High Plasma 5-Hydroxyindole-3-Acetic Acid Concentrations in Subjects With Metabolic Syndrome

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OBJECTIVE—Serotonin mediates vasoconstriction and induces the activation of platelets, which may promote atherosclerosis. The aim of this study was to investigate whether plasma 5-hydroxyindole-3-acetic acid (5-HIAA; a derivative end product of serotonin) concentrations are high in subjects with metabolic syndrome (MetS) and to investigate the relationship between plasma 5-HIAA concentrations and clinical and biochemical metabolic parameters.

RESEARCH DESIGN AND METHODS—Plasma 5-HIAA concentrations were measured in 311 subjects (152 men and 159 women) recruited from the Oike Clinic, which provides regular health check-ups for employees. We evaluated the relationship between plasma 5-HIAA concentrations and clinical and biochemical metabolic parameters, including waist circumference, serum lipid concentrations, fasting plasma glucose, or blood pressure.

RESULTS—Plasma 5-HIAA concentrations were higher in subjects with MetS than in those without, in both men (6.5 ± 4.4 vs. 4.9 ± 1.3 ng/mL, $P < 0.005$) and women (7.9 ± 6.5 vs. 5.2 ± 1.6 ng/mL, $P < 0.005$). In men, fasting plasma glucose ($r = 0.197$, $P = 0.0146$) was positively correlated, whereas HDL cholesterol ($r = -0.217$, $P = 0.0071$) was negatively correlated, with logarithmic (log) (plasma 5-HIAA concentrations). In women, triglycerides ($r = 0.252$, $P = 0.0013$) and fasting plasma glucose ($r = 0.344$, $P < 0.0001$) were positively correlated, whereas HDL cholesterol ($r = -0.328$, $P < 0.0001$) was negatively correlated, with log (5-HIAA concentrations). Furthermore, log (plasma 5-HIAA concentrations) were higher in subjects with more components of MetS.

CONCLUSIONS—Plasma 5-HIAA concentrations are high in subjects with MetS, suggesting the potential importance of serotonin in the development of cardiovascular disease in MetS.

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M etabolic syndrome (MetS), also known as insulin resistance syndrome, is defined by the clustering of several cardiovascular risk factors, including hyperglycemia, hypertension, dyslipidemia, and visceral obesity, in an individual subject. Cardiovascular disease (CVD) is the leading cause of mortality and morbidity in subjects with MetS (1) as well as in patients with type 2 diabetes (2). Serotonin (5-hydroxytryptamine; 5-HT), released from activated platelets, is a naturally occurring vasoactive substance involved in vascular inflammation and atherogenesis (3). 5-HT has various receptor subtypes (4), and it promotes vasoconstriction, vascular smooth muscle cell proliferation, and platelet aggregation (5,6). Plasma 5-HT concentrations have been reported to be high in diabetic patients (7), which may be one of the underlying mechanisms of diabetes complications. However, to our knowledge, plasma 5-HT concentrations have never been explored in MetS. It is difficult to determine plasma 5-HT concentrations because of their fluctuation within 24 h (circadian rhythm) and their acute elevation during the process of blood sampling. Therefore, we compared plasma levels of 5-hydroxyindole-3-acetic acid (5-HIAA; a derivative end product of 5-HT) concentrations in subjects with and without MetS and investigated the relationship between plasma 5-HIAA concentrations and clinical and biochemical metabolic parameters.

RESEARCH DESIGN AND METHODS

—Plasma 5-HIAA concentrations were measured in 311 subjects (152 men and 159 women) recruited from the Oike Clinic (Kyoto, Japan), which provides regular health check-ups for employees. Subjects were excluded if they were taking any medications that might affect plasma 5-HIAA concentrations (e.g., 5-HT receptor antagonists).

First, we compared plasma 5-HIAA concentrations between patients with and without MetS. Second, we evaluated the relationship between plasma 5-HIAA concentrations and clinical and biochemical metabolic parameters, including waist circumference, serum lipid concentrations, fasting plasma glucose, or blood pressure. Third, we compared plasma 5-HIAA concentrations between patients with and without components of MetS, including abdominal obesity, hypertriglyceridemia, low HDL cholesterol levels, hyperglycemia, and elevated blood pressure. Finally, we compared plasma 5-HIAA concentrations according to the number of components of MetS. This study was approved by the local research ethics committee and was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants.

Biochemical analysis

Fasting blood samples were obtained in the morning. Plasma 5-HIAA concentrations (normal range 1.8–6.1 ng/mL) were measured by high-performance liquid chromatography. The intra-assay coefficients of variation were 2.1, 2.0, and 0.9% for plasma 5-HIAA concentrations of 25.27, 41.30, and 95.09 ng/mL, respectively. The interassay coefficients of

variation were 3.9, 3.3, and 2.4% for plasma 5-HIAA concentrations of 7.45, 20.55, and 60.83 ng/mL, respectively. Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A_{1c} (expressed with the unit defined by the National Glycohemoglobin Standardization Program) was assayed using high-performance liquid chromatography.

Definition of MetS

The diagnosis of MetS was determined by a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; the National Heart, Lung, and Blood Institute; the American Heart Association; the World Heart Federation; the International Atherosclerosis Society; and the International Association for the Study of Obesity, using the criteria for Asians (8). The subjects were diagnosed with the presence of MetS when three or more of the following criteria were present: abdominal obesity (waist circumference ≥90 cm in men and ≥80 cm in women); hypertriglyceridemia (serum triglycerides ≥150 mg/dL and/or use of antihypertriglyceridemia medication, in both sexes); low HDL cholesterol levels (serum HDL cholesterol <40 mg in men and <50 mg in women); hyperglycemia (fasting glucose ≥100 mg/dL and/or use of antihyperglycemia medications, in both sexes); and elevated blood pressure (systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥85 mmHg and/or use of antihypertension medications, in both sexes).

Statistical analysis

Means and frequencies of potential confounding variables were calculated. Unpaired Student t tests or χ^2 tests were conducted to assess the statistical significance of differences between groups, using Stat View software (version 5.0; SAS Institute, Cary, NC). All continuous variables are presented as means ± SD. A P value <0.05 was considered statistically significant. Because plasma 5-HIAA concentrations showed skewed distributions, logarithmic (log) transformation was carried out before performing correlation analysis. The relationships between log (plasma 5-HIAA concentrations) and clinical and biochemical metabolic parameters, including waist circumference, serum lipid concentrations, fasting plasma glucose, or blood pressure, were examined by Pearson correlation analyses. One-way ANOVA, followed by the post hoc test with Scheffe, was conducted to assess the statistical significance of differences between groups according to the number of components of MetS, and ANCOVA was performed to adjust the effects of age on log (plasma 5-HIAA concentrations).

RESULTS—Clinical characteristics of the 311 subjects enrolled in this study are shown in Table 1. For both sexes, BMI, waist circumference, systolic blood pressure, diastolic blood pressure, triglycerides, fasting plasma glucose, hemoglobin A_{1c}, and plasma 5-HIAA concentrations were higher in those with MetS than in those without. For both sexes, HDL cholesterol was lower in those with MetS than in those without. In women, serum uric acid was significantly higher in those with MetS than in those without. In both sexes, age and total cholesterol were not different between those with and those without MetS. Relationships between log (plasma 5-HIAA concentrations) and clinical and biochemical metabolic parameters are shown in Table 2. In men, age, fasting plasma glucose, and hemoglobin A_{1c} were positively correlated with log (plasma 5-HIAA concentrations), whereas diastolic blood pressure and HDL cholesterol were negatively correlated with log (plasma 5-HIAA concentrations). In women, age, triglycerides, uric acid, fasting plasma glucose, and hemoglobin A_{1c} were positively correlated with log (plasma 5-HIAA concentrations), whereas HDL cholesterol was negatively correlated with log (5-HIAA concentrations). In men, log (plasma 5-HIAA concentrations) were significantly higher in those with low HDL cholesterol levels, hyperglycemia, or MetS than in those without, and in women log (plasma 5-HIAA concentrations) were significantly higher in those with hypertriglyceridemia, low HDL cholesterol levels, hyperglycemia, elevated blood pressure, or MetS than in those without (Table 3). In men, log (plasma 5-HIAA concentrations) were higher in those with four or five components of MetS than in those with one or two components of MetS, even after adjusting for age (Table 4). In women, log (plasma 5-HIAA concentrations) were higher in those with four or five components of MetS than in those with zero, one, two, or three components of MetS, even after adjusting for age.

CONCLUSIONS—In the current study, we found that plasma 5-HIAA concentrations were higher in subjects with MetS than in those without, for both sexes. Log (plasma 5-HIAA concentrations) correlated significantly with clinical and biochemical metabolic parameters. Furthermore, log (plasma 5-HIAA concentrations) were higher in subjects with more components of MetS.

Table 1—*Characteristics of subjects*

	Men		Women	
	Without MetS	With MetS	Without MetS	With MetS
n	71	81	75	84
Age (years)	56.7 ± 11.4	59.9 ± 11.6	56.4 ± 12.3	59.8 ± 9.9
BMI (kg/m^2)	22.3 ± 2.5	27.3 ± 3.5*	22.2 ± 3.3	26.1 ± 4.9*
Waist circumference (cm)	80.4 ± 7.8	94.9 ± 7.6*	78.7 ± 9.8	90.6 ± 8.9*
Systolic blood pressure (mmHg)	122 ± 14	134 ± 12*	122 ± 15	134 ± 16*
Diastolic blood pressure (mmHg)	77 ± 8	84 ± 9*	74 ± 10	81 ± 12*
Total cholesterol (mg/dL)	206 ± 31	209 ± 39	213 ± 29	210 ± 35
Triglycerides (mg/dL)	112 ± 74	226 ± 202*	84 ± 34	157 ± 74*
HDL cholesterol (mg/dL)	71 ± 13	49 ± 13*	80 ± 17	57 ± 13*
Uric acid (mg/dL)	5.9 ± 1.3	6.0 ± 1.4	4.4 ± 1.1	5.5 ± 1.1*
Fasting plasma glucose (mg/dL)	98 ± 24	117 ± 28*	90 ± 13	115 ± 30*
Hemoglobin A_{1c} (%)	5.2 ± 0.7	5.9 ± 1.0*	5.2 ± 0.4	6.0 ± 1.1*
Medication for hypertension (−/+)	57/14	40/41*	66/9	40/44*
Medication for diabetes (−/+)	67/4	54/27*	73/2	60/24*
Smoking (none/past/current)	44/18/9	48/19/14	68/4/3	73/10/1
Alcohol (−/+)	12/59	18/63	36/39	46/38
5-HIAA (ng/mL)	4.9 ± 1.3	6.5 ± 4.4†	5.2 ± 1.6	7.9 ± 6.5†

Data are means ± SD or n. −/+, no/yes. *P < 0.0001 vs. without MetS. †P < 0.005 vs. without MetS.

Table 2—Correlations between log (plasma 5-HIAA concentrations) and metabolic parameters

	Men		Women	
	r	P	r	P
Age	0.467	<0.0001	0.281	0.0003
BMI	−0.091	0.2685	−0.052	0.5128
Waist circumference	−0.041	0.6198	0.109	0.1738
Systolic blood pressure	0.029	0.7199	0.027	0.7401
Diastolic blood pressure	−0.159	0.0499	−0.154	0.0527
Total cholesterol	−0.076	0.3707	−0.109	0.1923
Triglycerides	0.008	0.9187	0.252	0.0013
HDL cholesterol	−0.217	0.0071	−0.328	<0.0001
Uric acid	−0.035	0.6758	0.244	0.0031
Fasting plasma glucose	0.197	0.0146	0.344	<0.0001
Hemoglobin A_{1c}	0.273	0.0006	0.441	<0.0001

Platelets contain large amounts of 5-HT that may be released during platelet aggregation and degranulation. Therefore, in the setting of vascular injury, endothelial damage and subsequent platelet activation may lead to increased plasma 5-HT concentrations. 5-HT induces the contraction, migration, and proliferation of vascular smooth muscle cells via the 5-HT_{2A} receptor followed by various intracellular signal transduction mechanisms (9–11). Watanabe and colleagues (12–14) demonstrated that 5-HT exerts a synergistic interaction with oxidized LDL, hydrogen peroxide, angiotensin II, endothein-1, thromboxane A2, thrombin, or monocyte chemoattractant protein-1 in inducing vascular smooth muscle cell proliferation. These findings indicate that 5-HT contributes to the deterioration of peripheral blood flow. Increased risk for CVD in MetS thus could be mediated partly through high concentrations of 5-HT.

Advanced age is one of the strongest predictors for coronary artery disease. Age correlated positively with log (plasma 5-HIAA concentrations) in the current study. The increase in plasma 5-HIAA concentrations with age may help to explain the age-related rise in the risk of CVD. In men, log (5-HIAA concentrations) were higher in subjects with four or five components of MetS than in subjects with one or two components of MetS, even after adjusting for age, and in women log (5-HIAA concentrations) were higher in subjects with four or five components of MetS than in subjects with zero, one, two, or three components of MetS, even after adjusting for age.

The 5-HT_{2A} receptor has been identified in glomerular mesangial cells (15), which suggests the involvement of 5-HT in the development of obesity-related nephropathy (16) through proliferation and matrix synthesis in mesangial lesions. In fact, frequencies of proteinuria were higher in subjects with MetS than in subjects without, in both men (17 of 81 vs. 1 of 71, P = 0.0005) and women (16 of 84 vs. 0 of 75, P = 0.0002) in the current study. Furthermore, log (5-HIAA concentrations) were higher in subjects with proteinuria than in subjects without, in both men (0.91 ± 0.29 vs. 0.63 ± 0.13, P < 0.0001) and women (1.11 ± 0.26 vs. 0.72 ± 0.16, P < 0.0001). Kasho et al. (17) demonstrated that 5-HT increased the production of type 4 collagen by cultured human mesangial cells through the 5-HT_{2A} receptor, which was mediated by the activation of protein kinase C and the subsequent increase in transforming growth factor-β activity. Currently, sarpogrelate hydrochloride, a potent 5-HT_{2A} receptor antagonist that inhibits 5-HT–induced vasoconstriction and platelet aggregation (18), is used clinically as an antiplatelet drug for the prevention of thrombosis in atherosclerotic disease. Takahashi et al. (19) reported that sarpogrelate hydrochloride reduced the degree of urinary albumin excretion, indicating the potential usefulness of this agent for the protection of the development and progression of obesity-related nephropathy.

Takahashi et al. (19) demonstrated that urinary 5-HIAA concentrations in diabetic patients were higher than those in normal subjects. They also demonstrated a positive correlation between urinary 5-HIAA concentrations and fasting plasma glucose, as in our study. Possible mechanisms of the positive association between hyperglycemia and 5-HIAA concentrations are as follows. Activated platelets release high amounts of 5-HT. Rapid alterations in platelet aggregability have been reported by acute hyperglycemia (20). Li et al. (21) reported that prolonged hyperglycemia in vitro can induce platelet Ca^{2+} abnormality and hyperactivity. Increased aggregation of human platelets was reported by advanced glycation end products (22). Moreover, increasing the production of oxygen free radicals (23) and reducing nitric oxide (24) contribute to the deleterious effects of high glucose on vascular endothelial function, which may promote platelet aggregability. Plasma 5-HIAA concentrations in subjects with MetS were higher than those in subjects without MetS in this study. Platelet hyperaggregability and the release of the granular contents of the platelets may contribute to the increased plasma concentrations of 5-HT and 5-HIAA.

Table 3—Comparison of log (plasma 5-HIAA concentrations) between groups

	Men		P	Women		P
Abdominal obesity (−/+)	0.72 ± 0.19	0.72 ± 0.16	0.9185	0.74 ± 0.16	0.77 ± 0.22	0.4415
Hypertriglyceridemia (−/+)	0.70 ± 0.13	0.74 ± 0.22	0.0897	0.73 ± 0.19	0.84 ± 0.22	0.0030
Low HDL cholesterol levels (−/+)	0.68 ± 0.13	0.87 ± 0.24	<0.0001	0.72 ± 0.16	0.92 ± 0.28	<0.0001
Hyperglycemia (−/+)	0.68 ± 0.12	0.75 ± 0.20	0.0166	0.72 ± 0.15	0.82 ± 0.25	0.0025
Elevated blood pressure (−/+)	0.68 ± 0.14	0.73 ± 0.19	0.0891	0.71 ± 0.13	0.79 ± 0.23	0.0200
MetS (−/+)	0.67 ± 0.12	0.76 ± 0.20	0.0021	0.70 ± 0.12	0.81 ± 0.25	0.0005

Data are means ± SD. −/+, no/yes.

Table 4—Log (plasma 5-HIAA concentrations) according to the number of components of MetS

	Number of components					
	0	1	2	3	4	5
Men	0.71 ± 0.03	0.65 ± 0.03	0.66 ± 0.04	0.71 ± 0.02	0.81 ± 0.03*	0.85 ± 0.05*
Age adjusted	0.74 ± 0.03	0.65 ± 0.03	0.65 ± 0.03	0.70 ± 0.02	0.80 ± 0.03*	0.82 ± 0.05*
Women	0.69 ± 0.04	0.69 ± 0.04	0.72 ± 0.04	0.75 ± 0.03	0.90 ± 0.04†	1.14 ± 0.09†
Age adjusted	0.73 ± 0.04	0.70 ± 0.04	0.70 ± 0.04	0.75 ± 0.02	0.89 ± 0.04†	1.13 ± 0.09†

Data are means ± SE. *$P < 0.05$ vs. one or two components. †$P < 0.05$ vs. zero, one, two, or three components.

In addition, the postprandial surge in 5-HT also may contribute to this increase because macronutrient intake in obese individuals is higher than that of normal individuals. Because insulin has an antiaggregatory effect on platelets (25,26), as well as an overall anti-inflammatory action (27,28), a state of insulin resistance would enhance platelet aggregation, and increased 5-HT would contribute to increased capillary permeability and inflammation. Several studies have demonstrated that sarpogrelate hydrochloride increases plasma adiponectin concentrations and insulin sensitivity in patients with type 2 diabetes (29) whose plasma adiponectin concentrations were reported to be lower than those in nondiabetic subjects (30). Sarpogrelate hydrochloride might ameliorate insulin resistance in subjects with MetS whose plasma adiponectin concentrations also were reported to be low (31).

Our findings suggest that increased plasma 5-HIAA concentrations may be involved in the pathogenesis and progression of atherosclerosis and obesity-related nephropathy in subjects with MetS. Limitations of our study include a cross-sectional design and relatively small number of subjects. However, to our knowledge, this is the first study comparing plasma 5-HIAA concentrations in subjects with and without MetS and investigating the relationship between plasma 5-HIAA concentrations and clinical and biochemical metabolic parameters. Large prospective trials and intervention studies are needed to better assess the effects of 5-HT on atherosclerosis and obesity-related nephropathy in subjects with MetS. In conclusion, plasma levels of 5-HT are high in subjects with MetS, suggesting the potential importance of 5-HT in the development of CVD in MetS.

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