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Inconvenient relationship of haemoglobin A1c level with endothelial function in type 2 diabetes in a cross-sectional study

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ABSTRACT

Objective The aim of this study was to determine the relationship of haemoglobin A1c (HbA1c) level with flow-mediated vasodilation (FMD) and nitroglycerine-induced vasodilation (NID) in patients with type 2 diabetes.

Design Cross-sectional study.

Setting 22 university hospitals and affiliated clinics in Japan.

Participants 1215 patients with type 2 diabetes including 349 patients not taking antidiabetic drugs.

Measures We evaluated FMD and HbA1c level. All patients were divided into four groups based on HbA1c level: <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%.

Results An inverted U-shaped pattern of association between HbA1c level and FMD was observed at the peak of HbA1c of about 7%. 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 (p<0.001 and p<0.001), and FMD values were similar in the HbA1c <6.5% group and HbA1c ≥8.0% group. There were no significant differences in NID values among the four groups. After adjustments for confounding factors, FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% and HbA1c 7.0%–7.9% group (p=0.002 and p=0.04). In patients not taking antidiabetic drugs, FMD was also significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% group and HbA1c 7.0%–7.9% group (p<0.001 and p=0.02), and there were no significant differences in NID values among the four groups.

Conclusions These findings suggest that there is an inverted U-shaped pattern of association between FMD and HbA1c and that a low HbA1c level of <6.5% is associated with endothelial dysfunction.

Trial registration number UMIN000012950, UMIN000012951, UMIN000012952 and UMIN000003409.

Strengths and limitations of this study

- ► The present study showed the relationship between haemoglobin A1c and flow-mediated vasodilation in patients with type 2 diabetes.
- ► The present study was conducted in multiple centres and had a large sample size.
- ► We did not have information on the duration of diabetes from onset.
- ► This study was a cross-sectional study and we were therefore not able to evaluate the causality between low haemoglobin A1c level and flow-mediated vasodilation.

INTRODUCTION

Diabetes is a risk factor for atherosclerosis and subsequent cardiovascular disease (CVD) and cardiovascular events.[1] Previous studies showed that adults with diabetes have twofold to fourfold higher rates of all-cause mortality and CVD mortality than in subjects without diabetes.[2 3] Therefore, prevention of CVD in patients with diabetes is clinically important. Haemoglobin A1c (HbA1c) level, an index of glycaemic control, is usually checked in patients with diabetes. However, HbA1c-guided diabetes treatment is still controversial.

Previous large clinical trials, including the Veterans Affairs Diabetes Trial (VADT), the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial, and the Kumamoto Study, have shown that intensive glucose control reduces the incidence of microvascular diseases such as retinopathy and nephropathy, but not the incidence of macrovascular diseases such as myocardial infarction and stroke in patients with type 2 diabetes.[4–7] The Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial showed that intensive therapy increased all-cause mortality in patients with type 2 diabetes.[8] The VADT and ADVANCE trials showed that severe hypoglycaemia increases death from CVD and any cause of death.[5 7] Unfortunately, the optimal target level of HbA1c in diabetes is unclear, and

it is still controversial whether intensive glucose control by HbA1c-guided therapy reduces the incidence of cardiovascular events.[5 7 8]

Endothelial dysfunction is well known as the initial step of atherosclerosis and plays a critical role in the development of atherosclerosis, leading to CVD.[9] Measurement of flow-mediated vasodilation (FMD) in the brachial artery is an established tool for assessment of endothelial function,[10] and it is well known as an independent predictor of cardiovascular events.[11] Endothelial function assessed by FMD is impaired by traditional cardiovascular risk factors such as hypertension, dyslipidaemia, smoking, chronic alcohol drinking and also diabetes.[12] FMD is reversible by several interventions such as lifestyle modifications and pharmacological treatment.[13 14] Therefore, FMD is a very useful tool for assessing current vascular function and cardiovascular risk.

Diabetes is associated with endothelial dysfunction.[15 16] Chronic hyperglycaemia is a major contributor to increased oxidative stress and causes endothelial dysfunction through inactivation of nitric oxide.[17] Several studies have shown that endothelial function is improved by antidiabetic therapy, including use of antidiabetic drugs.[13 18 19] However, there is little information on the relationship between HbA1c level and endothelial function.

Therefore, we evaluated the relationship between HbA1c level and endothelial function assessed by FMD in patients with type 2 diabetes.

METHODS

Study patients

A total of 10 260 subjects (7385 patients from the Flow-mediated Dilation-Japan (FMD-J) study and 2875 patients who underwent a health check-up at Hiroshima University Hospital between August 2007 and August 2016) were recruited in this study. The FMD-J study was a prospective multicentre registry. The design of the FMD-J study has been described in detail previously.[20] The protocol used for measurement of FMD was the same as in the FMD-J study and at Hiroshima University Hospital. Exclusion criteria are shown in online supplemental figure 1. Finally, we enrolled 1215 subjects in this study. Hypertension was defined as use of antihypertensive drugs or systolic blood pressure of more than 140 mm Hg or diastolic blood pressure of more than 90 mm Hg measured in a sitting position on at least three occasions. Dyslipidaemia was defined according to the third report of the National Cholesterol Education Program.[21] Diabetes was defined according to the American Diabetes Association recommendation.[22] Smokers were defined as those who were current smokers. CVD was defined as coronary heart disease and cerebrovascular disease. Coronary heart disease included angina pectoris, prior myocardial infarction and unstable angina. Cerebrovascular disease included ischaemic stroke, haemorrhagic stroke and transient ischaemic attack. Written informed consent for participation in this study was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

Study 1: HbA1c level and vascular function in patients with type 2 diabetes

In study 1, we assessed the relationships between HbA1c level and vascular function as assessed by measurement of FMD, an index of endothelium-dependent vasodilation, and by measurement of nitroglycerine-induced vasodilation (NID), an index of endothelium-independent vasodilation, in 1215 patients with type 2 diabetes. First, we divided the patients into two groups based on their HbA1c level: <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 HbA1c level: <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.

Study 2: HbA1c level and vascular function in patients with type 2 diabetes not taking antidiabetic drugs

We evaluated the relationship of HbA1c level with FMD and NID in 349 patients with type 2 diabetes who were not taking antidiabetic drugs by using the same protocol as that used in study 1.

Measurements of FMD and NID

High-resolution ultrasonography equipment specialised to measure FMD (UNEXEF18G, UNEX, Nagoya, Japan) was used to evaluate FMD. Additional details are available in the online supplemental methods. The intraclass correlation coefficient between each of the participating institutions and the core laboratory has been previously described.[23]

Statistical analysis

Results are presented as mean±SD. All reported probability values were two-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 χ^2 test. Continuous variables were compared using analysis of variance (ANOVA) multiple groups. Comparisons between the groups categorised according to HbA1c level 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 (<2.1%) and NID (<6.2%). Age, gender, body mass index (BMI), creatinine levels, current smoking, and the presence of hypertension, dyslipidaemia and CVD were entered into the multivariate logistic regression analysis. As a sensitivity analysis, propensity score analysis was used to minimise the selection bias for evaluation of the relationship between HbA1c level and vascular function. The propensity score was calculated for each patient on the basis of logistic regression analysis of the probability of not taking antidiabetic drugs within groups stratified by HbA1c level (<6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%) using clinical

Table 1 Clinical characteristics of patients with type 2 diabetes

Variables	Total (N=1215)	HbA1c <6.5% (n=474)	HbA1c ≥6.5% (n=741)	P value
Age, years	62±10	65±10	60±10	<0.001
Gender, male/female	870/345	301/173	569/172	<0.001
Body mass index, kg/m^2	25.3±4.3	24.7±4.0	25.7±4.4	<0.001
Heart rate, bpm	68±11	69±12	68±11	0.15
Systolic blood pressure, mm Hg	133±17	130±18	135±17	<0.001
Diastolic blood pressure, mm Hg	79±11	76±11	80±11	<0.001
Total cholesterol, mg/dL	188±37	180±33	192±38	<0.001
Triglycerides, mg/dL	148±109	130±81	159±123	<0.001
HDL-C, mg/dL	54±15	57±16	53±15	<0.001
LDL-C, mg/dL	107±32	101±29	111±33	<0.001
Creatinine, mg/dL	0.84±0.29	0.86±0.31	0.83±0.27	0.07
Uric acid, mg/dL	5.7±1.4	5.8±1.4	5.6±1.4	0.03
Glucose, mg/dL	138±46	119±27	150±51	<0.001
HbA1c, %	6.8±1.1	5.9±0.4	7.4±1.0	<0.001
Medical history, n (%)				
Hypertension	969 (79.8)	378 (79.8)	591 (79.8)	1.00
Dyslipidaemia	953 (78.4)	371 (78.3)	582 (78.5)	0.91
CVD, n (%)	409 (33.7)	150 (31.7)	259 (35.0)	0.23
Current smoking, n (%)	290 (24.1)	104 (21.9)	186 (25.6)	0.15
Medication, n (%)				
Antihypertensive drugs	852 (70.1)	365 (77.0)	487 (65.7)	<0.001
Lipid-lowering drugs	680 (56.0)	298 (62.9)	382 (51.6)	<0.001
Antidiabetic drugs	866 (71.3)	373 (78.7)	493 (66.5)	<0.001

bpm, beats per minute; CVD, cardiovascular disease; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

variables including age, sex, BMI, systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, uric acid levels, current smoking (yes or no), medication with antihypertensive drugs (yes or no), medication with lipid-lowering drugs (yes or no) and presence of CVD (yes or no). With these propensity scores using a calliper width of 0.25 SD 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 <2.1%, the division point for the lowest quartile of FMD in all participants. All data were processed using JMP Pro V.14.0 software (SAS Institute, Cary, North Carolina, USA).

RESULTS

Study 1

Relationships between HbA1c level and variables in patients with type 2 diabetes

The baseline characteristics of the 1215 patients are summarised in table 1. The mean FMD value was 4.2%±2.8% and the mean NID value was 10.6%±5.8%. The baseline characteristics of subjects with HbA1c <6.5% and those with HbA1c ≥6.5% are also summarised in table 1. FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c ≥6.5% group (3.5%±2.7% and 4.6%±2.7%, respectively, p<0.001; figure 1A). NID values were similar in the two groups (10.6%±5.8% in the HbA1c <6.5% group and 10.8%±5.6% in the HbA1c ≥6.5% group, p=0.73; figure 1B).

Next, the patients were divided into four groups based on HbA1c level: <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%. The baseline characteristics are summarised in online supplemental table 1. FMD values were 3.5%±2.7% in the HbA1c <6.5% group, 4.8%±2.9% in the HbA1c 6.5%–6.9% group, 4.5%±2.6% in the HbA1c 7.0%–7.9% group, and 4.2%±2.7% in the HbA1c ≥8.0% group (p<0.001). 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 (p<0.001 and p<0.001, respectively; online supplemental figure 2A). There was no significant difference in FMD between the HbA1c <6.5% group and HbA1c ≥8.0% group (p=0.055; online supplemental figure 2A). NID values were 10.6%±5.9% in the

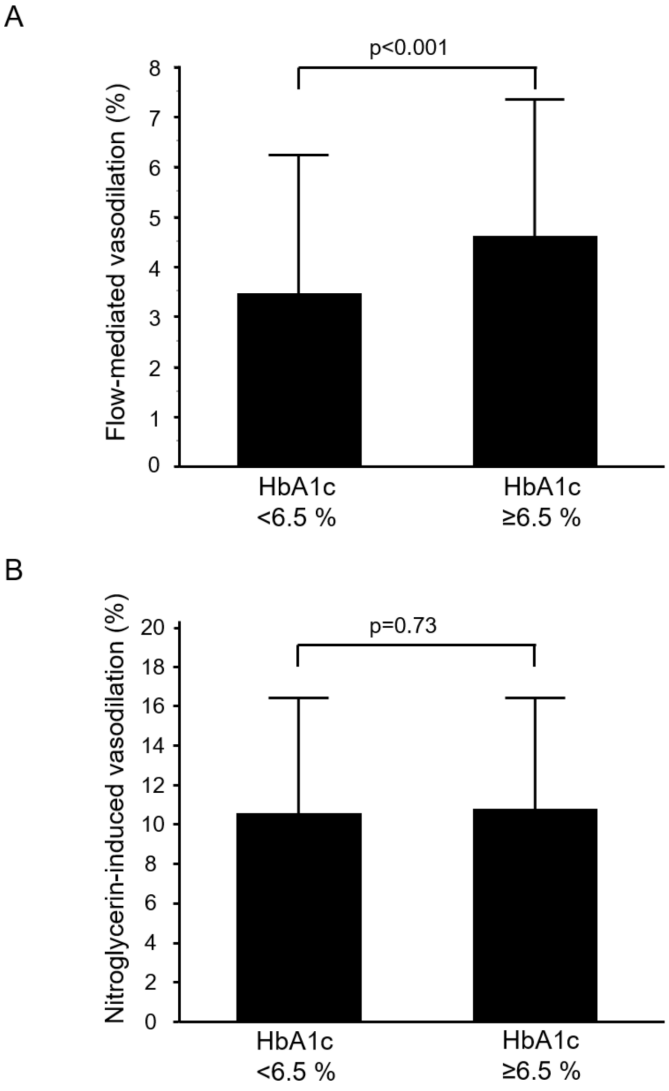

Figure 1 Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c <6.5% and patients with HbA1c ≥6.5%. HbA1c, haemoglobin A1c.

HbA1c <6.5% group, 11.2%±5.4% in the HbA1c 6.5%–6.9% group, 10.4%±5.2% in the HbA1c 7.0%–7.9% group, and 10.4%±6.8% in the HbA1c ≥8.0% group. There were no significant differences in NID values among the four groups (p=0.82; online supplemental figure 2B).

Univariate analysis of relationships among FMD, NID, HbA1c level and variables in patients with type 2 diabetes

Online supplemental table 2 shows the univariate relations among FMD, HbA1c level and variables. FMD was significantly correlated with age (r=−0.30, p<0.001), diastolic blood pressure (r=0.17, p<0.001), creatinine (r=−0.09, p=0.002), HbA1c level (r=0.08, p=0.004) and NID (r=0.33, p<0.001). HbA1c level was significantly correlated with age (r=−0.21, p<0.001), BMI (r=0.07, p=0.01), systolic blood pressure (r=0.13, p<0.001), diastolic blood pressure (r=0.14, p<0.001), total cholesterol (r=0.18, p<0.001), HDL cholesterol (r=−0.14, p<0.001), low-density lipoprotein (LDL) cholesterol (r=0.16, p<0.001), uric acid (r=−0.11, p<0.001), glucose level (r=0.57, p<0.001) and FMD (r=0.08, p=0.004). Linear regression analysis revealed that HbA1c level was significantly correlated with FMD (r=0.08, p=0.004; online supplemental figure 3A). A scatter plot between FMD and HbA1c level with a Lowess smoothed curve is shown in online supplemental figure 3B. FMD gradually increased with increase in HbA1c level to about 6.5%–6.9% and then decreased with increase in HbA1c level above 7.0%.

Multivariate analysis of relationships among low quartile of FMD, low quartile of NID, low HbA1c level and variables

The division points for the lowest quartile and second quartile were 2.1% for FMD and 6.2% for NID. Therefore, we defined small FMD as FMD of <2.1% and small NID as NID of <6.2%. We next examined whether low HbA1c (HbA1c <6.5%) was independently associated with small FMD by multiple logistic regression analysis. After adjustments for age, gender, BMI, current smoking, creatinine, and presence of hypertension, dyslipidaemia and CVD, HbA1c <6.5% was independently associated with a lower quartile of FMD (OR: 2.03, 95% CI 1.53 to 2.69; p<0.001) but was not associated with a lower quartile of NID (OR: 1.07, 95% CI 0.65 to 1.75; p=0.80) (online supplemental table 3).

Relationships among FMD, NID and HbA1c levels in patients with type 2 diabetes determined by using propensity score matching analysis

Propensity score matching analysis was used to create matched pairs between the HbA1c <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 level (HbA1c <6.5%) group and the other three groups are summarised in online supplemental tables 4–6. 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 (3.8%±2.6% vs 4.7%±3.0%, p=0.002; 3.9%±2.6% vs 4.5%±2.6%, p=0.04; online supplemental figure 4A,C), while there was no significant difference in FMD between the HbA1c <6.5% group and the HbA1c ≥8.0% group (4.5%±2.7% vs 4.1%±2.8%, p=0.36; online supplemental figure 4E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (11.0%±6.0% vs 11.2%±5.5% in the HbA1c <6.5% group vs the HbA1c 6.5%–6.9% group, p=0.84; 10.2%±5.8% vs 10.5%±5.6% in the HbA1c <6.5% group vs the HbA1c 7.0%–7.9% group, p=0.82; 12.8%±6.2% vs 11.6%±7.2%, in the HbA1c <6.5% group vs the HbA1c ≥8.0% group, p=0.52; online supplemental figure 4B,D,F).

Study 2

Baseline characteristics of patients with type 2 diabetes who were not taking antidiabetic drugs

Next, we evaluated the relationship between HbA1c level and FMD in patients with type 2 diabetes who were not taking antidiabetic drugs in order to eliminate possible effects of antidiabetic drugs and antidiabetic drug-induced hypoglycaemia on vascular function. The

Table 2 Clinical characteristics of patients with type 2 diabetes not taking antidiabetic drugs according to HbA1c level

Variables	Total (n=349)	HbA1c <6.5% (n=101)	HbA1c 6.5%–6.9% (n=149)	HbA1c 7.0%–7.9% (n=67)	HbA1c ≥8.0% (n=32)	P value
Age, years	61±10	66±10	60±10	61±9	57±10	<0.001
Gender, male/female	245/104	59/42	108/41	52/15	26/6	0.01
Body mass index, kg/m^2	25.4±4.2	24.6±4.1	25.5±4.2	26.0±4.6	25.8±4.2	0.1
Heart rate, bpm	69±11	70±11	68±11	68±10	69±11	0.21
Systolic blood pressure, mm Hg	133±17	128±18	133±16	136±16	138±19	0.004
Diastolic blood pressure, mm Hg	80±11	77±12	81±10	82±10	83±10	0.002
Total cholesterol, mg/dL	199±39	186±33	205±36	197±45	216±45	<0.001
Triglycerides, mg/dL	169±139	133±82	169±143	205±173	206±162	0.003
HDL-C, mg/dL	54±15	57±15	55±16	48±12	49±12	<0.001
LDL-C, mg/dL	116±32	110±30	119±31	115±36	127±30	0.04
Creatinine, mg/dL	0.8±0.3	0.8±0.4	0.8±0.2	0.8±0.2	0.8±0.3	0.33
Uric acid, mg/dL	5.8±1.5	6.0±1.7	5.8±1.5	5.5±1.4	5.5±1.7	0.23
Glucose, mg/dL	137±46	119±28	125±22	145±36	224±78	<0.001
HbA1c, %	6.8±1.0	5.9±0.4	6.7±0.1	7.3±0.3	9.4±1.2	<0.001
Medical history, n (%)						
Hypertension	266 (76.2)	75 (74.3)	112 (75.2)	56 (83.6)	23 (71.9)	0.45
Dyslipidaemia	275 (78.8)	79 (78.2)	116 (77.9)	57 (85.1)	23 (71.9)	0.46
CVD, n (%)	79 (22.6)	27 (26.7)	29 (19.5)	17 (25.4)	6 (18.8)	0.50
Current smoking, n (%)	79 (22.6)	20 (19.8)	34 (23.3)	17 (25.4)	8 (26.7)	0.79
Medication, n (%)						
Antihypertensive drugs	217 (62.2)	78 (77.2)	85 (57.1)	41 (61.2)	13 (40.6)	<0.001
Lipid-lowering drugs	144 (41.3)	59 (58.4)	57 (38.3)	24 (35.8)	4 (12.5)	<0.001
Antidiabetic drugs	0 (0)					

bpm, beats per minute; CVD, cardiovascular disease; HbA1c, haemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

baseline characteristics of those patients are summarised in table 2. The mean FMD value was 4.2%±2.8% and the mean NID value was 10.6%±5.8%.

Relationships among HbA1c level, FMD, NID and variables in patients with type 2 diabetes who were not taking antidiabetic drugs with HbA1c level <6.5% and HbA1c level ≥6.5%

The baseline characteristics of patients with type 2 diabetes not taking antidiabetic drugs who had HbA1c level <6.5% and HbA1c level ≥6.5% are summarised in online supplemental table 7. FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c ≥6.5% group (3.2%±2.9% and 4.8%±2.7%, respectively, p<0.001; online supplemental figure 5A). NID values were similar in the two groups (11.0%±6.0% in the HbA1c <6.5% group and 11.3%±4.7% in the HbA1c ≥6.5% group, p=0.79; online supplemental figure 5B).

Next, the patients were divided into four groups according to HbA1c level: <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%. The baseline characteristics are summarised in table 2. FMD values were 3.2%±2.9% in the HbA1c <6.5% group, 5.2%±2.9% in the HbA1c 6.5%–6.9% group, 4.4%±2.4% in the HbA1c 7.0%–7.9% group, and 3.9%±2.5% in the HbA1c ≥8.0% group (p<0.001; figure 2A). 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=0.02 and p=0.62, respectively; figure 2A). NID values were 11.0%±6.0% in the HbA1c <6.5% group, 12.6%±3.7% in the HbA1c 6.5%–6.9% group, 10.1%±5.7% in the HbA1c 7.0%–7.9% group, and 10.5%±4.0% in the HbA1c ≥8.0% group. There were no significant differences in NID values among the four groups (p=0.59; figure 2B).

Univariate analysis of relationships among FMD, NID, HbA1c level and variables in patients with type 2 diabetes who were not taking antidiabetic drugs

Online supplemental table 8 shows the univariate relationships among FMD, HbA1c level and variables. FMD was significantly correlated with age (r=−0.24, p<0.001), systolic blood pressure (r=0.10, p=0.048), diastolic blood pressure (r=0.19, p=0.02) and NID (r=0.36, p<0.001).

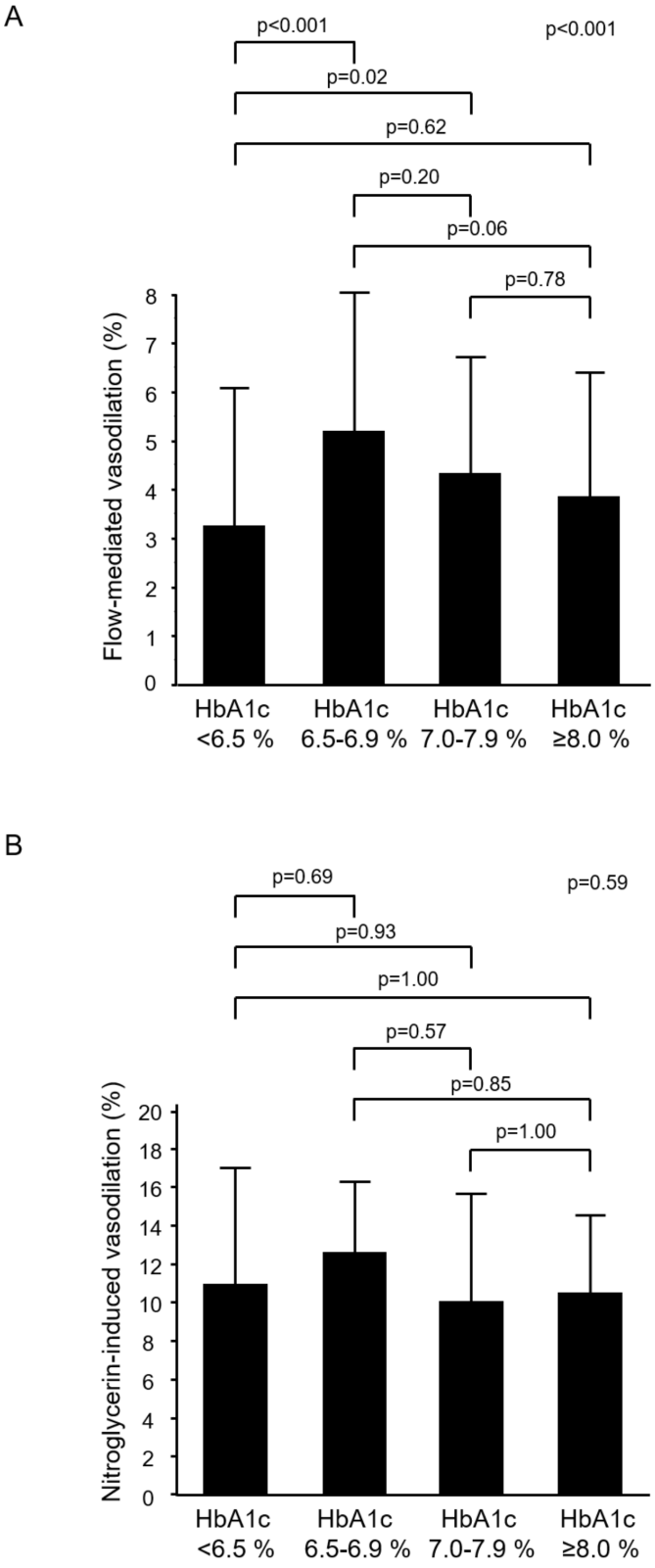

Figure 2 Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in four groups according to HbA1c level for patients not receiving antidiabetic drug treatment. HbA1c, haemoglobin A1c.

HbA1c level was significantly correlated with age (r=−0.2, p<0.001), systolic blood pressure (r=0.17, p=0.001), diastolic blood pressure (r=0.12, p=0.02), total cholesterol (r=0.22, p<0.001), triglycerides (r=0.23, p<0.001), HDL cholesterol (r=−0.19, p<0.001), LDL cholesterol (r=0.14, p=0.01) and glucose level (r=0.70, p<0.001). Linear regression analysis revealed that HbA1c level was not significantly correlated with FMD (r=0.05, p=0.40; online supplemental figure 6A). Scatter plots between FMD and HbA1c with a Lowess smoothed curve are shown in online supplemental figure 6B. FMD gradually increased with increase in HbA1c level to about 6.5%–6.9% and then decreased with increase in HbA1c level above 7.0%.

Multivariate analysis of relationships among low quartile of FMD, low quartile of NID, low HbA1c level and variables in patients with type 2 diabetes who were not taking antidiabetic drugs

Multiple logistic regression analysis revealed that after adjustments for age, gender, BMI, current smoking, creatinine, and presence of hypertension, dyslipidaemia and CVD, HbA1c level <6.5% was independently associated with a lower quartile of FMD (OR: 2.57, 95% CI 1.45 to 4.54; p=0.001), but was not associated with a lower quartile of NID (OR: 1.29, 95% CI 0.43 to 3.91; p=0.65) (table 3).

Relationships among FMD, NID and HbA1c level in patients with type 2 diabetes who were not taking antidiabetic drugs determined by using propensity score matching analysis

Propensity score matching analysis was used to create matched pairs between the HbA1c <6.5% group and the other groups (HbA1c 6.5%–6.9%, HbA1c 7.0%–7.9% and HbA1c of ≥8.0%). The baseline characteristics of matched pairs of the low HbA1c level <6.5% group and the other three groups are summarised in online supplemental tables 9–11. FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% group (3.1%±2.7% vs 4.6%±3.2%, p=0.02; online supplemental figure 7A), while there were no significant differences in FMD between the HbA1c <6.5% group, the HbA1c 7.0%–7.9% group and the HbA1c ≥8.0% group (3.2%±3.2% vs 4.0%±2.8%, p=0.35; 4.0%±3.0% vs 3.8%±2.4%, p=0.87; online supplemental figure 7C,E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (10.8%±5.6% vs 11.7%±4.0% in the HbA1c <6.5% group vs the HbA1c 6.5%–6.9% group, p=0.62; 11.8%±5.7% vs 7.8%±4.9% in the HbA1c <6.5% group vs the HbA1c 7.0%–7.9% group, p=0.10; 14.8%±5.5% vs 13.6%±3.9% in the HbA1c <6.5% group vs the HbA1c ≥8.0% group, p=0.78; online supplemental figure 7B,D,F).

DISCUSSION

In the present study, we demonstrated that a low HbA1c level of <6.5% was independently associated with small FMD in patients with type 2 diabetes. After adjustments for confounding factors, 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. In patients who were not taking antidiabetic drugs, FMD was also significantly

Table 3 Multivariate analysis of relationships among FMD, NID and low HbA1c level (<6.5%) in patients with type 2 diabetes not taking antidiabetic drugs

	Low quartile of FMD		Low quartile of NID	
Variables	OR (95% CI)	P value	OR (95% CI)	P value
Model 1	3.05 (1.80 to 5.14)	<0.001	1.33 (0.54 to 3.31)	0.53
Model 2	2.49 (1.44 to 4.33)	0.001	1.20 (0.46 to 3.13)	0.71
Model 3	2.57 (1.45 to 4.54)	0.001	1.29 (0.43 to 3.91)	0.65

Model 1: unadjusted model.
Model 2: adjusted for age, gender and body mass index.
Model 3: adjusted for age, gender, body mass index, current smoking, creatinine, presence of hypertension, dyslipidaemia and CVD.
Low quartile of FMD indicates less than 2.1%. Low quartile of NID indicates less than 6.2%.
CVD, cardiovascular disease; FMD, flow-mediated vasodilation; HbA1c, haemoglobin A1c; NID, nitroglycerine-induced vasodilation.

smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% group and HbA1c 7.0%–7.9% group. We also confirmed by using propensity score matching analysis that FMD was significantly smaller in the low HbA1c group than in the HbA1c 6.5%–6.9% group. To our knowledge, the present study is the first study showing detailed relationships between HbA1c level and endothelial function in patients with type 2 diabetes, including patients not taking antidiabetic drugs.

Interestingly, in the present study, HbA1c levels were not correlated with NID. There were no significant differences in NID values among the HbA1c groups of <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%. In patients with type 2 diabetes who were not taking antidiabetic drugs, there were also no significant differences in NID values among the four groups. These findings suggest that HbA1c level is not correlated with vascular smooth muscle function.

It is controversial whether endothelium-independent vasodilation assessed by NID as well as endothelium-dependent vasodilation assessed by FMD are impaired in individuals with cardiovascular risk factors and patients with CVD.[24 25] In the present study, although we found that there was an inverted U-shaped pattern of association between FMD and HbA1c, there was no significant relationship between NID and HbA1c. In a previous study, we showed that both NID and FMD were maintained in subjects without cardiovascular risk factors and that FMD was significantly smaller in subjects with cardiovascular risk factors than in subjects without cardiovascular risk factors, but that NID was significantly smaller in patients with CVD than in both subjects with and those without cardiovascular risk factors, whereas there was no significant difference in NID between subjects with and those without cardiovascular risk factors, suggesting that FMD values and NID values are different in relation to the grade of atherosclerosis.[23] The Hoorn Study showed that although FMD was significantly smaller in patients with type 2 diabetes than in subjects with normal glucose metabolism, NID values were similar in the two groups.[26] Kubota *et al*[27] showed that NID did not alter after treatment with sitagliptin in patients with type 2 diabetes and that changes in NID did not correlate with changes in HbA1c, while FMD improved in relation to decrease in HbA1c. These previous studies support our results showing that NID is not associated with HbA1c levels in patients with type 2 diabetes.

It is well known that the incidence of myocardial infarction increases in relation to HbA1c level.[28] It is thought that FMD, an index of endothelial function, decreases with increase in HbA1c level. However, in the present study, a low HbA1c level of <6.5% was found to be independently associated with endothelial dysfunction in patients with type 2 diabetes. To avoid the effects of antidiabetic drugs on HbA1c levels and to minimise the effect of hypoglycaemia, we evaluated the relationship between HbA1c level and FMD in patients with type 2 diabetes who were not taking antidiabetic drugs, and we found that the results were similar for patients taking and those not taking antidiabetic drugs.

The key finding of this study was that an inverted U-shaped pattern of association between HbA1c and FMD was observed at the peak of HbA1c of about 7% in patients with type 2 diabetes. This result may reflect the existence of a J-curve pattern of association between HbA1c and all causes of mortality. Diabetes is well known as a risk factor for endothelial function as well as for CVD.[15 26 29] However, the effect of intensive glucose control therapy on all causes of mortality is still controversial. Previous studies focused on the relationship between HbA1c and all causes of mortality. Some studies showed a positive linear relationship between HbA1c and all causes of mortality,[30 31] while other studies showed a J-shaped relationship between HbA1c and all causes of mortality.[32 33] The effects of intensive glucose control therapy on morbidity and mortality of cardiovascular events are also controversial.[33 34] TheUnited Kingdom Prospective Diabetes Study (UKPDS) 73 study showed that the frequency of hypoglycaemia in patients not taking antidiabetic drugs was 0.1%.[35] Hypoglycaemia during intensive glucose control is probably a predictor of morbidity and mortality of cardiovascular events. It has been shown that the HRs for all causes of mortality including cardiovascular events in patients with severe hypoglycaemia episodes are between 1.74 and 3.27.[36 37] It has been postulated that hypoglycaemia activates the sympathetic nervous system, resulting in release of catecholamines that cause increases in heart

rate and myocardial contractility,[38] and activates platelet aggregation, leading to acute coronary syndrome and fatal arrhythmia.[39] Although the precise mechanism by which a low HbA1c level impairs endothelial function is uncertain, activation of the sympathetic nervous system may play a critical role in endothelial dysfunction. We cannot deny the possibility that factors other than hypoglycaemia contribute to low HbA1c-induced endothelial dysfunction.

This study has some limitations. First, this study was a cross-sectional study, although it was conducted in multiple centres and had a large sample size. Therefore, we were able to evaluate the association but not causality between low HbA1c level and FMD. Second, unfortunately, we did not have information on the duration of diabetes from onset. The UKPDS 80 study has shown that CVD risk reduction was observed after 10 years of follow-up of intensive glucose therapy in patients with newly diagnosed type 2 diabetes. Assessment of information on duration of diabetes would enable more specific conclusions concerning the role of HbA1c in endothelial function to be drawn. Third, this study was conducted in Japan, and our results on the association between HbA1c and FMD might not be applicable to other races. However, the ACCORD trial was conducted in North America, and the ADVANCE trial was conducted in 20 countries including countries in Asia and Europe and in North America and Australia. The results of those studies suggest that an inverted U-shaped pattern of association between FMD and HbA1c, which was found in the present study, exists in all races. It is well known that HbA1c levels do not accurately reflect mean glucose values in patients with end-stage chronic kidney disease and in patients on dialysis. In the present study, we excluded those patients and we adjusted serum creatinine levels using propensity score matching analysis. Fourth, we did not have information on physical activity. Previous studies have shown that lifestyle per se and lifestyle modifications such as diet and physical activity influence endothelial function.[40–42] Assessment of the status of physical activity would enable more specific conclusions concerning the role of HbA1c in endothelial function to be drawn. Fifth, it is well known that hypertensive drugs, lipid-lowering drugs and antidiabetic drugs affect vascular function. Therefore, on the examination day, measurements of FMD and NID were conducted in the morning, all medications were withheld, and only drinking water was given to the patients. Patients in this study with HbA1c <6.5% had been taking large doses of antihypertensive drugs, lipid-lowering drugs and antidiabetic drugs. Unfortunately, we had no information on the kinds of drugs that were used in this study population. Therefore, we matched information on medications by propensity matched analysis. Even after adjustment for information on medications, patients with HbA1c <6.5% had lower FMD levels than did patients with HbA1c ≥6.5%. However, we cannot deny the possibility that differences in pharmacological interventions affected vascular function in this study population. In addition, since elderly patients often have malnutrition due to anorexia, which leads to low HbA1c, we excluded patients over 80 years of age. Even after excluding these confounding factors, a low HbA1c level was associated with endothelial dysfunction in patients with type 2 diabetes.

In conclusions, there is an inverted U-shaped pattern of association between FMD and HbA1c and a low HbA1c level (<6.5%) is associated with endothelial dysfunction in patients with type 2 diabetes, even in patients with type 2 diabetes who are not taking antidiabetic drugs.

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