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High serum high-sensitivity C-reactive protein concentrations are associated with relative cardiac sympathetic overactivity during the early morning period in type 2 diabetic patients with metabolic syndrome

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Abstract

Sympathetic activation is associated with metabolic syndrome (MS) and increased risk of cardiovascular disease. The aim of this study was to investigate whether cardiac autonomic activity or sympathovagal balance, as estimated by a 24-hour power spectral analysis of heart rate variation, is associated with serum concentrations of high-sensitivity C-reactive protein (hs-CRP), a sensitive predictor for cardiovascular events, in type 2 diabetic patients with and without MS. We studied 104 type 2 diabetic patients (50 female and 54 male). The diagnosis of MS was based on the National Cholesterol Education Program Adult Treatment Panel III criteria. Based on the serum hs-CRP, diabetic patients were also divided into 3 groups: low risk (CRP $<$ 1.0 mg/L), moderate risk (1.0 $\leq$ CRP $\leq$ 3.0), and high risk (CRP $>$ 3.0). Heart rate variation was determined automatically every 5 minutes over 24 hours using an ambulatory Holter electrocardiographic recording. Power spectral analysis of the R-R intervals was performed by fast Fourier transformation. Low frequency (LF, both sympathetic and parasympathetic activities), high frequency (HF, pure parasympathetic activity), and the ratio of LF to HF, an index of sympathovagal balance, were used as indices of cardiac autonomic activity. Blood concentrations of hs-CRP, interleukin 6, and plasminogen activator inhibitor 1 were higher in diabetic patients with than in those without MS ($P < .0001$, $P = .0056$, and $P < .0001$, respectively). Both the 24-hour mean LF and the LF-to-HF ratio were also significantly higher in diabetic patients with than in those without MS ($P = .0397$ and $P = .0483$, respectively). The LF-to-HF ratio at 6:00 AM was significantly higher in diabetic patients with a high CRP concentration than in those with a low or moderate CRP concentration ($P < .001$ and $P < .01$, respectively). Only urinary albumin and hs-CRP were independent factors predicting the LF-to-HF ratio at 6:00 AM in diabetic patients. In conclusion, type 2 diabetic patients with MS have elevated markers of inflammation and evidence of cardiac sympathetic predominance. High serum concentrations of hs-CRP are associated with relative cardiac sympathetic overactivity during the early morning in type 2 diabetic patients.

1. Introduction

Low heart rate variability (HRV) is associated with the development of coronary heart disease (CHD) as well as the metabolic syndrome (MS) [1,2]. Low HRV reflects a relative sympathetic predominance via vagal dysfunction or pure sympathetic overactivity in the setting of diabetes [3]. Power spectral analysis (PSA) of HRV simultaneously quantifies both cardiac sympathetic and parasympathetic activities, permitting the evaluation of the moment-to-moment balance between these activities (ie, sympathovagal balance) [4,5]. Twenty-four–hour PSA of HRV has shown that a relative sympathetic prevalence is present during the night in patients with type 2 diabetes mellitus [6]. Morning peaks in the occurrence of acute coronary syndrome and ischemic stroke are well documented [7]. Although this morning increase in cardiovascular events may reflect a number of diurnal physiologic rhythms, the importance of these includes increases in systemic blood pressure and heart rate, which are mediated partly by sympathetic activation [7].

Low HRV, a relative sympathetic overactivity, may be associated with the development of MS and its components

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[2,8-12]. Metabolic syndrome (ie, insulin resistance syndrome) is defined as the clustering of several cardiovascular risk factors in an individual, including impaired glucose tolerance (diabetes), hypertension, dyslipidemia, and visceral obesity [13,14]. Several studies have demonstrated that this syndrome is a strong predictor of cardiovascular disease, especially CHD, independent of the low-density lipoprotein cholesterol concentration [15-17].

Chronic inflammation plays a role in the pathogenesis of atherosclerosis [18]. Elevated blood concentrations of inflammatory markers, especially high-sensitivity C-reactive protein (hs-CRP), have been associated with mortality of CHD among the general population [19] and poor prognosis among patients with acute coronary syndromes [20]. Furthermore, obesity and MS represent states of chronic low-grade inflammation because several studies have reported that serum concentrations of hs-CRP are elevated in subjects with abdominal obesity or MS [21,22]. Several studies have reported that low HRV is associated with elevated blood markers of inflammation, including serum hs-CRP in both nondiabetic and diabetic subjects [23-28]. Thus, it is possible that sympathetic predominance in the sympathovagal balance may be associated with chronic inflammation in patients with MS or type 2 diabetes mellitus.

Hypothesizing that a high serum hs-CRP concentration is associated with sympathetic overactivity in patients with type 2 diabetes mellitus or MS, especially during the early morning, we investigated whether cardiac autonomic activity or sympathovagal balance, as estimated by PSA of HRV, is associated with serum concentrations of hs-CRP in type 2 diabetic patients with and without MS.

2. Subjects and methods

We studied 104 consecutive type 2 diabetic patients (50 female and 54 male). The age ranged from 26 to 82 years. The diabetic patients were referred to the diabetes outpatient clinic at the Dokkyo Medical University Hospital (Koshigaya, Saitama, Japan) and admitted for optimizing glycemic control. All patients received an optimal diet therapy (104.6 kJ/kg of ideal body weight; 50% carbohydrate, 20% protein, and 30% fat). We excluded patients with infections or connective tissue disease such as rheumatoid arthritis. The diabetic patients were controlled with diet alone (n = 12), with diet and oral hypoglycemic agents (n = 78), or with diet and insulin (n = 14).

The diagnosis of MS was based on the recent Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) criteria [29]. Metabolic syndrome was defined by the presence of 3 or more of the following 5 factors: obesity (body mass index [BMI] $\geq$ 25.0); triglyceride 150 mg/dL or higher and/or treatment with fibrates; high-density lipoprotein cholesterol (HDL-C) lower than 50 for women and lower than 40 for men; systolic blood pressure 130 mm Hg or higher, diastolic blood pressure 85 mm Hg or higher, and/or on antihypertensive medication; and fasting plasma glucose (FPG) 100 mg/dL or higher. We used a BMI cutoff value of 25.0 or higher for obesity because the value of waist circumference is not suitable for the detection of obesity in the Japanese population. In a recent review, Reaven [30] proposed that the measurement of BMI is a simpler and more effective way to quantify the degree of obesity because BMI and waist circumference are tightly correlated. The diabetic patients were then divided into 3 groups based on the serum CRP concentration [31], which is associated with cardiovascular risk: low risk (hs-CRP $<$ 1.0 mg/L), moderate risk (1.0 $\leq$ hs-CRP $\leq$ 3.0), and high risk (hs-CRP $>$ 3.0). All patients gave informed consent. The study was approved by the Dokkyo Medical University Institutional Review Board.

3. Power spectral analysis of HRV

During the first 3 or 4 days after the admission, the ambulatory electrocardiogram (ECG) was recorded continuously for 24 hours using a cassette-based 2-channel Holter monitor (Nihon Kohden, Tokyo, Japan). Electrocardiogram signals were digitized and stored using a commercially available PC-based system. Power spectral analysis of HRV was carried out by fast Fourier transformation (Cardinyzer; Kissei Komtec, Matsumoto, Japan) [32]. The PSA was calculated automatically from a series of 256 consecutive intervals between normal R waves for each 5-minute period during the 24-hour acquisition. This PSA software provided R-R variability data distributed in 2 bands: low-frequency (LF) power (0.04-0.15 Hz) and high-frequency (HF) power (0.15-0.40 Hz). Low-frequency power is thought to reflect both sympathetic and parasympathetic activity, whereas HF is determined solely by parasympathetic activity. An LF-to-HF ratio was calculated as a measure of sympathovagal balance that would reflect any shift toward sympathetic or parasympathetic activation. When a 5-minute period included more than 2 series of 256 consecutive intervals between R waves, the 2 LF and HF values were averaged for the 5-minute period. Power spectral analysis was evaluated for every 3-hour interval (6:00 AM, 9:00 AM, noon, 3:00 PM, 6:00 PM, 9:00 PM, midnight, and 3:00 AM).

Venous blood was obtained between 6 and 7 AM after an overnight fast. Serum concentrations of hs-CRP were determined by an immunonephelometric assay (N-High-sensitivity CRP; Dade Behring, Marburg, Germany), for which the intra- and interassay coefficients of variation (CVs) were 1.72% and 2.80%, respectively. Serum concentrations of interleukin 6 (IL-6) were measured by a chemiluminescent enzyme assay (Human IL-6 CLEIA; Fujirebio, Tokyo, Japan), with intra- and interassay CVs of 5.24% and 6.83%, respectively. Plasma concentrations of fibrinogen were determined by the Clauss method. Plasma plasminogen activator inhibitor 1 (PAI-1) was measured

Table 1
Demographic, clinical, and laboratory data for type 2 diabetic patients with or without MS

	No MS (n = 51)	MS (n = 53)	*P*
Female (postmenopausal)	24 (21)	26 (21)	
Age (y)	60.3 ± 10.7	57.0 ± 13.8	.1790
BMI (kg/m^2)	22.1 ± 3.4	27.0 ± 4.7	<.0001
Diabetes duration (y)	12.6 ± 8.0	8.1 ± 6.2	.0017
FPG (mmol/L)	10.2 ± 3.39	9.50 ± 3.02	.2718
HbA1c (%)	9.71 ± 1.79	9.90 ± 1.96	.6223
Total cholesterol (mmol/L)	5.40 ± 1.37	5.55 ± 1.25	.5642
Triglyceride (mmol/L)	2.30 ± 1.56	3.83 ± 2.62	.0132
HDL-C (mmol/L)	1.40 ± 0.41	1.07 ± 0.29	<.0001
HOMA-IR	2.65 ± 1.93	3.16 ± 2.10	.3305
Creatinine clearance (mL/min)	70.3 ± 29.8	78.2 ± 50.8	.3547
UAE (mg per 24 h)	42.0 (16.5-279.5)	44.0 (11.0-290.0)	.9533
Fibrinogen (mg/dL)	349.5 ± 87.47	369.3 ± 91.2	.2627
hs-CRP (mg/L)	0.31 (0.18-0.87)	1.15 (0.49-2.60)	<.0001
IL-6 (pg/mL)	1.90 (1.20-2.55)	2.60 (1.80-3.10)	.0056
PAI-1 (ng/mL)	20.0 (12.0-26.5)	38.0 (21.0-52.0)	<.0001
24-h mean LF (ln · ms^2)	10.33 ± 1.50	10.94 ± 1.28	.0397
24-h mean HF (ln · ms^2)	10.43 ± 1.25	10.76 ± 1.22	.2159
24-h mean LF/HF	2.86 (1.30-6.47)	4.05 (2.76-61.9)	.0483
Hypertension, n (%)	10 (19.6)	34 (64.2)	<.0001
Treatment (D/OHA/insulin)	23/42/12	5/13/8	NS

Data are expressed as mean ± SD or median and interquartile ranges, unless otherwise indicated. HbA_{1c} indicates hemoglobin A_{1c}; D, diet alone; OHA, oral hypoglycemic agents; NS, not significant.

by latex photometric immunoassay (LPIA tPAI-1 test; IATRON Laboratories, Tokyo, Japan). Intra- and interassay CVs were 2.01% and 2.38%, respectively. Plasma insulin concentrations were determined by radioimmunoassay. Insulin resistance was evaluated by homeostasis model assessment of insulin resistance (HOMA-IR), which is calculated as fasting plasma insulin (microunits per milliliter) × FPG/22.5. To apply the HOMA-IR as a measure of insulin resistance, we excluded patients who were receiving insulin treatment. Urinary albumin excretion (UAE, concentrations in a 24-hour collection specimen) was measured with an immunoturbidimetric assay.

4. Statistical analysis

Data are expressed as the mean ± SD or the median and interquartile ranges unless indicated otherwise. Differences were analyzed by an unpaired *t* test or a 1-way analysis of variance, with the Newman-Keuls multiple comparison test. For nonparametric data, differences between groups were analyzed by the Mann-Whitney *U* test or the Kruskal-Wallis test with Dunn multiple comparison test. Differences in prevalence between groups were assessed by the χ^2 test. A logarithmic transformation of urinary albumin, hs-CRP, LF, and HF values was used to render the distribution normal for the parametric tests. A *P* value less than .05 was accepted as indicating statistical significance.

5. Results

As shown in Table 1, BMI and triglyceride were significantly higher in diabetic patients with MS than in those without MS ($P < .0001$ and $P = .0132$, respectively). The serum concentrations of hs-CRP, IL-6, and PAI-1 were higher in diabetic patients with MS than in those without MS ($P < .0001$, $P = .0056$, and $P < .0001$, respectively). Both the 24-hour mean LF and the LF-to-HF ratio were also significantly higher in diabetic patients with MS than in those without MS ($P = .0397$ and $P = .0483$, respectively). The duration of diabetes and the HDL-C concentration were significantly lower in diabetic patients with MS than in

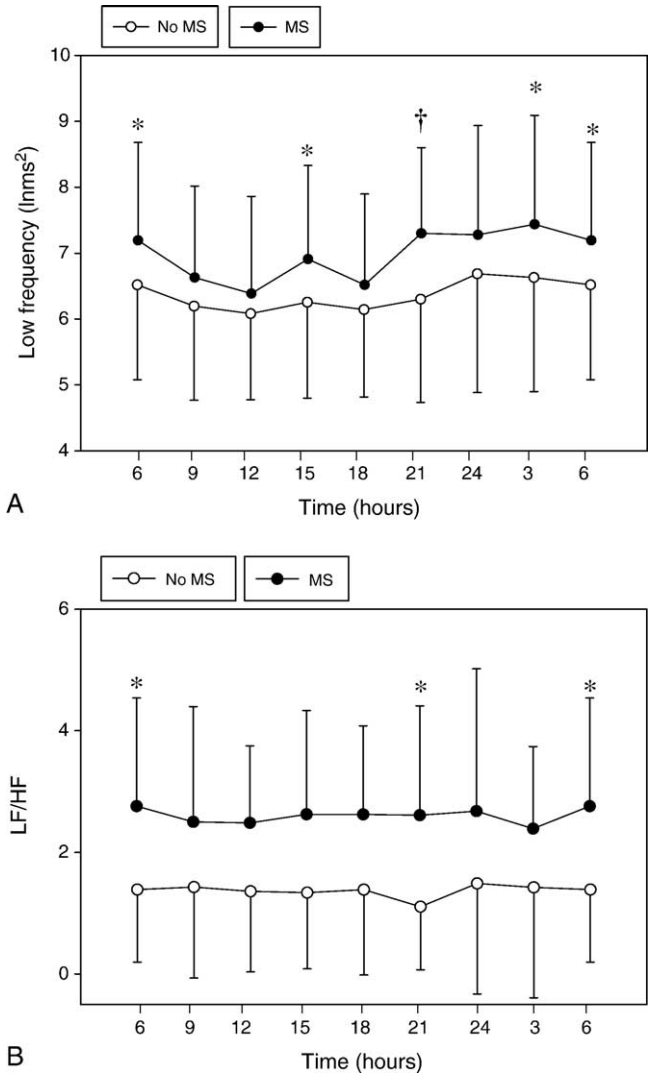

Fig. 1. Diurnal profile of LF (A) and LF-to-HF ratio (B) in diabetic patients with MS (closed circle) and in those without MS (open circle). Data are expressed as mean ± SD. *$P < .05$, †$P < .01$ vs no MS.

Table 2
Patient characteristics and laboratory data in subgroups defined by serum hs-CRP concentrations

Variable	Low	Intermediate	High
n (male/female)	60 (32/28)	29 (14/15)	15 (8/7)
Age (y)	59.5 ± 11.0	59.4 ± 12.9	53.9 ± 15.8
BMI	23.4 ± 4.2	25.6 ± 4.6	26.4 ± 6.2
Diabetes duration (y)	11.6 ± 8.0	8.0 ± 6.5	9.8 ± 6.3
FPG (mmol/L)	10.0 ± 3.43	9.90 ± 2.91	9.05 ± 2.88
HbA_{1c} (%)	9.75 ± 1.88	10.1 ± 2.00	9.57 ± 1.62
Total cholesterol (mmol/L)	5.16 ± 1.29	6.01 ± 1.14*	5.70 ± 1.37
Triglyceride (mmol/L)	1.84 (1.38-2.76)	2.77 (2.26-3.79)**	3.07 (2.22-4.12)***
HDL-C (mmol/L)	1.28 ± 0.44	1.22 ± 0.29	1.06 ± 0.34
Creatinine clearance (mL/min)	70.0 ± 29.6	80.2 ± 55.8	80.6 ± 52.7
HOMA-IR	2.66 ± 1.70	2.82 ± 2.02	3.72 ± 2.71
IL-6 (pg/mL)	1.60 (1.20, 2.35)	2.40 (1.80, 3.10)*	4.40 (2.65, 6.95)***
PAI-1 (ng/mL)	25.1 ± 14.4	45.3 ± 37.6**	49.3 ± 40.9**
24-h mean LF ($\ln \cdot ms^2$)	10.6 ± 1.52	10.7 ± 1.24	10.9 ± 1.44
24-h mean HF ($\ln \cdot ms^2$)	10.7 ± 1.29	10.5 ± 1.23	10.5 ± 1.15
24-h mean LF/HF	4.00 ± 2.83	4.60 ± 3.55	7.65 ± 6.44**.****
Hypertension, n (%)	15 (25.0%)	13 (40.6%)	17 (60.7%)*
Treatment (D/OHA/insulin)	6/45/9	2/23/4	3/11/1

Continuous variables are expressed as mean ± SD or median and interquartile ranges, unless otherwise indicated. Low risk, hs-CRP < 1.0 mg/L; moderate risk, 1.0 ≤ hs-CRP < 3.0; and high risk, hs-CRP ≥ 3.0.

* $P < .05$ vs low group.

** $P < .01$ vs low group.

*** $P < .001$ vs low group.

**** $P < .05$ vs intermediate.

those without MS ($P = .0017$ and $P < .0001$, respectively). We analyzed the circadian rhythms of sympathovagal balance in these diabetic patients. The LF components at 6:00 AM, 3:00 PM, 9:00 PM, and 3:00 AM were significantly higher in diabetic patients with MS than in those without MS (Fig. 1A). However, we found no significant differences in the HF component at any time between the 2 groups (data not shown). The LF-to-HF ratio at 6:00 AM and 9:00 PM was also significantly higher in diabetic patients with MS than in those without MS (Fig. 1B).

We next divided the diabetic patients into 3 groups based on the serum concentrations of hs-CRP. The American Heart Association suggests that concentrations of hs-CRP of lower than 1.0, 1 to lower than 3, and 3 mg/L or higher can be used to represent low, moderate, and high cardiovascular risk, respectively (Table 2). The serum triglyceride concentration was significantly higher in diabetic patients with moderate CRP ($P < .01$) or high CRP ($P < .001$) concentrations than in those with low CRP concentrations. The serum IL-6 concentration was significantly higher in diabetic patients with moderate CRP ($P < .05$) or high CRP concentrations ($P < .001$) than in those with low CRP concentrations. The plasma PAI-1 concentration was also significantly higher in diabetic patients with moderate CRP ($P < .01$) or high CRP ($P < .01$) concentrations than in those with low CRP concentrations. The 24-hour mean LF-to-HF ratio was significantly higher in diabetic patients with a high CRP concentration than in those with low or moderate CRP concentrations ($P < .01$ and $P < .05$, respectively). We then analyzed the circadian rhythms of sympathovagal balance in the 3 groups. The LF-to-HF ratio at 6:00 AM was significantly higher in diabetic patients with a high CRP concentration than in those with low or moderate CRP concentrations ($P < .001$ and $P < .01$, respectively) (Fig. 2). The LF-to-HF ratios at 3:00 and 9:00 PM were significantly higher in diabetic patients with a high CRP concentration than in those with a low CRP concentration ($P < .01$) (Fig. 2). However, we found no significant differences in LF or HF among the 3 groups at any time point (data not shown).

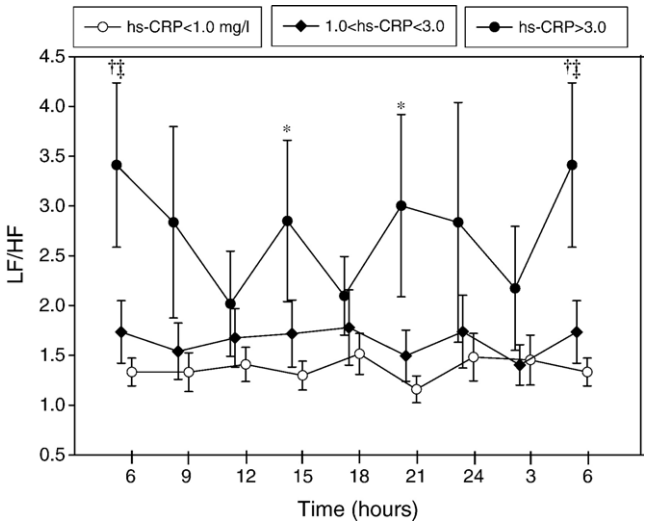

Fig. 2. Diurnal profile of LF-to-HF ratio in diabetic groups defined by serum concentrations of hs-CRP. Data are expressed as mean ± SEM. *$P < .01$, †$P < .001$ vs low risk, ‡$P < .01$ vs moderate risk.

Based on linear regression analysis (Table 3), the LF-to-HF ratio at 6:00 AM correlated positively with creatinine clearance ($r = 0.260$, $P = .013$) and the serum hs-CRP concentration ($r = 0.3473$, $P = .0001$). The LF-to-HF ratio at 6:00 AM also correlated negatively with age ($r = -0.257$, $P = .014$), duration of diabetes ($r = -0.335$, $P = .001$), and UAE ($r = -0.291$, $P = .005$). Because we found a positive correlation between serum hs-CRP and LF-to-HF ratio at 6:00 AM to be the strongest during the 24-hour period, we investigated the relationship between the LF-to-HF ratio at 6:00 AM and the clinical variables in diabetic patients to determine independent factors predicting the LF-to-HF ratio at 6:00 AM.

To determine the independent factors for the LF-to-HF ratio at 6:00 AM, we then performed multivariate analysis, including age, duration of diabetes, UAE, hs-CRP, and the presence or absence of MS. In a model that explained 52.8% of variation of the LF-to-HF ratio at 6:00 AM, only UAE ($\beta = -.306$, $P = .002$) and hs-CRP ($\beta = -.219$, $P = .048$) were independent factors predicting the LF-to-HF ratio at 6:00 AM in diabetic patients (Table 3). However, we could not find an independent relationship between the LF-to-HF ratio at 6:00 AM and the presence of MS. To further clarify a relative weighting of these independent factors to the ratio, we did a stepwise regression analysis. We found that hs-CRP was the strongest independent factor for the LF-to-HF ratio at 6:00 AM ($\beta = -.329$, $P = .001$).

We then investigated the effects of sex differences on HRV in all patients with type 2 diabetes mellitus. As shown in Table 4, both the 24-hour mean LF-to-HF ratio and the LF-to-HF ratio at 6:00 AM were significantly higher in male patients than in female patients ($P = .0467$ and $P = .0347$, respectively). However, we found no significant differences in the LF or HF component at 6:00 AM between male and female patients. Body mass index and plasma fibrinogen were significantly higher in female patients than in male patients ($P < .05$).

Table 3
Univariate and multivariate analyses of relationships between LF-to-HF ratio at 6:00 AM estimated by PSA of HRV and characteristics of patients with type 2 diabetes mellitus

Variable	Univariate analysis		Multivariate analysis	
	r	P	β	P
Age (y)	−0.257	.014	−.059	.592
BMI	0.136	.198	NE	
Diabetes duration (y)	−0.335	.001	−.170	.118
SBP (mm Hg)	−0.173	.101	NE	
DBP (mm Hg)	−0.094	.375	NE	
FPG (mmol/L)	−0.092	.386	NE	
HbA_{1c} (%)	0.055	.610	NE	
Total cholesterol (mmol/L)	0.001	.995	NE	
Triglyceride (mmol/L)	0.302	.004	.183	.103
HDL-C (mmol/L)	−0.121	.257	NE	
HOMA-IR	0.176	.193	NE	
Ccr (mL/min)	0.260	.013	NE	
UAE (log_{10}, mg per 24 h)	−0.291	.005	−.306	.002
hs-CRP (log_{10}, ng/mL)	0.3473	.001	.219	.048
IL-6 (pg/mL)	0.056	.607	NE	
Fibrinogen (mg/dL)	−0.102	.337	NE	
PAI-1 (ng/mL)	0.182	.083	NE	
MS (yes or no)			−.112	.913
R^2				.279

β indicates partial coefficient; NE, does not enter the final model; SBP, systolic blood pressure; DBP, diastolic blood pressure; Ccr, creatinine clearance.

Table 4
Demographic, clinical, and laboratory data for type 2 diabetic patients according to sex difference

	Male	Female	P
n	54	50	
Age (y)	57.1 ± 12.1	60.2 ± 12.7	.1968
BMI (kg/m^2)	23.6 ± 4.0	25.5 ± 5.3	.0474
Diabetes duration (y)	9.6 ± 7.7	11.1 ± 7.3	.3141
FPG (mmol/L)	9.9 ± 2.8	9.8 ± 3.6	.9657
Total cholesterol (mmol/L)	5.41 ± 1.23	5.55 ± 1.28	.6028
Triglyceride (mmol/L)	3.09 ± 2.72	2.61 ± 1.54	.2823
HDL-C (mmol/L)	1.20 ± 0.39	1.26 ± 0.40	.4243
Creatinine clearance (mL/min)	73.5 ± 29.5	75.1 ± 52.7	.8446
Fibrinogen (mg/dL)	341.9 ± 58.3	378.6 ± 111.1	.0369
hs-CRP (mg/L)	0.53 (0.24-1.73)	0.67 (0.24-1.61)	.7647
PAI-1 (ng/mL)	23.0 (16.5-42.0)	26.5 (18.0-41.0)	.6256
24-h mean LF ($ln \cdot ms^2$)	10.48 ± 1.60	10.82 ± 1.21	.2578
24-h mean HF ($ln \cdot ms^2$)	10.44 ± 1.43	10.77 ± 0.98	.2065
24-h mean LF/HF	4.40 (1.94-8.07)	3.12 (2.06-4.55)	.0467
LF at 6:00 AM ($ln \cdot ms^2$)	6.96 ± 1.55	6.78 ± 1.44	.5558
HF at 6:00 AM ($ln \cdot ms^2$)	6.59 ± 1.22	6.82 ± 1.23	.3612
LF/HF at 6:00 AM	1.51 (0.69-3.49)	1.04 (0.67-1.40)	.0374
Hypertension, n (%)	19 (35.2)	25 (50.0)	NS

Data are the mean ± SD or median and interquartile ranges, unless otherwise indicated.

6. Discussion

The present study investigated for the first time the relationship between the long-term (24-hour) sympathovagal balance, as estimated by PSA of HRV, and the MS in patients with type 2 diabetes mellitus. Both the 24-hour mean LF and the LF-to-HF ratio were significantly higher in diabetic patients with MS than in those without MS. Twenty-four–hour PSA of HRV also showed that both the LF component and the LF-to-HF ratio at 6:00 AM and 9:00 PM were significantly higher in diabetic patients with MS than in those without MS. Furthermore, the LF component at 3:00 AM and 3:00 PM was higher in diabetic patients with MS than in those without MS. The LF-to-HF ratio is considered to reflect sympathovagal balance. These findings suggest that a shift in the sympathovagal balance toward sympathetic activation is associated with the presence of MS in patients with type 2 diabetes mellitus. Thus, MS may shift a cardiac autonomic balance toward sympathetic predominance [2,8-12]. The Atherosclerosis Risk in Communities Study also demonstrated the association of MS with a reduction in HRV (increased sympathetic activity or reduced

parasympathetic activity) in the baseline examination, suggesting that cardiac autonomic imbalance favoring sympathetic predominance may contribute to the development of MS [2].

Several predictors of impaired HRV (increased sympathetic activity or reduced parasympathetic activity) have been proposed in subjects with MS. Obesity, especially visceral fat accumulation, is a main component of the MS [33]. In obesity, muscle sympathetic nerve activity is elevated in subjects with visceral obesity and correlates more closely with the level of abdominal visceral fat than total fat mass [34]. Although we found no significant correlation between the LF-to-HF ratio and BMI in diabetic patients, a previous study observed an increase in the LF-to-HF ratio in obese subjects compared with lean subjects [35]. Thus, visceral obesity may be associated with a relative cardiac sympathetic predominance and suggests a link between sympathetic predominance and MS. Another possibility is that sleep apnea, a component of MS, may contribute to impaired HRV because patients with obstructive sleep apnea have a number of autonomic abnormalities, including impaired HRV and increased heart rate [26]. We conclude that the MS is associated with a relative cardiac sympathetic predominance.

We found that blood inflammatory markers, such as CRP and IL-6, were significantly higher in diabetic patients with MS than in those without MS. Several studies have reported a chronic low-grade inflammation in subjects with MS [17,22]. The mechanisms responsible for the relationship between MS and elevated markers of inflammation remain unclear. Markers of inflammation, including hs-CRP, predict the development of type 2 diabetes mellitus and CHD [19,36], suggesting a common mechanism. It is well established that visceral fat accumulation, as well as insulin resistance, is a causal determinant for the MS [37]. Adipose tissue, which is considered an endocrine organ, produces a variety of secreted proteins, including the proinflammatory cytokines IL-6 and tumor necrosis factor α [38]. These adipocytokines may contribute to the development of the MS and atherosclerotic disease. Furthermore, recent studies demonstrate a high abundance of active macrophages in adipose tissue from obese individuals, which may contribute to the augmented production of inflammatory cytokines from adipose tissues in subjects with visceral obesity [39]. Thus, it is possible that the MS is an inflammatory disease.

We then investigated whether the serum hs-CRP concentration is associated with the cardiac autonomic activity and the sympathovagal balance in patients with type 2 diabetes mellitus. Based on the serum CRP concentration [31], which is related to cardiovascular risk, the 24-hour mean LF-to-HF ratio was significantly higher in diabetic patients with a high CRP concentration than in those with low or moderate CRP concentrations. The present study demonstrated that relative sympathetic activation was correlated positively with chronic low-grade inflammation, as reflected by the hs-CRP concentration, in diabetic patients. Furthermore, the LF-to-HF ratio at 6:00 AM was markedly elevated in diabetic patients with a high CRP concentration compared with those with low or moderate CRP concentrations. Multivariate analysis showed that serum hs-CRP was an independent factor predicting the LF-to-HF ratio at 6:00 AM in diabetic patients. These results suggest that chronic low-grade inflammation is associated with an autonomic imbalance favoring sympathetic overactivity, especially in the early morning. A previous study also reported that increased heart rate and reduced HRV are associated with subclinical inflammation estimated by serum hs-CRP in middle-aged and elderly subjects with no apparent heart disease [25]. On the other hand, a recent study could not find a significant positive correlation between serum hs-CRP and the LF-to-HF ratio in patients with type 2 diabetes mellitus [40]. One possible explanation for the discrepancy between these studies is a difference in sampling of R-R intervals. Anan et al [40] analyzed the short-term HRV using a 5-minute R-R interval at rest in the supine position, whereas we analyzed long-term HRV using a 24-hour Holter ECG. Because parasympathetic activity contributes largely to HRV under resting condition, it is difficult to accurately evaluate sympathovagal balance measured by the LF-to-HF ratio [41]. A circadian rhythm of the autonomic activity has been well documented [6,7]. We also found that among various different periods, the LF-to-HF ratio at 6:00 AM was markedly elevated in diabetic patients with a high CRP concentration compared with those with low or moderate CRP concentrations. Thus, we believe that long-term ECG recording is more helpful to evaluate sympathovagal balance.

Mechanisms responsible for the relationship between serum hs-CRP and relative cardiac sympathetic activation in type 2 diabetes mellitus remain unclear. There are several possible explanations for this association. First, an imbalance in the autonomic nervous system (ANS) favoring sympathetic activation could increase directly the inflammatory reaction because the bone marrow and lymphoid system are innervated by autonomic nerves [25]. Second, visceral fat accumulation may link serum hs-CRP to relative cardiac sympathetic activation. Third, air pollution could affect both HRV and inflammation, linking serum hs-CRP to relative cardiac sympathetic activation. Several studies have demonstrated that ambient particulate air pollution influences HRV and serum CRP in elderly subjects or in healthy young men [23,24]. Fourth, both high serum hs-CRP concentration and relative sympathetic activation may be epiphenomena of insulin resistance because insulin resistance plays a significant role in the development of MS or type 2 diabetes mellitus.

A large-scale prospective study has shown that baseline concentrations of CRP are an independent predictor of cardiovascular events among apparent healthy individuals [22]. This supports the hypothesis that atherosclerosis is a chronic low-grade inflammatory disease. Elevated serum concentrations of hs-CRP may reflect sympathetic overactivity

as well as subclinical inflammation, acting as a useful predictor of CHD. The increased LF-to-HF ratio and elevated concentrations of CRP could reflect the adverse effect of the interaction between sympathetic activation and inflammation on atherosclerosis. The combination of markers of both autonomic imbalance and inflammation could facilitate the identification of subjects at high risk for CHD.

As mentioned before, we found the strongest relationship between serum hs-CRP and LF-to-HF ratio at 6:00 AM among the various times of the day in patients with type 2 diabetes mellitus. The LF-to-HF ratio was significantly higher in diabetic patients with a high CRP concentration than with low or moderate CRP concentrations at 6:00 AM. These findings suggest that sympathetic overactivity during the early morning may contribute mainly to chronic inflammation in type 2 diabetes mellitus. Although this morning increase in cardiovascular events may reflect a number of diurnal physiologic rhythms, the most important factors are increases in systemic blood pressure and heart rate, which are mediated partly by sympathetic activation. Sympathetic overactivity may be a common mechanism responsible for the morning blood pressure peak in patients with hypertension. A previous study reported that the morning increase in LF-to-HF ratio may contribute to the morning rise in blood pressure in hypertensive patients [42]. We suspect that early morning sympathetic activation may contribute to an increase in serum hs-CRP, which, in turn, could be responsible for the excess of cardiovascular events in the morning.

As has been mentioned, both MS and high serum hs-CRP are associated with relative cardiac sympathetic overactivity, especially during the early morning, in patients with type 2 diabetes mellitus. It is also possible that the MS is an inflammatory disease. We therefore did multivariate analysis to further determine the interaction of hs-CRP and MS on the LF-to-HF ratio at 6:00 AM. The LF-to-HF ratio at 6:00 AM was associated independently with serum hs-CRP, but not with the presence of MS. We speculate that chronic low-grade inflammation may link MS to relative cardiac sympathetic overactivity.

We tried to determine the effects of sex differences or aging on cardiac ANS in the present study, as several studies have reported sex-related differences in the cardiac ANS [43,44]. The present study showed that both the 24-hour mean LF-to-HF ratio and the LF-to-HF ratio at 6:00 AM were significantly higher in male diabetic patients than in female diabetic patients. Our result is in agreement with previous studies using PSA of HRV [45,46]. These results suggest that men have a higher cardiac sympathetic activity than women. We speculate that the relative cardiac sympathetic activation may be responsible for the higher excess of cardiovascular events in men. However, we believe that in the present study, sex difference did not influence the relationship between high serum hs-CRP concentration and relative cardiac sympathetic overactivity in patients with type 2 diabetes mellitus because the proportion of male to female was similar among the diabetic groups according to serum hs-CRP concentrations.

It is well established that HRV declines continuously with aging in healthy subjects [47]. The present study showed a negative correlation between the LF-to-HF ratio at 6:00 AM and age, suggesting that aging can influence HRV in type 2 diabetes mellitus. However, because we found no significant difference in age between the diabetic groups, aging did not influence either the relationship between high serum hs-CRP and relative cardiac sympathetic overactivity in our study.

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