Race and Sex Differences in Small-Molecule Metabolites and Metabolic Hormones in Overweight and Obese Adults

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Abstract

In overweight/obese individuals, cardiometabolic risk factors differ by race and sex categories. Small-molecule metabolites and metabolic hormone levels might also differ across these categories and contribute to risk factor heterogeneity. To explore this possibility, we performed a cross-sectional analysis of fasting plasma levels of 69 small-molecule metabolites and 13 metabolic hormones in 500 overweight/obese adults who participated in the Weight Loss Maintenance trial. Principal-components analysis (PCA) was used for reduction of metabolite data. Race and sex-stratified comparisons of metabolite factors and metabolic hormones were performed. African Americans represented 37.4% of the study participants, and females 63.0%. Of thirteen metabolite factors identified, three differed by race and sex: levels of factor 3 (branched-chain amino acids and related metabolites, $p < 0.0001$), factor 6 (long-chain acylcarnitines, $p < 0.01$), and factor 2 (medium-chain dicarboxylated acylcarnitines, $p < 0.0001$) were higher in males vs. females; factor 6 levels were higher in Caucasians vs. African Americans ($p < 0.0001$). Significant differences were also observed in hormones regulating body weight homeostasis. Among overweight/obese adults, there are significant race and sex differences in small-molecule metabolites and metabolic hormones; these differences may contribute to risk factor heterogeneity across race and sex subgroups and should be considered in future investigations with circulating metabolites and metabolic hormones.

Introduction

OVER ONE BILLION PEOPLE in the world are overweight, and approximately one-third of these people are classified as obese (WHO, 2004). In the United States, obesity poses a public health challenge across all major population demographic subgroups, including race and sex-based subgroups (Flegal et al., 2012). Although a major cause of the obesity epidemic in all these subgroups is widely recognized to be a result of an energy imbalance, as the population consumes more calories than it expends, the propensity to develop obesity and obesity-related health consequences varies across race and sex subgroups. For instance, Caucasians and males have lower rates of obesity compared to African Americans and females, respectively; however, obese males have more visceral adiposity compared to females, particularly before menopause, and obese Caucasians have more atherogenic lipid profiles compared to African Americans (Cossrow and Falkner, 2004; Lovejoy and Sainsbury, 2009).

Understanding the factors contributing to these race and sex differences may help reduce disparities in outcomes observed across race- and sex-based subgroups. The underlying determinants of these differences are likely a combination of biologic and environmental factors, particularly regarding the differences observed across race subgroups, which poorly categorize an individual's unique ancestry and only weakly relate to biologic differences. In order to improve our mechanistic and clinical understanding of such differences, a more complete understanding of the metabolic differences across race- and sex-based subgroups is needed.

One emergent technology that provides the ability to investigate metabolic differences across race- and sex-based subgroups is the field of metabolomics. Metabolomics can be described as the comprehensive measurement of small-molecule metabolites, providing insight into changes in chemical "signature" that result from specific cellular processes and environmental exposures. Recent studies using metabolomics have identified novel metabolic biomarkers and mechanisms for insulin resistance, type 2 diabetes mellitus, coronary artery

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disease, and incident cardiovascular (CV) events (Huffman et al., 2009; Newgard et al., 2009; Shah et al., 2010, 2012a, 2012b, 2012c; Wang et al., 2011). One recent study found differences in metabolite levels and their underlying genetic associations between males and females in a population-based cohort (Mittelstrass et al., 2011).

The study of metabolic hormones may also provide unique insights into the metabolic differences associated with obesity across race- and sex-based subgroups. Indeed, prior studies have observed race and sex differences in select metabolic hormones such as leptin, insulin, and adiponectin (Basu et al., 2006; Bottner et al., 2004; Lu et al., 2007; Ong et al., 2006; Ostlund et al., 1996; Ryan et al., 2002).

However, no prior studies have performed a comprehensive evaluation of differences in circulating small-molecule metabolites across both race- and sex-based subgroups. Additionally, no studies have investigated such differences with a more comprehensive panel of metabolic hormones in a large overweight and obese population. The results of such analyses could inform our understanding of mechanistic differences by race and sex for cardiometabolic disease risk and could also provide critical information regarding identification of potential confounders and guidance for adjustment for race and sex differences in these profiles.

Therefore, in this study, we set out to describe the basic metabolic characteristics of an overweight and obese population in the Weight Loss Maintenance (WLM) clinical trial by race and sex subgroups (Svetkey et al., 2008). We describe not only conventional cardiometabolic risk factors, such as hypertension and dyslipidemia, but also evaluate an extensive panel of key metabolic hormones from adipose tissue, the central nervous system, and the gastrointestinal tract, and use mass spectrometry-based profiling to measure numerous intermediary metabolites, including amino acids, acylcarnitines, and free fatty acids.

Materials and Methods

Study participants

This study was ancillary to the WLM trial (Svetkey et al., 2008). As such, all analyses were conducted by the authors rather than by the WLM coordinating center. Details of the WLM study design have been reported previously (Svetkey et al., 2008). In brief, WLM was a two-phase multicenter trial studying different strategies for the maintenance of weight loss. To be eligible for WLM, individuals had to be overweight or obese (body mass index [BMI] 25–45 kg/m²), 25 years or older, and taking medication for hypertension and/or dyslipidemia. Major exclusion criteria included diabetes mellitus requiring pharmacologic therapy, a recent CV event or other medical or psychiatric conditions that would preclude full participation in the study, weight loss greater than 9 kg in the preceding 3 months, recent use of weight-loss medications, and prior weight loss surgery. Phase 1 consisted of a 6-month nonrandomized behavioral weight loss intervention standardized across all eligible participants. Participants losing at least 4 kg during this first phase were then randomized into one of three weight loss maintenance strategies in Phase 2, delivered over a 30-month period. The primary results of the overall WLM trial have been published (Svetkey et al., 2008). For this study, we randomly selected 500 participants who had lost at least 4 kg of weight in Phase 1 and were thus

randomized into Phase 2, and who had available biological samples for molecular analysis available at all time points, as previously described (Shah et al., 2012a). Metabolic profiling presented herein was performed on samples from these participants at entry into Phase 1 prior to the initiation of any weight-loss interventions. For purposes of this ancillary study, participants were categorized into subgroups based on self-reported race (Caucasian or African American) and sex.

Measurements

Demographics and health behavior. Age, race, sex, diet, smoking, and alcohol intake were assessed by self-report. Smokers were characterized as never, former, or current smokers. Alcohol intake was characterized as non-drinkers or drinkers. Diet was assessed using the Block Food Frequency questionnaire and reported as the Healthy Eating Index (HEI) (Block and Subbar, 1992). The HEI summarizes the overall dietary quality and has been associated with health outcomes (Guenther et al., 2005; McCullough et al., 2002). Physical activity levels were measured by having participants wear a tri-axial accelerometer for at least 10 hours per day for at least 4 days, including 1 weekend day. Accelerometer data were used to estimate total weekly minutes of moderate to vigorous physical activity (MVPA) (Chen et al., 2009; Jerome et al., 2009).

Physiologic characteristics. Height was measured at entry using a calibrated, wall-mounted stadiometer. Weight at entry was measured in duplicate with participants wearing light indoor clothing and using a calibrated digital scale. The BMI was subsequently calculated using the Quetelet index (kg/m²). Hypertension was defined by self-report of medication use, and types of anti-hypertensive agents used were recorded. Dyslipidemia was defined by self-report of medication use, and the types of agents used were recorded.

Biochemical measurements

Fasting glucose was measured as part of this sub-study and was used for identifying individuals with impaired fasting glucose (fasting plasma glucose 100–125 mg/dL). A Beckman-Coulter DxC600 clinical analyzer (Brea, CA) was used for analysis of glucose and highly-sensitive C-reactive protein (CRP) in EDTA-plasma (reagents also from Beckman). CRP values >3 mg/L were defined as abnormal.

Hormone analysis. Insulin, leptin, glucagon, and adiponectin were measured via electrochemiluminescent plate assay using an SI-2400 imager and reagents from Meso Scale Discovery (Gaithersburg, MD). Other enzyme-linked immunosorbent assays (ELISA) were performed on a Molecular Devices SpectraMax M2e platereader (Sunnyvale, CA) using kits for ghrelin and peptide YY (PYY) from Millipore (St. Charles, MO) and agouti-related protein (AgRP) from R&D Systems (Minneapolis, MN). Neuropeptide Y (NPY) was measured by radioimmunoassay (RIA) using a Wallac Wizard 1470-010 (Perkin-Elmer, Boston, MA) automatic gamma counter and reagent kits from Alpco (Salem, NH). Insulin-like growth factor binding proteins-1, -2, and -3 (IGFBP-1, -2, -3) were measured on a multiplex plate assay using a Searchlight chemiluminescent imager and assay kits from Thermo-Fisher (Woburn, MA).

Mass spectrometry-based metabolic profiling. Mass spectrometry was used to assay select acylcarnitines and amino acids, as previously described in detail (Newgard et al., 2009). Liquid-handling steps were routinely performed on a Genesis RSP 150/4 robotic sample processor (Tecan AG, Männedorf, Switzerland). Quantitative measurement of targeted analytes was achieved by spiking of plasma samples with cocktails of stable isotope-labeled standards specific to each assay module prior to sample extraction or other manipulations (Newgard et al., 2009). For the preparation of plasma acylcarnitines and amino acids, the proteins were first removed by precipitation with methanol. Aliquoted supernatant was dried and then esterified with hot, acidic methanol (acylcarnitines) or *n*-butanol (amino acids). Acylcarnitines and amino acids were then analyzed by tandem mass spectrometry (MS/MS) using a Quattro Micro instrument (Waters Corporation, Milford, MA).

Statistical analysis

Metabolites with >25% "0" values (i.e., those that were below the lower limits of quantification) were not analyzed further (consisting of two acylcarnitine species). Principal components analysis (PCA) was used to reduce the large number of correlated metabolites into clusters of fewer uncorrelated factors (Newgard et al., 2009; Shah et al., 2010; 2012b). Principal components analysis was performed using levels of total free fatty acids, ketones, β -hydroxybutyrate, 45 acylcarnitines, and 15 amino acids. Factors derived from PCA that had eigenvalues of greater than or equal to 1.0 were identified; the commonly used varimax rotation was performed to produce interpretable factors. Individual metabolites with a factor load ≥ 0.4 were reported as composing a given PCA-derived factor. Then, scoring coefficients were used to calculate baseline metabolite factor scores for each individual (consisting of the weighted sum of the standardized metabolites within that factor, weighted on the factor loading for each metabolite).

For analysis of metabolite factors and metabolic hormone levels, generalized linear regression models were constructed for each factor or hormone to assess the difference in factor/hormone levels between race and sex subgroups. Each model was adjusted for the other variable (i.e., race models were adjusted for sex and vice versa). Multivariable linear regression models were used to adjust for relevant clinical variables (age, race or sex, baseline BMI, HEI, MVPA, and alcohol intake) and to assess the independent relations of metabolite factors and metabolic hormone levels with race and sex subgroups. Continuous variables that were not normally distributed were log transformed to approximate normality. As all analyses were exploratory in nature and given the co-linearity of the metabolites, two-sided *p* values unadjusted for multiple comparisons are presented. Nominal statistical significance was defined as $p \leq 0.05$. Statistical analyses were performed using SAS version 9.1 (SAS Institute, Cary NC). This study was approved by the Duke University Institutional Review Board.

Results

The 500 individuals included in this analysis were randomly selected from 845 WLM participants who met our eligibility criteria (i.e., lost at least 4 kg in Phase I, were randomized into Phase 2, and had samples available at all

time points). This subset of individuals was similar in clinical characteristics to the overall WLM cohort (Shah et al., 2012a). Table 1 displays the baseline characteristics of these 500 participants, overall and by race and sex subgroups. Overall, the mean age of the population was 55.9 years (SD 8.7), the mean BMI at baseline (prior to weight loss) was 33.9 kg/m² (SD 4.7), 37.0% were males, and 62.6% were Caucasians. Caucasians were older than African Americans (mean age in years, 57.5 [SD 8.2] vs. 53.3 [SD 8.9], $p < 0.0001$), and males were older than females (mean age in years, 57.0 [SD 9.2] vs. 55.3 [SD 8.4], $p = 0.04$). Mean BMI was lower in Caucasians compared to African Americans (33.5 kg/m² [SD 4.6] vs. 34.7 kg/m² [SD 4.8], $p < 0.0001$) and males compared to females (mean BMI 34.2 kg/m² [SD 5.0] vs. 33.5 kg/m² [SD 4.1], $p < 0.001$).

Some traditional cardiometabolic risk factors were also found to differ across race and/or sex subgroups (Table 1). The prevalence of dyslipidemia was greater in Caucasians compared to African Americans (42.8% vs. 32.1%, $p = 0.02$) and males compared to females (46.5% vs. 34.3%, $p = 0.01$), respectively. In addition, males had a higher prevalence of impaired fasting glucose compared to females (48.1% vs. 32.7%, $p = 0.001$). Hypertension was highly prevalent in the overall population (87.6%), but no race or sex differences were observed with regard to this cardiometabolic risk factor.

Principal components analysis identified 13 factors, explaining 72.0% of the variance in the data. Individual metabolites clustered into factors reflective of plausible biological pathways and were similar in composition to our previous studies (Newgard et al., 2009; Shah et al., 2010, 2012a, 2012b). Table 2 displays the constituent individual small-molecule metabolites and the overall basic biologic descriptions of each of these factors. Given that factors 1–7 explain the majority of the variance of the data (59%) and each had Eigenvalues > 2.0 (factors with lower Eigenvalues explain less of the variability of the dataset), we chose to focus on factors 1–7 for the remainder of this publication.

Factors 1 and 7 are each composed of medium-chain acylcarnitines; factor 2 is comprised of medium-chain dicarboxylated acylcarnitines; factor 3 contains branched-chain amino acids (BCAA) and related metabolites; factor 4 contains ketone-related metabolites; factor 5 contains long-chain dicarboxylated acylcarnitines; and factor 6 contains long-chain acylcarnitines. Supplementary Figure S1 displays a heat map showing patterns of normalized metabolite factor levels across race and sex categories (Supplementary material is available online at www.liebertpub.com). Statistical comparisons of mean levels of factors 1–7, stratified by race and sex, are presented in Table 3. Statistically significant differences in levels of Factors 2, 3, and 6 were observed across race and/or sex subgroups. Specifically, males had higher levels of factors 2 and 3 when compared with females ($p < 0.0001$). For factor 6, both race and sex differences were observed. Caucasians had greater levels than African Americans ($p < 0.0001$) and males had greater levels than females ($p < 0.01$).

In addition to small-molecule metabolites, several race and sex differences were observed in metabolic hormones (Table 4). The levels of hormones involved in maintaining body weight homeostasis, namely AgRP, PYY, NPY, ghrelin, and leptin, were statistically significant different across race and/or sex subgroups. Compared to African Americans, Caucasians had higher levels of AgRP (74.8 pg/mL [SD 19.9] vs. 65.2 pg/mL [SD 17.6], $p < 0.0001$), higher levels of PYY

TABLE 1. BASELINE CHARACTERISTICS STRATIFIED BY RACE AND SEX SUBGROUPS

	All subjects N=500	Caucasian N=313	African American N=187	P value	Male N=185	Female N=315	P value
Age, years mean (SD)	55.9 (8.7)	57.5 (8.2)	53.3 (8.9)	<0.0001	57.0 (9.2)	55.3 (8.4)	0.04
BMI, kg/m ² mean (SD)	33.9 (4.7)	33.5 (4.6)	34.7 (4.8)	<0.0001	33.5 (4.1)	34.2 (5.0)	<0.001
Smoking, %				0.17			0.09
Never	0.6	54.0	62.6		54.1	59.1	
Former	38.8	41.9	33.7		43.8	35.9	
Current	4.0	4.2	3.7		2.2	5.1	
Alcohol Intake, %				<0.0001			0.06
Nondrinker	38.8	29.4	54.6		33.5	41.9	
Drinker	61.2	70.6	45.5		66.5	58.1	
HEI Score mean (SD)	62.1 (12.4)	63.7 (12.6)	59.5 (11.7)	<0.001	60.3 (12.0)	63.2 (12.6)	0.02
MVPA, min/week (SD)	123.1 (126.5)	133.3 (142.1)	105.6 (91.5)	0.08	162.4 (155.7)	99.5 (98.1)	<0.0001
Hypertension, %	87.6	85.6	90.9	0.08	85.4	88.9	0.25
Dyslipidemia, %	38.8	42.8	32.1	0.02	46.5	34.3	<0.01
Impaired fasting glucose, %	38.4	38.3	38.5	0.97	48.1	32.7	<0.001
Highly sensitive CRP ≥3 mg/L, %	47.8	43.5	55.1	0.01	27.0	60.0	<0.0001

BMI, body mass index; CRP, C-reactive protein; HEI, Healthy eating index; MVPA, moderate-vigorous intensity physical activity; SD, standard deviation.

(95.5 pg/mL [SD 52.4] vs. 71.4 pg/mL [SD 40.6], $p<0.0001$), and lower levels of NPY (67.1 pM [SD 29.1] vs. 77.8 pM [SD 34.7], $p<0.0001$). Compared to females, males had lower levels of ghrelin (761.3 pg/mL [SD 304.6] vs. 837.9 pg/mL [SD 291.0], $p=0.005$) and leptin (22946.3 ng/mL [SD 17853.9] vs. 67274.0 ng/mL [SD 36410.6], $p<0.0001$).

All statistically significant associations persisted after adjusting for age, race (for sex comparisons), sex (for race comparisons), BMI, HEI, MVPA, and alcohol intake (Tables 3 and 4).

Discussion

In this study, we report that in a diverse population of overweight and obese adults, levels of small-molecule me-

tabolites and metabolic hormones differ by race and sex. The significant differences in levels are independent of race (for sex comparisons), sex (for race comparisons), age, BMI, and other lifestyle factors. These small-molecule metabolites report on multiple pathways of lipid, protein, and glucose metabolism. Differences in these circulating metabolites and metabolic hormones likely reflect a combination of biologic and environmental differences across race and sex-based subgroups and may have important implications in the understanding of race and sex differences that have been observed in the epidemiology of obesity and obesity-related CV risk factors and disease.

While several differences in metabolic profiles across race and sex subgroups were noted, there were three major

TABLE 2. FACTORS OF SMALL-MOLECULE METABOLITES IDENTIFIED THROUGH PRINCIPAL COMPONENTS ANALYSIS

Factors	Metabolites within factor	Description	Eigenvalue	Variance	Cumulative variance
1	C8, C10:1, C10, C10-OH:C8-DC, C12:1, C12, C14:2, C14:1, C14, C8:1-DC, C16:2, C16:1 acylcarnitines	Medium-chain acylcarnitines	14.94	0.25	0.25
2	Ci4-DC/C4-DC, C6-DC, C10-OH/C8-DC, C12:1, C12-OH/C10-DC, C14:1-OH/C12:1-DC, C14-OH/C12-DC, C8:1-DC	Medium-chain dicarboxylated acylcarnitines	6.21	0.11	0.36
3	Ala, Pro, Val, Leu/Ile, Met, Phe, Tyr, Glx, Orn	Branched-chain amino acid related	3.58	0.06	0.42
4	C2, C4-OH, C16:1, Total Ketones, 3-OH Butyrate, Nonesterified Fatty Acid	Ketone-related	3.34	0.06	0.48
5	C18:1-OH/C16:1-DC, C18-OH/C16-DC, C20, C20:1-OH/C18:1-DC, C20-OH/C18-DC	Long-chain dicarboxyl-acylcarnitines	2.36	0.04	0.52
6	C14, C16, C18:1, C18, C16:1-OH/C14:1-DC	Long-chain acylcarnitines	2.21	0.04	0.56
7	C8:1, C10:3, C10:2	Medium-chain acylcarnitines	2.05	0.03	0.59
8	C3, C4/Ci4, C5s	Short-chain acylcarnitines	1.76	0.03	0.62
9	C18:2, C18:1, C20:4	Long-chain acylcarnitines	1.40	0.02	0.64
10	Gly, Ser, Arg	Amino acids	1.25	0.02	0.66
11	Orn, Cit, C5-DC	Urea cycle	1.16	0.02	0.68
12	C5:1	Miscellaneous	1.10	0.02	0.70
13	His (-), Asx	Miscellaneous	1.07	0.02	0.72

TABLE 3. RACE AND SEX COMPARISONS OF KEY PCA-DERIVED FACTORS OF SMALL MOLECULE METABOLITES

	Caucasian N=313	African American N=187	P value*	P value**	Men N=185	Women N=315	P value***	P value****
Factor 1 Medium-chain acylcarnitines, (SD)	0.03 (1.14)	-0.05 (0.70)	0.31	0.41	-0.07 (0.72)	0.04 (1.13)	0.21	0.23
Factor 2 Medium-chain dicarboxylated acylcarnitines, (SD)	0.03 (1.02)	-0.05 (0.97)	0.79	0.23	0.29 (1.13)	-0.17 (0.87)	<0.0001	<0.0001
Factor 3 Branched-chain amino acid related, (SD)	0.03 (1.03)	-0.04 (0.94)	0.86	0.97	0.50 (0.96)	-0.29 (0.91)	<0.0001	<0.0001
Factor 4 Ketone-related, (SD)	-0.02 (1.05)	0.03 (0.92)	0.79	0.62	-0.13 (1.15)	0.07 (0.90)	0.04	0.03
Factor 5 Long-chain dicarboxylated acylcarnitines, (SD)	0.03 (1.20)	-0.05 (0.52)	0.49	0.12	0.10 (0.73)	-0.06 (1.13)	0.11	0.20
Factor 6 Long-chain acylcarnitines, (SD)	0.19 (0.99)	-0.32 (0.93)	<0.0001	<0.0001	0.20 (0.93)	-0.12 (1.02)	<0.01	0.01
Factor 7 Medium-chain acylcarnitines, (SD)	-0.02 (1.00)	0.03 (1.00)	0.79	0.96	-0.11 (1.06)	0.07 (0.96)	0.06	0.06

PCA, principal components analysis; SD, standard deviation.

*adjusted for sex; **adjusted for sex, age, body mass index, healthy eating index, moderate to vigorous physical activity, and alcohol intake; ***adjusted for race; ****adjusted for race, age, body mass index, healthy eating index, moderate to vigorous physical activity, and alcohol intake.

observations that will be highlighted in this discussion. First, BCAA and related metabolites (factor 3 in this analysis), which have recently emerged as key metabolites related to insulin resistance and CV disease risks (Huffman et al., 2009; Newgard et al., 2009; Shah et al., 2010; 2012a; Wang et al., 2011), were found to be higher in males than females. Second, other small-molecule metabolites, such as long-chain acylcarnitines (factor 6) and medium-chain dicarboxylated acylcarnitines (factor 2), were found to differ by race and/or sex. And third, metabolic hormones regulating body weight were significantly different in race and/or sex subgroups. These three profiles are discussed below.

Branched-chain amino acids and related metabolites

In several prior studies on metabolic factors underlying CV disease and insulin resistance, we have reported principal components comprised of the BCAA and related metabolites, including the three BCAA (valine, leucine/isoleucine), glutamate (a product of the first step in BCAA catabolism), alanine, and the aromatic amino acids phenylalanine and tyrosine (Newgard et al., 2009; Shah et al., 2010). High-throughput metabolic profiling studies of targeted small-molecule metabolites have recently identified these metabolites as novel biomarkers for insulin resistance (Huffman et al., 2009; Newgard et al., 2009; Wang et al., 2011) and CV disease risk (Shah et al., 2010; 2012b), as well as reporting on novel mechanisms of disease development (Newgard et al., 2009) and markers of disease states (Lewis et al., 2008). We have also shown that these metabolites are highly heritable in families with premature coronary artery disease (Shah et al., 2009).

While these emerging studies provide evidence to support links between BCAA and insulin resistance and CV disease, questions remain as to whether BCAA are in the causal pathway of cardiometabolic disease or simply a marker of risk for these diseases. In fact, other metabolic diseases associated with increased BCAA levels, such as maple syrup urine disease, do not have these relationships. Maple syrup urine disease, also known as branched chain ketoaciduria, is caused by a genetic deficiency in branched-chain alpha-ketoacid dehydrogenase complex, a key enzyme in the degradation pathway of BCAA, and is characterized clinically by psychomotor retardation, feeding problems, and a maple syrup odor of the urine. Interestingly, rather than insulin resistance, there have been reports of hypoglycemia in patients with maple syrup urine disease, which has been speculated to be related to impairments in gluconeogenesis (Haymond et al., 1973).

The differences observed for this rare Mendelian disease versus the consistent observation of BCAA elevation in common insulin resistance may be due to differences in the degree of BCAA elevation. The increase in BCAA that is observed in obesity-related disorders is on the order of 50%, whereas maple syrup urine disease and transgenic mouse models cause increases of many fold. When such large changes happen, they are often accompanied by protein futile cycling (due to more shuttling of BCAA to protein synthesis, which is ATP consuming), and this masks the possible deleterious effects of BCAA on metabolic homeostasis that is observed in obesity-related conditions. In addition, the differences observed between maple syrup urine disease and obesity-related diseases may also be related to where in the

TABLE 4. RACE AND SEX COMPARISONS OF METABOLIC HORMONES

	Caucasian N = 313	African American N = 187	P value*	P value**	Male N = 185	Female N = 315	P value***	P value****
Glucagon, pg/mL (SD)	32.7 (35.0)	30.9 (47.0)	0.22	0.06	34.5 (45.4)	30.6 (36.2)	0.20	0.08
IGF-1, ng/mL (SD)	151.5 (68.6)	155.1 (71.7)	0.48	0.61	160.2 (66.4)	148.5 (71.3)	0.02	0.01
IGFBP-1, pg/mL (SD)	13430.1 (15274.6)	12159.3 (14997.4)	0.67	0.82	11356.5 (11451.9)	13895.8 (16918.9)	0.85	0.93
IGFBP-2, pg/mL (SD)	313575.8 (216080.8)	271022.1 (186206.6)	0.01	0.03	299020.4 (192497.1)	297054.1 (214317.5)	0.71	0.78
IGFBP-3, pg/mL (SD)	354936.2 (117159.5)	345042.5 (105723.3)	0.35	0.61	338253.8 (111717.3)	358877.3 (113295.9)	0.02	0.01
Insulin, μ UI/mL (SD)	396.0 (286.6)	423.5 (230.5)	0.03	0.70	464.3 (336.3)	372.5 (210.5)	<0.0001	<0.0001
Adiponectin (SD)	25260.3 (17977.4)	17531.4 (8993.5)	<0.0001	<0.0001	16841.3 (10189.5)	25587.6 (17365.0)	<0.0001	<0.0001
AgRP, pg/mL (SD)	74.8 (19.9)	65.2 (17.6)	<0.0001	<0.0001	74.9 (21.9)	69.1 (17.9)	0.02	0.01
Neuropeptide Y, pM (SD)	67.1 (29.1)	77.8 (34.7)	<0.0001	<0.0001	69.2 (30.6)	72.2 (32.3)	0.35	0.51
Peptide YY, pg/mL (SD)	95.5 (52.4)	71.4 (40.6)	<0.0001	<0.0001	93.4 (51.0)	82.5 (48.5)	0.03	0.03
Ghrelin, pg/mL (SD)	835.5 (308.0)	765.7 (276.1)	0.06	0.29	761.3 (304.6)	837.9 (291.0)	<0.01	0.01
Leptin, ng/mL (SD)	48624.0 (37430.8)	37586.3 (37586.3)	0.37	0.55	22946.3 (17853.9)	67274.0 (36410.6)	<0.0001	<0.0001

AgRP, Agouti-related protein; IGF-1, insulin-like growth factor binding protein-1; IGBP-2, insulin-like growth factor binding protein-2; IGBP-3, insulin-like growth factor binding protein-3; SD, standard deviation.

*adjusted for sex; **adjusted for sex, age, body mass index, healthy eating index, moderate to vigorous physical activity, and alcohol intake; ***adjusted for race; ****adjusted for race, age, body mass index, healthy eating index, moderate to vigorous physical activity, and alcohol intake.

BCAA pathway the pathology is manifest. Our previous work has shown a positive correlation between BCAA mitochondrial catabolite (C3 and C5 acylcarnitines) levels and BCAA levels (Huffman et al. 2009; Newgard et al. 2009), with both being associated with insulin resistance, suggesting that the pathologic mechanism for BCAA in common insulin resistance is through increased flux through the BCAA catabolic pathway, as opposed to the more proximal enzymatic deficit seen in maple syrup urine disease.

Few prior studies have evaluated the associations between demographic groups and BCAA levels. In a study of Chinese and Asian-Indian males with a mean BMI of 24 mg/kg², an association was observed between similar BCAA factors and insulin resistance (Tai et al., 2010). However, prior studies have not fully investigated race and sex influences on this metabolite cluster.

In our study, which included 500 overweight and obese individuals without diabetes mellitus, a similar BCAA metabolite factor was identified (factor 3) and was shown to have significantly higher levels in males as compared to females (i.e., males were found to have higher levels of BCAA and related metabolites, even after adjusting for baseline BMI and race). Consistent with our previous work showing association between peripheral blood BCAA levels and insulin resistance, in this study, males were more insulin resistant (i.e., higher HOMA-IR levels) and had higher fasting glucose and insulin levels when compared with females.

Our previous studies implicate BCAA directly in the pathogenesis of insulin resistance (and not just as bystanders in the disease process), including a feeding study in rats in which BCAA supplementation of a high-fat diet was shown to contribute to the development of obesity-associated insulin resistance (Newgard et al., 2009). Therefore, the sex-related heterogeneity we observe here in BCAA levels could be reflective of sex-related differences in BCAA intake and/or catabolism. To investigate this, we further adjusted models for BCAA dietary intake (as reported in the food frequency questionnaire), which showed no change in the association between factor 3 levels and sex (type III sum of squares 64.57 for full model without BCAA intake vs. 60.25 for full model including BCAA intake, both $p < 0.0001$), suggesting that the differences observed in peripheral blood BCAA levels are reporting on sex-related heterogeneity in BCAA catabolism. These results may be highlighting mechanistic underpinnings for differences in insulin resistance in males versus females.

Acylcarnitines and related metabolites

Acylcarnitines are derived from mitochondrial and peroxisomal acyl-CoA metabolites by substitution of the carnitine moiety for CoA. Factor 6 was comprised of long-chain acylcarnitines and in our study was found to be elevated in Caucasians as compared with African Americans and in males as compared with females. These metabolites are produced in the early stages of mitochondrial fatty acid oxidation. Their accumulation has previously been associated with skeletal muscle insulin resistance in both animal models and humans (Kien et al., 2011; Koves et al., 2008), and interventions that lower the levels of these metabolites, such as exercise or carnitine supplementation, simultaneously improve insulin sensitivity (Koves et al., 2005; Makowski et al., 2009; Noland et al., 2009). Higher levels of long-chain acylcarnitines

in males as compared with females may be reporting on impaired glucose homeostasis as evidenced by greater insulin resistance in males in our study as compared with females. Higher levels of these metabolites in Caucasians as compared with African Americans may also be related to racial differences in dyslipidemia (Cossrow and Falkner, 2004).

Another factor that was significantly elevated in males as compared with females was factor 2, comprised of medium-chain dicarboxylated acylcarnitines. Dicarboxylated acylcarnitines can be derived from mitochondrial lipid or amino acid oxidation or via omega-oxidation through P450 enzymes of the endoplasmic reticulum, followed by metabolism of these modified fatty acids to smaller CoA species in peroxisomes and their equilibration with cognate acylcarnitine species (Ferdinandusse et al., 2004). Interestingly, we have recently described a strong association of a similar small to medium-chain dicarboxylated acylcarnitine cluster with CV events (myocardial infarction and death) in individuals with coronary artery disease (Shah et al., 2010; 2012b). Given the known relationship between male sex and increased risk of CV events, especially in this relatively younger population, our results could be reporting on mechanistic heterogeneity in CV event risk by sex. The precise substrates and pathways that generate the dicarboxylated acylcarnitine metabolites and the specific factors that foster their production are currently under investigation.

Metabolic hormones regulating body weight homeostasis

In addition to circulating metabolites, our study observed race and sex differences in key metabolic hormones involved in regulating body weight homeostasis. Body weight homeostasis is evident in virtually all species after organisms reach a mature age (Kessey and Powley, 1986). While many factors help to accomplish body weight homeostasis, metabolic hormones play key roles in regulating appetite by moderating sensations of hunger and satiety and also in regulating energy expenditure by modulating the basal metabolic rate, the heat increment associated with food digestion, and physical activity levels (Kessey and Powley, 1986).

Our results demonstrate that levels of NPY, AgRP, and ghrelin (hormones that prevent weight loss) and PYY and leptin (hormones that prevent weight gain) vary across race and/or sex-based subgroups. The observed relationships do not provide clear-cut explanations for race or sex differences in obesity. For example, one hormone that prevents weight loss is higher in African Americans (NPY), one is higher in Caucasians (AgRP), and a third does not differ by race (ghrelin). Similarly, compared to men, women had higher levels of both leptin and ghrelin, which have opposite effects on appetite. Thus, the roles played by these hormones and their regulation in weight control require further study. Our data are consistent with the few prior studies that have investigated race and sex differences in a large panel of metabolic hormones. For instance, similar to our results, Bacha and Arslanian showed that PYY levels were elevated in Caucasians compared to African Americans (Bacha and Arslanian, 2006; Davis et al., 2005). Also, reduced levels of ghrelin and leptin in males compared to females have been observed previously (Makovey et al., 2007; Ostlund et al., 1996; Wong et al., 2004). Furthermore, consistent with prior studies, we

found higher insulin levels in men compared to women and higher adiponectin levels in Caucasians compared to African Americans (Basu et al., 2006; Bottner et al., 2004; Lu et al., 2007; Ong et al., 2006; Ryan et al., 2002).

Our findings of race and sex differences in hormones regulating body weight homeostasis suggest the possibility of unique mechanisms of weight regulation and variable propensities to develop obesity and obesity-related diseases across race and sex subgroups.

Broader implications of race differences

The race differences observed in this study should be interpreted cautiously, given the limitations of this demographic construct. While race is often used as a proxy for ancestral differences, significant genetic heterogeneity exists within race subgroups, particularly among African Americans (Gravlee, 2009). Similar concerns exist for demographic subgroups based upon ethnicity (generally used to distinguish cultural groups such as Hispanics vs. non-Hispanics). As a result, any race differences observed in this study cannot be strictly related to biologic differences and associated environmental factors across race-based subgroups must be accounted for. In our analyses, we found no differences in smoking across race groups and the metabolic differences observed in our study remained after adjustment for dietary, physical activity, and alcohol patterns. Future analyses will need to account more extensively for other environmental factors, such as stress, sleep patterns, and a broad array of social determinants of health. Differences between race groups require additional caution when considering potential treatment implications. While targeted, "personalized" intervention may be desirable, it is equally important to avoid racial stereotyping, even with regard to biological functions.

Study limitations

Certain study limitations need consideration when interpreting the results. First, the cross-sectional design of this analysis allows only for an analysis of associations and not cause and effect. As a result, the differences in small-molecule metabolites and metabolic hormones across race and sex subgroups may not be related to differences in cardiometabolic risk factors. Further, all samples were obtained in the fasting state, so race and sex differences in the postprandial excursion of these variables could not be determined.

Second, while this study performed one of the largest and most comprehensive analyses of metabolites in overweight and obese individuals, it targeted small-molecule metabolites; as a result, certain metabolites were not evaluated in this study. Third, given the exploratory nature of this study, there could be both false-positive and false-negative findings; however, the use of PCA allowed for an unbiased approach to investigating the large number of small-molecule metabolites and helped mitigate the effects of multiple comparisons inherent in investigating this large number of metabolites. Of note, our results for factor 3 would survive the highly conservative Bonferroni correction for multiple comparisons.

Fourth, this study did not address how other influences on metabolism (e.g., menopausal status) may influence the investigated metabolites and hormones. However, our study findings were independent of potential confounders such as age, BMI, diet, physical activity, and alcohol intake,

suggesting that these observed race and sex differences may be due to biological differences between the metabolism of males versus females and Caucasians versus African Americans. Prior studies showing the influence of genetics on metabolism also support the presence of inherent biological differences in metabolism (Gieger et al., 2008). Further, interactions between the environment and sex, race, and genes have yet to be adequately evaluated; such interactions may also be contributing to the heterogeneity of race and sex differences in metabolism and should be investigated in future studies.

Fifth, although the study population was large, diverse, and randomly selected from among WLM participants, it was not a random sample of the population at large. Indeed, prior analyses have revealed that sex differences in certain aspects of metabolism only manifest in patients who are obese (Würtz et al., 2012). Validation of our results is warranted before widely generalizing study findings.

Conclusions

While race and sex differences in the prevalence of obesity and its health consequences have long been appreciated, a poor understanding has remained for why these differences exist. Our results show that these demographic differences in obesity are associated with and may be explained by differences in levels of several small-molecule metabolites such as BCAA and metabolic hormones. These findings extend prior observations of race and/or sex-based differences in certain metabolites and metabolic hormones, and they bring to light newly identified differences in other metabolic parameters. Findings such as these highlight the need to consider demographics when evaluating the role of small-molecule metabolites and metabolic hormones in health and disease. More investigation is needed to validate these findings in larger cohorts and to elucidate their mechanistic and clinical significance.

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