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ORIGINAL ARTICLE

Association between insulin resistance and plasma amino acid profile in non-diabetic Japanese subjects

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ABSTRACT

Aims/Introduction: Elevation of the branched-chain amino acids (BCAAs), valine, leucine and isoleucine; and the aromatic amino acids, tyrosine and phenylalanine, has been observed in obesity-related insulin resistance. However, there have been few studies on Asians, who are generally less obese and less insulin-resistant than Caucasian or African-Americans. In the present study, we investigated the relationship between homeostasis model assessment of insulin resistance (HOMA-IR) and plasma amino acid concentration in non-diabetic Japanese participants.

Materials and Methods: A total of 94 healthy men and women were enrolled, and plasma amino acid concentration was measured by liquid chromatography/mass spectrometry after overnight fasting. The associations between HOMA-IR and 20 amino acid concentrations, and anthropometric and clinical parameters of lifestyle-related diseases were evaluated.

Results: The mean age and body mass index were 40.1 ± 9.6 years and 22.7 ± 3.9, respectively. Significantly positive correlations were observed between HOMA-IR and valine, isoleucine, leucine, tyrosine, phenylalanine and total BCAA concentration. Compared with the HOMA-IR ≤ 1.6 group, the HOMA-IR > 1.6 group showed significantly exacerbated anthropometric and clinical parameters, and significantly elevated levels of valine, isoleucine, leucine, tyrosine, phenylalanine and BCAA.

Conclusions: The present study shows that the insulin resistance-related change in amino acid profile is also observed in non-diabetic Japanese subjects. These amino acids include BCAAs (valine, isoleucine and leucine) and aromatic amino acids (tyrosine and phenylalanine), in agreement with previous studies carried out using different ethnic groups with different degrees of obesity and insulin resistance.

INTRODUCTION

Hyperaminoacidemia as a manifestation of the insulin resistance characteristic of obesity was first reported in 1969[1]. Of 20 plasma amino acids measured, the concentrations of valine, leucine, isoleucine, tyrosine and phenylalanine were elevated in obese subjects even after overnight fasting and correlated directly with serum insulin, which diminished after glucose infusion[1]. Since the late 2000s, metabolomic technologies have been applied to diabetes research[2], and alterations in metabolites, such as amino acids, fatty acids and organic acids, have been shown to be associated with future development of diabetes[3–5].

Branched-chain amino acids (BCAAs), namely valine, leucine and isoleucine, are among the nine essential amino acids (EAAs), accounting for 35% of the EAAs in muscle proteins and 40% of the preformed amino acids required by mammals[6].

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Several studies have shown that insulin resistance plays a key role in the underlying mechanism linking obesity with increased circulating concentrations of BCAAs[7–13]. Insulin is known as a regulator of branched-chain α-keto acid dehydrogenase (BCKDH) complex[14], a rate-limiting enzyme of BCAA catabolism, which catalyzes the irreversible oxidative decarboxylation of branched-chain a-keto acids generated by reversible transamination of BCAAs by branched-chain aminotransferase (BCAT)[15]. Reduced enzyme activity of BCKDH complex in obesity and/or diabetes has been shown[16–19], and suppression of BCAA catabolism by insulin resistance is considered as a plausible etiology of elevated BCAA concentration in obesity. However, although the degree of obesity and insulin resistance might differ among different ethnic groups, there have been few studies on insulin resistance and amino acid profiles in Asian populations. Furthermore, it is unclear whether obesity-related changes in specific amino acids are also observed in healthy Japanese people with lower body mass index (BMI) than Western people.

In the present study using 94 non-diabetic Japanese men and women, we investigated the relationship between homeostasis model assessment of insulin resistance (HOMA-IR) and plasma amino acid concentration, as well as the anthropometric and clinical parameters of lifestyle-related diseases.

Table 1 | Background characteristics of the study participants

	All	Men	Women
No. participants	94	48	46
Age (years)	40.1 ± 9.6	42.0 ± 10.2	38.1 ± 8.6
Bodyweight (kg)	63.2 ± 14.5	72.5 ± 12.4	53.4 ± 9.3
BMI (kg/m^2)	22.7 ± 3.9	24.1 ± 3.8	21.2 ± 3.5
Waist circumference (cm)	80.4 ± 10.6	85.4 ± 9.9	75.2 ± 8.7
Visceral fat (cm^2)	47.4 ± 40.9	70.5 ± 41.4	23.3 ± 22.3
Subcutaneous fat (cm^2)	136.3 ± 80.8	139.4 ± 90.2	133.0 ± 70.4
Body fat mass (kg)	15.6 ± 7.4	16.4 ± 8.3	14.8 ± 6.4
Fat free mass (kg)	44.9 ± 9.7	53.0 ± 5.5	36.3 ± 4.2
%Body fat (%)	24.3 ± 7.5	21.8 ± 7.0	26.9 ± 7.1
Systolic BP (mmHg)	119.6 ± 15.2	124.8 ± 12.2	114.1 ± 16.1
Diastolic BP (mmHg)	77.6 ± 13.2	82.5 ± 11.7	72.4 ± 12.8
Fasting glucose (mg/dL)	89.8 ± 8.7	93.2 ± 9.2	86.3 ± 6.7
HbA1c (%)	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.3
Fasting insulin (μU/mL)	4.97 ± 2.92	5.69 ± 3.41	4.21 ± 2.06
HOMA-IR	1.12 ± 0.72	1.33 ± 0.85	0.90 ± 0.46
LDL-C (mg/dL)	113.5 ± 32.2	120.2 ± 31.4	106.5 ± 31.9
HDL-C (mg/dL)	65.1 ± 16.8	57.3 ± 13.2	73.2 ± 16.4
Triglyceride (mg/dL)	107.7 ± 84.0	134.7 ± 99.6	79.5 ± 51.4
AST (U/L)	20.6 ± 5.6	22.9 ± 5.7	18.3 ± 4.5
ALT (U/L)	19.9 ± 12.4	25.6 ± 14.0	14.0 ± 6.5
γGT (U/L)	33.5 ± 30.7	45.9 ± 37.4	20.5 ± 12.0
Uric acid (mg/dL)	5.3 ± 1.3	6.1 ± 1.1	4.5 ± 0.9
Leptin (ng/mL)	7.92 ± 6.24	5.46 ± 3.78	10.49 ± 7.23
Adiponectin (μg/mL)	3.65 ± 2.46	2.28 ± 1.30	5.08 ± 2.58
hsCRP (ng/mL)	1055.3 ± 4929.6	1564.3 ± 6792.4	524.2 ± 1212.2

Data are mean ± SD. γGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

MATERIALS AND METHODS

Participants

Healthy volunteers aged between 20 and 65 years were recruited, and 94 individuals (48 men and 46 women) were enrolled in the present cross-sectional study. The examinations were carried out in February 2013. Written informed consent was obtained from all the participants to use their health records for analysis. The present study was approved by the Ethical Committee of Tokai University, and was carried out in accordance with the Declaration of Helsinki.

Table 2 | Average amino acid concentrations

(nmol/mL)	All	Men	Women
Glycine	209 ± 50.3	213.1 ± 43.9	206.0 ± 56.5
Alanine	329.0 ± 62.3	355.7 ± 59.0	301.2 ± 53.4
Serine	112.7 ± 20.3	109.6 ± 19.9	115.9 ± 20.3
Threonine	123.5 ± 24.7	123.2 ± 19.4	123.7 ± 29.5
Valine	206.7 ± 37.8	229.0 ± 29.1	183.3 ± 31.3
Isoleucine	59.6 ± 12.8	68.0 ± 10.6	50.9 ± 8.2
Leucine	118.3 ± 23.6	135.6 ± 15.5	100.3 ± 15.7
Lysine	172.0 ± 31.6	189.8 ± 25.1	153.4 ± 26.8
Arginine	51.0 ± 16.8	51.9 ± 15.8	50.1 ± 17.9
Histidine	78.9 ± 8.1	83.2 ± 7.3	74.4 ± 6.3
Tyrosine	57.7 ± 10.4	62.0 ± 9.2	53.2 ± 9.8
Phenylalanine	55.1 ± 8.4	59.8 ± 7.6	50.2 ± 6.1
Tryptophan	55.7 ± 8.8	59.6 ± 8.7	51.7 ± 7.1
Methionine	23.4 ± 4.0	25.1 ± 3.6	21.6 ± 3.7
Cysteine	12.7 ± 4.1	13.8 ± 4.4	11.5 ± 3.4
Proline	137.2 ± 39.7	157.6 ± 40.0	115.8 ± 26.0
Glutamine	507.2 ± 70.9	539.0 ± 58.5	474.0 ± 67.9
Glutamic acid	40.7 ± 18.4	50.2 ± 18.0	30.9 ± 13.0
Asparagine	47.0 ± 6.9	48.5 ± 7.4	45.5 ± 6.1
Aspartic acid	2.9 ± 0.8	3.0 ± 1.0	2.7 ± 0.5
Total AA	2570.7 ± 298.1	2759.4 ± 191.4	2373.7 ± 260.2
NEAA	1677.5 ± 209.6	1786.2 ± 148.3	1564.1 ± 205.0
EAA	893.1 ± 121.0	973.2 ± 83.2	809.6 ± 95.2
BCAA	384.6 ± 71.0	432.6 ± 50.8	334.5 ± 52.0

Data are mean ± SD. BCAA, branched-chain amino acid; EAA, essential amino acid; NEAA, non-essential amino acid; Total AA, total amino acid.

Anthropometric Measurements

All measurements were carried out after overnight fasting. Height and weight were measured in the standing position, and BMI was calculated as weight/height^2 (kg/m^2). Waist circumference was assessed at the end of expiration, measuring the minimum circumference at the level of the umbilicus. Blood pressure (BP) was measured in the sitting position. Visceral and subcutaneous fat area was measured at the level of the umbilicus in the spine position using computed tomography. Bioelectrical impedance analysis (Inbody 720; Biospace Co., Ltd., Tokyo, Japan) was used for evaluating body fat mass, fat-free mass and percentage body fat.

Biochemical Measurements

Fasting serum immunoreactive insulin was measured by chemiluminescent enzyme immunoassay (CLEIA). Glycated hemoglobin (HbA1c) was determined by the latex agglutination immunoassay. HOMA-IR was calculated as: fasting plasma glucose (in mg/dL) $\times$ insulin (in mU/mL)/405[20]. HOMA-IR $\leq$ 1.6 was considered as non-insulin-resistant according to the definition of the Japan Diabetes Society[21]. Low-density lipoprotein cholesterol (LDL-C) was measured directly, and high-density lipoprotein cholesterol (HDL-C) and triglycerides were determined enzymatically. Leptin was measured by double antibody radioimmunoassay. High molecular adiponectin was measured using the CLEIA method. High-sensitivity C-reactive protein (hsCRP) was measured by turbidimetric immunoassay.

Plasma amino acid concentrations were measured by liquid chromatography/mass spectrometry (LC/MC). Of 39 amino acids measured (taurine, aspartic acid, hydroxyproline, threonine, serine, asparagine, glutamic acid, glutamine, sarcosine, α-aminoadipic acid, proline, glycine, alanine, citrulline, α-aminobutyric acid, valine, cysteine, cystathionine, methionine, isoleucine, leucine, tyrosine, phenylalanine, γ-amino β-hydroxybutyric acid, β-alanine, β-amino-iso-butyric acid, γ-aminobutyric acid, monoethanolamine, homocysteine, histidine, 3-methylhistidine, 1-methylhistidine, carnosine, anserine, tryptophan, hydroxylysine, ornithine, lysine, arginine), 20 amino acids were at detectable levels and eligible for analysis. Levels of total amino acid (total AA), non-essential amino acid (NEAA), EAA and BCAA were also obtained and used for analysis.

Statistical Analyses

Data are expressed as mean ± SD. SPSS Statistics (version 22.0; SPSS Inc., Chicago, IL, USA) was used for the statistical analyses. Pearson's correlation coefficient was calculated as a

Table 3 | Correlations between homeostasis model assessment of insulin resistance and clinical parameters

	All		Men		Women	
	r	*P*	*r*	*P*	*r*	*P*
Age	0.045	0.667	0.013	0.930	–0.084	0.580
Bodyweight	0.471	<0.001	0.326	0.024	0.547	<0.001
BMI	0.511	<0.001	0.393	0.006	0.612	<0.001
Waist circumference	0.542	<0.001	0.479	0.001	0.510	<0.001
Visceral fat	0.626	<0.001	0.627	<0.001	0.430	0.003
Subcutaneous fat	0.374	<0.001	0.307	0.034	0.576	<0.001
Body fat mass	0.509	<0.001	0.478	0.001	0.585	<0.001
Fat free mass	0.299	0.003	0.006	0.968	0.301	0.042
%Body fat	0.374	<0.001	0.565	<0.001	0.534	<0.001
Systolic BP	0.410	<0.001	0.412	0.004	0.318	0.031
Diastolic BP	0.391	<0.001	0.357	0.013	0.289	0.051
Fasting glucose	0.435	<0.001	0.388	0.006	0.303	0.041
HbA1c	0.194	0.060	0.137	0.354	0.263	0.077
Fasting insulin	0.985	<0.001	0.985	<0.001	0.989	<0.001
LDL-C	0.132	0.206	0.096	0.514	0.038	0.802
HDL-C	–0.485	<0.001	–0.344	0.017	–0.597	<0.001
Triglyceride	0.595	<0.001	0.618	<0.001	0.303	0.041
AST	0.348	0.001	0.231	0.113	0.340	0.021
ALT	0.482	<0.001	0.365	0.011	0.586	<0.001
γGT	0.408	<0.001	0.381	0.008	0.048	0.750
Uric acid	0.319	0.002	0.348	0.015	–0.199	0.184
Leptin	0.392	<0.001	0.621	<0.001	0.792	<0.001
Adiponectin	–0.336	0.001	–0.305	0.035	–0.213	0.155
hsCRP	0.296	0.004	0.286	0.049	0.375	0.010

γGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

measure of association. Statistical significance of comparisons between the HOMA-IR ≤ 1.6 group and HOMA-IR > 1.6 group was determined by the chi squared-test or Student's *t*-test. All *P*-values were two-tailed, and $P < 0.05$ was considered significant.

RESULTS

The clinical characteristics of the participants are shown in Table 1. The mean age was 40.1 ± 9.6 years (42.0 ± 10.2 for men and 38.1 ± 8.6 for women). The age of the participants ranged from 22 to 62 years (24–62 for men and 22–55 for women). The participants were not obese on average (BMI 22.7 ± 3.9). Mean fasting glucose and HbA1c were within the range of normal glucose tolerance. There were no participants who exceeded both fasting glucose ≥ 126 mg/dL and HbA1c ≥ 6.5% or were under diabetes treatment. The mean HOMA-IR was 1.12 ± 0.2 (1.33 ± 0.85 for men and 0.90 ± 0.46 for women). A total of 38 men (79.1%) and 42 women (91.3%) were HOMA-IR ≤ 1.6. Average concentrations of 20 amino acids, total amino AA, NEAA, EAA and BCAA concentrations were within the normal ranges (Table 2).

The correlations between insulin resistance and clinical parameters are shown in Table 3. The influence of age on HOMA-IR was insignificant. HOMA-IR showed a significantly positive correlation with all the values of anthropometric measurements (bodyweight, BMI, waist circumference, fat-free mass and percentage body fat, visceral and subcutaneous fat area) in both men and women. HOMA-IR was also significantly positively associated with BP in both sexes. Insulin showed a higher correlation with HOMA-IR than fasting glucose. Among lipid parameters, HDL-C and triglycerides, but not LDL-C, were significantly positively correlated with HOMA-IR in men and women. Alanine aminotransferase, which is often used as a surrogate marker for fatty liver, was significantly positively correlated with HOMA-IR in both sexes. There was a significantly negative correlation between adiponectin and HOMA-IR in the whole population and in men. High-sensitivity C-reactive protein was significantly positively correlated with HOMA-IR.

Table 4 shows the correlations between HOMA-IR and amino acid levels. There were several amino acids that were significantly positively correlated with HOMA-IR. Most notably, BCAAs (valine, isoleucine and leucine) were positively associated with insulin resistance, although valine in men did not reach statistical significance. Furthermore, tyrosine and phenylalanine, which are aromatic amino acids, were significantly positively correlated with HOMA-IR. Total BCAA was also significantly positively associated with HOMA-IR in both men and women. Correlation diagrams for valine, isoleucine, leucine, tyrosine, phenylalanine and BCAA in the whole population are shown in Figure 1. We found that isoleucine, leucine, tyrosine, phenylalanine and BCAA showed significantly positive correlations with HOMA-IR ($P < 0.05$) even after adjusted for BMI. Valine tended to increase as HOMA-IR increased, although its level did not reach statistical significance ($P = 0.086$) after adjustment for BMI.

Next, we compared clinical characteristics between the HOMA-IR ≤ 1.6 group and HOMA-IR > 1.6 group (Tables 5 and 6). Compared with the HOMA-IR ≤ 1.6 group, the HOMA-IR > 1.6 group was significantly higher in weight, BP, fasting glucose and triglycerides. HDL-C and adiponectin were significantly lower, and hsCRP was significantly higher in the HOMA-IR > 1.6 group than in the HOMA-IR ≤ 1.6 group. Among the amino acids measured, valine, isoleucine, leucine, tyrosine, phenylalanine and BCAA were significantly elevated in the HOMA-IR > 1.6 group than in the HOMA-IR ≤ 1.6 group in both sexes.

DISCUSSION

In the present study, the association between HOMA-IR and plasma amino acid concentration was examined in 94 non-diabetic Japanese men and women. We identified that the insulin resistance-related change in amino acid profile was also observed in our participants, and BCAAs (valine, isoleucine and leucine) and aromatic amino acids (tyrosine and

Table 4 | Correlations between homeostasis model assessment of insulin resistance and plasma amino acid levels

	All		Men		Women	
	r	*P*	*r*	*P*	*r*	*P*
Glycine	−0.120	0.248	−0.326	0.024	0.084	0.578
Alanine	0.439	<0.001	0.478	0.001	0.140	0.354
Serine	−0.231	0.025	−0.374	0.009	0.114	0.451
Threonine	−0.113	0.276	−0.222	0.129	−0.016	0.918
Valine	0.384	<0.001	0.245	0.094	0.354	0.016
Isoleucine	0.495	<0.001	0.385	0.007	0.520	<0.001
Leucine	0.430	<0.001	0.323	0.025	0.383	0.009
Lysine	0.153	0.141	−0.017	0.908	−0.040	0.792
Arginine	0.192	0.063	0.265	0.068	0.079	0.600
Histidine	0.294	0.004	0.152	0.301	0.209	0.164
Tyrosine	0.462	<0.001	0.462	0.001	0.313	0.034
Phenylalanine	0.398	<0.001	0.292	0.044	0.304	0.040
Tryptophan	0.153	0.141	−0.021	0.886	0.132	0.383
Methionine	0.157	0.131	0.026	0.862	0.058	0.704
Cysteine	0.204	0.049	0.151	0.305	0.088	0.563
Proline	0.396	<0.001	0.312	0.031	0.249	0.095
Glutamine	0.075	0.474	−0.154	0.297	0.047	0.758
Glutamic acid	0.461	<0.001	0.448	0.001	0.195	0.195
Asparagine	−0.184	0.077	−0.229	0.117	−0.370	0.011
Aspartic acid	0.400	<0.001	0.383	0.011	0.303	0.087
Total AA	0.324	0.001	0.202	0.168	0.194	0.197
NEAA	0.266	0.010	0.162	0.271	0.128	0.396
EAA	0.336	0.001	0.176	0.230	0.254	0.089
BCAA	0.436	<0.001	0.319	0.027	0.410	0.005

BCAA, branched-chain amino acid; EAA, essential amino acid; NEAA, non-essential amino acid; Total AA, total amino acid.

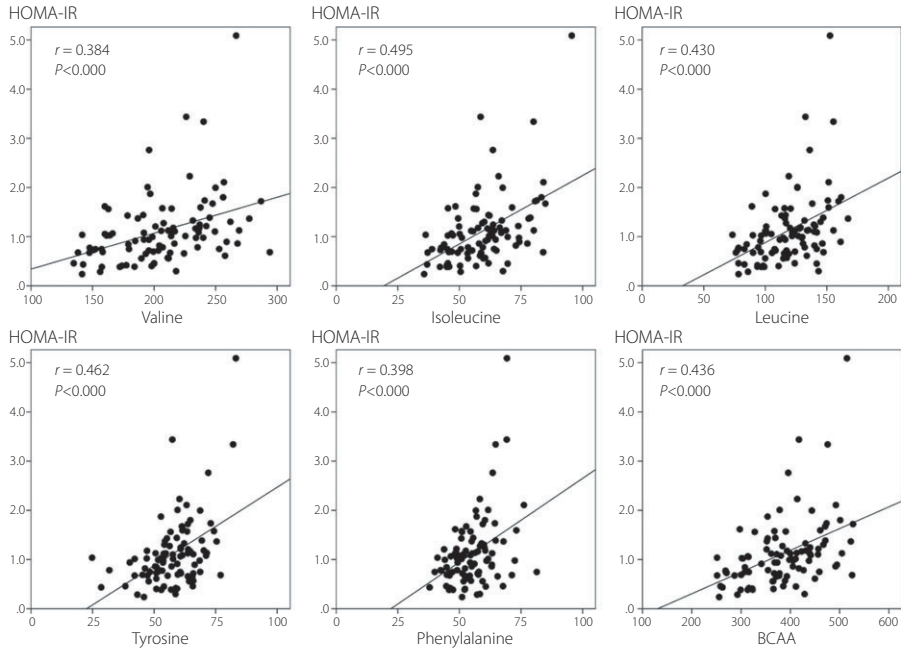

Figure 1 | Correlation between homeostasis model assessment of insulin resistance (HOMA-IR) and branched-chain amino acids (BCAAs) and aromatic amino acids. Significant correlations were observed ($P < 0.01$) between HOMA-IR and valine, isoleucine, leucine, tyrosine, phenylalanine and BCAA. Correlation diagrams for the whole population are shown.

phenylalanine) were found to be significantly related to insulin resistance.

Pioneering application of metabolomics technologies by Newgard *et al.*[7] has shown that a cluster of amino acids including BCAAs and aromatic amino acids was strongly associated with obesity-related insulin resistance, followed by several reports supporting the association between insulin resistance and increased circulating concentrations of BCAAs[8–12]. The degree of obesity and insulin resistance greatly differs among different ethnic groups; Asians are generally less obese and less insulin-resistant than Caucasian or African-Americans. In a study of Americans by Newgard *et al.*[7], the average BMI and HOMA-IR were 36.6 and 5.73 in the obese group, and 23.3 and 2.51 in the lean group. The HOMA-IR value of even the lean American subjects with normal BMI exceeds the Japanese criterion for insulin resistance (HOMA-IR $\geq$ 2.5)[21], which is comparable with the 97.5 percentile value of the Japanese reference individuals[22]. In the present study, the BMI and HOMA-IR values were 22.7 and 1.12 in the whole population, 21.8 and 0.90 in the HOMA-IR $\leq$ 1.6 group, and 27.5 and 2.38 in the HOMA-IR > 1.6 group. There is only one Asian study on the insulin resistance-related increase in amino acids using Chinese and Asian-Indian men living in Singapore. The mean BMI and HOMA-IR values for Chinese men were 23.9 and 0.75 for the low HOMA group (below the lower tertile), and 24.5 and 3.02 for the high HOMA group (above the upper tertile)[9]. We have investigated the insulin resistance-related change in amino acid profile in both men and women, whereas Tai *et al.* only studied in men. Although Tai *et al.* were the first who reported this phenotype in Asians, they emphasized the need to analyze Chinese and Asian-Indians separately to find the differences among the Asian ethnic groups. Ours is the first report using Japanese men and women, and has a significant role in the development for the studies of diabetes in Asians. Although heterogeneity in ethnicity might contribute to discrepancies in the degree of obesity and insulin resistance, the influence of insulin resistance on amino acids profile in our Japanese study was consistent with that of previous other studies[7–12], regardless of ethnic background. Furthermore, the extent of BCAA elevation was 10–20% between the low and high HOMA groups in the present study, comparable with other studies[7,9].

Currently, suppression of BCAA catabolism by insulin resistance is considered as a plausible etiology of elevated BCAA concentration in obesity. As BCAAs and phenylalanine are EAAs, and cannot be synthesized in the human body, it is highly doubtful whether dietary protein has any impact on their plasma concentration. Although we did not evaluate dietary nutrient intake in the present study, the limited numbers of previous reports showed that increased protein intake was not observed as evidence for higher BCAA levels in obese insulin-resistant subjects[3,9,12]. There is another intervention study supporting that BCAAs are uniquely correlated with insulin resistance, where BCAA levels were associated with improvement in insulin resistance with weight loss[11]. We and other investigators examined circulating free BCAAs, not whole-body storage of BCAAs, which are largely present as

Table 5 | Comparison of clinical parameters between homeostasis model assessment of insulin resistance ≤1.6 and homeostasis model assessment of insulin resistance >1.6 groups

	HOMA-IR ≤ 1.6	HOMA-IR > 1.6	*P*
No. men/women	38 (47%)/42 (53%)	10 (71%)/4 (29%)	0.086
Age (years)	40.5 ± 9.7	37.9 ± 9.1	0.360
Body weight (kg)	60.5 ± 11.9	78.6 ± 18.7	<0.001
BMI (kg/m^2)	21.8 ± 2.9	27.5 ± 5.1	<0.001
Waist circumference (cm)	78.1 ± 8.2	93.2 ± 13.6	<0.001
Visceral fat (cm^2)	39.2 ± 33.1	94.4 ± 50.1	<0.001
Subcutaneous fat (cm^2)	121.1 ± 57.7	222.9 ± 130.3	<0.001
Body fat mass (kg)	14.0 ± 5.2	25.1 ± 10.8	<0.001
Fat free mass (kg)	43.9 ± 9.3	50.5 ± 10.2	0.017
%Body fat (%)	23.1 ± 6.8	31.2 ± 7.7	<0.001
Systolic BP (mmHg)	117.4 ± 14.4	131.9 ± 13.7	0.001
Diastolic BP (mmHg)	75.8 ± 11.8	87.9 ± 16.2	0.001
Fasting glucose (mg/dL)	88.7 ± 8.1	96.2 ± 9.6	0.003
HbA1c (%)	5.29 ± 0.26	5.40 ± 0.33	0.164
Fasting insulin (μU/mL)	4.08 ± 1.47	10.00 ± 3.94	<0.001
HOMA-IR	0.90 ± 0.34	2.38 ± 0.98	<0.001
LDL-C (mg/dL)	111.8 ± 31.9	123.1 ± 33.5	0.226
HDL-C (mg/dL)	67.4 ± 16.7	51.9 ± 9.5	0.001
Triglyceride (mg/dL)	95.8 ± 65.9	175.4 ± 135.3	0.001
AST (U/L)	19.9 ± 5.0	25.0 ± 7.1	0.001
ALT (U/L)	17.9 ± 9.4	31.5 ± 19.8	<0.001
γGT (U/L)	29.2 ± 26.6	57.9 ± 40.8	0.001
Uric acid (mg/dL)	5.2 ± 1.2	5.9 ± 1.5	0.074
Leptin (ng/mL)	6.64 ± 4.13	15.25 ± 10.36	<0.001
Adiponectin (μg/mL)	3.90 ± 2.49	2.23 ± 1.78	0.019
hsCRP (ng/mL)	461.6 ± 945.2	4448.3 ± 12403.6	0.005

Data are mean ± SD, unless otherwise stated. γGT, γ-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol.

Table 6 | Comparison of amino acid profile by insulin resistance

(nmol/mL)	HOMA-IR ≤ 1.6	HOMA-IR > 1.6	*P*
Glycine	211.1 ± 51.7	201.1 ± 42.4	0.495
Alanine	321.8 ± 58.3	370.2 ± 70.8	0.007
Serine	114.2 ± 20.2	104.3 ± 19.1	0.092
Threonine	123.9 ± 26.2	120.8 ± 14.2	0.662
Valine	202.2 ± 36.8	232.0 ± 34.3	0.006
Isoleucine	57.5 ± 11.3	72.0 ± 14.0	<0.001
Leucine	115.2 ± 22.6	135.7 ± 22.0	0.002
Lysine	171.4 ± 31.9	175.1 ± 30.9	0.695
Arginine	49.4 ± 16.6	60.2 ± 15.8	0.027
Histidine	78.3 ± 8.1	82.7 ± 6.9	0.060
Tyrosine	56.2 ± 10.0	66.0 ± 8.9	0.001
Phenylalanine	53.9 ± 8.1	61.6 ± 7.2	0.001
Tryptophan	55.3 ± 8.4	58.2 ± 10.8	0.256
Methionine	23.2 ± 4.1	24.2 ± 3.8	0.418
Cysteine	12.5 ± 4.1	13.8 ± 3.6	0.272
Proline	131.9 ± 34.4	167.2 ± 54.3	0.002
Glutamine	505.6 ± 72.7	515.9 ± 61.2	0.619
Glutamic acid	38.5 ± 16.2	53.6 ± 25.1	0.004
Asparagine	47.6 ± 6.6	43.8 ± 7.7	0.057
Aspartic acid	2.8 ± 0.6	3.4 ± 1.4	0.014
Total AA	2544.0 ± 299.2	2723.1 ± 249.6	0.037
NEAA	1663.0 ± 211.7	1760.9 ± 182.1	0.107
EAA	881.0 ± 120.1	962.1 ± 105.4	0.020
BCAA	374.9 ± 67.7	439.7 ± 66.6	0.001

Data are mean ± SD. BCAA, branched-chain amino acid; EAA, essential amino acid; HOMA-IR, homeostasis model assessment of insulin resistance; NEAA, non-essential amino acid; Total AA, total amino acid.

muscle proteins. The pool size of free BCAAs is very small (accounting for ~0.1 g/kg muscle), and this free amino acid pool is quite constant because of the continuous supply from muscle when decreased and rapid degradation when increased[23]. Taken together, it is unlikely that circulating BCAAs are dominantly regulated by dietary intake, at least in the steady state.

Contrary to BCAAs, the relationships of other amino acids with insulin resistance remain to be elucidated. The aromatic amino acids, tyrosine and phenylalanine, have been reported to be elevated together with BCAAs in obesity-related insulin resistance[7–10,13], although the reason is not fully discussed in the literature. Phenylalanine is metabolized to catecholamines via tyrosine through a completely different pathway from that of BCAAs. Newgard *et al.* [7] explained in their report that the aromatic acids, phenylalanine and tyrosine, are elevated in obese subjects compared with lean subjects, because the 'large neutral amino acids' (valine, isoleucine and leucine, tyrosine, phenylalanine and tryptophan) compete for transport into mammalian cells by the large neutral amino acid transporter, assuming that chronic elevations in BCAAs might impair the transport of aromatic amino acids into cells and tissues. Alanine and glutamic acid also appeared to be associated with insulin resistance. A report from the Framingham offspring study showed that alanine and glutamic acid were positively associated with the HOMA index[24], which was perhaps observed in the present study. Alanine is metabolized to pyruvate and glutamic acid is derived from BCAAs catalyzed by BCAT in hepatic gluconeogenic pathways, suggesting some role in glucose homeostasis.

There were some limitations in the present study. First, the sample size was not large enough for conclusions regarding marginal insignificant *P*-values. Second, because of the cross-sectional nature of the study, the cause–effect relationship of our findings is uncertain. We conjecture that insulin resistance causes alteration of amino acid profile; however, other

controversial evidence indicates that increased amino acid levels might cause insulin resistance[7,25–27]. Third, we used HOMA-IR as an index of insulin resistance, which reflects the balance between hepatic glucose output and insulin secretion in the basal state and sometimes fails to show a close relationship with whole-body insulin resistance assessed by the hyperinsulinemic-euglycemic clamp technique. HOMA-IR has been validated by the gold standard method including Japanese subjects whose BMI range was close to that of the present study (BMI 22–24)[28,29], and therefore we consider it reasonable to use HOMA-IR as an alternative to the glucose clamp in this study. Finally, we simply carried out univariate analysis between HOMA-IR and the clinical parameters. Further investigations are necessary to examine the interrelations and also to elucidate the causal relationships among these factors.

In conclusion, the present study shows that the insulin resistance-related changes in amino acid profile are also observed in non-diabetic Japanese men and women. These amino acids include BCAAs (valine, isoleucine and leucine) and aromatic amino acids (tyrosine and phenylalanine), in agreement with previous studies carried out using different ethnic groups with different degrees of obesity and insulin resistance. Fasting measurement of these amino acids is expected to provide additional information on standard diabetes risk factors.

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