Bull Yamaguchi Med Sch 68(3-4):31-39, 2021

A Close Relationship between Plasma Concentrations of Branched-Chain and Aromatic Amino Acids and Uric Acid among Healthy Adults

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(Received August 4, 2021, accepted October 12, 2021)
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Abstract **Purpose:** The purpose of this study was to investigate the potential differences in plasma branched-chain and aromatic amino acid (BCAAs and AAAs) concentrations according to the levels of uric acid (UA) and determine the trends in such differences among apparently healthy subjects. **Methods:** Data from a total of 2804 healthy subjects were included in the current analysis and were categorized into three groups based on the tertiles of plasma UA level. The group differences for BCAAs and AAAs between UA tertiles were explored by analyses of variance (ANOVA) and covariance (ANCOVA). **Results:** There was a progressive increase in the concentrations of all BCAAs and AAAs from the lower to the upper tertiles. The group differences were significant for all amino acids investigated in this study ($P<0.001$) except for tryptophan. Overall, the differences were stronger in the higher quartile categories when compared with those for the corresponding lowest quartile category of individual BCAAs and AAAs. **Conclusions:** The current results suggest the potential existence of a close relationship of plasma levels of BCAAs and AAAs with UA, and warrant further research with elucidation of causal associations and interactions between them.

Key words: branched-chain, aromatic, amino acids, uric acid, relationship

Introduction

Under normal physiological conditions, individual circulating amino acids play different metabolic or biochemical roles in the human body.[1] As reported, the amino acids can make important contributions to the pathogenesis of specific diseases.[2,3] In recent years, there has been an increasing interest in investigating the association of specific plasma free amino acids (PFAAs) with different disease states. Among the PFAAs, branched-chain amino acids/BCAAs (Isoleucine/Ile, Leucine/Leu, and Valine/Val) and aromatic amino acids/AAAs (Phenylalanine/Phe, Tyrosine/Tyr, and Tryptophane/Trp) have been shown to play important roles in the pathogenesis of diseases like hypertension, metabolic syndrome, insulin resistance, type 2 diabetes (T2D), kidney disease etc.[3-7] On the other hand, elevated blood level of uric acid (UA) or hyperuricemia plays important roles in the

development and progression of a wide variety of human diseases.[8-11] Especially, these published studies suggested the association of hyperuricemia with cardiovascular conditions including hypertension, metabolic syndrome and coronary artery disease, and also with vascular diseases such as cerebrovascular disease, vascular dementia, preeclampsia, as well as with diabetes and kidney disease etc.

The findings of the above-mentioned studies indicate the potential role of BCAAs and AAAs, and also UA in the development and/or progression of a number of common disease states. Furthermore, we postulate that circulating levels of BCAAs and AAAs might be closely linked with UA, and probably play important roles—individually and/or interactively—in the development of relevant diseases.[12] Revealing the potential relationship between plasma concentrations of BCAAs and AAAs, and UA might help to better understand the development of disease pathophysiology, and undertake specific measures in the prevention and management of such diseases. However, to the best of our knowledge, such a relationship between circulatory levels of BCAAs and AAAs, and UA has not yet been investigated. For proper understanding of the mentioned relationship, at first, it is important to determine and understand whether there are specific patterns in the altered concentrations of circulatory levels of BCAAs and AAAs according to the levels of UA among healthy people.

Accordingly, the purposes of this cross-sectional study were to investigate the possible differences in BCAA and AAA concentrations according to the degree of plasma uric acid levels, as measured by its tertiles, and determine the trends in such differences among apparently healthy subjects.

Materials and methods

Study design and ethical issues

For this cross-sectional study, approval of the protocol was obtained from the relevant institutional review board of Yamaguchi University (H25-26-2) and Shimane University (20100129-3). The study was conducted in accordance with the Declaration of Helsinki and the study participants were briefed verbally about the detailed protocol. The participants provided written informed consent to participate in this study.

Study population

The flowchart of the participants included in this study has been depicted in Figure 1. Briefly, a total of 2804 adult subjects were finally considered for this study, who: a) underwent their annual health check-up at different health examination centers in Shimane Prefecture of Japan, b) were free from any known diseases, and c) not taking any medications. The annual health check-up included physical examinations, clinical and other laboratory tests. Also, data were collected on their personal and medical history using a self-administered questionnaire. The subjects were apparently free from any known health problems.

Measurement of BCAAs and AAAs

In the current study, we measured the absolute concentrations (in μmol/L) of BCAAs and AAAs. For this purpose, 5 ml of blood samples were collected and analyzed following the protocol previously described elsewhere.[13] Briefly, after overnight fasting, cubital venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA; Termo, Tokyo, Japan). The tubes with blood samples were immediately placed on ice. Then the tubes were centrifuged at 3,000 rpm under 4℃ for a period of 15 min and stored at −80℃. The tubes were kept there until the desired analysis. Before the measurements of PFAAs, the plasma samples were deproteinized using acetonitrile at a final concentration of 80%. After precolumn derivatization, the concentrations of PFAAs were measured by high-performance liquid chromatography–electrospray ionization–mass spectrometry (HPLC–ESI–MS), which allows such measurements with high accuracy.

Measurement of other laboratory variables

We determined fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) using the hexokinase method and latex agglutination immunoassay, respectively. Also, we measured high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol

(LDLC), and triglyceride (TG) in the serum enzymatically. To measure the plasma UA, we used the uricase-HMMPS method by L-type UA.M kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Statistical analyses

The continuous variables of this study did not show normal distributions as evaluated by Kolmogorov-Smirnov and Shapiro-Wilk tests. Therefore, statistical analyses were performed after logarithmic transformation of the collected data and the results have been reported in the original scale after back transformation. Summary statistics for the continuous variables have been expressed as geometric mean and corresponding 95% CI, and for the categorical variable, as number and percentage. We categorized the subjects of this study into three groups based on the tertiles of plasma UA concentrations.[14-16] The sex-specific cutoffs were: ⩽5.1 mg/dl, 5.2–6.1 mg/dl, and ⩾6.2 mg/dl in the men, and ⩽3.6 mg/dl, 3.7–4.3 mg/dl, and ⩾4.4 mg/dl in the women, for tertile 1, tertile 2, and tertile 3, respectively. Pearson correlation analysis was performed between the concentrations of BCAAs and AAAs separately in tertile categories. Differences for the variables between the UA tertiles were tested using the one-way analysis of variance (ANOVA) for the continuous variables, and by the Chi-square (χ^2) test for the categorical variable. Further analyses for individual PFAAs were conducted by using analysis of covariance (ANCOVA), with adjustments for the potential confounding demographic and clinical factors that differed between the UA tertiles. Multiple comparisons were performed with Bonferroni adjustments in the level of significance as necessary. To perform the statistical analyses of the data, the software package SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA) was used. All statistical tests in this study were considered as two-tailed, and the significance level was set at $P<0.05$.

Results

The data for a total of 2804 healthy subjects (1191 men, 1613 women) not taking any medications and apparently free from any diseases were included in the final analysis of this study (Figure 1).

All the tertile categories included more females, but the sex distribution was similar across the tertiles ($P=0.773$).

Demographic and clinical characteristics of study subjects

The demographic and clinical characteristics of the current study subjects have been presented in Table 1, according to the tertiles of plasma UA. Compared to the subjects in tertile 2, those in tertiles 1 and 3 were older ($P<0.001$). Subjects in the higher tertiles (with higher plasma UA concentrations) had increasing levels of HbA1c, TG, and DBP, and lower levels of HDLC and the group differences were significant ($P<0.05$ to 0.001). The

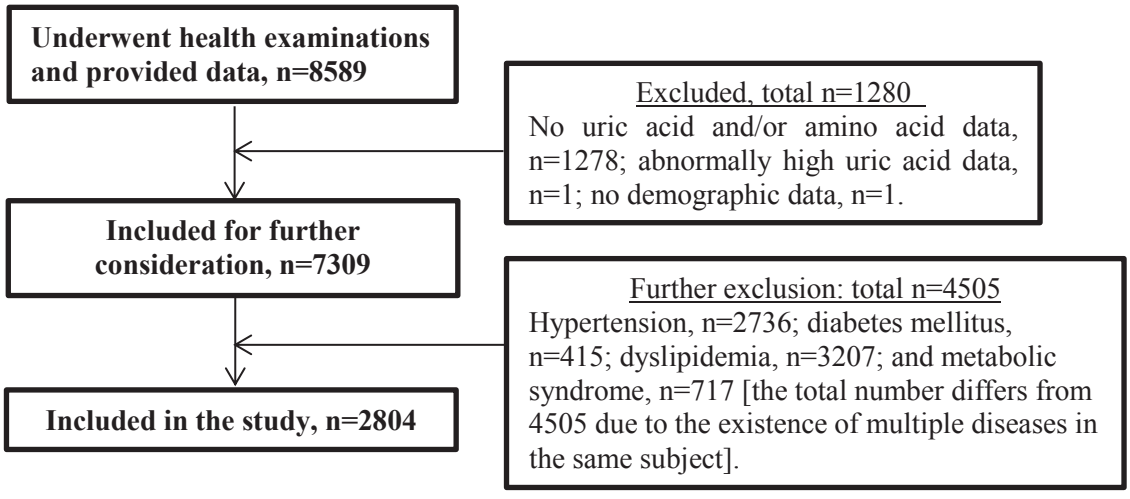

Fig. 1 Flowchart of current study participants.

values for FPG, LDLC and SBP did not differ significantly between the tertiles.

Correlation between BCAAs and AAAs

The relationship between the measured concentration of BCAAs and AAAs in different tertiles were examined with Pearson correlation analysis. As evident in Table 2, plasma concentrations of BCAAs and AAAs showed significant positive correlations with each other in all tertiles (r=0.42 to 0.58, 0.44 to 0.53, and 0.44 to 0.53 for tertiles 1, 2, and 3, respectively; P<0.001).

Table 1 Demographic and clinical characteristics of the study subjects by tertiles of uric acid. Values are expressed as geometric mean and 95% confidence interval (CI) for continuous variables, and number and percentage for the categorical variable

Variable	Tertile 1 (n=913)			Tertile 2 (n=1006)			Tertile 3 (n=885)			P-value
	Geometric mean or n (%)	95%CI Lower	Upper	Geometric mean or n (%)	95%CI Lower	Upper	Geometric mean or n (%)	95%CI Lower	Upper	
Age (Years)	45.6	44.6	46.7	42.7	41.8	43.6	44.2	43.2	45.3	<0.001
Sex										
Male	379 (41.5)	–	–	432 (42.9)	–	–	380 (42.9)	–	–	0.773
Female	534 (58.5)	–	–	574 (57.1)	–	–	505 (57.1)	–	–	
BMI (kg/m^2)	20.7	20.5	20.8	21.2	21.1	21.4	22.2	22.0	22.4	<0.001
FPG (mg/dL)	91.0	90.5	91.6	90.4	90.0	90.9	91.6	91.0	92.2	0.198
HbA1c (%)	5.4	5.4	5.5	5.4	5.4	5.4	5.5	5.4	5.5	0.032
HDLC (mg/dL)	68.8	67.9	69.8	68.7	67.7	69.7	66.7	65.7	67.8	<0.001
LDLC (mg/dL)	102.3	100.9	103.7	103.6	102.3	104.9	104.6	103.1	106.0	0.958
TG (mg/dL)	62.3	60.8	63.9	62.7	61.2	64.3	69.9	68.0	71.8	<0.001
SBP (mmHg)	115.8	115.1	116.5	116.0	115.3	116.7	118.3	117.5	119.0	0.404
DBP (mmHg)	71.4	70.8	72.0	71.7	71.1	72.3	73.5	72.9	74.1	<0.001
Waist (cm)	74.4	74.0	74.9	76.0	75.5	76.5	78.9	78.3	79.5	<0.001

Cutoff values for UA tertiles: ⩽5.1 mg/dl, 5.2-6.1 mg/dl, and ⩾6.2 mg/dl for men, and ⩽3.6 mg/dl, 3.7-4.3 mg/dl, and ⩾4.4 mg/dl for women for tertile 1, tertile 2, and tertile 3, respectively.
BMI, body mass index; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c; HDLC, high-density lipoprotein cholesterol; LDLC, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TG, triglycerides.
P-values indicate two-tailed group differences for the variables between the uric acid tertiles, tested by one-way analysis of variance (ANOVA) for the continuous variables, and Chi-square (χ^2) test for the categorical variable.
The total number of subjects in the tertiles represents the categorization of subjects into three groups based on sex-specific cutoffs values of uric acid.

Table 2 Correlation coefficients between the concentrations of branched-chain and aromatic amino acids across tertiles

Amino acids	Tertile 1			Tertile 2			Tertile 3		
	Phe	Tyr	Trp	Phe	Tyr	Trp	Phe	Tyr	Trp
Ile	0.48*	0.42*	0.52*	0.48*	0.45*	0.49*	0.48*	0.45*	0.49*
Leu	0.58*	0.47*	0.56*	0.53*	0.46*	0.53*	0.53*	0.46*	0.53*
Val	0.52*	0.45*	0.55*	0.47*	0.44*	0.49*	0.47*	0.44*	0.49*

Cutoff values for UA tertiles: ⩽5.1 mg/dl, 5.2-6.1 mg/dl, and ⩾6.2 mg/dl for men, and ⩽3.6 mg/dl, 3.7-4.3 mg/dl, and ⩾4.4 mg/dl for women for tertile 1, tertile 2, and tertile 3, respectively.
Ile, isoleucine; Leu, leucine; Val, valine; Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan.
*All P-values: <0.001

Differences in the concentrations of BCAAs and AAAs between tertiles of UA

Table 3 depicts the geometric mean and corresponding 95% CI values for the concentrations of individual BCAAs (Ile, Leu, and Val), and AAAs (Phe, Trp, and Tyr), according to the tertiles of UA. The differences in the concentration of BCAAs and AAAs across different tertiles were evaluated using ANOVA and ANCOVA with adjustments for potential confounders in the latter. The analyses revealed that all BCAAs and AAAs had a linear trend in their concentrations across the tertile categories. There was a progressive increase in the concentrations of all BCAAs and AAAs from the first to the last tertiles.

As the current analysis by ANOVA and ANCOVA shows, the group differences were significant for the PFAAs investigated in this study, except for Trp by ANCOVA. Subsequent multiple comparisons by both ANOVA and ANCOVA revealed that compared to the lowest tertile category, the BCAAs demonstrated consistently significant differences across higher tertile categories (P<0.001), except for Ile compared between tertiles 1 and 2 by ANCOVA (P=0.074). For the AAAs, multiple comparison revealed significant differences only between tertiles 1 and 3 in the analyses by ANOVA (P<0.01 to 0.001); on the other hand, the differences by ANCOVA demonstrated significant differences in the higher

Table 3 Differences in the concentrations of BCAAs and AAAs between tertiles without (ANOVA) and with (ANCOVA) adjustments for potential confounding factors. Values of amino acids are expressed as geometric mean and 95%CI

Amino acids	Tertiles	n	Geometric mean	95%CI		P1 (ANOVA)		P2 (ANCOVA)	
				Lower	Upper	Group	Tertile 1 vs.	Group	Tertile 1 vs.
Ile	Tertile 1	913	50.5	49.8	51.2				
	Tertile 2	1006	52.0	51.3	52.7	<0.001	<0.01	<0.001	0.074
	Tertile 3	885	54.0	53.2	54.8		<0.001		<0.001
Leu	Tertile 1	913	98.8	97.6	100.0				
	Tertile 2	1006	103.2	102.0	104.5	<0.001	<0.001	<0.001	<0.001
	Tertile 3	885	107.2	105.8	108.6		<0.001		<0.001
Val	Tertile 1	913	184.1	181.9	186.2				
	Tertile 2	1006	191.3	189.1	193.5	<0.001	<0.001	<0.001	<0.001
	Tertile 3	885	199.2	196.7	201.7		<0.001		<0.001
Phe	Tertile 1	913	53.0	52.5	53.6				
	Tertile 2	1006	53.9	53.4	54.3	<0.001	.056	<0.001	<0.005
	Tertile 3	885	56.1	55.5	56.6		<0.001		<0.001
Tyr	Tertile 1	913	56.1	55.4	56.8				
	Tertile 2	1006	57.0	56.3	57.6	<0.001	.283	<0.001	0.084
	Tertile 3	885	59.4	58.6	60.1		<0.001		<0.001
Trp	Tertile 1	913	50.4	49.9	51.0				
	Tertile 2	1006	51.1	50.6	51.7	<0.01	.216	0.235	0.551
	Tertile 3	885	51.8	51.2	52.4		<0.005		0.333

Cutoff values for UA tertiles: ⩽5.1 mg/dl, 5.2-6.1 mg/dl, and ⩾6.2 mg/dl for men, and ⩽3.6 mg/dl, 3.7-4.3 mg/dl, and ⩾4.4 mg/dl for women for tertile 1, tertile 2, and tertile 3, respectively.
ANOVA, analysis of variance; ANCOVA, analysis of covariance; CI, confidence interval.
Ile, isoleucine; Leu, leucine; Val, valine; Phe, phenylalanine; Tyr, tyrosine; Trp, tryptophan
P1 and P2 indicate the P-values for differences between tertile categories by two-tailed ANOVA and ANCOVA, respectively. Multiple comparisons were performed with Bonferroni adjustments. Results of ANCOVA were adjusted for age, Sex, BMI, HbA1c, HDLC, TG, DBP and waist circumference.

tertiles for Phe, and in the highest tertile for Tyr, compared to the lowest tertile category, respectively.

Overall, as observed, the significant differences were stronger in the higher quartile categories when compared with those for the corresponding lowest quartile category of individual PFAAs investigated in this study.

Discussion

In this study, we demonstrated that the level of plasma UA in its higher tertiles was accompanied by concomitant elevations in the levels of BCAAs and AAAs. The current findings indicate that circulating levels of BCAAs and AAAs, and UA are closely linked with each other. This is the first study of its kind to investigate the existence of such a relationship between the levels of BCAAs and AAAs with UA.

In our study, a progressive increase in HbA1c, TG, and DBP and a decrease in HDLC across UA tertiles simply represent the fact that the level in the mentioned parameters progressively changes with a change in the UA level. In general, these findings correspond to those of others reported in the existing literature.[14,17] Interestingly, there was a significant group difference in age across tertiles, and subjects in the higher tertiles were comparatively younger. However, this finding is also in agreement with previous observations in age distributions across UA tertiles.[14,17,18]

In this study, we observed significant positive correlations between BCAAs and AAAs. Our findings are consistent with earlier research works which also observed such positive correlations between BCAAs and AAAs.[13,19] Furthermore, our adjusted results showed significant group differences in the levels of BCAAs and AAAs across the UA tertiles (except Trp). As shown in our study, there was a progressive increase in the concentrations of BCAAs and AAAs from the lower to the upper tertiles. These indicate the presence of a relationship between the circulatory levels of BCAAs and AAAs, and UA.

There is a severe lack of similar studies that focused on the relationship of BCAAs and AAAs with UA, which makes it difficult to explain the underlying mechanisms behind such observations. BCAAs and AAAs, and UA probably influence each other via multiple direct and indirect mechanisms. In humans, UA is the end product of purine catabolism.[9] Certain amino acids take part in the biosynthesis of purine and subsequent formation of UA. For example, among the amino acids, BCAAs have been shown to contribute to the purine nucleotide cycle.[20] On the other hand, the existence of a close link between the circulatory levels of BCAAs and AAAs has been suggested in the literature.[13,20] Based on such observations, it is reasonable to postulate that altered circulatory levels of BCAAs and AAAs might cause a change in the level of UA (or vice versa). Also, it has been reported that BCAAs and AAAs (Phe and Tyr) can contribute to the development of insulin resistance.[21,22] Subsequently, insulin resistance can alter the level of circulatory UA by inhibiting uric acid excretion through increased renal tubular reabsorption and subsequent development of hyperuricemia.[23,24] All these findings from other studies and those of ours—taken together—suggest the potential existence of a close link between the altered concentrations of plasma BCAAs and AAAs, and UA. However, it is noteworthy to mention here that our study design does not allow us to explain whether the altered levels of PFAA in different tertiles of UA is a consequence of changes in the level of the latter, or vice versa. Future studies should clarify the temporal relationship between circulatory levels of BCAAs and AAAs, and UA.

Based on the findings and facts discussed above, we hypothesize that altered levels of BCAAs and AAAs triggered by lifestyle-related factors might induce a change in the plasma level of UA through multiple mechanisms, causing an increase in the latter.[4] Subsequently, such altered UA possibly induces further changes in BCAAs and AAAs. All these might lead to a vicious circle between these amino acids and UA, and be associated with profound effects on various biological mechanisms and functions in the human body including cell signaling, gene expression and neuroendocrine function.[1,25-28] Thus, alterations in BCAAs and AAAs, and UA might play major roles in the pathogenesis

and clinical course of patients with different disease states such as diabetes mellitus, metabolic syndrome, hypertension, cardiovascular disease, cerebrovascular disease and kidney disease etc, and other chronic diseases of public health importance.[4-7,28-35] However, the exact roles of BCAAs, AAAs and UA in the development of disease states remains to be properly established in future longitudinal studies. Clarification of such associations with explanations for underlying mechanisms might have important clinical implications for understanding the pathophysiology and diagnosis of the diseases caused by concomitant alterations in the levels of BCAAs and AAAs, and UA. Moreover, such knowledge on the association between altered levels of BCAAs and AAAs, and UA might be helpful in predicting the risk of developing a range of disease states. Furthermore, it might also be helpful in establishing intervention strategies aimed at manipulating BCAA, AAA, and/or UA concentrations for the treatment of relevant health disorders.

Limitations to the current study findings

This study had several potential methodological limitations and hence, caution is required while interpreting the current results. In this study, the data on background information such as diet, physical exercise, smoking status or alcohol consumption, family history of hyperuricemia etc were not available for the study population. However, the effects of these variables on the current results should be very limited as in this study, we included only the healthy subjects with similar socio-demographic characteristics. In the current study, we did not stratify our results by sex. However, we believe that this did not influence our study findings as we classified the study participants according to the tertiles of UA, separately for both men and women. Moreover, the sex distribution did not differ across the tertiles of UA. Furthermore, the results of ANCOVA have been presented after adjustments for potential confounders including the variable 'sex'. The current study design is cross-sectional in nature and does not allow us to speculate on any causality or temporality or mechanisms underlying the observed relationships between BCAAs, AAAs, and UA. Lastly, we agree that this study was conducted among healthy subjects and a part of the relevant discussion on the current findings are pure speculations only. Therefore, our findings warrant future research that should clarify how such elevated concentrations of BCAAs and AAAs, and UA play roles and interact with each other in the development of pathological conditions in humans.

Conclusions

Our study showed that the levels of BCAAs and AAAs progressively increased with a concomitant elevation in the level of UA. Also, the current findings suggest the potential existence of a close link between plasma levels of BCAAs and AAAs, and UA in healthy subjects. The results highlight the need for further investigations with elucidation of causal associations between alterations in the plasma levels of BCAAs, AAAs and UA, and their possible interactions that might provide new insights into the pathophysiology of relevant diseases and development of intervention strategies directed towards their prevention and management.

Conflict of Interest

TT received the research grant from Ajinomoto Co., Inc. HY and SK are employees of Ajinomoto Co., Inc. The funder had no role in the design of this study or its execution, collection and analysis or interpretation of data, preparation of the submitted manuscript, or decision to publish it.

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