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Research Article

Association of Plasma Small-Molecule Intermediate Metabolites With Age and Body Mass Index Across Six Diverse Study Populations

William E. Kraus,[1,2,3] Carl F. Pieper,[2,4] Kim M. Huffman,[1,2,5] Dana K. Thompson,[1,2] Virginia B. Kraus,[1,2,3] Miriam C. Morey,[1,2,5] Harvey J. Cohen,[1,2,5] Eric Ravussin,[6] Leanne M. Redman,[6] James R. Bain,[1,3] Robert D. Stevens,[3] and Christopher B. Newgard[2,3]

[1]Department of Medicine, [2]Claude D. Pepper Older Americans Independence Center/Center for the Study of Aging and Human Development, [3]Duke Molecular Physiology Institute, and [4]Department of Biostatistics and Bioinformatics, Duke University, Durham, North Carolina. [5]Durham VA Medical Center, North Carolina. [6]Pennington Biomedical Research Center, Baton Rouge, Louisiana.

Address correspondence to William E. Kraus, MD, Duke Molecular Physiology Institute, Duke University, 300 North Duke Street, Durham, NC 27701. E-mail: william.kraus@duke.edu

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Abstract

Background: Older age and obesity are associated with metabolic dysregulation; the mechanism by which these factors impact metabolism across the lifespan is important, but relatively unknown. We evaluated a panel of amino acids (AAs) and acylcarnitines (ACs) to identify effects of age and adiposity (body mass index) on circulating small-molecule metabolites in a meta-analysis of six diverse study populations.
Methods: Targeted metabolic profiling was performed in six independent studies, representing 739 subjects with a broad range of age, body mass index, health states, and ethnic origin. Principal components analysis was performed on log-normalized values for AAs and ACs separately, generating one AC factor and two AA factors for each study. A common AC factor consisted primarily of acetylcarnitine, medium-chain AC, and several long-chain AC. AA Factor 1 consisted primarily of large neutral AAs. Glycine was its own factor.
Results: Metabolic profiling and factor analysis identified clusters of related metabolites of lipid and AA metabolism that were consistently associated with age and body mass in a series of studies with a broad range of age, body mass index, and health status. An inverse association of glycine with body mass index and male gender supports its role as a marker of favorable metabolic health.
Conclusions: An important focus of future investigations should be to determine whether these clusters of metabolic intermediates are possible early predictors of health outcomes associated with body mass; are involved with accelerated aging; are involved in the causative pathway of aging; and how modification of these metabolic pathways impact the biology of aging.

Keywords: Metabolomics—Acylcarnitines—Branched-chain amino acids—Glycine

Metabolic dysfunction is characterized by an imbalance in the synthesis, concentration, and removal of small molecules of energy metabolism, including amino acids (AAs) and acylcarnitines (ACs). AAs are the agents of protein metabolism that can be derived from the diet (as in the essential branched-chain AAs [BCAA]) or synthesized de novo. ACs are derived from mitochondrial oxidation of fatty acids, carbohydrates, and AAs; they are intermediaries in the mitochondrial transport of these oxidation substrates. ACs bear acyl side chains of various lengths: most abundant short acyl chains (less than seven carbons); less abundant medium acyl chains (8–14 carbons); and long acyl (15–22 and longer carbons) chains. Plasma concentrations of specific AAs and ACs integrate the status of numerous enzymatic pathways across all bodily organs; as such, they may serve as reporters of the metabolic mechanisms underlying conditions such as obesity, diabetes, normal aging (1), and the interaction among them. Metabolic profiling (metabolomics) has revealed changes in metabolic regulation as a component of diseases such as coronary artery disease (2,3), diabetes (4,5), and the physical

functional decline seen with aging (6); they may be the harbinger of disease in as-yet asymptomatic individuals. The potential for metabolic profiling to predict disease risk provides the opportunity for disease prevention and the benefits of clinical interventions (2,5).

Obesity, age, and metabolic dysfunction are key components of many of the most pervasive and costly challenges to public health. Throughout the lifespan, dysregulation of AAs and ACs is associated with adiposity (high body mass index [BMI]) and diabetes mellitus (4). Indeed, with advancing age, metabolic dysfunction accompanies the onset of disease and functional decline; this ultimately results in disability and mortality. Furthermore, excess adiposity and age are independent risk factors for metabolic dysfunction. Ongoing studies seem only to reveal new complexities in the effects of both age and BMI on metabolic health and disease.

The relationship between body mass and age across the lifespan continues to be debated, as the presence or lack of an inverse U-shaped relationship between the two and the effect of this relationship on health remains controversial (7,8). The issue of an obesity paradox with respect to age-related morbidity and mortality has fueled another debate: whether to alter the advice to lose weight for some patients in scenarios where higher BMI is actually associated with reduced risk of mortality (9,10). It is possible that metabolic factors might be better indicators of health status than are body mass or adiposity; metabolic factors may assist in identifying subsets of individuals that are at higher risk for the development of chronic diseases of aging. As an initial foray into this arena, we undertook the present study.

Building on our interest in small-molecule metabolites as biomarkers and potential biological mediators of functional decline with age (6), we characterized the variation in small-molecule metabolites across an assembled study with a broad range of ages and body mass. We were guided by the hypothesis that such pathways could play a role in the physiologic changes seen in aging and age-related diseases. As part of our Claude Pepper Center, we purposefully assembled a set of clinical studies in which we could collect the same biological parameters relating to metabolic pathways. Including heterogeneous populations distributed across the BMI and age ranges, provided the opportunity to evaluate the effects of a broad range of ages and BMI on small molecule metabolites. We assembled a unique data set of 739 individuals combined from 6 diverse study studies to constitute an overall study population with a wide range of BMI, age, and ethnic origin. Applying a meta-analytic approach to this study set, we sought to evaluate the independent associations of BMI and age on circulating small-molecule metabolites. Ultimately such understanding will aid in the use of small-molecule metabolites for their ability to predict adverse health outcomes or guide therapeutic interventions to prevent or treat disease.

Methods

Contributing Studies

Six studies (summarized in Table 1 and Figure 1) contributed to this meta-analysis. They included one cross-sectional study; one longitudinal observational study; and single baseline samples from four longitudinal intervention studies. The combined data set included 431 women (58.3%); 441 members of ethnic or racial minorities (nonwhite, 59.7%); an overall mean age of 58.6 years; and a mean BMI of 30.7 kg/m^2. The age of the subjects spanned 18–98 years and BMI 19–61 kg/m^2 (Figure 1). Brief descriptions of the six contributing studies are provided below. The demographic and study characteristics are displayed in Table 1 and described in the Supplementary Text and Supplementary Table A1.

Table 1. Group Characteristics in Six Independent Studies Contributing to the Meta-analysis

	CALERIE (11)	STRRIDE (3)	POP (12)	POP Pilot (13)	LIFE (6)	CARRIAGE (14)	Entire Group
N	46	73	138	40	77	36.5	739
% women	56.5	48.0	73.2	65.0	0.0	66.6	58.3
% nonwhite	39.1	19.2	13.0	22.5	22.1	100.0	59.7
Age mean (25%, 75%)	38.5 (33, 44)	51.2 (46, 56)	65.4 (57, 75)	69.5 (61, 76)	79.2 (75, 83)	54.4 (43, 66)	58.6 (46, 72)
BMI mean (25%, 75%)	27.7 (26.7, 29.4)	30.5 (28.4,3 2.1)	31.3 (26.0, 35.2)	32.7 (27.1, 36.2)	28.4 (25.8, 31.3)	30.9[a] (25.7, 35.2)	30.7[b] (26.6, 33.9)
% BMI ≤ 25 (kg/m^2)	2	0	14	10	21	21	15
% BMI > 25–30	87	41	33	38	48	32	38
% BMI > 30	11	59	53	52	31	47	47

Note: BMI = body mass index. [a]Measure available for *N* = 314 (of 347). [b]Measure available for *N* = 688 (of 739).

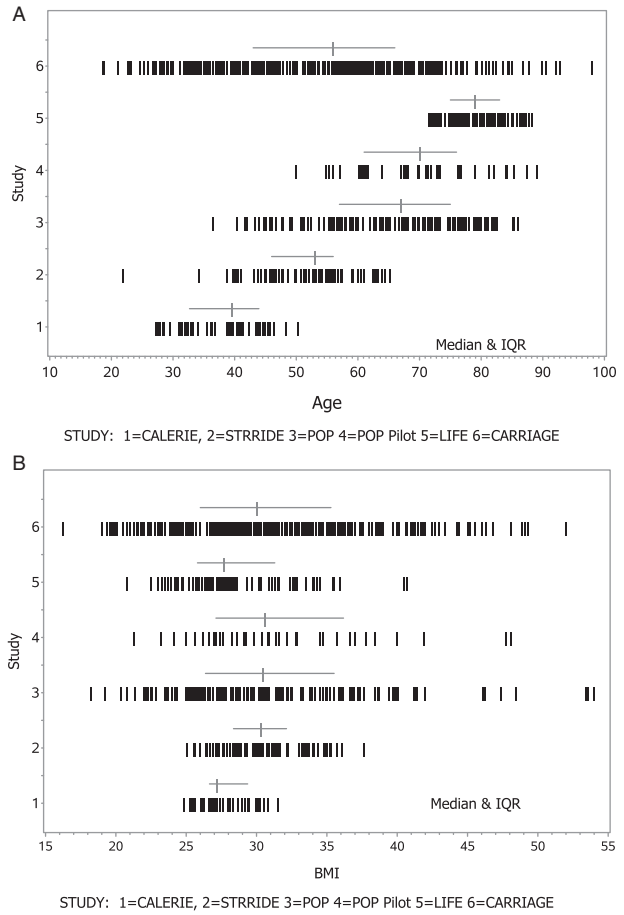

Figure 1. Value (median and interquartile range) for age (**A**) and body mass index (BMI; **B**) in six independent studies. Study (1) CALERIE, (2) STRRIDE, (3) POP, (4) POP Pilot, (5) LIFE, and (6) CARRIAGE. Median and *SD* are indicated.

Determination of Small-Molecule Metabolites

Plasma and serum samples were used for quantitative determination of targeted metabolite levels for 45 ACs species and 15 AAs by the Sarah W. Stedman Nutrition and Metabolism metabolomics core laboratory of the Duke Molecular Physiology Institute, as described previously (15). Briefly, proteins were first removed by precipitation with methanol, and supernatants were separated into aliquots, dried, and esterified with hot, acidic methanol (ACs) or *n*-butanol (AAs). Analysis was performed using tandem mass spectroscopy with a Quattro Micro instrument (Waters Corporation, Milford, MA). All mass-spectrometric analyses employed stable-isotope dilution for quantification of metabolite concentration. Quantification of the "targeted" intermediary metabolites involved addition of mixtures of known quantities of stable-isotope internal standards to samples; stable isotopes were obtained from Isotec (St. Louis, MO), Cambridge Isotope Laboratories (Andover, MA); and CDN Isotopes (Pointe-Claire, Quebec, Canada). Leucine and isoleucine were reported as a single analyte; they are not resolved by this tandem mass spectroscopy. The acidic conditions used to form butyl esters results in partial hydrolysis of glutamine to glutamic acid and of asparagine to aspartate; values reported as Glu/Gln or Asp/Asn are not meant to signify the molar sum of glutamate and glutamine, or of aspartate and asparagine, but rather measure the amount of glutamate or aspartate plus the contribution of the partial hydrolysis reactions of glutamine and asparagine.

Data Handling and Statistical Analysis

Principal components analysis (PCA) was used to create multicomponent factors of log-transformed values for AA and ACs separately. Using PCA relieves the burden of multiple comparisons by identifying the correlation structures of the individual analytes and reducing the dimensionality of the analysis from, in this case, 60 metabolites, to 3 factors. We have successfully used this strategy in dozens of previous studies from our group (2–4,16,17). Separately, for each individual study, normalized metabolite factors were constructed, and the results were aggregated and compared across sites. Consistency of factor elements and loadings was compared across the six study studies using the Cronbach's alpha statistic (unweighted). We were encouraged by the fact that the metabolite factors assembled from each of the cohorts have very similar structures. The summary factor score was computed by aggregation of the factor loadings for the individual study, *i*, using meta-analytic techniques for correlations (18). First, the individual factors loadings in the individual studies were transformed using the Fisher's Z-transform. Second, the average weighted (weight = $N_i - 3$) transformed score was computed across the studies, and, finally, this average score was used to calculate the average factor score by back transformation of Fisher's formula. The score for the aggregated population factor scores for the individual analytes was used to derive total factor scores across the studies going forward in the analytic strategy. Multivariable models evaluated the association of the two derived metabolic factors with the following independent variables: study, age, BMI, interaction of BMI and age, gender, and race (white vs nonwhite). Associations were considered significant at $p < .05$. Analyses presented here were exploratory and involved only three factors; no corrections for multiple comparisons were made. PCA creates normalized factors (with a mean of 0 and standard deviation [*SD*] of 1 across the study population); therefore, regression coefficients for relationships between variables and the PCA components can be directly used and compared to determine the direction and strength of the effect in units of the *SD* of the measure. Each derived PCA factor contains the common information of the elements (metabolites, e.g., 45 ACs or 15 AAs) that were input to create it. That is, each element has a coefficient based on its contribution or "loading" on the factor; when elements (metabolite concentrations) are multiplied by these coefficients and then combined in a linear or additive fashion, an individual PCA factor score is created. It is customary, however, to characterize a PCA factor with respect to the component elements that have an absolute normalized coefficient (ranges from −1 to +1) greater than 0.7; 0.7 as a normalized coefficient can be interpreted to mean that 50% of the variance in the variable is explained by the factor.

Interaction Between Age and BMI in the Study Populations

Some have reported an inverse U-shaped relationship of BMI with age and with BMI and disease/death. We thought it to be important to investigate this in our study population. Analysis of the relationship of age to BMI across our meta-population revealed a monotonic linear relationship between age and BMI with the factors both within and between studies; most notable was the lack of an inverted U-shaped relationship between BMI and age as well as the relationship of these two demographics with the outcome. Given that there were no curvilinear effects for age and BMI with the factors, we retained linear versions. Given that we did not observe an age-by-BMI interaction, we did not retain or report any interactions of age and BMI in further analyses.

Results

Factor Structure of Metabolic Intermediates Among Studies

PCA was performed separately for ACs and AAs, and generated one major factor for ACs and two major factors for AAs. Supplementary Table A2 show the factor loadings for each independent study, for Acylcarnitine Factor 1 (Supplementary Table A2a), Amino Acid Factor 1 (Supplementary Table A2b), and Amino Acid Factor 2 (Supplementary Table A2c). Acylcarnitine Factor 1 consisted primarily of acetyl carnitine (2-carbon acyl side chain), medium-chain ACs, and several ACs containing 16 and 18 carbons (C16, C18) and is referred to hereafter as the AC Factor. Amino Acid Factor 1 consisted primarily of the large neutral AAs (LNAA) that include the essential BCAA isoleucine, leucine, and valine; the sulfur-containing AA methionine; the aromatic AAs phenylalanine and tyrosine. It is referred to hereafter as the LNAA Factor. Amino Acid Factor 2 was loaded consistently heavily for glycine across all studies; therefore, glycine alone, as its own factor, was studied going forward. Cronbach's alpha was 0.926 for AC and 0.908 for LNAA indicating high consistency of the factor structure across all six contributing studies for these two factors. Because AA Factor 2 was a single variable, no Cronbach's alpha was calculated.

Multivariable Models to Distinguish Age, BMI, Gender, and Race Effects

In a multivariable model containing study, race, gender, BMI, and age (Table 2), the AC Factor was independently associated with age ($p < .0001$) and BMI ($p = .014$). The regression coefficients indicated that every 10-year increase in age was associated with a 0.208 unit increase in the normalized AC factor score, and every unit (kg/m^2) increase in BMI was associated with a 0.015 (*SD*) unit increase in the normalized AC factor score. Men had significantly higher AC factor scores than women (regression coefficient 0.277; $p = .0004$). Age and BMI associations were similar in both racial groups.

In a multivariable model containing study, race, gender, BMI, and age (Table 3), the LNAA factor was independently associated with age (regression coefficient per 10 years of age 0.094; $p = .0008$) and BMI (regression coefficient 0.012; $p = .038$). Men had significantly higher LNAA factor scores (regression coefficient 0.412; $p < .0001$). These relationships were observed in both racial groups. There were significant differences between the studies (omnibus $p = .0010$) in these relationships: Compared with the STRRIDE study as reference, all of the other studies had a greater LNAA factor score—from 0.52 to 1.36 *SD*s greater—in the following order: LIFE, POP Pilot, POP, CARRIAGE, and CALERIE.

In a multivariable model containing study, race, gender, BMI, and age (Table 4), glycine was independently and inversely associated with BMI (regression coefficient −0.012; $p < .0001$). Also, men had significantly lower levels of glycine (regression coefficient −0.061; $p = .0026$). There was no difference between the two racial groups or with age. There were significant differences between the studies (omnibus $p = .0001$) in glycine plasma concentration: Compared with the STRRIDE study as reference, the other studies had a glycine concentrations that differed—from 0.10 μM less to 0.30 μM greater—in the following order: LIFE, POP, STRRIDE, CARRIAGE, POP Pilot, and CALERIE. There were significant differences among the studies (omnibus $p < .0001$) in these relationships. There were no interaction effects between age and BMI on metabolic factors.

Table 2. Prediction of the AC Factor in a Multivariable Model Containing Study, Age, BMI, Race, and Gender

Item	Study Name	Estimate	*SE*	*p*
	Omnibus			.0580
	CALERIE	−0.26008	0.17704	.1423
	STRRIDE[a]	0.00000	—	—
	CARRIAGE	0.07528	0.15359	.6242
	POP	−0.17100	0.13921	.2197
	POP Pilot	0.25770	0.18716	.1690
	LIFE	0.07528	0.15359	.9674
Age	—	0.02076	0.00287	**<.0001**
BMI	—	0.01456	0.00589	**.0136**
Race[b]	—	−0.19102	0.12018	.1124
Gender[c]	—	−0.27746	0.07815	**.0004**

Note: AC = acylcarnitine; BMI = body mass index; *SD* = standard deviation. In this analysis, STRRIDE represents the reference group. Estimates are expressed as *SD* units of the factor score and *SE* is the standard error of these estimates; the AC factor is the log of the component elements. [a]Reference study. [b]White is reference. [c]Male is reference.

Table 3. Prediction of the LNAA Factor in a Multivariable Model Containing Study, Age, BMI, Race, and Gender

Item	Study Name	Estimate	*SE*	*p*
	Omnibus			***.0010***
	CALERIE	1.35968	0.17249	**<.0001**
	STRRIDE[a]	0.00000	—	—
	CARRIAGE	1.31931	0.14964	**<.0001**
	POP	1.24037	0.13563	**<.0001**
	POP Pilot	0.75964	1.18235	**<.0001**
	LIFE	0.52265	0.16800	**.0019**
Age	—	0.00943	0.00280	**.0008**
BMI	—	0.01193	0.00574	**.0380**
Race[b]	—	0.11709	0.11709	.1279
Gender[c]	—	−0.41215	0.07614	**<.0001**

Note: BMI = body mass index; LNAA = large neutral amino acids; *SD* = standard deviation. In this analysis, STRRIDE represents the comparative group. Estimates are expressed as *SD* units of the factor score and *SE* is the standard error of these estimates; the LNAA factor is composed from the log of the component elements. [a]Reference study. [b]White is reference. [c]Male is reference.

Discussion

The combination of targeted metabolic profiling with PCA is a powerful strategy that allows a large number of related molecules to be scrutinized simultaneously—taking into account BMI, age, and race—in order to discern and characterize metabolic pathways and their modulation across significant demographic and study (disease) strata. In this study, a comprehensive set of 15 AAs and 45 ACs were measured across 6 studies in a single laboratory according to the same standardized procedures. Despite the significant differences in age and BMI, there was high consistency of the AC Factor and the LNAA Factor across all six individual studies, demonstrated by high values for the Cronbach's alpha in these analyses; this consistency also indicates that there were minimal influences of fed versus fasted state or other unmeasured covariate effects on the metabolite associations observed across these studies. We have observed high

Table 4. Associations of Glycine (μM) in a Multivariable Model Containing Study, Age, BMI, Race and Gender

Item	Study Name	Estimate	*SE*	*p*
	Omnibus			***<.0001***
	CALERIE	0.30010	0.04593	**<.0001**
	STRRIDE[a]	0.00000	—	—
	CARRIAGE	0.05412	0.03985	.1749
	POP	−0.00349	0.03612	.9230
	POP Pilot	0.12821	0.04856	**.0085**
	LIFE	−0.10210	0.04474	**.0228**
Age	—	0.00054	0.00074	.4686
BMI	—	−0.01224	0.00153	**<.0001**
Race[b]	—	−0.01274	0.03118	.6829
Gender[c]	—	0.06140	0.02028	.0026

Note: BMI = body mass index; *SD* = standard deviation; *SE* = standard error. In this analysis, STRRIDE represents the comparative group. Estimates are in *SD* units of the factor score and are calculates as log of the glycine concentration. [a]Comparative study. [b]White is reference. [c]Male is reference.

heritability for many of these analytes (4), indicating that a genetic component is a substantial determinant of their circulating levels. Despite this, we detected significant effects of both age and BMI on these factors in this meta-analysis study.

The metabolite composition of the factors generated in this analysis is consistent with those identified in other independent human studies associated with metabolic dysregulation. In an assessment of the differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects (19), the same panel of metabolites (15 AAs and 45 ACs) subjected to unbiased PCA (pooling AC and AA) yielded an AC Factor composed primarily of AC, and an AA factor (loaded heavily by BCAA) as the two largest contributors to metabolite variance. Shah and coworkers (4) examined the heritability of metabolic profiles in families with high prevalence of coronary artery disease and identified numerous factors from unsupervised PCA, including two factors that loaded heavily for medium- and long-chain AC and BCAA and LNAA. Other studies have reported generation of similar factors of metabolites (2–4,6,11,20). The analysis described here overtly separated ACs from AAs before factoring, an analytic decision supported by the separate clustering of these two classes of molecules in unbiased factor analysis.

Creation of these three factors allowed natural clusters of metabolites to be considered in the aggregate when analyzing the effects of age, BMI, gender, and race within the studies and in a meta-analytic approach. Independent of BMI, gender, and race, age was significantly associated with both the AC factor and the LNAA factor; but not with glycine. Independent of age, gender, and race, BMI was significantly associated with all three factors. In contrast, there was no significant association of race (white vs nonwhite) with any of the factors. Independent of age, BMI, and race, gender showed significant associations with all three factors. Notably, when compared with women, men had significantly greater scores for the AC and LNAA factors, but lower concentrations of glycine. We observed a study-specific effect only on LNAA, the source of which is unclear but may be related to sampling environment or a study-specific factor not modeled in this analysis. Importantly, we failed to observe any age-by-BMI interaction effects on metabolite factors. The observation that LNAA were associated with age and BMI in our cohort but that glycine was only associated with BMI, emphasizes the relative specificity of the LNAA association with age and begs for further investigation.

The association of the metabolic factors with age and BMI confirmed the occurrence of metabolic dysregulation with aging and greater body mass. Based on these data, we speculate that older persons and persons with greater adiposity exhibit more systemic fatty acid oxidation by-products. This is based on our observation that even-chain ACs (loaded most heavily on the AC Factor) and the acetylcarnitine (C2) in Factor 1 are by-products of fatty acid catabolism and completion of fatty acid oxidation, respectively. These results might reflect either higher rates of fatty acid oxidation or fatty acid supply and oxidation that is outpacing energy need and downstream metabolic processes (TCA cycle and electron transport chain). Additional investigations would be necessary to differentiate these possibilities. The elevated AC scores with age and BMI are consistent with an increased cardiometabolic risk with increased age and body mass.

Elevations in circulating even-chain ACs have been associated with a number of metabolic states associated with both aging and increased BMI, including obesity (21); insulin resistance (3); type 2 diabetes (22); anemia (23); inflammation (22); and cardiovascular events and mortality (2). Although the age and BMI associations with elevated ACs might simply denote the presence of health problems, the extent to which the dysregulation of these metabolites mediates biological aging and its associated functional decline is currently the focus of growing interest. Lum and coworkers, in a separate analysis of the Veteran's LIFE study, reported that an AC factor associated inversely with several measures of physical performance in elderly community-dwelling men (6). Numerous objective measures of physical performance (including the Short Physical Performance Battery [SPPB], SPPB gait speed, SPPB chair stands, usual gait speed, and actual gait speed) correlated inversely and significantly with a factor composed of all 45 ACs, loaded most heavily on medium-chain ACs and acetylcarnitine.

Greater LNAA factor scores are associated with insulin resistance (3) independent of age, gender, and waist circumference, a marker of body composition similar to BMI. In a population of individuals undergoing cardiac catheterization, both LNAA and AC factors, similar to the one identified here, were associated with cardiac events and mortality in longitudinal analyses (2). In these articles and other epidemiologic studies, particularly those from the Framingham Heart Study (5) elevation of LNAA, specifically the BCAA, are associated with insulin resistance and risk for type 2 diabetes mellitus. The greater LNAA scores with age and BMI are consistent with an increased cardiometabolic risk with increased age and body mass.

Glycine was the dominant component of AA factor 2. Analyzed as a single metabolite, glycine demonstrated a significant inverse association with BMI. Furthermore, glycine was lower in men but showed no significant relation with age or race. The observation that men and individuals with greater BMI had lower glycine concentrations is of particular interest. From accumulating data, these findings would imply that adult men are at a higher metabolic risk than women of similar age. There is a developing literature about glycine and metabolic regulation. Almanza-Perez and coworkers correction have observed that glycine administration in animal models is anti-inflammatory—specifically reducing circulating

concentrations of tumor necrosis factor-alpha and interleukin-6—and protecting against metabolic syndrome in an animal model of diet-induced obesity (24). Deletion of the L-arginine:glycine amidinotransferase (*AGAT*) gene in animal models results in greater circulating glycine levels; it protects from diet-induced obesity and is associated with fewer adverse cardiovascular outcomes (25,26). Greater circulating glycine is associated with a more favorable body composition, greater lean mass, and more favorable insulin action in elderly adults (27).

It is notable, furthermore, that serine also loaded highly with glycine in three of the studies and in the average of the entire subject set. This could be expected, given that serine serves as a precursor in glycine synthesis, either via glycine synthase or serine hydroxymethyl transferase. Glycine catabolism occurs primarily on the inner mitochondrial membrane. Given the plurality of roles of glycine beyond its contribution to cellular proteins, including its requirement in the synthesis of purines, creatine, bile salts, porphyrins, and glutathione, it would be difficult to discern a singular reason for the association with body weight reported here. However, based on the paradigm of carnitine, one area of considerable interest is whether glycine might also covalently modify acyl groups destined for clearance. One might then expect an inverse relation between circulating ACs and glycine; for instance, conditions of high plasma ACs, such as mitochondrial dysfunction due to obesity, would be associated with lower free glycine concentrations due to glycine sequestration. This was observed in our study. The condensation reaction of arginine and glycine in the first step of creatine synthesis is also of interest.

In work from our group, we observed that plasma glycine concentrations were directly related to insulin sensitivity (3); furthermore, with exercise training, increased glycine concentrations corresponded to improved insulin sensitivity (28). We recently completed a study comparing BCAA turnover in lean and obese adults; its correlation with insulin sensitivity and its modulation with exercise training. We observed that circulating glycine levels were higher in the lean and obese training group; further urinary metabolic profiling suggested that exercise induces more efficient elimination of excess acyl groups derived from BCAA and aromatic AA metabolism via formation of urinary glycine adducts (29). In a very recent finding, reduced glycine concentrations are associated with subsequent myocardial infarction in individuals with suspected coronary ischemia (30). Although we failed to observe an association with age in our contributing studies here, further investigation is indicated for understanding the importance of the association of circulating glycine levels with obesity and metabolic dysregulation in aging populations.

As a strength of our study, we combined numerous diverse study studies to investigate the association of targeted metabolic intermediates with age and BMI. This approach necessitated including studies that differed on whether individuals were sampled in fed or fasted state, sampled in serum or plasma, or other differences. Fed-fasted state is a significant contributor to some metabolite levels (31). However, we are confident that the associations were not influenced by study-specific differences, including fed-fasted state. The composition of the principal component factors were consistent across studies; in multivariate analyses, group assignment (study membership) was never a significant contributor to the associations.

The findings we observed suggest that age and BMI should be considered in future studies of the association of small-molecule metabolites with disease. In particular, the effects of age and BMI on AC and LNAA, and additionally the effects of BMI on glycine, should be controlled. Most intriguingly, the study also revealed that there are effects of age on metabolic health that are independent of the tendency toward overweight with aging. Investigating the biomolecular mechanisms underlying these associations will be an important emphasis for future work.

Supplementary Material

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

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References

1. Fadini GP. Age-associated cardiovascular risk and metabolomics of mitochondrial dysfunction. *Atherosclerosis*. 2014;232:257–258. doi:10.1016/j.atherosclerosis.2013.10.038
2. Shah SH, Bain JR, Muehlbauer MJ, et al. Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circ Cardiovasc Genet*. 2010;3:207–214. doi:10.1161/circgenetics.109.852814
3. Huffman KM, Shah SH, Stevens RD, et al. Relationships between circulating metabolic intermediates and insulin action in overweight to obese, inactive men and women. *Diabetes Care*. 2009;32:1678–1683. doi:10.2337/dc08-2075
4. Shah SH, Hauser ER, Bain JR, et al. High heritability of metabolomic profiles in families burdened with premature cardiovascular disease. *Mol Syst Biol*. 2009;5:258. doi:10.1038/msb.2009.11
5. Wang TJ, Larson MG, Vasan RS, et al. Metabolite profiles and the risk of developing diabetes. *Nat Med*. 2011;17:448–453. doi:10.1038/nm.2307
6. Lum H, Sloane R, Huffman KM, et al. Plasma acylcarnitines are associated with physical performance in elderly men. *J Gerontol A Biol Sci Med Sci*. 2011;66:548–553. doi:10.1093/gerona/glr006
7. Gallagher D, Visser M, Sepulveda D, Pierson RN, Tamara Harris T, Heymsfield SB. How useful is the body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol*. 1996;143:228–239.
8. Jackson AS, Stanforth PR, et al. The effect of sex, age, and race on estimating percentage body fat from body mass index: The Heritage Family Study. *Int J Obes*. 2002;26:789–796. doi:10.1038/sj.ijo.0802006
9. Lavie C, Milani RV, Ventura HO. Obesity and cardiovascular disease. *J Am Coll Cardiol*. 2009;53:1925–1932. doi:10.1016/j.jacc.2008.12.068
10. Anker SD, von Haehling S. The obesity paradox in heart failure: accepting reality and making rational decisions. *Clin Pharmacol Ther*. 2011;90:188–190. doi:10.1038/clpt.2011.72
11. Redman LM, Huffman KM, Landerman LR, et al. Effect of caloric restriction with and without exercise on metabolic intermediates in non-

obese men and women. *J Clin Endocrinol Metab*. 2011;96:E312–E321. doi:10.1210/jc.2010-1971

12. Kraus VB, McDaniel G, Worrell TW, et al. Association of bone scintigraphic abnormalities with knee malalignment and pain. *Ann Rheum Dis*. 2009;68:1673–1679. doi:10.1136/ard.2008.094722
13. Thompson DK, Sloane R, Bain JR, et al. Daily variation of serum acylcarnitines and amino acids. *Metabolomics*. 2011;8:556–565. doi:10.1007/s11306-011-0345-9
14. Chen HC, Shah S, Stabler TV, Li YJ, Kraus VB. Biomarkers associated with clinical phenotypes of hand osteoarthritis in a large multigenerational family: the CARRIAGE family study. *Osteoarthr Cartilage*. 2008;16:1054–1059. doi:10.1016/j.joca.2007.12.010
15. Haqq AM, Lien LF, Boan J, et al. The Study of the Effects of Diet on Metabolism and Nutrition (STEDMAN) weight loss project: rationale and design. *Contemp Clin Trials*. 2005;26:616–625. doi:10.1016/j.cct.2005.09.003
16. Heilbronn LK, de Jonge L, Frisard MI, et al. Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial. *J Am Med Assoc*. 2006;295:1539–1548. doi:10.1001/jama.295.13.1539
17. Shah SH, Granger CB, Hauser ER, et al. Reclassification of cardiovascular risk using integrated clinical and molecular biosignatures: design of and rationale for the Measurement to Understand the Reclassification of Disease of Cabarrus and Kannapolis (MURDOCK) Horizon 1 Cardiovascular Disease Study. *Am Heart J*. 2010;160:371–379.e2.doi:10.1016/j.ahj.2010.06.051
18. Hedges L, Olkin I. *Statistical Methods for Meta-analysis*. Orlando, FL: Academic Press; 1985.
19. Laferrère B, Reilly D, Arias S, et al. Differential metabolic impact of gastric bypass surgery versus dietary intervention in obese diabetic subjects despite identical weight loss. *Sci Transl Med*. 2011;3:80re2. doi:10.1126/scitranslmed.3002043
20. Tai ES, Tan ML, Stevens RD, et al. Insulin resistance is associated with a metabolic profile of altered protein metabolism in Chinese and Asian-Indian men. *Diabetologia*. 2010;53:757–767. doi:10.1007/s00125-009-1637-8
21. Newgard CB, An J, Bain JR, et al. A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab*. 2009;9:311–326. doi:10.1016/j.cmet2009.02.002
22. Adams SH, Hoppel CL, Lok KH, et al. Plasma acylcarnitine profiles suggest incomplete long-chain fatty acid beta-oxidation and altered tricarboxylic acid cycle activity in type 2 diabetic African-American women. *J Nutr*. 2009;139:1073–1081. doi:10.3945/jn.108.103754
23. Reuter SE, Faull RJ, Ranieri E, Evans AM. Endogenous plasma carnitine pool composition and response to erythropoietin treatment in chronic haemodialysis patients. *Nephrol Dial Transplant*. 2009;24:990–996. doi:10.1093/ndt/gfn588
24. Almanza-Perez JC, Alarcon-Aguilar FJ, Blancas-Flores G, et al. Glycine regulates inflammatory markers modifying the energetic balance through PPAR and UCP-2. *Biomed Pharmacother*. 2010;64:534–540. doi:10.1016/j.biopha.2009.04.047
25. Choe CU, Atzler D, Wild PS, et al. Homoarginine levels are regulated by L-arginine:glycine amidinotransferase and affect stroke outcome: results from human and murine studies. *Circulation*. 2013;128:1451–1461. doi:10.1161/CIRCULATIONAHA.112.000580
26. Choe CU, Nabuurs C, Stockebrand MC, et al. L-arginine:glycine amidinotransferase deficiency protects from metabolic syndrome. *Hum Mol Genet*. 2013;22:110–123. doi:10.1093/hmg/dds407
27. Lustgarten MS, Price LL, Phillips EM, Fielding RA. Serum glycine is associated with regional body fat and insulin resistance in functionally-limited older adults. *PLoS One*. 2013;8:e84034. doi:10.1371/journal.pone.0084034
28. Huffman KM, Slentz CA, Bateman LA, et al. Exercise-induced changes in metabolic intermediates, hormones, and inflammatory markers associated with improvements in insulin sensitivity. *Diabetes Care*. 2011;34:174–176. doi:10.2337/dc10-0709
29. Glynn EL, Piner LW, Huffman KM, et al. Impact of combined resistance and aerobic exercise training on branched-chain amino acid turnover, glycine metabolism and insulin sensitivity in overweight humans. *Diabetologia*. 2015;58:2324–2335. doi:10.1007/s00125-015-3705-6
30. Ding Y, Svingen GFT, Pederen ER, et al. Plasma glycine and risk of acute myocardial infarction in patients with suspected stable angina pectoris. *J Am Heart Assoc*. 2016;5:e002621. doi:10.1161/JAHA.115.002621
31. Krug S, Kastenmüller G, Stückler F, et al. The dynamic range of the human metabolome revealed by challenges. *FASEB J*. 2012;26:2607–2619. doi:10.1096/fj.11-198093