

# Hypertension, a Low Ejection Fraction and Severe Angiographic Findings are Associated with Smooth Muscle Dysfunction in Patients with Coronary Atherosclerosis

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## ABSTRACT

**Background and Objectives:** Nitroglycerin-mediated arterial dilation (NMD) was shown to be preserved in most previous studies, and this is possibly due to using a single high dose of nitroglycerin (NTG), which causes maximal arterial dilation. We sought to evaluate the clinical factors of flow-mediated dilation (FMD) and NMD at different doses of NTG in the patients with coronary artery disease (CAD). **Subjects and Methods:** Thirty-two consecutive patients (mean age: 61 years old, 18 males) with angiographically proven CAD underwent FMD and NMD at total cumulative doses of 25  $\mu$ g, 175  $\mu$ g and 325  $\mu$ g with using high-resolution ultrasound for the imaging. **Results:** The FMD, NMD (25  $\mu$ g), NMD (175  $\mu$ g) and NMD (325  $\mu$ g) were  $4.72 \pm 1.82\%$ ,  $7.08 \pm 3.02\%$ ,  $13.33 \pm 6.14\%$  and  $15.89 \pm 7.24\%$ , respectively ( $p < 0.001$  compared with each other). Univariate analysis showed that the FMD is associated with the serum homocysteine level, the NMD (25  $\mu$ g) is associated with the body mass index, the NMD (175  $\mu$ g) is associated with the fasting blood sugar and the ejection fraction, and the NMD (325  $\mu$ g) is associated with the fasting blood sugar, while there was no significant difference of the FMD and NMD according to the presence of CAD risk factors. Multivariate analysis disclosed that the independent factors of FMD were the serum homocysteine and triglyceride levels, and those of NMD (25  $\mu$ g) were hypertension, a low ejection fraction and severe coronary angiographic findings, while there was no independent factor for NMD (175  $\mu$ g) and NMD (325  $\mu$ g). **Conclusion:** This study suggests that hypertension, a low ejection fraction and significant stenotic coronary lesion may be associated with endothelium-independent smooth muscle dysfunction at low dose NTG, while the serum homocysteine and triglyceride levels are associated with endothelium-dependent endothelial dysfunction in the patients with CAD. Using low-dose NTG is important when measuring the NMD. (Korean Circulation J 2007;37:470-474)

**KEY WORDS:** Muscle, smooth ; Endothelium ; Nitroglycerin ; Coronary atherosclerosis.

## Introduction

Most studies have reported that nitroglycerin (NTG)-mediated brachial arterial dilation (NMD), that is, the smooth muscle function, is preserved in the patients with coronary artery disease (CAD) or in patients who have cardiovascular risk factors. Yet the flow-mediated brachial arterial dilation (FMD), which is an endothelium

dependent endothelial function, is impaired in those populations, and this is part of the early process of atherosclerosis.<sup>1-3)</sup> However, the NMD showed significant correlation with FMD in several studies<sup>4,5)</sup> and the insignificant findings of NMD in those study patients might have been caused by an inappropriate NTG dose; a single high-dose of NTG probably masked the difference between those patients and the healthy subjects, while the dose required to produce 50% of the maximum response was significantly greater in the patients than in the controls.<sup>6)</sup> These studies showed that NMD is also impaired in the patients with CAD or in the patients having some of the risk factors for CAD.<sup>5,6)</sup> Furthermore, it has been recently reported that the determinants of NMD in children are reduced endothelial function,

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increased oxidative stress and preclinical carotid atherosclerosis, and those in the CAD patients with risk factors are diabetes, a larger vessel size and reduced endothelial function. However, there is still uncertainty about the clinical factors of NMD, as well as those for FMD, in the CAD patients and especially when using different doses of NTG for the patients with NMD, so as to avoid masking effect due to a high single dose of NTG, induced maximal vasodilation.

The objectives of this study are to assess the FMD and NMD at different doses of NTG, and to evaluate the clinical factors of FMD and NMD at different NTG doses in the patients with angiographically proven CAD.

## Subjects and Methods

### Study population and the test sequence

The study population consisted of 32 consecutive patients with angiographically proven CAD (mean age: 61 years, 18 males). The clinical characteristics of the study patients are shown in Table 1. Written informed consent was obtained from all the patients and the study was approved by the hospital ethics committee. This study was also in accordance with the Declaration of Helsinki.

At first, we measured the flow-mediated endothelium-dependent brachial artery dilation (FMD) and nitroglycerin (NTG)-induced endothelium-independent brachial artery dilation (NMD) with using different cumulative doses of NTG, with the study patients having undergone overnight fasting one day before or at the day of coronary angiography (Fig. 1).

### Measurement of endothelial function

The method we used for measuring the flow-mediated endothelium-dependent brachial artery dilation (FMD) and the nitroglycerin-induced endothelium-independent brachial artery dilation (NMD) was described previously.<sup>17)</sup> We measured FMD by using high-resolution ultrasound (Hewlett-Packard Sonos 5500) with a

7.5 MHz probe for evaluating the endothelial function. The excellent reproducibility and repeatability of the brachial artery diameter measurements were documented in our previous report.<sup>8)</sup>

In brief, the FMD was measured, in a dark and quiet room, during the early morning in the study subjects after at least 10 hours of overnight fasting. At first, the brachial artery was defined by the optimal gain control of the high resolution-ultrasound at three to five centimeters above the elbow. Then, the media to media distance of the brachial artery was measured at end diastole. After measuring the brachial artery diameter, the blood pressure cuff was inflated to approximately 300 mmHg at the forearm for 5 minutes. Hyperemic blood flow then resulted from decompression of the blood pressure cuff. The media-to-media distance at the same brachial artery site was measured 1 minute after decompression of the blood pressure cuff. The FMD was expressed as percent diameter change of the hyperemic brachial artery diameter relative to the baseline brachial artery diameter.

After 15 minutes of rest, the brachial artery diameter was measured again as a baseline brachial artery diameter of the NMD. Then, 25  $\mu$ g NTG was administered with using a spray bottle (Pohl-Boskamp GmbH, Hohenlockstedt, Germany). The brachial artery diameter was measured again 4 minutes after the NTG spray. Then, a 150  $\mu$ g NTG tablet was given sublingually, and the artery diameter was measured 4 minutes after that. Finally, an additional 150  $\mu$ g NTG tablet was given again, and the artery diameter was measured 4 minutes after that (Fig. 1). The NMD1, NMD2 and NMD3 were expressed as percent diameter changes of the 25  $\mu$ g, 175  $\mu$ g and 325  $\mu$ g cumulative NTG-induced brachial artery diameters relative to the baseline brachial artery diameter, respectively. The difference of the NMD between the healthy subjects and the CAD patients was evident at a 300  $\mu$ g cumulative NTG dose.<sup>6)</sup> So, we used the above NTG doses in our study.

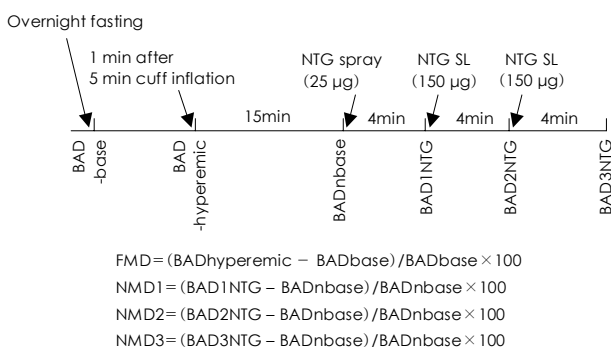
### Statistical analysis

All the analyses were done using SPSS (version 12.0; SPSS Inc., Chicago, Illinois). Continuous variables are summarized as means  $\pm$  SDs. Pearson's correlation coefficient was used to evaluate the association between variables. Comparison between groups was performed using an independent t-test. Backward Linear regression analysis was done to determine the independent factors for FMD, NMD1, NMD2 and NMD3. Statistical significance was set at  $p < 0.05$ .

## Results

### Patient population and the ultrasonographic results

The clinical characteristics of our study population



**Fig. 1.** Schematic diagram showing the study sequence and the definition of FMD, NMD25  $\mu$ g, NMD175  $\mu$ g and NMD325  $\mu$ g. BAD: brachial artery diameter, NTG: nitroglycerin, SL: sublingual, min: minutes, FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation.

are summarized in Table 1. The FMD, NMD1, NMD2 and NMD3 of the study patients were  $4.72 \pm 1.82\%$ ,  $7.08 \pm 3.02\%$ ,  $13.33 \pm 6.14\%$  and  $15.89 \pm 7.24\%$ , respectively and those were all significantly different with each other (Fig. 2). The ultrasonographic results were also correlated with each other (Table 2).

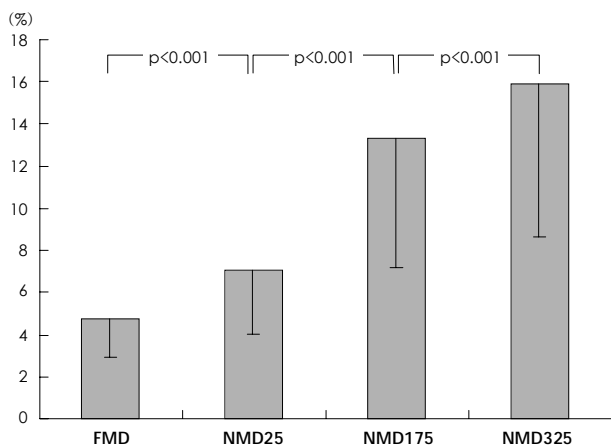
### Univariate analysis

The FMD was negatively correlated with the level of serum homocysteine, and NMD1 was positively correlated with the body mass index, while NMD2 was negatively correlated with the fasting blood glucose level

**Table 1.** Demographics of the study patients (n=32)

Variables	
Age (yrs)	61 $\pm$ 10
Male, n (%)	18 (56.3%)
Body mass index (kg/m <sup>2</sup> )	25.2 $\pm$ 3.0
Hypertension, n (%)	18 (56.3%)
Diabetes, n (%)	8 (25.0%)
Smoker, n (%)	7 (21.9%)
Dyslipidemia, n (%)	19 (59.4%)
Fasting blood sugar (mg/dL)	132 $\pm$ 53
Total cholesterol (mg/dL)	192 $\pm$ 40
Triglyceride (mg/dL)	174 $\pm$ 111
HDL-cholesterol (mg/dL)	40.4 $\pm$ 7.9
LDL-cholesterol (mg/dL)	117 $\pm$ 31
Homocysteine (mg/dL)	10.1 $\pm$ 2.8
Hs-CRP (mg/dL)	0.24 $\pm$ 0.23
Ejection fraction (%)	65 $\pm$ 11
Numbers of vessel $\geq$ 50% stenosed	
0 ( $>$ 10%, $<$ 50% stenosed)	15 (46.9%)
1	8 (25.0%)
2	7 (21.9%)
3	2 (6.3%)
Clinical diagnosis	
Stable angina	24 (75.0%)
Unstable angina	4 (12.5%)
Myocardial infarction	4 (12.5%)

H(L)DL: high (low) density lipoprotein, Hs-CRP: high-sensitivity C-reactive protein



**Fig. 2.** Comparison of FMD, NMD25  $\mu$ g, NMD175  $\mu$ g and NMD325  $\mu$ g in the study patients. FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation.

and it was positively correlated with the low density lipoprotein level and the left ventricular ejection fraction; the NMD3 was negatively correlated with the serum fasting blood glucose level (Table 3). However, those factors did not show any significant differences between the presence and absence of cardiovascular risk factors (Table 4).

### Multivariate analysis

The serum homocysteine level and triglyceride level were the independent factors of FMD, while hypertension, the left ventricular ejection fraction and the coronary angiographic findings were associated with the

**Table 2.** Pearson correlation coefficients between each variable

Variables	FMD	NMD1	NMD2
FMD			
NMD1	0.444*		
NMD2	0.648†	0.813†	
NMD3	0.638†	0.799†	0.969†

\*p=0.010, †p<0.001. FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation

**Table 3.** Univariate analysis of the ultrasonographic results

Variables	FMD	NMD1	NMD2	NMD3
Age	-0.097	0.217	0.068	0.058
BMI	0.169	0.461†	0.234	0.193
FBS	-0.169	-0.325	-0.438‡	-0.405§
TC	0.210	0.299	0.330	0.281
TG	-0.178	0.027	-0.113	-0.116
HDL-C	0.213	0.232	0.241	0.153
LDL-C	0.257	0.307	0.360§	0.311
Homocysteine	-0.514*	-0.148	-0.069	-0.042
Hs-CRP	-0.230	0.139	-0.045	0.043
Ejection fraction	0.203	0.346	0.373§	0.339

Values are Pearson correlation coefficients. \*p=0.003, †p=0.008, ‡p=0.012, §p<0.05. FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation, BMI: body mass index, FBS: fasting blood sugar, TC: total cholesterol, TG: triglyceride, H(L)DL-C: high (low) density lipoprotein cholesterol, Hs-CRP: high sensitivity C-reactive protein

**Table 4.** Comparison of the ultrasonographic findings according to the presence of risk factors

Variables	FMD	NMD1	NMD2	NMD3
<b>Hypertension</b>				
Yes (n=18)	4.38 $\pm$ 1.1	6.79 $\pm$ 2.7	12.39 $\pm$ 5.7	14.68 $\pm$ 6.7
No	5.16 $\pm$ 2.4	7.45 $\pm$ 3.5	14.5 $\pm$ 6.6	17.43 $\pm$ 7.9
<b>Diabetes</b>				
Yes (n=8)	5.09 $\pm$ 1.6	7.79 $\pm$ 4.3	12.14 $\pm$ 6.9	14.80 $\pm$ 8.3
No	4.60 $\pm$ 1.9	6.84 $\pm$ 2.5	13.72 $\pm$ 6.0	16.25 $\pm$ 7.0
<b>Smoking</b>				
Yes (n=7)	4.67 $\pm$ 1.5	7.45 $\pm$ 2.6	14.27 $\pm$ 5.4	17.35 $\pm$ 5.5
No	4.74 $\pm$ 1.9	6.98 $\pm$ 3.2	13.07 $\pm$ 6.4	15.48 $\pm$ 7.7
<b>Dyslipidemia</b>				
Yes (n=19)	4.83 $\pm$ 1.7	6.84 $\pm$ 3.1	13.23 $\pm$ 6.3	15.25 $\pm$ 7.1
No	4.57 $\pm$ 2.0	7.43 $\pm$ 3.0	13.48 $\pm$ 6.2	16.82 $\pm$ 7.6

Values are percents. FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation

**Table 5.** Independent factors of the FMD, NMD1, NMD2 and NMD3 in CAD patients

Variables	FMD $\beta$ (p)	NMD1 $\beta$ (p)	NMD2 $\beta$ (p)	NMD3 $\beta$ (p)
Homocysteine	-0.592 (p=0.001)	NS	NS	NS
Triglyceride	-0.341 (p=0.034)	NS	NS	NS
Hypertension	NS	-0.472 (p=0.005)	NS	NS
EF	NS	0.688 (p<0.001)	NS	NS
Minimal lesion	NS	-0.379 (p=0.007)	NS	NS

All the models included age, gender, body mass index, hypertension, diabetes, smoking, dyslipidemia, fasting blood glucose, total cholesterol, triglyceride, high (low)-density lipoprotein cholesterol, homocysteine, high sensitivity C-reactive protein, ejection fraction and coronary angiographic findings as independent variables. Only those variables that remained significant after backward stepwise elimination are shown in this table. FMD: flow-mediated dilation, NMD: nitroglycerin-mediated arterial dilation, CAD: coronary artery disease, EF: ejection fraction, NS: not significant

NMD1 in the patients with CAD. However, none of the clinical variables were associated with NMD2 or NMD3 (Table 5).

## Discussion

The main findings of this study are: i) Documentation of smooth muscle dysfunction was only possible with using low-dose NTG; ii) hypertension, a low ejection fraction and significant luminal narrowing were associated with smooth muscle dysfunction, while the serum homocystein and triglyceride levels were associated with endothelial dysfunction in the patients with CAD; iii) The smooth muscle function was associated with the endothelial function.

### Endothelial function

Endothelial dysfunction is quite apparent in the patients who are at risk for CAD as well as for the patients with CAD.<sup>1-3)</sup> Furthermore, it is associated with the prognosis for the CAD patients.<sup>4)9-11)</sup> However, the determinants of endothelial function in CAD patients are not certain. This study showed that the serum homocysteine and triglyceride levels are associated with endothelial function in those patients. Homocysteine can increase plasma asymmetric dimethylarginine (an endogenous NO synthase inhibitor) and so decrease endothelial function in healthy subjects as well as in patients with atherosclerotic disease.<sup>12)</sup> Serum triglyceride can also decrease endothelial function in healthy subjects as well as in the patients with CAD, via increased oxidative stress.<sup>8)13)14)</sup>

### Smooth muscle function

NMD is endothelium-independent, NTG-induced, smooth muscle dependent arterial dilation.<sup>7)</sup> The impaired dilator response to NTG in CAD patients is due to dysfunction at the level of the smooth muscle cells.<sup>6)</sup> Thus, NMD can be thought to reflect the smooth muscle function instead of the endothelial function. This study showed, for the first time, the determinants of smooth muscle function in CAD patients, and these were not evident when using a 175-325  $\mu$ g

dose of NTG. Hypertension, the ejection fraction and the angiographic findings are associated with smooth muscle dysfunction in CAD patients. To date, the independent factors of NMD are endothelial dysfunction, increased oxidative stress and preclinical carotid atherosclerosis in children, while diabetes, endothelial dysfunction and vessel size are those for the adults at risk for atherosclerosis.<sup>5)15)</sup> We did not test the ultrasonographic findings on multivariate analysis to determine the independent clinical factors and this might have caused the difference between our study results and the other study results. Differences of the study populations might also help to explain the difference.

### Low dose nitroglycerin in nitroglycerin-mediated arterial dilation

Our study revealed the association between NMD and the clinical factors only at the NTG dose of 25  $\mu$ g, and not 175  $\mu$ g or 325  $\mu$ g, in the patients with CAD. This dose is quite low compared with the previously used one,<sup>6)</sup> which was used to compare NMD between healthy subjects and patients with CAD, and a difference of the NMD was seen for NTG doses of 150-450  $\mu$ g. That study suggested that the reason why there was no significant impairment, but only mild impairment of the NMD in the patients with atherosclerosis or the patients was the use of a single high dose of NTG that elicited maximal dilation of an artery when measuring the NMD. Our study suggests that a lower dose such as 25  $\mu$ g should be used for NMD measurements in order to assess the smooth muscle function, and especially when the study population consists of only CAD patients, rather than conducting a comparative study that includes patients with CAD and healthy subjects. In other words, the sensitivity of the arterial responses to NTG, instead of the smooth muscle dysfunction, is impaired in CAD patients or especially when this is combined with hypertension, a low ejection fraction and more severe angiographic findings.

### Correlation between flow-mediated dilation and nitroglycerin-mediated arterial dilation

Although many previous studies have not shown

any significant difference of the NMD in various study groups and the FMD was found to be consistently impaired in the patients with atherosclerosis or those who were at risk of atherosclerosis, the NMD in those studies showed significant correlation with the FMD, like our study results did at any dose of NTG.<sup>14)5)15)</sup> These findings suggest that the atherosclerosis risk factors, as well as atherosclerosis, cause possible smooth muscle dysfunction as well as endothelial dysfunction. Yet the smooth muscle dysfunction was not evident with a single high dose of NTG and it should be assessed in terms of the sensitivity of the arterial dilator response to low dose NTG.

### Study limitations

This study did not show the clinical significance of those independent factors of NMD such as hypertension, a low ejection fraction and more severe angiographic findings, but those factors are associated with each other for CAD patients. There is the possibility of the effects of vasoactive drugs on the FMD and NMD in our study. However, recent administration of commonly used nonnitrate vasoactive drugs has no significant effect on brachial reactivity.<sup>16)</sup> In addition, the study was performed in the same manner for all the study subjects, although nitrate as well as nonnitrate drugs were also discontinued for at least 10 hours before the study. A future study is required to evaluate the discrepancy of the clinical factors for determining the FMD and NMD in this study population and to ascertain the association between the FMD and NMD.

### Clinical implications

1) Decreased sensitivity of the arterial dilator response to NTG in CAD patients is associated with hypertension, a low ejection fraction and more severe angiographic findings; thus, this patient population is subject to developing primary nitrate tolerance. 2) Low dose NTG should be used for measuring the NMD and the proper dose-response curve should be obtained in a future clinical study. 3) The serum homocysteine and triglyceride levels should be controlled when we examine the prognostic significance of endothelial function and the association between endothelial function and the serum homocysteine and triglyceride levels.

### Conclusions

This study is the first to evaluate the clinical factors associated with endothelial function and smooth muscle function in CAD patients. Hypertension, a low ejection fraction and more severe angiographic findings are associated with endothelium-independent smooth muscle dysfunction at low dose NTG, while the serum homocysteine and triglyceride levels are associated with the endothelium-dependent endothelial dysfunction. Us-

ing low-dose NTG is important when measuring the NMD in order to avoid masking subtle differences of the patients' sensitivity to NTG.

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