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Altered Levels of Plasma Free Amino Acids in Hyperuricemia among Male Smokers

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Abstract **Purpose:** The purpose was to explore the existence of distinct patterns of alterations in plasma free amino acids (PFAAs) among smokers with hyperuricemia. **Methods:** Venous blood samples from 169 male subjects (95 smokers and 74 nonsmokers) were analyzed for the measurements of 19 PFAAs and uric acid. Mann-Whitney U-test was applied for comparison of PFAA levels between two groups. **Results:** Evaluations of PFAAs made separately in smokers and non-smokers show that compared to the corresponding values for the subjects without hyperuricemia, subjects with it had significantly higher levels of isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine and valine ($P<0.05$ to 0.01) and significantly lower levels of glutamine, glycine and serine ($P<0.05$ to 0.001) in the smoker group, and a significantly higher level of phenylalanine ($P<0.05$) in the nonsmoker group. **Conclusions:** The findings suggest that hyperuricemia may have remarkable effects on the PFAA profiles in smokers, which underscores the importance of investigations into the mechanisms underlying the effects of cigarette smoking on the possible interactive relationship between the levels of plasma uric acid and PFAAs.

Key words: Amino acids, plasma profile, cigarette smoking, Brinkman index, hyperuricemia

Introduction

Cigarette smoking is an established causal risk factor for multiple diseases of the lung and many other chronic and degenerative diseases affecting various organ systems which cause enormous burdens and challenges to the health care system globally.[1] It is also considered to be the single most significant cause of preventable deaths in the world.[2] On the other hand, the prevalence of hyperuricemia (HU) has been increasing worldwide.[3] A growing number of published studies reported a strong association of elevated uric acid (UA) as an independent risk factor and predictor for the development and progression of various lifestyle-related diseases.[4,5] Despite some conflicting reports, published literature suggests the existence of an association between smoking and an elevated blood UA level or increased prevalence of HU.[6,7]

In recent years, plasma free amino acids (PFAAs) profiles are increasingly being used in the evaluation of various diseases. It has been observed that cigarette smoking can significantly influence the PFAAs and cause

alterations in the levels of the amino acids.[8,9] On the other hand, some published studies reported a clear association between HU and PFAAs among patients with various lifestyle-related diseases, and suggested the possibility of interplay between PFAAs and UA in the pathophysiology of those diseases.[10,11]

It is understandable from the available literature that both cigarette smoking and HU can cause alterations in PFAAs, and distinct patterns of differences in the concentrations of the latter might exist among smokers with and without HU. A better understanding of the specific patterns of changes in PFAA levels among smokers with HU might contribute towards a better understanding of any associated pathophysiology, and provide useful information for the prevention and/or early detection and treatment of diseases caused by the presence of HU among smokers. However, any such potential changes in the concentrations of PFAAs remain unknown as no study, to the best of our knowledge has hitherto investigated it among smokers with and without HU. Therefore, one purpose of this study was to examine the differences in the concentrations of PFAAs and UA amongst smokers versus non-smokers. Another purpose was to explore the possible differences in PFAAs with and without HU in both groups of subjects in an attempt to clarify the existence of any distinct differences in alterations of PFAAs among smokers with and without HU.

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

A total of 327 male subjects undergoing their annual health check-up between June and July 2012 at different health examination centers in Shimane Prefecture, Japan were initially considered for this study. As described elsewhere, the health examination included physical examination, clinical and laboratory tests, and a self-administered questionnaire containing personal and medical history.[10] A total of 158 subjects aged over 75 years were excluded from the study as they did not have the data on UA (not measured), leaving 169 subjects for final inclusion in this study. The subjects were free from any serious health problems such as heart diseases, cancer or renal failure etc.

Measurement of PFAAs

In the current study, we measured the absolute concentrations (in μmol/L) of 19 PFAAs: alanine (Ala), arginine (Arg), asparagine (Asn), Citrulline (Cit), glutamine (Gln), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), ornithine (Orn), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tryptophan (Trp), tyrosine (Tyr), and valine (Val). For this purpose, 5 ml of blood samples were collected and analyzed following the protocol previously described elsewhere.[11] Briefly, after overnight fasting, cubital venous blood samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA; Terumo, 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%. The concentrations of PFAAs were measured by high-performance liquid chromatography-electrospray ionization-mass spectrometry followed by precolumn derivatization.

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 the uricase-HMMPS method by L-type UA.M kit (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

Smoking history

For collection of data on smoking history from the study subjects, the original questionnaire used Brinkman Index/BI (number of cigarettes smoked per day x number of years of smoking) as described by Brinkman and Coates.[12] Based on the collected data, we classified all subjects with a BI=0 as non-smokers (n=74) and the rest subjects as smokers (n=95, including current and past smokers; median and IQR values for BI, 640 and 540, respectively).

Data analyses and statistical methods

As our study included only male subjects, we defined HU as a plasma UA ≥7 mg/dL [11]. The continuous variables in this study showed a non-normal distribution by Kolmogorov-Smirnov and Shapiro-Wilk tests. Hence, nonparametric statistical analyses of the study data were performed. The continuous variables of this study were presented as the median and interquartile range (IQR), and the categorical variables as number and percent. The differences were assessed by the Mann-Whitney U-test for the continuous variables and Chi-square (χ^2) test, for the categorical variables. The statistical analyses were performed with the software package SPSS version 22 for Windows (SPSS Inc., Chicago, IL, USA). For all statistical tests, the significance level was set at a two-tailed $P<0.05$.

Results

Table 1 shows the demographic and clinical characteristics of the study subjects for both smoker and non-smoker groups. Most subjects in both groups were the elderly males. As the results show, no significant differences in terms of the demographic and clinical variables existed between the two groups except for the plasma level of UA which showed a significantly higher value for the smokers (median and IQR values for the smoker and non-smoker groups were 6.0 mg/dl and 1.4 mg/dl, and 5.7 mg/dl and 1.4 mg/dl, respectively; P=0.009). Also, no statistically significant differences between the

Table 1 Demographic and clinical characteristics of the study populations including smokers and nonsmokers and further divided according to the presence or absence of hyperuricemia (HU). Values are expressed as median and interquartile range (IQR) for all continuous variables

Variables	All					Smokers (n = 95)					Nonsmokers (n = 74)				
	Smokers (n = 95)		Nonsmokers (n = 74)		P1-value	Without HU (n = 61)		With HU (n = 34)		P2-value	Without HU (n = 58)		With HU (n = 16)		P3-value
	Median	IQR	Median	IQR		Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Age (Years)	68.0	8.0	68.0	7.3	0.855	68.0	8.5	68.5	8.3	0.560	69.0	7.5	66.0	8.3	0.519
BMI (kg/m^2)	23.0	3.5	23.4	4.2	0.739	22.6	3.4	23.6	4.7	0.080	23.0	4.3	23.6	3.4	0.287
FPG (mg/dL)	88.0	13.0	88.0	12.0	0.453	88.0	12.5	87.0	10.5	0.638	88.0	12.3	88.0	12.5	0.921
HbA1c (%)	5.3	0.5	5.3	0.5	0.386	5.3	0.6	5.3	0.5	0.876	5.2	0.6	5.5	0.3	0.045
HDLC (mg/dL)	58.0	21.0	56.5	20.3	0.610	60.0	25.0	55.5	19.0	0.197	57.5	20.5	54.5	17.5	0.278
LDLC (mg/dL)	111.0	34.0	113.0	32.5	0.624	106.0	33.5	119.0	41.5	0.049	108.5	30.5	124.5	25.5	0.039
TG (mg/dL)	93.0	73.0	91.5	62.0	0.169	89.0	54.5	125.0	97.5	0.049	88.5	61.5	104.0	74.3	0.196
SBP (mmHg)	125.0	19.0	121.5	17.3	0.105	124.0	21.5	127.0	15.3	0.263	122.5	17.3	121.0	27.0	0.895
DBP (mmHg)	76.0	13.0	75.5	12.0	0.617	75.0	12.0	76.0	14.5	0.310	75.0	11.5	78.5	12.5	0.411
UA (mg/dL)	6.0	1.4	5.7	1.4	0.009	—	—	—	—	—	—	—	—	—	—

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; UA, uric acid.

P-values indicate statistically significant difference between groups by Mann-Whitney U Test: P1, smokers versus nonsmokers; P2, without HU versus with HU in smokers; P3, without HU versus with HU in non-smokers.

smokers and nonsmokers were observed for alcohol drinking status (χ^2=0.035, P=0.874). The medications commonly used by the study subjects were prescribed for the treatment of hypertension (smokers, 26/95 or 27.4%; nonsmokers, 21/74 or 28.4%), diabetes mellitus (smokers, 14/95 or 14.7%; non-smokers, 8/74 or 10.8%), and dyslipidemia (smokers, 21/95 or 22.1%; non-smokers, 15/74 or 20.3%). However, the smoker and non-smoker groups did not differ significantly with respect to the use of medications for any of the mentioned diseases (hypertension, χ^2=0.021, P=0.884; diabetes mellitus, χ^2=0.566, P=0.452; dyslipidemia, χ^2=0.084, P=0.773).

As we observed, the prevalence of HU among smokers and nonsmokers were 35.8% and 21.6%, respectively. When the demographic and clinical characteristics of the study subjects were compared between subjects without and with HU separately in both smoker and non-smoker groups, no statistically significant differences were observed except for marginally significant differences (P=0.049) for LDLC and TG among the smokers, and significant differences for HbA1c (P=0.045) and LDLC (P=0.039) among the nonsmokers (Table 1).

For the individual amino acids, at first, we investigated the existence of any significant differences in their concentrations between the smoker and non-smoker groups, as presented in Table 2. However, no significant differences between these two groups could be revealed for the level of any PFAAs except for Lys, the plasma concentration of which was

Table 2 Plasma amino acid concentrations (μmol/L) in smokers and nonsmokers

Amino acids	Smokers (n = 95)		Nonsmokers (n = 74)		P-value
	Median	IQR	Median	IQR	
Ala	360.7	113.6	360.8	110.2	0.882
Arg	97.6	23.3	100.5	27.8	0.217
Asn	48.2	7.8	49.6	8.7	0.270
Cit	36.2	10.9	33.3	10.5	0.101
Gln	605.4	89.1	628.6	86.5	0.155
Gly	212.7	63.4	211.3	44.6	0.761
His	80.6	13.4	82.3	11.9	0.464
Ile	62.4	19.2	65.7	17.3	0.632
Leu	125.3	30.4	125.2	23.0	0.874
Lys	184.7	36.5	196.6	41.5	0.017
Met	27.6	5.3	27.0	5.9	0.566
Orn	54.4	16.0	52.3	14.6	0.255
Phe	62.3	9.2	62.8	12.7	0.864
Pro	138.7	49.7	146.9	52.6	0.235
Ser	111.4	28.7	110.2	25.1	0.584
Thr	123.8	27.3	127.6	37.9	0.498
Trp	56.7	14.1	59.1	12.0	0.152
Tyr	68.5	20.6	67.3	17.7	0.314
Val	226.9	58.6	231.9	52.3	0.678

Ala, alanine; Arg, arginine; Asn, asparagine; Cit, citrulline; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine, Trp, tryptophan, Tyr, tyrosine; Val, valine.

P-values indicate statistically significant difference between smoker and nonsmoker groups by Mann-Whitney U Test.

significantly lower among the smokers compared to that for the nonsmokers (P=0.017).

Next, to investigate the differences between the subjects with and without HU, the statistical tests were performed in both smokers and nonsmokers separately (Table 3). For the smokers, the concentrations of 10 out of 19 PFAAs demonstrated significant differences between the subjects without and with HU. Among those 10 PFAAs, compared to the corresponding values for the subjects without HU, subjects with HU had significantly higher levels of Ile, Leu, Met, Phe, Trp, Tyr and Val (P<0.05 to 0.01), and significantly lower levels for Gln, Gly and Ser (P<0.05 to 0.001). In contrast, in the nonsmoker group, no individual PFAAs showed any significant difference in their concentration between the subjects with and without HU except for Phe, the plasma concentration of which was found to be higher among the subjects with HU (P=0.039).

Discussion

To our knowledge, this is the first study in the literature that examined the differences in PFAAs among smokers with and without HU. We also included nonsmoker subjects with and without HU and examined the

Table 3 Differences in plasma concentrations of amino acids (μmol/L) between the subjects without and with hyperuricemia (HU) in smokers and nonsmokers

Amino acids	Smokers (n = 95)					Nonsmokers (n = 74)				
	Without HU (n = 61)		With HU (n = 34)		P1-value	Without HU (n =58)		With HU (n = 16)		P2-value
	Median	IQR	Median	IQR		Median	IQR	Median	IQR	
Ala	356.3	105.0	366.0	133.2	0.302	359.4	110.7	377.9	181.2	0.128
Arg	102.2	22.7	91.5	17.9	0.101	97.3	27.5	106.3	27.6	0.684
Asn	33.7	7.5	48.0	11.5	0.305	49.1	8.8	50.6	12.1	0.288
Cit	36.2	9.0	35.7	13.7	0.981	33.3	9.9	32.7	12.4	0.609
Gln	626.1	88.7	573.6	80.9	0.007	626.5	85.7	631.5	104.2	0.743
Gly	224.8	74.2	186.1	45.6	0.000	211.6	59.1	211.3	27.0	0.416
His	80.4	12.5	82.2	14.8	0.368	81.4	12.2	84.8	6.6	0.115
Ile	57.7	19.6	70.3	14.6	0.004	64.7	16.2	69.6	23.0	0.243
Leu	116.9	29.4	135.1	28.4	0.009	122.5	25.7	132.7	17.7	0.066
Lys	181.8	40.4	185.1	27.0	0.499	195.5	47.4	212.7	44.1	0.106
Met	26.9	5.0	28.7	4.4	0.026	27.0	5.7	28.2	7.3	0.276
Orn	55.8	15.5	53.2	19.8	0.424	51.9	14.6	53.1	18.9	0.990
Phe	61.5	9.5	64.9	16.5	0.013	62.2	11.1	71.7	15.7	0.039
Pro	134.2	48.1	155.7	49.9	0.126	141.2	57.0	165.5	30.7	0.085
Ser	113.8	29.3	103.7	20.5	0.027	109.8	25.2	110.8	28.4	0.854
Thr	125.7	25.7	120.1	35.0	0.364	127.3	39.1	135.6	45.2	0.581
Trp	55.9	9.4	60.8	14.3	0.007	58.5	12.3	61.4	12.3	0.282
Tyr	64.4	17.2	77.2	20.4	0.002	67.2	17.4	69.6	20.8	0.627
Val	210.4	54.5	247.5	56.0	0.006	226.2	56.2	238.0	39.9	0.217

Ala, alanine; Arg, arginine; Asn, asparagine; Cit, citrulline; Gln, glutamine; Gly, glycine; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Orn, ornithine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine, Trp, tryptophan, Tyr, tyrosine; Val, valine.

P1 and P2-values indicate statistically significant differences between subjects without and with HU by Mann-Whitney U Test in the smoker and nonsmoker groups, respectively.

differences in PFAAs amongst them.

Overall, in our study, the smoker and nonsmoker study populations (also, considering the presence or absence of HU) were similar with respect to the demographic and clinical variables. The only difference between the smokers and nonsmokers was for the concentration of UA, which was detected to be significantly higher among smokers compared with the nonsmokers. This latter finding does not support the inverse relationship between smoking and plasma level of UA reported by a number of studies in the literature.[13,14] Conversely, our findings are consistent with the findings of other previously published studies which showed higher blood UA levels among the smokers.[6,7] The presence of higher prevalence of HU among smokers observed in our study is probably due to the fact that both smoking and ageing is individually associated with increased UA levels especially in men, and that the elderly subjects were predominant in this study which comprised exclusively male subjects.[15,16]

Little is known about the changes in PFAA profiles among smokers as a limited number of research works has been conducted on this issue. Furthermore, those studies performed measurements of various PFAAs that differ from PFAAs measured in our study. This makes the comparison of our study findings with those of others difficult. In a cohort study, it has been demonstrated that compared with the former smokers and never smokers, the concentrations of Arg, Orn, and Ser were higher among the current smokers.[9] In contrast, Tomoda et al. (2014) in an animal study, exposed rats to cigarette smoke for a period of four-weeks and observed a significant reduction in the levels of branched chain amino acids/BCAAs (Ile, Leu, Val) levels in the rats.[17] However, in our study, only the plasma concentration of Lys was significantly lower among the smokers compared to that for the nonsmokers. Differences in the amount of alcohol consumption by the study populations,[9] the selection of study subjects (human or animal),[17] and the differing doses and duration of exposure to cigarette smoking or cigarette smoke etc might have contributed to the observed inconsistency between the study findings.

The patterns of changes in PFAA profiles in subjects with and without HU among smokers remain uncertain as, to the best of our knowledge, no other study has hitherto investigated it. As observed in our study, the concentration of Phe differed significantly between the subjects without and with HU (higher in HU group) in both smokers and nonsmokers. This finding is consistent with the finding of our recently published study, where the healthy control subjects and patients with 4 types of lifestyle-related diseases showed significantly higher level of Phe among the subjects with HU compared with the subjects without it.[11] On the other hand, among the smokers compared with the subjects without HU, subjects with HU had significantly altered levels in a number of PFAAs: subjects with HU had significantly higher levels of Ile, Leu, Met, Trp, Tyr and Val, and significantly lower levels of Gln, Gly and Ser than those without HU. Based on our observed findings of the similarities and dissimilarities in the patterns of changes in PFAA concentrations amongst the smokers and nonsmokers with and without HU and also the findings from previously published studies considered together, it can be assumed that a close relationship probably exists between smoking and plasma UA and their interaction probably induces further changes in the plasma PFAAs. We found a lower concentration of several PFAAs (Gln, Gly and Ser) in the smokers with HU compared with those without it, which might have been caused by the fact that these amino acids, particularly of Gly and Ser, play important roles in the biosynthesis of purine and are increasingly being utilized in the formation of UA among the smokers with HU.

Furthermore, we found elevated plasma concentrations of BCAAs (Ile, Leu, Val) and aromatic amino acids/AAAs (Phe, Tyr, Trp) among the smokers with HU. In a nested case-control study Wang et al. (2011) observed that that a cluster of BCAAs (Ile, Leu, Val) and AAAs (Phe, Tyr) predicted the future development of type 2 diabetes up to 12 years prior to onset of it.[18] The authors of that study suggested that increased levels of circulating BCAAs may promote insulin resistance possibly via the disruption of insulin

signalling in skeletal muscles through activation of the mTOR, JUN and IRS1 signalling pathways. Furthermore, higher levels of BCAAs in the blood may lead to increased levels of AAAs as both BCAAs and AAAs compete for transport into mammalian cells by the common large neutral amino acid transporter (LAT1).[19] Also, in our previously published study, the patients with lifestyle-related diseases including DM demonstrated significantly higher concentration of Trp and Tyr compared with those for apparently healthy controls; the concentrations were also significantly higher among the subjects with HU when the comparison was made between the latter subjects and subjects without HU.[11] Moreover, in the current study, the concentration of Met showed an elevation amongst the smokers with HU. Met is a precursor of homocysteine which has been found to correlate with insulin resistance.[20] Although the findings of our study cannot be directly compared with those of other studies mentioned here, however, our findings of elevated plasma concentrations of PFAAs among the smokers with HU are consistent with the existing published literature as epidemiologic studies demonstrated a clear association of cigarette smoking and HU with an increased risk of diabetes and effects on insulin sensitivity.[21,22] However, future studies investigating the effects of smoking on PFAA profiles among subjects with HU should include the measurements of insulin sensitivity/resistance in the study populations.

Limitations to the current study

The findings of this study should be interpreted in light of several potential methodological limitations. First, we did not collect data on dietary habits of the study populations. However, the existence of any such effects on our study findings should be limited as we included a nonsmoker control group for comparison of results. Second, we included current and past smokers as the smoker population and the question may arise on the interpretation of current results as it is unlikely that past smoking might influence amino acid metabolism for years. Such a classification of smokers was unavoidable as the original questionnaire included BI for collection of data on the smoking status of the study subjects. Also, we firmly believe that this does not influence the outcome of our study because inclusion of past smokers as smokers means an underestimation of the current results. Third, we did not collect data on the medications such as those blocking UA production, lowering the blood UA level and/or promoting the excretion of uric acid However, we believe that the findings of our study were not influenced by the intake of any such medications as it would mean an underestimation of the observed results. Fourth, only male Japanese subjects were included in this study which limits the generalization of the study findings to some extent. Fifth, this study was cross-sectional in nature which does not allow us to speculate any causality or temporality of the association between altered levels of PFAAs with HU among the cigarette smokers.

Conclusions

The findings of our study demonstrated that among the smokers with HU compared with those without HU, there was an elevation in BCAAs (Ile, Leu, Val) and AAAs (Phe, Trp, Tyr) and Met, and a reduction in the levels of Gln, Gly and Ser. The findings suggest that HU among smokers may have remarkable effects on the PFAA profiles. The findings may provide new insights into the biological mechanisms of cigarette smoking and HU-related diseases. However, for validation of the current study findings and a comprehensive understanding of the causal association between the alterations in the levels of PFAAs with HU among smokers and the underlying mechanisms, prospective cohort studies should be conducted in future with inclusion of age-matched large and diverse populations.

Conflict of Interest

HY and SK are employees of Ajinomoto Co., Inc. TT received the research grants from Ajinomoto Co., Inc. However, this does not alter the authors' adherences to all of the journal policies. The remaining authors declare that they have no conflict of interest.

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