An Inconvenient Relationship of Hemoglobin A1c Level with Endothelial Function in Type 2 Diabetes

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Original investigation

Keywords: hemoglobin A1c, type 2 diabetes, endothelial function, flow-mediated vasodilation, nitroglycerine-induced vasodilation

Posted Date: April 30th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-23581/v1

Abstract

Background Diabetes is associated with endothelial dysfunction. However, there is little information on the relationship between hemoglobin A1c (HbA1c) level and endothelial function. This study evaluated the relationship between HbA1c level and flow-mediated vasodilation (FMD).

Methods We measured FMD in 1215 patients with type 2 diabetes including 349 patients not taking antidiabetic drugs and 866 patients taking antidiabetic drugs. The patients were divided into four groups based on HbA1c levels: <48 mmol/mol, 48–52 mmol/mol, 53–63 mmol/mol, and ≥ 64 mmol/mol. (< 6.5%, 6.5–6.9%, 7.0-7.9%, ≥ 8.0%)

Results An inverted U-shaped pattern of association between HbA1c level and FMD was observed at the peak of HbA1c of about 53 mmol/mol (7%). FMD was significantly smaller in the HbA1c < 48 mmol/mol (6.5%) group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) group and HbA1c 53–63 mmol/mol (7.0-7.9%) group ($p < 0.001$ and $p < 0.001$), and FMD values were similar in the HbA1c < 48 mmol/mol (6.5%) group and HbA1c ≥ 64 mmol/mol (8.0%) group. There were no significant differences in nitroglycerine-induced vasodilation (NID) values among the four groups. After adjustments for confounding factors, FMD was significantly smaller in the HbA1c < 48 mmol/mol (6.5%) group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) and HbA1c 53–63 mmol/mol (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 < 48 mmol/mol (6.5%) group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) group and HbA1c 53–63 mmol/mol (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 a low HbA1c level of < 48 mmol/mol (6.5%) is associated with endothelial dysfunction. An HbA1c level of 48–52 mmol/mol (6.5–6.9%) may be appropriate for maintenance of endothelial function.

Background

Diabetes is a risk factor for atherosclerosis and subsequent cardiovascular disease (CVD) and CV events. [1] Previous studies showed that adults with diabetes have 2-4-fold higher rates of all-cause mortality and CVD mortality than those in subjects without diabetes.[2] [3] Therefore, prevention of CVD in patients with diabetes is clinically important. HbA1c level, an index of glycemic 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: Preter Ax and Diamicron MR Controlled Evaluation (ADVANCE) trial, and the Kumamoto study, have shown that intensive glucose control reduces the incidences 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 the ADVANCE trial showed that severe hypoglycemia increases death from cardiovascular disease 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 CV events.

Endothelial dysfunction is well known as the initial step of atherosclerosis, and it 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, dyslipidemia, smoking, chronic alcohol drinking and also diabetes. FMD is reversible by several interventions such as life-style modifications and pharmacological treatment. [12, 13] Therefore, FMD is a very useful tool for assessing the current vascular function and cardiovascular risk.

Diabetes is associated with endothelial dysfunction. [14] Chronic hyperglycemia is a major contributor to increased oxidative stress and causes endothelial dysfunction through inactivation of nitric oxide. [15] Several studies have shown that endothelial function is improved by antidiabetic therapy including use of antidiabetic drugs. [12, 16] 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 10260 subjects (7385 patients from the FMD-J study and 2875 patients who underwent a health checkup at Hiroshima University Hospital between August 2007 and August 2016) were recruited in this study. The FMD-J study was a prospective multicenter registry. The design of FMD-J study has been described in detail previously. [17] The protocol used for measurement of FMD was the same in the FMD-J study and at Hiroshima University Hospital. Exclusion criteria was listed on Online Figure S1. Finally, we enrolled 1215 subjects in this study. Hypertension was defined as the use of antihypertensive drugs or systolic blood pressure of more than 140 mmHg or diastolic blood pressure of more than 90 mmHg measured in a sitting position on at least 3 occasions. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program. [18] Diabetes was defined according to the American Diabetes Association recommendation. [19] 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 ischemic stroke, hemorrhagic stroke, and transient ischemic attack. The ethics committee in Hiroshima University approved the study protocol. Written informed consent for participation in this study was obtained from all participants.

Study 1. HbA1c levels 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 levels: <48 mmol/mol (6.5%) and ≥48 mmol/mol (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 levels: <48 mmol/mol (6.5%), 48-52 mmol/mol (6.5-6.9%), 53-63 mmol/mol (7.0-7.9%), and ≥64 mmol/mol (8.0%). We next assessed the relationships of HbA1c levels with FMD and NID using propensity score matching.

Study 2. HbA1c levels and vascular function in patients with type 2 diabetes not taking antidiabetic drugs

We evaluated the relationships of HbA1c levels 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

A high-resolution ultrasonography equipment specialized to measure FMD (UNEXEF18G, UNEX Co., Nagoya, Japan) was used to evaluate FMD. Additional details are available in the Online methods. The intraclass correlation coefficient between each participating institutions and the core laboratory has been previously described. [20]

Statistical analysis

Results are presented as means±SD. 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 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 (<2.1%) and NID (<6.2%). Age, gender, body mass index, creatinine levels, current smoking, and the presence of hypertension, dyslipidemia and CVD 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 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 levels ((< 48 mmol/mol (6.5%), 48-52 mmol/mol (6.5-6.9%), 53-63 mmol/mol (7.0-7.9%), and ≥64 mmol/mol (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 presence of CVD (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 prevalences 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 the JMP Pro. Ver 14.0 software (SAS Institute, Cary, NC, USA)

Results

Study 1.

Baseline characteristics of patients with type 2 Diabetes

The baseline characteristics of the 1215 patients are summarized in Table 1. The mean age was 62 years. The 1215 patients included 870 men (71.6%) and 345 women (28.4%). The Mean blood glucose level was 7.7±2.6 mmol/L and the mean HbA1c level was 50.7±12.2 mmol/mol (6.8±1.1%). Among the subjects, 969 (79.8%) had hypertension, 953 (78.4%) had dyslipidemia, 409 (33.7%) had previous CVD, 290 (24.1%) were current smokers, and 866 (71.3%) were being treated with antidiabetic drugs. The mean FMD value was 4.2±2.8% and the mean NID value was 10.6±5.8%.

Relationships between HbA1c level and variables in Patients with type 2 Diabetes

The baseline characteristics of subjects with HbA1c of <48 mmol/mol (6.5%) and those with HbA1c of ≥48 mmol/mol (6.5%) are also summarized in Table 1. There were significant differences in age, gender, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, HDL-C, LDL-C, uric acid, glucose, HbA1c level, and use of antihypertensive drugs, lipid lowering drugs and antidiabetic drugs between the two groups. FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c ≥48 mmol/mol (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 <48 mmol/mol (6.5%) group and 10.8±5.6% in the HbA1c ≥48 mmol/mol (6.5%) group, p=0.73; Figure 1B).

Next, the patients were divided into four groups based on HbA1c levels: <48 mmol/mol (6.5%), 48-52 mmol/mol (6.5-6.9%), 53-63 mmol/mol (7.0-7.9%), and ≥64 mmol/mol (8.0%). The baseline characteristics 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, uric acid, glucose, use of antihypertensive drugs, lipid lowering drugs, and antidiabetic drugs, and prevalence of CVD among the four groups. FMD values were 3.5±2.7% in the HbA1c <48 mmol/mol (6.5%) group, 4.8±2.9% in the HbA1c 48-52 mmol/mol (6.5-6.9%) group, 4.5±2.6% in the HbA1c 53-63 mmol/mol (7.0-7.9%) group, and 4.2±2.7% in the HbA1c ≥64 mmol/mol (8.0%) group ($p<0.001$). FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c 48-52 mmol/mol (6.5-6.9%) group and HbA1c 53-63 mmol/mol (7.0-7.9%) group ($p<0.001$ and $p<0.001$, respectively; Figure 2A). There was no significant difference in FMD between the HbA1c <48 mmol/mol (6.5%) group and HbA1c ≥64 mmol/mol (8.0%) group. ($p=0.055$; Figure 2A). NID values were 10.6±5.9% in the HbA1c <48 mmol/mol (6.5%) group, 11.2±5.4% in the HbA1c 48-52 mmol/mol (6.5-6.9%) group, 10.4±5.2% in the HbA1c 53-63 mmol/mol (7.0-7.9%) group, and 10.4±6.8% in the HbA1c ≥64 mmol/mol (8.0%) group. There were no significant differences in NID values among the four groups ($p=0.82$; Figure 2B).

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

Online Table S1 shows 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 ($r=-0.14$, $p<0.001$), 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 ($r=0.08$, $p=0.004$; ESM Figure 2A). A scatter plot between FMD and HbA1c level with a Lowess smoothed curve is shown in Online Figure S2B. FMD gradually increased with increase in HbA1c level to about 48-52 mmol/mol (6.5-6.9%) and the decreased with increase in HbA1c level above 53 mmol/mol (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% FMD and 6.2% 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 of <48 mmol/mol (6.5%)) was independently associated with small FMD by multiple logistic regression analysis. After adjustments for age, gender, BMI, current smoking, creatine, and

presence of hypertension, dyslipidemia and CVD, HbA1c <48 mmol/mol (6.5%) was independently associated with a lower quartile of FMD (OR: 2.03, 95% CI: 1.53-2.69; p<0.001) but was not associated with a lower quartile of NID (OR: 1.07, 95% CI: 0.65-1.75; p=0.80) (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 <48 mmol/mol (6.5%) group and the other three groups (HbA1c of 48-52 mmol/mol (6.5-6.9%), HbA1c of 53-63 mmol/mol (7.0-7.9%), and HbA1c of ≥64 mmol/mol (8.0%)). Baseline characteristics of matched pairs of the low HbA1c level (HbA1c of <48 mmol/mol (6.5%)) group and the other three groups are summarized in Online Tables S2, S3, S4. FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c 48-52 mmol/mol (6.5-6.9%) group and the HbA1c 53-63 mmol/mol (7.0-7.9%) group (3.8±2.6% versus 4.7±3.0%, p=0.002; 3.9±2.6% versus 4.5±2.6%, p=0.04; Online Figures S3A and S3C), while there was no significant difference in FMD between the HbA1c <48 mmol/mol (6.5%) group and the HbA1c ≥64 mmol/mol (8.0%) group (4.5±2.7% versus 4.1±2.8%, p=0.36; Online Figure S3E). There were no significant differences in NID between the HbA1c <48 mmol/mol (6.5%) group and the other three groups (11.0±6.0% versus 11.2±5.5% in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c 48-52 mmol/mol (6.5-6.9%) group, p=0.84; 10.2±5.8% versus 10.5±5.6% in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c 53-63 mmol/mol (7.0-7.9%) group, p=0.82; 12.8±6.2% versus 11.6±7.2%, p=0.5, in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c ≥64 mmol/mol (8.0%) group, p=0.82; Online Figures S3B, S3D, and S3F).

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 hypoglycemia on vascular function. The baseline characteristics of those patients are summarized in Table 4. Of those 349 patients, 245 (70.2%) were men and 104 (29.8%) were women. The mean blood glucose level was 7.6±2.6 mmol/L and the mean HbA1c level was 50.8±11.4 mmol/mol (6.8±1.0%). Among those patients, 266 (76.2%) had hypertension, 275 (78.8%) had dyslipidemia, 79 (22.6%) had previous CVD, and 79 (23.0%) were current smokers. 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 levels <48 mmol/mol (6.5%) and HbA1c levels ≥48 mmol/mol (6.5%)

The baseline characteristics of patients with type 2 diabetes not taking antidiabetic drugs who had HbA1c levels <48 mmol/mol (6.5%) and HbA1c levels ≥48 mmol/mol (6.5%) are summarized in Table 4. There were significant differences in age, gender, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, HDL-C, LDL-C, glucose, HbA1c level, and use of antihypertensive drugs and lipid lowering drugs between the two groups. FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c ≥48 mmol/mol (6.5%) group (3.2±2.9% and 4.8±2.7%, respectively, p<0.001; Figure 3A). NID values were similar in the two groups (11.0±6.0% in the HbA1c <48 mmol/mol (6.5%) group and 11.3±4.7% in the HbA1c ≥48 mmol/mol (6.5%) group, p=0.79; Figure 3B)

Next, the patients were divided into four groups according to HbA1c levels: <48 mmol/mol (6.5%), 48-52 mmol/mol (6.5-6.9%), 53-63 mmol/mol (7.0-7.9%), and ≥64 mmol/mol (8.0%). The baseline characteristics are summarized in Online Table S5. There were significant differences in age, gender, systolic blood pressure, diastolic blood pressure, total cholesterol, triglycerides, HDL-C, LDL-C, glucose, and use of antihypertensive drugs and lipid lowering drugs among the four groups. FMD values were 3.2±2.9% in the HbA1c <48 mmol/mol (6.5%) group, 5.2±2.9% in the HbA1c 48-52 mmol/mol (6.5-6.9%) group, 4.4±2.4% in the HbA1c 53-63 mmol/mol (7.0-7.9%) group, and 3.9±2.5% in the HbA1c ≥64 mmol/mol (8.0%) group (p<0.001; Figure 4A). FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c 48-52 mmol/mol (6.5-6.9%) group and HbA1c 53-63 mmol/mol (7.0-7.9%) group, while there was no significant difference in FMD between the HbA1c <48 mmol/mol (6.5%) group and the HbA1c ≥64 mmol/mol (8.0%) group (p<0.001, p=0.02 and p=0.62 respectively; Figure 4A). NID values were 11.0±6.0% in the HbA1c <48 mmol/mol (6.5%) group, 12.6±3.7% in the HbA1c 48-52 mmol/mol (6.5-6.9%) group, 10.1±5.7% in the HbA1c 53-63 mmol/mol (7.0-7.9%) group, and 10.5±4.0% in the HbA1c ≥64 mmol/mol (8.0%) group. There were no significant differences in NID values among the four groups (p=0.59; Figure 4B).

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

Online Table S6 shows 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). 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 Figure S4A). Scatter plots between FMD and HbA1c with a Lowess smoothed curve are shown in Online Figure S4B. FMD gradually

increased with increase in HbA1c level to about 48-52 mmol/mol (6.5-6.9%) and then decreased with increase in HbA1c level above 53 mmol/mol (7.0%) (Online Figure S4B).

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, creatine, and presence of hypertension, dyslipidemia and CVD, HbA1c level of <48 mmol/mol (6.5%) was independently associated with a lower quartile of FMD (OR: 2.57, 95% CI: 1.45-4.54; p=0.001) but was not associated with a lower quartile of NID (OR: 1.29, 95% CI: 0.43-3.91; p=0.65) (Online Table S7).

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 <48 mmol/mol (6.5%) group and the other groups (HbA1c of 48-52 mmol/mol (6.5-6.9%), HbA1c of 53-63 mmol/mol (7.0-7.9%), and HbA1c of ≥64 mmol/mol (8.0%)). Baseline characteristics of matched pairs of the low HbA1c level (HbA1c of <6.5%) group and the other three groups are summarized in Online Tables S8, S9, and S10. FMD was significantly smaller in the HbA1c <48 mmol/mol (6.5%) group than in the HbA1c 48-52 mmol/mol (6.5-6.9%) group (3.1±2.7% versus 4.6±3.2%, p=0.02; Online Figure S5A), while there were no significant differences in FMD between the HbA1c <48 mmol/mol (6.5%) group, the HbA1c 53-63 mmol/mol (7.0-7.9%) group and the HbA1c ≥64 mmol/mol (8.0%) group (3.2±3.2% versus 4.0±2.8%, p=0.35; 4.0±3.0% versus 3.8±2.4%, p=0.87; Online Figures S5C and S5E). There were no significant differences in NID between the HbA1c <48 mmol/mol (6.5%) group and the other three groups (10.8±5.6% versus 11.7±4.0% in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c 48-52 mmol/mol (6.5-6.9%) group, p=0.62; 11.8±5.7% versus 7.8±4.9% in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c 53-63 mmol/mol (7.0-7.9%) group, p=0.10; 14.8±5.5% versus 13.6±3.9% in the HbA1c <48 mmol/mol (6.5%) group versus the HbA1c ≥64 mmol/mol (8.0%) group, p=0.78; Online Figures 5B, 5D, and 5F).

Discussion

In the present study, we demonstrated that a low HbA1c level of < 48 mmol/mol (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 < 48 mmol/mol (6.5%) group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) group and HbA1c 53–63 mmol/mol (7.0-7.9%) group. In patients who were not taking antidiabetic drugs, FMD was also significantly smaller in the HbA1c < 48 mmol/mol

(6.5%) group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) group and HbA1c 53–63 mmol/mol (7.0-7.9%) group. In addition, we confirmed that FMD was significantly smaller in the low HbA1c group than in the HbA1c 48–52 mmol/mol (6.5–6.9%) group by using propensity score matching analysis. To our knowledge, the present study is the first study showing the detailed relationships between HbA1c levels and endothelial function in patients with type 2 diabetes including patients not taking antidiabetic drugs.

Interestingly, in the present study, HbA1c levels were not corelated with NID. There were no significant differences in NID values among the HbA1c groups of < 48 mmol/mol (6.5%), 48–52 mmol/mol (6.5–6.9%), 53–63 mmol/mol (7.0-7.9%), and ≥ 64 mmol/mol (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 well known that the incidence of myocardial infarction increases in relation to HbA1c level. [21] 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 < 48 mmol/mol (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 minimize the effect of hypoglycemia, we evaluated the relationships between HbA1c levels 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 53 mmol/mol (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. [14, 22, 23] 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 [24, 25], while other studies showed a J-shaped relationship between HbA1c and all causes of mortality [26, 27]. The effects of intensive glucose control therapy on morbidity and mortality of CV events are also controversial. [27, 28] The UKPDS 73 study showed that the frequency of hypoglycemia in patients not taking antidiabetic drugs was 0.1%. [29] Hypoglycemia during intensive glucose control is probably a predictor of morbidity and mortality of CV events. It has been shown that the hazard ratios for all causes of mortality including CV events in patients with severe hypoglycemia episodes are between 1.74 and 3.27. [30, 31] It has been postulated that hypoglycemia activates the sympathetic nervous system, releases catecholamines that cause increase heart rate and myocardial contractility [32], and activates platelet aggregation, leading to acute coronary syndrome and fatal arrythmia. [33] Although the precise mechanisms 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 hypoglycemia contribute to low HbA1c-induced endothelial dysfunction.

Study Limitations

This study has some limitations. First, this study was a cross-sectional study, although the study was conducted in multiple centers 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 UKPDS80 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. Third, this study was conducted in Japan, and our results for 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 the association of FMD with HbA1c that found in the present study is observed 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 with dialysis. In the present study, we excluded those patients and we adjusted serum creatinine levels using propensity score matching analysis. In addition, since elderly patients often have malnutrition due to anorexia that 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.

Conclusions

A Low HbA1c level (< 48 mmol/mol (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. Control of HbA1c level in the range of 48–52 mmol/mol (6.5–6.9%) may be appropriate for maintenance of vascular function in patients with type 2 diabetes.

Abbreviations

BMI = body mass index

CVD = cardiovascular disease

FMD = flow-mediated vasodilation

HbA1c = hemoglobin A1c

HDL-C = high-density lipoprotein cholesterol

LDL-C = high-density lipoprotein cholesterol

NID = nitroglycerine-induced vasodilation

Declarations

Ethics approval and consent to participate

The ethical committees of the participating institutions approved the study protocol. All participants provide written informed consent before date collection.

Consent for publication

Not applicable

Availability of date and material

Not applicable

Competing interests

The authors declared that they do not have anything to disclose regarding conflict of interest with respect to this manuscript

Funding sources

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).

Author's contributions

T.Y. and Y.Higashi, drafting the article and conception of the study; T.H., Y.H., Y.T., M.K., Y.Han, T.M., H.H., S.K., T.H., C.G., Y.A., A.N., and F.M.Y. acquiring subjects and/or data; E.H., K.C. and Y.K., revising the article critically for important intellectual content. Y.H. is the guarantor of this work and, as such, had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of data analysis.

Acknowledgments

The authors would like to thank all of the patients who participated in this study. In addition, 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).

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Tables

Table 1. Clinical Characteristics of the Patients with Type 2 Diabetes

Variables	Total (n=1215)	HbA1c <48 mmol/mol <6.5% (n=474)	HbA1c ≥48 mmol/mol ≥6.5% (n=741)	P value
Age, yr	62±10	65±10	60±10	<0.001
Gender, men/women	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, mmHg	133±17	130±18	135±17	<0.001
Diastolic blood pressure, mmHg	79±11	76±11	80±11	<0.001
Total cholesterol, mmol/L	4.9±1.0	4.7±0.9	5.0±1.0	<0.001
Triglycerides, mmol/L	1.7±1.2	1.5±0.9	1.8±1.4	<0.001
HDL-C, mmol/L	1.4±0.4	1.5±0.4	1.4±0.4	<0.001
LDL-C, mmol/L	2.8±0.8	2.6±0.8	2.9±0.9	<0.001
Creatinine, µmol/L	74.3±25.6	76.0±27.4	73.4±23.9	0.07
Uric acid, µmol/L	339±83	345±83	333±83	0.03
Glucose, mmol/L	7.7±2.6	6.6±1.5	8.3±2.8	<0.001
Hemoglobin A1c, mmol/mol	50.7±12.2	40.7±3.9	57.0±11.5	<0.001
Hemoglobin A1c, %	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
Dyslipidemia	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

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CVD, cardiovascular disease; FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation.

Table 2. Clinical Characteristics of Patients with Type 2 Diabetes in Four Groups on the Basis on HbA1c Level

Variables	Total (n=1215)	HbA1c <48 mmol/mol <6.5% (n=474)	HbA1c 48-52 mmol/mol 6.5-6.9% (n=333)	HbA1c 53-63 mmol/mol 7.0-7.9% (n=272)	HbA1c ≥64 mmol/mol ≥8.0% (n=136)	P value
Age, yr	62±10	65±10	61±10	62±10	57±11	<0.001
Gender, men/women	870/345	301/173	240/93	220/52	119/22	<0.001
Body mass index, kg/m^2	25.3±4.3	24.7±4.0	25.7±4.3	25.7±4.4	25.7±4.3	0.002
Heart rate, bpm	68±11	69±12	68±11	67±11	69±12	0.3
Systolic blood pressure, mmHg	133±17	130±18	134±16	135±18	136±17	<0.001
Diastolic blood pressure, mmHg	79±11	76±11	80±11	80±11	81±13	<0.001
Total cholesterol, mmol/L	4.9±1.0	4.7±0.9	5.0±0.9	4.8±1.0	5.2±1.2	<0.001
Triglycerides, mmol/L	1.7±1.2	1.5±0.9	1.7±1.3	1.9±1.5	2.0±1.5	<0.001
HDL-C, mmol/L	1.4±0.4	1.5±0.4	1.4±0.4	1.3±0.4	1.3±0.4	<0.001
LDL-C, mmol/L	2.8±0.8	2.6±0.8	2.9±0.8	2.7±0.8	3.1±1.0	<0.001
Creatinine, µmol/L	74.3±25.6	76±27	72±21	74±27	73±27	0.26
Uric acid, µmol/L	339±83	345±83	345±83	333±83	315±83	<0.001
Glucose, mmol/L	7.7±2.6	6.6±1.5	7.2±1.4	8.3±2.1	11.2±4.3	<0.001
Hemoglobin A1c, mmol/mol	50.7±12.2	40.7±3.9	49.5±1.5	56.7±3.0	76.1±13.7	<0.001
Hemoglobin A1c, %	6.8±1.1	5.9±0.4	6.7±0.1	7.3±0.3	9.1±1.2	<0.001
Medical history, n (%)						
Hypertension	969 (79.8)	378 (79.8)	266 (79.9)	226 (83.1)	99 (72.8)	0.12
Dyslipidemia	953 (78.4)	371 (78.3)	251 (75.4)	226 (83.1)	105 (77.2)	0.13
CVD, n (%)	409 (33.7)	150 (31.7)	98 (29.4)	104 (38.2)	57 (41.9)	0.02
Current Smoking, n (%)	290 (24.1)	104 (21.9)	73 (22.3)	73 (27.3)	40 (29.6)	0.12
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	866 (71.3)	373 (78.7)	184 (55.3)	205 (75.4)	104 (76.5)	<0.001

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CVD, cardiovascular disease; FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation.

Table 3. Multivariate Analysis of Relationships among Low Quartiles of FMD and NID and Low HbA1c Level

	Low quartile of FMD		Low quartile of NID	
Variables	OR (95% CI)	P value	OR (95% CI)	P value
Model 1	2.50 (1.93-3.27)	<0.001	1.17 (0.74-1.84)	0.50
Model 2	2.04 (1.55-2.69)	<0.001	1.00 (0.61-1.61)	0.99
Model 3	2.03 (1.53-2.69)	<0.001	1.07 (0.65-1.75)	0.80

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, creatine, and presence of hypertension, dyslipidemia and CVD

FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation; OR, odds ration; CI, confidence interval; CVD, cardiovascular disease.

Low quartile of FMD indicates less than 2.1%. Low quartile of NID indicates less than 6.2%.

Table 4. Clinical Characteristics of Patients with Type 2 Diabetes Not Taking Antidiabetic Drugs

Variables	Total (n=349)	HbA1c <48 mmol/mol <6.5% (n=101)	HbA1c ≥48 mmol/mol ≥6.5% (n=248)	P value
Age, yr	61±10	66±10	60±10	<0.001
Gender, men/women	245/104	59/42	186/62	0.002
Body mass index, kg/m^2	25.4±4.2	24.6±4.1	25.7±4.3	0.03
Heart rate, bpm	69±11	70±11	68±11	0.06
Systolic blood pressure, mmHg	133±17	128±18	135±16	<0.001
Diastolic blood pressure, mmHg	80±11	77±12	82±10	<0.001
Total cholesterol, mmol/L	5.2±1.2	4.8±0.9	5.3±1.0	<0.001
Triglycerides, mmol/L	1.9±1.1	1.5±0.9	2.1±1.7	0.002
HDL-C, mmol/L	1.4±0.4	1.5±0.4	1.3±0.4	0.005
LDL-C, mmol/L	3.0±0.8	2.8±0.8	3.1±0.8	0.02
Creatinine, µmol/L	71±27	75±33	70±18	0.07
Uric acid, µmol/L	345±89	357±101	339±89	0.10
Glucose, mmol/L	7.6±2.6	6.6±28	7.9±2.8	<0.001
Hemoglobin A1c, mmol/mol	50.8±11.4	40.6±3.9	54.9±10.9	<0.001
Hemoglobin A1c, %	6.8±1.0	5.9±0.4	7.2±1.0	<0.001
Medical history, n (%)				
Hypertension	266 (76.2)	75 (74.3)	191 (77.0)	0.58
Dyslipidemia	275 (78.8)	79 (78.2)	196 (79.0)	0.87
CVD, n (%)	79 (22.6)	27 (26.7)	52 (21.0)	0.24
Current Smoking, n (%)	79 (22.6)	20 (19.8)	59 (23.8)	0.37
Medication, n (%)				
Antihypertensive drugs	217 (62.2)	78 (77.2)	139 (56.1)	<0.001
Lipid lowering drugs	144 (41.3)	59 (58.4)	85 (34.3)	<0.001
Antidiabetic drugs	0 (0)	0 (0)	0 (0)	

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CVD, cardiovascular disease; FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation.

Figures

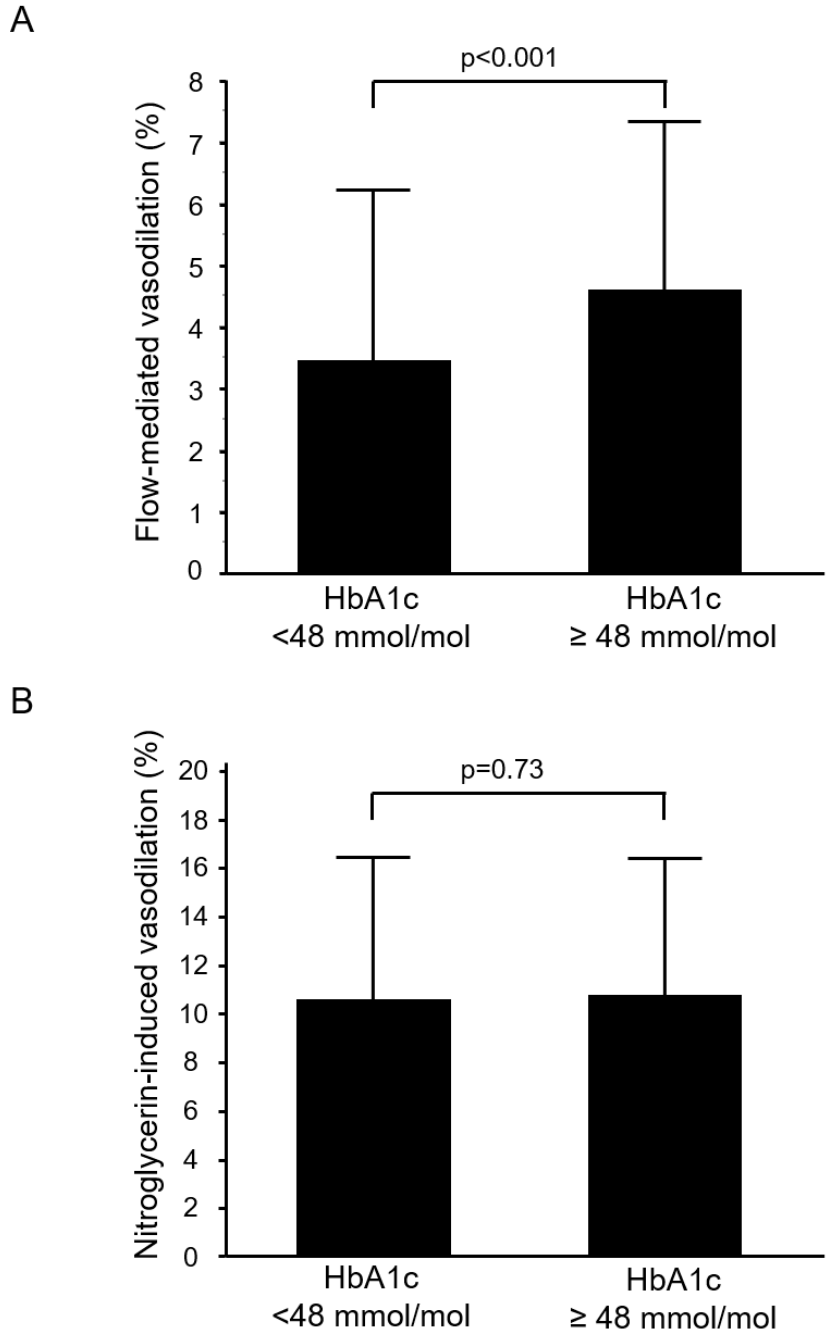

Figure 1

Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <48 mmol/mol (6.5%) and patients with HbA1c of ≥48 mmol/mol (6.5%).

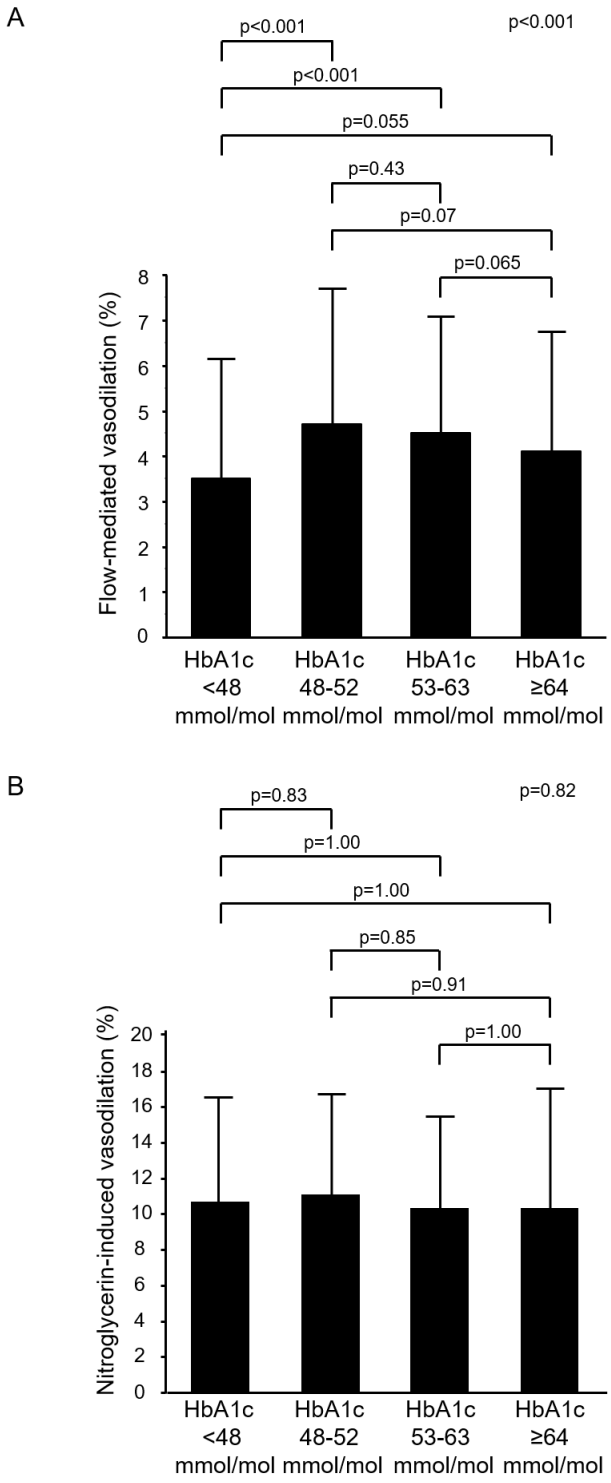

Figure 2

Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in 4 groups according to HbA1c levels.

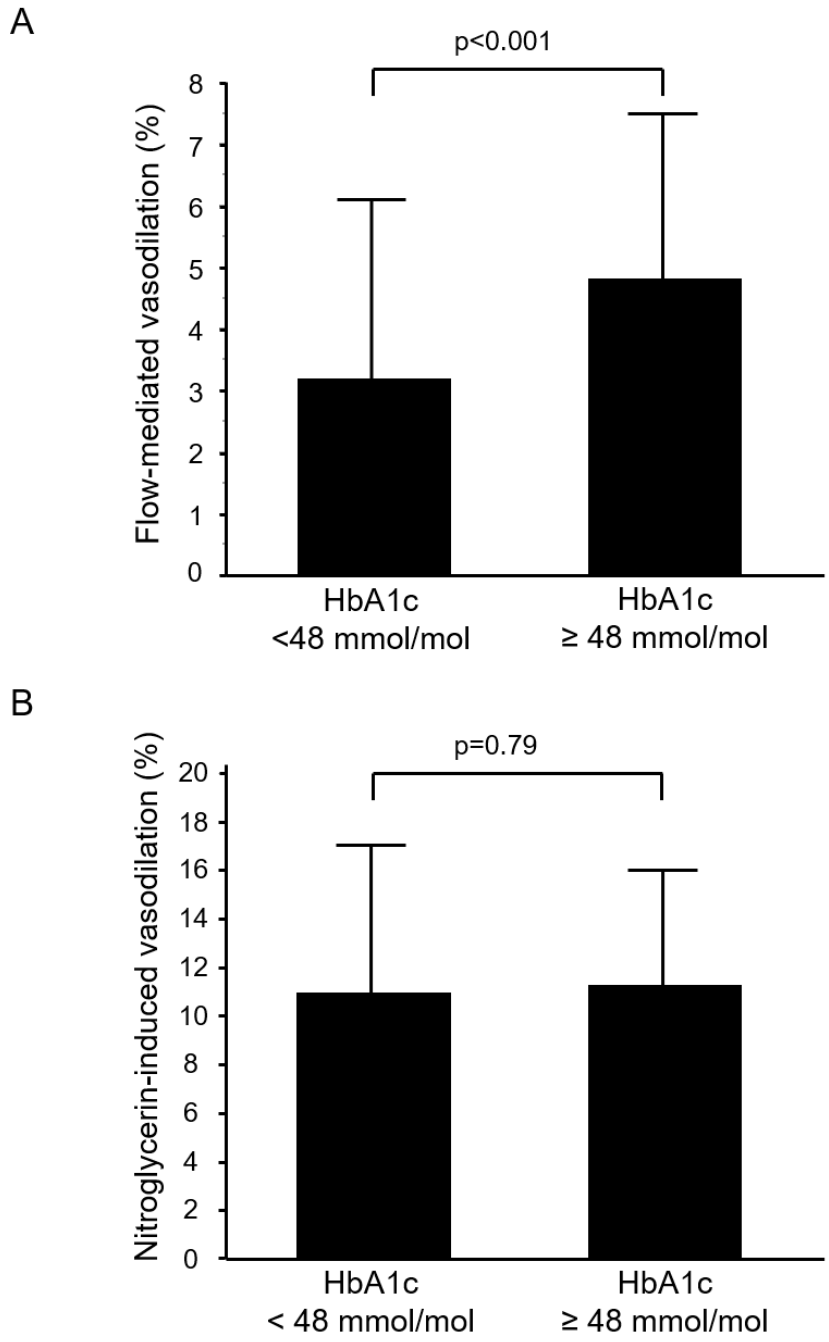

Figure 3

Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <48 mmol/mol (6.5%) and patients with HbA1c of ≥48 mmol/mol (6.5%) who were not receiving antidiabetic drug treatment.

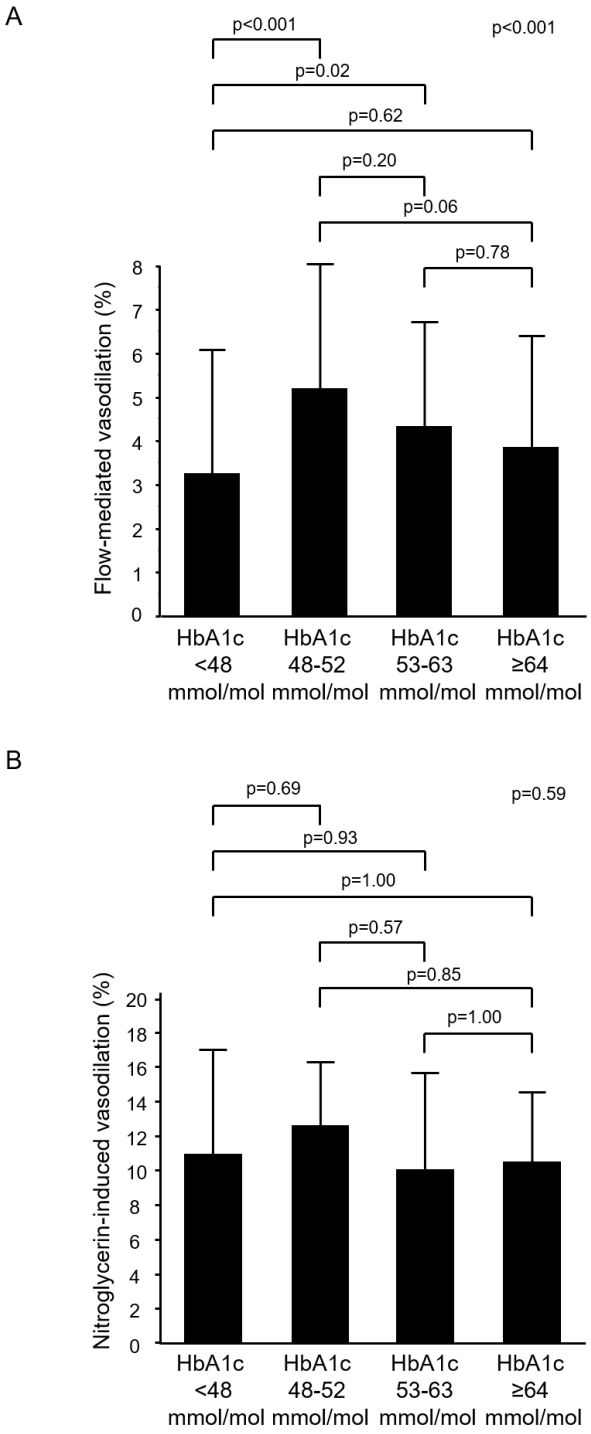

Figure 4

Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in 4 groups according to HbA1c levels for patients not receiving antidiabetic drug treatment.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- HbA1cSuppleCardiovasculardiabetologyV1.docx
- HbA1cSuppleCardiovasculardiabetologyV1.docx