Original Article

The Relationships of Cardiovascular Disease Risk Factors to Flow-Mediated Dilatation in Japanese Subjects Free of Cardiovascular Disease

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We examined the relationship of several cardiovascular disease (CVD) risk factors to flow-mediated vasodilatation of the brachial artery (FMD) and the clustering effect of risk factors on FMD in Japanese subjects free of CVD. In 819 Japanese subjects free of CVD (age 45 ± 10 years, 611 men and 208 women), FMD correlated significantly with the Framingham risk points score (FRS) ($r = -0.27$, $p < 0.01$). FMD was higher in subjects in the 1st tertile of the FRS range than in patients in either the 2nd or 3rd tertiles of the FRS range, but it was similar between the subjects in the 2nd and 3rd tertiles. FMD was found to have a significant independent negative correlation with age ($\beta = -0.19$, $p < 0.01$), gender ($\beta = -0.024$, $p < 0.01$) and smoking habit ($\beta = -0.08$, $p = 0.02$). In subjects ≥ 50 years of age, the FMD in men with one CVD risk factor, excluding smoking, was similar to that in men with no CVD risk factors. CVD risk factors did not attenuate FMD in women. Thus, in Japanese subjects free of CVD, FMD may be a useful marker of CVD risk, but it may not be a robust marker for endothelial damage related to clusters of CVD risk factors. Age, gender and smoking were independent variables related to the impairment of FMD, which therefore appears to be less applicable in subjects aged 50 or more, and especially in women. (*Hypertens Res* 2008; 31: 2019–2025)

Key Words: flow-mediated dilatation, age, gender, risk factors

Introduction

Endothelial dysfunction is observed in the early stages of atherosclerosis, and this abnormality can be assessed by measuring flow-mediated dilatation of the brachial artery (FMD) (1–3). FMD is thought to be a marker of vascular damage and/or a predictor of future cardiovascular events in subjects with cardiovascular disease (CVD) risk factors (1–3). Some large-cohort studies have demonstrated heterogeneities in the relationships of CVD risk factors and FMD (4–10), as well as

racial differences in FMD (8, 11). A recent study suggested that the augmentation of the FMD impairment caused by the cluster of CVD risk factors is modest (12). However, in Japanese healthy subjects, the heterogeneities of the relationship of each CVD risk factor to FMD, and the clustering effect of CVD risk factors on FMD, have not been fully clarified.

The present study was conducted to examine the relationships and clustering of CVD risk factors with respect to FMD, and the relationship of FMD to Framingham risk points score (FRS), an established marker of CVD risk, in Japanese subjects free of CVD.

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Table 1. Clinical Characteristics of the Study Subjects

Variable	All subjects	Men	Women
Number	819	611	208
Age (years)	45±10	46±10	44±10
BMI	23.0±3.2	23.7±3.1	21.1±2.7
Smoking (%)	195 (24)	172 (28)	23 (11)
SBP (mmHg)	120±15	124±14	111±14
DBP (mmHg)	74±11	75±11	68±10
TC (mmol/L)	5.3±0.8	5.3±0.8	5.3±0.9
HDL (mmol/L)	1.6±0.4	1.5±0.4	1.9±0.4
TG (mmol/L)	1.4±1.0	1.5±1.1	0.9±0.7
FPG (mmol/L)	5.3±0.6	5.4±0.6	5.0±0.5

BMI, body mass index; Smoking, number of subjects with a history of smoking; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, serum total cholesterol; HDL, serum high-density lipoprotein cholesterol; TG, serum triglycerides; FPG, fasting plasma glucose.

Methods

Design and Subjects

This cross-sectional study was conducted in the health care centers of a food product company and an electronics company, as well as a health care clinic. At each location, each participant was informed about the measurement of FMD and the examination of its relationship with CVD risk factors. After obtaining written informed consent, measurement of FMD was conducted in addition to the routine annual health checkup, which included an evaluation of atherosclerotic risk factors. All measurements were performed from May to September 2007. A total of 1,015 subjects aged ≥ 30 years agreed to enter the study. We excluded 22 female subjects who were menstruating at the time of the annual health check-up, 23 patients who were receiving treatment for CVD (19 subjects with coronary heart disease and 4 subjects with stroke), 2 who were receiving treatment for thyroid disease and 2 who were receiving treatment for collagen diseases. No female subject was on hormonal replacement therapy. In addition, 147 patients who were receiving treatment for hypertension, hypercholesterolemia or diabetes mellitus were excluded. In all, data from 819 subjects (age 47 ± 10 [30–84] years old, 611 men and 208 women) were included in the present analysis. None of the subjects had a serum creatinine value greater than $114 \mu\text{mol/L}$. The protocol of this study conformed to the principles of the Declaration of Helsinki of 1964, and the study was conducted with the approval of the ethical guidelines committee of each investigators' institutes.

Assessment of the Flow-Mediated Dilatation of Brachial Artery

The subjects were instructed to fast for at least 4 h before test-

Fig. 1. Flow-mediated dilatation of the brachial artery in subject groups classified by the tertile ranges of Framingham risk score. FMD, flow-mediated dilatation of the brachial artery. * $p < 0.05$ vs. the value in the group with the first tertile range of Framingham risk score points (as assessed by one-way analysis of variance).

ing and to abstain from smoking and from ingesting alcohol, caffeine or antioxidant vitamins for at least 12 h prior to testing. The patients were asked to rest in the sitting position in a quiet, dark, air-conditioned room (22 to 25°C) for 5 min, after which their blood pressure was assessed using oscillometric methods (Omron Healthcare Co., Ltd., Kyoto, Japan). Then, after the subjects had rested again for at least 15 min in a supine position in the same room, measurement of the FMD was conducted. We performed ultrasound measurements according to the guidelines for ultrasound assessment of the FMD of the brachial artery (3).

Using a 10-MHz linear array transducer probe, the longitudinal image of the right brachial artery was recorded at baseline and then continuously from 30 s before to at least 2 min after the cuff deflation that followed suprasystolic compression (50 mmHg above systolic blood pressure [SBP]) of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter (Unex Co. Ltd., Nagoya, Japan). Briefly, the continuous recording of a 2D gray-scale image and A-mode waves of the brachial artery in the longitudinal plane was conducted with a novel stereotactic probe-holding device. A segment with clear anterior (media-adventitia) and posterior (intima-media) interfaces was manually determined. Then, these border interfaces were identified automatically on the A-mode waves. The diastolic per-beat diameter of the brachial artery was synchronized with the electrocardiographic R-wave and tracked automatically. The changes in the diastolic diameter were continuously recorded. Then, FMD was estimated as the percent change in the diameter over the baseline value at maximal dilatation during reactive hyperemia.

The reproducibility of the FMD measurement (on visits 1

Table 2. Results of Univariate and Multivariate Linear Regression Analyses Conducted to Assess the Relationship of Flow-Mediated Dilatation of the Brachial Artery to Cardiovascular Disease Risk Factors

Variable	Univariate		Multivariate ($r^2=0.12$)		
	<i>r</i>	<i>p</i> value	β	<i>t</i> value	<i>p</i> value
Age	-0.21	<0.01	-0.19	-5.13	<0.01
Gender	-0.27	<0.01	-0.24	-6.01	<0.01
BMI	-0.14	<0.01	-0.04	-0.87	0.38
Smoking	-0.14	<0.01	-0.08	-2.44	0.02
SBP	-0.14	<0.01	0.01	0.03	0.98
DBP	-0.10	<0.01	—	—	—
TC	-0.07	0.05	0.01	0.19	0.85
HDL	0.11	<0.01	-0.02	-0.46	0.65
TG	-0.12	<0.01	0.01	0.30	0.77
FPG	-0.12	<0.01	-0.02	-0.54	0.59

BMI, body mass index; Smoking, subjects with a history of smoking; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, serum total cholesterol; HDL, serum high-density lipoprotein cholesterol; TG, serum triglycerides; FPG, fasting plasma glucose.

and 2) was assessed in 39 subjects. Pearson's correlation coefficient of the FMD between visits 1 and 2 was 0.86, $p<0.01$, and the coefficient of variation was 11.2%.

Laboratory Measurements and Framingham Risk Score

Blood samples were collected from fasting subjects at least 1 h after the assessment of FMD. The serum triglycerides (TG), high-density lipoprotein cholesterol (HDL), total cholesterol (TC), and fasting blood glucose (FPG) levels were measured using enzymatic methods at each testing location.

Based on risk factors (*i.e.*, age, blood pressure, smoking status and laboratory measurements), FRS was calculated for each participant (13).

Statistical Analysis

Data were expressed as means $\pm$ SD. The relationships between the variables were assessed by univariate and multivariate linear regression analyses. For assessment of the differences in the status of each variable among the groups, unpaired *t*-test, one-way analysis of variance (ANOVA) or a general linear model (GLM) univariate analysis was applied, with adjustments for continuous variables. All the analyses were conducted using SPSS software for Windows, version 11.0J (SPSS, Chicago, USA); *p* values of <0.05 were considered to denote statistical significance.

Results

Table 1 shows the clinical characteristics of all study subjects, men and women. FRS significantly correlated with FMD ($r=-0.27$, $p<0.01$). Whereas FMD was higher in subjects with the 1st tertile range of FRS (FRS: -19-0) than it was in those with either the 2nd (FRS: 1-5) or the 3rd tertile (FRS:

6-13), FMD of the latter two groups did not differ when assessed by AVOVA (Fig. 1). The results of the univariate linear regression analysis revealed significant negative correlations between the CVD risk factors and FMD (Table 2). The results of the multivariate linear regression analysis revealed that age, gender and smoking were independently associated with FMD, but other CVD risk factors were not (Table 2). In addition, the effects of smoking and other CVD risk factors on FMD were evaluated separately according to age and gender. Celermajer *et al.* reported that age-related decline of FMD started at 40-50 years of age (14). In the present data, FMD was less pronounced in the subjects aged 50 years or over than in those under age 50 in both genders, as assessed by ANOVA (Fig. 2). Therefore, for further analysis, we divided the study subjects into those aged under 50 and those 50 or over.

The CVD risk factors were defined as follows: obesity: BMI $\geq$ 25; smoking: current smoker; hypertension: blood pressure levels at the time of measurement of FMD $\geq$ 140/90 mmHg; hypercholesterolemia: TC $\geq$ 6.22 mmol/L; diabetes mellitus: FPG 6.99>mmol/L. We have classified subjects into four groups according to their CVD risk factors (*i.e.*, without CVD risk factors, with only smoking, with one CVD risk factor other than smoking and with two or more CVD risk factors).

Table 3 shows the differences in FMD and baseline diameter of the brachial artery between subjects with and without CVD risk factors in men and women aged under 50 and 50 or over. Among those without CVD risk factors, the unpaired *t*-test showed FMD to be less pronounced in those $\geq$ 50 years of age than in those <50 years of age, in both sexes ($p<0.05$). In addition, FMD was less pronounced in men than in women among subjects under aged 50 who lacked CVD risk factors, even after adjustment for age by a GLM univariate analysis (Table 3). However, in subjects aged 50 or more and having no CVD risk factors, FMD values were similar in men and

Fig. 2. Flow-mediated dilatation of the brachial artery in subject groups classified by age in both genders. FMD, flow-mediated dilatation of the brachial artery. * $p < 0.05$ vs. the value in the group aged 30–39 (as assessed by one-way analysis of variance); † $p < 0.05$ vs. the value in the group aged 40–49 (as assessed by one-way analysis of variance).

women (Table 3).

In male subjects under 50, FMD was less pronounced in subjects with one or more risk factors than in subjects without CVD risk factors, even after adjustment for age by a GLM univariate analysis (Table 3). However, FMD in subjects with two or more CVD risk factors did not differ from the FMD in subjects with any single factor, including smoking. In contrast, in men over 50, FMD was less pronounced in subjects who only smoked or had two or more CVD risk factors than in subjects without CVD risk factors but did not differ from those with one CVD risk factor other than smoking, even after adjustment for age by a GLM univariate analysis (Table 3).

In women under 50, FMD was less marked in subjects who had any one CVD risk factor than in those with no CVD risk factors, even after adjustment for age by a GLM univariate analysis (Table 3). The number of subjects with two or more CVD risk factors was too small for statistical analysis ($n=6$). In contrast, in women aged 50 or over, FMD was similar among the four groups (Table 3).

Discussion

To the best of our knowledge, the present study is the first to assess the relationship and clustering of CVD risk factor with respect to FMD in a large number of Japanese subjects free of CVD.

Celermajer *et al.* demonstrated that age, gender and smoking were independent significant factors related to FMD in healthy subjects (mean age=36 years), but the ethnicity of their study subjects was not mentioned (14). In the NOMAS study, in addition to age and gender, body mass index, hypertension and plasma glucose levels, but not smoking, were shown to be independent variables affecting FMD in a multi-ethnic (white, black and Hispanic) non-diabetic population free of overt CVD (mean age=66 years) (15). Thus, even in healthy subjects, race and age may affect the relationship of

each CVD risk factor to FMD. In the present study, univariate linear regression analysis demonstrated significant negative associations of each of the CVD risk factors with FMD, and in subjects aged under 50, but not in those 50 or more, CVD risk factors lowered FMD. However, the results of multivariate linear regression analyses in the present study revealed that only age, gender and smoking were independent variables affecting FMD. In the management of CVD risk factors, therefore, while CVD risk factors may act to impair FMD, the present results emphasize the importance of quitting smoking even at early stages of atherosclerosis in Japanese subjects aged 30 years or more.

Because each CVD risk factor acts additively or synergistically to increase the risk of CVD events (16), the cluster of CVD risk factors was thought to augment the impairment of FMD. However, Witte *et al.* suggested that the effect of the cluster of CVD risk factors on FMD is modest (12). The present finding is that, in subjects aged under 50, FMD measurements were lower in subjects with any one CVD risk factor, but the clustering of CVD risk factors did not significantly induce additional impairment of FMD. Furthermore, while FMD was greater in subjects with the 1st tertile range of FRS than that in subjects with either the 2nd or the 3rd tertiles, FMD was similar between the latter two groups. Therefore, in Japanese subjects, FMD may not be a robust marker for the exacerbation of endothelial damage related to the cluster of CVD risk factors.

Some studies have demonstrated an age-related decline in FMD (14, 17), and Taddei *et al.* reported that reduction of nitric oxide availability and increased oxidative stress were the causal factors for age-related decline in endothelial function (18). In contrast, previous large-cohort studies to assess the relationship of FMD to CVD risk factors showed age heterogeneity in the subjects. For example, the Young Finns Study was conducted in subjects aged 24–39 years (4, 7), the PIVUS study and Cardiovascular Health Study were con-

Table 3. Differences in Flow-Mediated Dilatation of the Brachial Artery and Baseline Diameter of Brachial Artery between Subjects with and without Cardiovascular Risk Factors in Men and Women Aged under 50 and 50 or over

	CVD-risk status			
	No risk	Smoking only	One risk, but not smoking	Two or more risks
Men				
Age<50 years				
Number	177	48	77	83
Smoking		48		54
BMI>25			40	66
HyperTC			25	30
Hypertens			11	30
DM			1	2
FMD (%)	6.5±3.5	5.5±2.9*	5.6±2.7*	5.4±2.6*
Diameter (mm)	4.0±0.4	4.1±0.4	4.2±0.5*	4.3±0.5*
Age≥50 years				
Number	66	44	58	58
Smoking		44		26
BMI>25			29	44
HyperTC			16	18
Hypertens			10	40
DM			3	7
FMD (%)	5.6±2.7 [†]	4.0±3.6*	5.3±2.4	4.6±2.8*
Diameter (mm)	4.3±0.5 [†]	4.2±0.5	4.3±0.5	4.3±0.4
Women				
Age<50 years				
Number	113	10	17	6
Smoking		10		4
BMI>25			6	3
HyperTC			8	4
Hypertens			3	3
DM			0	0
FMD (%)	8.8±3.2 [‡]	7.0±3.5*	7.0±3.4*	7.5±4.1
Diameter (mm)	3.1±0.3 [‡]	3.2±0.3	3.2±0.3	3.2±0.3
Age≥50 years				
Number	26	4	24	8
Smoking		4		5
BMI>25			6	4
HyperTC			13	5
Hypertens			5	4
DM			0	0
FMD (%)	5.5±3.1 [‡]	4.0±2.4	6.2±3.0	5.4±3.8
Diameter (mm)	3.3±0.3 [‡]	3.2±0.3	3.3±0.3	3.4±0.3

CVD-risk status, status of cardiovascular disease risk factors; No risk, subjects without cardiovascular disease risk factors; Smoking only, subjects with only smoking; One risk, but not smoking, subjects with one cardiovascular disease risk factor other than smoking; Two or more risks, subjects with two or more cardiovascular disease risk factors; Smoking, number of subjects with smoking; BMI>25, number of subjects whose body mass index over 25; HyperTC, number of subjects with hypercholesterolemia; Hypertens, number of subjects with hypertension; DM, number of subjects with diabetes mellitus; FMD, flow-mediated dilatation of the brachial artery; Diameter, baseline diameter of the brachial artery. * $p<0.05$ vs. subjects without cardiovascular risk factors adjusted for age; [†] $p<0.01$ vs. male subjects aged under 50 with no cardiovascular risk factors assessed by unpaired t -test; [‡] $p<0.01$ vs. female subjects aged under 50 with no cardiovascular risk factors assessed by unpaired t -test; [§] $p<0.05$ vs. male subjects aged under 50 with no cardiovascular risk factors adjusted for age.

ducted in elderly subjects (5, 9), most study subjects in the Framingham study were aged over 50 years (6) and the subjects in Celermajer's study were relatively young and included children (mean age=36 years) (10). Thus, it has not been fully clarified whether aging itself may affect the relationships of other CVD risk factors to FMD. In the present study, in men aged 50 or more, the FMD in subjects having one CVD risk factor other than smoking was similar to that in subjects with no CVD risk factors, and in women aged 50 or more, the FMD was similar in all four groups. Therefore, the effect of aging may partially supersede that of other CVD risk factors on FMD in subjects aged 50 or more, and this effect may be more prominent in women than in men.

The present study has some major limitations. First, the baseline diameter of the brachial artery has been reported as one of the major determinants of FMD (4, 15), and it has even been suggested as a marker to predict future cardiovascular events similar to FMD (19). In the present study, the baseline diameter of the brachial artery also had a significant correlation with FMD ($r=-0.45$, $p<0.01$). A study to evaluate the role of baseline diameter of the brachial artery in the relationship of CVD risk factors to FMD is now under way in our institute. Second, nitroglycerin-induced (*i.e.*, endothelium-independent) dilatation of the brachial artery was not assessed in this study (1–3); therefore, the influence of age-related structural changes in the brachial artery on FMD, and any gender-related difference in this influence, could not be evaluated. Third, we used a commercially available computer-assisted semi-automatic device for the measurement of FMD. However, this device was not equipped with pulse-wave Doppler. Therefore, we were unable to confirm complete occlusion of forearm arterial inflow. Fourth, the number of female subjects in the present study was relatively small, and therefore confirmation of the present results is needed with a larger number of female study subjects. Fifth, in the present study, multivariate linear regression analysis failed to confirm the significance of the effect of CVD risk factors other than smoking on FMD. However, except in smokers, FMD was lower in those with one CVD risk factor than in those with no CVD risk factors. Therefore, the number of study subjects who had CVD risk factors other than smoking may not be sufficient to reach statistical significance of an effect on FMD. Further study is necessary to clarify the significance of each CVD risk factor other than smoking in impairing FMD. Sixth, in the present study, the number of subjects treated for CVD or CVD risk factors was relatively small, because the cohort was drawn mostly from company employees rather than the general population. The next logical step is to clarify whether FMD is a marker of the prognosis in subjects with either CVD or its associated risk factors.

Conclusion

In Japanese subjects free of CVD, FMD may serve as a marker of the risk for CVD, but it may not be a robust marker

for the endothelial damage related to the cluster of CVD risk factors. Among these risk factors, age, gender and smoking were independent variables related to the impairment of FMD. Therefore, the utility of this marker may be smaller in subjects, especially women, aged 50 or more.

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