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Profiling of plasma metabolites in postmenopausal women with metabolic syndrome

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Abstract

Objective: The aim of the study was to investigate the associations of amino acids and other polar metabolites with metabolic syndrome (MetS) in postmenopausal women in a lean Asian population.

Methods: The participants were 1,422 female residents enrolled in a cohort study from April to August 2012. MetS was defined according to the National Cholesterol Education Program Adult Treatment Panel III modified for Japanese women. Associations were examined between MetS and 78 metabolites assayed in fasting plasma samples using capillary electrophoresis-mass spectrometry. Replication analysis was performed to confirm the robustness of the results in a separate population created by random allocation.

Results: Analysis was performed for 877 naturally postmenopausal women, including 594 in the original population and 283 in the replication population. The average age, body mass index, and levels of high- and low-density lipoprotein cholesterol of the entire population were 64.6 years, 23.0 kg/m^2, 72.1 mg/dL, and 126.1 mg/dL, respectively. There was no significant difference in low-density lipoprotein cholesterol levels between women with and without MetS. Thirteen metabolites were significantly related to MetS: multiple plasma amino acids were elevated in women with MetS, including branched-chain amino acids, alanine, glutamate, and proline; and alpha-aminoadipate, which is generated by lysine degradation, was also significantly increased.

Conclusions: Our large-scale metabolomic profiling indicates that Japanese postmenopausal women with MetS have abnormal polar metabolites, suggesting altered catabolic pathways. These results may help to understand metabolic disturbance, including in persons with normal body mass index and relatively high levels of high-density lipoprotein cholesterol, and may have clinical utility based on further studies.

Key Words: Amino acids – Aminoadipate – Branched-chain amino acids – Menopause – Metabolic syndrome – Metabolomics.

Metabolic syndrome (MetS) is a cluster of conditions that increase the risk of development of cardiovascular disease (CVD) as a major residual risk factor beyond low-density lipoprotein cholesterol (LDL-C). Each of the five components of MetS, abdominal obesity, high blood pressure (BP), elevated triglycerides (TG), decreased high-density lipoprotein cholesterol (HDL-C), and elevated glucose is an independent predictor for CVD. Given that CVD accounts for one of every three deaths in the United States,[1] tackling MetS is a key to CVD prevention.

The risk of CVD attributed to MetS is reported to be higher in women than in men.[2-5] A large Japanese cohort study showed that MetS in women had a stronger association with future coronary artery disease than LDL-C levels.[6] In women,

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loss of estrogen with menopause promotes accumulation of visceral fat and changes in lipid metabolism,[7,8] putting women after menopause at greater risk of MetS and thus CVD. As the average life expectancy continues to increase around the globe, implementation of preventive strategies to decrease or delay MetS among postmenopausal women is crucial for graceful aging and maintenance of independent living without disability.

Recently, metabolomics has been increasingly applied to disease research, including CVD.[9] Measurement of multiple small-molecule metabolites, such as sugars, lipids, fatty acids, amino acids, and cofactors, which are downstream products of genetic and environmental variations, provides an integrated measure of phenotypes, and thus leads to identification of mechanisms and biomarkers of many diseases. Low sample throughput and data preprocessing remain major hindrances, but improvements in sample preparation and automated data processing along with reductions in cost have allowed adaptation of metabolomics to large-scale epidemiological studies.[10-14]

Evidence from such studies has suggested that amino acids play key roles in obesity-related diseases. For example, amino acids are independently associated with CVD from conventional factors.[15-20] Elevation of plasma amino acids precedes any alteration in insulin action detectable by standard measures, and their associations with future diabetes are stronger than those of age or body mass index (BMI).[21] A recent metabolomics study highlighted the ability to distinguish between healthy obese and unhealthy obese participants after a caloric challenge using amino acid profiles.[22] Interestingly, 6.2% of women in the United States were reported to have MetS even at normal weight, that is BMI of 18.5 to $<25\ kg/m^2$.[23] This is referred to as "metabolically obese, normal weight (MONW)" and individuals with this condition have a higher CVD risk.[24-27] Because MONW is often undetected and thus untreated for years because of the normal BMI, identifying individuals with MONW is important for early preventive measures. Most female participants in previous studies, however, have been overweight or obese, and whether such metabolic changes appear in women with normal weight has not been explored. In addition, these issues have not been investigated in postmenopausal women. There have been a few studies showing the association of altered amino acid metabolism with insulin resistance in normal-weight men.[28,29]

We thus performed a large-scale metabolomic profiling in Japanese postmenopausal women, which is a unique population with lean body mass, relatively high HDL-C, and a lower incidence of coronary artery disease than that in the Western populations.[30] The goal of the study was to reveal the associations of amino acids and other polar metabolites with MetS. Capillary electrophoresis-mass spectrometry (CE-MS) was used for metabolomics measurements. There are several technologies used to analyze metabolites, including nuclear magnetic resonance, gas chromatography-mass spectrometry, liquid chromatography-mass spectrometry, and CE-MS. Although CE-MS is performed less frequently compared with the other methods and has limitations of metabolome coverage, including water-insoluble lipids and neutral steroids, its superiority in measuring charged metabolites including amino acids simultaneously and its extremely high resolution made this method suitable for use in the current study.[31]

METHODS

Study population

The study base was the participants of the ongoing Tsuruoka Metabolomic Cohort Study, initiated in April 2012 (Yamagata Prefecture, Japan), comprising individuals aged 35 to 74 years who were recruited among attendees of annual municipal or worksite health check-up programs in the city. A total of 1,422 women consented to participate during the first few months of the baseline period, and plasma metabolomic profiling in these women was completed by the end of 2014. Only postmenopausal female participants were ultimately included in the analysis due to the small number of premenopausal women. Because there are known metabolic differences compared with natural menopause, participants with surgical or medical menopause were excluded.[32] In addition, women who had used hormone therapy within 1 year or had undergone oophorectomy after menopause were excluded to eliminate the possible effect on the metabolic profile of administered estrogen and hormones excreted from postmenopausal ovaries.[33] Menopause was defined as having ceased menstruation for at least 1 year. A detailed flow of the participants is shown in Figure 1. The study was approved by the Medical Ethics Committee of the School of Medicine, Keio University, Tokyo, Japan (Approval No. 20110264), and all participants gave voluntary written informed consent.

Data collection, anthropometric measurements, and biochemical examinations

All data and samples were obtained in the baseline study, including anthropometrics, clinical biochemistry, and blood specimens for metabolomic profiling. Detailed information was collected through a standardized self-administered questionnaire on medical history, including treatment of hypertension, dyslipidemia, and diabetes; gynecological and reproductive history, including menopause status (pre/peri/post), ages at menarche and menopause, reason for menopause (natural/medical/others), number of pregnancies and deliveries, age at first delivery, use of hormone therapy (if yes, number of years used and when it was used last), and whether oophorectomy was ever performed; and lifestyle parameters such as smoking habits, alcohol intake, diet, stress, and physical activity. Anthropometric measurements included body weight, height, and waist circumference (WC). WC was measured to the nearest 0.1 cm at the umbilicus at the end of a normal breath. If the umbilicus drooped down, the measurement was made midway between the inferior margin of the last rib and the top of the iliac crest in a horizontal plane. BP was measured twice on one occasion in the sitting position using an automated sphygmomanometer (Omron HBP-T105S-N), and the mean of the two measurements was used for analysis.

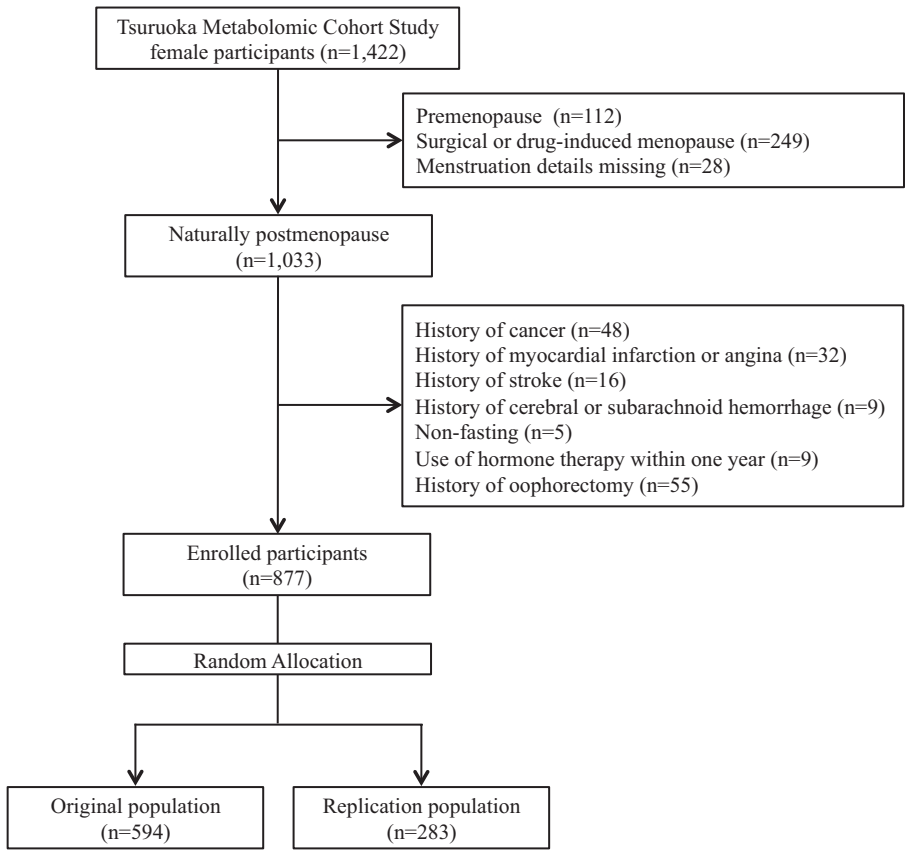

FIG. 1. Flow diagram of included and excluded participants.

Blood samples were collected in the morning between 8:30 and 10:30 after overnight fasting to avoid variation due to fasting and circadian rhythm. Plasma samples were collected with ethylenediaminetetraacetic acid-2Na as an anticoagulant and kept at 4°C immediately after collection. The samples were centrifuged for 15 minutes (1,500*g* at 4°C) within 1 hour of collection, divided into aliquots, and kept for a maximum of 6 hours at 4°C until extraction of metabolites. Serum samples were collected with serum-separating medium and kept at room temperature after collection. Serum levels of total cholesterol, TG, and fasting plasma glucose were analyzed using enzymatic methods, and glycated hemoglobin (HbA1c) was determined by immunoassay. HDL-C values were measured by a direct method. LDL-C levels were calculated using the Friedwald equation.[34]

Definition of MetS

MetS was defined using the modified National Cholesterol Education Program Adult Treatment Panel III definition with 100 mg/dL as the cutoff for the glucose level.[35] Because lower overall adiposity has been associated with an increased risk of medical conditions such as type 2 diabetes in Asian countries,[36] the threshold for WC was set according to the recommendation for Asians established by several organizations, including the National Cholesterol Education Program.[37,38] Specifically, central obesity was defined as WC at least 80 cm, high BP as mean systolic/diastolic BP at least 130/85 mm Hg or currently on antihypertensive therapy, high serum TG as at least 1.7 mmol/L (150 mg/dL), low HDL-C as less than 1.3 mmol/L (50 mg/dL), and high fasting plasma glucose as at least 5.5 mmol/L (100 mg/dL) or current use of antidiabetic medication. Women with three or more of these components were defined as having MetS.

Metabolomics measurement

Nontargeted mass spectrometry-based metabolomic profiling was performed with fasting plasma samples via capillary electrophoresis time-of-flight mass spectrometry. Metabolite extraction from plasma was completed within 6 hours after collection to minimize the effect of metabolic changes in plasma.[39] The extraction method has been described in detail elsewhere.[40] Capillary electrophoresis time-of-flight mass spectrometry analysis of cationic and anionic metabolites was performed as described previously.[41,42] Raw data were processed using our proprietary software (MasterHands).[43] As a preliminary study, we identified 290 metabolite peaks (131 cations and 159 anions) in plasma: 154 known with standard compounds and 136 unknown. We decided a priori to measure absolute concentrations of 115 metabolites (63 cations and 52 anions) that were expected to be observed stably in most human plasma samples and matched with standard compounds. To monitor the stability of metabolome analysis,

TABLE 1. *Characteristics of the original study population (n = 594)*

Variables	non-MetS (n = 445)	MetS (n = 149)	P^f
Age, y	64.1 (5.2)	65.5 (5.1)	0.004
Age at menarche, y	13.7 (1.5)	14.0 (1.6)	N.S.
Age at menopause, y	50.6 (3.2)	50.3 (3.7)	N.S.
Years after menopause, y	13.5 (6.3)	15.2 (6.7)	0.004
No. of deliveries, times	2.4 (0.6)	2.4 (0.6)	N.S.
Age at first delivery, y	23.8 (3.0)	23.5 (3.5)	N.S.
Body mass index, kg/m^2	22.1 (2.9)	25.6 (3.0)	<0.0001
Waist circumference, cm	80.0 (8.2)	90.1 (7.1)	<0.0001
Systolic blood pressure, mm Hg	124.4 (18.9)	141.4 (18.0)	<0.0001
Diastolic blood pressure, mm Hg	71.5 (10.9)	79.2 (9.8)	<0.0001
Triglyceride, mg/dL[a]	76.0 (27-226)	111.0 (45-441)	<0.0001
LDL-cholesterol, mg/dL	124.9 (29.3)	126.1 (31.5)	N.S.
HDL-cholesterol, mg/dL	75.3 (16.3)	64.8 (15.6)	<0.0001
Non–HDL-cholesterol, mg/dL[b]	141.4 (31.1)	150.0 (33.3)	0.004
Fasting plasma glucose, mg/dL[a]	95.0 (57-173)	104.0 (77-230)	<0.0001
Hemoglobin A1c (NGSP), %[a]	5.6 (4.9-8.6)	5.8 (5.1-9.9)	<0.0001
On hypertensive medication, yes[c]	109 (24.5%)	80 (53.7%)	<0.0001
On lipid-lowering medication, yes[c]	118 (26.5%)	55 (36.9%)	0.02
On diabetic medication, yes[c]	18 (4.0%)	30 (20.1%)	<0.0001
Current smoker, yes[c]	9 (2.0%)	2 (1.3%)	N.S.
Any current alcohol intake, yes[c,d]	89 (20.4%)	33 (22.3%)	N.S.
Dietary energy intake, kcal/d	1598.2 (249.9)	1592.4 (249.0)	N.S.
Daily physical activity, METs × h/d[c,e]	12.1 (0-79.5)	11.1 (0-69.0)	N.S.

Reported as mean (SD) unless stated otherwise.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; MetS, metabolic syndrome; METs, Metabolic Equivalent of Tasks; NGSP, National Glycohemoglobin Standardization Program; N.S., not significant.
[a]Reported as median (range). Range (minimum-maximum).
[b]Non–HDL-cholesterol was calculated by subtracting HDL-cholesterol from total cholesterol.
[c]Reported as numbers (percentage).
[d]Information on alcohol intake was missing in nine participants.
[e]Information on daily physical activity was missing in four participants.
[f]Student's *t* test was used for comparisons of group means. Wilcoxon rank-sum test was used for comparisons of triglyceride, fasting plasma glucose, hemoglobin A1c, and daily physical activity. Fisher's exact test was used to compare proportions. $P < 0.05$ was considered significant.

quality control samples were injected every 10 samples and assessed at the start of the analytical run and at intervals throughout the analysis. The technical variability and between-subject variability for each metabolite assayed is shown in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/MENO/A158).

Statistical analysis

Participants were randomly allocated into two groups (2:1) to create two independent "original" and "replication" populations. Analyses were first performed in the original population, and then the same analyses were performed in the replication population to confirm the robustness of the study outcome. The two groups were stratified for age and batch number to eliminate any confounding effects of age and analytical variability between multiple batches.

Out of 115 metabolites, 37 had plasma levels below the assay limits of detection (LOD) in more than 90% of the participants, and therefore were excluded from further analyses. The remaining 78 metabolites were analyzed. For samples with levels below LOD, which comprised less than 1% of all samples, values were input using half of the LOD.[44] The distribution of each metabolite concentration was tested for normality using quantile-quantile plots. On the basis of the shape of the distribution, most of the metabolite concentrations were log-transformed.

The characteristics of participants with and without MetS were compared by *t* test and χ^2 test for continuous and categorical variables, respectively. Age-adjusted Spearman correlation coefficients were calculated for each metabolite pair in the original population. Linear regression analysis was performed between the MetS and non-MetS groups with each metabolite concentration used as the outcome to examine the association between plasma metabolite concentrations and MetS. Analysis of covariance was performed to adjust for possible confounders using age, LDL-C levels, current smoking status, current alcohol consumption, physical activity, and dietary intake level. We calculated *P* values using the Benjamini and Hochberg false discovery rate method ($\alpha = 0.05$) for analysis of the original population to adjust for independent multiple comparisons.[45] Adjusted mean concentrations of each metabolite were calculated in both groups. Sensitivity analyses were performed by excluding (1) women with BMI at least 25 kg/m^2 and (2) those taking medications for hypertension, dyslipidemia, and diabetes. Both sensitivity and replication analyses were conducted using the metabolites shown to be significantly associated with MetS in the original population (unadjusted false discovery rate $P < 0.05$). SAS 9.3 (SAS Institute Inc, Cary, NC) was used for all analyses.

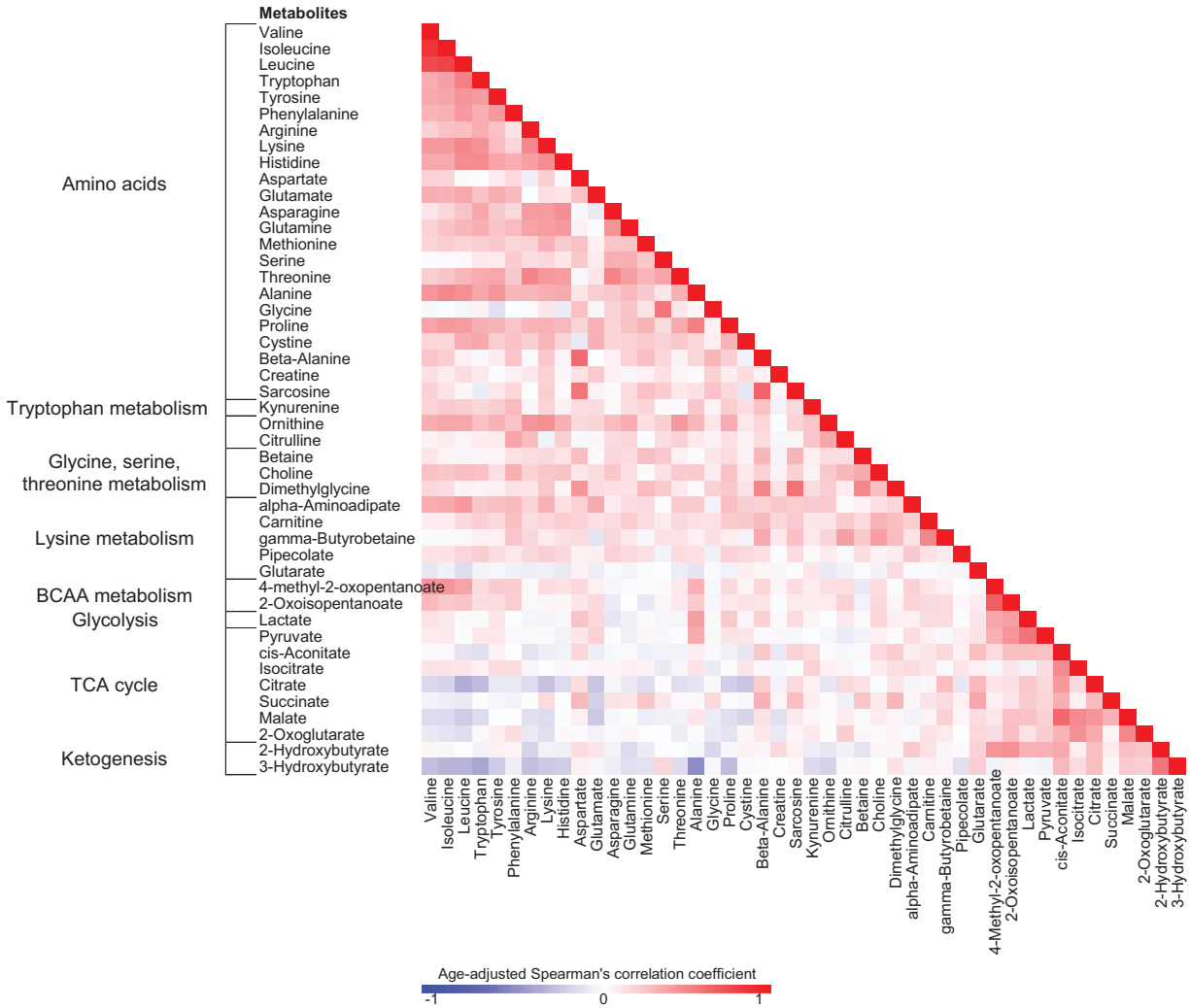

FIG. 2. Correlation matrix for plasma metabolite concentrations in the original population (n = 594). Age-adjusted Spearman coefficients were calculated for each pair of metabolite levels in the original population. Metabolites with correlation coefficients more than 0.40 are listed. BCAA, branched-chain amino acid; TCA, tricarboxylic acid.

RESULTS

Population characteristics

The final data set included 877 participants, with 594 women in the original population and 283 in the replication population. In the original population, 149 women were diagnosed with MetS and 445 women did not meet the criteria (non-MetS). In the replication population, 72 women were diagnosed with MetS and 211 were free of MetS. The ratio of MetS to non-MetS participants was similar in the two populations (1:3). The characteristics of the original population are shown in Table 1. Women with MetS were older than those without MetS (65.5 vs 64.1 y, $P = 0.004$), and had higher TG (111.0 vs 76.0 mg/dL) and lower HDL-C (64.8 vs 75.3 mg/dL) levels. There was no significant difference in serum LDL-C levels (126.1 vs 124.9 mg/dL) or in lifestyle characteristics (current smoking, current alcohol consumption, calorie intake, and physical activity) between the two groups. Most characteristics of the replication population were similar to those of the original population (data not shown). The only discrepancy between the two populations was for alcohol intake, with significantly fewer women with MetS having current alcohol intake in the replication population (7.0% vs 51.0%, $P = 0.01$). Thus, women with MetS in this population may have refrained from alcohol use.

Potential biomarkers of MetS in plasma metabolites

High correlations within groups of related metabolites were observed for amino acids and their metabolites, and for intermediates of the tricarboxylic acid (TCA) cycle, suggesting the importance of these metabolites in revealing the network of biochemical reactions in humans (Fig. 2). We discovered a total of 19 metabolites with plasma concentrations that differed significantly between MetS and non-MetS in the original population, of which 11 remained

TABLE 2. *Associations of metabolites with metabolic syndrome*

			Original population (n = 594)						Replication population (n = 283)					
			Model 1			Model 2			Model 1			Model 2		
Pathways		Metabolites[a]	% Change[b]	95% CI	P[c]	% Change[d]	95% CI	P[c]	% Change[b]	95% CI	P[e]	% Change[d]	95% CI	P[e]
Branched-chain amino acid metabolism	log	Valine	10.3	6.5-14.3	1.1×10^{-6}	10.7	6.8-14.8	5.3×10^{-6}	12.1	6.6-17.9	1.1×10^{-5}	11.8	6.1-17.8	3.6×10^{-5}
	log	Isoleucine	10.6	6.5-14.7	1.9×10^{-6}	10.9	6.8-15.2	1.2×10^{-5}	14.6	8.7-20.8	7.0×10^{-7}	14.1	8.1-20.5	3.0×10^{-6}
	log	Leucine	10.2	6.6-14.0	3.4×10^{-7}	10.8	7.1-14.6	2.1×10^{-6}	9.8	4.8-15.1	1.2×10^{-4}	10.7	5.4-16.2	6.2×10^{-5}
	log	4-Methyl-2-oxopentanoate	7.4	3.2-11.8	0.004	8.0	3.7-12.5	0.009	15.2	8.8-21.9	1.9×10^{-6}	15.1	8.5-22.1	4.1×10^{-6}
		2-Oxoisopentanoate	8.2	3.6-12.8	0.004	8.0	3.4-12.6	0.02	10.9	4.1-17.7	0.002	11.1	3.2-18.9	0.01
Aromatic amino acid metabolism	log	Phenylalanine	5.0	1.5-8.7	0.02	4.6	1.0-8.3	0.16	5.3	0.7-10.2	0.02	4.3	−0.3 to 9.2	0.07
	log	Tyrosine	5.6	2.2-9.0	0.007	4.3	1.0-7.8	0.15	4.8	−0.1 to 9.8	0.05	3.6	−1.3 to 8.6	0.15
Alanine, aspartate, and glutamate metabolism	log	Alanine	13.3	8.9-17.8	8.5×10^{-8}	12.2	7.8-16.8	3.7×10^{-6}	19.9	13.5-26.6	3.3×10^{-10}	19.4	12.9-26.3	1.7×10^{-9}
Alanine-glucose cycle	log	Glutamate	26.2	16.8-36.4	2.6×10^{-7}	26.8	17.1-37.3	2.1×10^{-6}	28.2	14.4-43.6	2.3×10^{-5}	28.0	13.8-44.0	4.7×10^{-5}
Glycine, serine, and threonine metabolism	log	Threonine	5.2	1.3-9.2	0.04	5.2	1.2-9.3	0.15	−3.3	−8.5 to 2.1	0.22	−2.7	−7.9 to 2.8	0.33
Lysine metabolism	log	Alpha-aminoadipate	11.9	6.4-17.6	2.0×10^{-4}	12.6	7.0-18.5	5.2×10^{-4}	16.1	7.2-25.7	2.7×10^{-4}	15.8	6.7-25.6	4.7×10^{-4}
Cysteine and methionine metabolism	log	Cystine	10.3	6.6-14.2	4.4×10^{-7}	9.8	6.1-13.7	1.2×10^{-5}	4.4	−1.0 to 10.1	0.11	3.4	−2.0 to 9.2	0.22
Proline and arginine metabolism	log	Proline	12.4	6.3-18.9	4.0×10^{-4}	11.8	5.7-18.4	0.006	11.5	3.3-20.4	0.005	12.8	4.5-21.8	0.002
TCA cycle; glycolysis/gluconeogenesis; involved in metabolism of multiple amino acids		Pyruvate	9.9	3.6-16.3	0.01	8.7	2.8-14.7	0.07	20.3	10.0-30.6	1.2×10^{-4}	19.0	8.8-29.2	2.9×10^{-4}
TCA cycle	log	cis-Aconitate	7.2	1.8-12.8	0.04	5.8	0.5-11.3	0.28	8.8	1.0-17.3	0.03	6.6	−1.3 to 15.0	0.10
Glycolysis/gluconeogenesis, pyruvate metabolism	log	Lactate	10.8	4.6-17.5	0.004	9.4	3.1-16.0	0.06	16.9	7.1-27.6	5.3×10^{-4}	14.5	4.7-25.3	0.003
Ketones	log	3-Hydroxybutyrate	−17.9	−28.4 to −5.7	0.02	−19.8	−30.2 to −7.8	0.048	−20.6	−35.4 to −2.3	0.03	−24.8	−39.0 to −7.5	0.007
Glucuronic acid derivatives	log	Mucate	−9.3	−14.4 to −3.8	0.007	−8.9	−14.2 to −3.3	0.05	−14.8	−26.4 to −1.3	0.03	−12.7	−25.1 to 1.7	0.08
Uremic toxins	log	Guanidinosuccinate	−22.6	−34.5 to −8.7	0.01	−20.9	−33.2 to −6.4	0.11	−33.2	−47.3 to −15.3	0.001	−28.2	−43.6 to −8.5	0.008

Model 1: unadjusted. Model 2: adjusted for age, LDL-C levels, current smoker or not, current alcohol drinker or not, physical exercise level (high/low), and calorie intake (high/low).
TCA, tricarboxylic acid.
[a]Metabolites labeled "log" were log-transformed.
[b]For log-transformed metabolites, regression coefficients were back-transformed. For normal metabolites, regression coefficients were divided by the means of the non-MetS group.
[c]False discovery rate P values are shown. $P < 0.05$ is considered significant.
[d]Same as (b) for log-transformed metabolites. For normal metabolites, coefficients were divided by the adjusted means of the non-MetS group.
[e]Raw P values are shown. $P < 0.05$ is considered significant.

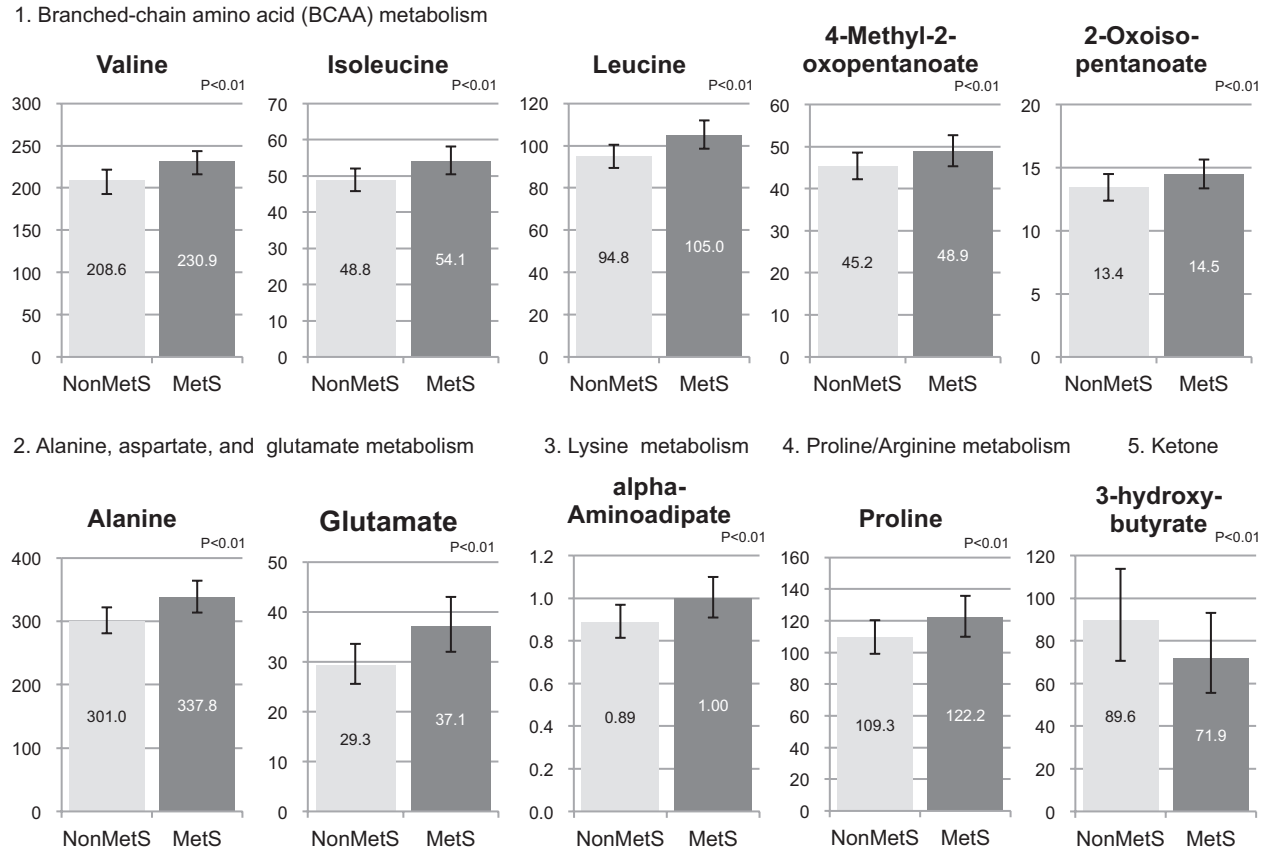

FIG. 3. Comparisons of plasma metabolite adjusted mean concentrations in women with and without metabolic syndrome. The adjusted mean concentration of each metabolite was calculated using the fully adjusted model of the original population. Concentrations of metabolites are in μmol/L units. Numbers in the middle of each bar reflect the adjusted mean concentrations. Error bars reflect 95% confident intervals. False discovery rate *P* values are shown. MetS, metabolic syndrome.

significant after adjusting for possible confounders (Table 2). Plasma levels of branched-chain amino acids (BCAAs) and their derivatives were 7% to 10% higher in MetS. Levels of phenylalanine and tyrosine tended to be slightly higher in MetS, but significance disappeared after adjustments. Other metabolites that showed significantly higher concentrations in MetS included alanine, glutamate, alpha-aminoadipate, cystine, and proline. A suggestive positive trend could also be seen for lactate and pyruvate ($P = 0.06$ and 0.07, respectively). 3-Hydroxybutyrate levels were 18% lower in the MetS group compared with the non-MetS group. The results for all metabolites are shown in Supplementary Table 2 (Supplemental Digital Content 2, http://links.lww.com/MENO/A159).

Out of the 19 metabolites, 16 (84.2%) could be replicated (Table 2), including those related to BCAA metabolism, aromatic amino acid metabolism, alanine, aspartate and glutamate metabolism, glucose-alanine cycle, lysine metabolism, proline and arginine metabolism, and the TCA cycle. Among these, 13 remained significant after adjustments. Comparisons of the adjusted means of the 13 metabolites between non-MetS and MetS in the original population are shown in Figure 3. Sensitivity analyses excluding (1) participants with BMI at least 25 kg/m^2 and (2) those taking medications gave similar results (data not shown).

DISCUSSION

To the best of our knowledge, this is the first population-based cross-sectional study to report associations between polar metabolites and MetS in postmenopausal women in a lean Asian population (average BMI 23 kg/m^2) with average HDL-C levels of 72.1 mg/dL. Our data indicate that alterations in amino acid metabolism are present in both obese individuals and in women with normal BMI with MetS after menopause.

4-Methyl-2-oxopentaonate and 2-oxoisopentanoate, which are degradation products of BCAAs, were significantly elevated in MetS, in addition to BCAAs themselves. The significant increase of BCAAs in plasma in MetS is consistent with many prior experimental and physiological studies.[28,46-49] In a study of lean and obese individuals, BCAAs accounted for the largest amount of variance in principal components analysis of metabolite data, along with derivatives from oxidation products of BCAAs.[16] This increase in levels of downstream metabolites indicates that the associations with MetS are related to an alteration in the flux of BCAAs through this catabolic pathway.

A leading theory of the mechanism behind this physiological change is suppression of BCAA catabolism under insulin resistance. Diabetic rats and humans have significantly lower levels of branched-chain keto acid dehydrogenase (BCKDH) enzyme activity, which is the rate-limiting step in overall catabolism of BCAAs.[45,48,50,51] Several theories have been proposed for the mechanisms underlying suppressed BCKDH activity, including increased activity of BCKDH kinase that inactivates BCKDH,[48] and reduction of adiponectin signaling via an adenosine monophosphate-activated protein kinase α2-dependent pathway.[47,49] The increase of BCAAs has also been explained based on amino acid breakdown products being released into blood because insulin resistance causes excess protein breakdown in skeletal muscle.[52] A causal role for BCAAs in metabolic disease via the mammalian target of rapamycin pathway or inhibition of glucose transport/phosphorylation has also been suggested.[53-55] Whether the increase in BCAAs reflects the consequence of diabetes pathogenesis or has a causal role in disease development has yet to be proven because many large epidemiological studies have had a cross-sectional or case-control design. Further work is needed to determine the mechanisms through which increased amino acid levels might contribute to metabolic diseases.

In our study, several metabolites other than amino acids were significantly associated with MetS in postmenopausal women. Alpha-aminoadipate levels were 6.4% to 17.6% higher in plasma of women with MetS compared with those without MetS. Aminoadipate was found to be the strongest biomarker of diabetes risk out of 70 metabolites screened in the Framingham Heart Study, in which participants with the highest quartile of plasma aminoadipate had fourfold higher odds of developing diabetes over a 12-year follow-up period compared with those in the lowest quartile.[56] There were no correlations between aminoadipate and BCAAs. Because aminoadipate is generated by lysine degradation and may also serve as a substrate for enzymes downstream of tryptophan metabolism, the current and previous findings collectively suggest that the mechanism behind MetS and insulin resistance involves alterations in these metabolic pathways, distinct from pathways of BCAAs.

3-Hydroxybutyrate, a ketone body, was significantly lower in MetS compared with non-MetS participants (5.7%-28.4%). This is in contrast to previous studies showing that alpha-hydroxybutyrate is a positive predictor of type 2 diabetes.[57-60] The reason for this discrepancy is unclear. Alpha-hydroxybutyrate is generated in the liver under increased oxidative stress; therefore, the different results and the suggestive trends in lactate and pyruvate concentrations may be indicative of down- and up-regulation of ketogenesis and its relationship to the TCA cycle. Another hypothesis is that the phenotype of MetS in our population differs from those in previous studies, especially with respect to the very high levels of HDL-C. A recent review article on BCAAs in insulin resistance described two types of obesity in rodents with regard to impairment of BCAA metabolism.[61] Similarly, the alpha-hydroxybutyrate levels might be regulated in a more complex manner. Further studies of the mechanism behind the altered levels of 3-hydroxybutyrate are required.

One of the strengths of our study is the characteristics of the participants. To our knowledge, metabolomic profiling of MetS on a population of lean, older females has not been performed previously. The only study in women with normal BMI (average 24.4 kg/m^2) was reported by Würtz et al[62]; however, the average age of the participants was 32.1 years. Metabolism changes significantly with age and data should be carefully interpreted, even with adjustment of statistical models for age. In addition, the average HDL-C level of our participants was much higher than those in other populations. HDL-C levels are indeed high among Japanese people in general, and have increased in the past few decades.[63] As one of the components of MetS, decreased HDL-C is an independent risk factor for CVD, and the TG/HDL-C ratio has a positive correlation with the insulin resistance index.[64,65] Our data suggest that alterations in plasma amino acids may precede the reduction of HDL-C levels in the development of insulin resistance.

The study has several limitations. When interpreting metabolic profiling data in a cross-sectional study, the temporality of cause-effect relationships is not assured and possible confounding of unmeasured factors may exist, even after adjustments. Equally of concern is that our data were derived from a single site, lacking test marker performance in an independent cohort. Therefore, follow-up and intervention studies along with those at different facilities are needed. Comparisons between pre- and postmenopausal women could not be performed because of the few premenopausal women enrolled in the study. Completion of a separate ongoing cohort with a working age population is awaited to investigate the impact of menopause on these metabolic changes. Measurement of WC at the umbilicus might also have affected the diagnosis of MetS. Other common sites for WC measurements include the midpoint between the lowest rib and the iliac crest; narrowest or widest WC; just below the lowest rib; and just above the iliac crest.[66] However, there is no consensus on the optimal protocol for measurement of WC.[66,67] An effort was made to eliminate conditions that could affect the metabolic profile, such as history of cancer and nonfasting status, but multiple drugs for treated participants may have acted as confounders. Various factors might also have influenced measurement variability in the metabolomics analysis, potentially causing nondifferential misclassification of metabolomics data. To minimize these variabilities, we set a uniform fasting condition for participants and standardized the quality control procedures for metabolomics analysis.[39]

CONCLUSIONS

This study shows that Japanese postmenopausal women who develop MetS may have elevated concentrations of multiple amino acids, including BCAAs, alanine, glutamate, lysine, proline, and other polar metabolites such as alpha-aminoadipate. These findings may not be translatable to populations of non-Japanese women without very high HDL-C levels, but identifying these metabolic changes

may be useful for detection of high-risk individuals, including those with normal BMI and relatively high HDL-C levels. A follow-up survey is needed to examine whether these candidate metabolites provide better prediction than standard measures for future development of MetS.

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