from a minimum of $r = |.22|$ for all measures to $r = .37$ (valine) for BMI, $r = -.33$ (acetylglycine) for % fat, $r = .37$ (C52:3 triacylglycerol [TAG]) for % trunk fat, and $r = .39$ (valine) for ALM. The number of metabolites associated with each body composition measure by taxonomy subclass is presented in Table 2. Metabolites with strong associations that were generally common among the body composition measures were also observed and correspond to branched-chain amino acids (BCAAs), TAGs (e.g., C56:7, C56:8, C52:3), and a carnitine (C5). Correlations with additional adjustment for prevalent disease and cystatin C (Model 2) are shown in Supplementary Figure S1. In general, correlations were minimally attenuated. The mean absolute change in correlation for BMI, percent fat, percent trunk fat, and ALM were: 0.03, 0.02, 0.03, and 0.02, respectively.

Table 1. Characteristics of the Sample Population of Black Men ($N = 319$) at the 12-mo Health ABC Study Visit

Characteristic	Mean (*SD*) or *N* (%)
Age, y	72 (2.4)
Pittsburgh site	171 (53.6)
Education	
<High school	161 (50.6)
High school	72 (23.0)
>High school	84 (26.4)
Smoking status	
Never	99 (31.0)
Former	59 (18.5)
Current	161 (50.5)
Cancer	44 (13.8)
Diabetes	87 (27.3)
Hypertension	247 (77.4)
Coronary heart disease	81 (25.4)
Physical activity[a], kcal/kg/wk	6.61 (11.8)
Body composition measures[b]	Median (IQR)
Body mass index, kg/m^2	26.8 (23.8–30.0)
Total fat, %	28.2 (24.9–32.0)
Trunk fat, %	14.4 (11.8–16.7)
Appendicular lean mass, kg/m^2	8.24 (7.53–9.00)

Note: IQR = interquartile range.
[a]kcal/kg/wk walking and stairs. [b]Numbers vary due to missing values.

Metabolites Correlated With BMI

There were significant correlations between 92 metabolites and BMI in age, smoking, and site-adjusted models (Table 2). These were predominately lipids (N = 72) and organic acids (N = 11) including BCAAs (positively correlated) as well as two alkaloids (urate and a negative correlation with cotinine), three intermediates in tryptophan metabolism (3-hydroxyanthranilic acid, kynurenic acid, and xanthurenate), one organooxygen metabolite (hexose), and three unclassified metabolites. The top 10 ranked metabolites correlated with BMI included valine, C5 carnitine, 7 triglycerides, and acetylglycine.

Metabolites Correlated With Percent Fat

Statistically significant age-, smoking-, and site-adjusted associations between 48 metabolites and % fat were identified, including lipids (N = 38), and organic acids (N = 6) including 1 BCAA (valine), 1 organoheterocyclic compound, and unclassified metabolites (N = 3).

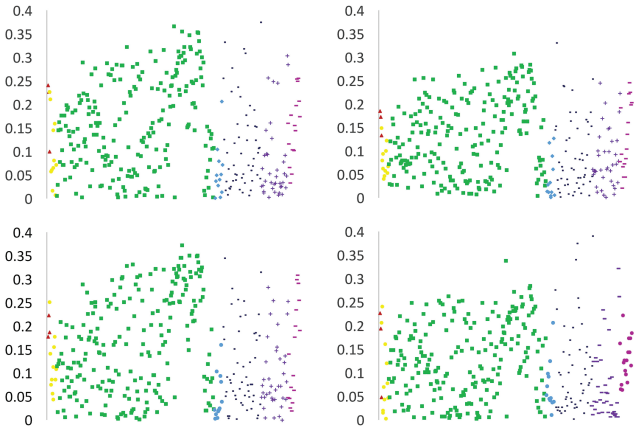

Figure 1. Each point represents the absolute Pearson partial correlation between a body composition measure and a metabolite in the Health ABC Study sample. Triangles (red online) represent alkaloids, circles (yellow online) are benzenoids, squares (green online) are lipids, diamonds (blue online) are nucleosides, short dashes (navy online) are organic acids, crosses (purple online) are organoheterocyclics, long dashes (magenta online) are unclassified. Correlations are adjusted for study site, age and smoking.

The top 10 ranked metabolites correlated with % fat included acetylglycine, 7 triglycerides, and 2 phosphatidylcholines.

Metabolites Correlated With Percent Trunk Fat

There were significant correlations between 96 metabolites with percent trunk fat in age-, smoking- and site-adjusted models. The majority of correlated metabolites were lipids (N = 75) and organic acids (N = 11) including the BCAAs. One alkaloid (urate), one benzenoid (3-hydroxyanthranilic acid), three organoheterocyclics (kynurenic acid, xanthurenate, and quinolate), and five unclassified metabolites were also correlated with percent trunk fat. Seven TAG, two diacylglycerols (DAGs), and acetylglycine were among the top metabolites correlated with percent trunk fat.

Metabolites Correlated With ALM

Significant age-, smoking- and site-adjusted associations between 53 metabolites and ALM were identified. Most metabolites were lipids (N = 39) and organic acids (N = 10) in addition to a negative correlation with the alkaloid cotinine and positive correlations with tryptophan metabolites (3-hydroxyanthranilic acid and xanthurenate) and one unclassified metabolite. BCAAs (valine, leucine, and isoleucine) were the top ALM-correlated metabolites. Phenylalanine and 2-aminoadipate were also among the top metabolites and were positively correlated with ALM as was a carnitine (C5), three TAG, and one DAG.

Unique Correlations With ALM

Metabolites associated with measures of percent fat were, in some cases, the same as metabolites associated with ALM as seen in Supplementary Tables S2–S5. This may reflect correlations between adiposity measures and ALM; the correlations of BMI, percent fat, and percent trunk fat with ALM were r = .83, r = .35, and r = .56, respectively. However, of the metabolites correlated with ALM, seven (13%) were solely correlated with ALM and not any adiposity measures at p less than .00014. These consisted of three lipids and lipid-like molecules (C14:1 monoacylglycerol [MAG] and C18:0 MAG and C36:1 phosphatidylcholine plasmalogen), one benzenoid (4-hydroxymandelate), and three organic acids (lysine, methionine, and tryptophan). A further four metabolites were correlated with ALM and BMI but not other adiposity measures (C36:0 DAG and C38:7 phosphatidylethanolamine

Table 2. Number of Metabolites Detected by Taxonomy Subclass and Number (%) Significantly Associated With Body Composition Measures

Taxonomy Super Class	Total N of Metabolites	Correlated With BMI	Correlated With Percent Fat	Correlated With Percent Trunk Fat	Correlated With ALM
All	350	92	48	96	43
Alkaloids and derivatives	3	2	0	1	1
Benzenoids	11	1	0	1	1
Lipids and lipid-like molecules	217	72	38	75	29
Nucleosides, nucleotides, and analogues	12	0	0	0	0
Organic acids and derivatives	52	11	6	11	10
Organoheterocyclic compounds	18	2	1	3	1
Organonitrogen compounds	6	0	0	0	0
Organooxygen compounds	9	1	0	0	0
Organophosphorus compounds	3	0	0	0	0
Phenylpropanoids and polyketides	2	0	0	0	0
Unclassified	17	3	3	5	1

Note: Super class based on HMDB 3.0 (37). Additional information on subclasses within each super class can be found in Supplementary Table S1. ALM = appendicular lean mass; BMI = body mass index.

plasmalogen, both lipids, aminoadipate an organic acid, and cotinine an alkaloid).

Metabolic Pathways as Determined by GGM

In our GGM (Figure 2), we evaluated interrelationships of the top 25 ALM and top 25 percent trunk fat-associated metabolites. Eleven metabolites overlapped between ALM and percent trunk fat, thus 39 metabolites are included in the GGM. All of the TAGs, DAGs, cholesterol esters, and phosphatidylcholine metabolites in this set clustered into a single large interrelated network of 25 metabolites. These metabolites were primarily associated with percent trunk fat, with especially strong associations for the C52 TAGs. We also observed a cluster of five BCAAs; these metabolites were primarily associated with ALM. Additional clusters included three plasmalogens and two carnitines. The remaining four metabolites were not related to any of the other metabolites with conditional correlation of 0.3 and above and may represent distinct phenomena.

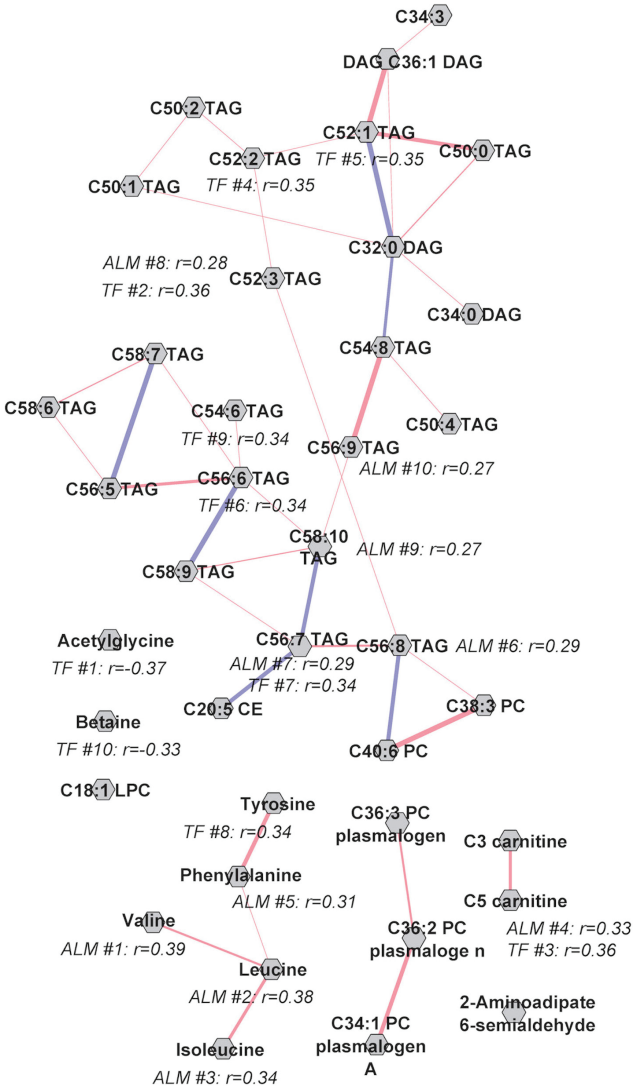

Figure 2. Gaussian Graphical Model of the top 25 metabolites correlated with percent trunk fat and appendicular lean mass. The width of the line represents the strength of the correlation. Metabolites that are connected represent direct conditional correlations = 0.3. Online version: blue lines represent inverse correlations and red lines positive correlations. Abbreviations: ALM = appendicular lean mass; TAG = triacylglycerols; TF = percent trunk fat.

Discussion

In this analysis of older black men, BMI, percent fat, percent trunk fat, and ALM were correlated with 92, 48, 96, and 43 metabolites, respectively. BCAAs, which play a crucial role in regulating protein structure, were found to be significantly correlated with each body composition measure as were carnitine metabolites, which are markers of energy production and lipid catabolism. In general, amino acids were most strongly correlated with BMI and ALM, while TAGs were most strongly correlated with percent fat and percent trunk fat. This is consistent with the general picture of perturbed lipid homeostasis with obesity and physiologic muscle metabolism actions.

Our findings are consistent with prior evidence of metabolic associations with body composition. For example, we replicated eight metabolites (seven amino acids and kynurenic acid) reported by Moore and colleagues (34) to be associated with BMI and another six metabolites Cheng and colleagues (9) found to be associated with BMI. Associations of BMI, amino acids, nucleotides, and kynurenic acid (9,16) were also replicated. Similar to our study, Newgard and colleagues (8) reported elevated levels of valine, leucine, phenylalanine, tyrosine, glutamate, and intermediates and byproducts of BCAA catabolism—C3 and C5 acylcarnitines—in obese versus lean subjects. The positive associations we detected between BCAAs and ALM were also similar to a study that reported a positive and strong association between BCAAs and muscle cross-sectional area and fat-free mass in a small sample of mobility limited older men and women (12).

The identification of seven metabolites associated with ALM but not any measure of adiposity is a novel finding. Of these, three are essential amino acids (lysine, methionine, and tryptophan) that have well-established roles in nitrogen balance and maintenance of muscle mass (35,36). The other four metabolites (C14:1 MAG, C18:0 MAG, C36:1 phosphatidylcholine plasmalogen, and 4-hydroxymandelate) have not, to our knowledge, been previously identified as correlates of ALM. The implication of this finding is unclear—the first three metabolites are involved in cell signaling pathways, are energy sources, and membrane components while 4-hydroxymandelate is a phenylacetic acid derivative found in the retina and vitreous humor (37). Additional study of these metabolites may help clarify the significance for ALM.

Recent research has highlighted that BCAAs may play an important role in the initiation and onset of diabetes. A BCAA-related metabolite cluster was correlated with insulin resistance in humans and induced insulin resistance when BCAAs were supplemented in animals (8). Two large studies found that amino acid metabolites (in addition to other metabolites) predicted diabetes risk independent of established risk factors such as BMI and fasting glucose (14,15). It is also possible that the correlations between amino acids, BCAAs, lean mass, and measures of body fat observed in our study have implications for disease and dynamic processes of lean tissue and insulin resistance (e.g., skeletal muscle catabolism), rather than simply being markers of absolute amounts of lean tissue or adiposity. Exploring metabolites in relation to changes in ALM and health outcomes would help clarify the implications of our findings.

Multiple correlations with lipids for each body composition measure were observed. The most prominent correlations were for acetylglycine, C5 carnitine, and the TAG subclass. Fatty acid oxidation is elevated in individuals with insulin resistance (8) and C5

carnitine, a known marker of fatty acid oxidation was positively correlated with adiposity measures. TAGs are major components of very-low-density lipoprotein and chylomicrons. Elevated TAG is associated with diabetes (38) and delayed clearance of plasma TAG, very-low-density lipoprotein–TAG, and chylomicron TAG, especially in the postprandial period, have been observed in obese subjects (39). Although correlations for TAGs were generally stronger than the other lipid subclasses, the GGM for percent trunk fat suggests that TAGs, DAGs, cholesterol ester, and phosphatidylcholine metabolites are metabolically related. The GGM also highlighted the C52 TAGs (52:1, 52:2, and 52:3) as particularly strong correlates of percent trunk fat. Glycerol molecules of the C52 TAGs are esterified with a combination of palmitic, stearic, oleic, and linoleic fatty acids that have been linked with diabetes and cardiovascular disease (40). It is possible then that these TAGs are biologically important for chronic disease risk beyond their association with adiposity.

Several other findings are potentially important, including positive correlations between tryptophan metabolites and BMI, percent fat, and percent trunk fat. The main pathway of tryptophan degradation is via kynurenine, which can be further metabolized to xanthurenate, kynurenic acid, quinolate, and 3-hydroxyanthranilic acid. Elevated adipose tissue gene expression of enzymes in the kynurenine pathway has been reported in people with obesity (41). Positive associations between serum levels of kynurenic acid and quinolinic acid and BMI have also been identified (42). Increased xanthurenic acid production has been proposed as one factor that may predispose individuals to insulin resistance (43) and high levels of xanthurenic acid in urine has been observed in people with diabetes (44). These results combined with ours, suggest altered tryptophan metabolism with obesity that may have implications for metabolic disease. Correlations between urate and BMI and percent trunk fat, and ALM and 4-hydroxymandelate may also be important. However, little is known regarding whether the biological functions of these metabolites have significance for metabolism and/or disease risk.

The main strengths of this study include the multiple measures of body composition from dual-energy x-ray absorptiometry, an objective, gold-standard method for measuring adiposity, which provides novel insight into metabolites associated with adipose and lean tissue, and the use of a well-characterized sample of older black men, a population with increased risk of many obesity-related health outcomes (45,46). To our knowledge, this is the first study of metabolomics correlates of body composition in black men and, thus, provides important insight into this population. Our results replicated a number of associations between metabolites and body composition previously observed in predominately white populations (12,13,35,47), which suggests commonality. However, it is possible that the novel associations observed and incomplete replication of associations from prior studies reflect metabolic differences unique to our study population.

A potential limitation is our sample size, which is small for epidemiologic studies, but relatively large for metabolic studies. Nevertheless, this pilot study that was developed to explore metabolic correlates of body composition identified a large number of metabolites that were significantly associated with ALM and adiposity even with strict control for multiple statistical testing. This suggests that metabolites are robustly associated with body composition measures, although additional correlates may be discovered in larger sample sizes. We did not mutually adjust models for body composition measures because of substitution effects and the subsequent complex interpretation of such a model. For example, if modeling BMI and ALM, ALM is a component of BMI. ALM is also a component of overall body size, which serves as the denominator for percent fat mass and percent trunk fat. Future methodologic studies are needed to best determine how to address these interrelated measures. It is also important to note that metabolites are influenced by behaviors such as diet and physical activity as well as disease. Although disease conditions minimally affected associations in this analysis, we cannot discount the potential effects of other metabolite-influencing factors. Due to the study design, our results may not be generalizable. The analytical sample was healthier than the overall population of black men in Health ABC because the 12-month visit was used as the study baseline and attrition occurred within the first year. As the body composition of individuals may vary by race/ethnicity, gender, and age, it is unclear whether these results are generalizable. However, the replication of prior associations with lean mass and BMI (8,11–13,16,34) suggests that not all of the findings are not specific to our population demographics.

Conclusions

We identified numerous metabolites correlated with adiposity and ALM, which notably include strong associations for BCAAs, carnitine, and TAGs. Future studies should consider these as potential metabolic mediators or targets for furthering the understanding of adiposity-related disease such as cardiovascular disease, diabetes, and cancer and ALM-related health outcomes such as frailty and diabetes (48,49).

Supplementary Material

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

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