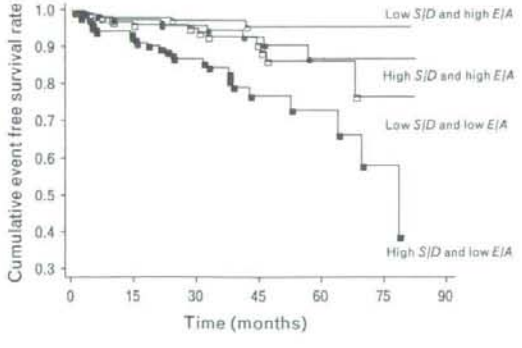
Table 2 Baseline clinical characteristics of study subjects

Variables	*E/A* ratio ≥ median Male ≥ 0.84 Female ≥ 0.82		*E/A* ratio < median Male < 0.84 Female < 0.82	
	Low *S/D* Male < 1.31 Female < 1.51	High *S/D* Male ≥ 1.31 Female ≥ 1.51	Low *S/D* Male < 1.77 Female < 1.81	High *S/D* Male ≥ 1.77 Female ≥ 1.81
n	177	178	175	175
Male (%)	50.9	50.6	50.3	50.3
Age (years)	52.7 ± 14.2§	60.5 ± 9.4†,§	65.7 ± 9.0†	67.6 ± 8.3†
Body mass index (kg/m^2)	24.1 ± 3.7	24.3 ± 3.3	24.4 ± 3.3	24.2 ± 3.0
Duration of hypertension (years)	11.9 ± 10.2§	12.9 ± 9.9§	16.3 ± 10.2†	17.9 ± 10.4†
Smoking status (%)				
Never/past/current	54.0/27.8/18.2	55.4/23.2/21.5	54.3/30.9/14.9	50.3/37.7/12.0
Pulse pressure (mmHg)	60.3 ± 15.1‡	61.0 ± 11.2	64.0 ± 13.7*	65.4 ± 15.0†
Heart rate (bpm)	65.9 ± 8.9	65.9 ± 9.4	67.3 ± 8.5	68.1 ± 7.5
Diabetes (%)	18.6	17.4‡	28.0	29.9*
Total cholesterol (mmol/l)	5.28 ± 0.88	5.25 ± 0.77	5.13 ± 0.79	5.23 ± 0.84
Triglycerides (mmol/l)	1.59 ± 1.53	1.45 ± 0.89	1.49 ± 0.85	1.50 ± 0.76
High-density lipoprotein cholesterol (mmol/l)	1.40 ± 0.44§	1.34 ± 0.40	1.27 ± 0.34†	1.24 ± 0.39†
LAD (cm)	3.54 ± 0.44‡	3.65 ± 0.49	3.67 ± 0.48*	3.66 ± 0.45
LVMI (g/m^2)	123.34 ± 36.28	124.58 ± 31.12	127.35 ± 35.23	132.19 ± 32.58*
Ejection fraction (%)	70.54 ± 8.64	71.76 ± 7.10	71.88 ± 7.74	72.59 ± 7.91*
E/A ratio	1.15 ± 0.28§	1.02 ± 0.17†,§	0.71 ± 0.09†	0.65 ± 0.11†,‡
DcT (ms)	206.2 ± 36.5§	216.7 ± 38.0§	243.0 ± 43.1†	263.7 ± 52.0†,§
S/D ratio	1.19 ± 0.20§	1.66 ± 0.24†,§	1.54 ± 0.19†	2.14 ± 0.33†,§
Peak PV_a-velocity (m/s)	0.26 ± 0.06§	0.28 ± 0.05	0.30 ± 0.08†	0.31 ± 0.10†
ARdur − A_d (ms)	−28.9 ± 30.8	−29.6 ± 28.3	−31.4 ± 28.1	−28.9 ± 26.6
Diastolic filling patterns (%)				
Normal filling	53.7§	44.9§	0†	0†
Impaired relaxation	39.6§	55.1†§	100.0†	100.0†
Pseudonormal filling	5.6§	0†	0†	0†
Restrictive filling	1.1	0	0	0
Antihypertensive medication (%)	67.2§	79.9†	85.9†	85.9†
Calcium channel blocker	52.9§	62.8§	78.7†	71.8†
Beta-blocker	29.9	31.5	22.3	23.4
ACEI or ARB	32.4	32.0	34.9	38.3
Diuretic	13.5	11.1	18.8	25.6†
Number of CVD events	5	10	13	28†,§

ACEI, angiotensin-converting enzyme inhibitor; A_d, the duration of atrial filling wave; ARB, angiotensin II receptor blocker; *ARdur*, the duration of flow at atrial contraction; A-velocity, the peak of atrial diastolic phase filling; CVD, cardiovascular disease; *DcT*, the deceleration time of early diastolic LV filling; *E/A*, the ratio of peak early to late diastolic filling velocity; E-velocity, the peak of early diastolic phase filling; LAD, left atrial dimension; LVMI, left ventricular mass index; PV_a, pulmonary vein atrial reversal; *S/D*, the ratio of the pulmonary venous systolic velocity to diastolic velocity. Data are mean ± SD or percentage. *$P < 0.05$ and †$P < 0.01$ versus low *S/D* ratio and high *E/A*. ‡$P < 0.05$ and §$P < 0.01$ versus low *S/D* ratio and low *E/A*.

Fig. 2



Cardiovascular event-free survival in four groups stratified by both baseline peak velocity ratio of the pulmonary venous systolic to diastolic wave (*S/D*) and peak transmitral velocity ratio of early diastolic to atrial filling (*E/A*) (log-rank $\chi^2 = 28.064$, $P < 0.01$).

Discussion

The present study demonstrated that the relationship between a high *S/D* ratio and CVD risk is significant, and persisted after multivariate Cox regression analysis including traditional risk factors. The combination of high *S/D* and low *E/A* was a powerful independent predictor of CVD events. Moreover, even in the subgroup with low *E/A*, high *S/D* was a significant predictor of CVD events.

Our results were partially in accordance with a previous report [13] that more than 95% of our study subjects had 'normal diastolic function' or 'mild diastolic dysfunction'. In addition, only 0.3 and 1.4% of the subjects were identified as having a 'restrictive pattern' and 'moderate diastolic dysfunction (pseudonormal pattern)', respectively. Therefore, pseudonormal or restrictive physiology is unlikely to affect the results observed in the present study to a significant degree.

Our results showed that a high *S/D* ratio is independently associated with CVD risk, and suggest that the assessments of PVF by transthoracic echocardiography, simple

Table 3 Combined effects of the peak velocity ratio of the pulmonary venous systolic to diastolic wave (*S/D*) and peak transmitral velocity ratio of early diastolic to atrial filling (*E/A*) ratios as predictors of cardiovascular disease events

	Crude		Risk factor-adjusted model*	
Variables	HR (95% CI)	*P*	HR (95% CI)	*P*
S/D and *E/A*				
Low *S/D* and high *E/A*	1 (reference)		1 (reference)	
High *S/D* and high *E/A*	1.480 (0.88–2.65)	0.140	1.402 (0.83–2.53)	0.211
Low *S/D* and low *E/A*	1.720 (1.05–3.04)	0.029	1.390 (0.84–2.48)	0.206
High *S/D* and low *E/A*	2.662 (1.72–4.57)	<0.001	2.158 (1.40–3.70)	0.001
Age, 1 year	1.059 (1.03–1.09)	<0.001	1.032 (1.00–1.06)	0.040
Diabetes, yes	1.449 (1.10–1.89)	0.010	1.301 (0.98–1.70)	0.067
Pulse pressure, 1 mmHg	1.031 (1.02–1.05)	<0.001	1.015 (1.00–1.03)	0.116
LAD, 0.1 cm	1.010 (1.00–1.01)	<0.001	1.002 (1.00–1.01)	0.428
LVMI, 1 g/m^2	1.017 (1.01–1.02)	<0.001	1.015 (1.01–1.02)	<0.001

CI, confidence interval; HR, hazard ratio; LAD, left atrial dimension; LVMI, left ventricular mass index. *Adjusted by age, diabetes, pulse pressure, LAD, and LVMI.

methods of assessing left atrial diastolic filling [29,30], are useful for predicting the risk of CVD in essential hypertension. The precise mechanisms by which the risk for CVD becomes higher with increasing *S/D* ratio are unclear; there are, however, several hypothetical mechanisms: LVMI, an established independent predictor of CVD in hypertension [31], was higher in patients with higher *S/D* ratio, but the association between a higher *S/D* ratio and CVD was statistically independent of LVMI in multivariate analysis; in normal LV function, the *S/D* ratio positively correlates with left atrial reservoir function [32], which may reflect the cumulative effect of filling pressures over time; and the activated renin–angiotensin–aldosterone system and brain natriuretic peptide, which are importantly involved in the development of hypertension and CVD, strongly promote myocardial remodeling, resulting in increased *S/D* ratio.

One notable result of this study is that, in essential hypertension, the combination of a high *S/D* and low *E/A* was a powerful independent predictor of CVD events, and this is especially noteworthy because of the relatively short follow-up of this study. More importantly, in the group with low *E/A*, the risk of CVD became higher with increasing *S/D* ratio, and thus, the assessment of *S/D* ratio adds prognostic information especially in subjects with low *E/A*. The fact that the association between the group with high *S/D* and low *E/A* and an increased risk for CVD was present even in those with 'normal diastolic function' or 'mild diastolic dysfunction' suggests that evaluation of both mitral valve flow (MVF) and PVF may help identify essential hypertensive subjects without clinical evidence of CVD who are predisposed to adverse outcomes. This result may have been introduced because of advanced age, higher pulse pressure, and longer duration of hypertension, which are established risk factors for CVD, in subjects with high *S/D* and low *E/A*. On the other hand, the diastole phase of PVF resembles early mitral flow, while systolic forward flow is influenced by left atrial compliance, atrial relaxation, mean left atrial pressure, descent of annuals toward the left ventricular apex, and right ventricular contraction [33]. Previous reports have shown that PV_s associates with LV preload [34] and left atrial pressure [35]. In addition, the *S/D* ratio positively correlates with left atrial pressure in subjects with normal LV function [32]. Thus, in subjects with *E/A* under median values, an increased *S/D* ratio may suggest the presence of worse left atrial function, increased LV preload, and worse right ventricular contraction. Evaluation of pulmonary *S/D* in addition to mitral *E/A* may help to assess not only LV diastolic function, but also left atrial and right ventricular function, and thus may provide clinically sensitive prognostic information in patients with essential hypertension. A previous study [36] as well as the present study found that it was possible to obtain high-quality recordings of PVF in more than 80% of the patients by transthoracic echocardiography with daily practice, and thus, we suggest routine evaluation of not only MVF, but also PVF. With respect to the *ARdur* and peak PV_a velocity, we could not find a significant association between these variables and CVD risk, possibly because these variables are usually normal in mild diastolic dysfunction [37].

A previous report showed that control of hypertension and regression of cardiac hypertrophy improved LV diastolic dysfunction [38]. Because our study population included patients with treated essential hypertension at the beginning of the study, our results suggest the importance of evaluating diastolic dysfunction to assess CVD risk, even in patients receiving antihypertensive medication. These results could, however, underestimate the involvement of blood pressure or PVF itself in the development of diastolic dysfunction and CVD events. Another limitation was the lack of control over occasional changes in the antihypertensive regimens over time. The deceleration time of PV_d, which is also useful for estimating pulmonary capillary wedge pressure as a measure of left atrial pressure [39], was not included in this study because it is useful only in patients with a relatively slow heart rate [40]. Severe mitral regurgitation or severe systolic dysfunction can influence PVF, and our findings may not be applicable to hypertensive patients with these other concomitant conditions.

In conclusion, our findings suggest that impaired diastolic function evaluated by increased *S/D* or decreased *E/A* on the baseline Doppler echocardiography is associated with an increased risk of CVD, and the combination of high *S/D* and low *E/A* may be a powerful predictor of CVD in essential hypertension. PVF evaluation by Doppler echocardiography may provide clinically important prognostic information in patients with essential hypertension.

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Original Article

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Decreased coronary flow reserve in haemodialysis patients

Shinnichiro Niizuma[1], Shin Takiuchi[1], Sadanori Okada[2], Takeshi Horio[1], Kei Kamide[1], Hiroto Nakata[1], Fumiki Yoshihara[1], Satoko Nakamura[1], Yuhei Kawano[1], Hajime Nakahama[1], Yoshitaka Iwanaga[3] and Satoshi Nakatani[3]

[1]Division of Hypertension and Nephrology, [2]Division of Atherosclerosis and Diabetes and [3]Division of Cardiology, National Cardiovascular Center, Fujishirodai 5-7-1, Suita 565-8565, Japan

Abstract

Background. Coronary flow reserve (CFR) reflects the functional capacity of microcirculation to adapt to blood demand during increased cardiac work.

Methods. Forty-one patients who had already undergone coronary angiography were studied. They consisted of 21 haemodialysis patients with no significant left anterior descending coronary artery (LAD) stenosis and 20 non-renal failure patients without LAD stenosis. We performed transthoracic Doppler recording of diastolic coronary flow velocity in the LAD at baseline and after maximal vasodilatation by adenosine triphosphate (ATP) infusion. CFR was defined as the ratio of hyperaemic to basal averaged peak flow velocity.

Results. Although the peak coronary velocities during hyperaemia were similar between the two groups, CFR was smaller in haemodialysis (HD) patients than in control subjects (1.96 ± 04 versus 2.3 ± 0.5, $P = 0.001$) due to the higher baseline peak coronary velocities in the former.

Conclusions. The elevated baseline peak coronary velocity may be caused by cardiac hypertrophy and anaemia in HD patients.

Keywords: anaemia; coronary flow reserve; echocardiography; haemodialysis; left ventricular

Introduction

Ischaemic heart disease is by far the leading cause of morbidity and mortality in haemodialysis (HD) patients. Since prevention is not easy, early detection of the disease is the key issue. While coronary angiography is a definitive diagnostic tool, its invasiveness makes it not suitable for all patients. Non-invasive exercise thallium single-photon emission computed tomography is expensive and furthermore not available at all facilities. Stress echography, which has gained increasing popularity, requires skilled interpreters. Recently, a method was developed for assessing coronary flow reserve in the left anterior descending coronary artery (LAD) non-invasively using transthoracic Doppler echography [1]. Coronary flow reserve (CFR) reflects the functional capacity of microcirculation to adapt to blood demand during increased cardiac work. It has been shown to be highly sensitive and specific in the general population [2]. It has also been shown to be as effective as Tl-201-SPECT for physiologic estimation of the severity of LAD stenosis [3]. However, there have been few reports on coronary flow reserve in HD patients. The purpose of the present study was to determine the prevalence and mechanism of abnormal CFR in HD patients without significant LAD stenosis.

Correspondence and offprint requests to: Hajime Nakahama, Division of Hypertension and Nephrology, National Cardiovascular Center, Fujishirodai 5-7-1, Suita 565-8565, Japan. E-mail: hnakaham@hsp.ncvc.go.jp

Methods

Study population

Between 1 June 2003 and 31 August 2003, 196 patients (HD 47, non-HD 149) were hospitalized in our facility because of clinically suspected coronary artery disease. Of the 72 patients who had no significant LAD stenosis on coronary angiography, 41 gave their consent to undergo CFR measurement using transthoracic Doppler echocardiography. The final study population consisted of 21 haemodialysis patients and 20 patients with normal renal function. The exclusion criteria for the present study included the presence of old myocardial infarction, dilated cardiomyopathy, valvular heart disease, hypertrophic cardiomyopathy, congenital heart disease and insufficient echo imaging. Patients with arrhythmias, including atrioventricular blocks or atrial fibrillation, and bronchial asthma were also excluded because the administration of adenosine triphosphate (ATP) might have worsened their symptoms. All subjects gave their informed consent, and the institutional ethics committee approved the study protocol.

CFR measurements by transthoracic Doppler echocardiography

Echocardiographic examinations were performed with a Siemens Sequoia digital ultrasound system (Siemens

Table 1. Patient characteristics

	Total	Haemodialysis	Normal	*P*-value
N	41	21	20	
Female (%)	21	5	30	0.05
Age	66.8 ± 8.7	64.0 ± 12.4	67.5 ± 9.7	0.34
BMI (kg/mm^2)	21.9 ± 0.5	21.6 ± 0.3	22.7 ± 0.6	0.11
HT (%)	76	100	55	0.03
DM (%)	55	68	44	0.53
HLP (%)	44	25	61	0.01
Laboratory data				
Cr (mg/dl)		7.65 ± 2.49	0.76 ± 0.02	0.0001
BUN (mg/dl)		65.6 ± 13.6	16.0 ± 4.13	0.0001
Hb (g/dl)		10.3 ± 1.59	12.7 ± 1.38	0.0001
Etiology				
DM (%)		38		
Nephrosclerosis (%)		43		
PCKD (%)		6		
CGN (%)		13		

Values are mean ± SD or %.
HT = hypertension; DM = diabetes mellitus; HLP = hyperlipidaemia; PCKD = polycystic kidney disease; CGN = chronic glomerulonephritis; Cr = serum creatinine; BUN = blood urea nitrogen; Hb = haemoglobin.

Medical Solutions Inc., Mountain View, CA, USA) with a frequency of 7.0 MHz. The left ventricular mass index (LVMI) was estimated according to the formula of Devereux *et al.* [4]. The pulsed Doppler transmitral flow velocity was recorded to measure the ratio of peak mitral E-wave velocity to peak mitral A-wave velocity (E/A ratio) and the deceleration time of mitral E-wave velocity.

The baseline spectral Doppler signals in the distal portion of the LAD were recorded first. ATP was then administered (140 μg/kg/min i.v.) for 3 min to record spectral Doppler signals during hyperaemic conditions. All patients had continuous heart rate and ECG monitoring throughout the study. Blood pressure was recorded at baseline, every minute during ATP infusion, and at recovery.

Measurements were performed off-line by tracing the contour of the spectral Doppler signal using the computer incorporated in the ultrasound system. Peak diastolic velocity was measured at baseline and under peak hyperaemic conditions. The values in three cardiac cycles were averaged. CFR was defined as the ratio of hyperaemic to basal peak diastolic velocity. Inter- and intraobserver variabilities for the measurement of Doppler velocity recordings were 4.1% and 4.2%, respectively.

Coronary angiography and lesion morphology

Coronary angiography was performed following a standard technique. Before angiography, all of the patients received an intracoronary bolus injection of nitroglycerin (0.125–0.25 mg). Coronary stenosis was evaluated using a computer-assisted quantitative analysis system (CMS-QCA versus 4.0 MEDIS, The Netherlands) based on multiple projections by an experienced investigator who was unaware of the echocardiographic data. A percent diameter stenosis of >50% was defined as significant stenosis [5].

Statistical analysis

For all statistical studies, we used the computer software application StatView (Abacus Concepts Inc., Berkeley, CA, USA). Values are expressed as the means ± SD. Differences were analysed by ANOVA followed by Fisher's protected least significant difference test for continuous variables and with the χ^2 test for categorical variables. $P < 0.05$ was considered statistically significant.

Table 2. Coronary angiography results

	Normal	Haemodialysis
Single vessel disease, *n*	2	6
Double vessel disease, *n*	7	6
Triple vessel disease, *n*	1	2
Left main disease, *n*	0	0
Left anterior descending artery stenosis, *n*	0	0
Left circumflex artery stenosis, *n*	8	8
Right coronary artery stenosis, *n*	10	11
Significant stenosis (−), *n*	10	7

Results

Patient characteristics and clinical findings

The clinical and demographic findings are summarized in Table 1. The percentage of females in the control group was greater than that in the haemodialysis group. There was no significant difference in age or the prevalence of diabetes mellitus between the two groups. The prevalence of hypertension was significantly higher in the haemodialysis group. The prevalence of hyperlipidaemia and the blood haemoglobin level were both significantly lower in the haemodialysis group.

Coronary angiography results

Table 2 shows the results of coronary angiography. Significant stenosis was found in the LAD in 0 patients, in the LCX in 16 patients (normal 8, HD 8) and in the RCA in 21 patients (normal 10, HD 11). Sixteen patients (normal 8,

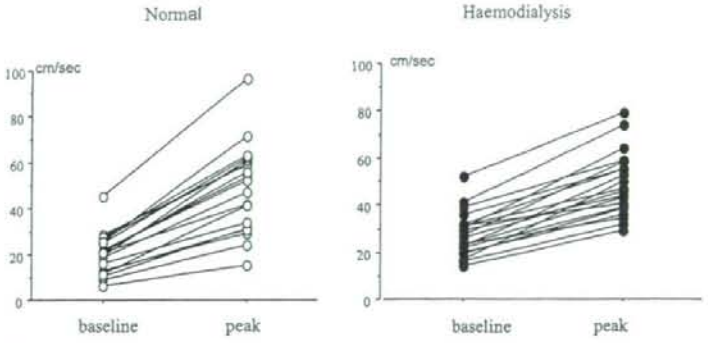

Fig. 1. Peak coronary flow velocity at baseline and hyperaemia.

Table 3. Echocardiography

	Haemodialysis	Normal	*P*-value
IVST (mm)	12.1 ± 1.8	9.8 ± 1.6	0.006
PWT (mm)	12.2 ± 1.8	9.8 ± 1.3	0.001
LVDd (mm)	54.1 ± 6.1	45.7 ± 3.9	0.0001
LVDs (mm)	38.4 ± 4.1	30.1 ± 5.4	0.003
FS (%)	29.6 ± 3.7	34.1 ± 10.2	0.20
E/A	1.0 ± 0.59	0.85 ± 0.32	0.32
DCT (ms)	223.6 ± 54.5	223.1 ± 59.6	0.69
LVMI (g/m^2)	206.7 ± 68.8	118.6 ± 33.7	0.0001

Values are mean ± SD.
IVST = interventricular septal thickness; PWT = posterior wall thickness; E/A = ratio of peak mitral E-wave velocity to peak mitral A-wave velocity; DCT = deceleration time of early diastolic filling; FS = fractional shortening; LVDd = left ventricular diastolic dimension; LVDS = left ventricular systolic dimension; LVMI = left ventricular mass index.

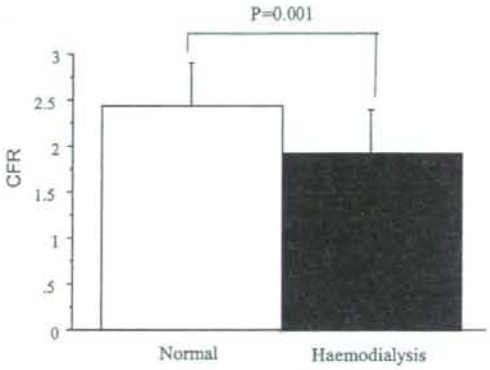

Fig. 2. CFR in normal and haemodialysis patients. Values are mean ± SD.

HD 8) had multivessel disease and no patient had left main trunk stenosis.

Echocardiographic parameters

The echocardiographic data are summarized in Table 3. The prevalence of left ventricular hypertrophy was significantly greater in the haemodialysis patients.

Haemodynamics and coronary flow findings

The haemodynamic data and coronary flow data are summarized in Table 4. Both systolic and diastolic blood pressures at baseline were significantly higher in the haemodialysis patients. The average peak coronary flow velocity at baseline in HD patients was significantly greater than that in control subjects. The average peak coronary flow velocity during hyperaemia tended to be greater in the haemodialysis group than in control subjects, but this difference was not statistically significant (Figure 1). Consequently, the coronary flow reserve, defined as the ratio of hyperaemic to basal peak diastolic velocity, was significantly lower in the haemodialysis group than in the control group (Figure 2).

Relationship between baseline coronary blood flow and left ventricular mass index or blood haemoglobin level

There was a significant positive correlation between left ventricular hypertrophy as assessed by the LVMI and the average peak coronary flow velocity at baseline (Figure 3a). There was a significant negative correlation between the blood haemoglobin level and the average peak coronary flow velocity at baseline (Figure 3b). There was no significant correlation ($r = 0.28$, $P = 0.08$) between LVMI and the haemoglobin level.

Discussion

Previous studies have shown that coronary flow reserve is reduced in various disorders. In this study, diminished coronary flow reserve was observed in haemodialysis patients. Diminished coronary flow reserve may account for several symptoms that are often encountered in haemodialysis patients. Chest pain, arrhythmia and hypotension during haemodialysis sessions may be caused by diminished coronary flow reserve.

The impaired coronary flow reserve observed here was caused primarily by an elevation of the peak coronary flow velocity at baseline. The elevated peak coronary flow

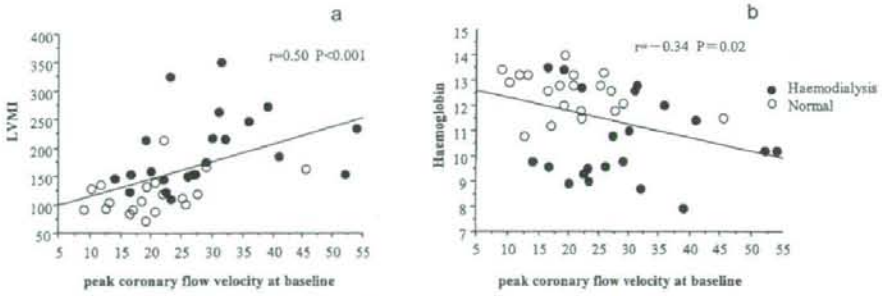

Fig. 3. Correlations of basal peak coronary flow velocity with LVMI (**a**) and haemoglobin (**b**).

Table 4. Haemodynamics and coronary flow findings

	Haemodialysis	Normal	*P*-Value
Baseline			
SBP (mmHg)	143 ± 20	116 ± 22	0.0008
DBP (mmHg)	75 ± 18	62 ± 10	0.01
HR (beats/min)	65 ± 12	66 ± 11	0.50
Average peak flow velocity (cm/s)	29.7 ± 11.4	19.1 ± 8.9	0.005
Hyperaemia			
SBP (mmHg)	124 ± 24	116 ± 20	0.28
DBP (mmHg)	65 ± 15	59 ± 9.0	0.12
HR (beats/min)	67 ± 11	66 ± 11	0.77
Average peak flow velocity (cm/s)	56.6 ± 27.2	45.8 ± 20.5	0.19
Coronary flow reserve	1.95 ± 0.49	2.44 ± 0.52	0.001

Values are mean ± SD.

velocity at baseline may be attributed to an increase in left ventricular mass due to long-standing hypertension and anaemia, which are common in this patient population.

Impaired coronary flow reserve in hypertensive patients has been reported previously. Hamasaki *et al.* studied coronary flow reserve by intravascular ultrasound examination in hypertensive subjects with normal or mildly diseased coronary arteries at angiography [6]. They demonstrated that coronary blood flow at baseline was enhanced and its response to both acetylcholine and adenosine was significantly reduced in patients with left ventricular hypertrophy. Takiuchi *et al.* demonstrated that there was a significant negative correlation between coronary flow reserve and the serum concentration of asymmetric dimethylarginine (ADMA) [7]. ADMA is an endogenous competitive inhibitor of nitric oxide synthases, and serum ADMA levels have been suggested to be markers of endothelial dysfunction. Ravani *et al.* studied the relationship among plasma levels of ADMA, renal function and the risk for progression to end-stage renal disease (ESRD) in 131 patients with chronic kidney disease. They demonstrated that in patients with mild to advanced chronic kidney disease, plasma ADMA was inversely related to glomerular filtration rate and represented a strong and independent risk marker for progression to ESRD and mortality [8]. This study suggests that plasma ADMA level is high in haemodialysis patients. As we demonstrated, anaemia is also associated with high resting basal coronary flow.

To our knowledge, there have been only two reports on the effect of renal failure on coronary flow reserve. Ragosta *et al.* studied coronary flow reserve with a Doppler ultrasound scanning wire in a normal coronary in 32 patients without diabetes mellitus, 11 patients with diabetes mellitus without renal failure and 21 patients with both diabetes mellitus and renal failure [9]. They demonstrated that coronary flow reserve was attenuated in 9% of patients without diabetes mellitus, 18% of patients with diabetes mellitus without renal failure and 57% of patients with diabetes mellitus and renal failure. In the latter cases, abnormal coronary flow reserve was caused by an elevation of baseline coronary flow at baseline. The univariate predictors of abnormal CFR included left ventricular hypertrophy but not the haematocrit level. The baseline heart rate and the presence of diabetes mellitus with renal failure were independent predictors of attenuated coronary flow reserve by multivariate analysis. Their definition of renal failure is ambiguous since they merely state that end-stage renal failure was diagnosed by a nephrologist. Furthermore, they did not have a group of patients with end-stage renal disease who did not have diabetes mellitus. Nevertheless, the notion that elevated coronary flow at baseline is a factor that should always be considered is important. In our present study, there was no difference in the prevalence of diabetes mellitus between the haemodialysis group and the control group. We speculate that an elevation of baseline coronary flow at baseline is independent of diabetes mellitus.

Tok *et al.* reported that coronary flow reserve was impaired in haemodialysis patients [10]. There was no difference in LVMI between the haemodialysis and control groups. There was also no difference in LVMI between

subjects with high and low coronary flow reserve subjects, even among the haemodialysis group. This implies that left ventricular hypertrophy is not a determinant of coronary flow reserve, which contradicts our findings and those in other previous studies. They found a strong inverse correlation between mitral-septal corner mean diastolic velocities during atrial contractions (Am) and coronary flow reserve. In addition, a positive correlation was found between the mitral-septal corner mean diastolic velocity during early diastole (Em):Am ratio, the lateral Em:Am ratio and coronary flow reserve. They concluded that decreased coronary flow reserve might contribute to the impairment of diastolic function, or vice versa. Since no data on baseline coronary flow at baseline were presented, a direct comparison with our study is not possible.

In conclusion, coronary flow reserve was diminished in haemodialysis patients. This impaired coronary flow reserve was caused primarily by an elevation of the peak coronary flow velocity at baseline. The elevated peak coronary flow velocity at baseline may be attributed to an increase in left ventricular mass due to long-standing hypertension and anaemia.

Conflict of interest statement. None declared.

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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

Hidenori Nishida [a], *Takeshi Horio* [a,*], *Yoshihiko Suzuki* [b], *Yoshio Iwashima* [c], *Kei Kamide* [a], *Kenji Kangawa* [d], *Yuhei Kawano* [a]

[a] *Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*

[b] *Department of Internal Medicine, Circulatory and Fluid Regulation, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan*

[c] *Department of Geriatric Medicine, Osaka University Graduate School of Medicine, Suita, Japan*

[d] *National Cardiovascular Center Research Institute, Suita, Japan*

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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

* *Corresponding author.* Tel.: +81 6 6833 5012; fax: +81 6 6872 8091.
E-mail address: thorio@ri.ncvc.go.jp (T. Horio).

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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.

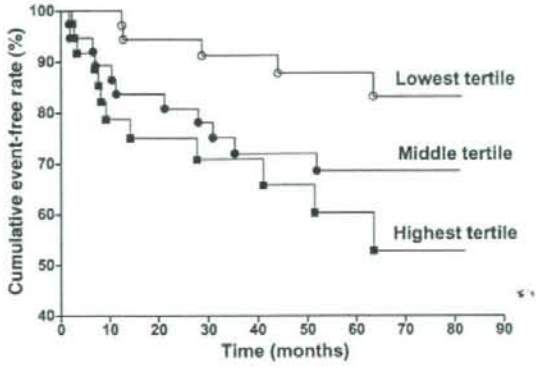

Fig. 1 – CV event-free curves obtained with the Kaplan–Meier method in the three groups divided by tertiles of basal AM levels. Cumulative event-free rates in the lowest, middle, and highest tertiles were 83.2%, 68.6%, and 52.8%, respectively (log-rank test, $P = 0.033$). Lowest tertile, basal AM <10.1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

Tomonori Okamura[a,*], Yoshihiro Kokubo[a], Makoto Watanabe[a], Aya Higashiyama[a], Yoshihiro Miyamoto[b], Yasunao Yoshimasa[b], Akira Okayama[c]

[a] Department of Preventive Cardiology, National Cardiovascular Center, 5-7-1, Fujishiro-dai, Suita, Osaka 565-8565, Japan
[b] Department of Atherosclerosis and Diabetes, National Cardiovascular Center, Osaka, Japan
[c] The First Institute for Health Promotion and Health Care, Japan Anti-tuberculosis Association, Tokyo, Japan

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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,

* Corresponding author. Tel.: +81 6 6833 5012x2228/2188; fax: +81 6 6833 5300.
E-mail address: okamurat@hsp.ncvc.go.jp (T. Okamura).

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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		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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