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Circulating CD34-Positive Cell Number Is Associated With Brain Natriuretic Peptide Level in Type 2 Diabetic Patients

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Patients with type 2 diabetes often suffer from asymptomatic left ventricular (LV) injury, including increased LV mass, without apparent myocardial ischemia. The mechanisms underlying diabetic LV injury remain unclear; however, it has been suggested that endothelial dysfunction plays a role. Accumulating evidence indicates that bone marrow–derived endothelial progenitor cells (EPCs) contribute to neovascularization of ischemic tissue and endothelialization of denuded endothelium. Recent studies have shown that circulating bone marrow–derived immature cells, including $CD34^+$ cells, contribute to the maintenance of the vasculature, both as a pool of EPCs and as the source of growth/angiogenesis factors (1). We hypothesized that circulating $CD34^+$ cells might be associated with LV dysfunction in patients with type 2 diabetes. Therefore, we studied the correlation between circulating $CD34^+$ cell levels and plasma brain natriuretic peptide (BNP) levels, an LV dysfunction marker, in type 2 diabetic patients.

RESEARCH DESIGN AND METHODS

The institutional review board of the National Cardiovascular Center approved this study, and all subjects provided informed consent. We examined 26 patients with type 2 diabetes (12 men and 14 women, duration of diabetes 16.1 ± 10.7 years) who were over 60 years of age (70.5 ± 6.4 years). Statin was given to nine subjects. ACE inhibitor or angiotensin receptor blocker was given to nine subjects, and thiazolidinedione was given to two subjects. Subjects were excluded from the study if they had known cardiovascular disease or chronic renal failure (defined as serum creatinine ≥180 μmol/l). No study subject showed hypokinesis by echocardiography or electrocardiogram change, indicating myocardial ischemia. Systolic (SBP) and diastolic (DBP) blood pressure and anthropometric parameters were determined. Blood samples were taken after 12-h fasting to measure circulating $CD34^+$ cells, plasma BNP, fasting plasma glucose (FPG), and A1C. Circulating $CD34^+$ cells were quantified by flow cytometry according to the manufacturer's protocol (ProCOUNT; Becton Dickinson Biosciences) as previously reported (2). BNP was quantified by enzyme immunoassay (Tohso, Tokyo, Japan). We further examined LV fractional shortening (LVFS), LV mass index (LVMI) (3), and peak flow velocity of the early filling wave (E), the late filling wave (A), and the E/A-wave ratio (E/A) by echocardiography. All echocardiograms were performed by several expert physicians who were blinded to $CD34^+$ cell level.

All statistical analyses were performed using JMP version 5.1.1 software (SAS Institute). Data are expressed as means ± SD. Comparisons of number of $CD34^+$ cells by sex were made using the two-tailed unpaired *t* test. Correlations between number of $CD34^+$ cells and clinical parameters were assessed by univariate liner regression analysis and multiple regression analysis. LVMI and plasma BNP concentrations were analyzed after logarithmic transformation.

RESULTS

FPG levels, A1C levels, and BMIs in the study subjects were measured to be 9.5 ± 2.6 mmol/l, 9.2 ± 1.8%, and 26.4 ± 4.3 kg/m^2, respectively. A total of 88% of the patients had hypertension (SBP 142 ± 18 mmHg, DBP 75.7 ± 13.5 mmHg). Plasma BNP levels were measured to be 95 ± 319 pg/ml. Although it has been reported that the level of BNP ≥100 pg/ml has a sensitivity of 90% of diagnosing congestive heart failure (CHF) in patients with CHF symptoms (4), none of the subjects in this study, including subjects with ≥100 pg/ml of BNP, showed symptoms of CHF. The level of circulating $CD34^+$ cells was measured to be 0.76 ± 0.39 cells/μl, and there was no significant difference between sexes. The range of LVMI was 73.3–340.2, and 11 subjects applied to the definition of LV hypertrophy (LVMI ≤131 in men and ≤100 in women) (3).

Plasma BNP levels had a significant inverse correlation with the number of circulating $CD34^+$ cells (Fig. 1*A*), whereas FPG, A1C, BMI, SBP, DBP, and age showed no significant correlations. There was a significant correlation between the number of circulating $CD34^+$ cells and LVMI by echocardiography (Fig. 1*B*). LVFS and E/A were not associated with circulating $CD34^+$ cell numbers (LVFS $r = -0.07$, $P = 0.72$; E/A $r = -0.11$, $P = 0.59$). There was also a significant correlation between BNP levels and LVMI ($r = 0.59$, $P = 0.001$).

In multiple regression analysis, the

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Abbreviations: BNP, brain natriuretic peptide; CHF, congestive heart failure; DBP, diastolic blood pressure; EPC, endothelial progenitor cell; FPG, fasting plasma glucose; LV, left ventricular; LVFS, LV fractional shortening; LVMI, LV mass index; SBP, systolic blood pressure.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

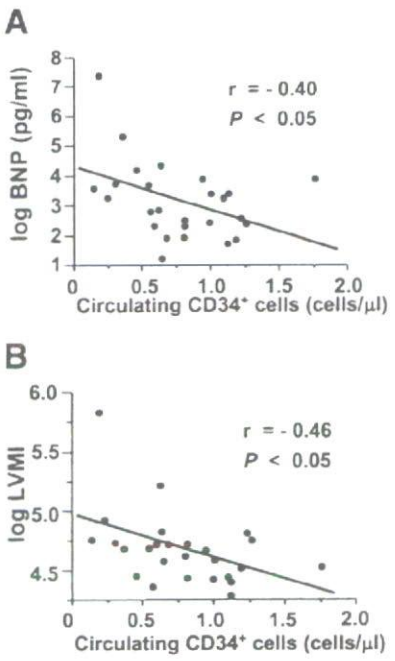

Figure 1—*Correlation between CD34$^+$ cell numbers and plasma BNP levels* (A) *and correlation between CD34$^+$ cell numbers and LVMI* (B) *in type 2 diabetic patients* (n = 26).

level of CD34$^+$ cells was an independent correlate of both BNP ($\beta = -1.64$, $P = 0.017$) and LVMI ($\beta = -0.337$, $P = 0.031$) in the model including age, A1C, SBP, BMI, and medication (ACE inhibitor/angiotensin receptor blocker, statin, and thiazolidinedione).

CONCLUSIONS — In this study, circulating CD34$^+$ cell number was found to significantly correlate with plasma BNP level, a marker of LV dysfunction. To the best of our knowledge, this is the first report that circulating bone marrow–derived cells are associated with diabetic LV abnormality. Circulating CD34$^+$ cell numbers also significantly correlated with LVMI, whereas they did not correlate with LVFS (an LV systolic function marker) or E/A (an LV diastolic function marker). LV hypertrophy is a well-known predictor of cardiovascular events independent of coronary artery disease. The Framingham Heart Study identified an association between diabetes and increased LV wall thickness and mass (5). Although the precise mechanisms underlying the association between diabetes and LV hypertrophy remain unknown, our results suggest that reduced circulating CD34$^+$ cell numbers may be involved in the progression of LV hypertrophy in diabetic patients. However, further investigations are necessary to demonstrate this hypothesis.

We measured the level of CD34$^+$ cells in this study but not the levels of circulating CD34$^+$/kinase insert domain receptor (KDR)$^+$ cells that are regarded as EPCs. Circulating CD34$^+$ cell levels are associated with ischemic stroke (6), and administration of CD34$^+$ cells ameliorates cerebral ischemia in mice (7). This indicates that CD34$^+$ cells may be involved in cardiovascular disease. Indeed, another recent report indicated that levels of circulating CD34$^+$ cells are more strongly correlated with cardiovascular risk than levels of EPCs (8). Therefore, our results suggest that measurement of CD34$^+$ cells may provide an indicator for diabetic LV hypertrophy.

Our study had several limitations. First, the study was performed only by cross sectional analysis; therefore, a prospective study is needed to clarify whether circulating CD34$^+$ cell numbers predict LV injury in diabetic patients. Second, although systemic blood pressure did not significantly associate with CD34$^+$ cell numbers, further investigation of normotensive diabetic patients is needed to exclude the possible effects of hypertension on circulating CD34$^+$ cell numbers, as most of the subjects in this study were hypertensive. Despite this caveat, these results may be of practical use in elderly patients with type 2 diabetes, as hypertension is a very common comorbid condition in this population.

In conclusion, reduced circulating CD34$^+$ cell numbers are significantly associated with plasma BNP concentration and LVMI in elderly patients with type 2 diabetes. These results suggest that decreased circulating CD34$^+$ cells may be involved in LV hypertrophy and that measurement of circulating CD34$^+$ cell numbers may be useful for the identification of diabetic patients at high risk of LV injury.

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Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study

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ABSTRACT

Objective: Only a small number of population-based cohort studies have directly compared the predictive value of low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDLC) for coronary artery disease in Asian populations, such as Japan.
Methods: We performed an 11.9-year cohort study of 4694 men and women, aged 30–74 years, selected randomly from an urban general population in Japan. Baseline LDL-C levels were estimated using the Friedewald formula. The predictive values of LDL-C and non-HDLC for myocardial infarction (MI) and stroke were compared.
Results and conclusion: During the follow-up period, there were 80 incident cases of MI and 139 of stoke, comprised of 23 intracerebral hemorrhages, 85 cerebral infarctions and 31 other types of stroke. The Hazard ratio (HR) for MI was highest in the top quintile of LDL-C (HR: 3.03, 95% CI, 1.32–6.96) when male and female data were combined. The HR for MI was also highest in the top quintile of non-HDLC (HR: 2.97, 95% CI, 1.26–6.97). Analysis of trends showed a significant positive relationship between MI incidence and serum LDL-C and non-HDLC levels (both $P = 0.02$). However, there was no relationship between the incidence of any subtype of stroke and either LDL-C or non-HDLC. The predictive value of LDL-C and non-HDLC for MI, assessed by calculating the differences in the −2 logarithm likelihood (−2 ln [L]) and area under the curve (AUC), were almost similar.

1. Introduction

The causal relationship between high levels of serum low-density lipoprotein cholesterol (LDL-C) and coronary artery disease (CAD) is well established [1–5]. Blood LDL-C levels are therefore the main target for lipid management in the majority of guidelines of developed countries for preventing atherosclerotic disease [3–5]. Some US cohort studies have also suggested that non-high-density lipoprotein (non-HDLC) may be a better predictor of CAD [6,7]. However, to our knowledge, only one population-based cohort study has directly compared the predictive value of these lipid markers for CAD in an Asian population [8], which have a lower incidence of coronary artery disease, but a higher risk of stroke than Western populations [9–12]. Furthermore, although it has not been shown that there is a positive relationship between the risk of any type of stroke and high serum levels of total cholesterol (TC) in the Japanese population [9,10], the effects on stroke incidence of the closely related lipid fractions, LDL-C and non-HDLC, have not been evaluated.

The purpose of this study was therefore to investigate the predictive value of LDL-C and non-HDLC for the incidence of CAD and stroke in a Japanese urban population over an 11.9-year period. Our *a priori* hypothesis was that both LDL-C and non-HDLC may be useful predictors of CAD risk, but not of stroke risk.

2. Methods

2.1. Populations

The Suita study [13,14], a cohort study of cardiovascular disease, was established in 1989 and included 12,200 Japanese urban residents of Suita City, Osaka. The participants, aged 30–79 years,

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were selected randomly from the municipality population registry. Of these, 6485 men and women had a baseline medical examination at the National Cardiovascular Center between September 1989 and March 1994 (participation rate: 53.2%). Of the 6485 participants, a total of 1791 were excluded for the following reasons: past history of coronary heart disease or stroke ($n = 208$), nonperiodical participation in baseline survey ($n = 79$), aged 75 or older ($n = 343$), non-fasting visit ($n = 153$), use of lipid-lowering agents such as statins ($n = 106$), serum triglyceride ≥4.5 mmol/l (400 mg/dl) ($n = 98$) and missing information at the baseline survey or lost to follow-up ($n = 804$). The data of the remaining 4694 participants (2169 men and 2525 women) were then analyzed. Informed consent was obtained from all participants. This cohort study was approved by the Institutional Review Board of the National Cardiovascular Center.

2.2. Baseline examination

Blood samples were collected at the National Cardiovascular Center (NCVC) after the participants had fasted for at least 12 h. The samples were centrifuged immediately and a routine blood examination that included serum total cholesterol (TC), HDL cholesterol, triglyceride and glucose levels then carried out. LDL-C was estimated using the Friedewald formula [15]. Non-HDLC was calculated by subtracting HDL-C from TC.

Blood pressures were measured in triplicate on the right arm in the seated position after 5 min rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used in the analyses. Hypertension was defined as either a systolic blood pressure ≥140 mmHg, a diastolic blood pressure ≥90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥7.0 mmol/l (126 mg/dl), the use of anti-diabetic agents, or both. Height in stockings and weight in light clothing were measured. Public health nurses obtained information on the smoking, drinking and medical histories of the participants.

2.3. Endpoint determination

The participants were followed until December 31, 2005. The first step in the survey involved checking the health status of all participants by repeated clinical visits every 2 years and yearly questionnaires sent by mail or conducted by telephone. Informed consent for review of in-hospital medical records was obtained from 86.2% participants who were suspected of having had a myocardial infarction (MI) or stroke. The medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information.

The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [16], which requires evidence from an electrocardiogram (ECG), cardiac enzymes and/or autopsy. Stroke was defined according to the National Survey of Stroke criteria [17], which requires the rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. The strokes were classified as either ischemic stroke (thrombotic or embolic), intracerebral hemorrhage, subarachnoid hemorrhage or undetermined type. A definite stroke was defined by autopsy or on the basis of diagnostic imaging, such as computed tomography or magnetic resonance imaging.

Cases with typical clinical symptoms, detected in the clinical visit during follow-up surveillance, but without informed consent for an in-hospital medical records survey, were defined as possible MI or stroke. Furthermore, to complete the surveillance for fatal MI and stroke, we conducted a systematic search for death certificates. All death certificates in Japan are forwarded to the Ministry of Health, Welfare, and Labor and coded for National Vital Statistics. We classified fatal MI and stroke listed on the death certificate, but not registered on our surveillance system, as possible MI and stroke.

2.4. Statistical analysis

Sex-specific analysis was performed. We set the cut-off points for serum LDL-C and non-HDLC according to the quintile ranges. For baseline characteristics, analysis of variance for means or Chi-square tests for proportions were used. The multivariable-adjusted hazard ratio (HR) of LDL-C and non-HDLC for MI or stroke was calculated using proportional hazards model adjusted for age, hypertension, diabetes, HDL-C, body mass index (BMI), smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker). Sex-combined analysis with further adjustment for sex was also carried out.

Separate models with LDL-C or non-HDLC levels as ordinal variables (median of LDL-C or non-HDLC quintile) were fitted to the other risk factor adjusted models (test for trend). The differences between the −2 logarithm likelihood (−2 ln [L]) in each lipid added model and the −2 ln [L] in other risk factor adjusted models were calculated. These differences had an approximate χ^2 distribution with 1 d.f. These χ^2 values assess which lipid had the greatest predictive value in other risk factor adjusted models. The ability to predict which people developed cardiovascular disease was also assessed by calculating the area under the receiver-operating characteristic (ROC) curve (AUC). This curve showed the predictive probability of the variables using logistic regression analysis and the same covariates used in the multivariable model of test for trend. Furthermore, the predictive values of the ratio of LDL-C to HDL-C (LDL-C/HDL-C) and the ratio of non-HDLC to HDL-C (non-HDLC/HDL-C) for myocardial infarction (MI) and stroke were also compared.

All confidence intervals were estimated at the 95% level and significance was set at a P value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 15.0J, Tokyo, Japan) was used for all the analyses.

3. Results

The mean and standard deviation of serum LDL-C in the baseline survey was 3.23 ± 0.82 mmol/l (124.9 ± 31.7 mg/dl) in men and 3.49 ± 0.90 mmol/l (134.8 ± 34.9 mg/dl) in women. The mean baseline serum non-HDLC was 3.90 ± 0.89 mmol/l (151.1 ± 34.5 mg/dl) in men and 4.01 ± 1.01 mmol/l (155.2 ± 39.1 mg/dl) in women.

Table 1 shows the baseline characteristics of the participants in each LDL-C quintile. In both sexes, there were significant differences in the mean values for age, non-HDLC, HDL-C and BMI. These variables, with the exception of HDL-C, tended to be higher in the higher LDL-C groups. Serum HDL-C levels were lower in the higher LDL-C groups. There was no significant difference in the prevalence of hypertension and diabetes in the quintiles for men, whereas the prevalence of these conditions in women was higher in the higher LDL-C groups. In both sexes, the proportion of current drinkers was lower in the higher LDL-C groups, whereas the proportion of current smokers was highest in the lowest LDL-C group. The relationships between non-HDLC quintiles and the above-mentioned baseline characteristics were almost similar (data not shown in the table).

The total person-years studied was 56,196 (25,420 for men and 30,776 for women), with a mean follow-up period of 11.9 years. During the follow-up period, there were 80 incident cases of MI (41 definite and 39 probable MIs) and 139 of stoke (102 definite and 37

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Table 1
Sex-specific mean and prevalence of risk characteristics at baseline in an 11.9-year prospective study of 4694 Japanese men and women

LDL cholesterol quintiles	Q1	Q2	Q3	Q4	Q5	P-values
Men						
Numbers	447	435	427	438	422	
LDL cholesterol (Stratum Mean), mmol/l	2.13	2.80	3.22	3.66	4.40	
Age, year	54.0 (12.7)	53.8 (12.6)	52.5 (12.4)	54.7 (12.1)	55.6 (11.0)	0.005
Non-HDL cholesterol, mmol/l	2.84 (0.52)	3.44 (0.39)	3.87 (0.34)	4.31 (0.32)	5.13 (0.56)	<0.001
HDL cholesterol, mmol/l	1.33 (0.39)	1.29 (0.36)	1.29 (0.32)	1.26 (0.30)	1.21 (0.28)	<0.001
BMI, kg/m^2	22.1 (2.9)	22.6 (2.8)	22.9 (2.8)	23.2 (2.6)	23.4 (2.7)	<0.001
Hypertension, %	29.5	27.4	30.4	31.3	33.6	0.364
Diabetes, %	8.1	4.6	4.4	4.6	5.9	0.091
Drinking						
Usual/ex-/never-, %	81.9/2.7/15.4	78.2/2.8/19.1	79.6/1.6/18.7	71.7/5.3/23.1	70.4/4.7/24.9	<0.001
Smoking						
Current/ex-/never-, %	59.3/25.5/15.2	55.4/26.9/17.7	46.6/31.1/22.2	46.6/31.1/22.4	48.1/31.8/20.1	0.002
Women						
Numbers	524	498	513	498	492	
LDL cholesterol (Stratum Mean), mmol/l	2.33	2.98	3.44	3.92	4.82	
Age, year	45.5 (11.4)	49.9 (11.9)	52.7 (11.3)	56.3 (10.6)	57.8 (9.1)	<0.001
Non-HDL cholesterol, mmol/l	2.77 (0.42)	3.47 (0.32)	3.96 (0.31)	4.50 (0.32)	5.46 (0.71)	<0.001
HDL cholesterol, mmol/l	1.54 (0.36)	1.49 (0.36)	1.48 (0.35)	1.45 (0.33)	1.40 (0.31)	<0.001
BMI, kg/m^2	21.0 (2.7)	21.8 (3.2)	22.3 (3.3)	22.6 (3.2)	23.2 (3.3)	<0.001
Hypertension, %	12.8	19.3	23.4	29.9	37.8	<0.001
Diabetes, %	1.5	2.8	3.1	4.0	4.7	0.050
Drinking						
Usual/ex-/never-, %	41.8/2.3/55.9	36.5/1.0/62.4	32.7/1.4/65.9	28.3/1.8/69.9	29.1/1.6/69.3	<0.001
Smoking						
Current/ex-/never-, %	16.4/4.6/79.0	12.7/3.8/83.5	9.6/2.1/88.3	10.8/3.4/85.7	11.6/3.7/84.8	0.015

HDL means high-density lipoprotein. LDL means low-density lipoprotein. S.D. means standard deviations. Brackets indicate standard deviation. Analysis of variance was used for comparisons of multiple group means and the Chi-square test was used to compare frequencies.

probable strokes), comprised of 23 intracerebral hemorrhages, 85 cerebral infarctions and 31 other types of stroke.

Table 2 shows the number of incident cases and multivariable-adjusted HRs for MI and cerebral infarction stratified by LDL-C quintile. In women, the bottom and second quintiles and the third and fourth quintiles were combined into two categories due to the small number of cardiovascular events. In both sexes, the HR for MI was highest in the top quintile of LDL-C, although the value in women was not statistically significant (HR 3.73; 95% CI 1.25–11.1 for men: HR 1.78; 95% CI 0.66–4.77 for women). In the test for trend, serum LDL-C showed a significant positive association with MI when the data from men and women were combined

Table 2
The numbers of cases and multivariable-adjusted HRs and 95% C.I.s for myocardial infarction and cerebral infarction according to serum LDL cholesterol level in an 11.9-year prospective study of 4694 Japanese men and women

LDL cholesterol quintiles	LDL-C range (mmol/l)	No. of persons	Person-years	Myocardial infarction			Cerebral infarction		
				No. of events	HR[a]	95% C.I.	No. of events	HR[a]	95% C.I.
Men									
Q1	<2.54	447	5,129	4	1.00		14	1.00	
Q2	2.54–3.03	435	5,122	15	3.56	1.18, 10.8	9	0.61	0.26, 1.42
Q3	3.04–3.43	427	4,945	9	2.60	0.80, 8.5	15	1.31	0.63, 2.72
Q4	3.44–3.90	438	5,201	10	2.25	0.70, 7.2	13	0.90	0.42, 1.94
Q5	3.91–	422	5,023	18	3.73	1.25, 11.1	6	0.42	0.16, 1.10
					P for trend	0.08		*P* for trend	0.22
Women									
Q1 + Q2[b]	<3.21	1022	12,473	6	1.00		7	1.00	
Q3 + Q4[b]	3.22–4.22	1011	12,279	5	0.45	0.14, 1.49	11	0.82	0.31, 2.15
Q5	4.23	492	6,023	13	1.78	0.66, 4.77	10	1.13	0.42, 3.02
					P for trend	0.14		*P* for trend	0.88
Men and women combined									
Q1		971	11,548	7	1.00		19	1.00	
Q2		933	11,176	18	2.37	0.97, 5.61	11	0.53	0.25, 1.12
Q3		940	11,102	11	1.57	0.61, 4.08	18	0.95	0.49, 1.82
Q4		936	11,323	13	1.40	0.56, 3.55	21	0.84	0.44, 1.59
Q5		914	11,046	31	3.03	1.32, 6.96	16	0.63	0.32, 1.24
					P for trend	0.02		*P* for trend	0.47

LDL means low-density lipoprotein.

[a] HR means hazard ratio and 95% C.I. means 95% confidence interval. The HR was adjusted for age, body mass index, diabetes, HDL cholesterol, cigarette smoking category and alcohol intake category by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] These groups were combined due to small number of cardiovascular event. The cut-off points were 2.73 between Q1 and Q2, and 3.68 between Q3 and Q4, respectively.

[c] Sex-specific quintiles were used for analysis.

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Table 3
The numbers of cases and multivariable-adjusted HRs and 95% C.I.s for myocardial infarction and cerebral infarction according to serum non-HDL cholesterol level in an 11.9-year prospective study of 4694 Japanese men and women

Non-HDL cholesterol quintiles	Non-HDLC range (mmol/l)	No. of persons	Person-years	Myocardial infarction			Cerebral infarction		
				No. of events	HR[a]	95% C.I.	No. of events	HR[a]	95% C.I.
Men									
Q1	<3.18	445	5,123	6	1.00		11	1.00	
Q2	3.18–3.68	450	5,195	14	2.34	0.89, 6.16	13	1.21	0.54, 2.73
Q3	3.69–4.12	426	5,077	7	1.21	0.40, 3.64	12	1.26	0.54, 2.91
Q4	4.13–4.63	428	5,041	10	1.49	0.53, 4.16	11	0.97	0.41, 2.31
Q5	4.64	420	4,982	19	2.61	1.00, 6.80	10	0.98	0.40, 2.40
					P for trend	0.12		*P* for trend	0.79
Women									
Q1 + Q2[b]	<3.70	1043	12,821	4	1.00		7	1.00	
Q3 + Q4[b]	3.71–4.87	1010	12,205	7	0.76	0.21, 2.72	11	0.67	
Q5	4.88	472	5,750	13	1.77	0.50, 6.25	10	0.80	
					P for trend	0.10		*P* for trend	
Men and women combined									
Q1		998	11,931	7	1.00		15	1.00	
Q2		940	11,208	17	2.35	0.97, 5.69	16	1.03	0.50, 2.10
Q3	[c]	947	11,412	11	1.38	0.53, 3.60	14	0.83	0.40, 1.76
Q4		917	10,911	13	1.40	0.55, 3.57	20	1.03	0.51, 2.06
Q5		892	10,732	32	2.97	1.26, 6.97	20	0.99	0.48, 2.03
					P for trend	0.02		*P* for trend	0.96

HDL means high-density lipoprotein.

[a] HR means hazard ratio and 95% C.I. means 95% confidence interval. The HR was adjusted for age, body mass index, hypertension, diabetes, HDL cholesterol, cigarette smoking category and alcohol intake category by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] These groups were combined due to small number of cardiovascular event. The cut-off points were 3.21 between Q1 and Q2, and 4.26 between Q3 and Q4, respectively.

[c] Sex-specific quintiles were used for analysis.

($P = 0.02$). A similar trend was observed when the endpoint was limited to definite MIs by the criteria of the MONICA project ($P = 0.01$, data not shown in the table). The incidence for cerebral infarction was not related to LDL-C levels in either sex. The incidences of intra-cerebral hemorrhage, other types of stroke and total stroke were also not associated with LDL-C levels (data not shown in the table).

Table 3 shows the results stratified by non-HDLC. The HR for MI was highest in the top quintile of non-HDLC in both sexes, although in women the value did not reach statistical significance (HR 2.61; 95% CI 1.00–6.8 for men: HR 1.77; 95% CI 0.50–6.25 for women). In men, the HR for MI was highest in the top quintile of non-HDLC (HR 2.61; 95% CI 1.00–6.80). In the test for trend, serum non-HDLC showed a significant positive association with MI when the data of men and women were combined ($P = 0.02$). A similar trend was observed when the endpoint was limited to define MIs ($P = 0.01$, data not shown in the table). The incidence of cerebral infarction was not associated with non-HDLC levels in either sex. The other types of stroke and total stroke were also not associated with non-HDLC level (data not shown in the table).

To determine the predictive values of LDL-C and non-HDLC, the difference between the $-2\ln[L]$ of model including each lipid and the $-2\ln[L]$ of other variable-adjusted models was calculated. The χ^2 values for LDL-C and non-HDLC were almost the same at 5.71 ($P = 0.02$) for LDL-C and 5.49 ($P = 0.02$) for non-HDLC. Furthermore, the AUC of the ROC curves based on predictive probability targeting for MI were also estimated. The AUC of LDL-C and non-HDLC were the same at 0.82.

We calculated the hazard ratios of LDL-C/HDL-C and non-HDLC/HDL-C, and compared the predictive values of these for the incidence of MI and stroke. Both ratios were significantly associated with the increased risk for MI but not with any types of stroke. The multivariable HRs of LDL-C/HDL-C and non-HDLC/HDL-C for MI were 1.32 [95% CI, 1.07–1.61] and 1.25 [95% CI, 1.07–1.47], respectively. Furthermore, the χ^2 values between the $-2\ln(L)$ of each lipid added model and non-added model for LDL-C/HDL-C and non-HDLC/HDL-C were almost the same at 7.34 ($P = 0.01$) for LDL-C/HDL-C and 7.06 ($P = 0.01$) for non-HDLC/HDL-C. The AUC of the ROC curves based on predictive probability were also the same. Apparently, because non-HDLC/HDLC was expressed as [(TC/HDLC) − 1], the HR and predictive value for TC/HDLC were just the same as those of non-HDLC/HDLC.

When the participants were divided in two groups using the median value of serum triglycerides (1.12 mmol/l, 99 mg/dl), the results of all the analyses listed above were similar.

4. Discussion

This 11.9-year cohort study of a Japanese urban population showed a positive association between serum LDL-C or non-HDLC levels and increased risk of MI, but not with any type of stroke. Furthermore, we found there was no substantial difference in the predictive value for MI incidence between LDL-C and non-HDLC. To our knowledge, this is the first cohort study in an urban Japanese population on the relationship between serum lipids and cardiovascular events.

The role of LDL-C in the development of atherosclerosis and the beneficial effect of LDL-C lowering therapy are well established, especially in Western populations [1–4] Our study indicated there is also a positive relationship between serum LDL-C and CAD events in community-dwelling Japanese with no history of cardiovascular disease or use of lipid-lowering agents, such as statins. A recent large clinical trial in Japan [18], the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA study), also have shown an 18% reduction in mean LDL-C (from 4.05 mmol/l to 3.31 mmol/l) was associated with a 33% decreased risk for CAD. These results suggested strongly that management of serum LDL-C levels is as effective for reducing CAD in Japan as it is in Western countries.

Non-HDLC levels are thought to be an alternative predictor that can substitute for LDL-C in patients with hypertriglycemia

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[3]. Non-HDLC reflects the total cholesterol concentration of all atherogenic lipoproteins. Several previous studies in US communities [6,7,9,19,20] or patients with type 2 diabetes [21,22] showed that the non-HDLC level was a stronger predictor for CAD risk than LDL-C. In the Lipid Research Clinics Program Follow-up Study [6], differences of 0.78 mmol/l (30 mg/dl) in non-HDLC and LDL-C levels corresponded to increases in CVD risk of 19% and 15% in men, and 11% and 8% in women, respectively. In contrast, Chien et al. showed that the hazard ratio of the top quintile and area under the ROC curve for CAD incidence were almost similar for LDL-C and non-HDLC in ethnic Chinese living in Taiwan [8].

Our results are consistent with the Taiwan study described above [8], which to date represents the only report from a non-Western community. As we calculated serum LDL-C levels using the Friedewald formula, our results were not applicable to the population with serum triglyceride levels equal to or greater than 4.5 mmol/l (≥400 mg/dl). However, even if the predictive values of LDL-C and non-HDLC are similar in the Japanese population, non-HDLC may be the more convenient indicator to use for primary prevention in the community. Both TC and HDL-C are included in routine biochemistry measurements because of convenience and low cost, and can be measured directly even in non-fasting serum. Accordingly, non-HDLC may be a good serum marker for risk assessment of CAD in a community-based setting.

In the present study, the positive association between serum lipids levels and MI in women was less evident than that in men. We believe it was mainly due to small number of MI in women. Continued community surveillance in Japan showed that incidence of MI for women was about one third of men [23]. In the present study, incidence of MI for women was only 0.78 per 1000 person-years. Because most MI cases (22 of 24) were post-menopausal women, the low incidence of MI in pre-menopausal women was one reason for sex-difference. However, it was difficult to perform further analysis because of small sample size of MI cases.

Similar to previous studies that have explored the relationship between TC and stroke in Japan [9,24,25], we found no association between LDL-C or non-HDLC levels and stroke events. A large meta-analysis of individual data from 61 prospective studies [26], the majority of which were from the US, European and Japanese populations, showed an absence of an independent positive association between TC or non-HDLC and ischemic and total stroke mortality. Recently, the death probability over a 10-year period due to MI and stroke have been calculated and displayed as color risk score charts by combining 10-year age, systolic blood pressure, smoking, and serum total cholesterol and glucose levels by NIPPON DATA (National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged) Research group [27]. NIPPON DATA Risk chart for MI clearly showed the positive relationship between TC and MI, however, the risk chart for stroke showed the color gradient, which was shown death probability, for stroke was not affected by TC levels.

The lack of a relationship between TC and ischemic stroke in Japanese studies may be due to a lower prevalence of thrombotic type cortical infarctions (large-artery occlusive) than in Western populations [28], a condition that is associated with atherosclerosis secondary to hypercholesterolemia. Furthermore, the Atherosclerosis Risk in Communities (ARIC) Study also indicated that TC was associated with increased risk of non-lacunar, non-embolic stroke (thrombotic type cortical infarction), but not with lacunar or embolic stroke [29]. The effect of LDL-C or non-HDLC on ischemic stroke may be weak in populations with a low prevalence of large-artery occlusive infarctions, such as in Japan. However, a meta-analysis of randomized control trials by statin therapy has indicated a reduction of stroke [30]. Even in Japanese patients with hypercholesterolemia, statin therapy showed a non-significant but inverse association with cerebral infarction [18]. Accordingly, high serum levels of LDLC or non-HDLC should be dealt with caution as a potential risk factor for ischemic stroke.

Previous studies indicated that CAD or MI morality in Japanese people was still lower than in Westerners [9–12]. However, recently, there were evidences that serum levels of TC and LDL-C in Japanese were as high as those reported in the US population [31]. However, CAD mortality has been shown to be higher in large urbanized areas in Japan such as Tokyo and Osaka compared to the rest of Japan [32]. These two cities are among the most urbanized areas in Asia. The present study therefore provides additional evidence supporting the usefulness of LDL-C and non-HDLC as predictors of future risk for MI in screening of the urbanized Japanese population. Although in Asian countries hypertension rather than LDL-C remains the most important manageable cardiovascular risk factor [33], the present study showed that, at least in urbanized areas, lowering of LDL-C levels should also be considered as an important public health issue.

The present study had some limitations. Firstly, the single LDL-C or non-HDLC measurement at the baseline survey may have underestimated the relationship between these lipids and CAD due to regression dilution bias. Secondly, we did not measure serum apolipoprotein B (apoB), which some previous studies have shown as a stronger predictor for CAD than non-HDLC [8,20]. Furthermore, measurement of apoB is not required fasting status and is estimated to be cost-efficient [34]. Further cohort studies with measurement of apoB are needed in Japanese community-dwelling populations. Thirdly, in order to accurately compare the predictive value of non-HDLC and LDL-C, serum levels of LDL-C should be measured by direct measurement of LDL-C, rather than by the Friedewald formula. Exclusion of participants with a high serum triglyceride level (≥400 mg/dl) may reduce the predictive potential of non-HDLC. Finally, the relationship between serum lipids and cerebral infarction warrants further investigation, as we did not evaluate the effect of serum LDL-C and non-HDLC on each subtype of cerebral infarction due to small sample size, especially for thrombotic type cortical infarctions.

In conclusion, higher levels of serum LDL-C and non-HDLC are both associated with an increased risk of MI, but not with cerebral infarction in a Japanese urban population. Although the predictive value of non-HDLC for MI is almost similar to that of LDL-C calculated by the Friedewald formula, non-HDLC may be recommended as an alternative screening marker for primary prevention of CAD in the community, as it is less expensive and more convenient.

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Masked Hypertension: Subtypes and Target Organ Damage

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Masked hypertension has been drawing attention recently because this condition is often seen in untreated and treated individuals and is associated with target organ damage and a poor cardiovascular prognosis. Although masked hypertension is defined as normal office blood pressure with elevated ambulatory or home blood pressure, there are several subtypes. Morning hypertension is the most common form of masked hypertension, and is caused by natural circadian variation, evening alcohol consumption, and the use of short-acting antihypertensive drugs. Daytime hypertension may be caused by lifestyle factors such as habitual smoking and mental or physical stress. Nighttime hypertension is seen in various conditions that produce non-dipping status, including a high salt intake, renal dysfunction, obesity, sleep apnea, and autonomic failure. Advanced target organ damage such as increases in the left ventricular mass, carotid artery intima-media thickness, and urinary albumin excretion, is often present both in untreated and treated subjects with masked hypertension. In our study, the presence of the reverse white-coat effect is independently associated with those indices of organ damage among treated hypertensive patients. It is important to identify individuals with masked hypertension, to evaluate them with including the search for the subtype, and to treat each patient appropriately according to the cause of this condition.

Keywords masked hypertension, target organ damage, ambulatory blood pressure monitoring, home blood pressure

Introduction

Masked hypertension, which is also called reverse white-coat hypertension or isolated ambulatory hypertension, has been drawing attention recently (1–3). Masked hypertension is defined as normal office blood pressure (BP) with elevated ambulatory or home BP. Although the term of masked hypertension was originally applied to untreated subjects, this condition is also frequently seen in treated hypertensive

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patients. The prevalence of masked hypertension has been reported to be about 10% in normotensive (defined by casual BP) subjects and about 20% among treated hypertensive patients (1,4–6). There is increasing evidence that masked hypertension is associated with advanced target organ damage and a poor cardiovascular prognosis (7–11).

Masked hypertension can be classified into several subtypes according to the pattern of ambulatory BP and underlying mechanisms. These subtypes include morning, daytime, and nighttime hypertension (3). Detecting the subtype and underlying mechanism may be helpful for the appropriate management of each patient with masked hypertension. Regarding the target organ damage in masked hypertension, obtained information may not be enough, especially for treated patients. In this review, we describe the subtypes and organ damage of masked hypertension, including the results of our studies.

Subtypes of Masked Hypertension

Morning Hypertension

Morning hypertension is the most common form of masked hypertension (see Table 1). The circadian rhythm of BP is well known. Usually, BP elevates sharply with waking in the early morning, decreases slightly from the late morning to early afternoon, increases again in the early evening, decreases in the late evening, and then falls largely with sleeping. It has been shown that home BP in the early morning is somewhat higher than that in the late evening (6,12). It is possible that this physiological change in BP causes masked hypertension, if office BP is measured in the late morning or early afternoon in the absence of the white-coat effect. Morning hypertension is also caused by lifestyle-related factors such as habitual alcohol intake. We observed that evening alcohol consumption decreases nighttime BP but increases daytime BP in

Table 1
Subtypes of masked hypertension

Subtypes	Causes	Management
Morning hypertension (morning surge)	Natural circadian rhythm	Alcohol restriction
	Alcohol	Long-acting drug
	Antihypertensive drug (short-acting)	Evening drug administration
		Alpha blockers (evening)
Daytime hypertension (worksite hypertension)	Smoking	Smoking cessation
	Stress (mental, physical)	Stress management
		Beta blockers (morning)
Nighttime hypertension (non-dipper)	Salt, renal dysfunction	Salt restriction
	Obesity, sleep apnea	Weight reduction
	Autonomic failure	Diuretics
		Treatment of sleep apnea

hypertensive patients (12,13). This alcohol-induced BP elevation is most obvious in the early morning.

Morning hypertension is often seen among treated hypertensive patients, particularly in those who are taking short-acting antihypertensive drugs in the morning. Such medication does not maintain the antihypertensive efficacy for 24 hours, resulting in BP elevation in the early morning. The use of long-acting drugs or evening administration of antihypertensive drugs is helpful to control morning hypertension. Because the sympathetic nervous system plays an important role in the morning BP elevation through alpha receptor-mediated vasoconstriction, the administration of alpha blockers in the evening may also be effective to attenuate the morning BP surge (14).

Daytime Hypertension

Daytime hypertension is caused by lifestyle-related factors such as habitual smoking and daily stress (see Table 1). Smoking cigarettes acutely elevates BP, and smokers show a higher daytime BP on a smoking day compared with nonsmokers or a nonsmoking day (15). Mental or physical stress also acts to elevate daytime BP, particularly during working (16). We also observed that daytime BP but not nighttime BP is higher during usual daily life than during a hospital stay in hypertensive patients (17). When habitual smokers or subjects experiencing stress visit clinics, their BP may be normal because they can take a rest without smoking in the waiting room. The cessation of smoking and control of daily stress is recommended for subjects with daytime hypertension. Beta blocker usage may be effective to control stress-related hypertension.

Nighttime Hypertension

Although BP usually falls at night, the nighttime BP dip is blunted or absent in a considerable portion of normotensive and hypertensive subjects. Some individuals show a rise in BP during sleep. This non-dipper pattern is often seen in salt-sensitive subjects on a high-salt diet; patients with renal dysfunction; obese subjects, particularly those with sleep apnea; and patients with autonomic failure; and may cause masked hypertension (see Table 1). It should be mentioned that many non-dippers also show morning hypertension because their BP continues to increase during the night until waking up.

Previous studies by our institute have shown that treatment with a low-salt diet or a diuretic decreases nighttime BP effectively in hypertensive patients (18,19). Weight reduction is recommended for obese subjects. Continuous positive airway pressure treatment is effective to lower nighttime as well as 24-hour BP in patients with sleep apnea (20). It is also important to use long-acting antihypertensive drugs to control nighttime BP.

Identifying the Subtypes

The diagnosis of masked hypertension is obtained by the use of ambulatory BP monitoring (ABPM) or home BP measurement in comparison with office BP. The Japanese guidelines for the management of hypertension (JSH 2004) support the use of ABPM and home BP measurement, particularly for the diagnosis of white-coat hypertension and masked hypertension (21).

To identify the subtypes of masked hypertension, ABPM is superior to home BP measurement because it provides multiple BP readings throughout 24 hours. However, the

application of ABPM to all hypertensive subjects is not practical, and a single ABPM may not be enough to represent the individual's 24-hour BP profile. Self-measurement of BP in the morning and evening at home appears to detect morning hypertension. Daytime hypertension can be detected through additional BP measurement at home or worksite during the daytime. ABPM is particularly suitable for the diagnosis of nighttime hypertension. The detection of nighttime hypertension by home BP measurement is difficult; however, new devices with timers, such as OMRON HEM-747IC, can determine BP during sleep. The widespread application of such devices may easily identify the subtypes of masked hypertension without using ABPM.

Target Organ Damage in Masked Hypertension

Numerous studies have examined the relationship between ambulatory BP or home BP and cardiovascular complications. It has been shown that ambulatory BP and home BP are more closely related to hypertensive organ damage and cardiovascular prognosis than office BP (22–26). Therefore, it is not surprising that subjects with masked hypertension are prone to develop target organ damage.

Untreated Subjects

It has been shown that subjects with masked hypertension have advanced target organ damage and a poor cardiovascular prognosis compared to normotensive subjects. Liu et al. measured target organ abnormality by echocardiography and arterial ultrasonography in untreated subjects with sustained normotension, masked hypertension, and sustained hypertension (27). They demonstrated that left ventricular mass and carotid wall thickness are greater in subjects with masked hypertension compared to those with sustained normotension, and are similar to those with sustained hypertension. Lurbe et al. also showed that young patients with masked hypertension have a higher left ventricular mass index than normotensive subjects (28). It is likely that a majority of masked hypertensives are overlooked because of normal office BP, resulting in the progression of target organ damage.

Treated Patients

Advanced target organ damage is also seen in treated patients with masked hypertension. We determined the left ventricular mass index, carotid artery intima-media thickness, and urinary albumin excretion in 332 treated hypertensive patients (29,30). In our study, all of these indices of target organ damage in patients with masked hypertension were significantly higher than those with controlled hypertension or white coat hypertension, and were even higher than those with sustained hypertension (see Figure 1). Cuspidi et al. examined left ventricular mass index and urinary albumin excretion in treated hypertensive patients at baseline and after an average follow-up of 30 months (31). They observed that these parameters decreased in patients with controlled ambulatory BP but not in those with masked hypertension.

Subtypes and Organ Damage

A number of studies have shown that the non-dipper pattern or the level of nighttime BP is associated with advanced organ damage and a poor prognosis (22–24,32,33). In the PAMELA study, nighttime BP was the best predictor of future cardiovascular death

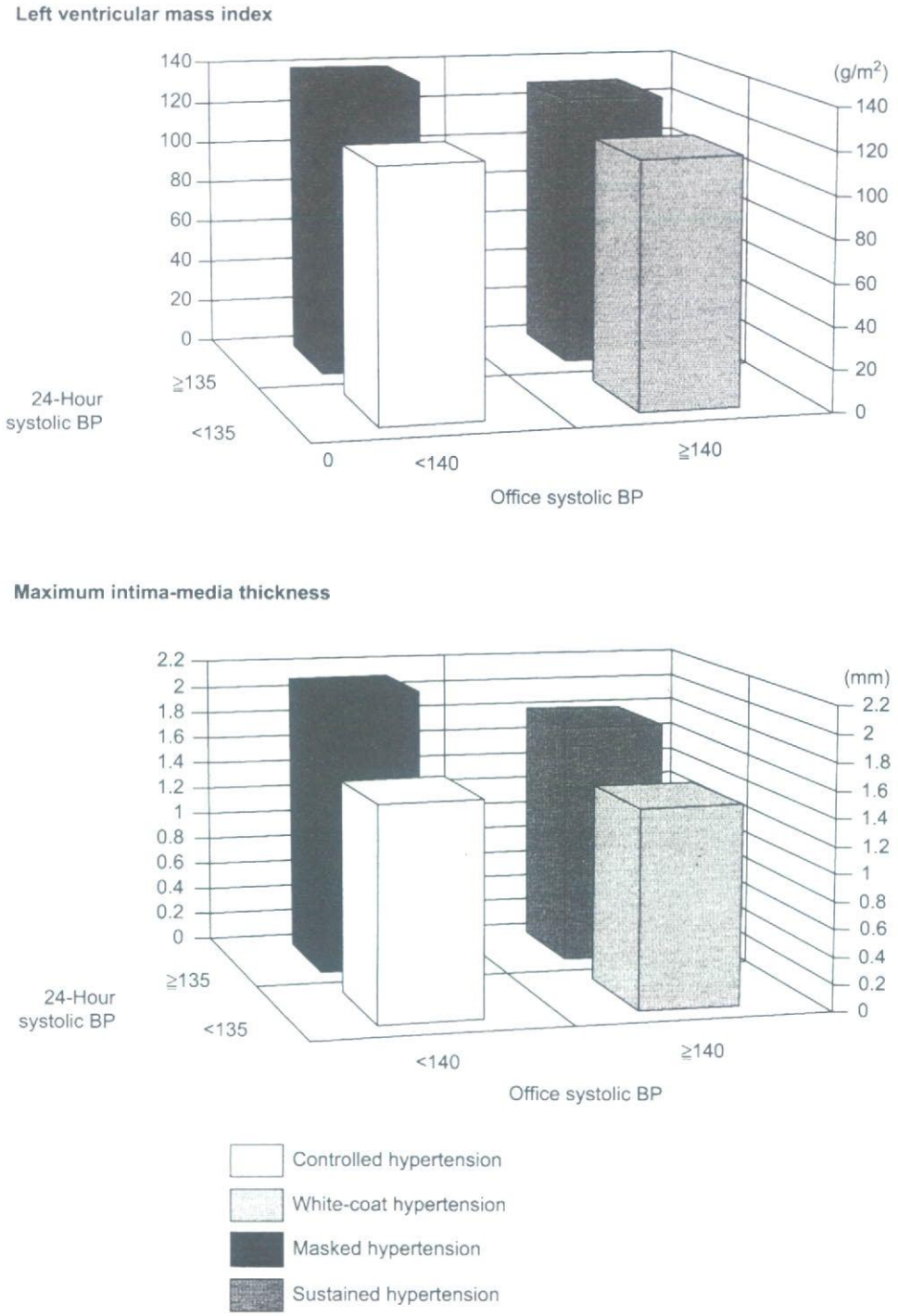

Figure 1. Left ventricular mass index and carotid artery maximum intima-media thickness in treated patients with controlled hypertension, white-coat hypertension, masked hypertension, and sustained hypertension, adopted from (29).

among office BP, home BP, and ambulatory BP parameters (24). Therefore, it is likely that nighttime hypertension is prone to develop organ damage such as left ventricular hypertrophy, carotid atherosclerosis, and impaired renal function.

It is well known that cardiovascular events occur frequently in the early morning when BP increases rapidly. Kario et al. have shown that the morning surge in BP is independently associated with silent and clinical cerebrovascular disease, and morning hypertension is the strongest independent risk factor for stroke in elderly hypertensives (34,35). It is also reported that the morning rise in BP correlates with the left ventricular mass index or hypertrophy in hypertensive patients (36,37), and high morning BP is associated with a loss of functional independence in elderly subjects (38). Therefore, morning hypertension appears to play a role in the target organ damage and cardiovascular events.

The association of daytime BP with organ damage and prognosis is less recognized, although daytime BP is a main determinant of average 24-hour BP. In the PAMELA study, the contribution of daytime BP to cardiovascular mortality was relatively weak compared with nighttime BP (24). However, it has been shown that mental stress is related to the progression of carotid atherosclerosis and cardiovascular mortality (39,40). It is possible that subjects with daytime hypertension are also susceptible to the development of target organ damage.

Conclusion

There are several subtypes of masked hypertension. Morning hypertension is caused by natural circadian variation, evening alcohol consumption, and short-acting antihypertensive drugs. Daytime hypertension may be caused by smoking and stress. Nighttime hypertension is seen in various conditions that lead to a non-dipping status. Advanced target organ damage is often present both in untreated and treated subjects with masked hypertension. All three subtypes of masked hypertension seem to be associated with organ damage, although the relative risk of those subtypes remains to be clarified. It is important to identify individuals with masked hypertension, evaluate them (including identifying the subtype), and treat each patient appropriately according to the cause of this condition.

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Plasma adrenomedullin as an independent predictor of future cardiovascular events in high-risk patients: Comparison with C-reactive protein and adiponectin

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ABSTRACT

This study investigated the predictive power of plasma adrenomedullin (AM) for future cardiovascular (CV) events. In 121 patients with multiple CV risk factors and/or disease, plasma concentrations of AM, high sensitive C-reactive protein (hs-CRP), and adiponectin were measured. During follow-up periods (mean, 3.5 years) after the baseline assessment, 28 patients newly experienced CV events such as stroke/transient ischemic attack, acute coronary syndrome, and congestive heart failure. The plasma level of AM, but not hs-CRP or adiponectin, was significantly higher in patients who had CV events than in event-free subjects. When the patients were divided into three groups by tertiles of basal levels of AM (<10.1, 10.1–13.1, and ≥13.1 fmol/mL), cumulative event-free rates by the Kaplan–Meier method were decreased according to the increase in basal AM levels (83.2%, 68.6%, and 52.8% in the lowest, middle, and highest tertiles of AM, respectively; log-rank test, $P = 0.033$). By univariate Cox regression analysis, previous coronary artery disease, creatinine clearance, and plasma AM and hs-CRP levels were significantly associated with CV events during follow-up. Among these possible predictors, high plasma AM ($P = 0.004$) and low creatinine clearance ($P = 0.043$) were independent determinants for morbidity in multivariate analysis. These findings indicate that plasma AM is a powerful independent predictor of future CV events in high-risk patients, suggesting its predictive value is superior to that of hs-CRP or adiponectin.

1. Introduction

Adrenomedullin (AM) is a potent vasodilator peptide that was originally isolated from human pheochromocytoma [14]. Subsequent studies have revealed that AM is widely distributed in various organs and tissues including the cardiovascular (CV) system [6,38,39]. Plasma levels of AM are elevated in various CV disorders, such as essential hypertension [8,17,24], chronic renal failure [8,24], coronary artery disease [15,22,41], congestive heart failure [11,25], ischemic

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stroke [7], and peripheral artery disease [40,41], and the degree of increase in AM levels is shown to be in proportion to the clinical severity of the disease [8,15,17,22,24,25,40]. These previous findings suggest that plasma AM may be a biochemical marker reflecting the presence and severity of CV complications in patients with CV risk factors. However, it remains unclear whether plasma AM levels have a predictive value for the occurrence of future CV events in such patients.

It is currently recognized that low-grade inflammation and insulin resistance contribute importantly to the initiation and progression of CV lesions [19,20]. In fact, many studies have shown that a mild increase in C-reactive protein (CRP), a sensitive inflammatory marker, is an independent predictor of future CV events [1,31–34,36]. It has also been shown that decreased blood levels of adiponectin, an adipocytokine with insulin sensitizing, anti-inflammatory, and anti-atherogenic properties, are a novel predictive factor for atherosclerotic CV disease [5,9,16,37,47]. In the present study, we aimed to determine whether an elevated level of plasma AM is a significant predictor of future CV events in high-risk patients, comparing its predictive power with those of CRP and adiponectin.

2. Methods

2.1. Study subjects

A total of 121 patients with two or more CV risk factors and/or diseases were enrolled in the present study. All subjects were inpatients who were admitted to the National Cardiovascular Center, Suita, Japan, for examination and treatment of hypertension, diabetes mellitus, and CV diseases including stable coronary artery disease. Patients with acute coronary syndrome (i.e., acute myocardial infarction and unstable angina pectoris) or congestive heart failure were excluded from the study. Hypertension was defined as a systolic blood pressure of ≥140 mmHg and/or a diastolic blood pressure of ≥90 mmHg by repeated measurements or when subjects had already been treated with antihypertensive drugs. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria (a fasting plasma glucose of ≥126 mg/dL and/or a plasma glucose level at 2 h after 75 g oral glucose load of ≥200 mg/dL), or when medication was taken for treatment of hyperglycemia. Diagnosis of hyperlipidemia required a serum total cholesterol level of ≥220 mg/dL and/or a serum triglyceride level of ≥150 mg/dL or the use of lipid-lowering drugs. Coronary artery disease was diagnosed by electrocardiographic, radioisotope cardiographic, and coronary angiographic criteria. All subjects gave their informed consent to participate in the present study. All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies.

2.2. Biochemical measurement

Peripheral blood samples were obtained at rest in the supine position. Blood for AM measurement was immediately transferred into ice-chilled glass tubes containing disodium EDTA (1 mg/mL) and aprotinin (500 U/mL) and centrifuged for 10 min at 4 °C. Plasma samples were frozen and stored at −80 °C until assayed. Human AM concentration was measured by immunoradiometric assay using a specific kit (AM RIA SHIONOGI, Shionogi Pharmaceutical Co. Ltd., Osaka, Japan), as described previously [27].

Plasma adiponectin was determined by a sandwich ELISA system (Adiponectin ELISA Kit, Otsuka Pharmaceutical Co. Ltd.), as previously reported [9,10]. High sensitive CRP (hs-CRP) was measured by nephelometry (SRL Inc., Tokyo, Japan). Fasting plasma glucose, hemoglobin A1c, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and serum creatinine were determined by standard laboratory measurements. Creatinine clearance was calculated from the Cockcroft–Gault formula [3].

2.3. Follow-up

After the initial assessment, all patients periodically visited our hospital for the treatment of risk factors (hypertension, diabetes mellitus, and/or hyperlipidemia) and CV diseases. CV events as clinical endpoints were stroke and transient ischemic attack confirmed by clinical symptoms, computed tomography, magnetic resonance angiography, and/or cerebrovascular angiography findings, acute coronary syndrome confirmed by electrocardiographic changes, coronary angiography, and/or myocardial scintigraphy findings, and congestive heart failure requiring hospitalization. Congestive heart failure was defined as clinical symptoms and signs (dyspnea, pulmonary rale, and/or leg edema), hypoxemia, and findings of chest radiography (pulmonary congestion and/or pleural effusion). Diagnosis of heart failure and need for admission were determined by clinical physicians who were blind to the basal level of AM, hs-CRP, or adiponectin. For patients who experienced multiple episodes, the analysis included only the first event. For patients without any CV event mentioned above, the date of censor was that of the last contact with the subject. The mean follow-up period was 42.0 months (0.3–81.3 months).

2.4. Statistical analysis

Statistical analysis was performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, CA). Values were expressed as mean ± S.D. An unpaired Student's *t*-test was used for comparison between the two groups. The significance of differences among the three groups was evaluated by an unpaired ANOVA with subsequent Scheffe's multiple comparison test. Event-free curves were derived by means of the Kaplan–Meier method and were compared by log-rank test. The predictive value for CV events was tested by univariate Cox proportional hazards regression analysis. Then, a multivariate analysis using stepwise regression model was applied to identify independent predictors and their prognostic power. A value of $P < 0.05$ was accepted as statistically significant.

3. Results

Baseline clinical characteristics of total study subjects are shown in Table 1. The present subjects had a high percentage

Table 1 – Clinical characteristics of total subjects (n = 121)

Variable	
Age (years)	67.6 ± 9.5
Sex (men) (%)	68.6
Body mass index (kg/m^2)	23.6 ± 4.4
Hypertension (%)	84.3
Diabetes mellitus (%)	44.6
Hyperlipidemia (%)	57.0
Smokers (current or past) (%)	76.0
Previous coronary artery disease (%)	48.8
Systolic blood pressure (mmHg)	136 ± 18
Diastolic blood pressure (mmHg)	73 ± 11
Heart rate (beats/min)	65 ± 8
Fasting plasma glucose (mg/dL)	106 ± 31
Hemoglobin A1c (%)	6.2 ± 1.6
Total cholesterol (mg/dL)	191 ± 30
Triglycerides (mg/dL)	114 ± 51
HDL cholesterol (mg/dL)	45.1 ± 13.4
Creatinine clearance (mL/min)	78.6 ± 35.5

Values are mean ± S.D. or percentage.

of CV risk factors such as hypertension, diabetes mellitus, hyperlipidemia, and smoking habit, although their blood pressure, plasma glucose, and serum lipid levels were controlled by adequate treatments. In addition, 59 patients (48.8%) had a history of coronary artery disease.

During follow-up periods after the baseline assessment, 28 patients newly experienced major CV events. There were six subjects with cerebral infarction, one with cerebral hemorrhage, five with transient ischemic attack, six with unstable angina pectoris, one with acute myocardial infarction, and nine with congestive heart failure. The plasma AM level was significantly higher in patients who had CV events than in event-free subjects (Table 2). There was no significant difference in hs-CRP or adiponectin level between the two groups.

Table 2 – Association of basal AM, hs-CRP, and adiponectin levels with the following CV events

Variable	CV event		P
	(−) (n = 93)	(+) (n = 28)	
AM (fmol/mL)	11.6 ± 3.3	14.6 ± 6.3	<0.001
Hs-CRP (mg/dL)	0.23 ± 0.30	0.31 ± 0.65	0.359
Adiponectin (μg/mL)	5.8 ± 4.7	7.2 ± 5.6	0.214

Values are mean ± S.D.

All subjects were divided into three groups according to tertiles of basal AM levels (<10.1, 10.1–13.1, and ≥13.1 fmol/mL). Mean plasma levels of basal AM in the lowest, middle, and highest tertile groups were 8.3 ± 1.1, 11.5 ± 1.0, and 16.9 ± 4.1 fmol/mL, respectively (Table 3). Age, sex, body mass index, prevalence of hypertension, diabetes mellitus, and hyperlipidemia, smoking habit, blood pressure, heart rate, and glucose and lipid parameters did not differ among the three groups. The group in the highest tertile of AM had a significantly higher rate of past history of coronary artery disease, and lower creatinine clearance compared with the other two groups. Hs-CRP and adiponectin levels were also elevated in the highest tertile than in the lowest and/or middle tertiles. CV event-free Kaplan–Meier curves in the three groups are presented in Fig. 1. Cumulative event-free rates in the lowest, middle, and highest tertiles of AM were 83.2%, 68.6%, and 52.8%, respectively. These curves showed that higher basal levels of plasma AM were significantly associated with higher rate of CV events during follow-up (log-rank test, P = 0.033).

Table 3 – Clinical characteristics of three groups divided by tertiles of basal AM levels

Variable	Lowest tertile (n = 40)	Middle tertile (n = 40)	Highest tertile (n = 41)
Age (years)	66.6 ± 8.4	66.8 ± 10.6	69.3 ± 9.3
Sex (men) (%)	75.0	60.0	70.7
Body mass index (kg/m^2)	24.3 ± 3.9	24.3 ± 5.6	22.1 ± 3.0
Hypertension (%)	80.0	77.5	95.1
Diabetes mellitus (%)	37.5	55.0	41.5
Hyperlipidemia (%)	67.5	55.0	48.8
Smokers (current or past) (%)	77.5	67.5	82.9
Previous coronary artery disease (%)	35.0	40.0	70.7*,**
Systolic blood pressure (mmHg)	133 ± 14	135 ± 22	139 ± 16
Diastolic blood pressure (mmHg)	74 ± 9	73 ± 12	71 ± 11
Heart rate (beats/min)	65 ± 9	64 ± 8	65 ± 8
Fasting plasma glucose (mg/dL)	109 ± 30	103 ± 27	107 ± 35
Hemoglobin A1c (%)	6.4 ± 2.1	6.2 ± 1.3	6.0 ± 1.2
Total cholesterol (mg/dL)	191 ± 31	199 ± 29	183 ± 29
Triglycerides (mg/dL)	125 ± 59	109 ± 51	108 ± 39
HDL cholesterol (mg/dL)	44.6 ± 12.6	47.7 ± 14.7	43.0 ± 12.6
Creatinine clearance (mL/min)	87.4 ± 26.1	85.4 ± 39.1	63.3 ± 35.4*,**
AM (fmol/mL)	8.3 ± 1.1	11.5 ± 1.0*	16.9 ± 4.1*,**
Hs-CRP (mg/dL)	0.11 ± 0.14	0.17 ± 0.23	0.47 ± 0.60*,**
Adiponectin (μg/mL)	4.7 ± 3.5	6.4 ± 4.4	7.5 ± 6.2*

Values are mean + S.D. or percentage.
* $P < 0.05$ vs. lowest tertile.
** $P < 0.05$ vs. middle tertile.