Combined examination of glyceryl trinitrate-mediated vascular dilation with flow-mediated vascular dilation is essential for assessment of vascular function in type 2 diabetes

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ABSTRACT

Aims/Introduction: In order to characterize the impaired vascular function in type 2 diabetes (DM) patients, we evaluated the flow-mediated vascular dilation (FMD) with glyceryl trinitrate-mediated vascular dilation (NMD) using ultrasonography.

Materials and Methods: A total of 111 DM patients and 42 healthy control participants were studied. The maximal dilatation of FMD and NMD (%FMD and %NMD, respectively), the beginning time (T) of dilatation after stimulation and the velocity (V) of the vascular dilatation were also measured.

Results: Among DM patients, 49% had impaired %NMD, which affects the results of %FMD. In DM patients with normal %NMD, the %FMD was also significantly lower than that in control participants, although the *T* and the *V* were not impaired. In contrast, both the *T* and the *V* were disturbed in the DM patients with low %NMD. Multiple linear regression analysis showed that %NMD was independently correlated with albuminuria. Our results indicate that the impaired FMD in DM is be affected by low NMD, and impaired endothelial function already exists even in DM patients whose vascular smooth muscle function is still retained, and also albuminuria is the clinical feature of DM with low %NMD.

Conclusions: Examination of NMD, not only FMD, should be carried out as it offers the possibility of clarifying vascular function in DM patients. (J Diabetes Invest doi: 10.1111/jdi.12021, 2013)

KEY WORDS: Flow-mediated vascular dilatation, Glyceryl trinitrate-mediated vascular dilatation, Type 2 diabetes

INTRODUCTION

Vascular endothelium is located in the innermost layer of the arterial wall, and it has been clarified that its dysfunction is an early step in the development of atherosclerosis[1]. To estimate vascular endothelial function, several clinical examinations have been developed. Among them, flow-mediated vascular dilation (FMD), an index of the responsiveness to nitric oxide (NO) derived from vascular endothelium, is widely accepted for evaluating the endothelial function as a non-invasive examination, because endothelial dysfunction is mainly characterized by the reduction in the bioavailability of nitric oxide[2]. FMD is thus regarded as a parameter of atherosclerosis, and clinical and experimental studies suggest that endothelial dysfunction is an important marker of cardiovascular disorders[3]. In addition, glyceryl trinitrate (GTN)-mediated vascular dilation (NMD), which is known as endothelium-independent vascular dilatation, is also used as a non-invasive examination for vascular dilation. In NMD, GTN as an exogenous NO donor is used to evaluate the responsiveness of the vascular smooth muscle to NO. Thus, in a patient with impaired NMD, interpretation of the results of FMD would be difficult, and FMD should be carried out only on patients with normal NMD. In the present study, we evaluated FMD in conjunction with NMD in order to characterize the impaired vascular function in patients with type 2 diabetes (DM)[4], using an ultrasonographic apparatus that has the function to draw a trend gram of the changes in vascular diameter.

METHODS

Participants

A total of 111 DM patients (62 males, 63.3 ± 11.5 years) and 42 age- and body mass index (BMI)-matched healthy control participants (22 males, 62.7 ± 13.7 years) were recruited for the present study (Table 1).

Evaluated Parameters

The presence of ischemic heart disease (IHD) was documented by angina pectoris or myocardial infarction based on symptoms and ischemic changes in an electrocardiogram, and/or on

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Table 1 | Clinical characteristics, the beginning time of vascular dilation and the velocity of vascular dilation on flow-mediated vascular dilation and glyceryl trinitrate-mediated vascular dilation in type 2 diabetes patients and control participants

	DM patients	Control participants	*P*-value
n	111	42	–
Age (years)	63.3 ± 11.5	62.7 ± 13.7	NS
Sex (% men)	55.9	52.4	NS
BMI (kg/m^2)	22.8 ± 2.40	22.0 ± 2.30	NS
Duration of diabetes (years)	19.7 ± 11.1	0	–
A1C (%)	7.7 ± 1.3	5.0 ± 0.4	<0.001
Systolic blood pressure (mmHg)	136.8 ± 15.4	127.0 ± 9.7	<0.001
Diastolic blood pressure (mmHg)	77.2 ± 7.6	69.8 ± 7.0	<0.001
Fasting plasma glucose (mg/dL)	132.3 ± 43.1	90.7 ± 8.6	<0.001
LDL cholesterol (mg/dL)	133.8 ± 21.0	134.3 ± 24.7	NS
HDL cholesterol (mg/dL)	34.9 ± 16.5	40.6 ± 9.3	NS
Triglyceride (mg/dL)	145.4 ± 102.0	143.7 ± 42.9	NS
Prevalence of complicated with			
IHD (%)	19.0	0	<0.001
Retinopathy (%)	31.5	0	<0.001
Albuminuria (%)	23.8	0	<0.001
eGFR (mL/min/1.73 m^2)	93.9 ± 30.5	91.7 ± 27.9	NS
%FMD (%)	4.52 ± 3.04	6.67 ± 2.78	<0.001
T on FMD (s)	23.3 ± 15.2	16.9 ± 10.0	0.005
V on FMD (mm/s)	0.017 ± 0.013	0.019 ± 0.016	0.030
%NMD (%)	10.7 ± 5.30	16.3 ± 6.37	<0.001
T on NMD (s)	74.3 ± 25.2	57.2 ± 11.6	<0.001
V on NMD (mm/s)	0.004 ± 0.002	0.006 ± 0.003	<0.001

Data are presented as mean ± standard deviation. A1C, hemoglobin A1C; Albuminuria, macroalbuminuria (albumin creatinine ratio ≥300 mg/g of creatinine); BMI, body mass index; eGFR, estimated glomerular filtration rate; FMD, flow-mediated vascular dilation; *h*, maximum diameter; HbA_{1c}, glycated hemoglobin; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; NMD, glyceryl trinitrate-mediated vascular dilation; NS, not significant; Retinopathy, preproliferative or proliferative diabetic retinopathy; *t*, elapsed time from the point of beginning of dilation to the point of maximum dilation; *T*, beginning time of vascular dilation after stimulation in flow-mediated vascular dilation or glyceryl trinitrate-mediated vascular dilation; *V*, velocity of vascular dilation.

positive findings at coronary angiography. Albuminuria was defined by the presence of macroalbuminuria[5] (urine albumin concentration ≥300 mg/g of creatinine in spot urine collected in the morning). Patients with a history of non-diabetic renal disease were excluded. Estimated glomerular filtration rate (eGFR) was calculated with the calculus equation provided by the Japanese Society of Nephrology[6]. The value for A1C is estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the following formula: A1C (%) = 1.02 × glycated hemoglobin (HbA_{1c}; Japan Diabetes Society [JDS]) (%) + 0.25%, which expresses the relationship between HbA_{1c} (JDS) (%) measured by the Japanese reference system and HbA_{1c} measured by NGSP (%)[7]. No participants in the control group had been diagnosed as having cardiovascular disease or risk factors, such as dyslipidemia or hypertension. Participants who had a smoking habit were excluded from the study. The study procedures were approved by the Ethics Committee of Wakayama Medical University.

FMD and NMD

Brachial artery ultrasonography was carried out after resting in the supine position for more than 10 min. All the examinations were carried out in a fasting state on the day of the examination. All participants refrained from drinking beverages containing caffeine or alcohol for 12 h before the examination, and were also advised not to take antihypertensive or vasodilation drugs on the day of examination. Patients were examined in a quiet and temperature controlled room (20–25°C). The right arm was extended and immobilized with an angle of approximately 60° from the trunk of the body. A 10-MHz linear transducer, connected to an ultrasound device[8] (UNEX Corporation, Nagoya, Japan) was placed on the brachial artery at approximately 1–2 cm proximal to the elbow joint. After scanning the baseline artery diameter, the cuff was rapidly inflated to 50 mmHg above systolic blood pressure and kept for 5 min. By rapid deflation of the cuff, reactive hyperemia was induced and scanning was continued for 2 min to obtain maximum arterial dilation (%FMD)[9]. As more than 20 min of bedrest was required to evaluate NMD after obtaining %FMD, participants were rested in bed for 20 min; subsequently, baseline diameter of the brachial artery was obtained again. Then GTN, as an exogenous NO donor, was sprayed sublingually and the arterial dilation was subsequently recorded for 5 min to evaluate maximum arterial dilation (%NMD). %FMD and %NMD were calculated as the percentage rise of this peak diameter from the baseline diameter[10]. FMD and NMD were examined by a qualified investigator, and the mean coefficient of variation of %FMD did not exceed 5%. As the ultrasound devise that we used can record the trend gram of alteration of the vascular diameter, the time of beginning of dilation (*T*) and the vascular dilation velocity (*V*) were also evaluated[11]. *T* was calculated from the point of cuff deflation or administration of GTN sublingually to the beginning of vascular dilation, and *V* was calculated as maximum diameter (*h*) divided by elapsed time (*t*): lasting time from the point of beginning of dilation to the point of maximum dilation (Figure 1). *T* and *V* were measured in both FMD and NMD.

Statistical Analyses

Results are expressed as mean ± standard deviation (SD). Comparisons of clinical variables between two groups were analyzed by Student's *t*-test, and Scheffé's method was adapted for

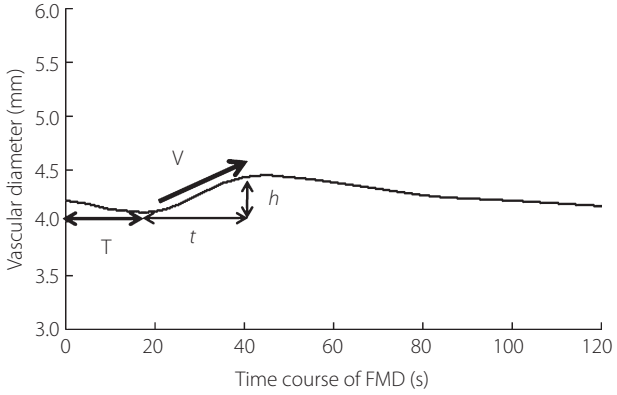

Figure 1 | Trend gram of the vascular diameter during the examination of flow-mediated vascular dilation (FMD). Time course and diameter of brachial artery are shown. *T*, beginning time of vascular dilation after stimulation (rapid deflation of the cuff on FMD or sublingual administration of glyceryl trinitrate on glyceryl trinitrate-mediated vascular dilation [NMD]). *h*, maximum diameter; *t*, elapsed time from the point of beginning of dilation to the point of maximum dilation; *V*, velocity of vascular dilation.

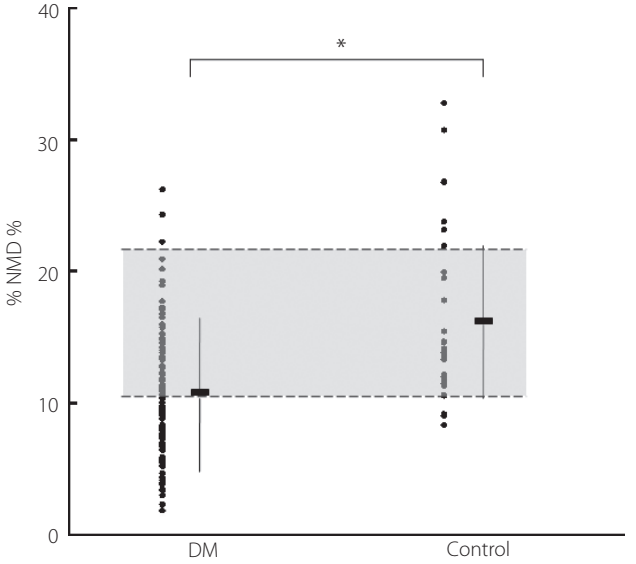

Figure 2 | The maximal dilation of glyceryl trinitrate-mediated vascular dilation (%NMD) of type 2 diabetes (DM) patients and control participants (Control). The range of 1 standard deviation of %NMD in control participants is shown by the shaded area. *$P < 0.001$ vs control participants.

comparisons among three groups. Correlation coefficients were derived by multiple linear regression analysis, and a value of $P < 0.05$ was considered statistically significant.

RESULTS

The clinical characteristics of the whole group of DM patients and the control participants are shown for comparison in Table 1. The duration of DM patients was approximately 20 years (19.7 ± 11.1 years). Systolic and diastolic blood pressure were higher in DM patients than control participants ($P < 0.001$), and lipid profiles were almost similar in both groups. Both %FMD and %NMD in DM patients were significantly lower compared with the control participants, respectively (Table 1 and Figure 2). *T* and *V* on FMD and NMD in DM patients were also significantly delayed or decreased compared with the control participants (Table 1).

In all 111 DM, 55 patients (49%) had impaired %NMD (less than mean-1SD of control participants; Figure 2). In order to exclude the influence of the vascular smooth muscle dysfunction (impaired %NMD) on %FMD, we selected DM patients who had normal %NMD (equal to or more than mean-1SD of the control participants), and the %FMD, *T* and *V* of this group (DM with normal %NMD) were compared with those of the control participants and also DM patients with impaired % NMD group (Table 2). The %FMD in the DM with normal % NMD and impaired %NMD group were also significantly lower than control participants, respectively. On the other hand, *T* and *V* of FMD were not significantly different between the DM patients with normal %NMD group and control participants, in contrast with the significantly disturbed *T* and *V* on FMD in the DM with impaired %NMD (Table 2).

In regard to the clinical feature of DM with impaired % NMD, the frequency of hypertension and albuminuria were significantly increased compared with those of the patients with normal %NMD, although eGFR was not significantly different between the two groups (Table 3). Multiple linear regression analysis also showed that %NMD, not eGFR, was independently associated with albuminuria (Table 4).

DISCUSSION

In our former examination[12] for the assessment of vascular responses in DM, we used 15 seconds interval measurement of the arterial diameter to determine the post-deflation arterial responses in FMD and post-GTN responses in NMD in accordance with other articles[13–17]. As the time course of vascular dilation was different among each participants, true maximum diameters for calculating %FMD and %NMD might have been missed using the 15 seconds intervals examination. From this aspect, in the present study we used an ultrasonographic apparatus that has the function to draw the trend gram of the changes in vascular diameter. This apparatus gives the possibility to observe the continuous changes of the vascular diameter, and we have considered that it measures the accurate maximum diameter or the peak of dilation.

Several articles reported that %FMD in DM was decreased, but usually they assessed only %FMD[18–20], not combined with %NMD, which reflects vascular smooth muscle function[9]. The feature of atherosclerosis in DM is considered as an increased arterial stiffness[21,22], with both endothelial and vascular smooth muscle dysfunction. In our data obtained from the present

Table 2 | Comparison of parameters in vascular dilation on flow-mediated vascular dilation and glyceryl trinitrate-mediated vascular dilation among type 2 diabetes patients and control subjects

	(A) DM patients with normal %NMD	(B) DM patients with impaired %NMD	(C) Control participants	*P*-value		
				(A) vs (C)	(B) vs (C)	(A) vs (B)
n	56	55	42	–	–	–
%FMD (%)	4.86 ± 2.43	3.79 ± 3.10	6.67 ± 2.78	<0.001	<0.001	NS
T on FMD (s)	19.5 ± 10.2	24.9 ± 15.7	16.9 ± 10.0	NS	0.003	0.025
V on FMD (mm/s)	0.021 ± 0.035	0.012 ± 0.011	0.019 ± 0.016	NS	0.005	NS
%NMD (%)	14.75 ± 4.75	6.69 ± 2.33	16.3 ± 6.37	NS	<0.001	<0.001
T on NMD (s)	67.5 ± 22.7	81.1 ± 26.6	58.9 ± 11.6	NS	<0.001	0.007
V on NMD (mm/s)	0.005 ± 0.001	0.003 ± 0.002	0.006 ± 0.003	NS	<0.001	<0.001

%FMD, maximal dilation of flow-mediated vascular dilation; %NMD, maximal dilation of glyceryl trinitrate-mediated vascular dilation; DM, type 2 diabetes patients; FMD, flow-mediated vascular dilation; *h*, maximum diameter; NMD, glyceryl trinitrate-mediated vascular dilation; NS, not significant; *t*, elapsed time from the point of beginning of dilation to the point of maximum dilation; *T*, beginning time of vascular dilation after stimulation in flow-mediated vascular dilation or glyceryl trinitrate-mediated vascular dilation; *V*, velocity of vascular dilation.

Table 3 | Clinical characteristics of type 2 diabetes patients divided into two groups according to maximal dilation of glyceryl trinitrate-mediated vascular dilation value

Characteristic	DM patients with normal %NMD	DM patients with impaired %NMD	*P*-value
n	56	55	–
Age (years)	64.2 ± 9.4	62.4 ± 11.7	NS
Sex (% men)	58.9	52.7	NS
BMI (kg/m^2)	22.8 ± 5.1	24.1 ± 3.3	NS
Duration of diabetes (years)	20.0 ± 13.0	19.6 ± 9.8	NS
Systolic blood pressure (mmHg)	132.4 ± 14.7	142.4 ± 14.4	0.004
Diastolic blood pressure (mmHg)	75.7 ± 7.88	79.1 ± 6.93	0.017
A1C (%)	7.59 ± 1.00	8.04 ± 1.23	NS
Fasting plasma glucose (mg/dL)	129.9 ± 44.2	149.0 ± 39.1	0.027
LDL cholesterol (mg/dL)	131.3 ± 20.4	137.2 ± 21.5	NS
HDL cholesterol (mg/dL)	35.7 ± 17.6	34.1 ± 15.3	NS
Triglyceride (mg/dL)	139.7 ± 115.1	152.8 ± 82.7	NS
Therapy for diabetes (%)			
Diet	4 (6.8)	1 (1.8)	NS
Sulfonylurea	32 (57.1)	26 (46.2)	NS
Insulin	29 (38.9)	23 (51.8)	NS
Other medications			
Antihypertensive drugs (%)	25 (44.6)	42 (76.3)	<0.001
ARB	19 (33.9)	30 (45.5)	<0.001
Calcium channel blocker	20 (17.9)	30 (54.5)	0.015
β-Blocker	3 (5.4)	3 (5.5)	NS
Statins (%)	10 (17.9)	17 (30.9)	0.030
IHD (%)	25.4	41.5	NS
Retinopathy (%)	23.4	39.5	NS
Albuminuria (%)	17.0	35.8	0.036
eGFR (mL/min/1.73 m^2)	93.9 ± 30.5	91.7 ± 27.9	NS

%NMD, maximal dilation of glyceryl trinitrate-mediated vascular dilation; A1C, hemoglobin A1C; ARB, angiotensin II receptor blocker; BMI, body mass index; IHD, ischemic heart disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; IHD, ischemic heart disease; LDL, low-density lipoprotein; NS, not significant.

study, 51% of the whole group of DM had low %NMD (less than mean-1SD of the control participants). The vascular smooth muscle in those patients was considered to also have poor contraction in response to endothelium-derived relaxing factors, such as NO[23–25]. In order to exclude the influence of impaired vascular smooth muscle function on the measurement

Table 4 | Multiple linear regression analyses of independent determinants of maximal dilation of glyceryl trinitrate-mediated vascular dilation in type 2 diabetes patients

	β	*P*-value
Age	−0.118	0.031
Sex (males: 0, females: 1)	0.809	NS
BMI (kg/m^2)	−0.102	NS
Duration of diabetes	−0.375	NS
A1C (%)	−0.089	NS
Absence (0) or presence (1) of		
Hypertension	1.042	NS
IHD	−2.164	NS
Retinopathy	−0.556	NS
Albuminuria	−2.574	0.001
eGFR (mL/min/1.73 m^2)	0.021	NS

A1C, hemoglobin A1C; Albuminuria, macroalbuminuria (albumin creatinine ratio ≥ 300 mg/g creatinine); β, standard regression coefficient; BMI, body mass index; eGFR, estimated glomerular filtration rate; Hypertension, systolic blood pressure 140 mmHg or diastolic blood pressure ≥ 90 mmHg; IHD, ischemic heart disease; Retinopathy, preproliferative or proliferative diabetic retinopathy.

of %FMD, we selected DM patients who had normal %NMD (equal or more than mean-1SD of the control participants). Using these patients with normal %NMD, we could clarify exactly the impaired %FMD in DM patients (Table 3). Thus, the impaired endothelial function is already present, even in DM patients whose vascular smooth muscle function is still retained. These results showed that combined examination of NMD with FMD should be necessary to obtain accurate results for vascular function in DM patients.

The trend gram that we used in the present study makes it possible to assess *T* and *V* during the examination of FMD and NMD. As shown in Table 2, *T* and *V* during examination of FMD in DM patients with normal %NMD were not significantly impaired compared with those in the control participants, despite the significant decrease in %FMD (maximal dilation). Generally, pathophysiology of endothelial dysfunction was considered to be caused by a reduction of NO and the other factors[23–25]. In the present study, it is more likely that the impairment of endothelial function that was observed in DM patients with normal %NMD would be a result of the low production of NO in the endothelium, because the maximal dilation in response to reactive hyperemia (%FMD) was disturbed, although their responsiveness and reactivity indicated as *T* and *V* of FMD were normal, but further investigation is required to settle this issue.

In the whole group of DM patients, approximately half of the patients (51%) were thought to have vascular smooth muscle dysfunction (impaired %NMD). When clinical characteristics of patients with impaired %NMD were compared with those of patients with normal %NMD, an increased trend of A1C, and a significant high prevalence of albuminuria and hypertension were detected, although the duration of diabetes was almost the same between the two groups. Multiple regression analysis also showed that albuminuria was independently correlated with %NMD in a negative manner when corrected with eGFR. Some articles reported that impairment of vascular dilation had often been shown to be associated with albuminuria in the non-diabetic patients with hypertension or dyslipidemia[26–28]. As impaired %FMD was found in diabetes alone[29], it is not surprising that vascular dysfunction might occur in DM patients with albuminuria. Our data also showed hyporesponsiveness of vascular dilation to the exogenous NO in DM patients with albuminuria. Campuzano[30] reported that the main cause of hyporesponsiveness of vascular dilation to exogenous NO might be related to the vascular smooth muscle dysfunction. This is why DM patients with albuminuria are considered to have vascular smooth muscle dysfunction.

The present data, taken together, strongly suggest that both vascular endothelial function and vascular smooth muscle function have been impaired in DM, and in particular, vascular smooth muscle function is considered to be a key factor for vascular dilation in DM. Also, impaired %NMD in DM is related to albuminuria. Thus, it is necessary to examine not only FMD, but also NMD in evaluating vascular function of DM, especially in those with albuminuria. Further long-term prospective studies could address the issue of impairment in vascular function shown by FMD and NMD.

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