Original Article

Nitroglycerin-mediated vasodilatation of the brachial artery may predict long-term cardiovascular events irrespective of the presence of atherosclerotic disease

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Aim: **Nitroglycerin-mediated vasodilatation (NMD) provides insight into the NTG-induced bioactivity of smooth muscle. It is plausible that in dysfunctional smooth muscle cells, the response to nitroglycerin may become blunted. The relationship between impaired brachial artery NMD and subsequent cardiovascular events is not well established.**

Methods: **We examined brachial artery flow-mediated dilatation (FMD) and NMD using ultrasound in 93 subjects (71 ± 7 years, including 26 with peripheral artery disease (PAD), 37 with aortic aneurysms, 10 with PAD complicated with aneurysms, and 20 without evident arterial disease). Brachial artery responses to hyperemia and nitroglycerin were measured every minute after cuff deflation and nitroglycerin administration. Time courses of vasodilatation were assessed and maximal FMD and NMD were measured.**

Results: **The time courses in response to NTG were sigmoidal and maximal diameter reached 7.2 ± 1.6 minutes after NTG was administered sublingually. The mean FMD was 2.3±2.0% and the mean NMD was 17.6 ± 7.1%. Subjects were prospectively followed for an average of 47 ± 13 months. Eighteen subjects had an event during follow-up; events included myocardial infarction (five), unstable angina pectoris (four), stroke (two), aortic dissection (one), ruptured aortic aneurysm (three), symptomatic abdominal aortic aneurysm (two), and lower limb ischemia requiring revascularization (one). NMD and FMD were significantly lower in subjects with events than in those without an event. In a Cox proportional-hazards model, lower FMD as well as lower NMD independently predicted future cardiovascular events.**

Conclusion: **Brachial artery nitroglycerin-mediated vasodilatation may add information to conventional risk stratification.**

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Key words; nitroglycerin-mediated vasodilatation, flow-mediated vasodilatation, endothelial dysfunction, smooth muscle dysfunction

Introduction

Atherosclerosis is a progressive disease that initially involves endothelial dysfunction, the accumulation and peroxidation of intimal lipids, and the release of inflammatory cytokines and growth factors, resulting in vascular smooth muscle cell proliferation and collagen matrix production. Intensified inflammatory activity may lead to local proteolysis, plaque rupture, and thrombus formation, resulting in ischemia and infarction[1, 2]. There is currently increasing interest into a new method for predicting cardiovascular

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events. Some researchers have reported that endothelial dysfunction predicts cardiovascular events[3-9]. Endothelial function is non-invasively assessed by using flow-mediated vasodilatation (FMD), to determine the endothelium-dependent vasodilatory response in the brachial artery[7, 10-12]. Impaired FMD has been suggested as a prognostic tool to predict future cardiovascular events[3-5, 13], and so FMD may be a marker of cardiovascular atherosclerosis.

The brachial ultrasound test to determine vascular function also assesses the artery's vasodilatory response to nitroglycerin (NTG), which has direct effects upon the smooth muscle producing a endothelium-independent vasodilatation[14]. Weaker responses to nitroglycerin-mediated vasodilatation (NMD) have been observed in patients with coronary heart disease (CHD)[15, 16] and in subjects with CHD risk factors[17]. Recently, a weaker response to NMD, not FMD, has been associated with the presence and quantity of calcium within the coronary artery in asymptomatic adults. This suggests NMD to be a marker of coronary anatomical abnormality[18].

The power of brachial NMD to be used as a clinical marker of cardiovascular disease is unclear. Our previous study showed that brachial responses to NTG reached a maximal limit approximately 7 minutes after the administration, and that NMD correlated with atherosclerotic risk factors in patients displaying evidence of atherosclerosis[19]. The current study was undertaken to investigate whether NMD can provide prognostic information on cardiovascular disease.

Methods

Study population

A total of 93 subjects including 73 patients with justifiable evidence of atherosclerosis and 20 without evidence of peripheral artery disease (PAD) or aneurysm were studied using brachial ultrasound tests. The 73 patients comprised 26 with PAD, 37 displaying on abdominal or thoracic aortic aneurysm and the remaining 10 exhibiting complicated PAD with an aortic aneurysm (**Table 1**). The subjects with atherosclerosis were all ambulatory, and all agreed to be enrolled in this study. All PAD patients displayed symptoms and voluntarily checked in to our hospital to receive the appropriate treatment. PAD was diagnosed by duplex scanning or computed tomography (CT) based on an ABI <0.9. An aneurysm was diagnosed when localized dilatation of the aorta was at least 50% larger than an adjacent normal portion detected by either ultrasonography or CT. At the time of vascular evaluation, none of the subjects displayed decompensated heart failure, glaucoma, ischemic gangerene, malignant neoplasma, or evidence of hepatic, renal or inflammatory disease. The 20 adults without evidence of arterial disease were chosen from the general physical examination list and all agreed to be enrolled in this study. They all had an ABI >0.9, normal electrocardiogram, normal chest X-ray, and normal ultrasonographic results from the abdominal aorta to the iliac artery, as well as no history of previous cardiovascular events, or the taking of vasoactive medications. This investigation was approved by the Tohoku University ethics committee, with all participants giving written consent.

Table 1. Baseline Characterisitics

Age	71 ± 7
Sex, female/male	7/86
Aortic Aneurysm	37 (40)
PAD	26 (28)
PAD complicated with aneurysm	10 (11)
Hypertension	59 (63)
Dyslipidemia	20 (22)
Diabetes mellitus	25 (27)
Smoking history	70 (75)
Previous history of CAD	18 (19)
Previous history of stroke	14 (15)
Atrial fibrillation	5 (5.4)
Medications at entry	
ACE inhibitors	26 (28)
Calcium antagonists	49 (53)
ARBs	20 (22)
Statins	13 (14)
Insulin	7 (7.5)
Oral hypoglycemic agents	5 (5.4)
NO donors	13 (14)

The numbers in parentheses represent percentages.
ACE: angiotensin-converting enzyme
ARB: angiotensin 2 receptor blocker

Brachial ultrasound tests

After an overnight fast (14 hours), FMD and NMD were measured using an ultrasound (Aplio SSA-700A 5-12-MHz linear array transducer, Toshiba) as described previously[10, 11]. Briefly, the brachial artery was scanned in longitudinal sections 2-10 cm above the elbow (control scan) after 15 min of rest in the supine position. The depth and gain settings were optimized to identify the anterior and posterior intimal interfaces between the lumen and vessel wall. Hyperemia was induced by inflating a forearm blood pressure cuff 50 mmHg above the systolic pressure for

5 minutes before deflating it. The vasodilator response to hyperemia was recorded for 5 minutes after deflation. Following at least 10 minutes' rest, a further control scan was performed and recorded. A single 300-μg dose of NTG was administered sublingually. Upon the NTG administration, a drop of water was poured over the tablet and the drug solution checked, as dissolution was found to be delayed in older patients due to dentures or dryness of the mouth[20]. Thereafter, the vasodilator response to NTG was recorded for 15 minutes.

Measurements were recorded at end-diastole, coinciding with the R-wave viewed on ECG. Mean diameter measurements were calculated from three different cardiac cycles. FMD and NMD were expressed as a percent change relative to the diameter before cuff inflation and before drug administration, respectively. Brachial artery responses to reactive hyperemia and NTG were measured every minute after cuff deflation and NTG administration. Maximal diameter responded to hyperemia and NTG was used to calculate FMD and NMD. Brachial diameter from three minutes after NTG administration was used for NMD3. The intra-observer variability for repeated measurements of resting arterial diameter was 0.02 ± 0.01 mm, variability in FMD was 0.49 ± 0.27 (%), and variability for NMD was 0.44 ± 0.34 (%). Brachial intima-media thickness (brachial IMT) was also measured as described[21]. The same segment of the brachial artery used to analyze vasodilatory response was measured for brachial IMT. The scan was focused on the posterior wall, and eight measurements were taken at end-diastole, coinciding with the R-wave, at even intervals. The average of these measurements was used as the mean brachial IMT for comparative analysis. Before the brachial ultrasound test commenced, a venous blood sample was taken, and the plasma was separated from the blood cells by centrifugation. The total plasma homocysteine level was determined using high-pressure liquid chromatography (SRL, Tokyo, Japan). The serum concentration of advanced glycation end products (AGEs) was measured with an enzyme-linked immunosorbent assay (SRL, Tokyo, Japan). Serum triglyceride, and total cholesterol levels were also measured using commercially available kits.

Follow-up and cardiovascular events

The majority of patients visited the outpatient clinic periodically. To improve long-term follow-up, a questionnaire was mailed to primary physicians and telephone interviews were undertaken to assess the incidence of cardiovascular events. All information regarding cardiovascular events was validated by obtaining source data, including hospital records, death certificates, and any other original documents available.

The following cardiovascular events were assessed during long-term follow-up. Sudden cardiac death, fatal and nonfatal myocardial infarction, fatal and nonfatal stroke, unstable angina, coronary revascularization procedures, symptomatic or ruptured aortic aneurysm, and newly developed aortic dissection or symptomatic aortoiliac occlusive disease were defined as cardiovascular events. Myocardial infarction was defined as an increase in creatine kinase and ST elevation and coronary angiography. Unstable angina was defined as chest pain associated with ischemic ECG changes and coronary angiography. Ischemic stroke was defined as evidence of a stroke without intracranial hemorrhage determined by brain imaging scans. Peripheral bypass revascularization was defined as the need for surgical revascularization of a de novo stenosis or occlusion of peripheral arteries. In patients with aortic aneurysm, the aneurysm diameter was assessed by ultrasonography every 3 months and by CT every 6- 12 months. A symptomatic or ruptured aneurysm and/or an aneurysm more than 5 cm in diameter were recommended for surgery.

Statistical analysis

Data are expressed as the mean $\pm$SD. Comparisons were made using the Student's t test if data were normally distributed; otherwise, they were analyzed using a nonparametric, Mann-Whitney *U*-test. Categorical variables were compared with the χ^2 test or Fisher's exact probability test. Univariate associations between variables were analyzed by calculating the Pearson's correlation coefficient. Cumulative event numbers were estimated using Kaplan Meier survival curves for categorical variables. Each measured value was altered to a categorical value based on the median. Probability values were determined by the use of the log-rank statistic. A Cox proportional-hazards model was used to examine whether FMD, NMD and NMD3 were each separately predictive of cardiovascular events. Data was first assessed using the Kaplan Meier survival curves for variables, the variables were then entered into the model based on the selection criteriaun of a log-rank probability value $\leqq 0.10$. Based on this, the following variables were tested: age, brachial diameter, suffering PAD and/or aneurysm, brachial IMT, plasma total homocysteine, serum levels of AGEs, triglyceride, total cholesterol, medications at entry (angiotensin-converting enzyme inhibitors, angiotensin 2 receptor blockers, calcium antagonists, Statins, NO donor) as well as a previous history of CAD, stroke, hypertension, atrial fibrillation, hyperlipidemia and diabetes mellitus. A p value of <0.05 was considered significant. All statistical analysis was performed using SAS software.

Results

The mean age of participants was 71±7 (range 51-86) years and the mean follow-up period was 47±13 (range, 6-63; median, 49.8) months. A total of 59 (63.4%) participants were hypertensive and 14 (15.1%) had previously suffered a stroke. Eighteen (19.4%) participants had a previous history of CAD; among these, 13 subjects received percutaneous coronary intervention (PCI) for angina pectoris (AP), one subject received coronary artery bypass grafting (CABG) for AP, two subjects received PCI for acute myocardial infarction (AMI) and two subjects received CABG for AMI. Five subjects suffered atrial fibrillation and three subjects received anticoagulant therapy.

The mean FMD was 2.3±2.0% (range −1.2-8.7%), mean NMD was 17.6±7.1% (range 2.6-37.1%), and mean NMD3 was 8.4±5.7% (range −0.6-24.8%). FMD was directly correlated with NMD (r=0.459, $p<0.001$) and NMD3 (r=0.351, $p<0.001$).

Maximal diameter was reached at 7.2±1.6 (range 4-12) minutes after 0.3 mg of NTG was administered sublingually. The time courses in response to NTG were sigmoidal (**Fig. 1**), displaying a gradual increase from 0- 2 minutes, then rapidly increasing to approximately 5 minutes, again followed by a gradual increase.

FMD (2.1±1.9% versus 3.1±2.4%, $p=0.06$) and NMD3 (7.9±5.3% versus 10.5±6.6%, $p=0.07$) were lower but not significantly different when comparing subjects with established arterial disease to those without arterial disease at entry. NMD was similar between the two groups (17.4±7.3% versus 18±6.3%, $p=0.73$).

During the follow-up, there were 18 new cardiovascular events including five participants suffering MI (three of which died), four with unstable angina pectoris (all four underwent coronary revascularization), one operated on for ruptured abdominal aortic aneurysm (AAA), two operated on for symptomatic AAA, two with a ruptured thoracic aortic aneurysm (both of when died), one thoracic aortic dissection (Stanford A, fatal), two non-fatal strokes, and one displaying new onset lower limb ischemia that underwent revascularization. Among these, the one nonfatal unstable angina pectoris patient and the one patient that developed lower limb ischemia developed these events despite no sign of vascular disease at the beginning of the study. In addition, three participants died

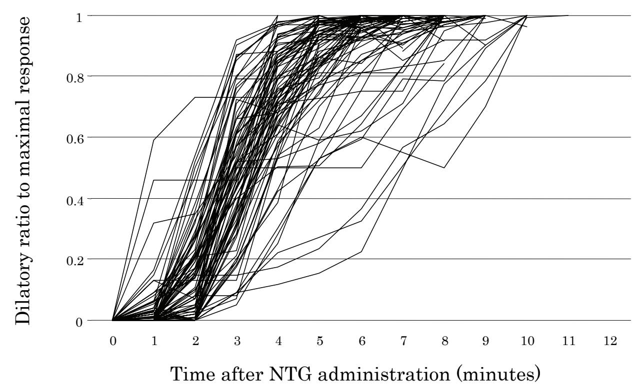

Fig. 1.

Time course of brachial artery responses to NTG. The time courses are sigmoidal curves.

of gastric cancer (23, 25 and 14 months after brachial tests), and two died of pneumonia (6 and 48 months after tests).

FMD, NMD and NMD3 were all significantly lower in the Event (+) group than Event (−) group. Total levels of homocysteine, AGEs, and highly sensitive CRP did not differ significantly between the two groups (**Table 2**).

Multivariate analysis

In the univariate analysis using Kaplan-Meier curves, a previous history of coronary artery disease ($p=0.006$) was found to be significantly associated with the occurrence of cardiovascular events. Although not significant, a history of stroke ($p=0.07$) had a tendency to be associated with occurrence of the events. A history of smoking ($p=0.877$), hypertension ($p=0.678$), hyperlipidemia ($p=0.492$), diabetes mellitus ($p=0.818$), or atrial fibrillation ($p=0.11$), suffering from an arterial disease including PAD and/or aortic aneurysm ($p=0.216$), and having a larger brachial diameter (>4.3 mm, $p=0.20$), larger brachial IMT (>0.45 mm, $p=0.942$), higher total homocysteine levels (>11.75 nmol/ml, $p=0.951$), or higher serum levels of AGEs (>2.8 mU/ml, $p=0.430$), higher levels of hs-CRP (>0.98 mg/L, $p=0.121$), higher levels of total cholesterol (>220 mg/dl, $p=0.253$), and higher levels of TG (>150 mg/dl, $p=0.818$) were not associated with a poor outcome. Regarding medications at entry, using NO donors was associated with occurrence of the events ($p=0.039$) but such a relationship was not observed for other drugs. Participants with lower levels of NMD (<18.6%, $p<0.001$) differed significantly from those with higher levels of NMD (>18.6%) (**Fig. 2**). Participants with lower levels of

Table 2. Clinical characterisitics of subjects with cardiovascular events and those without event

	Event (+) ($n=18$)	Event (−) ($n=75$)	p-value
Age	73 ± 7.4	71 ± 6.9	0.09
Sex, female/male	18/0	68/7	0.18
Smoking history	14	56	0.78
Body mass index (kg/m^2)	22.9 ± 3.3	24.2 ± 3.3	0.21
Without evident arterial disease	2	18	0.23
Aneurysm	8	29	0.65
PAD	6	20	0.57
PAD complicated with aneurysm	2	8	0.95
Hypertension	12	47	0.75
Dyslipidemia	5	15	0.47
Diabetes mellitus	4	21	0.61
Previous history of CAD	7	11	0.02
Previous history of stroke	5	9	0.09
Atrial fibrillation	2	3	0.25
Brachial diameter (mm)	4.5 ± 0.6	4.3 ± 0.7	0.12
FMD (%)	1.4 ± 1.5	2.5 ± 2.0	0.04
NMD (%)	12 ± 6.4	18.9 ± 6.6	0.0006
NMD3 (%)	4.1 ± 4.4	9.5 ± 5.5	0.0001
Brachial IMT (mm)	0.46 ± 0.07	0.45 ± 0.08	0.53
Total cholesterol (mg/dL)	200 ± 35	194 ± 36	0.52
Triglycerides (mg/dL)	122 ± 59	118 ± 64	0.73
hs-CRP (mg/L)	2.51 ± 2.06	2.31 ± 3.89	0.16
Total homocysteine (nmol/mL)	12.5 ± 3.3	12.2 ± 3.8	0.59
AGEs (mU/ml)	2.9 ± 0.66	2.8 ± 0.63	0.31
Medications at entry			
ACE inhibitors	5	21	0.98
ARBs	3	17	0.58
Calcium antagonists	11	38	0.42
Statins	4	9	0.26
NO donors	5	8	0.06

Data are expressed as the mean ± S.D.
ACE : angiotensin-converting enzyme; ARB : angiotensin 2 receptor blocker

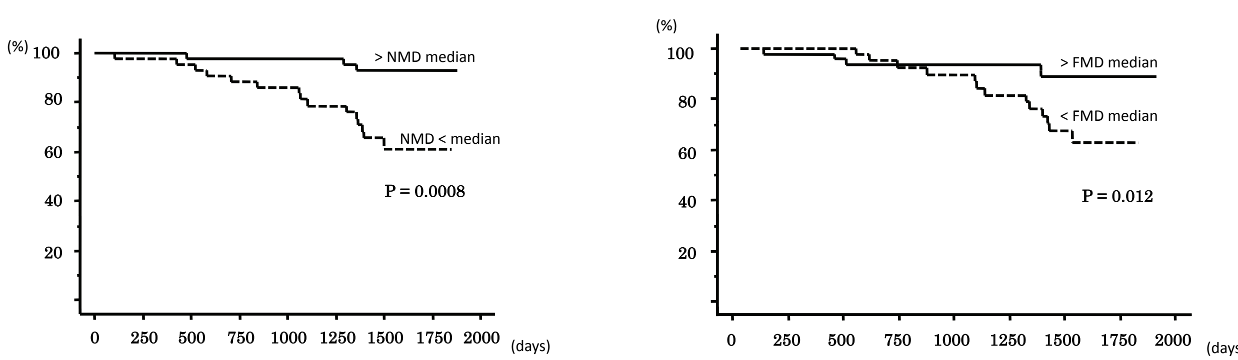

Fig. 2.

Kaplan-Meier event-free survival curves for participants. The left panel displays event-free survival classified on the basis of median NMD (=18.6%). Subjects with a value higher than the media differed significantly from those with a value lower than the median ($p<0.001$). The right panel displays event free-survival classified on the basis of median FMD (1.9%). Subjects with a value higher than the median differed significantly from those with a value lower than the median ($p=0.012$).

Table 3. Univariate and multivariate analyses for cardiovascular events

variables	*p*-value of log-rank test	Multivariate cox regression analysis							
		Model 1		Model 2		Model 3		Model 4	
		RR (95%CI)	*p*	RR (95%CI)	*p*	RR (95%CI)	*p*	RR (95%CI)	*p*
age	-	1.0 (0.95-1.1)	0.458	1.1 (0.99-1.2)	0.097	1.0 (0.95-1.1)	0.45	1.1 (0.98-1.2)	0.16
previous CAD	0.006	0.3 (0.1-1.3)	0.112	0.2 (0.04-0.89)	0.035	0.7 (0.2-3.1)	0.63	0.18 (0.04-0.9)	0.03
previous stroke	0.07	0.4 (0.1-1.2)	0.1	0.7 (0.23-1.9)	0.441	0.6 (0.2-1.7)	0.3	0.57 (0.2-1.7)	0.3
using NO donor	0.039	1.3 (0.3-5.6)	0.716	2.7 (0.5-13)	0.233	0.5 (0.1-2.4)	0.41	2.8 (0.6-14)	0.2
FMD	0.012	4.3 (1.4-13.0)	0.009	-	-	-	-	2.1 (0.6-6.6)	0.23
NMD	0.001>	-	-	10.4 (2.6-42)	0.001	-	-	7.1 (1.6-32)	0.01
NMD3	0.003	-	-	-	-	4.5 (1.4-14)	0.01	-	-

A log-rank probability value ≦0.1 was used as the criterion for entry into the multivariate analysis.

FMD (<1.9%) and NMD3 (<8.2%) also differed significantly from those with higher levels of FMD (>1.9%, $p=0.012$, **Fig. 2**) and NMD3 (>8.2%, $p=0.003$).

A multivariate Cox regression analysis was used to examine whether FMD, NMD and NMD3 were each separately predictive of cardiovascular events. By the selection criteriun of a log-rank probability value ≦0.10, a previous history of coronary artery disease, previous stroke, and using NO donors were entered into the model in addition to FMD, NMD and NMD3. As shown in **Table 3**, lower NMD, lower NMD3 and lower FMD each separately predicted cardiovascular events. When NMD and FMD were entered into the same model (**Table 3**, model 4), NMD significantly predicted events, but FMD was not associated with events. The same results were obtained when 20 subjects without evidence of PAD or aneurysm were excluded.

A scatter graph of NMD v.s FMD is shown in **Fig. 3**. Thirteen of 18 subjects with cardiovascular disease were distributed under the line of median FMD ($n=48$, 13/48, 27%). Fifteen were distributed under the line of median NMD ($n=47$, 15/47, 32%). Twelve cases of cardiovascular disease were framed by median FMD and median NMD ($n=31$, 12/31, 39%).

When the subjects taking NO donors were excluded ($n=80$), 13 subjects had an event during follow-up. NMD (19.4±6.5 versus 11.7±6.6%, $p=0.001$) and NMD3 (9.9±5.5 versus 3.9±4.6%, $p<0.001$) were significantly lower in subjects with an event than in those without an event. However, FMD was not significantly lower in subjects without an event (2.5±2.1 versus 1.6±1.6%, $p=0.20$). A previous history of CAD ($p<0.001$), NMD ($p=0.011$) and NMD3 ($p=0.004$) were significantly associated

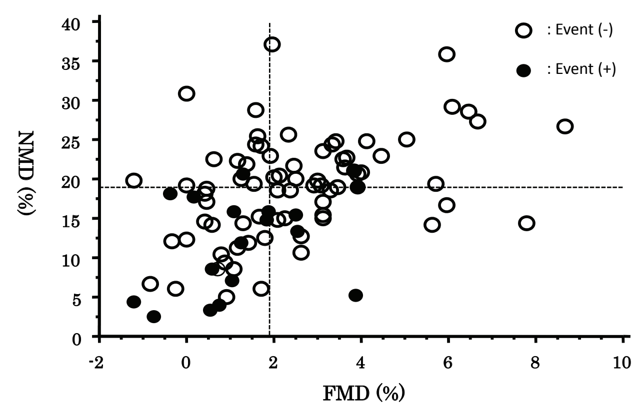

Fig. 3.

Scatter graph of NMD v.s FMD. Twelve of 18 subjects suffering cardiovascular events were distributed within the lower FMD (<1.9%) and lower NMD (<18.6%) group. Thirty-one out of 93 subjects were distributed within the lower FMD (<1.9%) and lower NMD (<18.6%) group.

with an event in the univariate analysis, although, lower FMD did not significantly differ from higher FMD ($p=0.16$). In the multivariate analysis, NMD ($p=0.003$) and NMD3 ($p=0.03$) were each separately associated with cardiovascular events.

In addition, we excluded aortic diseases from the endpoint analysis. Twelve subjects developed events. In the univariate analysis, a previous history of CAD ($p=0.01$), a previous history of CVD ($p=0.04$), using NO donors ($p=0.002$), FMD ($p=0.02$), NMD ($p=0.007$), and NMD3 ($p=0.02$) were associated with cardiovascular events. In the multivariate analysis, NMD ($p=0.002$) predicted the events, but FMD ($p=0.07$) and NMD3 ($p=0.05$) were not significantly associated with the events.

Discussion

In this small, prospective study, nitroglycerin-mediated vasodilatation was found to predict cardiovascular events, in subjects with a mean age of 71 years including patients with atherosclerosis and healthy participants displaying no evidence of atherosclerotic disease. Impaired FMD was also associated with an increased risk of cardiovascular events; however, NMD was rather closely associated with such a risk. FMD is a method used to measure brachial smooth muscle dilation induced by endogenous nitric oxide, while NMD is induced by stimulation with exogenous nitric oxide[14]. Endothelial function is important in FMD as shear stress stimulates NO synthesis, dilating surrounding vascular smooth muscle[10], while NMD provides insight into NTG-induced bioactivity of the smooth muscle, inducing relaxation[14]. However, a comparison of FMD does not indicate true endothelial function when downstream smooth muscle reactivity differs between individuals. Hence, the relationship between FMD and NMD would be complementary. Although FMD and NMD are directly correlated, twelve of 18 subjects suffering cardiovascular events distributed within the region framed by the lower FMD($<1.9\%$) and lower NMD($<18.6\%$) value in the scatter graph, so a parameter combining FMD and NMD may serve as a more effective predictor of cardiovascular events.

Previously published data concerning the association between brachial ultrasound examinations and cardiovascular events suggest that FMD, and not NMD, was associated with incidents of cardiovascular events[6, 7], while some studies either did not measure NMD[4, 5] or did not provide sufficient statistical data[3]. These discrepancies may due to technical differences between research groups, and differences between test subjects. Some studies calculated NMD at 3 or 4 minutes after NTG administration[6], or measured vessel diameter between the media and adventitia. We measured the brachial diameter between the lumen and vessel wall, and recorded the entire time course following NTG administration. Over the time course, the brachial artery responded with a sigmoidal pattern, and reached maximal diameter at 7.2 ± 1.6 minutes. NMD3 is suggested to underestimate the extent of vasodilatation induced by the sublingual application of NTG; however, NMD3 was able to predict cardiovascular events in the current study. Our previous report suggested that NMD was associated with atherosclerotic risk factors, but that NMD3 displayed no such association[19]. NMD displays the dilative potency of NTG, while NMD3 provides a measure of the speed of response to NTG, or provides a limited measure of dilatation. In our population, NMD3 or NMD4 would be poorly reproducible as 3- 4 minutes post-NTG treatment the brachial artery is only midway through its increase in diameter as shown in **Fig. 1**.

NMD was similar between those with and without arterial disease. Long-term use of medication may have an influence, as most patients with established arterial disease were taking antihypertensives or antiplatelet agents or receiving treatment for hyperlipidemia for long periods.

FMD and NMD were significantly lower in individuals that experienced cardiovascular events than in the non-event group. The reason why NMD predicted cardiovascular events remains speculative. Some studies have demonstrated NMD to be associated with high blood pressure[13], diabetes mellitus[17], high oxidized LDL expression[21], and great carotid IMT[21]. NMD reflects NTG-induced dilatation of the brachial smooth muscle, not mechanical forces limiting the ability of the vessel to relax, as confirmed by the observation that brachial IMT did not significantly correlate with NMD[18]. Smooth muscle cells play a pivotal role in atherosclerosis, and it is plausible that in dysfunctional smooth muscle cells, the response to nitroglycerin may become blunted. Recently, J. Kullo *et al.*[18] suggested the vasodilative response to nitroglycerin, but not FMD, to be associated with the presence and level of coronary atherosclerosis in 441 asymptomatic adults. Although this study was in subjects with subclinical atherosclerosis, an association between coronary atherosclerosis and impaired smooth muscle relaxation in the brachial artery may be evident.

Yeboah *et al.*[5] studied the relationship between impaired brachial FMD and clinical cardiovascular events in 2791 elderly adults (mean age; 78.6 ± 4.4, range; 72 to 98 years). In this study, event-free survival rates for cardiovascular events were significantly higher in subjects with FMD greater than the sex-specific medians than in subjects with FMD less than or equal to the medians, but added only $\approx 1\%$ to the prognostic accuracy of the best Cox model. Shimbo *et al.*[4] also studied the relationship between FMD and future cardiovascular events in 842 elderly participants free of stroke or myocardial infarction, and concluded that FMD predicts incidents of cardiovascular events, though its predictive value was not independent of cardiovascular risk factors. These studies suggest that the clinical utility of brachial FMD is very limited in the elderly with diminished FMD. Impaired smooth muscle function may also be the underlying mechanism responsible for the diminished FMD in elderly subjects. There is a need to evaluate the relationship between brachial vascular function (both FMD and NMD) and CVD risk prediction by age group.

NMD and NMD3 were each separately associated with cardiovascular events but FMD was not, when subjects taking NO donors were excluded from the analysis. A previous study showed that GTN induces tolerance to nitrate and endothelial dysfunction[22]. In the present study, long-term use of NO donors would have affected NMD as well as FMD, and lessened the predictive value of FMD when excluding subjects using NO donors.

Our study does have some limitations: it examined male subjects comprising asymptomatic participants drawn directly from the community, almost exclusively. Although the sample group gathered does represent a population of particular interest for the purpose of primary prevention, risk identification and reduction of cardiovascular disease, we cannot rule out sub-clinical coronary atherosclerosis, because not all the subjects underwent coronary angiography at the start of the study. The patients with PAD used in the current study displayed mild atherosclerosis without gangrene, ensuring that the effects of inflammation or infection were excluded. However, this also resulted in deselection of severe or uncontrolled diabetes mellitus. Thus, the results reported here should not be extrapolated to whole populations, or those with low risk or severe atherosclerosis. Although it remains controversial whether atherosclerosis is a causative factor of aneurysms, we included patients with aneurysms because most exhibit risk factors for atherosclerosis. Our sample size is small, but larger study samples may enable diabetes mellitus, hypertension, the presence of PAD and/or aneurysm, dyslipidemia, and higher levels of hsCRP to also become independent predictors of cardiovascular events, as reported in earlier studies[4-6, 23]. Although this study included only Asian subjects, these findings might be applicable to subjects of other ethnicities.

In summary, brachial FMD as well as NMD is an independent predictor of long-term cardiovascular events in subjects with a mean age of 71 years, including patients with atherosclerosis and healthy participants displaying no evidence of atherosclerotic disease. Further research is required to clarify the predictive value of FMD and NMD over various levels of cardiovascular risk factors.

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