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ORIGINAL ARTICLE

Serial assessment of arterial stiffness by cardio-ankle vascular index for prediction of future cardiovascular events in patients with coronary artery disease

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Arterial stiffness is a significant predictor of cardiovascular disease (CVD), the risk of which is modified by medications for atherosclerotic risk factors and life-style changes. Cardio-ankle vascular index (CAVI) provides noninvasive, objective information on arterial stiffness, independent of blood pressure. This study aimed to investigate changes in CAVI after management of atherosclerotic risk factors, and the impact of these changes on future CVD outcomes in patients with coronary artery disease (CAD). The study consisted of 211 CAD patients (65 ± 10 years, 118 men) with impaired CAVI. CAVI examination was repeated 6 months later. Impaired CAVI was defined as greater than the mean plus 1 s.d. of the age- and gender-specific normal CAVI values, according to results obtained in 5188 healthy subjects. All patients were followed for >1 year or until the occurrence of a CVD event. Of the 211 patients, CAVI improved in 106 (50%) patients after 6 months, but remained high in 105 (50%) patients. During follow-up (2.9 ± 1.0 years), CVD events occurred in 28 (13%) patients. Persistently impaired CAVI was an independent predictor of future CVD events ($P=0.01$), independent of baseline CAVI. CVD outcomes were worse in patients with persistently impaired CAVI than in those with improved CAVI ($P<0.001$). Among patients with a normalized CAVI after treatment ($n=22$) only one suffered a CVD event. This study was the first to demonstrate that persistent impairment of arterial stiffness was an independent risk factor of future CVD events. Serial measurements of CAVI provide important prognostic information regarding patients with CAD in clinical practice.
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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality in most developed countries. Among markers of CVD, arterial stiffness has proven to be an important parameter in the assessment of cardiovascular risk and an independent strong predictor of future CVD events. Arterial stiffness correlates primarily with arterial structural changes, including elastin fragmentation and degeneration, collagen accumulation, thickening of the arterial wall and progressive arterial dilation.[1,2] A number of investigations have reported the reversibility of arterial stiffness after reduction of atherosclerotic risk factors by pharmacological intervention and life-style modifications.[3–6] We hypothesized that serial assessments of arterial stiffness after comprehensive treatment of atherosclerotic risk factors, rather than a one-time assessment, provide an accurate estimate of the risk of future CVD events. This study was designed to investigate improvement in impaired arterial stiffness induced by treatment of atherosclerotic risk factors in association with future CVD events in patients with coronary artery disease (CAD).

Among the different methods currently available for assessing arterial stiffness, the cardio-ankle vascular index (CAVI) was used in this study for its noninvasive, quantitative and objective nature, and its independence from arterial blood pressure.[7,8] This study also used measurement of carotid artery intima-media thickness (IMT) as a surrogate marker for the early stage of structural changes of atherosclerosis.

METHODS

A total of 371 consecutive patients with newly diagnosed CAD who underwent coronary computed tomographic angiography (CCTA) between March 2008 and April 2011 were initially enrolled in this study. The presence of CAD was defined as coronary artery segments exhibiting plaque with a luminal diameter stenosis of 50% or more on CCTA. Patients with a history of percutaneous coronary intervention were not enrolled in this study. Of the 371 patients, 184 patients (50%) were referred for exercise or pharmacological stress myocardial perfusion imaging. One hundred and twenty patients (65%) were found to have normal perfusion imaging, and 64 patients (35%) were referred for invasive coronary angiography. The other 32 patients (8.6%) were referred for

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subsequent invasive coronary angiography without stress myocardial perfusion imaging because of the presence of severe CAD. The remaining 155 patients (42%) received medical therapy without stress myocardial perfusion imaging, defined by the patient's attending physician after taking into consideration the patient's condition, including age, comorbid diseases and severity of CAD. A total of 96 patients (26%) underwent invasive coronary angiography and 15 patients (4.0%) underwent subsequent coronary revascularization. These patients had their first CAVI examination at the time of diagnosis, and patients with an impaired CAVI (defined as greater than the mean plus 1 s.d. of the age- and gender-specific normal CAVI values) were included. The exclusion criteria were follows: (1) CVD event within 4 weeks of enrollment and during the 6-month period between the first and second CAVI tests; (2) left ventricular ejection fraction <30% on echocardiography; (3) history of peripheral arterial disease; (4) chronic kidney disease (estimated glomerular filtration rate $<60\,ml\,min^{-1}$ per $1.73\,m^2$), including maintenance hemodialysis; (5) atrial fibrillation and (6) the presence of other serious systemic diseases.

Among the 371 patients with CAD, 160 patients were excluded: 132 patients were excluded because of an unimpaired CAVI ($\leqslant$ age- and gender-specific cutoff value of control subjects): 6 patients suffered a CVD event between their first and second CAVI tests, and 22 patients met one of the other exclusion criteria. Therefore, the final population was 211 patients (65 ± 10 years, 118 men). The second CAVI test was performed 6 months after the first, and patients were then classified as having improved CAVI (the second CAVI was better than the first), or persistently impaired CAVI. Blood pressure, body mass index, serum cholesterol, C-reactive protein, fasting glucose, hemoglobin (Hb)-A1c and carotid ultrasound examination for IMT measurements were also assessed at the times of the first and second CAVI tests.

This study also included 5188 healthy subjects (43 ± 11 years, 1872 men) with no history of CVD and no risk factors based on a cardiovascular screening program to define the age- and gender-specific normal CAVI values: 4988 subjects were selected from an epidemiological study[9] and 200 subjects were chosen from a cardiovascular screening program at Osaka Ekisaikai Hospital. Written informed consent was obtained from all patients and control subjects before the study. This study was approved by the Institutional Review Board of Osaka Ekisaikai Hospital. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the institution's human research committee.

CAVI was measured after 12 h of fasting, 3 days (first CAVI) before the CCTA examination and repeated 6 months after the first CAVI test (second CAVI) in all study patients. After the first CAVI test, all patients received optimized therapy for atherosclerotic risk factors and life-style modifications according to AHA/ACC guidelines.[10,11] The target values were as follows: (1) hypertension (blood pressure of <140/90 mm Hg or 130/80 mm Hg in the presence of diabetes or CAD); (2) low-density lipoprotein cholesterol $<100\,mg\,dl^{-1}$ ($2.6\,mmol\,l^{-1}$); (3) HbA1c <7.0% for diabetes control. Framingham risk score (FRS) was calculated at the time of the second examination.[12] Patients were encouraged to quit smoking and to continue an appropriate balance of physical activity and caloric intake.[10]

After the second CAVI test, all 211 patients were followed every month at our institution for >1 year or until the occurrence of one of the following CVD events: cardiac death, non-fatal myocardial infarction, unstable angina pectoris, recurrent angina pectoris requiring coronary revascularization or stroke. Non-fatal myocardial infarction was defined by the European Society of Cardiology/American College of Cardiology Committee, and unstable angina was defined according to the Braunwald classification. The diagnosis of stroke was made by neurological examination and brain magnetic resonance imaging. The same medications prescribed during the 6 months between the first and second CAVI tests, as well the as the recommended diet and life-style modifications, were continued in each patient throughout the follow-up period.

CAVI, a measure of arterial stiffness, was calculated with the patient in the supine position. CAVI was assessed with an automatic device, the VaSera (Fukuda Denshi, Tokyo, Japan), which measures the time delay between the rapid upstroke of simultaneously recorded pulse waves in the brachial and ankle arteries. The distance between the recording sites on the brachial and ankle arteries was measured with a tape over the body surface. CAVI was calculated by substituting the stiffness parameter, β, into the following equation.[7,8,13] Stiffness parameter β, recognized as a blood pressure-independent parameter of arterial stiffness, was calculated as follows:

$$\text{Stiffness parameter } \beta = \ln(Ps/Pd) \times (D/DD)$$

On the other hand, pulse wave velocity (PWV) was derived from Bramwell–Hill's equation as follows:

$$(D/DD) = 2\rho \times PWV^2/DP$$

On the basis of these two equations, CAVI was calculated as follows:

$$\text{Stiffness parameter } \beta = \ln(Ps/Pd) \times 2\rho \times PWV^2/DP = CAVI$$

(D, diameter; Ps, systolic blood pressure; Pd, diastolic blood pressure; ΔP, pulse pressure (Ps–Pd); ρ, blood density).

$$CAVI = a[(2\rho/DP) \times \ln(Ps/Pd) \times PWV^2] + b$$

The values of *a* and *b* are constants in order to make the units of CAVI comparable to those of PWV.

Carotid IMT was measured by B-mode ultrasonography using a 7.5-MHz linear array transducer.[14] The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of each measurement. The average of IMT of each of three frozen images was calculated. For each individual, IMT was determined as the average of near- and far-wall measurements of both the left and right common carotid arteries.

CCTA was performed using a SOMATOM Sensation 64 system (Siemens Medical Systems, Forchheim, Germany), with the following scan parameters: 64×0.6 mm collimation, tube voltage of 120 kV, gantry rotation time of 330 ms and tube current rotation time of 770–850 mAs. CCTA was performed in accordance with the protocol in our previous reports.[15,16]

All CCTA data sets were analyzed on a per-segment basis by two experienced readers. Coronary arteries were divided into 15 separate segments that were 1.5 mm or more in diameter as measured by CCTA. Coronary atherosclerotic lesions were quantified for stenosis by visual estimation. The severity of luminal-diameter stenosis was divided into non-obstructive plaques (<50% luminal stenosis) and obstructive plaques (>50% luminal stenosis). Two vessels, three vessels and left main CAD were defined as a multi-vessel CAD.

Categorical variables are presented as a number (%), and continuous variables, as the mean ± s.d. The χ^2 test was used for comparison of categorical variables. Continuous variables were compared by unpaired *t*-test or Mann–Whitney *U*-test, according to the data distribution. The baseline characteristics for patients with and without impaired CAVI, and for those excluded because of other exclusion criteria, were compared using one-way analysis of variance for parametric data distribution or Kruskal–Wallis test for nonparametric data distribution. The values of CAVI before and 6 months after optimized therapy were compared between groups with improved CAVI and persistently impaired CAVI using two-way repeated-measures analysis of variance. Cox proportional hazard analysis was performed to identify predictors of CVD events. Baseline variables that were considered clinically relevant or showed a univariate relationship with outcome were entered into the analysis. The Kaplan–Meier survival method was used to compare survival during follow-up, using the log-rank test. A *P*-value <0.05 was considered statistically significant.

RESULTS

The characteristics and age- and gender-specific CAVI values of the control subjects are shown in Table 1. Age-specific CAVI values became higher in both genders as their ages increased by 10-year intervals. Table 2 shows baseline characteristics, which compared the clinical variables of patients with and without impaired CAVI, as well as those patients who were excluded because of other exclusion criteria, including the six patients with a CVD event between their first and second CAVI tests. There were no significant differences in clinical characteristics among the three groups, except for CAVI value ($P=0.002$), estimated glomerular filtration rate ($P=0.04$) and left ventricular ejection fraction ($P<0.001$) by the definition. The CAD vessel number was similar among the three groups. The characteristics

Table 1 Characteristics of atherosclerotic risk factors and the age- and gender-specific normal CAVI values in healthy subjects

Gender	*Men*					*Women*				
Age	*30–39*	*40–49*	*50–59*	*60–69*	*70–79*	*30–39*	*40–49*	*50–59*	*60–69*	*70–79*
Number	*885*	*430*	*326*	*176*	*55*	*1319*	*1090*	*708*	*159*	*40*
Systolic BP, mm Hg	117±10	119±9	120±10	124±11	124±11	108±10	112±11	115±11	119±11	119±12
Diastolic BP, mm Hg	70±8	74±8	76±8	77±8	79±9	64±7	66±8	69±8	71±8	72±8
Total cholesterol, mg dl^{-1}	185±19	189±25	189±23	196±25	190±26	182±21	189±20	197±17	202±18	191±21
Triglyceride, mg dl^{-1}	80±31	86±27	84±27	93±38	85±30	62±26	65±25	70±28	80±29	94±34
HDL cholesterol, mg dl^{-1}	61±13	61±13	64±13	60±14	64±16	73±13	74±13	74±13	68±16	65±15
LDL cholesterol, mg dl^{-1}	106±21	111±21	110±21	115±19	111±23	96±20	103±19	110±18	115±19	109±20
CAVI	7.10±0.68	7.59±0.7	8.06±0.76	8.63±0.81	8.90±0.96	6.97±0.63	7.29±0.66	7.81±0.7	8.25±0.89	8.59±1.04
Cutoff value	7.78	8.29	8.82	9.44	9.86	7.6	7.95	8.51	9.14	9.63

Abbreviations: BP, blood pressure; CAVI, cardio-ankle vascular index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data are mean±s.d.

Table 2 Comparison of baseline patient characteristics among patients with and without impaired CAVI, and excluded patients with other confounding factors at first CAVI test

	Patients without impaired CAVI (n = 132)	*Patients with impaired CAVI (n = 211)*	*Patients excluded by other confounding factors (n = 28)*	P-*value*
Age, years	63±7	65±10	63±7	0.10
Male, *n* (%)	63 (48)	118 (56)	18 (64)	0.16
Hypertension, *n* (%)	96 (74)	153 (73)	14 (50)	0.20
Hyperlipidemia, *n* (%)	88 (68)	141 (67)	12 (48)	0.16
Diabetes, *n* (%)	54 (42)	114 (55)	14 (50)	0.68
One-vessel CAD, *n* (%)	96 (73)	133 (63)	19 (68)	0.17
Two-vessel CAD, *n* (%)	23 (17)	40 (19)	4 (14)	0.81
Three-vessel or left main CAD, *n* (%)	13 (10)	38 (18)	5 (18)	0.11
Systolic BP, mm Hg	140±21	145±23	139±21	0.10
Diastolic BP, mm Hg	81±9	84±10	82±13	0.15
Heart rate, b.p.m.	67±13	69±12	65±11	0.10
Body mass index, kg m^{-2}	23.8±3.4	24.3±2.8	24.2±4.1	0.12
Current smoker, *n* (%)	26 (29)	56 (26)	6 (21)	0.12
LDL cholesterol, mg dl^{-1}	120±33	117±32	103±33	0.50
HDL cholesterol, mg dl^{-1}	51±14	50±13	49±12	0.86
Fasting glucose, mg dl^{-1}	126±38	134±38	125±95	0.21
HbA1c, %	6.1±0.7	6.3±0.9	6.2±0.5	0.39
CRP, mg l^{-1}	1.8±2.1	1.6±1.7	1.8±1.1	0.58
eGFR, ml min^{-1} per 1.73 m^2	59±15	57±14	48±29	0.04
Left ventricular ejection fraction, %	60±9	59±9	49±17	0.002
First CAVI	8.95±0.46	9.96±0.72	9.14±0.72	<0.001

Abbreviations: BP, blood pressure; CAD, coronary artery disease; CAVI, cardio-ankle vascular index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Values are mean±s.d. or *n* (percentage). The column showed *P*-values for the comparison between patients with persistently impaired CAVI and those with improved CAVI.

of the study patients at study enrollment are shown in Table 3. Among 211 patients with impaired CAVI, CAVI improved in 106 (50%) patients after 6 months, whereas impaired CAVI persisted in the remaining 105 (50%) patients (Figure 1). CAVI was normalized in 22 (10%) patients.

Table 3 Comparison of patient characteristics at first CAVI test in patients with and without impaired CAVI

	Patients with persistently impaired CAVI (n = 105)	*Patients with improved CAVI (n = 106)*	P-*value*
Age, years	64±11	66±8	0.2
Male, *n* (%)	63 (60)	55 (52)	0.2
Hypertension, *n* (%)	74 (71)	79 (75)	0.6
Hyperlipidemia, *n* (%)	68 (65)	73 (69)	0.5
Diabetes, *n* (%)	61 (59)	53 (50)	0.2
Multi-vessel CAD, *n* (%)	35 (33)	43 (41)	0.3
Systolic BP, mm Hg	148±26	142±21	0.1
Diastolic BP, mm Hg	85±10	83±10	0.1
Heart rate, b.p.m.	69±12	70±13	0.3
Body mass index, kg m^{-2}	24.4±3.2	24.3±2.4	0.8
Current smoker, *n* (%)	25 (30)	31 (35)	0.5
LDL cholesterol, mg dl^{-1}	119±30	114±35	0.5
HDL cholesterol, mg dl^{-1}	51±12	49±13	0.5
Fasting glucose, mg dl^{-1}	129±38	130±39	0.9
HbA1c, %	6.4±0.8	6.3±1.1	0.4
CRP, mg l^{-1}	1.5±1.5	1.7±1.9	0.6
Mean IMT, mm	0.86±0.18	0.86±0.19	0.9
First ba-PWV, m s^{-1}	17.1±2.4	17.3±3.0	0.5
First CAVI	9.87±0.65	10.05±0.78	0.07

Abbreviations: ba-PWV, brachial-ankle pulse wave velocity; BP, blood pressure; CAD, coronary artery disease; CAVI, cardio-ankle vascular index; CRP, C-reactive protein; Hb, hemoglobin; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein.
Values are mean±s.d. or *n* (percentage). The column showed *P*-values for the comparison between patients with persistently impaired CAVI and those with improved CAVI.

The baseline characteristics, including blood pressure, body mass index and cholesterol profiles, were comparable between the two groups (Table 3). At the second CAVI examination, HbA1c was higher in patients with persistently impaired CAVI than in patients with improved CAVI ($P=0.006$; Table 4). However, the two groups demonstrated equal improvement in atherosclerotic risk factors, including blood pressure and cholesterol profiles, at the second CAVI test, and the percent change in risk status between the first and second CAVI tests was comparable. We performed univariate and multivariate analyses to determine the regression of CAVI. There were no significant differences in any variables between patients with or without regression of CAVI (Tables 3 and 4). In addition, we performed a multivariate analysis including the following variables

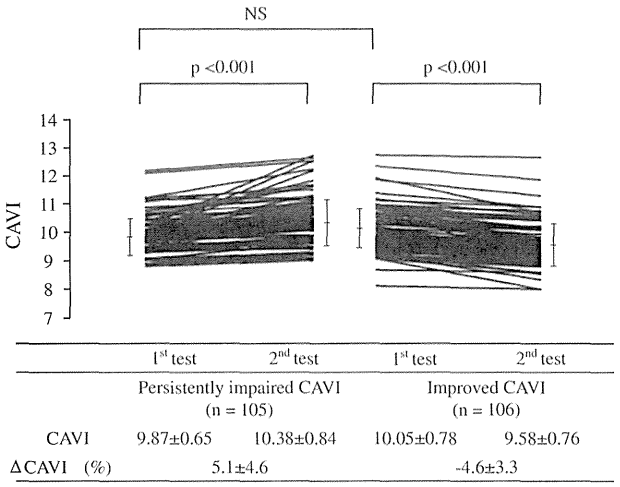


Figure 1 CAVI values measured at baseline and 6 months after comprehensive management of atherosclerotic risk factors in patients with persistently impaired CAVI (left) and improved CAVI (right). CAVI, cardio-ankle vascular index; NS, not significant.

Table 4 Comparison of clinical characteristics at the second CAVI test and percent change in risk status from the first to the second CAVI test

	Patients with persistently impaired CAVI	*Patients with improved CAVI*	P-*value*
The clinical characteristics at second CAVI test			
Systolic BP, mm Hg	143±23	138±18	0.1
Diastolic BP, mm Hg	85±9	83±8	0.08
Heart rate, b.p.m.	69±12	68±11	0.7
Body mass index, kg m^{-2}	24±3.5	23±3.0	0.3
Current smoker, *n* (%)	19 (22)	20 (22)	0.9
LDL cholesterol, mg dl^{-1}	104±27	96±26	0.1
HDL cholesterol, mg dl^{-1}	53±11	51±14	0.5
Fasting glucose, mg dl^{-1}	122±29	121±33	0.8
HbA1c, %	6.4±0.9	6.0±0.6	0.006
CRP, mg l^{-1}	1.0±1.1	1.2±1.3	0.4
Mean IMT, mm	0.86±0.19	0.87±0.19	0.8
Second ba-PWV, m s^{-1}	17.9±2.7	16.2±3.2	<0.01
Second CAVI	10.38±0.84	9.58±0.76	<0.01
Percent change in risk status from the first to the second CAVI test			
Systolic BP, %	−4.1±23	−3.8±25	0.9
Diastolic BP, %	−3.2±11	−3.5±10	0.7
Heart rate, %	0.6±13	−2.0±14	0.1
Body mass index, kg m^{-2}, %	−1.8±5.9	−3.1±2.9	0.2
Current smoker, *n*	−6	−11	—
LDL cholesterol, %	−9.8±20	−11±32	0.9
HDL cholesterol, %	2.1±7.3	2.3±7.7	0.8
Fasting glucose, %	−2.0±24	−4.9±17	0.3
HbA1c, %	−1.3	−2.0	0.6
CRP, %	−17±61	−6.9±57	0.4
Δba-PWV, %	5.3±11	−5.4±13	<0.001
Δ CAVI, %	5.1±4.6	−4.6±3.3	<0.001
Δ IMT, %	2.4±18	4.1±28	0.6

Abbreviations: ba-PWV, brachial-ankle pulse wave velocity; BP, blood pressure; CAD, coronary artery disease; CAVI, cardio-ankle vascular index; CRP, C-reactive protein; Hb, hemoglobin; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein.
Values are mean ± s.d.

Table 5 Comparison of medication usage

	Patients with persistently impaired CAVI	*Patients with improved CAVI*	P-*value*
Medications at first CAVI test, n *(%)*			
Statins, *n* (%)	50 (48)	55 (52)	0.5
ACEIs/ARBs, *n* (%)	48 (46)	57 (55)	0.2
CCBs, *n* (%)	35 (34)	31 (30)	0.5
Beta-blockers, *n* (%)	27 (26)	21 (20)	0.3
Aspirin, *n* (%)	61 (58)	63 (61)	0.7
Oral antidiabetic agents, *n* (%)	45 (43)	32 (30)	0.06
Insulin, *n* (%)	11 (10)	9 (8.5)	0.6
Medications at second CAVI test, n *(%)*			
Statins, *n* (%)	77 (73)	84 (80)	0.3
ACEIs/ARBs, *n* (%)	56 (50)	66 (63)	0.3
CCBs, *n* (%)	40 (38)	37 (35)	0.6
Beta-blockers, *n* (%)	35 (33)	27 (25)	0.2
Aspirin, *n* (%)	61 (58)	63 (61)	0.2
Oral antidiabetic agents, *n* (%)	46 (44)	35 (33)	0.1
Insulin, *n* (%)	13 (12)	10 (9.4)	0.5

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; CAVI, cardio-ankle vascular index; CCB, calcium channel blocker.
Values are *n* (percentage).

that were considered clinically relevant: age, male gender, hypertension, hyperlipidemia, diabetes, heart rate and changes in heart rate. As a result, multivariate analysis failed to show an independent determinant for the regression of CAVI (*P*-values did not reach statistical significance).

All medications used at baseline and at the second CAVI test (for example, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, beta-blockers, calcium channel blockers, statins, antiplatelet drugs, oral antidiabetic drugs and insulin) were comparable between the two groups (Table 5). In addition, 53 patients were prescribed one or more of these medications between first and second CAVI tests. CAVI values at the first (10.17 *vs.* 9.92, $P=0.07$) and second CAVI tests (10.16 *vs.* 9.95, $P=0.09$), as well as the changes in these values (-0.01 *vs.* 0.03, $P=0.7$), were similar between patients who started using statins and other medications.

During a mean follow-up period of 2.9 ± 1.0 years, which ranged from 6 months to 4.9 years (median 3.4 years) after second CAVI examination, 28 of the following CVD events were registered: cardiac death in 2 patients, non-fatal myocardial infarction in 4 patients, unstable angina in 12 patients, recurrent angina pectoris requiring coronary revascularization in 5 patients and stroke in 5 patients. Initial CAVI values were not predictive of event occurrence in the univariate analysis (Table 6). In addition, there was no significant prognostic difference between patients with CAVI values above or below the median in the first CAVI test ($P=0.32$), as shown in Figure 2a. By contrast, patients with persistently impaired CAVI had 21 (20%) CVD events, whereas patients with improved CAVI had only 7 (6.6%) CVD events ($P<0.001$). Kaplan–Meier analysis demonstrated significantly worse CVD outcomes for patients with persistently impaired CAVI compared with the other group ($P<0.001$; Figure 2b). Only 1 of 21 patients in whom CAVI normalized after treatment developed a CVD event during the follow-up period.

Table 6 shows the results of a comparison of patient clinical characteristics at the first CAVI test and the percent change in risk

Table 6 Comparison of clinical characteristics at first and second CAVI tests and percent change in risk status from the first to second CAVI test in patients with and without future CVD

	Patients with CVD events (n=28)	*Patients without CVD events (n=183)*	P-value
Clinical characteristics at first CAVI test			
Age, years	66±8	65±10	0.4
Male, *n* (%)	13 (48)	105 (57)	0.4
Hypertension, *n* (%)	22 (81)	131 (72)	0.3
Hyperlipidemia, *n* (%)	22 (81)	119 (65)	0.1
Diabetes, *n* (%)	21 (78)	93 (51)	0.01
Multi-vessel CAD, *n* (%)	15 (56)	63 (34)	0.03
Systolic BP, mm Hg	146±29	144±22	0.7
Diastolic BP, mm Hg	84±11	84±10	0.7
Heart rate, b.p.m.	72±11	69±13	0.2
Body mass index, kg m^{-2}	24.8±3.7	24.2±2.7	0.4
LDL cholesterol, mg dl^{-1}	122±28	116±33	0.5
HDL cholesterol, mg dl^{-1}	50±10	50±13	0.9
Fasting glucose, mg dl^{-1}	126±28	130±40	0.6
HbA1c, %	6.8±0.9	6.3±0.9	0.003
CRP, mg l^{-1}	1.6±1.0	1.6±1.9	0.9
Mean IMT, mm	0.87±0.17	0.86±0.19	0.8
First ba-PWV, m s^{-1}	17.3±2.8	17.1±2.7	0.7
First CAVI	10.07±0.76	9.94±0.71	0.4
Clinical characteristics at second CAVI test			
Systolic BP, mm Hg	138±20	141±21	0.5
Diastolic BP, mm Hg	83±8	83±9	0.9
Heart rate, b.p.m.	72±10	68±11	0.05
Body mass index, kg m^{-2}	24.3±4.4	23.6±3.1	0.3
LDL cholesterol, mg dl^{-1}	110±33	98±25	0.1
HDL cholesterol, mg dl^{-1}	51±15	52±12	0.8
Fasting glucose, mg dl^{-1}	117±26	122±32	0.4
HbA1c, %	6.5±0.8	6.1±0.7	0.03
CRP, mg l^{-1}	1.3±1.3	1.1±1.2	0.4
Mean IMT, mm	0.87±0.17	0.86±0.19	0.8
Second ba-PWV	18.2±3.1	16.9±3.0	0.03
Second CAVI	10.55±0.83	9.89±0.87	<0.001
Percent change in risk status from first to second CAVI test			
Systolic BP, %	−5.8±14	−3.8±19	0.6
Diastolic BP, %	−0.3±11	0.9±10	0.6
Heart rate, %	1.6±14	−1.1±11	0.3
Body mass index, kg m^{-2}, %	−1.0±4.0	−2.8±8.4	0.3
Current smoker, *n*	−3	−14	—
LDL cholesterol, %	−9.3±18	−10±28	0.9
HDL cholesterol, %	3.6±25	6.2±14	0.6
Fasting glucose, %	−4.9±18	−3.0±21	0.6
HbA1c, %	−3.8±10	−1.3±9.1	0.2
CRP, %	−8.0±70	−13±56	0.7
Δ mean IMT, %	4.9±18	3.2±25	0.7
Δ ba-PWV, %	6.5±14	−1.0±12	<0.001
Δ CAVI, %	4.9±6.8	−0.5±5.9	<0.001
Persistently impaired CAVI, *n* (%)	21 (78)	84 (46)	0.002

Abbreviations: ba-PWV, brachial-ankle pulse wave velocity; BP, blood pressure; CAD, coronary artery disease; CAVI, cardio-ankle vascular index; CRP, C-reactive protein; CVD, cardiovascular disease; Hb, hemoglobin; HDL, high-density lipoprotein; IMT, intima-media thickness; LDL, low-density lipoprotein.
Values are mean±s.d. or *n* (percentage).

status from the first to the second test in patients with and without CVD events. Patients with CVD events were more likely to have diabetes ($P=0.01$), multi-vessel CAD ($P=0.03$) and persistently impaired CAVI ($P=0.002$) than patients without CVD events. Other atherosclerotic risk factors such as blood pressure, body mass index and cholesterol profiles were comparable between the two groups.

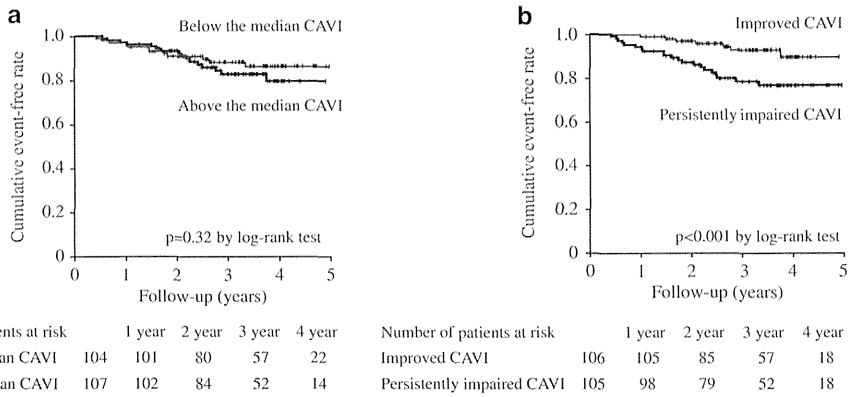
Patients with persistently impaired CAVI tended to have higher FRS compared with patients with improved CAVI (15.0±4.4 *vs.* 14.1±4.7, $P=0.13$). Patients who suffered a CVD event also tended to have higher FRS compared with patients who did not (15.7±2.7 *vs.* 14.3±4.8, $P=0.14$), but these values were not statistically significant. Furthermore, we performed a multivariate Cox proportional hazard analysis, which included diabetes, multi-vessel disease, second brachial-ankle PWV, persistently impaired CAVI and FRS, in order to predict future CVD events. HbA1c was excluded from the covariates in this analysis because it was initially associated with diabetes. ΔCAVI was also excluded because it was initially associated with persistently impaired CAVI. Cox proportional hazards model analysis showed that multi-vessel CAD (hazard ratio: 2.2, 95% confidence interval: 1.02–4.89, $P=0.04$), and persistently impaired CAVI (hazard ratio: 3.3, 95% confidence interval: 1.47–8.59, $P<0.01$) were independent predictors of future CVD events (model 1, Table 7). When multivariate analysis was repeated with the addition of the absolute value of the second CAVI instead of 'persistent impairment of arterial stiffness,' the second CAVI value was found to be an independent predictor of future CVD events (hazard ratio: 1.8, 95% confidence interval: 1.18–2.74, $P<0.01$; model 2, Table 7). Patients with CVD events had higher brachial-ankle PWV values at the second test than those without CVD events (Table 6). However, brachial-ankle PWV at the second test was found to be insignificant in the multivariate analysis (Table 7).

DISCUSSION

This study demonstrated that arterial stiffness, as estimated by CAVI, did not improve even after comprehensive treatment of atherosclerotic risk factors in approximately half of the patients with CAD. This is the first study focusing on persistently impaired arterial stiffness, which primarily reflected CAVI after treatment, as an independent risk for poor CVD outcome. Only one of the patients in whom CAVI normalized after treatment suffered a CVD event.

Arterial stiffness develops from interactional changes involving structural and cellular elements of the arterial walls.[1,2] Several previous studies demonstrated the importance of assessing arterial stiffness as a predictor of all-cause and CVD mortality for various diseases, independent of classical CVD risk factors. The results of these observations stressed the importance of arterial stiffness as an index for assessing the severity of atherosclerosis. Furthermore, increased arterial stiffness is also reportedly associated with endothelial dysfunction and inflammation, an association linked to future CVD events.[17]

CAVI has been validated by the stiffness parameter β in the thoracic descending aorta and the carotid artery.[18] The correlations of CAVI with other atherosclerotic parameters (age, carotid IMT and CAD severity) were superior to those between CAVI and PWV. In addition, the independence of CAVI from arterial blood pressure has been reported.[3,18] On the other hand, several studies have already shown that life-style modification and medical therapies, such as smoking cessation,[4] blood glucose control,[5] control of hypertension[6] and lipid-lowering therapies, improved CAVI.[19,20] To our knowledge, however, there are no studies regarding the prognostic value of CAVI on long-term CVD outcomes.



Number of patients at risk		1 year	2 year	3 year	4 year
Below the median CAVI	104	101	80	57	22
Above the median CAVI	107	102	84	52	14

Number of patients at risk		1 year	2 year	3 year	4 year
Improved CAVI	106	105	85	57	18
Persistently impaired CAVI	105	98	79	52	18

Figure 2 (a) Comparison of Kaplan–Meier curves of event-free survival between patients above the median and below the median CAVI value in the first CAVI test. (b) Comparison of Kaplan–Meier curves of event-free survival between patients with persistently impaired CAVI and improved CAVI. CAVI, cardio-ankle vascular index.

Table 7 Multivariate Cox proportional hazard analysis for predictors of CVD event

Variables	*Hazard ratio*	*95% CI*	P-*value*
Model 1			
Diabetes	1.6	0.73–4.4	0.2
Multi-vessel CAD	2.2	1.02–4.89	0.04
Second ba-PWV	1.0	0.88–1.18	0.7
Persistently impaired CAVI	3.3	1.47–8.59	<0.01
Model 2			
Diabetes	1.9	0.83–4.89	0.1
Multi-vessel CAD	1.8	0.87–4.20	0.1
Second ba-PWV	1.1	0.92–1.23	0.3
Second CAVI	1.8	1.18–2.74	<0.01

Abbreviations: ba-PWV, brachial-ankle pulse wave velocity; CAD, coronary artery disease; CAVI, cardio-ankle vascular index; CI, confidence interval; CVD, cardiovascular disease.

In this study, impaired CAVI did not improve after 6 months of comprehensive treatment of atherosclerotic risk factors in 50% of patients with CAD. This finding suggests that CAVI may reflect irreversible organ damage and functionally reversible arterial stiffness in patients with advanced atherosclerosis. CAVI values before the treatment of atherosclerotic risks could not distinguish between irreversible and reversible arterial stiffness after managing atherosclerotic risks. In fact, the value of the first CAVI at study enrollment did not correlate with future CVD events in this study. All patients enrolling in our study had impaired CAVI and concomitant CAD. Serial measurements of CAVI, particularly after treatment, seem to help identifying high-risk patients requiring more aggressive management, as well as providing the pathogenic mechanisms of arterial stiffness in patients with advanced atherosclerosis in clinical practice.

Another important finding of this study was that all clinical variables, including, medication usage and risk factor modification after comprehensive therapy, were similar between patients with persistently impaired CAVI and those with improved CAVI. In addition, the clinical variables and changes in these variables did not predict the improvement of CAVI. This may be explained as follows: first, individual risk factors, such as blood pressure, lipid profile and blood glucose, may fluctuate over time, and their values may not reflect their true impact on the arterial wall; second, the response to medications varies among individuals and multiple parameters have been reported that contribute to this variation (age, smoking status and insulin resistance).[21,22] Finally, there may be class effects of specific medications, such as antihypertensive agents causing the regression of structural changes to the arterial wall, and statins modifying both endothelial function and arterial stiffness.[22–24] CAVI is an integrated parameter that reflects the severity of atherosclerosis of the arterial wall, and serves as an additional prognostic importance to stratify CAD patients at risk of developing CVD, beyond the assessment of traditional atherosclerotic risk factors.

Furthermore, as expected, carotid IMT did not change during the 6-month follow-up period of this study. Controversy still exists on the use of IMT in both the management and prediction of CAD.[14,25] A recent meta-analysis also showed no association between IMT progression and 7-year CVD outcome.[26] These findings suggest the superiority of CAVI over IMT as a surrogate maker of CVD risk in response to management of atherosclerosis, particularly over short-term follow-up periods. Overcoming inherent methodological limitations of current IMT measurement via novel echocardiographic techniques, such as an automatic tracking system or three-dimensional image acquisition, may enable the combination of IMT and CAVI to be useful for more detailed risk stratification based on their representation of different aspects of atherosclerosis.

This study has limitations that need to be acknowledged. First, this study was based on a relatively small sample size. Furthermore, patients with normal CAVI values at the beginning of the study were not enrolled because this study was designed to investigate the potential of CAVI for further risk stratification of CAD by evaluating impaired arterial stiffness in relation to the treatment of atherosclerotic risk factors. Future studies with a larger number of patients are required to fully explore the factors associated with improvement of CAVI and the role of persistently impaired arterial stiffness in the pathogenesis and progression of atherosclerosis. Second, central blood pressure is noted as a factor associated with future CVD events,[27] which was not accounted for by CAVI measurements. In addition, heart-femoral PWV was not measured. CAVI has been validated in prior studies against other markers of arterial stiffness (stiffness parameter β); however, these studies were based on relatively small sample sizes.[28,29] Therefore, the validity of CAVI as a maker of arterial stiffness should be confirmed either in future studies with a larger number of subjects or in comparison with pathological findings. Third, excellent reproducibility of CAVI was reported in previous studies.[17] Unfortunately, this study was not designed to confirm the reproducibility of CAVI. Therefore, it was unclear whether the

reproducibility of CAVI affected the results of this study. Finally, we used CCTA to enroll a more general patient population, and patients who underwent CCTA may not be representative of average patients with CAD scheduled for invasive coronary angiography. Careful attention may be necessary to extrapolate the results of this study. CCTA is an established modality that provides non-invasive diagnosis of CAD, especially for the exclusion of CAD, although CCTA may overestimate the severity of CAD. Therefore, other imaging modalities, such as invasive coronary angiography, should be used to confirm the presence of CAD in future investigations.

This study is the first to demonstrate that persistent impairment of arterial stiffness was associated with future CVD events, even after comprehensive management of traditional atherosclerotic risk factors. Serial measurements of CAVI, especially after treatment, provided important prognostic information on patients with CAD in clinical practice.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Original Contribution

RELATIONSHIP BETWEEN CHADS$_2$ SCORE AND COMPLEX AORTIC PLAQUES BY TRANSESOPHAGEAL ECHOCARDIOGRAPHY IN PATIENTS WITH NONVALVULAR ATRIAL FIBRILLATION

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Abstract—The CHADS$_2$ score is widely used for risk stratification of thromboembolism in patients with nonvalvular atrial fibrillation (NVAF). Although the correlation of CHADS$_2$ score with left atrial (LA) abnormality as detected by transesophageal echocardiography (TEE) has been reported in previous studies, the relationship between CHADS$_2$ score and complex aortic plaque, which is also a significant risk factor for thromboembolism, has not been fully investigated. We assessed aortic plaques by TEE in 150 patients age ≥ 55 y with NVAF. The prevalence of complex aortic plaques increased along with increases in CHADS$_2$ score (p = 0.001). In a multivariate analysis that included atherosclerotic risk factors and LA abnormality, a CHADS$_2$ score ≥2 was independently associated with the presence of complex aortic plaques (odds ratio [OR] 3.39; 95% confidence interval [CI], 1.29–8.90). A high CHADS$_2$ score is closely associated with the presence of complex aortic plaques, which explains, in part, the increased risk of thromboembolism in NVAF patients with high CHADS$_2$ score. (E-mail: k-sugioka@med.osaka-cu.ac.jp) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Atrial fibrillation, Aortic plaques, CHADS$_2$ score, Transesophageal echocardiography.**

INTRODUCTION

Currently, the CHADS$_2$ score (congestive heart failure, hypertension, age ≥75 y, diabetes mellitus, and stroke or transient ischemic attack [2 points]) (Gage et al. 2001) is the most commonly used method of stroke and thromboembolic risk stratification in patients with nonvalvular atrial fibrillation (NVAF) (Furie et al. 2012). Several studies have reported that a high CHADS$_2$ score is associated with the risk of left atrial (LA) abnormality such as LA thrombus or spontaneous echo contrast assessed by transesophagel echocardiography (TEE), which is established marker of thromboembolic risk (Puwanant et al. 2009; Rader et al. 2007; Yarmohammadi et al. 2013). Accordingly, these data mainly support the concept that high CHADS$_2$ score can identify patients eligible for oral anticoagulation.

Nonvalvular atrial fibrillation and systemic atherosclerosis are closely related and often coexist (Chang et al. 2002; Heeringa et al. 2007; Willeit et al. 2013). Blackshear et al. (1999) previously showed that aortic plaques as detected by TEE are common in NVAF patients. Furthermore, it has been reported that, in addition to LA abnormality, the presence of complex aortic plaques such as large plaques (>4 mm), ulcerated plaques, or mobile plaques is an important risk factor for stroke and thromboembolism in high-risk patients with NVAF (Zabalgoitia et al. 1998). Because most components of the CHADS$_2$ score consist of atherosclerotic risk factors, a high CHADS$_2$ score may predict the presence of severe aortic plaques. However, previous studies have focused on only the association of CHADS$_2$ score with LA abnormality, and the relationship between CHADS$_2$ score and complex aortic plaques has not been fully investigated. The purpose of the present study, therefore, was to

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evaluate the potential relationship between $CHADS_2$ scores and the presence of complex aortic plaques as detected by TEE in patients with NVAF.

METHODS

Study population

The study population included 221 consecutive patients age ≥55 y with atrial fibrillation who were referred for TEE from March 2010 to August 2013 in Osaka City University Hospital. We excluded 41 patients with significant primary valvular heart disease (≥moderate range) and 25 patients who underwent valvular surgery. We also excluded 5 patients in whom the large portion of the aortic arch could not be visualized adequately on TEE, although TEE has an inherent blind spot in the distal ascending aorta and the small portion of the aortic arch because of interfering tracheal air column (Kronzon and Tunick. 2006). Ultimately, 150 patients with NVAF were enrolled in the study (114 men; mean age, 67 ± 8 y). A total of 72 age-matched patients without atrial fibrillation who were referred for TEE served as controls (mean age, 67 ± 8 y). The study protocol was approved by the hospital's ethics committee, and written informed consent was obtained from each patient.

Clinical variables

Clinical variables, including risk factors such as age, hypertension, diabetes mellitus, a history of congestive heart failure, stroke or transient ischemic attack and vascular disease, were collected for each patient. Hypertension was defined as a systolic blood pressure ≥140 mm Hg or a diastolic blood pressure ≥90 mm Hg on two separate occasions, a patient's self-report of a history of hypertension or the use of anti-hypertensive medications. Diabetes mellitus was determined by the presence of an existing diagnosis, a fasting blood glucose level ≥126 mg/dL, glycohemoglobin A1c level ≥ 6.5% (NGSP) as defined by the Japan Diabetes Society (Seino et al. 2010) or the use of anti-diabetes medications or insulin. These clinical variables were used to calculate the $CHADS_2$ score with 1 point assigned to a history of congestive heart failure, hypertension, age ≥75 y and diabetes mellitus, and 2 points assigned to a history of stroke or transient ischemic attack (Gage et al. 2001), and the CHA_2DS_2-VASc score with 1 point assigned to a history of congestive heart failure, hypertension, diabetes mellitus, vascular disease (previous myocardial infarction, peripheral vascular disease or complex aortic plaques), age 65–74 and female gender, and 2 points assigned to age ≥75 y and a history of stroke or transient ischemic attack (Lip et al. 2010).

Information regarding the use of medications such as anti-coagulants, anti-platelet drugs, statins, angiotensin-converting enzyme inhibitors, and angiotensin II receptor blockers was obtained. Data on hypercholesterolemia and smoking status were also collected. Hypercholesterolemia was defined as a serum cholesterol value ≥220 mg/dL or low-density lipoprotein cholesterol ≥ 140 mg/dL by Japan Atherosclerotic Society 2007 guidelines (Teramoto et al. 2007), or the use of cholesterol-lowering medication. Patients were classified as non-smokers if they had never smoked or if they had stopped smoking for ≥10 y before the study. All other patients were classified as smokers.

Transesophageal echocardiographic analysis of aortic plaques

The method used for the assessment of aortic plaques in the thoracic aorta by performing TEE has been described in previous publications (Ito et al. 2013; Sugioka et al. 2002, 2011). Briefly, TEE was performed by using a commercially available ultrasound imaging system (iE33, Philips Medical Systems, Andover, MA, USA) with a 3-D matrix-array transesophageal transducer (X7-2t). After routine examinations of cardiac structures by TEE, the transducer was gradually withdrawn from the descending aorta to the level of the aortic arch. We evaluated the presence, thickness and characteristics of aortic plaques in the thoracic aorta. Plaques were defined as discrete protrusions of the intimal surface of the vessel, ≥2 mm in thickness and different in appearance and echogenicity from the adjacent intact intimal surface. Plaque thickness was measured in the horizontal plane, perpendicular to the major axis of the aortic lumen. In cases of multiple plaques, the most advanced lesion was considered. Ulceration was defined as a discrete indentation of the luminal surface of the plaque with a base width and maximum depth of at least 2 mm each (Di Tullio et al. 2000, 2009). Complex plaques were defined as large plaques (≥4 mm in thickness), plaques with ulceration, or plaques with mobile components (Kronzon and Tunick 2006; Sugioka et al. 2011) (Fig. 1). Echocardiographic studies were interpreted by an experienced echocardiographer who was blinded to patient information (K.S.).

Statistical analysis

The results are expressed as mean ± SD. When two groups were compared, the unpaired *t*-test or Mann-Whitney *U* test was used, as appropriate. Categorical variables were compared using a chi-square test or Fisher's exact test. Prevalence of aortic plaques and complex plaques were compared across the level of $CHADS_2$ score. Univariate logistic regression analysis was used to assess the association of clinical factors and $CHADS_2$ scores with the presence of complex plaques. The association between $CHADS_2$ scores and the presence of complex

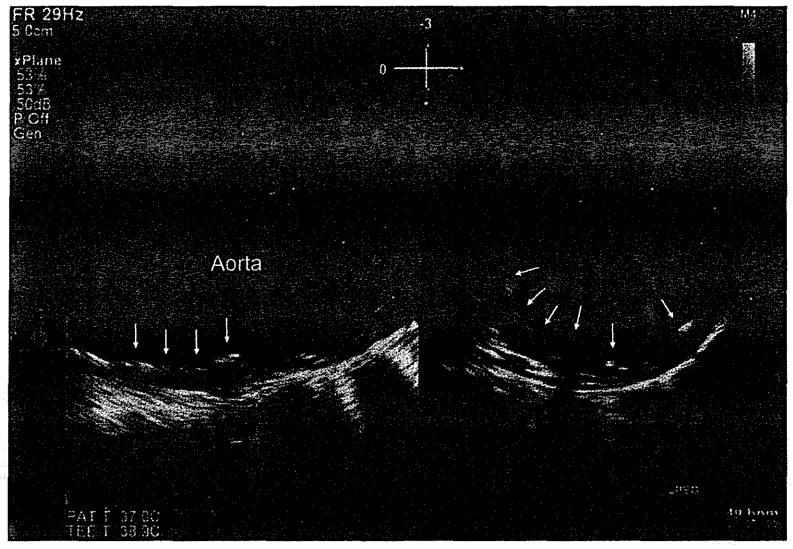

Fig. 1. Transesophageal echocardiography (TEE) images of complex aortic plaques in a nonvalvular atrial fibrillation (NVAF) patient with a $CHAD_2$ score of 4. Simultaneous multi-plain imaging by real-time 3-D TEE provided both long-axis view *(left panel)* and short-axis view *(right panel)* of the aortic arch and showed large plaques with ulcerations *(arrows)*.

plaques was then investigated by using multivariate logistic regression analysis. Only variables with a significant value on univariate analysis were included in the multivariate model. Age and $CHADS_2$ score were entered into the regression analysis as continuous variables. p Values <0.05 were considered significant.

RESULTS

Patient characteristics

The characteristics of the 150 patients with NVAF and 72 controls are shown in Table 1. There was no significant difference between NVAF patients and controls with respect to the prevalence of male, hypercholesterolemia, smoking, congestive heart failure, hypertension or diabetes mellitus. NVAF patients had a significantly higher prevalence of previous stroke or transient ischemic attack ($p = 0.03$), vascular disease ($p = 0.01$) and the use of anti-coagulants ($p < 0.001$) and a lower left ventricular ejection fraction ($p < 0.001$) than controls.

Transesophageal echocardiographic findings

Among the 150 NVAF patients, 104 patients (69%) received anti-coagulant therapy. LA thrombus was detected by TEE in 8 patients (5%) and spontaneous echo contrast in 42 patients (28%). Furthermore, aortic plaques were detected in 102 patients (68%), including complex plaques in 45 patients (30%) (large plaques in 41 [27%], ulcerated plaques in 32 [21%] and mobile plaques in 7 patients [5%]). In 72 control subjects, aortic plaques were detected in 33 patients (46%), including complex plaques in 8 patients (11%) (large plaques in 8 [11%], ulcerated plaques in 7 [10%] and mobile plaques in 0 patients [0%]). Patients with NVAF had a significantly greater prevalence of any plaques (68% vs. 46%; $p = 0.001$) and complex plaques (30% vs. 11%; $p = 0.002$) compared with controls (Fig. 2).

$CHADS_2$ score and aortic plaques

The overall mean $CHADS_2$ score in patients with NVAF was 1.6 ± 1.3. Aortic plaques were detected in 14 of 31 patients with a $CHADS_2$ score of 0 (45%), 29 of 46 patients with a $CHADS_2$ score of 1 (63%), 28 of 38 patients with a $CHADS_2$ score of 2 (74%) and 31 of 35 patients with a $CHADS_2$ score ≥ 3 (89%). Complex plaques were detected in 5 patients with a $CHADS_2$ score of 0 (16%), 8 patients with a $CHADS_2$ score of 1 (17%), 14 patients with a $CHADS_2$ score of 2 (37%) and 18 patients with a $CHADS_2$ score ≥ 3 (51%). The mean $CHADS_2$ score was significantly higher in patients with aortic plaques (1.9 ± 1.3 vs. 1.1 ± 1.1, $p < 0.001$) and complex plaques (2.2 ± 1.4 vs. 1.4 ± 1.2, $p < 0.001$) than in those without them. The prevalence of aortic plaques and complex plaques increased with increases in $CHADS_2$ score in NVAF patients ($p = 0.001$, each) (Fig. 3a).

In control group, aortic plaques were detected in 4 of 18 patients with a $CHADS_2$ score of 0 (22%), 8 of 23 patients with a $CHADS_2$ score of 1 (35%), 14 of 22 patients with a $CHADS_2$ score of 2 (64%), and 7 of 9 patients with a $CHADS_2$ score ≥ 3 (78%). Complex plaques were detected in 0 patients with a $CHADS_2$ score of 0 (0%),

Table 1. Patient characteristics in NVAF patients and controls

	NVAF (n = 150)	Controls (n = 72)	*p*
Age (y)	67 ± 8	67 ± 8	—
Males	114 (76%)	49 (68%)	0.21
Hypercholesterolemia	51 (34%)	25 (35%)	0.88
Smoking	55 (37%)	22 (31%)	0.29
Congestive heart failure	39 (26%)	17 (24%)	0.63
Hypertension	95 (63%)	44 (61%)	0.72
Diabetes mellitus	41 (27%)	15 (21%)	0.28
Stroke or TIA	27 (18%)	5 (7%)	0.03
$CHADS_2$ score	1.6 ± 1.3	1.3 ± 1.1	0.08
0	31 (21%)	18 (25%)	
1	46 (31%)	23 (32%)	
2	38 (25%)	22 (31%)	
≥3	35 (23%)	9 (13%)	
Vascular disease	51 (34%)	13 (18%)	0.01
CHA_2DS_2-VASc score	2.8 ± 1.8	2.3 ± 1.5	0.06
Framingham risk score (%)	13 ± 2	13 ± 2	0.80
Paroxysmal AF	73 (49%)	—	—
LVEF (%)	53 ± 11	59 ± 10	<0.001
LA diameter (mm)	45 ± 9	42 ± 5	0.08
LA abnormality			
LA thrombus	8 (5%)	0 (0%)	—
Spontaneous echo contrast	42 (28%)	0 (0%)	—
Medications			
Antiplatelet	33 (22%)	10 (14%)	0.19
Anticoagulants	104 (69%)	5 (7%)	<0.001
Statin	36 (24%)	15 (21%)	0.48
ACEI/ARB	68 (45%)	37 (51%)	0.30

ACEI = angiotensin-converting enzyme inhibitors; AF = atrial fibrillation; ARB = angiotensin II receptor blockers; LA = left atrial; LVEF = left ventricular ejection fraction; NVAF = nonvalvular atrial fibrillation; TIA = transient ischemic attack.

Categorical data are expressed as n (%) and continuous data as mean ± SD.

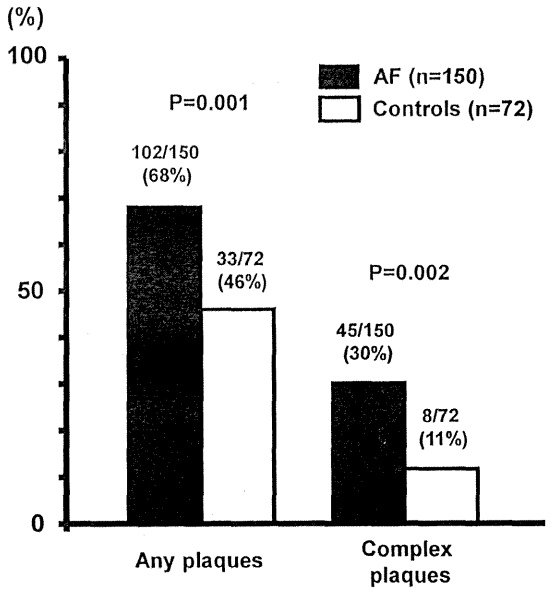

Fig. 2. Prevalence of aortic plaques in nonvalvular atrial fibrillation (NVAF) patients and controls. Complex plaques were defined as large plaques (≥4 mm in thickness), ulcerated plaques or mobile plaques. Among the 150 NVAF patients, aortic plaques were detected in 102 patients (68%), including complex plaques in 45 patients (30%) (large plaques in 41 [27%], ulcerated plaques in 32 [21%] and mobile plaques in 7 patients [5%]). In 72 control subjects, aortic plaques were detected in 33 patients (46%), including complex plaques in 8 patients (11%) (large plaques in 8 [11%], ulcerated plaques in 7 [10%] and mobile plaques in 0 patients [0%]). The prevalence of any plaques ($p = 0.001$) and complex plaques ($p = 0.002$) was greater in NVAF patients than in controls.

1 patients with a $CHADS_2$ score of 1 (4%), 4 patients with a $CHADS_2$ score of 2 (18%), and 3 patients with a $CHADS_2$ score ≥ 3 (33%). The prevalence of aortic plaques ($p = 0.006$) and complex plaques ($p = 0.03$) increased with increases in $CHADS_2$ score in controls (Fig. 3b).

Relation of $CHADS_2$ score to the presence of complex aortic plaques

In NVAF patients, univariate logistic analyses showed that age (odds ratio [OR] 1.06 per y increase; 95% confidence interval [CI], 1.01–1.11; $p = 0.01$), smoking (OR 2.31; 95% CI, 1.12–4.80; $p = 0.02$), left ventricular ejection fraction <40% (OR 3.17; 95% CI, 1.23–8.14; $p = 0.02$), LA abnormality (OR 2.16; 95% CI, 1.03–4.54; $p = 0.041$), $CHADS_2$ score (OR 1.60 per unit increase; 95% CI, 1.21–2.12; $p = 0.001$), history of congestive heart failure (OR 2.17; 95% CI, 1.01–4.63; $p = 0.046$), diabetes mellitus (OR 3.72; 95% CI, 1.74–7.97; $p < 0.001$) and $CHADS_2$ score ≥2 (OR 5.02; 95% CI, 2.29–11.02; $p < 0.001$) were associated with the presence of complex plaques. In multivariate logistic analyses adjusted for these significant variables, a $CHADS_2$ score ≥2 was found to be an independent predictor of complex plaques in patients with NVAF (OR 3.39; 95% CI, 1.29–8.90; $p = 0.01$) (Table 2).

In control subjects, univariate logistic analyses showed that hypercholesterolemia (OR 7.17; 95% CI, 1.32–38.93; $p = 0.02$), congestive heart failure (OR 7.22; 95% CI, 1.51–34.48; $p = 0.01$), $CHADS_2$ score (OR 2.72 per unit increase; 95% CI, 1.28–5.76; $p = 0.009$) and $CHADS_2$ score ≥2 (OR 11.67; 95% CI, 1.35–100.73; $p = 0.03$) were associated with the presence of complex aortic plaques. In multivariate logistic analyses adjusted for these significant variables, a $CHADS_2$ score ≥2 was not an independent predictor of complex plaques in controls (OR 5.10; 95% CI, 0.48–54.56; $p = 0.18$) (Table 3).

DISCUSSION

To the best of our knowledge, this is the first study to demonstrate the correlation between $CHADS_2$ score and complex aortic plaques as detected by TEE in patients with NVAF. In the present study, we found that the

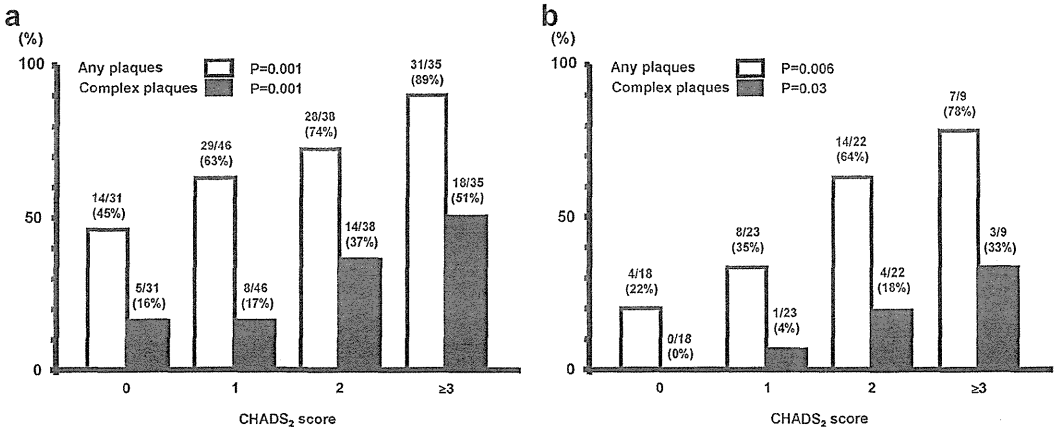

Fig. 3. (a) Prevalence of aortic plaques according to the $CHADS_2$ score in patients with nonvalvular atrial fibrillation (NVAF). The prevalence of aortic plaques and complex plaques increased with increases in $CHADS_2$ score ($p = 0.001$, each). (b) Prevalence of aortic plaques according to the $CHADS_2$ score in controls. The prevalence of aortic plaques ($p = 0.006$) and complex plaques ($p = 0.03$) increased with increases in $CHADS_2$ score.

prevalence of complex aortic plaques increased along with increases in $CHADS_2$ score, and $CHADS_2$ score ≥ 2 was independently associated with the presence of complex aortic plaques after adjustment for atherosclerotic risk factors and LA abnormality in NVAF patients. A high $CHADS_2$ score is closely related to the presence of complex aortic plaques, suggesting that the increased risk of thromboembolism in NVAF patients with high $CHADS_2$ score may be partly explained by high prevalence of complex aortic plaques.

Previous studies have reported an association between AF and manifestations of systemic atherosclerosis such as carotid atherosclerosis (Chang et al. 2002; Heeringa et al. 2007; Willeit et al. 2013), coronary artery disease (Benjamin et al. 1994; Krahn et al. 1995) or aortic atherosclerosis (Agmon et al. 2001; Blackshear et al.1999). In addition, our study found that there is a significantly higher prevalence of complex aortic plaques in patients with NVAF compared with controls, which is consistent with previous studies reporting a close relationship between NVAF and systemic atherosclerosis. The mechanisms of the relationship between AF and systemic atherosclerosis have been speculated (Willeit et al. 2013). Aortic atherosclerosis may induce elevated aortic stiffness or pulse pressure, which increases systolic cardiac afterload. This condition leads to left ventricular hypertrophy, increased LA pressure, and LA dysfunction which predisposes to the development of AF (Mitchell et al. 2007). Moreover, atherosclerosis in the coronary arteries may also directly cause ischemia or transitory hypoperfusion in the atrium, resulting in fibrosis and the occurrence of AF (Nucifora et al. 2009).

Table 2. Logistic regression analyses regarding the presence of complex aortic plaques in patients with NVAF (n = 150)

	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (per y)	1.06 (1.01–1.11)	0.01	1.05 (1.00–1.11)	0.07
Males	2.07 (0.83–5.16)	0.12		
Hypercholesterolemia	0.94 (0.45–1.98)	0.88		
Smoking	2.31 (1.12–4.80)	0.02	2.58 (1.10–6.05)	0.03
LVEF < 40%	3.17 (1.23–8.14)	0.02	2.40 (0.80–6.98)	0.12
LA abnormality	2.16 (1.03–4.54)	0.041	1.58 (0.68–3.66)	0.29
$CHADS_2$ score (per unit)	1.60 (1.21–2.12)	0.001		
Congestive heart failure	2.17 (1.01–4.63)	0.046		
Hypertension	1.91 (0.89–4.11)	0.10		
Age ≥75 y	2.02 (0.87–4.73)	0.10		
Diabetes mellitus	3.72 (1.74–7.97)	<0.001	1.40 (0.55–3.59)	0.48
Stroke/TIA	1.59 (0.66–3.84)	0.30		
$CHADS_2$ score ≥ 2	5.02 (2.29–11.02)	<0.001	3.39 (1.29–8.90)	0.01

CI = confidence interval; LA = left atrial; LVEF = left ventricular ejection fraction; NVAF = nonvalvular atrial fibrillation; OR = odds ratio; TIA = transient ischemic attack.

Table 3. Logistic regression analyses regarding the presence of complex aortic plaques in patients with controls (n = 72)

	Univariate		Multivariate	
	OR (95% CI)	*p*	OR (95% CI)	*p*
Age (per y)	1.09 (0.98–1.21)	0.12		
Males	3.67 (0.42–31.73)	0.24		
Hypercholesterolemia	7.17 (1.32–38.93)	0.02	7.49 (1.15–48.62)	0.03
Smoking	2.65 (0.59–11.79)	0.20		
$CHADS_2$ score (per unit)	2.72 (1.28–5.76)	0.009		
Congestive heart failure	7.22 (1.51–34.48)	0.01	4.81 (0.73–31.83)	0.10
Hypertension	4.86 (0.56–42.01)	0.15		
Age ≥75 y	1.61 (0.29–9.03)	0.59		
Diabetes mellitus	0.53 (0.06–4.68)	0.57		
Stroke/TIA	6.78 (0.94–48.90)	0.06		
$CHADS_2$ score ≥ 2	11.67 (1.35–100.73)	0.03	5.10 (0.48–54.56)	0.18

CI = confidence interval; OR = odds ratio; TIA = transient ischemic attack.

The $CHADS_2$ score is widely used in clinical practice to guide decisions regarding the need for anticoagulants in AF patients, and oral anticoagulation is recommended for NVAF patients with a $CHADS_2$ score ≥2 (Furie et al. 2012). Several studies have demonstrated that $CHADS_2$ score is associated with the presence of LA abnormality (Puwanant et al. 2009; Rader et al. 2007; Yarmohammadi et al. 2013). By contrast, the association between $CHADS_2$ score and systemic atherosclerosis has recently been found in NVAF patients because most $CHADS_2$ components are risk factors for atherosclerosis. Recently, Kim et al. (2011) reported that a high $CHADS_2$ score is correlated with intra-cerebral atherosclerosis in patients with NVAF. In the present study, we found that a $CHADS_2$ score ≥2 is independently associated with complex aortic plaques in NVAF patients after adjustment for LA abnormality. The presence of complex aortic plaques has been reported to be a significant marker for cerebral embolism, especially in high-risk patients with NVAF (Zabalgoitia et al. 1998) or in elderly patients with atrial fibrillation (Shinokawa et al. 2001). Therefore, our findings indicate that increased risk of thromboembolism in NVAF patients with high $CHADS_2$ scores is partly explained by an increased frequency of complex aortic plaques. The combination of NVAF and vascular diseases has been recognized as having an unfavorable impact on stroke risk (Olesen et al. 2012). A meta-analysis has confirmed that coexistence of NVAF and complex aortic plaques as detected by TEE is a significant predictor of stroke, thromboembolism and mortality (Anandasundaram et al. 2013). Moreover, the recently developed CHA_2DS_2-VASc score incorporates complex aortic plaques as a new category of vascular disease and has been proposed as an improvement to the $CHADS_2$ score, specifically for discriminating risk in lower risk patients (Lip et al. 2010).

The present study has some limitations. First, our patient population may not be representative of the NVAF patients as a whole because it included only NVAF patients who were referred to TEE. Second, in this study, age and hypercholesterolemia had the low ORs for the prediction of aortic complex plaques in NVAF group. Our study, however, focused on the patients aged ≥55 years who appeared to have aortic plaques. Furthermore, approximately 70% of the patients with hypercholesterolemia received statin treatment, which has been reported to be effective on the regression of aortic plaques (Corti et al. 2002; Lima et al. 2004). These may affect the low ORs of age and hypercholesterolemia in the present study. Third, we performed logistic regression analyses in control group as well as in NVAF group; however, these analyses may be limited by the small sample size (n = 72) and the low number of complex aortic plaques (n = 8) in control group. Finally, we investigated only Japanese patients with NVAF. The prevalence of aortic atherosclerosis may vary between Japan and Western countries.

In conclusion, this study found a close relationship between complex aortic plaques as detected by TEE and high $CHADS_2$ scores, which are widely used for the stratification of stroke and thromboembolic risk in patients with NVAF. These findings suggest that, in addition to LA abnormality, complex aortic plaques should also be considered among the mechanisms of stroke and thromboembolism in NVAF patients with high $CHADS_2$ scores.

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Short Communication

A Noninvasive Metabolic Syndrome Model Using an Extremely Small Minipig, the Microminipig

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Abstract. Metabolic syndrome (MetS) induces serious complications; therefore, we developed a noninvasive MetS model using an extremely small minipig, the Microminipig. For 8 weeks, Microminipigs were administrated a high-fat and high-cholesterol diet (HFCD) for atherosclerosis and N^G-nitro-L-arginine methyl ester (L-NAME) for inhibiting nitric oxide synthase. HFCD significantly increased serum low-density lipoprotein levels, L-NAME increased blood pressure and cardiac hypertrophy, and HFCD-induced aortal arteriosclerosis was accelerated by L-NAME administration. Endothelium-dependent relaxation of the coronary artery was remarkably decreased by L-NAME administration. This model may be useful for elucidating the mechanisms of MetS and developing new therapeutic medicines for its treatment.

Keywords: metabolic syndrome, Microminipig, nitric oxide

Metabolic syndrome (MetS) is a significant risk factor for cardiovascular diseases and increased morbidity and mortality (1). Recent westernization of the Japanese lifestyle has increased the number of MetS patients in Japan. Because this increase is strongly related to atherosclerosis, various animal atherosclerosis models have been developed using mice, rabbits, and swine. Atherosclerosis is influenced by several genetic and environmental factors. Mice were originally resistant to a high-fat and high-cholesterol diet (HFCD) that induces atherosclerosis. Mice lacking apolipoprotein-E and Watanabe heritable hyperlipidemic rabbits lacking low-density lipoprotein (LDL) receptor have been reported as models of atherosclerosis with genetic abnormality (2, 3). In contrast, nutritional manipulation by HFCD has been used to develop swine atherosclerosis models with environmental factors because their physiology and sleep and feeding habits are similar to those of humans (4). Recently, the world's smallest pig, the Microminipig™, was developed (5) as has a hyperlipidemia-induced atherosclerosis model (6, 7). In the present study, we established a MetS model by feeding Microminipigs HFCD and administering the nitric oxide synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME; Sigma Chemical Co., St. Louis, MO, USA).

Male 3.5- to 4.5-month-old Microminipigs (Fuji Micra, Inc., Shizuoka) were used in this study. All procedures were performed in accordance with Osaka

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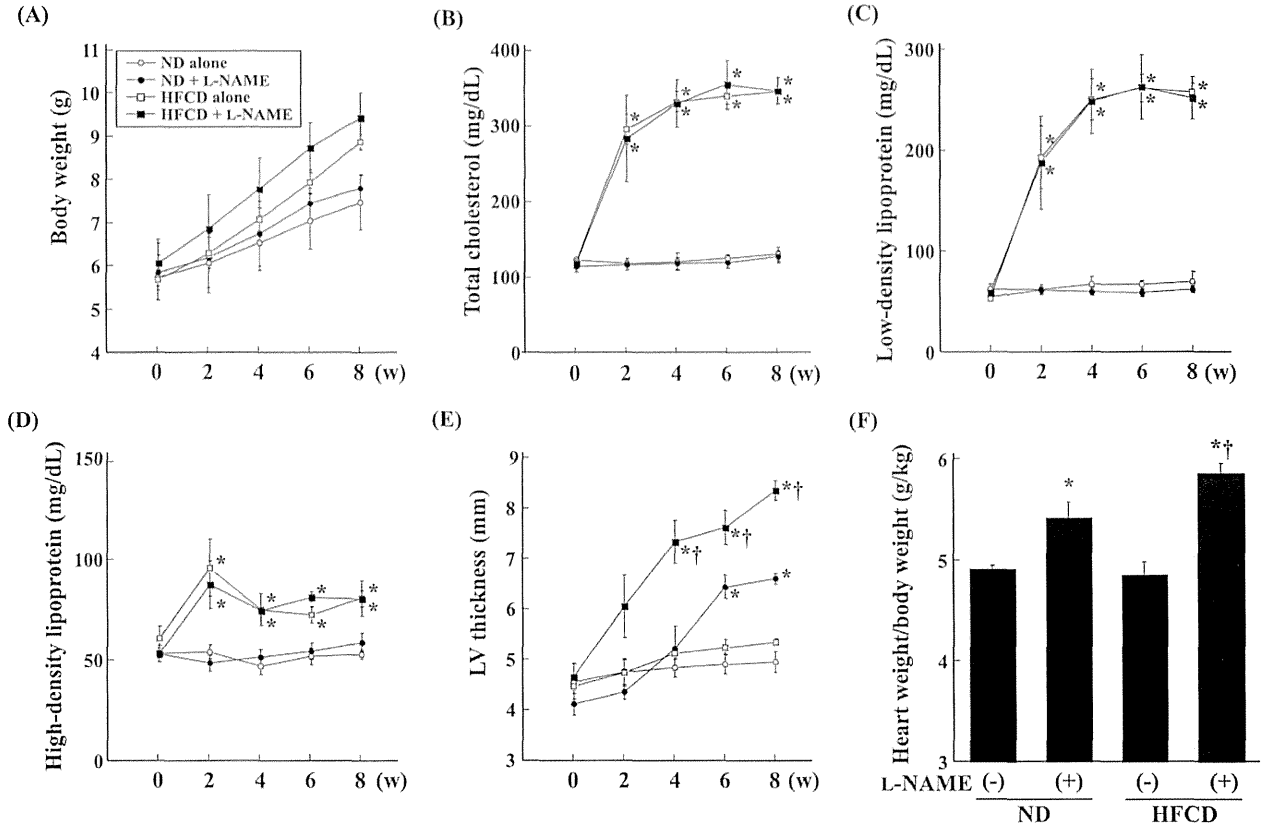

Fig. 1. Time course of body weight (A), serum lipoprotein levels (B – D), left ventricular thickness (E), and left ventricular weight after 8 weeks (F). All values are reported as the mean ± S.E.M. $^{*}P < 0.05$ vs. ND alone; $^{\dagger}P < 0.05$, ND + L vs. HFCD + L. ND alone, normal diet alone; ND + L, normal diet and L-NAME; HFCD alone, high-fat and high-cholesterol diet alone; HFCD + L, high-fat and high-cholesterol diet and L-NAME; L-NAME, N^{G}-nitro-L-arginine methyl ester.

City University animal care guidelines, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Microminipigs were divided into 4 groups: groups fed a normal chow diet (ND; Kodakara 73; Marubeni Nisshin Feed, Tokyo) without (ND alone, n = 3) or with L-NAME administration (80 $mg \cdot kg^{-1} \cdot day^{-1}$) (ND + L, n = 2) and groups fed HFCD (Kodakara 73 including 12% fat and 0.5% cholesterol) without (HFCD alone, n = 3) or with L-NAME administration (HFCD + L, n = 3). The bait and L-NAME were given twice every day for 8 weeks. Serum lipoprotein level measurements and echocardiography by a Xario ultrasound device (Toshiba Medical Systems, Tokyo) were performed every 2 weeks according to previously described methods (8).

After 8 weeks, blood pressure was measured by cannulation into the Microminipigs' femoral artery, with 10 mg/kg of intramuscular tiletamine anesthesia (United States Pharmacopeial Convention, Inc., Rockville, MD, USA); animals then were sacrificed under deep anesthesia with an additional 40 mg/kg of intravenous sodium pentobarbital (Kyoritsu Seiyaku, Tokyo). The isolation and preparation for relaxation of the proximal right coronary arteries were performed as previously described (9). Isometric mechanical responses of the coronary artery strips were displayed on a pen recorder, as previously reported (9, 10).

The aortas were longitudinally incised and fixed with formalin, followed by staining with Oil-red O stain for an *en face* analysis (6). All data are presented as the mean ± S.E.M. Student's *t*-test was used for differences between groups. The differences were considered statistically significant at $P < 0.05$.

Body weight gain in the HFCD-fed groups tended to be accelerated, but no significant differences were observed among the groups (Fig. 1A). Systolic blood pressure was higher in the L-NAME-administered groups (186 ± 11 mmHg) than in the groups not administered L-NAME (135 ± 3 mmHg).

HFCD induced hypercholesterolemia in the Microminipigs (Fig. 1: B – D). Serum total cholesterol and

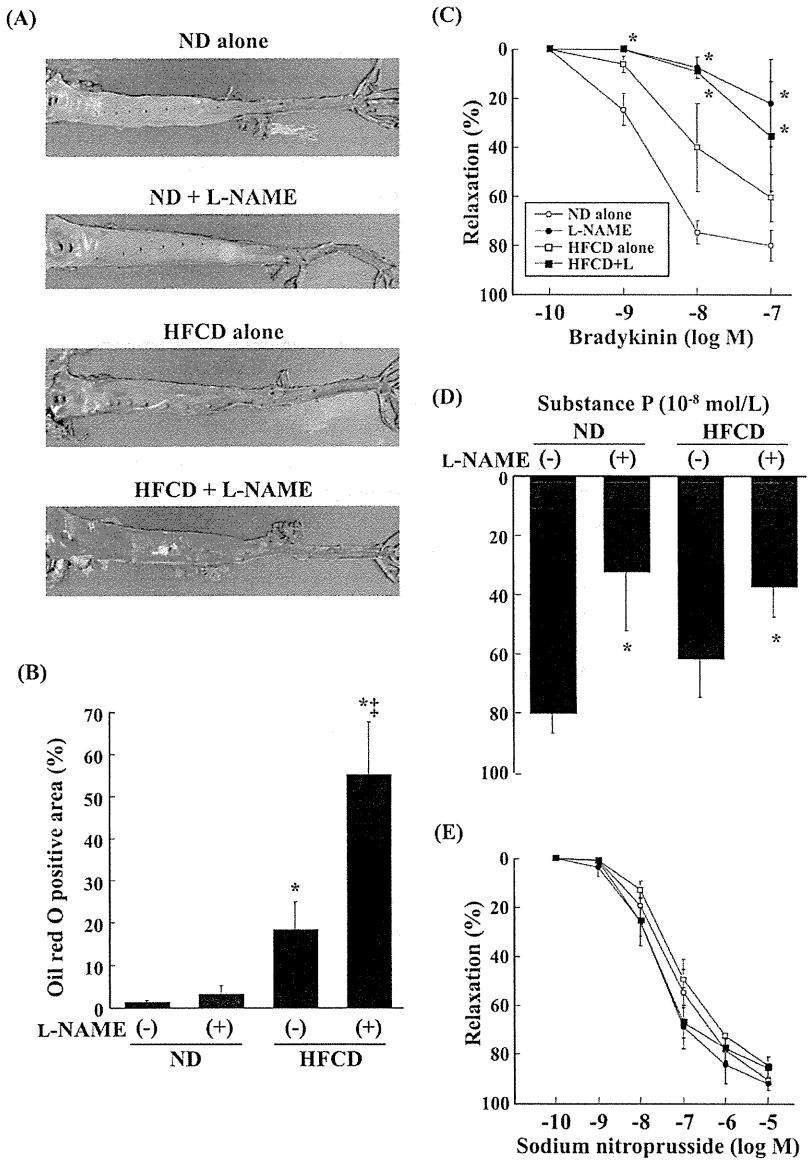

Fig. 2. Representative Oil-red O-stained atherosclerotic lesion (A) and the percentage of atherosclerotic areas (B) in each group. The relaxation responses of the coronary arteries by bradykinin (C), substance P (D), and sodium nitroprusside (E). All values are reported as the mean ± S.E.M. $^{*}P < 0.05$ vs. ND alone; $^{\ddagger}P < 0.05$, HFCD alone vs. HFCD + L. See the Fig. 1 legend for abbreviations.

LDL levels were significantly increased in the HFCD groups compared with the ND-fed group. Serum high-density lipoprotein levels were also increased in the HFCD-fed groups. In contrast, serum triglyceride levels were similar in all groups. The fasting blood glucose level after 8 weeks of HFCD and L-NAME administration was similar to that of the ND alone group (54 ± 1 vs. 55 ± 3 $mg \cdot dL^{-1}$, respectively).

Left ventricular (LV) wall thickness and heart weight were significantly increased in the ND + L group compared with the ND-alone group (Fig. 1: E and F). Interestingly, LV wall thickness and heart weight were greater in the HFD + L group than in the ND + L group, whereas there were no differences between the ND-alone and HFCD-alone groups. These results suggest that HFCD accelerates L-NAME-induced LV hypertrophy. LV ejection fraction was kept in all four groups during the experimental period.

The *en face* analysis of aortas demonstrated that aortic atherosclerotic lesions were significantly increased in the HFCD-alone group (Fig. 2: A and B). L-NAME enhanced more HFCD-induced atherosclerotic lesions, although there was no difference between the ND-alone and ND + L groups.

The addition of bradykinin produced a dose-dependent relaxation in the coronary arteries of the ND-alone group (Fig. 2C). Bradykinin- and substance P-induced relaxation of the coronary arteries was remarkably suppressed in the L-NAME-administered groups (Fig. 2: C and D). On the other hand, sodium nitroprusside-induced

relaxation did not differ among all four groups. These results suggest that L-NAME administration reduced endothelium-dependent relaxation. Compared with the ND + L group, a weaker response in the HFCD + L group was not observed in the present study, possibly because the amount of deterioration by L-NAME alone was considerably strong.

We conclude that the nitric oxide synthase inhibitor L-NAME accelerates atherosclerosis when given with a HFCD. This Microminipig model, although it does not include diabetes mellitus which is a component of MetS, can be useful for elucidating the mechanisms of MetS and developing the therapeutic medicines for its treatment.

Acknowledgment

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Conflicts of Interest

None declared.

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RAPID COMMUNICATION

Differences Between Rosuvastatin and Atorvastatin in Lipid-Lowering Action and Effect on Glucose Metabolism in Japanese Hypercholesterolemic Patients With Concurrent Diabetes

– Lipid-Lowering With Highly Potent Statins in Hyperlipidemia With Type 2 Diabetes Patients (LISTEN) Study –

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Background: Little is known about the differences between standard-dose statins effects on glucose level and lipids in Japanese patients with diabetes mellitus (DM).

Methods and Results: The 1,049 patients were randomly assigned to either the rosuvastatin group or atorvastatin group. There were no significant differences between the 2 groups in the effect on non-high-density lipoprotein cholesterol (non-HDL-C) and HbA1c at 12 months. However, physicians tended to switch to more intensive therapy for DM in the atorvastatin group.

Conclusions: Rosuvastatin 5 mg and atorvastatin 10 mg have a similar lowering effect on non-HDL-C, but might be different in terms of adverse effect on glucose levels. (*Circ J* 2014; **78:** 2512–2515)

Key Words: Hypercholesterolemia; Statins; Type 2 diabetes mellitus

The clinical benefit of preventing cardiovascular events by using statins in hypercholesterolemic patients is well established.[1,2] The benefit in hypercholesterolemic patients with diabetes mellitus (DM) has also been demonstrated in several randomized trials,[3–5] but recent data showed that statins are associated with an increased risk of new-onset DM[6,7] and that the risk is dose dependent.[8] Some reports suggested the suppressive effect of statin on cardiovascular events outweighs the risk of DM onset.[9–11] The guidelines show the rationale for statin therapy to prevent cardiovascular events based on risk stratification for each patient, which includes DM.[12,13] However, few prospective, randomized, controlled studies have been conducted to investigate the effect of statin therapy on glucose levels in patients with DM, although such data would greatly contribute to decision making in the clinical setting. We conducted this study to examine the effects of statins on both glucose and lipid levels in Japanese patients with DM. The final result will be presented in a hot-line session of the ESC congress 2014.

Methods

This study was a 12-month multicenter open-label randomized, comparative study. The protocol was approved by the institutional review board. All patients provided written informed consent.

The study included hypercholesterolemic patients with type

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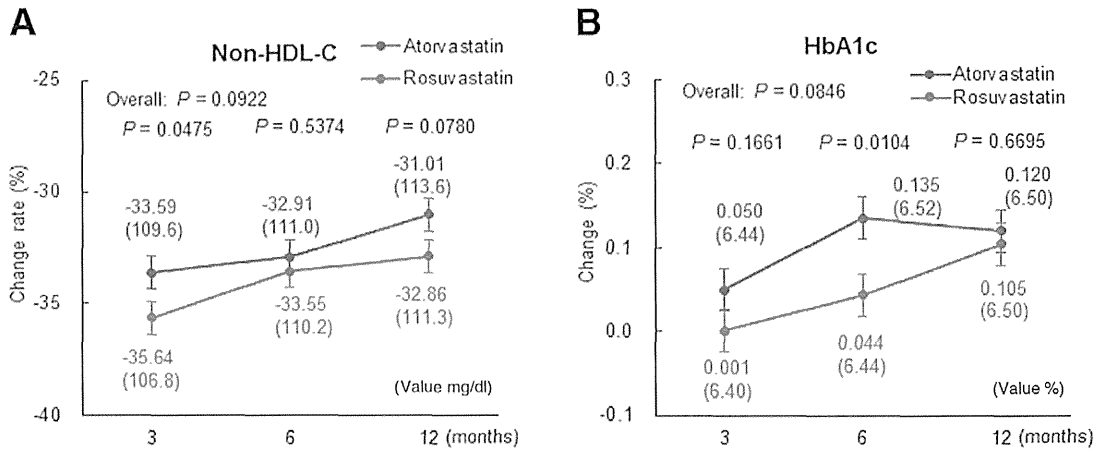

Figure 1. Percent change in non-HDL-C (A) and change in HbA1c (B). The analyses were performed using repeated measures ANOVA for overall comparison between the 2 statin treatment groups. The unpaired t-test adjusted by Holm's method was used for intergroup comparison to avoid multiplicity at multiple time points. Non-HDL-C, non-high-density lipoprotein cholesterol.

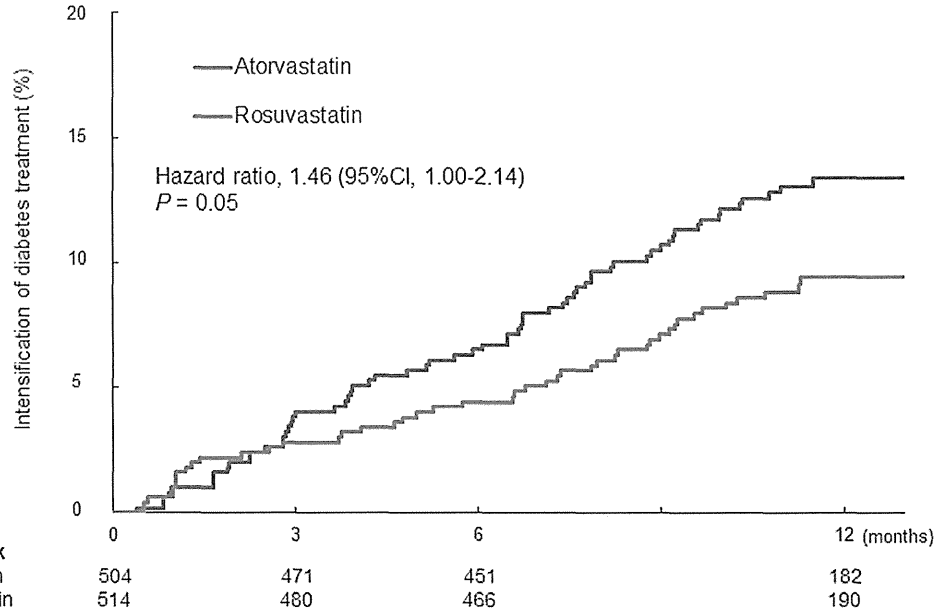

Figure 2. Cumulative Kaplan-Meier estimates of the incidence of the patients who were switched to more intensive therapy for diabetes mellitus. The atorvastatin group had therapy intensified more often than the rosuvastatin group during the study period (P=0.05). CI, confidence interval.

2 DM whose HbA1c (Japan Diabetes Society [JDS]) was <7.0% (or <7.4%: National Glycohemoglobin Standardization Program [NGSP]). Patients were excluded if they had received rosuvastatin or atorvastatin before registration. Patients were randomly assigned to the rosuvastatin 5 mg group or the atorvastatin 10 mg group at registration.

The primary endpoints were the percentage change in non-high-density lipoprotein cholesterol (non-HDL-C) and the change in HbA1c. Secondary endpoints were changes in other lipids, glucose metabolism parameters, and any intensification of DM treatment (ie, drugs were added and/or increased for DM), which was assessed independently by 2 investigators. The original agreement on κ score was 0.6316, which was fairly good, and discrepancies were fixed by direct discussion.