JDI Journal of Diabetes Investigation Open access
Official Journal of the Asian Association for the Study of Diabetes
ORIGINAL ARTICLE

Relationship between hemoglobin A1c level and flow-mediated vasodilation in patients with type 2 diabetes mellitus receiving antidiabetic drugs

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Keywords
Diabetes mellitus, Flow-mediated vasodilation, Hemoglobin A1c

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J Diabetes Investig 2022; 13: 677–686

doi: 10.1111/jdi.13705

ABSTRACT

Aims/Introduction: Diabetes mellitus is associated with endothelial dysfunction. However, it is still controversial as to whether antidiabetic drug treatment affects endothelial function. The purpose of this study was to evaluate the relationships of the hemoglobin A1c (HbA1c) level with flow-mediated vasodilation (FMD) and nitroglycerine-induced vasodilation (NID) in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs.

Materials and Methods: The FMD was measured in 866 patients with type 2 diabetes mellitus who were receiving antidiabetic drugs (625 men and 241 women; mean age: 62 ± 10 years). The patients were divided into four groups according to HbA1c levels: <6.5, 6.5–6.9, 7.0–7.9, and ≥8.0%.

Results: There was an inverted U-shaped pattern of association of the HbA1c level with the FMD at an HbA1c level of about 7% of the peak of FMD in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs. The FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5–6.9% group and the HbA1c 7.0–7.9% group ($P < 0.001$ and $P < 0.001$, respectively). The FMD values were similar in the HbA1c <6.5% group and HbA1c ≥8.0% group ($P = 0.10$). There were no significant differences in NID among the four groups ($P = 0.98$).

Conclusions: These findings suggest that a low HbA1c <6.5% as well as a high HbA1c ≥8.0% is associated with endothelial dysfunction in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs and that vascular smooth muscle function is similar in such patients regardless of the HbA1c level.

INTRODUCTION

Endothelial dysfunction is established as an initial factor of atherosclerosis and plays a critical role in the onset of cardiovascular events[1,2]. Measurement of flow-mediated vasodilation (FMD) in the brachial artery is a widely used noninvasive tool for the assessment of endothelial function[3–5]. Endothelial function assessed by FMD is an independent marker of cardiovascular events[6–10].

It is well known that diabetes mellitus (DM) is a risk factor for endothelial dysfunction, subsequent cardiovascular disease, and cardiovascular mortality[11,12]. Physicians usually treat diabetes mellitus using hemoglobin A1c (HbA1c) as a marker for

Received 4 June 2021; revised 13 October 2021; accepted 31 October 2021

glucose control. However, unfortunately, HbA1c is not established as a predictor for future cardiovascular events. Recently, the American College of Physicians committee recommended that an HbA1c level of 7.0%–8.0% is appropriate for DM control, that if the HbA1c level is lower than 6.5% in patients treated with antidiabetic drugs, the doses of antidiabetic drugs should be reduced since there is a high risk for hypoglycemia, and that a target HbA1c level is not needed in patients with diabetes mellitus who are 80 years of age or more[13].

Although previous studies showed that reduction in HbA1c level induced by antidiabetic drugs improves endothelial function in patients with type 2 diabetes mellitus[14,15], there has been little information on the association of the HbA1c level with FMD in patients with type 2 diabetes mellitus in a large-scale clinical trial. Recently, we have shown that there is an inverted U-shaped pattern of association of the HbA1c level with FMD at the peak of HbA1c of about 7% in patients with type 2 diabetes mellitus who are not receiving antidiabetic drugs[16].

However, the relationship between the HbA1c level and FMD in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs is still unknown. Therefore, we evaluated the relationships of HbA1c level with endothelial function assessed by FMD and vascular smooth muscle function assessed by nitroglycerine-induced vasodilation (NID) in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs.

METHODS

Study subjects

A total of 10,260 subjects, including 7,385 subjects from the Flow-mediated Dilation Japan Registry (FMD-J) and 2,875 subjects who underwent a health checkup at Hiroshima University Hospital between August 2007 and August 2016, were enrolled in this study. The FMD-J study was a prospective multicenter registry with the aim of establishing the usefulness of FMD. The design of the FMD-J study has been described in detail previously[17]. The protocol used for the measurement of FMD was the same in the FMD-J study and in the medical checkups at Hiroshima University Hospital. We excluded the following subjects: subjects with unclear images of the brachial artery (n = 12), subjects without information on HbA1c level (n = 2,010), subjects without DM (n = 6,932), patients with type 2 diabetes mellitus who were not receiving antidiabetic drugs (n = 349), subjects on dialysis or with end-stage chronic kidney disease (n = 12), subjects with inadequate medical information (n = 2), and subjects over 80 years of age (n = 77). Finally, we enrolled 866 subjects (625 men and 241 women; mean age: 62 ± 10 years) in this study (Figure S1). Hypertension was defined as the use of antihypertensive drugs or a systolic blood pressure of more than 140 mmHg or a diastolic blood pressure of more than 90 mmHg measured in a sitting position on at least three occasions. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program[18]. Diabetes mellitus was defined according to the American Diabetes Association recommendation[19]. Cardiovascular disease was defined as coronary heart disease and cerebrovascular disease. Coronary heart disease included angina pectoris, prior myocardial infarction, and unstable angina. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack. The Ethics Committee of Hiroshima University approved the study protocol (ID number: E-1902). Written informed consent for participation in this study was obtained from all participants.

Study protocol

Vascular function was assessed in all patients by using measurements of FMD and NID. The patients fasted overnight and abstained from alcohol, smoking, caffeine, and antioxidant vitamins for at least 12 h before the study. The participants were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 22–25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the deep antecubital vein to obtain blood samples. After maintaining the supine position for 30 min, FMD and NID were measured. The observers were blind to the form of examination.

We evaluated the relationships of HbA1c levels with FMD and NID in the 866 patients with type 2 diabetes mellitus who were receiving antidiabetic drugs. First, we divided the patients into two groups based on their HbA1c levels: <6.5 and ≥6.5%. Multivariate regression analysis was performed to identify independent variables associated with vascular function. Next, we divided the patients into four groups according to the HbA1c levels: <6.5, 6.5–6.9, 7.0–7.9, and ≥8.0%. We next assessed the relationships of HbA1c levels with FMD and NID using propensity score matching.

Measurements of FMD and NID

We measured the vascular response to reactive hyperemia in the brachial artery for assessment of endothelium-dependent FMD. A high-resolution linear artery transducer was coupled to computer-assisted analysis software (UNEXEF18G; UNEX Co., Nagoya, Japan) that used an automated edge detection system for measurement of the brachial artery diameter[20]. A blood pressure cuff was placed around the forearm of each subject. The brachial artery was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and vessel wall was obtained, the transducer was held at the same point throughout the scan by using a special probe holder (UNEX Co.) to ensure consistency of the imaging. Depth and gain setting were set to optimize the images of the arterial lumen wall interface. When the tracking gate was placed on the intima, the artery diameter was automatically tracked, and the waveform of diameter changes over the cardiac cycle was displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak

hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. The blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time. Baseline longitudinal images of the artery were acquired for 30 s, and then the blood pressure cuff was inflated to 50 mmHg above systolic pressure for 5 min. The longitudinal image of the artery was recorded continuously until 5 min after cuff deflation. Pulsed Doppler velocity signals were obtained for 20 s at baseline and for 10 s immediately after cuff deflation. Changes in the brachial artery diameter were immediately expressed as the percentage change relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. The percentage of FMD [(Peak diameter − Baseline diameter)/Baseline diameter] was used for analysis. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area ($-r^2$). Reactive hyperemia was calculated as the maximum percentage increase in flow after cuff deflation compared with the baseline flow. The correlation coefficient between FMD analyzed at the core laboratory and participant institutions was 0.84 ($P < 0.001$).

The response to nitroglycerine was used for assessment of endothelium-independent vasodilation[20]. After acquiring baseline rest images for 30 s, a sublingual tablet (nitroglycerine, 75 μg) was given and imaging of the artery was done continuously for 5 min. NID was automatically calculated as a percentage change in the peak vessel diameter from the baseline. The percentage of NID [(Peak diameter − Baseline diameter)/Baseline diameter] was used for analysis. Inter- and intra-coefficients of variation for the brachial artery diameter were 1.6 and 1.4%, respectively, in our laboratory.

Statistical analysis

The results are presented as mean ± SD or median (interquartile range). All reported probability values were 2-sided, and a probability value of <0.05 was considered statistically significant. An association between FMD and HbA1c level was explored visually using a locally weighted regression smoothing (Lowess) plot. Categorical values were compared by means of the chi-square test. Continuous variables were compared by using ANOVA of multiple groups. Comparisons between the groups categorized according to HbA1c levels were carried out using repeated measures ANOVA with Tukey's post hoc test. Univariate linear regression analyses were performed to assess the relationships among the variables. Multivariate logistic regression analysis was performed to identify independent variables associated with lower quartiles of FMD (<2.0%) and NID (<6.0%). Age, gender, body mass index, estimated glomerular filtration rate (e-GFR), current smoking, and the presence of hypertension, dyslipidemia, and cardiovascular disease were entered into the multivariate logistic regression analysis. As a sensitivity analysis, propensity score analysis was used to minimize the selection bias for evaluation of the relationships among antidiabetic drugs, HbA1c level, and vascular function. The propensity score was calculated for each patient on the basis of logistic regression analysis of the probability of taking antidiabetic drugs within groups stratified by HbA1c levels (<6.5, 6.5–6.9, 7.0–8.0, and ≥8.0%) using clinical variables including age, sex, body mass index (BMI), systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, triglycerides, high-density lipoprotein (HDL-C), uric acid levels, current smoking (yes or no), medication with antihypertensive drugs (yes or no), medication with lipid-lowering drugs (yes or no), and the presence of cardiovascular disease (yes or no). With these propensity scores using a caliper width of 0.25 standard deviations of the logit of the propensity score, two well-matched groups based on clinical characteristics were created for comparison of the prevalence of endothelial dysfunction defined as FMD of <2.0%, the division point for the lowest quartile of FMD in all participants. As a post hoc analysis, we fitted a cubic spline curve relationship between HbA1c and endothelial dysfunction assessed by FMD and vascular smooth muscle dysfunction assessed by NID. All data were processed using JMP Pro. Ver 14.0 software (SAS Institute, Cary, NC, USA).

RESULTS

Baseline characteristics of patients with type 2 diabetes mellitus who were receiving antidiabetic drugs

The baseline characteristics of patients are summarized in Table 1. Of the 866 patients, 625 (72.2%) were men and 241 (27.8%) were women. The mean fasting blood glucose level in the patients was 139 ± 46 mg/dL and the mean HbA1c level was 6.8 ± 1.1%. Among the patients, 703 (81.2%) had hypertension, 678 (78.3%) had dyslipidemia, 330 (38.1%) had previous cardiovascular disease, and 211 (24.6%) were current smokers. The mean FMD value in patients was 4.1 ± 2.8% and the mean NID value was 10.4 ± 5.9% (Table 1).

FMD, NID, HbA1c level, and variables in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs

Table S1 shows univariate relations among FMD, HbA1c level, and variables. FMD was significantly correlated with age ($r = -0.31$, $P < 0.001$), diastolic blood pressure ($r = 0.15$, $P < 0.001$), creatinine ($r = -0.11$, $P = 0.002$), e-GFR ($r = 0.13$, $P = 0.005$), HbA1c level ($r = 0.10$, $P = 0.004$), and NID ($r = 0.31$, $P < 0.001$). HbA1c level was significantly correlated with age ($r = -0.22$, $P < 0.001$), BMI ($r = 0.08$, $P = 0.02$), systolic blood pressure ($r = 0.12$, $P < 0.001$), diastolic blood pressure ($r = 0.15$, $P < 0.001$), total cholesterol ($r = 0.17$, $P < 0.001$), HDL-C ($r = -0.12$, $P < 0.001$), low-density lipoprotein cholesterol (LDL-C) ($r = 0.17$, $P < 0.001$), e-GFR ($r = 0.20$, $P < 0.001$), uric acid ($r = -0.12$, $P < 0.001$), fasting blood glucose level ($r = 0.53$, $P < 0.001$), and FMD ($r = 0.10$, $P = 0.004$). Linear regression analysis revealed that the HbA1c level was significantly correlated with FMD ($r = 0.10$, $P = 0.004$; Figure S2a). A scatter plot between FMD and HbA1c level with a Lowess smoothed curve is shown in Figure

Table 1 | Clinical characteristics of patients with type 2 diabetes mellitus receiving antidiabetic drugs

Variable	Total (n = 866)	HbA1c <6.5% (n = 373)	HbA1c 6.5–6.9% (n = 184)	HbA1c 7.0–7.9% (n = 205)	HbA1c ≥8.0% (n = 104)	P value
Age, year	62 ± 10	65 ± 9	62 ± 10	62 ± 10	56 ± 11	<0.001
Gender, men/women	625/241	242/131	132/52	168/37	83/21	<0.001
Body mass index, kg/m^2	25.3 ± 4.3	24.7 ± 4.0	25.8 ± 4.5	25.6 ± 4.4	25.6 ± 4.4	0.01
Heart rate, bpm	68 ± 12	68 ± 12	68 ± 11	67 ± 12	68 ± 13	0.63
Systolic blood pressure, mmHg	133 ± 18	130 ± 18	134 ± 16	135 ± 18	135 ± 17	0.001
Diastolic blood pressure, mmHg	78 ± 11	76 ± 10	79 ± 11	80 ± 11	80 ± 13	<0.001
Total cholesterol, mg/dL	183 ± 35	178 ± 33	185 ± 31	182 ± 35	197 ± 46	<0.001
Triglycerides, mg/dL	134 (92, 199)	111 (80, 160)	112 (78, 154)	124 (86, 179)	137 (85, 191)	<0.001
HDL-C, mg/dL	55 ± 15	57 ± 16	55 ± 15	52 ± 14	52 ± 15	0.003
LDL-C, mg/dL	104 ± 31	99 ± 28	107 ± 29	104 ± 31	115 ± 41	<0.001
Creatinine, mg/dL	0.85 ± 0.3	0.86 ± 0.30	0.85 ± 0.28	0.86 ± 0.32	0.84 ± 0.32	0.82
e-GFR, mL/min/1.73 m^2	71 ± 19	68 ± 18	72 ± 19	73 ± 18	79 ± 24	<0.001
Uric acid, mg/dL	5.7 ± 1.3	5.7 ± 1.2	5.8 ± 1.3	5.6 ± 1.4	5.2 ± 1.2	<0.001
Fasting blood glucose, mg/dL	139 ± 46	118 ± 27	134 ± 28	150 ± 37	196 ± 77	<0.001
HbA1c, %	6.8 ± 1.1	5.9 ± 0.4	6.7 ± 0.1	7.4 ± 0.3	9.0 ± 1.3	<0.001
Medical history, n (%)						
Hypertension	703 (81.2)	303 (81.2)	154 (83.7)	170 (82.9)	76 (70.1)	0.13
Dyslipidemia	678 (78.3)	292 (78.3)	135 (73.4)	169 (82.4)	82 (78.9)	0.2
CVD	330 (38.1)	123 (33.0)	69 (37.5)	87 (42.4)	51 (49.0)	0.01
Current smoking, n (%)	211 (24.6)	84 (22.5)	39 (21.6)	56 (28.0)	32 (31.1)	0.15
Medication, n (%)						
Antihypertensive drugs	852 (70.1)	365 (77.0)	227 (68.2)	181 (66.5)	79 (58.1)	<0.001
Lipid lowering drugs	680 (56.0)	298 (62.9)	168 (50.5)	154 (56.6)	60 (44.1)	0.001
Antidiabetic drugs						
Sulfonylurea	82 (21.6)	33 (14.2)	18 (26.9)	23 (43.4)	8 (28.6)	<0.001
Glinide	20 (5.3)	14 (6.0)	3 (4.5)	2 (3.8)	1 (3.57)	<0.001
α-Glucosidase inhibitors	63 (16.6)	33 (14.2)	17 (25.4)	10 (18.9)	3 (10.7)	0.13
DPP-4 inhibitors	282 (74.2)	186 (80.2)	44 (65.7)	38 (71.7)	14 (50)	0.001
Biguanide	58 (15.3)	31 (13.4)	12 (17.9)	13 (24.5)	2 (7.14)	0.12
Pioglitazone	44 (11.61)	28 (12.1)	8 (11.9)	6 (11.32)	2 (7.14)	0.88
Insulin	41 (10.8)	10 (4.3)	8 (11.9)	9 (17.0)	14 (50)	<0.001
FMD, %	4.1 ± 2.8	3.6 ± 2.7	4.5 ± 2.9	4.5 ± 2.6	4.3 ± 2.7	<0.001
NID, %	10.4 ± 5.9	10.4 ± 5.8	10.7 ± 5.9	10.5 ± 5.1	10.4 ± 7.4	0.98

CVD, cardiovascular disease; DPP-4, dipeptidyl peptidase-4; e-GFR, estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NID, nitroglycerine-induced vasodilation.

S2b. FMD gradually increased with increase in the HbA1c level to about 6.5–7.0% and decreased with an increase in the HbA1c level above 7.0%. A cubic spline curve described the relationship between HbA1c and odds ratio for endothelial function assessed by FMD and vascular smooth muscle function assessed by NID (Figure S3).

FMD, NID, HbA1c level, and variables in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs with HbA1c levels <6.5% and HbA1c levels ≥6.5%

The baseline characteristics of patients with type 2 diabetes mellitus who were receiving antidiabetic drugs and who had HbA1c levels of <6.5% and HbA1c levels of ≥6.5% are summarized in Table 2. There were significant differences in age, gender, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, HDL-C, LDL-C, e-GFR, fasting blood glucose, HbA1c level, past cardiovascular disease, and the use of antihypertensive drugs between the two groups. FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c ≥6.5% group (3.6 ± 2.7% and 4.5 ± 2.7%, respectively, $P < 0.001$; Figure 1a). The NID values were similar in the two groups (10.4 ± 5.8% in the HbA1c <6.5% group and 10.6 ± 5.9% in the HbA1c ≥6.5% group, $P = 0.77$; Figure 1b).

Next, the patients were divided into four groups according to the HbA1c levels: <6.5, 6.5–6.9, 7.0–7.9, and ≥8.0%. The baseline characteristics of the patients in the four groups are summarized in Table 1. There were significant differences in age, gender, BMI, systolic blood pressure, diastolic blood pressure,

Table 2 | Clinical characteristics of patients with type 2 diabetes mellitus receiving antidiabetic drugs on the basis of serum HbA1c level (<6.5% vs ≥6.5%)

Variable	HbA1c <6.5% (*n* = 373)	HbA1c ≥6.5% (*n* = 493)	*P* value
Age, year	65 ± 9	61 ± 11	<0.001
Gender, men/women	242/131	383/110	<0.001
Body mass index, kg/m^2	24.7 ± 4.0	25.7 ± 4.4	0.001
Heart rate, bpm	68 ± 12	68 ± 12	0.47
Systolic blood pressure, mmHg	130 ± 18	135 ± 17	<0.001
Diastolic blood pressure, mmHg	76 ± 10	79 ± 12	<0.001
Total cholesterol, mg/dL	178 ± 33	186 ± 36	<0.001
Triglycerides, mg/dL	111 (80, 160)	120 (83, 177)	0.01
HDL-C, mg/dL	57 ± 16	53 ± 15	0.001
LDL-C, mg/dL	99 ± 28	107 ± 33	<0.001
Creatinine, mg/dL	0.86 ± 0.30	0.85 ± 0.30	0.48
e-GFR, mL/min/1.73 m^2	68 ± 18	74 ± 20	<0.001
Uric acid, mg/dL	5.7 ± 1.2	5.6 ± 1.3	0.09
Fasting blood glucose, mg/dL	118 ± 27	154 ± 51	<0.001
HbA1c, %	5.9 ± 0.4	7.5 ± 1.1	<0.001
Medical history, *n* (%)			
Hypertension	303 (81.2)	400 (81.1)	0.97
Dyslipidemia	292 (78.3)	386 (78.3)	1.00
CVD, *n* (%)	123 (33.0)	207 (42.0)	0.01
Current smoking, *n* (%)	84 (22.5)	127 (26.2)	0.21
Medication, *n* (%)			
Antihypertensive drugs	287 (76.9)	348 (70.6)	0.04
Lipid lowering drugs	239 (64.1)	297 (60.2)	0.25
Antidiabetic drugs	373 (100)	162 (100)	1.00
FMD, %	3.6 ± 2.7	4.5 ± 2.7	<0.001
NID, %	10.4 ± 5.8	10.6 ± 5.9	0.77

CVD, cardiovascular disease; DM, diabetes mellitus; e-GFR, estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; NID, nitroglycerine-induced vasodilation.

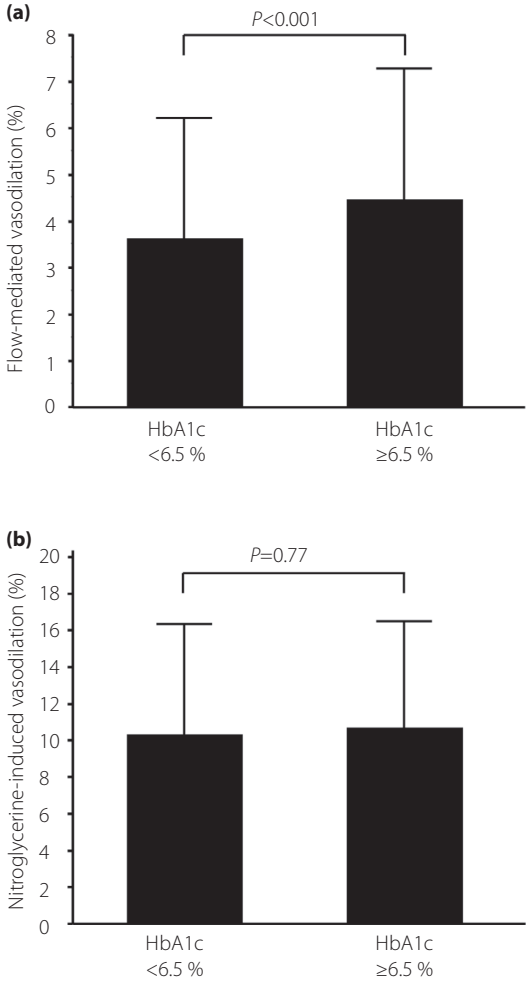

Figure 1 | Bar graphs show flow-mediated vasodilation (a) and nitroglycerine-induced vasodilation (b) in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs in the HbA1c <6.5% group and in the HbA1c ≥6.5% group.

total cholesterol, triglycerides, HDL-C, LDL-C, e-GFR, uric acid, fasting blood glucose, past cardiovascular disease, and the use of antihypertensive drugs, lipid-lowering drugs, sulfonylureas, glinide and dipeptidyl peptidase-4 (DPP-4) inhibitors among the four groups. The FMD values were 3.6 ± 2.7% in the HbA1c <6.5% group, 4.5 ± 2.9% in the HbA1c 6.5–6.9% group, 4.5 ± 2.6% in the HbA1c 7.0–7.9% group, and 4.3 ± 2.7% in the HbA1c ≥8.0% group ($P < 0.001$; Figure 2a). The FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5–6.9% group and HbA1c 7.0–7.9% group, while there was no significant difference in FMD between the HbA1c <6.5% group and the HbA1c ≥8.0% group ($P < 0.001$, $P < 0.001$ and $P = 0.11$ respectively; Figure 2a). The NID values were 10.4 ± 5.8% in the HbA1c <6.5% group, 10.7 ± 5.9% in the HbA1c 6.5–6.9% group, 10.5 ± 5.1% in the HbA1c 7.0–7.9% group, and 10.4 ± 7.4% in the HbA1c ≥8.0% group. There were no significant differences in NID values among the four groups ($P = 0.98$; Figure 2b).

We next determined whether a low HbA1c level of <6.5% was independently associated with a small FMD by multiple logistic regression analysis. After adjustments for age, gender, BMI, current smoking, e-GFR, and the presence of hypertension, dyslipidemia and cardiovascular disease, a HbA1c level of <6.5% was independently associated with a lower quartile of FMD (OR: 1.95, 95% CI: 1.39–2.74; $P < 0.001$) but was not associated with a lower quartile of NID (OR: 1.01, 95% CI: 0.56–1.83; $P = 0.98$; Table 3).

Propensity score matching analysis was used to create matched pairs between the HbA1c level of <6.5% group and the other three groups (HbA1c 6.5–6.9%, HbA1c 7.0–7.9%, and HbA1c ≥8.0%). The baseline characteristics of matched pairs of the low HbA1c <6.5% group and the other three groups are summarized in Tables S2–S4. The FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5–6.9% group (3.9 ± 2.8% vs 4.6 ± 3.0%, $P = 0.04$; Figure 3a) and the

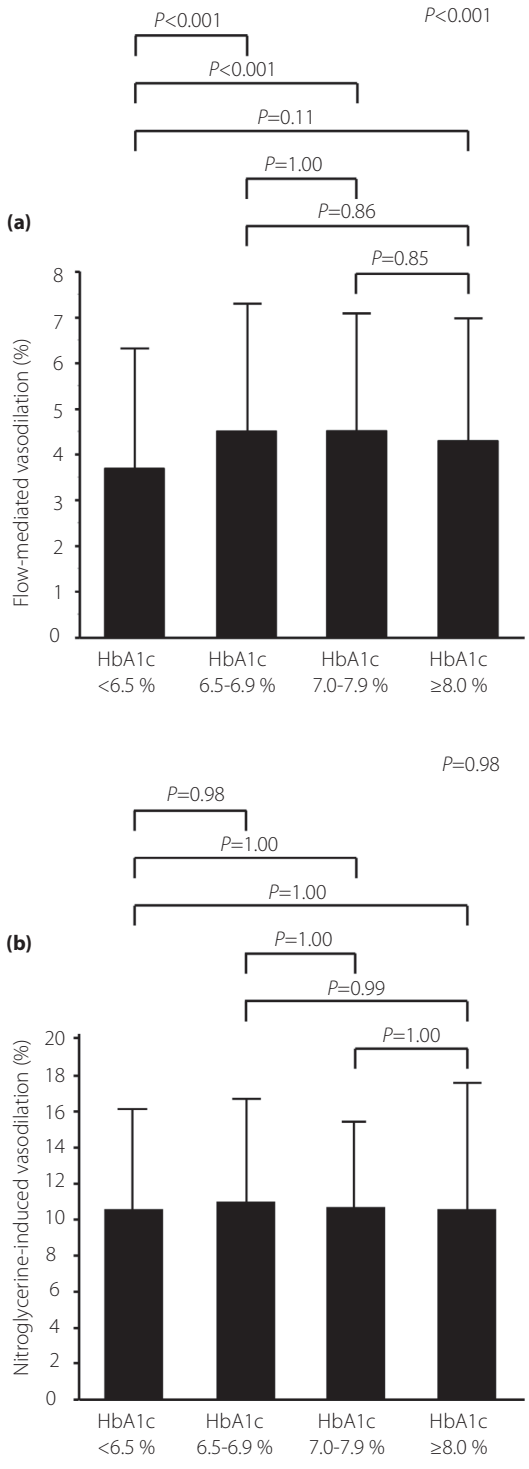

Figure 2 | Bar graphs show flow-mediated vasodilation (a) and nitroglycerine-induced vasodilation (b) in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs in four groups according to HbA1c levels.

Table 3 | Multivariate analysis of the relationships between FMD, NID, and low HbA1c levels (<6.5%) in patients with type 2 diabetes mellitus receiving antidiabetic drugs

Variables	Low quartile of FMD		Low quartile of NID	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Model 1	2.35 (1.71–3.23)	<0.001	1.13 (0.66–1.91)	0.66
Model 2	1.95 (1.39–2.74)	<0.001	1.01 (0.56–1.83)	0.98

Model 1: unadjusted model; Model 2: adjusted for age, gender, body mass index, current smoking, e-GFR, presence of hypertension, dyslipidemia, and CVD. Low quartile of FMD was defined as flow-mediated vasodilation of <2.0%. Low quartile of NID was defined as flow-mediated vasodilation of <6.0%. CI, confidence interval; CVD, cardiovascular disease; e-GFR, estimated glomerular filtration rate; FMD, flow-mediated vasodilation; HbA1c, hemoglobin A1c; NID, nitroglycerine-induced vasodilation; OR, odds ratio.

HbA1c 7.0–7.9% group (3.8 ± 2.6% vs 4.5 ± 2.7%, $P = 0.02$; Figure 3b), while there was no significant difference in FMD between the HbA1c <6.5% group and the HbA1c ≥8.0% group (4.8 ± 2.3% vs 4.0 ± 2.9%, $P = 0.06$; Figure 3c). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups: 10.3 ± 5.4% in the HbA1c <6.5% group and 10.9 ± 6.1% in the HbA1c 6.5–6.9% group ($P = 0.58$; Figure 3d), 9.9 ± 5.9% in the HbA1c level of <6.5% group and 10.3 ± 5.2% in the HbA1c 7.0–7.9% group ($P = 0.71$; Figure 3e), and 11.5 ± 6.6% in the HbA1c <6.5% group and 11.4 ± 7.8% in the HbA1c ≥8.0% group ($P = 0.96$; Figure 3f).

DISCUSSION

The present study demonstrated for the first time that even after adjustments of confounding factors for FMD, the FMD values were smaller in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs and who had an HbA1c level of <6.5% than in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs and who had an HbA1c level of 6.5–6.9% and an HbA1c level of 7.0–7.9% and that there were no significant differences in NID in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs among the HbA1c levels.

In a previous study, to avoid the pharmacological effects of antidiabetic drugs on vascular function, we evaluated the relationships of HbA1c level with FMD and NID in patients with type 2 diabetes mellitus who were not receiving antidiabetic drugs[16]. Interestingly, we found that there was an inverted U-shaped pattern of the association between HbA1c level and FMD at the peak of HbA1c of about 7% and that FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5–6.9% group and HbA1c 7.0–7.9% group and FMD

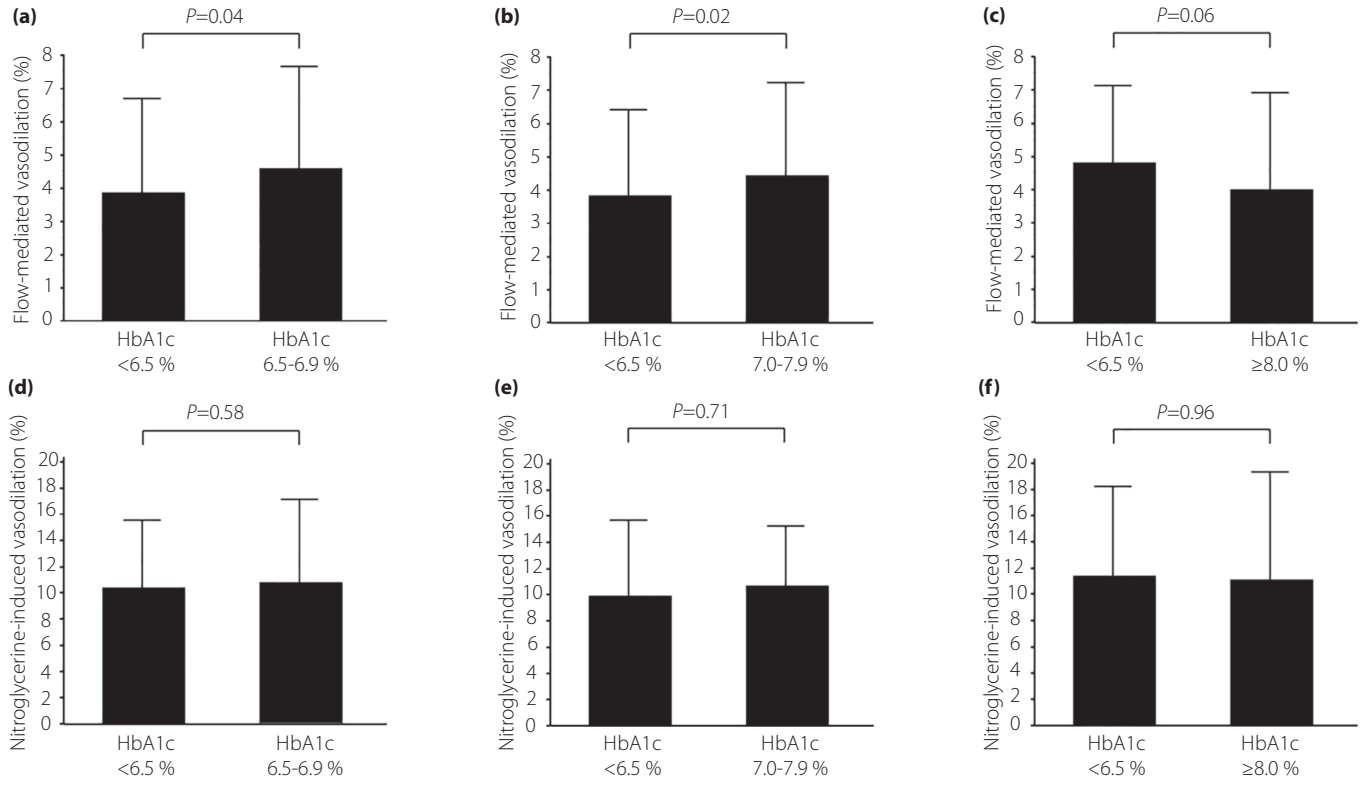

Figure 3 | Bar graphs show flow-mediated vasodilation in patients with HbA1c <6.5% and patients with HbA1c 6.5–6.9% (a), patients with HbA1c <6.5% and patients with HbA1c 7.0–7.9% (b), and patients with HbA1c <6.5% and patients with HbA1c ≥8.0% (c) and nitroglycerine-induced vasodilation in patients with HbA1c <6.5% and patients with HbA1c level of 6.5–6.9% (d), patients with HbA1c <6.5% and patients with HbA1c 7.0–7.9% (e), and patients with HbA1c <6.5% and patients with HbA1c ≥8.0% (f) in a propensity score-matched population.

values were similar in the HbA1c <6.5% group and HbA1c ≥8.0% group, suggesting that a low HbA1c level of <6.5% is associated with endothelial dysfunction in patients with type 2 diabetes mellitus who are not receiving antidiabetic drugs. The FMD values were similar among the HbA1c levels after adjustment of confounding factors by using propensity score matching analysis. There were no significant differences in NID among the HbA1c levels. Also in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs, endothelial function, but not vascular smooth muscle function, was impaired and a low HbA1c level of <6.5% as well as a high HbA1c level of ≥8.0% was associated with endothelial dysfunction. These findings suggest that a low HbA1c level of <6.5% is associated with endothelial dysfunction both in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs and in patients with type 2 diabetes mellitus who are not receiving antidiabetic drugs.

Some studies, but not all studies, have shown that some kinds of antidiabetic drugs improve endothelial function. Indeed, it has been shown that antidiabetic drugs including an α-glucosidase inhibitor and metformin improve endothelial function[14,15]. A meta-analysis revealed that DPP-4 inhibitors did not improve endothelial function[21]. Therefore, it is thought that endothelial function may be different in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs and in patients with type 2 diabetes mellitus who are not receiving antidiabetic drugs. Even in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs, endothelial function was impaired in the HbA1c <6.5% group as well as in the HbA1c ≥8.0% group compared with that in the HbA1c 6.5–6.9% and HbA1c 7.0–7.9% groups. Although we do not know the precise mechanisms by which endothelial function is impaired in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs and who have an HbA1c level of <6.5%, antidiabetic drug-induced hypoglycemia may contribute to endothelial dysfunction in those patients. In the present study, about 74% of the type 2 diabetes mellitus patients were being treated with DPP-4 inhibitors, about 17% were treated with α-glucosidase inhibitors, and about 15% were being treated with biguanide. The kinds and doses of antidiabetic drugs differ between countries and regions, and differences in the kinds and doses of antidiabetic drugs may affect vascular function.

Study limitations

This study has some limitations. First, even though this study had a large sample size and was conducted in multiple centers,

this study was a cross-sectional study. Therefore, we did not establish a causal relationship between low HbA1c and vascular function. Second, although biguanide is used as a first-line drug in most countries, about 70% of the patients with type 2 diabetes mellitus in Japan are treated with DPP-4 inhibitors and the frequency of biguanide use is relatively low in Japan[22]. In addition, doses of antidiabetic drugs used in Japan are small compared with those in Western countries. In the present study, there were no patients who were receiving sodium glucose co-transporter 2 (SGLT2) inhibitors. It is well known that SGLT2 inhibitors have multiple effects including reductions in fasting blood glucose level, blood pressure, and body weight[23–25]. A previous study showed that SGLT2 inhibitors improve endothelial function as assessed by FMD[26]. In addition, large-scale clinical trials have clearly shown that SGLT2 inhibitors reduce the incidences of major cardiovascular adverse events and all causes of mortality[23–25]. Recently, the frequency of use of SGLT2 inhibitors has also been increasing in Japan. Future studies are needed to evaluate vascular function in patients with type 2 diabetes mellitus who are receiving and those not receiving SGLT2 inhibitors who have similar HbA1c levels. In addition, in the present study, there were no patients who were being treated with a glucagon-like peptide-1 receptor agonist. Third, unfortunately, not all information on antidiabetic drugs that were used in this study population was available. The purpose of this study was to evaluate the associations of HbA1c level with FMD and NID in patients with type 2 diabetes mellitus who were receiving antidiabetic drugs. Future studies are needed to confirm the associations of HbA1c level with FMD and NID in different types of antidiabetic drug groups of patients with type 2 diabetes mellitus who are receiving antidiabetic drugs. Fourth, hypoglycemia is one of the common adverse effects of antidiabetic drugs. It is thought that antidiabetic drug-induced hypoglycemia plays a critical role in vascular dysfunction. It is well known that the incidence of hypoglycemia is only a few percent in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs in Japan. However, in the present study, we did not obtain data on the prevalence of hypoglycemia. We assessed FMD and NID in insulin, sulfonylurea, and other antidiabetic drugs groups of patients in whom the use of those drugs was confirmed. There were no significant differences in FMD and NID between the three groups (Figure S4). These findings suggest that there is no significant association of hypoglycemia with FMD or NID in this study population. Fifth, in the present study, we did not have data for the duration of diabetes. Assessment of duration of diabetes would enable more specific conclusions concerning the role of HbA1c in endothelial function to be drawn.

A low HbA1c level of <6.5% as well as a high HbA1c level of ≥8.0% is associated with endothelial dysfunction in patients with type 2 diabetes mellitus who are receiving antidiabetic drugs.

ACKNOWLEDGMENTS

We thank Miki Kumiji, Megumi Wakisaka, Ki-ichiro Kawano, and Satoko Michiyama for their excellent secretarial assistance; FMD-J investigators Takayuki Hidaka, MD, PhD; Shuji Nakamura, MD, PhD; Junko Soga, MD, PhD; Yuichi Fujii, MD, PhD; Naomi Idei, MD; Noritaka Fujimura, MD, PhD; Shinsuke Mikami, MD, PhD; Yumiko Iwamoto, MD; Akimichi Iwamoto, MD, PhD; Takeshi Matsumoto, MD, PhD; Nozomu Oda, MD, PhD (Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Kana Kanai, PhD; Haruka Morimoto, PhD (Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan); Tomohisa Sakashita, MD, PhD; Yoshiki Kudo, MD, PhD (Department of Obstetrics and Gynecology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Taijiro Sueda, MD, PhD (Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Hirofumi Tomiyama, MD, PhD, FAHA; Akira Yamashina, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Bonpei Takase, MD, PhD, FAHA (Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan); Takahide Kohro, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Toru Suzuki, MD, PhD (Cardiovascular Medicine, University of Leicester, Leicester, UK); Tomoko Ishizu, MD, PhD (Cardiovascular Division, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan); Shinichiro Ueda, MD, PhD (Department of Clinical Pharmacology and Therapeutics, University of the Ryukyu School of Medicine, Okinawa, Japan); Tsutomu Yamazaki, MD, PhD (Clinical Research Support Center, Faculty of Medicine, The University of Tokyo, Tokyo, Japan); Tomoo Furumoto, MD, PhD (Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Hokkaido, Japan); Kazuomi Kario, MD, PhD (Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Tochigi, Japan); Teruo Inoue, MD, PhD (Department of Cardiovascular Medicine, Dokkyo Medical University, Mibu, Tochigi, Japan); Shinji Koba, MD, PhD (Department of Medicine, Division of Cardiology, Showa University School of Medicine, Tokyo, Japan); Kentaro Watanabe, MD, PhD (Department of Neurology, Hematology, Metaboism, Endocrinology and Diabetology (DNHMED), Yamagata University School of Medicine, Yamagata, Japan); Yasuhiko Takemoto, MD, PhD (Department of Internal Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka, Japan); Takuzo Hano, MD, PhD (Department of Medical Education and Population-based Medicine, Postgraduate School of Medicine, Wakayama Medical University, Wakayama, Japan); Masataka Sata, MD, PhD (Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima, Japan); Yutaka Ishibashi, MD, PhD (Department of General Medicine, Shimane University Faculty of Medicine, Izumo, Japan); Koichi Node, MD, PhD (Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan); Koji Maemura, MD, PhD (Department of Cardiovascular

Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan); Yusuke Ohya, MD, PhD (The Third Department of Internal Medicine, University of the Ryukyus, Okinawa, Japan); Taiji Furukawa, MD, PhD (Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan); Hiroshi Ito, MD, PhD (Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan); and Hisao Ikeda, MD, PhD (Faculty of Fukuoka Medical Technology, Teikyo University, Omuta, Japan). Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (18590815 and 21590898 to Y. Higashi) and a Grant-in-Aid of Japanese Arteriosclerosis Prevention Fund (to Y. Higashi).

DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409).

Informed consent: Written informed consent for participation in this study was obtained from all participants.

Registry and the registration no. of the study/trial: The name of the institutional Ethics Committee that approved the research: The Ethics Committee of Hiroshima University (approval no.: E-1902; date: 2020/2/5).

Animal studies: N/A.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Univariate analysis of relationships among FMD, NID, HbA1c level, and variables in patients with type 2 diabetes mellitus receiving antidiabetic drugs

Table S2 | Clinical characteristics of patients with type 2 diabetes mellitus receiving antidiabetic drugs on the basis of serum HbA1c level (6.5% vs 6.5–6.9%)

Table S3 | Clinical characteristics of patients with type 2 diabetes mellitus receiving antidiabetic drugs on the basis of serum HbA1c level (6.5% vs 7.0–7.9%)

Table S4 | Clinical characteristics of patients with type 2 diabetes mellitus receiving antidiabetic drugs on the basis of serum HbA1c level (6.5% vs ≥8.0%)

Figure S1 | Flow chart of the study design.

Figure S2 | Scatter plots show the relationship between flow-mediated vasodilation and serum hemoglobin A1c (HbA1c) levels in patients receiving antidiabetic drugs (a) and locally weighted regression smoothing (Lowess) plot (b) in patients with type 2 diabetes mellitus receiving antidiabetic drugs.

Figure S3 | Adjusted cubic spline of the relationship between HbA1c and odds ratio for endothelial dysfunction (a) and vascular smooth muscle function (b).

Figure S4 | Bar graphs show flow-mediated vasodilation and nitroglycerine-induced vasodilation in patients with type 2 diabetes mellitus receiving insulin, sulfonylurea (SU) and other antidiabetic drugs.