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

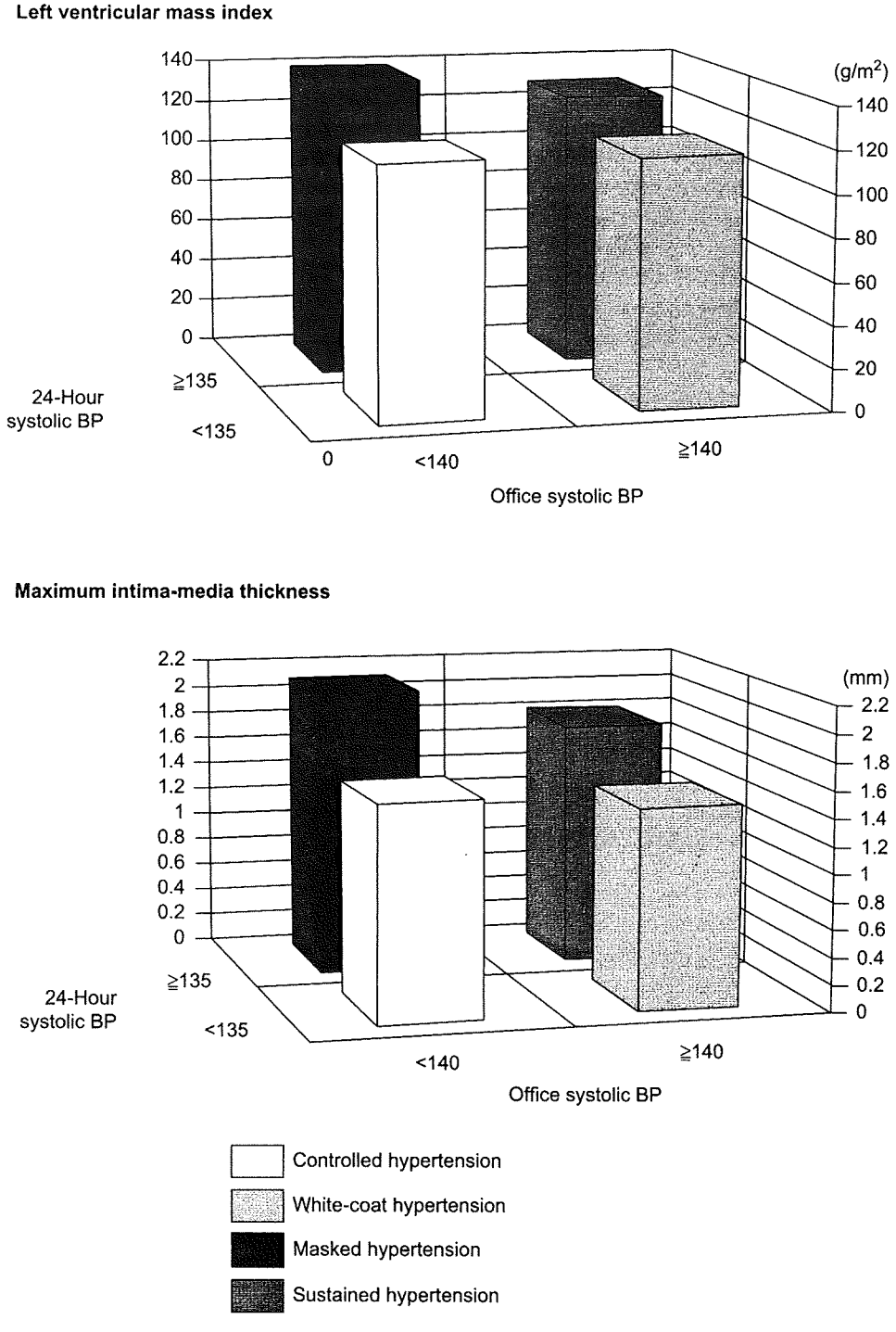

Figure 1. Left ventricular mass index and carotid artery maximum intima-media thickness in treated patients with controlled hypertension, white-coat hypertension, masked hypertension, and sustained hypertension, adopted from (29).

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.

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.

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.

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.

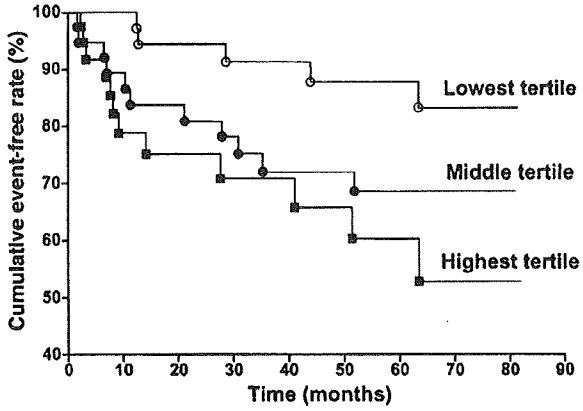

Fig. 1 – CV event-free curves obtained with the Kaplan–Meier method in the three groups divided by tertiles of basal AM levels. Cumulative event-free rates in the lowest, middle, and highest tertiles were 83.2%, 68.6%, and 52.8%, respectively (log-rank test, P = 0.033). Lowest tertile, basal AM <10.1 fmol/mL (n = 40); middle tertile, basal AM ≥10.1 and <13.1 fmol/mL (n = 40); highest tertile, basal AM ≥13.1 fmol/mL (n = 41).

Cox regression analysis was performed to examine the predictive power of plasma AM for future CV events, comparing with those of hs-CRP and adiponectin. In the univariate analysis, past history of coronary artery disease, creatinine clearance, and plasma hs-CRP in addition to plasma AM were significantly related to the incidence of CV events during the follow-up periods (Table 4). Among these possible predictive factors, high plasma AM and low creatinine clearance were independent predictors of CV events in the multivariate analysis, and the predictive value of AM for morbidity was most significant (+10% per 1-fmol/mL increase in AM, P = 0.004). Furthermore, even when the multivariate regression was reanalyzed after excluding subjects with previous coronary artery disease, the predictive value of AM for CV events was still significant, independently of creatinine clearance and other variables (hazard ratio 1.20 (per 1 fmol/mL increase), 95% confidence interval 1.06–1.35, P = 0.004).

4. Discussion

Plasma AM levels are known to be elevated in various pathological states, including several CV diseases [7,8,11, 15,17,22,24,25,40,41]. In addition, some studies showed that AM level was a predictor of survival in patients with acute myocardial infarction and chronic heart failure [12,23,29,30]. However, there have been no reports examining whether plasma AM can predict the occurrence of CV events in subjects with CV risk factors. Thus, the present study has demonstrated for the first time that an increased level of plasma AM becomes a significant predictor of future CV events in high-risk patients, independently of a variety of influencing factors.

In this study, we compared the predictive power of AM with those of hs-CRP and adiponectin. Our findings showed that neither hs-CRP nor adiponectin was an independent

Table 4 – Predictors of future CV events by univariate and multivariate Cox regression analysis

	Hazard ratio (95% CI)	P
Univariate analysis		
Age, 10 years	1.34 (0.88–2.04)	0.174
Sex, male	1.13 (0.50–2.56)	0.772
Body mass index, 1 kg/m^2	0.97 (0.88–1.07)	0.523
Hypertension, yes	2.02 (0.61–6.71)	0.249
Diabetes mellitus, yes	1.90 (0.89–4.06)	0.097
Hyperlipidemia, yes	1.00 (0.47–2.11)	0.999
Smoking (current or past), yes	1.65 (0.63–4.33)	0.313
Previous coronary artery disease, yes	2.90 (1.31–6.43)	0.009
Systolic blood pressure, 10 mmHg	1.03 (0.83–1.27)	0.799
Diastolic blood pressure, 10 mmHg	0.90 (0.65–1.24)	0.509
Heart rate, 5 beats/min	1.02 (0.83–1.27)	0.828
Fasting plasma glucose, 10 mg/dL	1.07 (0.97–1.19)	0.196
Hemoglobin A1c, 1%	1.14 (0.96–1.36)	0.126
Total cholesterol, 10 mg/dL	0.90 (0.80–1.02)	0.102
Triglycerides, 10 mg/dL	1.00 (0.94–1.07)	0.960
HDL cholesterol, 5 mg/dL	1.02 (0.90–1.16)	0.769
Creatinine clearance, 10 mL/min	0.80 (0.70–0.93)	0.003
AM, 1 fmol/mL	1.13 (1.06–1.19)	<0.001
Hs-CRP, 0.1 mg/dL	1.08 (1.00–1.18)	0.047
Adiponectin, 1 μg/mL	1.08 (0.99–1.16)	0.054
Multivariate analysis		
Creatinine clearance, 10 mL/min	0.87 (0.76–0.99)	0.043
AM, 1 fmol/mL	1.10 (1.03–1.18)	0.004

CI: confidence interval. In the multivariate analysis using stepwise regression model, all factors that had a significant association in the univariate analysis, i.e., previous coronary artery disease, creatinine clearance, AM, and hs-CRP, were included as possible independent variables.

predictor of future CV events, in contrast to the powerful prognostic value of AM. Several large epidemiological studies have suggested that CRP measurement predicts the risk of future CV events [1,31–34,36], whereas others have failed to identify CRP as a significant independent risk factor, especially after using multivariate analysis [28,42,44]. Hs-CRP was one of the significant predictors of CV events in univariate Cox regression analysis of the present study. However, since there was a close correlation between hs-CRP and AM levels (data not shown) and the predictive power of hs-CRP was weaker than that of AM in univariate analysis, hs-CPR might not become an independent predictor in multivariate analysis. As for adiponectin, it has been shown that low levels of plasma adiponectin are a predictor of CV events and mortality [4,5,9,16,37,47], but some studies reported that adiponectin did not predict future risk of coronary artery disease after adjusted for classical risk factors [18,35]. In addition, recent studies revealed that high, rather than low, adiponectin levels were associated with increased mortality and incidence of myocardial infarction in patients with chronic heart failure, chronic kidney disease, and stable angina [2,13,21]. Thus, the value of adiponectin as an independent risk marker for CV events and mortality remains controversial at present.

Although the exact reason behind the superiority of plasma AM over hs-CRP and adiponectin as a predictor of CV events in the present study remains to be elucidated, a number of mechanisms may be involved. AM is produced in various organs and tissues, but the main source of circulating AM is the blood vessels (especially vascular endothelial cells) [38], in contrast to the major sites of the production of CRP and adiponectin. Therefore, AM may directly reflect vascular inflammation and endothelial injury during the initiation and development of atherosclerosis. In fact, increased plasma levels of AM were reported to be associated with the progression of atherosclerotic lesions [7,40]. Furthermore, since several studies have shown that ischemic and hypoxic conditions stimulate the production and secretion of AM [26,43,46], it is possible that the increase in baseline AM might be induced by silent cerebral or cardiac ischemia before attack. Plasma AM has also been shown to increase in response to left ventricular systolic and diastolic dysfunction [23,25,45], suggesting the possibility that baseline AM in our subjects could detect latent cardiac disorders. Therefore, as AM comprehensively reflects vascular inflammation and injury, atherosclerotic change, systemic and myocardial ischemia, and cardiac dysfunction, plasma AM might become a sensitive marker of future CV disease.

There were some limitations in the present study. The sample size of our subjects was small to evaluate the predictive power of AM discretely for cerebrovascular, coronary, and heart failure events. In addition, the prognostic value of AM for all-cause and CV death could not be investigated. As another limitation of this study, we did not consider the influence of medication during follow-up on the occurrence of CV disease. Therefore, the use of statin, aspirin, renin angiotensin system inhibitors, and β-blockers and the alteration of dosage of these drugs after the initial assessment might bias the outcome of the present study. Furthermore, we did not examine the change of plasma AM levels during follow-up periods. It is possible that the prognostic potential of AM may be raised by serially evaluating its plasma level in high-risk patients.

In conclusion, the present findings indicate that plasma AM is a powerful independent predictor of future CV events in patients with multiple CV risk factors, and suggest that its prognostic value is superior to that of hs-CRP or adiponectin. However, further investigations using larger population of high-risk patients will be required to establish the usefulness of AM as a novel predictive marker for CV diseases.

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Fasting Plasma Glucose Cutoff for Diagnosis of Diabetes in a Japanese Population

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Objective: We examined the relationship between fasting plasma glucose (FPG) and 2-h post-load glucose (PG) levels, and the optimal FPG cutoff level to correspond to a 2-h PG of 11.1 mmol/liter, the gold standard diagnostic criterion, in a general Japanese population.

Design: Cross-sectional study populations of 2421 subjects in 1988 and 2698 subjects in 2002, aged 40–79 yr and without antidiabetic medication, were tested with an oral glucose tolerance test. The relationship between FPG and 2-h PG was investigated by various regression models and a receiver operating characteristic curve.

Results: The best-fit model for the relationship between FPG and 2-h PG was a quadratic regression model. The FPG cutoff levels corresponding to the 2-h PG of 11.1 mmol/liter by this model were 6.2 mmol/liter in 1988 and 6.3 mmol/liter in 2002. In the combined populations, the FPG cutoff point was 6.3 mmol/liter; the sensitivity and specificity of this cutoff point for detecting a 2-h PG of 11.1 mmol/liter were 75.2 and 88.6%, respectively. The receiver operating characteristic curve analysis confirmed that the corresponding FPG point was 6.2 mmol/liter in both the 1988 and 2002 populations. In a stratified analysis, the FPG cutoff level increased with increasing body mass index levels; however, even in subjects with body mass index more than or equal to 30 kg/m^2, the FPG cutoff level was lower than 7.0 mmol/liter.

Conclusions: Our findings suggest that the FPG cutoff level corresponding to the 2-h PG of 11.1 mmol/liter in the general Japanese population is lower than the current diagnostic criterion. ***(J Clin Endocrinol Metab* 93: 3425–3429, 2008)**

A 2-h post-load glucose (PG) cutoff level of 11.1 mmol/liter is considered to be the gold standard diagnostic criterion for diabetes mellitus. This cutoff point was originally adopted for several reasons (1). First, 11.1 mmol/liter has been found to approximate the cutoff point separating the two components of the bimodal distribution of 2-h PG levels. Second, according to several epidemiological studies, including our own, the prevalence of microvascular disease sharply increases in patients having a 2-h PG above 11.1 mmol/liter (1–4). Third, a great number of clinical and epidemiological studies have used this criterion. By contrast, fasting plasma glucose (FPG) has not been adequately justified as a diagnostic criterion. The FPG cutoff point for diagnosing diabetes was revised by the Expert Committee of the American Diabetes Association (ADA) (1) in 1997; namely, the cutoff point defining diabetes was reduced from more than or equal to 7.8 mmol/liter to more than or equal to 7.0 mmol/liter, though the ADA itself has recognized that this new cutoff point is not the best equivalent of the 2-h value of 11.1 mmol/liter (1, 5). The World Health Organization adopted an FPG of 7.0 mmol/liter as a diagnostic criterion of diabetes in 1998 (6). This lowering was based on the following findings from several studies, primarily with cohorts of high body mass index (BMI) subjects: 1) the prevalence and incidence of diabetic retinopathy increased at an FPG of approximately 7.0 mmol/liter (1, 3, 4); 2) the discrepancy in the detection rate of diabetes between FPG and 2-h PG values was reduced when an FPG of 7.0 mmol/liter was

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Abbreviations: ADA, American Diabetes Association; BMI, body mass index; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; PG, post-load glucose; ROC, receiver operating characteristic.

used; and 3) the prevalence of diabetes by a 2-h PG cutoff point of 11.1 mmol/liter was identical to that of an FPG of approximately 7.0 mmol/liter in several populations. However, the Diabetes Prevention Program Research Group has recently shown that the retinopathy characteristic of diabetes was present in persons whose FPG was below the diabetic range and who had no known history of diabetes (7). Furthermore, an integrated study of three general populations suggested that although the prevalence of retinopathy increased with FPG concentration, there was no clear diagnostic cutoff (8). These findings imply that data of diabetic retinopathy alone are not adequate to determine an FPG cutoff point. Thus, another approach, such as a regression analysis, is needed to validate the FPG cutoff point.

On the other hand, it remains controversial whether the FPG of 7.0 mmol/liter is adequately diagnostic for diabetes in Asian populations, which tend to be leaner than Western populations. For instance, FPG cutoff levels corresponding to a 2-h PG of 11.1 mmol/liter were also lower than 7.0 mmol/liter in other Asian populations (9–11). There have been very few reports on this issue in the Japanese population, in which the prevalence of diabetes has been increasing rapidly in recent years. The purposes of this study were to determine the FPG cutoff value corresponding to a 2-h PG of 11.1 mmol/liter, and to check whether this cutoff value varied according to changes in the society over time by examining the relationship between FPG and 2-h PG values in a general Japanese population at two different time points separated by an interval of 14 yr.

Subjects and Methods

A population-based prospective study of cardiovascular disease has been underway since 1961 in the Town of Hisayama, a suburb in the Fukuoka metropolitan area on Kyushu Island, in Japan. Based on data from the national census, the age and occupational distributions for Hisayama have been almost identical to those of Japan as a whole from 1961 to the present. As a part of the study, two cross-sectional diabetes surveys of Hisayama residents were conducted in similar fashion in 1988 and 2002. A detailed description of the surveys has been published previously (12, 13); briefly, of the total of 3227 residents in 1988 aged 40–79 yr in the town registry, 2587 (participation rate, 80.2%) consented to take part in a comprehensive assessment, including a 75-g oral glucose tolerance test (OGTT) and an interview covering both medical histories (including items on diabetes, hypertension, and other chronic diseases) and current medical treatments with insulin and oral hypoglycemic agents. After excluding participants who had already had breakfast, those who were receiving insulin therapy for diabetes, and those who refused the OGTT due to complaints of nausea or general fatigue during the ingestion of glucose, we successfully completed the OGTT on 2480 subjects. An additional 59 subjects were excluded because they were taking oral hypoglycemic agents; thus, the final 1988 study group comprised 2421 subjects (1045 men and 1376 women) (Fig. 1). In 2002, we established another study population of 2698 (1162 men and 1536 women) using the same methods and criteria.

In both the 1988 and 2002 surveys, clinical evaluation and laboratory measurements were performed in a similar manner. The study subjects underwent the OGTT between 0800 and 1030 h after an overnight fast of at least 12 h. Blood for the glucose assay was obtained by venipuncture into tubes containing sodium fluoride at fasting and at 2-h post-load, and was separated into plasma and blood cells within 20 min. Plasma glucose levels were determined by the glucose-oxidase method. The between-assay and within-assay coefficients of variance of glucose measurement in our laboratory were 0.96 and 0.81% at 5.6 mmol/liter, and 0.81 and 0.56% at 16.7 mmol/liter, respectively. Total cholesterol and triglycerides were determined enzymatically. Blood pressure was obtained three times using a mercury sphygmomanometer with the subject in a sitting position; the average values were used in the analyses. Hypertension was defined as systolic blood pressure more than or equal to 140 mm Hg and/or diastolic blood pressure more than or equal to 90 mm Hg and/or current treatment with antihypertensive agents. The height and weight of each subject, wearing light clothes without shoes, were recorded, and the BMI (kg/m^2) was calculated. The interview investigated smoking habits and alcohol intake. Both were classified as either currently habitual or not. Subjects engaging in sports at least three times per week during their leisure time were classified into a regular exercise group.

SAS (SAS Institute Inc., Cary, NC) was used to perform all statistical analyses. Various regression models, including linear, quadratic, logarithmic, inverse, power, and exponential models, without covariates were examined to determine which best fit the relationship between FPG and 2-h PG levels. Furthermore, an FPG cutoff point corresponding to the 2-h PG of 11.1 mmol/liter was calculated from each regression equation. The sensitivity of the FPG cutoff point was defined as its ability to identify correctly individuals who had a 2-h PG of 11.1 mmol/liter or higher, and the specificity was its ability to identify correctly individuals who did not have a 2-h PG of 11.1 mmol/liter or higher. To compare the ability of FPG measurements to detect the presence or absence of a 2-h PG of 11.1 mmol/liter or higher across a range of values, we plotted receiver operating characteristic (ROC) curves. The diagnostic properties of specific cutoff levels of FPG were defined by maximizing the sensitivity and specificity to identify a 2-h PG of 11.1 mmol/liter or higher.

This study was conducted with the approval of the Ethics Committee of the Faculty of Medicine, Kyushu University, and written informed consent was obtained from the participants.

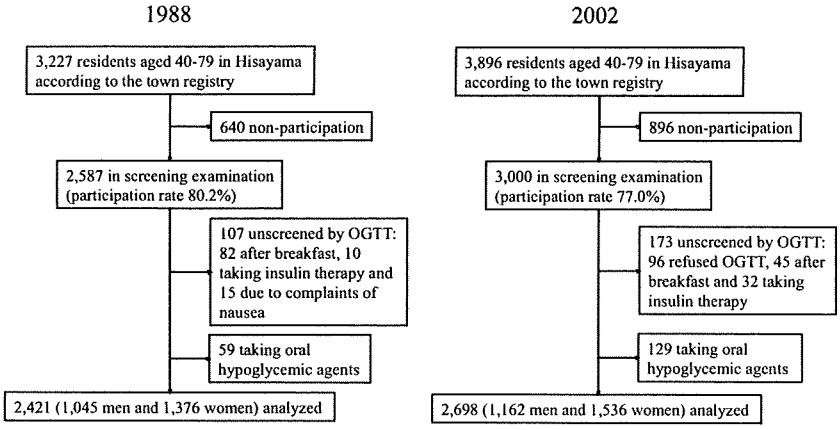

FIG. 1. Flow diagram of the study.

Results

The clinical characteristics of the subjects in 1988 and 2002 are summarized in Table 1. Mean values of age, FPG, 2-h PG, and BMI were higher in 2002 than 1988, whereas the frequency of men was not different between the populations.

To elucidate the relationship between FPG and 2-h PG, we analyzed their interrelationships using the various regression models listed in Table 2. FPG values corresponding to a 2-h PG of 11.1 mmol/liter and R^2 values were calculated for the combined populations

TABLE 1. Clinical characteristics of subjects: the Hisayama study in 1988 and 2002

	1988 (n = 2421)	2002 (n = 2698)	*P* value
Age (yr)	57 (10)	59 (11)	<0.001
Men (%)	43.2	43.1	0.94
FPG (mmol/liter)	5.7 (1.1)	6.0 (1.0)	<0.001
2-h PG (mmol/liter)	7.3 (3.2)	7.7 (3.1)	<0.001
BMI (kg/m^2)	23.0 (3.1)	23.3 (3.3)	<0.001

Values are means (SD).

of 1988 and 2002. The R^2 value was larger for the quadratic regression model, indicating that it is a better fit than the other models; the relevant FPG point in this model was 6.3 mmol/liter.

Figure 2 depicts the relationship between the FPG and 2-h PG in 1988 and 2002 considered separately. The quadratic model analyses were still the best fit among the various models for both the 1988 and 2002 populations (data not shown), with R^2 values of 64.0 in 1988 and 61.3 in 2002. The FPG point corresponding to a 2-h PG of 11.1 mmol/liter was 6.2 mmol/liter in 1988 and 6.3 mmol/liter in 2002.

To confirm the cutoff point of FPG corresponding to the 2-h PG of 11.1 mmol/liter, we plotted ROC curves and calculated the optimal cutoff points defined as the maximum combination of sensitivity and specificity, and their area under the ROC curves (Fig. 3). In the 1988 subjects, the corresponding FPG point was 6.2 mmol/liter. The sensitivity and specificity of this cutoff point were 81.2 and 88.7%, respectively; and the area under the curve was 91.0%. In the 2002 subjects, the cutoff point was 6.2 mmol/liter; the sensitivity, specificity, and area under the curve were 77.9, 81.3, and 86.7%, respectively.

Finally, we performed a stratified analysis by sex, age, and BMI levels in the combined population using both the quadratic regression model and ROC analysis (Table 3). The FPG level corresponding to the 2-h PG of 11.1 mmol/liter was slightly higher in men than women by both the quadratic regression model and ROC analysis. Higher FPG levels corresponding to a 2-h PG of 11.1 mmol/liter were observed in the younger age groups in the quadratic regression model analysis. However, in ROC analysis there was no association between age and FPG level. The FPG level corresponding to a 2-h PG of 11.1 mmol/liter increased with increasing BMI levels in both the quadratic regression model and ROC analysis. However, even in subjects with a BMI more than or equal to 30 kg/m^2, the FPG cutoff level was still lower than the diagnostic criterion of 7.0 mmol/liter.

Discussion

We examined the association between FPG and 2-h PG levels in a general Japanese population at two time points separated by a 14-yr interval, and using the quadratic model, which proved to be the best fit for the data, demonstrated that the FPG level corresponding to a 2-h PG of 11.1 mmol/liter, the gold standard for diagnosis of diabetes, was 6.2 mmol/liter for the 1988 data and 6.3 mmol/liter for the 2002 data. The FPG points derived from the ROC analyses corroborated these findings. It has been reported that the corresponding FPG cutoff level by the quadratic model was 5.7 mmol/liter in Chinese (9) and 6.3 mmol/liter in Taiwanese (10). Together with the findings of these other studies, our results suggest that, in relatively lean Asian populations, including the Japanese, the FPG cutoff level is clearly lower than the FPG value of 7.0 mmol/liter, which is currently used in various diagnostic criteria for diabetes (1, 6), and that this situation did not change over the course of 14 yr in the Japanese population.

Although a method using FPG values corresponding to the gold standard of 2-h PG levels for diagnosis of diabetes has not yet been established, regression analysis appears to be a useful method for detecting the FPG cutoff value. Two previous epidemiological studies determined FPG cutoff points by analyzing the relationship between FPG and 2-h PG using linear or exponential models (14, 15). However, in our study the quadratic model showed the highest positive correlation between FPG and 2-h PG, and, thus, was the best-fitted model. This is consistent with the findings of studies in Taiwanese (9) and Chinese (10) populations.

The ADA recommends the use of the FPG instead of 2-h PG for diagnosing diabetes because it is difficult to perform an OGTT in routine clinical practice (1). Thus, it is very important to determine the appropriate FPG cutoff value for the diagnosis of diabetes in different populations. The FPG of 7.0 mmol/liter for diagnosing diabetes is based on several population studies examining the relationship between the glycemic threshold and diabetic retinopathy (1, 3, 4); however, optimal cutoff levels of plasma glucose for defining diabetes depend on ethnicity. In a Pima Indian study, the ROC curve analysis in a diabetic retinopathy study identified the optimal FPG cutoff level as 6.8 mmol/liter (3). The National Health and Nutrition Examination Survey III study of the U.S. population also reported that the prevalence of retinopathy increased dramatically at FPG levels of 6.7 mmol/liter (1). These findings were apparently confirmed by a similar study in Egypt (4), in which the optimal FPG cutoff level

TABLE 2. Relationship between FPG (Y) and 2-h PG (X) in various regression models for the combined population of 1988 and 2002

Model	Equation	FPG corresponding to 2-h PG of 11.1 mmol/liter (mmol/liter)	R^2 (%)
Quadratic	$Y = 0.0149X^2 - 0.102X + 5.621$	6.3	62.3
Linear	$Y = 0.243X + 4.024$	6.7	51.8
Exponential	$Y = 2.718^{(0.0323X + 1.511)}$	6.5	48.6
Power	$Y = 3.512X^{0.255}$	6.5	36.6
Logarithmic	$Y = 1.831 \log X + 2.277$	6.7	35.6
	$\text{Log}(Y) = 0.255 \log X + 1.256$	6.5	36.6
	$\text{Log}(Y) = 0.243 (\log X)^2 - 0.748 \log X + 2.260$	6.5	50.2
Inverse	$Y = 7.265 - 9.416/X$	6.4	20.1

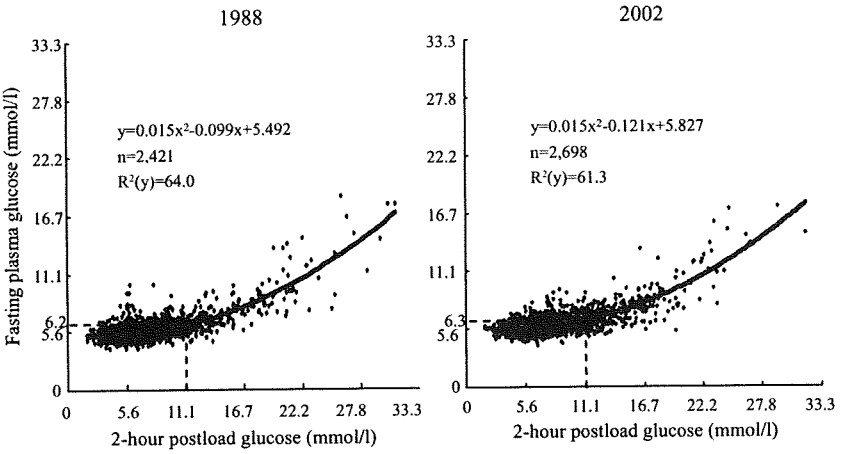

FIG. 2. The relationship between FPG and 2-h PG by a 75-g OGTT in Hisayama residents aged 40–79 yr in 1988 (*left panel*) and 2002 (*right panel*). *Solid line* represents the regression line by the quadratic regression model.

for detecting diabetic retinopathy was 6.9–7.2 mmol/liter. However, these three populations have higher BMI levels compared with Asian populations. We previously reported that although the glycemic threshold of 2-h PG for retinopathy in Japanese was 11.1 mmol/liter, that of FPG was only 6.4 mmol/liter (2). Other Asian population studies have reported optimal FPG cutoff levels for retinopathy ranging between 5.6 and 6.0 mmol/liter (16, 17). These findings suggest that FPG cutoff levels are lower in Asian populations than in other populations.

In our subjects the FPG cutoff levels corresponding to a 2-h PG of 11.1 mmol/liter increased with increasing BMI levels. However, even in subjects with a BMI more than or equal to 30 kg/m^2, the FPG cutoff level using the quadratic model was 6.4 mmol/liter, much lower than the diagnostic criterion of 7.0 mmol/liter. It is not clearly understood why FPG cutoff levels differ among ethnic groups. One possible explanation is that the capacity for acute insulin response to glucose load may influence the FPG cutoff level. The acute insulin response is known to be lower in Asian populations than other populations (18). In some clinical studies, the loss of acute insulin response by somatostatin was associated with a marked impairment in the initial suppression of hepatic glucose production, which led to higher 2-h PG concentrations (19, 20). Thus, impairment of acute insulin response may lead to a wide gap between FPG and 2-h PG; in other words, much lower FPG cutoff levels correspond to the 2-h PG diagnostic standard level. These findings might explain why the FPG cutoff level for the diagnosis of diabetes is lower in Asian populations, including ours, even in those with high BMI.

In the present study, the R^2 value in the quadratic model and the sensitivity, specificity, and area under the curve in the ROC analysis were all lower in 2002 than 1988. Although this phenomenon was not clearly understood, one possible reason may be that individuals in 2002 had more diverse lifestyles compared with those in 1988. Nevertheless, it is noteworthy that the FPG cutoff value corresponding to a 2-h PG of 11.1 mmol/liter was similar in the two populations.

Two limitations of our study should be discussed. First, in our study we determined the FPG cutoff level that corresponded to a 2-h PG of 11.1 mmol/liter, the gold standard for the diagnosis of diabetes, rather than that corresponding directly to diabetic complications. However, our previous study showed that the glycemic threshold of FPG for retinopathy is 6.4 mmol/liter (2), a result very similar to that of the present study. These findings suggest that the quadratic model precisely predicts the relationship between FPG and 2-h PG levels, making the FPG cutoff level nearly as accurate as the 2-h PG level, as well as more useful in clinical settings. Second, it is known that 2-h PG values in a 75-g OGTT have lower reproducibility than FPG (21, 22). It might be reasonable to propose FPG as the "gold standard." However, in the National Health and Nutrition Examination Survey III, 2-h PG was more specific for diabetic retinopathy than FPG (1). In several epidemiological studies, 2-h PG was also a stronger predictor of cardiovascular disease and total death compared with FPG (23–27). In addition, a 2-h PG of 11.1 mmol/liter was established in some revised processes for the diagnosis of diabetes. Based on these studies, then, a 2-h PG of 11.1 mmol/liter remains the "gold standard." Nevertheless, the present study found that two cross-sectional populations in 1988 and 2002 had nearly the same cutoff FPG values. This suggests that the high variability in 2-h PG values did not invalidate the present findings.

In conclusion, we have shown that the quadratic regression model is best fitted for the relationship between FPG and 2-h PG in a general Japanese population. The FPG cutoff level corresponding to a 2-h PG of 11.1 mmol/liter was 6.3 mmol/liter, and this result did not change over the course of 14 yr. Furthermore, the FPG cutoff levels were higher in subjects with higher BMI levels. The findings of the present study together with those of previous studies examining diabetic retinopathy sug-

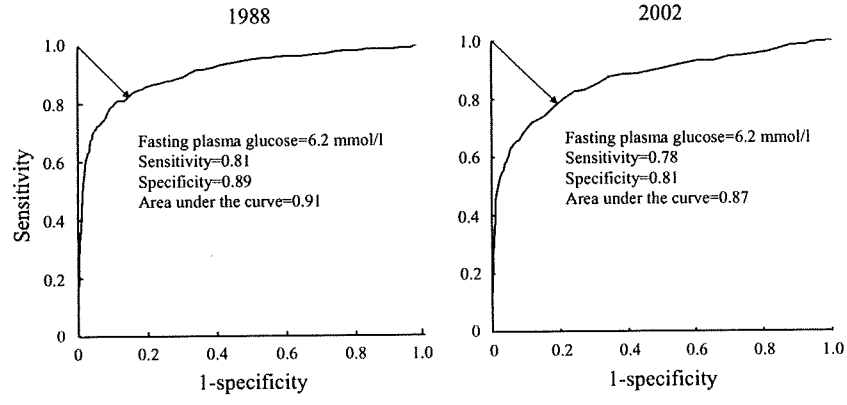

FIG. 3. ROC curves for FPG for predicting the 2-h PG of 11.1 mmol/liter using 1988 (*left*) and 2002 (*right*) data sets. The *arrow* shows the optimal cutoff point for detecting the 2-h PG of 11.1 mmol/liter defined as the maximum combination of sensitivity and specificity.

TABLE 3. FPG cutoff points corresponding to the 2-h PG of 11.1 mmol/liter by quadratic regression model and receiver operating curve analysis in the combined population of 1988 and 2002

Factors	No.	Cutoff point defined by quadratic regression analysis (mmol/liter)	Cutoff point defined by ROC analysis (mmol/liter)
Sex			
Men	2207	6.4	6.3
Women	2912	6.3	6.1
Age (yr)			
40–49	1341	6.4	6.0
50–59	1569	6.4	6.2
60–69	1363	6.3	6.2
70–79	846	6.2	6.1
BMI (kg/m^2)			
<20	818	6.1	5.9
20–24.9	2978	6.3	6.1
25–29.9	1192	6.3	6.2
≥30	131	6.4	6.7

gest that in Asian populations, the FPG cutoff level corresponding to a 2-h PG of 11.1 mmol/liter is lower than 7.0 mmol/liter, the current diagnostic criterion for diabetes. Considering the growing importance of the FPG test in screening for diabetes, further investigations are required to clarify the optimal FPG cutoff level in Asian and other ethnic populations.

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High-Sensitivity C-Reactive Protein and Coronary Heart Disease in a General Population of Japanese

The Hisayama Study

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Objective—The purpose of this study was to investigate the effects of high-sensitivity C-reactive protein (hs-CRP) on the risks of coronary heart disease (CHD) in a general population of Japanese.

Methods and Results—The Hisayama study is a population-based prospective cohort study. A total of 2589 participants aged 40 years or older were followed up for 14 years. Outcomes are incident CHD (myocardial infarction, coronary revascularization, and sudden cardiac death). The median hs-CRP level was 0.43 mg/L at baseline. During the follow-up period, 129 coronary events were observed. Age- and sex-adjusted annual incidence rates of CHD rose progressively with higher hs-CRP levels: 1.6, 3.3, 4.5, and 7.4 per 1000 person-years for quartile groups defined by hs-CRP levels of <0.21, 0.21 to 0.43, 0.44 to 1.02, and >1.02 mg/L, respectively ($P<0.0001$ for trend). The risk of CHD in the highest quartile group was 2.98-fold (95% CI, 1.53 to 5.82) higher than that in the lowest group even after controlling for other cardiovascular risk factors.

Conclusions—hs-CRP levels were clearly associated with future CHD events in a general population of Japanese. In Japanese populations, the hs-CRP cut-off point for high-risk of future development of CHD is likely to be >1.0 mg/L, which is much lower than that for Western populations. (*Arterioscler Thromb Vasc Biol.* **2008;28:1385-1391**)

Key Words: inflammation ■ C-reactive protein ■ coronary heart disease ■ prospective cohort study ■ general population

Coronary heart disease (CHD) is estimated to be one of the leading causes of death in Japan as well as other countries around the world, placing a burden on the community.[1] Although the burden of CHD has been reduced in several developed countries in the past few decades,[2] its incidence rates have not declined in Japan.[3] Effective prevention will require a strategy based on knowledge of the importance of novel and traditional risk factors for CHD in Japan.

See accompanying article on page 1222

Recently, inflammation has emerged as an important factor in atherosclerosis,[4] and high-sensitivity C-reactive protein (hs-CRP) has attracted clinical attention as a novel risk factor for CHD. However, current knowledge of the importance of hs-CRP as a risk factor for CHD is derived mainly from studies done in Western populations,[5–12] and it is unclear to what extent these findings apply to Japanese populations. The Hisayama Study is a prospective cohort study of a general Japanese population. A previous report from the Hisayama Study showed a positive association between hs-CRP levels and the risks of ischemic stroke among Japanese men.[13] The objective of the present analysis is to examine the relationship between serum hs-CRP levels and future development of coronary heat disease in a general population of Japanese.

Methods

Study Design and Participants

Since 1961, we have been conducting a long-term prospective cohort study of cardiovascular disease in the town of Hisayama, a suburb of Fukuoka City in Southern Japan.[3,14] In 1988, a screening survey for the present study was performed in the town. A total of 2736 residents aged 40 years or older (80.9% of the total population of this age group) consented to participate in the examination.[13,15] After the exclusion of 102 subjects with a history of stroke or CHD and 45 subjects whose frozen blood samples were of insufficient quantity for the measurement of serum hs-CRP, the remaining 2589 individuals were enrolled in this study.

The ethics committee of Kyushu University approved this study, participants provided written informed consent, and the procedures followed were in accordance with national guidelines.

Follow-Up Survey

The subjects were followed up prospectively from December 1988 to November 2002 by repeated health examinations. A detailed description of the study methods has been published previously.[3,13,15] In

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brief, the health status of any subject who had not undergone a regular examination or who had moved out of town was checked yearly by mail or telephone. We also established a daily monitoring system among the study team and local physicians or members of the town's Health and Welfare Office. When a subject died, an autopsy was performed at the Departments of Pathology of Kyushu University. During the follow-up period, 545 subjects died, of whom 412 (75.6%) underwent autopsy. Only one participant was lost to follow-up.

Outcomes

The primary outcome of the present analysis was CHD. The criteria for a diagnosis of CHD included first-ever acute myocardial infarction (MI), silent MI, sudden cardiac death within 1 hour after the onset of acute illness, or coronary artery disease followed by coronary artery bypass surgery or angioplasty.[3,14] Acute MI was diagnosed when a subject met at least 2 of the following criteria: (1) typical symptoms, including prolonged severe anterior chest pain; (2) abnormal cardiac enzymes more than twice the upper limit of the normal range; (3) evolving diagnostic electrocardiographic (ECG) changes; (4) morphological changes including local asynergy of cardiac wall motion on echocardiography, a persistent perfusion defect on cardiac scintigraphy, or myocardial necrosis or scars >1 cm long accompanied by coronary atherosclerosis at autopsy. Silent MI was defined as myocardial scarring without any historical indication of clinical symptoms or abnormal cardiac enzyme changes. The secondary outcomes of the present investigation were deaths attributable to any cardiovascular disease (ICD-10[16] codes I00-I99), deaths attributable to noncardiovascular disease, and total deaths.

Risk Factors

Plasma glucose levels were determined by the glucose-oxidase method, and diabetes was defined by a 75-g oral glucose tolerance test and by fasting (≥7.0 mmol/L) or postprandial (≥11.1 mmol/L) blood glucose levels or by the use of hypoglycemic agents. Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride levels were determined enzymatically. Low-density lipoprotein (LDL) cholesterol level was estimated using the Friedewald formula.[17] Hypercholesterolemia was defined as a serum cholesterol level of 5.69 mmol/L or higher. Serum specimens collected at the time of CRP measurement were stored at −20°C until they were used in 2002. Serum hs-CRP levels were analyzed using a modification of the Behring latex-enhanced CRP assay on a BN-100 nephelometer (Dade Behring) with a 2% interassay coefficient of variation. Sitting blood pressure (BP) was measured 3 times at the right upper arm using a sphygmomanometer after 5 minutes of rest; an average of 3 measurements was used for the analysis. Hypertension was defined as BP levels of ≥140/90 mm Hg or current treatment with antihypertensive agents. The waist circumference was measured at the umbilical level in a standing position. Height and weight were measured in light clothes without shoes, and body mass index (BMI, kg/m^2) was calculated. Obesity was defined as a BMI of ≥25kg/m^2. ECG abnormalities were defined as Minnesota code 3-1 or 4-1,2,3. Information on smoking habits, alcohol intake, and physical activity during leisure time was obtained using a standard questionnaire. Smoking habits and alcohol intake were classified as either current or not. Subjects engaging in sports or other forms of exertion ≥3 times a week during their leisure time made up a regular exercise group. Metabolic syndrome was defined using criteria recommended in the National Cholesterol Education Program Adult Treatment Panel III guideline[18] with a modification of abdominal obesity, which was defined as a waist circumference ≥90 cm in men and ≥80 cm in women according to the International Obesity Task Force central obesity criteria for Asia.[19]

Statistical Analysis

We used quartiles of hs-CRP levels for the analysis of the effects of hs-CRP on the risks of CHD. The contributions of relevant factors to an elevated hs-CRP level, which was defined as the highest quartile, were examined using a logistic regression model, with an estimated odds ratio (OR) and 95% confidence interval (95% CI). The cumulative incidence of CHD was estimated using Cox's proportional hazards model. The incidence rates were calculated by the person-year method and standardized for age and sex distribution of the world standard population by the direct method using 10-year age groupings. The age- and sex-adjusted or multivariate-adjusted hazard ratio (HR) and 95% CI were estimated using Cox's proportional hazard model. Comparison of the effects hs-CRP between participants with and without other cardiovascular risk factors was done, and the probability value for homogeneity was estimated by adding an interaction term to the statistical model. All analyses were performed using the SAS software package (SAS Institute).

Results

Among the 2589 participants, the median hs-CRP level was 0.43 mg/L. The baseline characteristics of the subjects by hs-CRP quartile groups are shown in Table 1. Subjects with higher hs-CRP levels were older and less frequently women. The age- and sex-adjusted logistic regression analysis revealed that hypertension (OR, 1.40; 95% CI, 1.16 to 1.69), diabetes (OR, 1.67; 95% CI, 1.29 to 2.16), obesity (OR, 1.80; 95% CI, 1.47 to 2.22), hypercholesterolemia (OR, 1.32; 95% CI, 1.09 to 1.60), metabolic syndrome (OR, 2.04; 95% CI, 1.67 to 2.50), and smoking habits (OR, 1.96; 95% CI 1.56 to 2.47) were significantly associated with elevated hs-CRP levels, which were defined as the highest quartile (>1.02 mg/L).

During the 14 years of follow up, 129 coronary events were observed. The Figure shows the age- and sex-adjusted cumulative incidence of CHD according to hs-CRP quartiles. The cumulative incidence of CHD clearly increased with rising hs-CRP levels. The age- and sex-adjusted incidence rates of CHD according to hs-CRP quartiles are shown in Table 2. The incidence rates rose progressively with higher hs-CRP levels: 1.6, 3.3, 4.5, and 7.4 per 1000 person-years from the first to the fourth quartile groups, respectively ($P<0.0001$ for trend). Table 2 also shows age- and sex-adjusted and multivariate-adjusted HRs and 95% CIs for the development of CHD according to the hs-CRP quartiles. The risks of CHD significantly increased with rising hs-CRP levels even after controlling for age, sex, systolic BP, ECG abnormalities, diabetes, BMI, total and HDL cholesterol, smoking habits, alcohol intake, and regular exercise ($P=0.0002$ for trend). The risk of CHD in the highest quartile group was significantly higher than that in the lowest group (multivariate-adjusted HR, 2.98; 95% CI, 1.53 to 5.82).

During the follow-up period, 545 participants died (158 died of cardiovascular disease and 387 died of noncardiovascular disease). The age- and sex-adjusted total and cause-specific mortality rates are shown in Table 3. The age-and sex-adjusted all-cause mortality rates rose progressively with higher hs-CRP levels ($P<0.0001$ for trend). The age- and sex-adjusted and multivariate-adjusted HRs also increased with rising hs-CRP levels even after controlling for other risk factors (Table 3; $P<0.0001$ for trend). When causes of death were divided into cardiovascular and noncardiovascular diseases, the relationship of hs-CRP to cardiovascular deaths was stronger than that to noncardiovascular deaths.

Age- and sex-adjusted hazard ratios of hs-CRP (highest versus lowest quartiles) for the development of CHD among