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

Short title: Low HbA1c and endothelial function

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Abstract

Objectives: The aim of this study was to evaluate the relationship between HbA1c level and flow-mediated vasodilation (FMD) in patients with type 2 diabetes.

Design: Cross-sectional study.

Setting: 22 University hospitals and affiliated clinics in Japan.

Participants: 1215 patients (870 men and 345 women; mean age: 62±10 years) with type 2 diabetes including 349 patients not taking antidiabetic drugs and 866 patients taking antidiabetic drugs.

Measures: We evaluated FMD and HbA1c level. All patients were divided into four groups based on HbA1c levels: <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 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. 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 $p=0.02$).

Conclusions: These findings suggest that a low HbA1c level of <6.5% is associated with endothelial dysfunction. An HbA1c level of 6.5-6.9% may be appropriate for maintenance of endothelial function.

Trial registration number: The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409)

Strengths and limitations of this study

- The present study shows the relationship between HbA1c and FMD in patients with type 2 diabetes.
- The present study was conducted in multiple centers 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, therefore, we were not able to evaluate the causality between low HbA1c level and FMD.

INTRODUCTION

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. Hemoglobin A1c (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. [5 7,8]

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 supplementary Figure 1. 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. All methods were performed in accordance with the relevant guidelines and regulations.

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: <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 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.

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 supplementary method. 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 (<6.5%, 6.5-6.9%, 7.0-7.9%, 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 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)

Patient involvement

Patients and the public were not involved in the design or planning of the study.

RESULTS

Study 1.

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

The baseline characteristics of the 1215 patients are summarized 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 of <6.5% and those with HbA1c of ≥6.5% are also summarized 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 levels: <6.5%, 6.5-6.9%, 7.0-7.9%, and ≥8.0%. The baseline characteristics are summarized in online supplementary 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 supplementary Figure 2A). There was no significant difference in FMD between the HbA1c <6.5% group and HbA1c ≥8.0% group. (p=0.055; online supplementary Figure 2A). NID values were 10.6±5.9% in the 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 supplementary Figure 2B).

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

Online supplementary Table 2 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; online supplementary Figure 3A). A scatter plot between FMD and HbA1c level with a Lowess smoothed curve is shown in online

supplementary Figure 3B. FMD gradually increased with increase in HbA1c level to about 6.5-6.9% and the 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% 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 <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 <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) (online supplementary 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 of 6.5-6.9%, HbA1c of 7.0-7.9%, and HbA1c of ≥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 supplementary Tables 4, 5, and 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% versus 4.7±3.0%, p=0.002; 3.9±2.6% versus 4.5±2.6%, p=0.04; online supplementary Figures 4A and 4C), while there was no significant difference in FMD between the HbA1c <6.5% group and the HbA1c ≥8.0% group (4.5±2.7% versus 4.1±2.8%, p=0.36; online supplementary Figure 4E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (11.0±6.0% versus 11.2±5.5% in the HbA1c <6.5% group versus the HbA1c 6.5-6.9% group, p=0.84; 10.2±5.8% versus 10.5±5.6% in the HbA1c <6.5% group versus the HbA1c 7.0-7.9% group, p=0.82; 12.8±6.2% versus 11.6±7.2%, p=0.5, in the HbA1c <6.5% group versus the HbA1c ≥8.0% group, p=0.82; online supplementary Figures 4B, 4D, and 4F).

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 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 levels <6.5% and HbA1c levels ≥6.5%

The baseline characteristics of patients with type 2 diabetes not taking antidiabetic drugs who had HbA1c levels <6.5% and HbA1c levels ≥6.5% are summarized in online supplementary 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 supplementary 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 supplementary Figure 5B).

Next, the patients were divided into four groups according to HbA1c levels: <6.5%, 6.5-6.9%, 7.0-7.9%, and ≥8.0%. The baseline characteristics are summarized 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 supplementary Table 8 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 supplementary Figure 6A). Scatter plots between FMD and HbA1c with a Lowess smoothed curve are shown in online supplementary 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, creatine, and presence of hypertension, dyslipidemia and CVD, HbA1c level of <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) (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 of 6.5-6.9%, HbA1c of 7.0-7.9%, and HbA1c of ≥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 supplementary Tables 9, 10, and 11. FMD was significantly smaller in the HbA1c < 6.5% group than in the HbA1c 6.5-6.9% group (3.1±2.7% versus 4.6±3.2%, p=0.02; online supplementary 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% versus 4.0±2.8%, p=0.35; 4.0±3.0% versus 3.8±2.4%, p=0.87;

online supplementary Figures 7C and 7E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (10.8±5.6% versus 11.7±4.0% in the HbA1c <6.5% group versus the HbA1c 6.5-6.9% group, p=0.62; 11.8±5.7% versus 7.8±4.9% in the HbA1c <6.5% group versus the HbA1c 7.0-7.9% group, p=0.10; 14.8±5.5% versus 13.6±3.9% in the HbA1c <6.5% group versus the HbA1c ≥8.0% group, p=0.78; online supplementary Figures 7B, 7D, and 7F).

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 smaller in the HbA1c <6.5% group than in the HbA1c 6.5-6.9% group and HbA1c 7.0-7.9% group. In addition, we confirmed that FMD was significantly smaller in the low HbA1c group than in the HbA1c 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 <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 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 <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 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.

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.

In conclusions, 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. Control of HbA1c level in the range of 6.5-6.9% may be appropriate for maintenance of vascular function in patients with type 2 diabetes.

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Contributors
T.Y. and Y.Higashi, drafting the article and conception of the study; T.H., Y.Hashimoto., Y.T., M.K., Y.Han, T.M., S.K., H.H., C.G., 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.Higashi. 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.

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Competing interest
All authors have no conflicts of interests to report.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or dissemination plans of this research.

Patient consent for publication
Not required.

Ethics approval
This study protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409). The protocol of this study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of Hiroshima University.

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Refferences

1. Standl E, Balletshofer B, Dahl B, et al. Predictors of 10-year macrovascular and overall mortality in patients with NIDDM: the Munich General Practitioner Project. *Diabetologia* 1996;39(12):1540-5. [published Online First: 1996/12/01]
2. Khaw KT, Wareham N, Bingham S, et al. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Ann Intern Med* 2004;141(6):413-20. doi: 10.7326/0003-4819-141-6-200409210-00006 [published Online First: 2004/09/24]
3. Stamler J, Vaccaro O, Neaton JD, et al. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor

Intervention Trial. *Diabetes Care* 1993;16(2):434-44. doi: 10.2337/diacare.16.2.434 [published Online First: 1993/02/01]

4. Chew EY, Ambrosius WT, Davis MD, et al. Effects of medical therapies on retinopathy progression in type 2 diabetes. *The New England journal of medicine* 2010;363(3):233-44. doi: 10.1056/NEJMoa1001288 [published Online First: 2010/07/01]

5. Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *The New England journal of medicine* 2008;358(24):2560-72. doi: 10.1056/NEJMoa0802987 [published Online First: 2008/06/10]

6. Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995;28(2):103-17. [published Online First: 1995/05/01]

7. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *The New England journal of medicine* 2009;360(2):129-39. doi: 10.1056/NEJMoa0808431 [published Online First: 2008/12/19]

8. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine* 2008;358(24):2545-59. doi: 10.1056/NEJMoa0802743 [published Online First: 2008/06/10]

9. Higashi Y, Noma K, Yoshizumi M, et al. Endothelial function and oxidative stress in cardiovascular diseases. *Circulation journal : official journal of the Japanese Circulation Society* 2009;73(3):411-8. [published Online First: 2009/02/06]

10. Soga J, Noma K, Hata T, et al. Rho-associated kinase activity, endothelial function, and cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 2011;31(10):2353-9. doi: 10.1161/atvbaha.111.227892 [published Online First: 2011/07/09]

11. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based

study: the multi-ethnic study of atherosclerosis. *Circulation* 2009;120(6):502-9. doi: 10.1161/circulationaha.109.864801 [published Online First: 2009/07/29]

12. Martens FM, Rabelink TJ, op 't Roodt J, et al. TNF-alpha induces endothelial dysfunction in diabetic adults, an effect reversible by the PPAR-gamma agonist pioglitazone. *Eur Heart J* 2006;27(13):1605-9. doi: 10.1093/eurheartj/ehl079 [published Online First: 2006/06/10]
13. Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther* 2004;102(1):87-96. doi: 10.1016/j.pharmthera.2004.02.003 [published Online First: 2004/04/02]
14. Maruhashi T, Soga J, Fujimura N, et al. Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart* 2013;99(24):1837-42. doi: 10.1136/heartjnl-2013-304739 [published Online First: 2013/10/25]
15. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414(6865):813-20. doi: 10.1038/414813a [published Online First: 2001/12/14]
16. Nakamura K, Oe H, Kihara H, et al. DPP-4 inhibitor and alpha-glucosidase inhibitor equally improve endothelial function in patients with type 2 diabetes: EDGE study. *Cardiovasc Diabetol* 2014;13:110. doi: 10.1186/s12933-014-0110-2 [published Online First: 2014/07/31]
17. Tomiyama H, Kohro T, Higashi Y, et al. A multicenter study design to assess the clinical usefulness of semi-automatic measurement of flow-mediated vasodilatation of the brachial artery. *Int Heart J* 2012;53(3):170-5. [published Online First: 2012/07/14]
18. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *Jama* 2001;285(19):2486-97. [published Online First: 2001/05/23]
19. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018;41(Suppl 1):S13-s27. doi: 10.2337/dc18-S002 [published Online First: 2017/12/10]

20. Maruhashi T, Soga J, Fujimura N, et al. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. *Arterioscler Thromb Vasc Biol* 2013;33(6):1401-8. doi: 10.1161/atvbaha.112.300934 [published Online First: 2013/03/23]

21. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Bmj* 2000;321(7258):405-12. doi: 10.1136/bmj.321.7258.405 [published Online First: 2000/08/11]

22. Williams SB, Cusco JA, Roddy MA, et al. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of the American College of Cardiology* 1996;27(3):567-74. [published Online First: 1996/03/01]

23. Henry RM, Ferreira I, Kostense PJ, et al. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not; The Hoorn Study. *Atherosclerosis* 2004;174(1):49-56. doi: 10.1016/j.atherosclerosis.2004.01.002 [published Online First: 2004/05/12]

24. Palta P, Huang ES, Kalyani RR, et al. Hemoglobin A1c and Mortality in Older Adults With and Without Diabetes: Results From the National Health and Nutrition Examination Surveys (1988-2011). *Diabetes Care* 2017;40(4):453-60. doi: 10.2337/dci16-0042 [published Online First: 2017/02/23]

25. Sluik D, Boeing H, Montonen J, et al. HbA1c measured in stored erythrocytes is positively linearly associated with mortality in individuals with diabetes mellitus. *PLoS One* 2012;7(6):e38877. doi: 10.1371/journal.pone.0038877 [published Online First: 2012/06/22]

26. Arnold LW, Wang Z. The HbA1c and all-cause mortality relationship in patients with type 2 diabetes is J-shaped: a meta-analysis of observational studies. *Rev Diabet Stud* 2014;11(2):138-52. doi: 10.1900/rds.2014.11.138 [published Online First: 2014/11/15]

27. Wan EYF, Fung CSC, Wong CKH, et al. Association of Hemoglobin A1c Levels With Cardiovascular Disease and Mortality in Chinese Patients With Diabetes. *Journal of the American College of Cardiology*

2016;67(4):456-58. doi: 10.1016/j.jacc.2015.11.020 [published Online First: 2016/01/30]

28. Rawshani A, Rawshani A, Franzen S, et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *The New England journal of medicine* 2018;379(7):633-44. doi: 10.1056/NEJMoa1800256 [published Online First: 2018/08/16]
29. Wright AD, Cull CA, Macleod KM, et al. Hypoglycemia in Type 2 diabetic patients randomized to and maintained on monotherapy with diet, sulfonylurea, metformin, or insulin for 6 years from diagnosis: UKPDS73. *J Diabetes Complications* 2006;20(6):395-401. doi: 10.1016/j.jdiacomp.2005.08.010 [published Online First: 2006/10/31]
30. Zoungas S, Patel A, Chalmers J, et al. Severe hypoglycemia and risks of vascular events and death. *The New England journal of medicine* 2010;363(15):1410-8. doi: 10.1056/NEJMoa1003795 [published Online First: 2010/10/12]
31. Mellbin LG, Ryden L, Riddle MC, et al. Does hypoglycaemia increase the risk of cardiovascular events? A report from the ORIGIN trial. *Eur Heart J* 2013;34(40):3137-44. doi: 10.1093/eurheartj/eht332 [published Online First: 2013/09/04]
32. Fisher BM, Gillen G, Hepburn DA, et al. Cardiac responses to acute insulin-induced hypoglycemia in humans. *Am J Physiol* 1990;258(6 Pt 2):H1775-9. doi: 10.1152/ajpheart.1990.258.6.H1775 [published Online First: 1990/06/01]
33. Wright RJ, Frier BM. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes Metab Res Rev* 2008;24(5):353-63. doi: 10.1002/dmrr.865 [published Online First: 2008/05/08]

Figure Legends

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

Figure 2. 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.

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

Variables	Total (n=1215)	HbA1c <6.5% (n=474)	HbA1c ≥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, 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
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 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, yr	61±10	66±10	60±10	61±9	57±10	<0.001
Gender, men/women	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, mmHg	133±17	128±18	133±16	136±16	138±19	0.004
Diastolic blood pressure, mmHg	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
Hemoglobin A1c, %	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
Dyslipidemia	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)					

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 FMD, NID and Low HbA1c level (<6.5%) in Patients with Type 2 DM 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 (1.80-5.14)	<0.001	1.33 (0.54-3.31)	0.53
Model 2	2.49 (1.44-4.33)	0.001	1.20 (0.46-3.13)	0.71
Model 3	2.57 (1.45-4.54)	0.001	1.29 (0.43-3.91)	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, creatine, presence of hypertension, dyslipidemia and CVD
FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease.
Low quartile of FMD indicates less than 2.1%. Low quartile of NID indicates less than 6.2%.

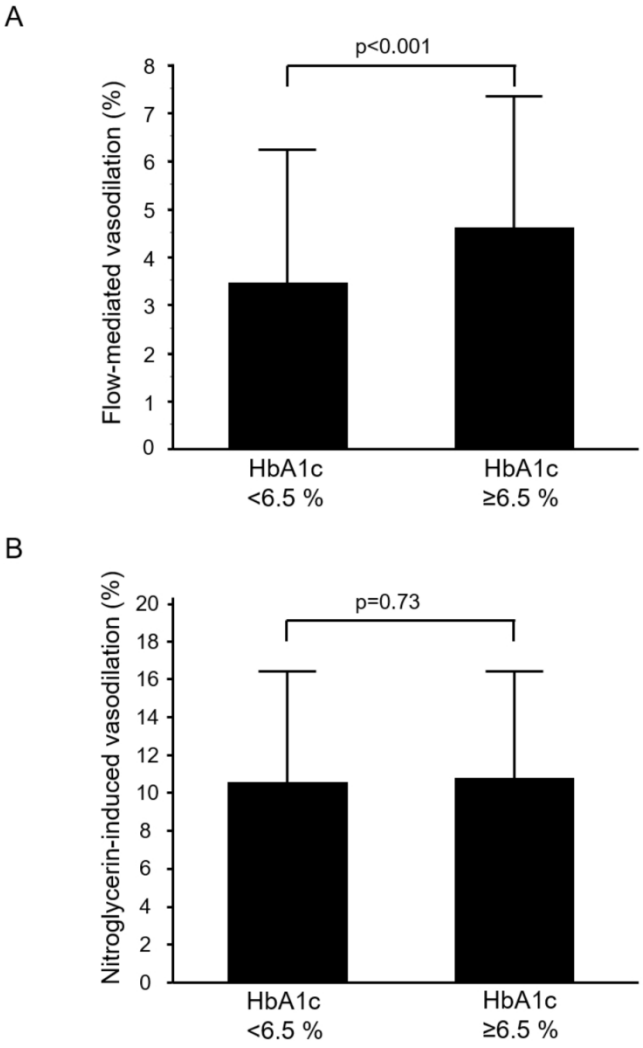

Figure 1

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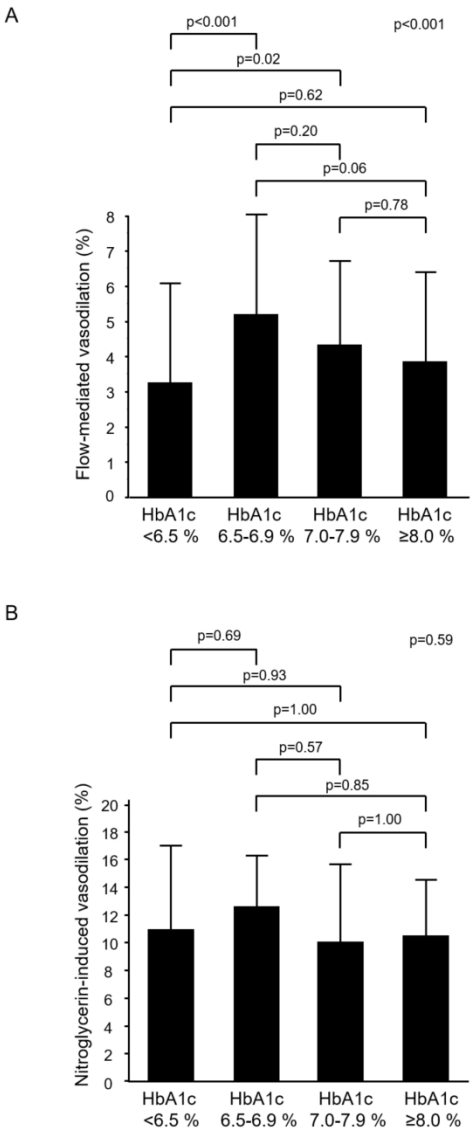

Figure 2

127x272mm (300 x 300 DPI)

Supplementary Material

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

Short title: Low HbA1c and endothelial function

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Supplementary Methods

Measurement of FMD and NID

Vascular response to reactive

A blood pressure cuff was placed around the forearm of each subjects. The brachial artery was scanned longitudinally 5 to 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 seconds after cuff deflation. 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 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. 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 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. After acquiring baseline rest images for 30 seconds, a sublingual tablet (nitroglycerine, 75 μg) was given and imaging of the artery was done continuously for 5 minutes. NID was automatically calculated as a percentage change in peak vessel diameter from the baseline. Percentage of NID [(Peak diameter-Baseline diameter)/Baseline diameter] was used for analysis.

Online Supplementary Tables

Table 1. Clinical Characteristics of Patients with Type 2 Diabetes in Four Groups on the Basis on HbA1c Level

Variables	Total (n=1215)	HbA1c <6.5% (n=474)	HbA1c 6.5-6.9% (n=333)	HbA1c 7.0-7.9% (n=272)	HbA1c ≥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, mg/dL	188±37	180±33	194±35	186±38	202±46	<0.001
Triglycerides, mg/dL	148±109	130±81	147±111	164±130	177±133	<0.001
HDL-C, mg/dL	54±15	57±16	55±15	51±14	51±14	<0.001
LDL-C, mg/dL	107±32	101±29	112±31	106±32	118±39	<0.001
Creatinine, mg/dL	0.84±0.29	0.86±0.31	0.82±0.24	0.84±0.30	0.83±0.31	0.26
Uric acid, mg/dL	5.7±1.4	5.8±1.4	5.8±1.4	5.6±1.4	5.3±1.4	<0.001
Glucose, mg/dL	138±46	119±27	130±26	149±37	202±78	<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 2. Univariate Analysis of Relationships among FMD, HbA1c Level and Variables

Variables	FMD		HbA1c	
	r	P value	r	P value
Age, yr	-0.30	<0.001	-0.21	<0.001
Body mass index, kg/m^2	0.01	0.63	0.07	0.01
Heart rate, bpm	-0.03	0.27	-0.01	0.73
Systolic blood pressure, mmHg	0.04	0.13	0.13	<0.001
Diastolic blood pressure, mmHg	0.17	<0.001	0.14	<0.001
Total cholesterol, mg/dL	0.03	0.33	0.18	<0.001
Triglycerides, mg/dL	-0.04	0.15	0.18	<0.001
HDL-C, mg/dL	-0.01	0.6	-0.14	<0.001
LDL-C, mg/dL	0.04	0.2	0.16	<0.001
Creatinine, mg/dL	-0.09	0.002	0.004	0.88
Uric acid, mg/dL	-0.01	0.62	-0.11	<0.001
Glucose, mg/dL	-0.02	0.44	0.57	<0.001
HbA1c, %	0.08	0.004		
FMD, %			0.08	0.004
NID, %	0.33	<0.001	-0.02	0.70

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation.
Univariate analysis of the relations among FMD, HbA1c levels and variables (Pearson's correlation analysis).

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 Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 6.5-6.9%

Variables	HbA1c <6.5% (n=238)	HbA1c 6.5-6.9% (n=238)	P value
Age, yr	63±10	63±10	0.94
Gender, men/women	171/67	162/76	0.37
Body mass index, kg/m^2	25.2±4.2	25.3±4.1	0.75
Heart rate, bpm	67±11	68±11	0.6
Systolic blood pressure, mmHg	132±17	132±16	0.92
Diastolic blood pressure, mmHg	78±10	78±11	0.96
Total cholesterol, mg/dL	188±33	189±34	0.63
Triglycerides, mg/dL	126±60	130±69	0.51
HDL-C, mg/dL	56±14	56±16	0.84
LDL-C, mg/dL	108±29	109±30	0.94
Creatinine, mg/dL	0.82±0.21	0.82±0.26	0.58
Uric acid, mg/dL	5.8±1.3	5.8±1.4	0.95
Glucose, mg/dL	119±26	130±26	<0.001
Hemoglobin A1c, %	5.9±0.4	6.7±0.1	<0.001
Medical history, n (%)			
Hypertension	191 (80.3)	192 (80.7)	0.91
Dyslipidemia	174 (73.1)	183 (76.9)	0.34
CVD, n (%)	76 (31.9)	76 (31.9)	1.00
Current Smoking, n (%)	53 (22.3)	56 (23.5)	0.74
Medication, n (%)			
Antihypertensive drugs	39 (79.6)	36 (73.4)	0.47
Lipid lowering drugs	131 (55.0)	135 (56.7)	0.71
Antidiabetic drugs	186 (78.2)	140 (58.8)	<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 5. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 7.0-7.9%

Variables	HbA1c <6.5% (n=192)	HbA1c 7.0-7.9% (n=192)	P value
Age, yr	63±10	63±9	0.96
Gender, men/women	163/29	151/41	0.11
Body mass index, kg/m^2	25.3±3.9	25.4±4.2	0.80
Heart rate, bpm	67±11	67±12	0.99
Systolic blood pressure, mmHg	133±18	133±16	0.94
Diastolic blood pressure, mmHg	79±11	79±10	0.74
Total cholesterol, mg/dL	178±33	181±37	0.36
Triglycerides, mg/dL	135±76	132±84	0.72
HDL-C, mg/dL	52±13	53±15	0.54
LDL-C, mg/dL	101±29	104±32	0.29
Creatinine, mg/dL	0.88±0.30	0.84±0.29	0.16
Uric acid, mg/dL	5.8±1.4	5.7±1.4	0.32
Glucose, mg/dL	118±21	146±36	<0.001
Hemoglobin A1c, %	5.9±0.4	7.3±0.3	<0.001
Medical history, n (%)			
Hypertension	162 (84.4)	158 (82.3)	0.58
Dyslipidemia	150 (78.1)	157 (81.8)	0.37
CVD, n (%)	75 (39.1)	77 (40.1)	0.84
Current Smoking, n (%)	49 (25.5)	45 (23.4)	0.64
Medication, n (%)			
Antihypertensive drugs	138 (71.9)	135 (70.3)	0.74
Lipid lowering drugs	111 (57.8)	117 (60.9)	0.53
Antidiabetic drugs	159 (82.8)	146 (76.0)	0.10

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 6. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of ≥8.0 %

Variables	HbA1c <6.5% (n=93)	HbA1c ≥8.0% (n=93)	P value
Age, yr	58±11	58±10	0.78
Gender, men/women	68/25	71/22	0.61
Body mass index, kg/m^2	24.9±4.9	25.1±4.1	0.78
Heart rate, bpm	68±12	67±12	0.76
Systolic blood pressure, mmHg	136±18	134±17	0.61
Diastolic blood pressure, mmHg	80±11	80±13	0.86
Total cholesterol, mg/dL	193±40	190±39	0.58
Triglycerides, mg/dL	129±59	139±72	0.28
HDL-C, mg/dL	54±14	53±15	0.67
LDL-C, mg/dL	114±34	110±35	0.41
Creatinine, mg/dL	0.85±0.36	0.85±0.33	1.00
Uric acid, mg/dL	5.3±1.2	5.4±1.1	0.88
Glucose, mg/dL	118±22	189±72	<0.001
Hemoglobin A1c, %	5.9±0.4	9.0±1.2	<0.001
Medical history, n (%)			
Hypertension	62 (66.7)	65 (69.9)	0.64
Dyslipidemia	65 (69.9)	67 (72.0)	0.75
CVD, n (%)	33 (35.5)	39 (41.9)	0.37
Current Smoking, n(%)	25 (26.9)	27 (29.0)	0.74
Medication, n (%)			
Antihypertensive drugs	54 (58.1)	59 (63.4)	0.45
Lipid lowering drugs	43 (46.2)	43 (46.2)	1.00
Antidiabetic drugs	80 (86.0)	73 (78.5)	0.18

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

Variables	Total (n=349)	HbA1c <6.5% (n=101)	HbA1c ≥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, mg/dL	199±39	186±33	204±40	<0.001
Triglycerides, mg/dL	169±139	133±82	183±154	0.002
HDL-C, mg/dL	54±15	57±15	52±15	0.005
LDL-C, mg/dL	116±32	110±30	119±32	0.02
Creatinine, mg/dL	0.8±0.3	0.85±0.37	0.79±0.20	0.07
Uric acid, mg/dL	5.8±1.5	6.0±1.7	5.7±1.5	0.10
Glucose, mg/dL	137±46	119±28	143±50	<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.

Table 8. Univariate Analysis of Relationships among FMD, HbA1c Level and Variables in Patients with Type 2 Diabetes Not Taking Antidiabetic Drugs

Variables	FMD		HbA1c	
	r	P value	r	P value
Age, yr	-0.24	<0.001	-0.2	<0.001
Body mass index, kg/m^2	0.09	0.08	0.06	0.28
Heart rate, bpm	0.05	0.33	-0.01	0.82
Systolic blood pressure, mmHg	0.10	0.048	0.17	0.001
Diastolic blood pressure, mmHg	0.19	<0.001	0.12	0.02
Total cholesterol, mg/dL	0.02	0.66	0.22	<0.001
Triglycerides, mg/dL	-0.02	0.64	0.23	<0.001
HDL-C, mg/dL	-0.05	0.30	-0.19	<0.001
LDL-C, mg/dL	0.03	0.59	0.14	0.01
Creatinine, mg/dL	-0.03	0.62	-0.07	0.20
Uric acid, mg/dL	0.08	0.15	-0.10	0.07
Glucose, mg/dL	-0.08	0.12	0.70	<0.001
HbA1c, %	0.05	0.40		
FMD, %			0.05	0.40
NID, %	0.36	<0.0001	-0.02	0.78

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

Table 9. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 6.5-6.9%

Variables	HbA1c <6.5% (n=49)	HbA1c 6.5-6.9% (n=49)	P value
Age, yr	64±9	66±7	0.29
Gender, men/women	29/20	26/23	0.54
Body mass index, kg/m^2	25.3±3.7	24.8±3.6	0.51
Heart rate, bpm	69±9	68±10	0.5
Systolic blood pressure, mmHg	131±17	129±16	0.46
Diastolic blood pressure, mmHg	78±11	76±9	0.30
Total cholesterol, mg/dL	189±35	190±28	0.95
Triglycerides, mg/dL	126±48	132±86	0.66
HDL-C, mg/dL	56±13	58±16	0.47
LDL-C, mg/dL	112±30	108±24	0.52
Creatinine, mg/dL	0.79±0.22	0.76±0.18	0.49
Uric acid, mg/dL	5.6±1.2	5.3±1.3	0.32
Glucose, mg/dL	117±18	124±22	0.14
Hemoglobin A1c, %	5.8±0.4	6.7±0.2	<0.001
Medical history, n (%)			
Hypertension	39 (79.6)	39 (79.6)	1.00
Dyslipidemia	37 (75.5)	40 (81.6)	0.46
CVD, n (%)	12 (24.5)	12 (24.5)	1.00
Medication, n (%)			
Antihypertensive drugs	39 (79.6)	36 (73.4)	0.47
Lipid lowering drugs	29 (59.2)	30 (61.2)	0.84
Antidiabetic drugs	0 (0)	0 (0)	
Current Smoking, n (%)	12 (24.5)	10 (20.4)	0.63

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 10. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 7.0-7.9%

Variables	HbA1c <6.5% (n=29)	HbA1c 7.0-7.9% (n=29)	P value
Age, yr	63±10	65±8	0.37
Gender, men/women	19/10	20/9	0.78
Body mass index, kg/m^2	24.9±3.8	24.8±3.9	0.91
Heart rate, bpm	70±10	68±11	0.4
Systolic blood pressure, mmHg	133±17	134±14	0.74
Diastolic blood pressure, mmHg	80±12	81±8	0.78
Total cholesterol, mg/dL	189±31	183±39	0.51
Triglycerides, mg/dL	168±116	146±143	0.51
HDL-C, mg/dL	49±12	51±15	0.47
LDL-C, mg/dL	114±28	108±32	0.48
Creatinine, mg/dL	0.80±0.24	0.78±0.22	0.68
Uric acid, mg/dL	5.7±1.4	5.4±1.5	0.40
Glucose, mg/dL	119±23	136±32	0.02
Hemoglobin A1c, %	5.8±0.4	7.3±0.3	<0.001
Medical history, n (%)			
Hypertension	25 (86.2)	27 (93.1)	0.39
Dyslipidemia	23 (79.3)	23 (79.3)	1.00
CVD, n (%)	8 (27.6)	9 (31.0)	0.77
Current Smoking, n (%)	8 (27.6)	5 (17.2)	0.34
Medication, n (%)			
Antihypertensive drugs	24 (82.7)	19 (65.5)	0.13
Lipid lowering drugs	18 (62.1)	15 (51.7)	0.43
Antidiabetic drugs	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.

Table 11. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of ≥8.0%

Variables	HbA1c <6.5% (n=20)	HbA1c ≥8.0% (n=20)	P value
Age, yr	59±11	58±11	0.82
Gender, men/women	15/5	16/4	0.71
Body mass index, kg/m^2	24.9±4.5	25.4±4.6	0.71
Heart rate, bpm	72±10	67±8	0.11
Systolic blood pressure, mmHg	138±20	136±21	0.80
Diastolic blood pressure, mmHg	84±14	82±11	0.65
Total cholesterol, mg/dL	199±40	213±40	0.31
Triglycerides, mg/dL	149±73	203±142	0.13
HDL-C, mg/dL	56±16	48±14	0.10
LDL-C, mg/dL	126±38	125±32	0.89
Creatinine, mg/dL	0.76±0.16	0.85±0.34	0.30
Uric acid, mg/dL	5.8±1.4	6.0±1.7	0.67
Glucose, mg/dL	116±17	212±63	<0.001
Hemoglobin A1c, %	6.0±0.4	9.4±1.2	<0.001
Medical history, n (%)			
Hypertension	14 (70.0)	15 (75.0)	0.72
Dyslipidemia	13 (65.0)	16 (80.0)	0.29
CVD, n (%)	5 (25.0)	3 (15.0)	0.43
Medication, n (%)			
Antihypertensive drugs	12 (60.0)	11 (55.0)	0.75
Lipid lowering drugs	5 (25.0)	4 (20.0)	0.71
Antidiabetic drugs	0 (0)	0 (0)	
Current Smoking, n (%)	6 (30.0)	4 (20.0)	0.47

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

Online Supplementary Figures

Figure 1

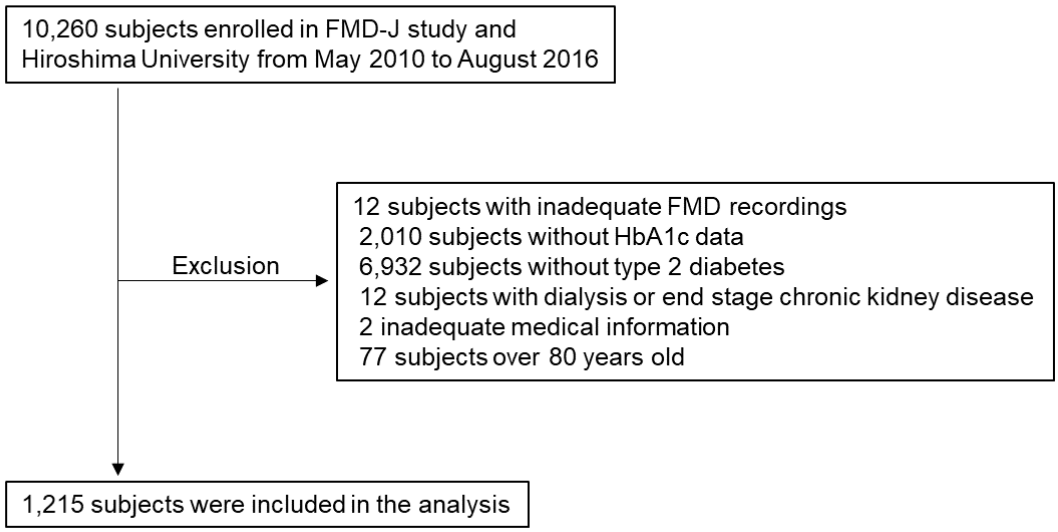

Figure 1. Flow chart of the study design.

Figure 2

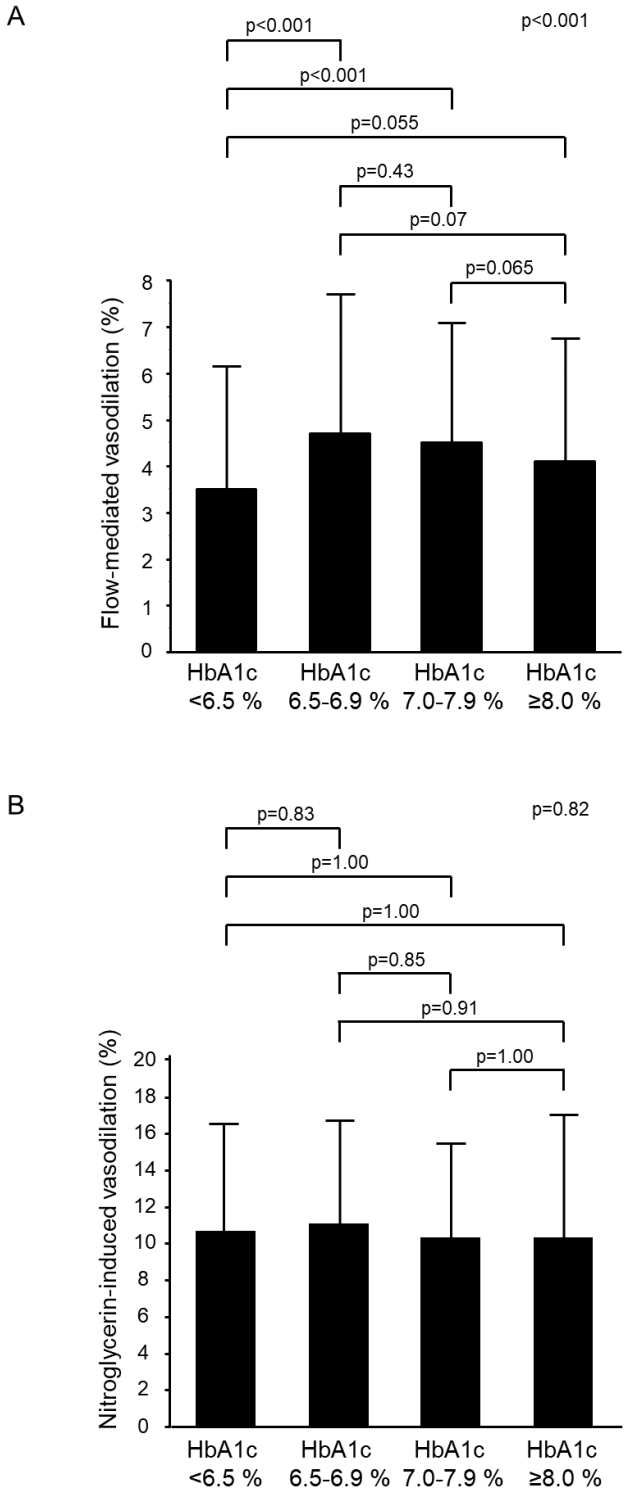

Figure 2. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in 4 groups according to HbA1c levels.

Figure 3

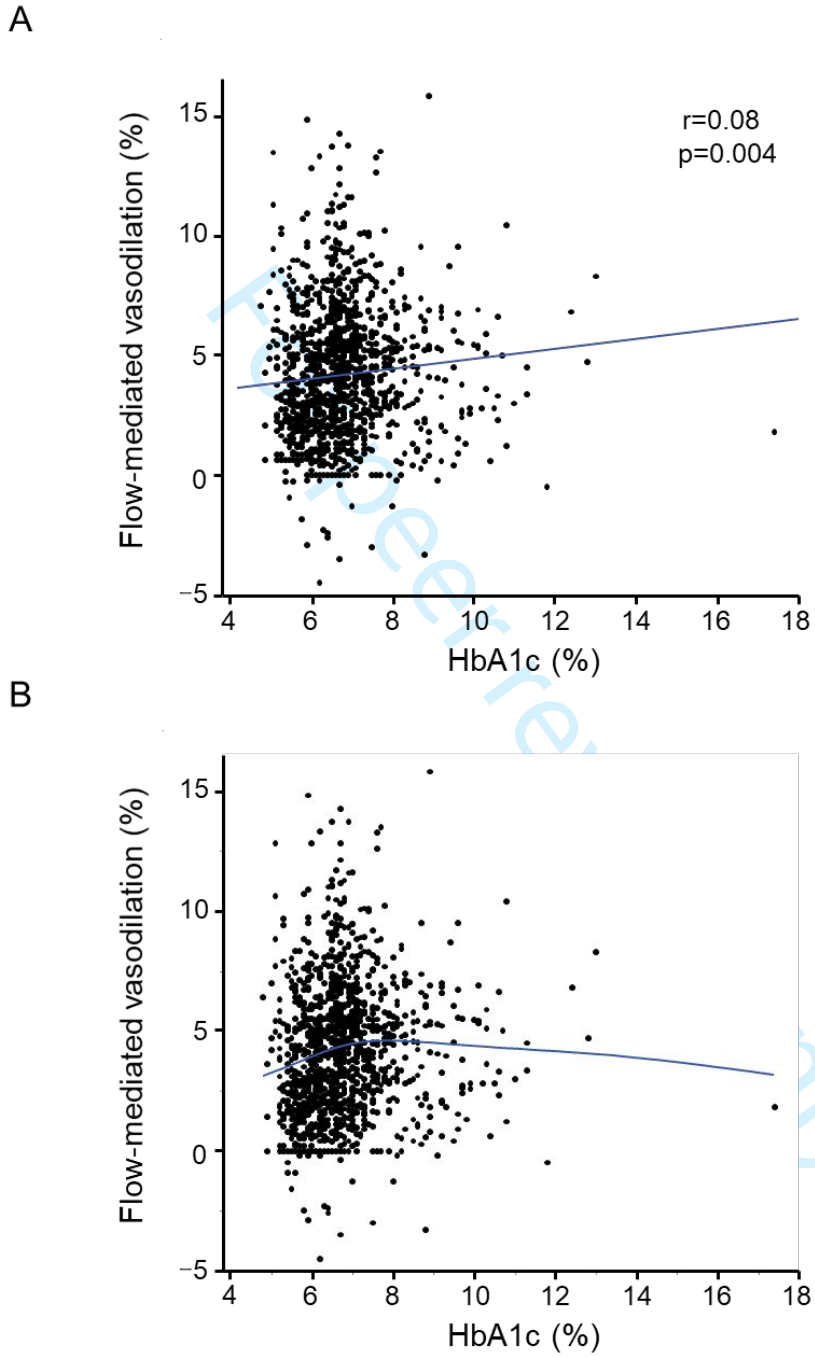

Figure 3. Scatter plots show the relationship between flow-mediated vasodilation and serum HbA1c levels in all patients (A) and locally weighted regression smoothing (Lowess) plot (B)

Figure 4

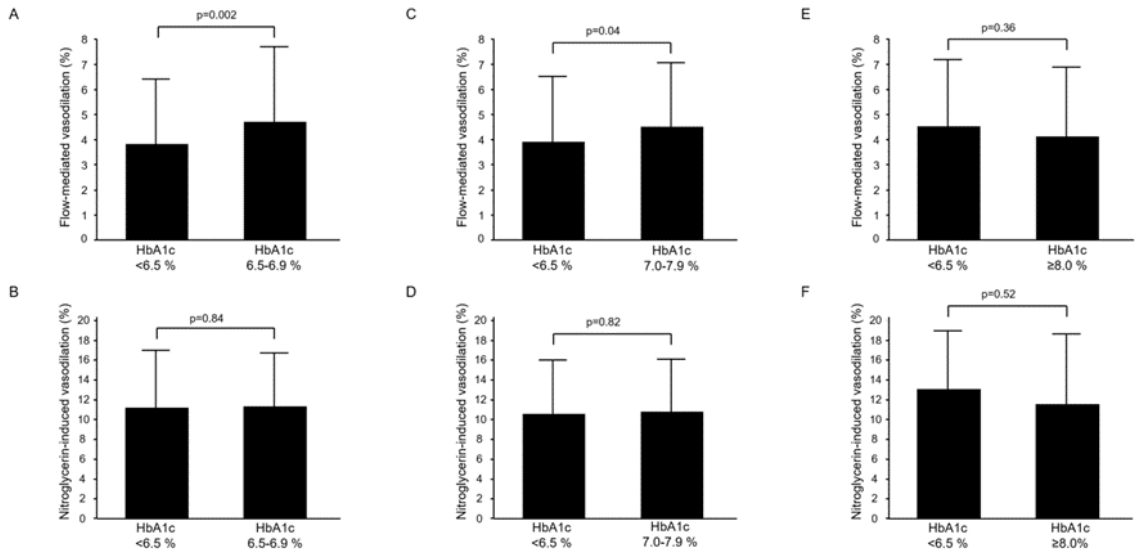

Figure 4. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of 6.5%-6.9%, flow-mediated vasodilation (C) and nitroglycerine-induced vasodilation (D) in patients with HbA1c of <6.5% and patients with HbA1c of 7.0%-7.9%, and flow-mediated vasodilation (E) and nitroglycerine-induced vasodilation (F) in patients with HbA1c of <6.5% and patients with HbA1c of ≥8.0% in a propensity score-matched population.

Figure 5

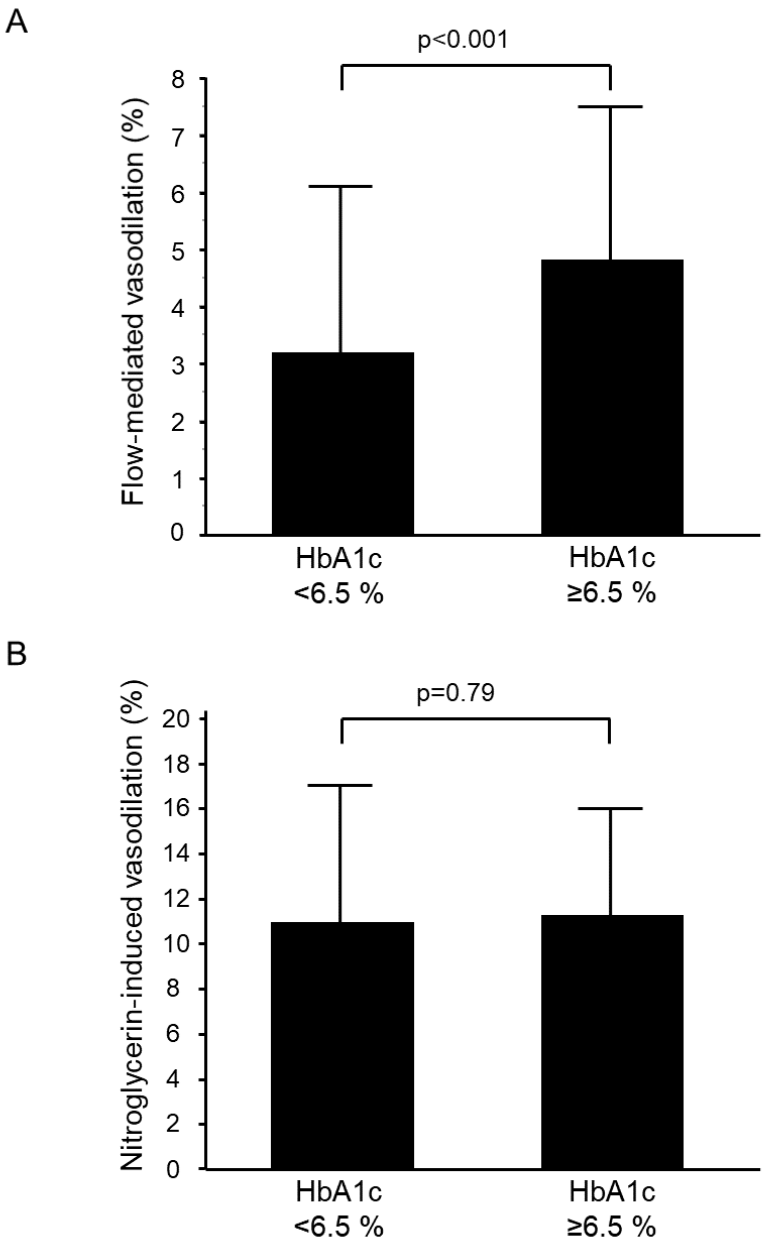

Figure 5. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of ≥6.5% who were not receiving antidiabetic drug treatment.

Figure 6

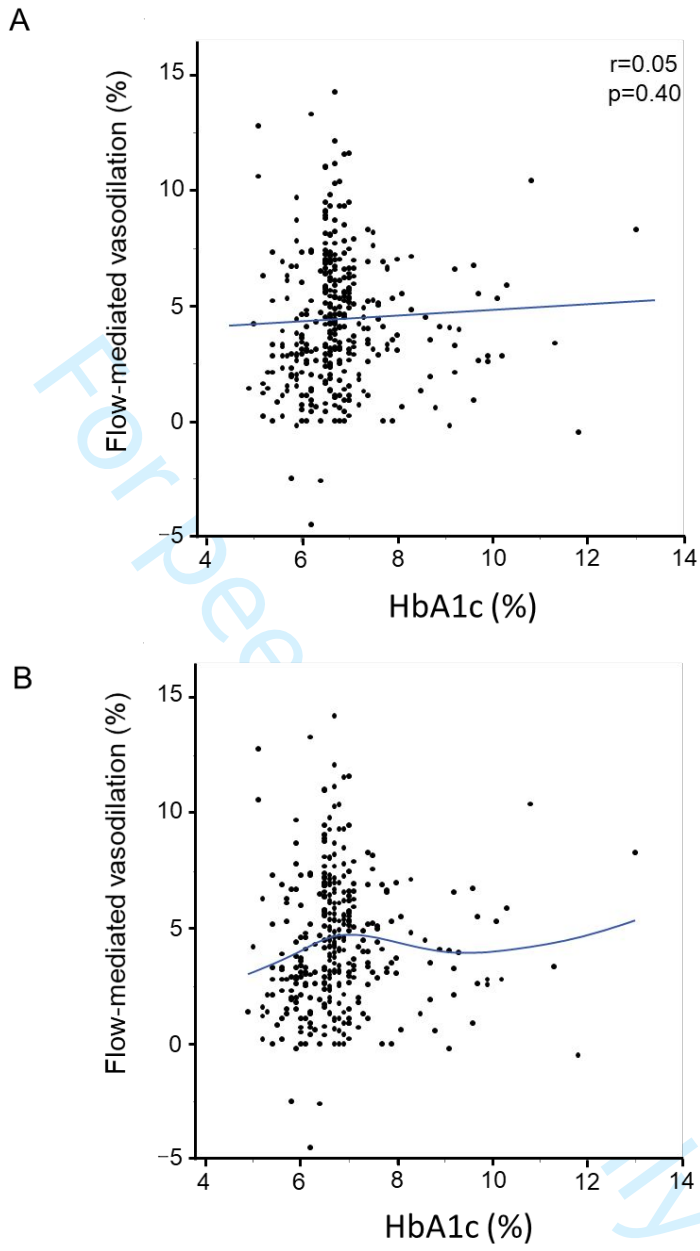

Figure 6. Scatter plots show the relationship between flow-mediated vasodilation and serum HbA1c levels in patients not receiving antidiabetic drug treatment (A) and locally weighted regression smoothing (Lowess) plot (B）.

Figure 7

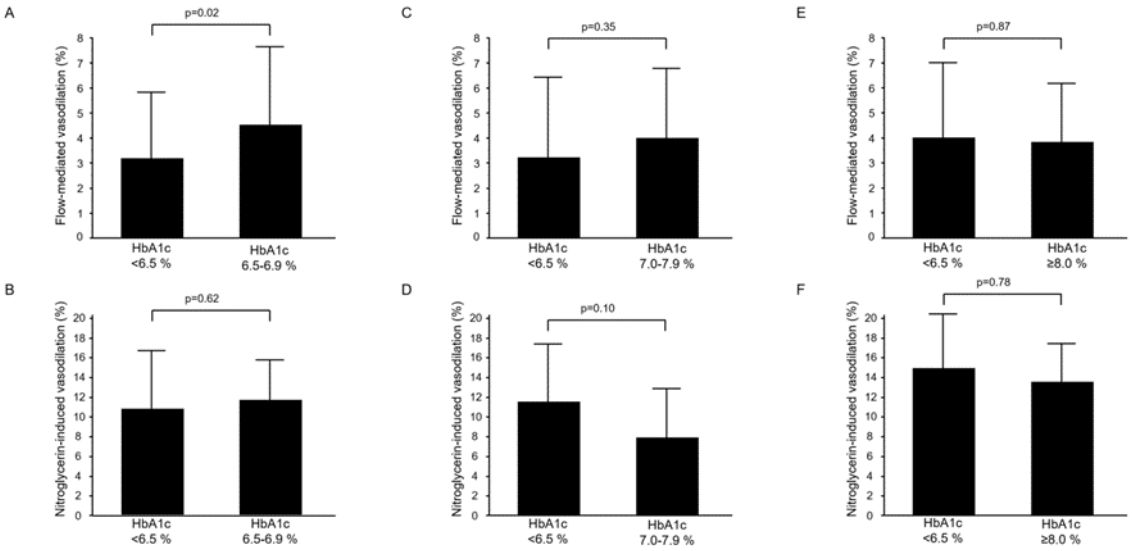

Figure 7. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of 6.5%-6.9% not receiving antidiabetic drug treatment, flow-mediated vasodilation (C) and nitroglycerine-induced vasodilation (D) in patients with HbA1c of <6.5% and patients with HbA1c of 7.0%-7.9% not receiving antidiabetic drug treatment, and flow-mediated vasodilation (E) and nitroglycerine-induced vasodilation (F) in patients with HbA1c of <6.5% and patients with HbA1c of ≥8.0% not receiving antidiabetic drug treatment antidiabetic drugs treatment in a propensity score-matched population.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4-5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-7
		(b) Indicate number of participants with missing data for each variable of interest	5-7
Outcome data	15*	Report numbers of outcome events or summary measures	5-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-8

		(*b*) Report category boundaries when continuous variables were categorized	6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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An Inconvenient Relationship of Hemoglobin A1c Level with Endothelial Function in Type 2 Diabetes in A Cross-sectional Study

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An Inconvenient Relationship of Hemoglobin A1c Level with Endothelial Function in Type 2 Diabetes in A Cross-sectional Study

Short title: Low HbA1c and endothelial function

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Number of tables: 3; Number of figures: 2; Number of references: 33

Abstract

Objectives: The aim of this study was to determine the relationships of 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 levels: <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 the association of FMD with HbA1c and that a low HbA1c level of <6.5% is associated with endothelial dysfunction.

Trial registration number: The protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409)

Strengths and limitations of this study

- The present study showed the relationship between HbA1c and FMD in patients with type 2 diabetes.
- The present study was conducted in multiple centers 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 HbA1c level and FMD.

INTRODUCTION

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. Hemoglobin A1c (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. [5 7,8]

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.[12] FMD is reversible by several interventions such as life-style 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 hyperglycemia 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 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 the FMD-J study has been described in detail previously. [20] The protocol used for measurement of FMD was the same in the FMD-J study and at Hiroshima University Hospital. Exclusion criteria are shown in online supplementary Figure 1. 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. [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 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. All methods were performed in accordance with the relevant guidelines and regulations.

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: <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 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.

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

High-resolution ultrasonography equipment specialized to measure FMD (UNEXEF18G, UNEX Co., Nagoya, Japan) was used to evaluate FMD. Additional details are available in the supplementary methods section. 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 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 (<6.5%, 6.5-6.9%, 7.0-7.9%, 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 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 JMP Pro. Ver 14.0 software (SAS Institute, Cary, NC, USA)

RESULTS

Study 1.

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

The baseline characteristics of the 1215 patients are summarized 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 of <6.5% and those with HbA1c of ≥6.5% are also summarized 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 levels: <6.5%, 6.5-6.9%, 7.0-7.9%, and ≥8.0%. The baseline characteristics are summarized in online supplementary 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 supplementary Figure 2A). There was no significant difference in FMD between the HbA1c <6.5% group and HbA1c ≥8.0% group. (p=0.055; online supplementary Figure 2A). NID values were 10.6±5.9% in the 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 supplementary Figure 2B).

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

Online supplementary Table 2 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; online supplementary Figure 3A). A scatter plot between FMD and HbA1c level with a Lowess smoothed curve is shown in online supplementary 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% 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 <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 <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) (online supplementary 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 of 6.5-6.9%, HbA1c of 7.0-7.9%, and HbA1c of ≥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 supplementary Tables 4, 5, and 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% versus 4.7±3.0%, p=0.002; 3.9±2.6% versus 4.5±2.6%, p=0.04; online supplementary Figures 4A and 4C), while there was no significant difference in FMD between the HbA1c <6.5% group and the HbA1c ≥8.0% group (4.5±2.7% versus 4.1±2.8%, p=0.36; online supplementary Figure 4E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (11.0±6.0% versus 11.2±5.5% in the HbA1c <6.5% group versus the HbA1c 6.5-6.9% group, p=0.84; 10.2±5.8% versus 10.5±5.6% in the HbA1c <6.5% group versus the HbA1c 7.0-7.9% group, p=0.82; 12.8±6.2% versus 11.6±7.2%, p=0.5, in the HbA1c <6.5% group versus the HbA1c ≥8.0% group, p=0.82; online supplementary Figures 4B, 4D, and 4F).

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 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 levels <6.5% and HbA1c levels ≥6.5%

The baseline characteristics of patients with type 2 diabetes not taking antidiabetic drugs who had HbA1c levels <6.5% and HbA1c levels ≥6.5% are summarized in online supplementary 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 supplementary 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 supplementary Figure 5B).

Next, the patients were divided into four groups according to HbA1c levels: <6.5%, 6.5-6.9%, 7.0-7.9%, and ≥8.0%. The baseline characteristics are summarized 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 supplementary Table 8 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 supplementary Figure 6A). Scatter plots between FMD and HbA1c with a Lowess smoothed curve are shown in online supplementary 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, creatine, and presence of hypertension, dyslipidemia and CVD, HbA1c level of <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) (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 of 6.5-6.9%, HbA1c of 7.0-7.9%, and HbA1c of ≥8.0%). Baseline characteristics of matched pairs of the low HbA1c level of <6.5% group and the other three groups are summarized in online supplementary Tables 9, 10, and 11. FMD was significantly smaller in the HbA1c < 6.5% group than in the HbA1c 6.5-6.9% group (3.1±2.7% versus 4.6±3.2%, p=0.02; online supplementary 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% versus 4.0±2.8%, p=0.35; 4.0±3.0% versus 3.8±2.4%, p=0.87; online supplementary Figures 7C and 7E). There were no significant differences in NID between the HbA1c <6.5% group and the other three groups (10.8±5.6% versus 11.7±4.0% in the HbA1c <6.5% group versus the HbA1c 6.5-6.9% group, p=0.62; 11.8±5.7% versus 7.8±4.9% in the HbA1c <6.5% group versus the HbA1c 7.0-7.9% group, p=0.10; 14.8±5.5%

versus 13.6±3.9% in the HbA1c <6.5% group versus the HbA1c ≥8.0% group, p=0.78; online supplementary Figures 7B, 7D, and 7F).

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 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 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 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 is impaired in individuals with cardiovascular risk factors and patients with cardiovascular disease.[24 25] In the present study, although we found that there was an inverted U-shaped pattern of the association of FMD with 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 cardiovascular disease 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. 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.[27] 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 minimize the effect of hypoglycemia, we evaluated the relationship 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 the 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 the 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 CV events are also controversial. [33 34] The UKPDS 73 study showed that the frequency of hypoglycemia in patients not taking antidiabetic drugs was 0.1%. [35] 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. [36 37] It has been postulated that hypoglycemia 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 hypoglycemia contribute to low HbA1c-induced endothelial dysfunction.

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. 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 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, 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 with 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 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.

In conclusions, there is an inverted U-shaped pattern of the association of FMD with 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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Contributors
T.Y. and Y.Higashi, drafting the article and conception of the study; T.H., Y.Hashimoto., Y.T., M.K., Y.Han, T.M., S.K., H.H., C.G., 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.Higashi. 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.

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Competing interest
All authors have no conflicts of interests to report.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or dissemination plans of this research.

Patient consent for publication
Not required.
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Ethics approval
This study protocol was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409). The protocol of this study conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committee of Hiroshima University.

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Data are available on reasonable request. All data generated analysed during the current study are available from the corresponding author on reasonable request.

References

1. Standl E, Balletshofer B, Dahl B, et al. Predictors of 10-year macrovascular and overall mortality in patients with NIDDM: the Munich General Practitioner Project. *Diabetologia* 1996;39(12):1540-5. [published Online First: 1996/12/01]
2. Khaw KT, Wareham N, Bingham S, et al. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. *Ann Intern Med* 2004;141(6):413-20. doi: 10.7326/0003-4819-141-6-200409210-00006 [published Online First: 2004/09/24]

3. Stamler J, Vaccaro O, Neaton JD, et al. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care* 1993;16(2):434-44. doi: 10.2337/diacare.16.2.434 [published Online First: 1993/02/01]

4. Chew EY, Ambrosius WT, Davis MD, et al. Effects of medical therapies on retinopathy progression in type 2 diabetes. *The New England journal of medicine* 2010;363(3):233-44. doi: 10.1056/NEJMoa1001288 [published Online First: 2010/07/01]

5. Patel A, MacMahon S, Chalmers J, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *The New England journal of medicine* 2008;358(24):2560-72. doi: 10.1056/NEJMoa0802987 [published Online First: 2008/06/10]

6. Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. *Diabetes Res Clin Pract* 1995;28(2):103-17. [published Online First: 1995/05/01]

7. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. *The New England journal of medicine* 2009;360(2):129-39. doi: 10.1056/NEJMoa0808431 [published Online First: 2008/12/19]

8. Gerstein HC, Miller ME, Byington RP, et al. Effects of intensive glucose lowering in type 2 diabetes. *The New England journal of medicine* 2008;358(24):2545-59. doi: 10.1056/NEJMoa0802743 [published Online First: 2008/06/10]

9. Higashi Y, Noma K, Yoshizumi M, et al. Endothelial function and oxidative stress in cardiovascular diseases. *Circulation journal : official journal of the Japanese Circulation Society* 2009;73(3):411-8. [published Online First: 2009/02/06]

10. Soga J, Noma K, Hata T, et al. Rho-associated kinase activity, endothelial function, and cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 2011;31(10):2353-9. doi: 10.1161/atvbaha.111.227892 [published Online First: 2011/07/09]

11. Yeboah J, Folsom AR, Burke GL, et al. Predictive value of brachial flow-mediated dilation for incident cardiovascular events in a population-based study: the multi-ethnic study of atherosclerosis. *Circulation* 2009;120(6):502-9. doi: 10.1161/circulationaha.109.864801 [published Online First: 2009/07/29]

12. Yugar-Toledo JC, Tanus-Santos JE, Sabha M, et al. Uncontrolled hypertension, uncompensated type II diabetes, and smoking have different patterns of vascular

dysfunction. *Chest* 2004;125(3):823-30. doi: 10.1378/chest.125.3.823 [published Online First: 2004/03/10]

13. Martens FM, Rabelink TJ, op 't Roodt J, et al. TNF-alpha induces endothelial dysfunction in diabetic adults, an effect reversible by the PPAR-gamma agonist pioglitazone. *Eur Heart J* 2006;27(13):1605-9. doi: 10.1093/eurheartj/ehl079 [published Online First: 2006/06/10]
14. Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther* 2004;102(1):87-96. doi: 10.1016/j.pharmthera.2004.02.003 [published Online First: 2004/04/02]
15. Maruhashi T, Soga J, Fujimura N, et al. Relationship between flow-mediated vasodilation and cardiovascular risk factors in a large community-based study. *Heart* 2013;99(24):1837-42. doi: 10.1136/heartjnl-2013-304739 [published Online First: 2013/10/25]
16. Tacito LHB, Pires AC, Yugar-Toledo JC. Impaired flow-mediated dilation response and carotid intima-media thickness in patients with type 1 diabetes mellitus with a mean disease duration of 4.1 years. *Arch Endocrinol Metab* 2017;61(6):542-49. doi: 10.1590/2359-3997000000281 [published Online First: 2017/07/21]
17. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414(6865):813-20. doi: 10.1038/414813a [published Online First: 2001/12/14]
18. Nakamura K, Oe H, Kihara H, et al. DPP-4 inhibitor and alpha-glucosidase inhibitor equally improve endothelial function in patients with type 2 diabetes: EDGE study. *Cardiovasc Diabetol* 2014;13:110. doi: 10.1186/s12933-014-0110-2 [published Online First: 2014/07/31]
19. Moreno B, de Faria AP, Ritter AMV, et al. Glycated hemoglobin correlates with arterial stiffness and endothelial dysfunction in patients with resistant hypertension and uncontrolled diabetes mellitus. *J Clin Hypertens (Greenwich)* 2018;20(5):910-17. doi: 10.1111/jch.13293 [published Online First: 2018/05/08]
20. Tomiyama H, Kohro T, Higashi Y, et al. A multicenter study design to assess the clinical usefulness of semi-automatic measurement of flow-mediated vasodilatation of the brachial artery. *Int Heart J* 2012;53(3):170-5. [published Online First: 2012/07/14]
21. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood

Cholesterol In Adults (Adult Treatment Panel III). *Jama* 2001;285(19):2486-97. [published Online First: 2001/05/23]

22. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. *Diabetes Care* 2018;41(Suppl 1):S13-s27. doi: 10.2337/dc18-S002 [published Online First: 2017/12/10]

23. Maruhashi T, Soga J, Fujimura N, et al. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilation. *Arterioscler Thromb Vasc Biol* 2013;33(6):1401-8. doi: 10.1161/atvbaha.112.300934 [published Online First: 2013/03/23]

24. Iwamoto Y, Maruhashi T, Kajikawa M, et al. Chronic kidney disease is associated with vascular smooth muscle dysfunction but not with endothelial dysfunction. *Int J Cardiol* 2018;254:284-90. doi: 10.1016/j.ijcard.2017.10.122 [published Online First: 2018/02/07]

25. Matsui S, Kajikawa M, Maruhashi T, et al. Decreased frequency and duration of tooth brushing is a risk factor for endothelial dysfunction. *Int J Cardiol* 2017;241:30-34. doi: 10.1016/j.ijcard.2017.03.049 [published Online First: 2017/03/28]

26. Henry RM, Ferreira I, Kostense PJ, et al. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not; The Hoorn Study. *Atherosclerosis* 2004;174(1):49-56. doi: 10.1016/j.atherosclerosis.2004.01.002 [published Online First: 2004/05/12]

27. Kubota Y, Miyamoto M, Takagi G, et al. The dipeptidyl peptidase-4 inhibitor sitagliptin improves vascular endothelial function in type 2 diabetes. *J Korean Med Sci* 2012;27(11):1364-70. doi: 10.3346/jkms.2012.27.11.1364 [published Online First: 2012/11/21]

28. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Bmj* 2000;321(7258):405-12. doi: 10.1136/bmj.321.7258.405 [published Online First: 2000/08/11]

29. Williams SB, Cusco JA, Roddy MA, et al. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. *Journal of the American College of Cardiology* 1996;27(3):567-74. [published Online First: 1996/03/01]

30. Palta P, Huang ES, Kalyani RR, et al. Hemoglobin A1c and Mortality in Older Adults With and Without Diabetes: Results From the National Health and Nutrition Examination

Surveys (1988-2011). *Diabetes Care* 2017;40(4):453-60. doi: 10.2337/dci16-0042 [published Online First: 2017/02/23]

31. Sluik D, Boeing H, Montonen J, et al. HbA1c measured in stored erythrocytes is positively linearly associated with mortality in individuals with diabetes mellitus. *PLoS One* 2012;7(6):e38877. doi: 10.1371/journal.pone.0038877 [published Online First: 2012/06/22]
32. Arnold LW, Wang Z. The HbA1c and all-cause mortality relationship in patients with type 2 diabetes is J-shaped: a meta-analysis of observational studies. *Rev Diabet Stud* 2014;11(2):138-52. doi: 10.1900/rds.2014.11.138 [published Online First: 2014/11/15]
33. Wan EYF, Fung CSC, Wong CKH, et al. Association of Hemoglobin A1c Levels With Cardiovascular Disease and Mortality in Chinese Patients With Diabetes. *Journal of the American College of Cardiology* 2016;67(4):456-58. doi: 10.1016/j.jacc.2015.11.020 [published Online First: 2016/01/30]
34. Rawshani A, Rawshani A, Franzen S, et al. Risk Factors, Mortality, and Cardiovascular Outcomes in Patients with Type 2 Diabetes. *The New England journal of medicine* 2018;379(7):633-44. doi: 10.1056/NEJMoa1800256 [published Online First: 2018/08/16]
35. Wright AD, Cull CA, Macleod KM, et al. Hypoglycemia in Type 2 diabetic patients randomized to and maintained on monotherapy with diet, sulfonylurea, metformin, or insulin for 6 years from diagnosis: UKPDS73. *J Diabetes Complications* 2006;20(6):395-401. doi: 10.1016/j.jdiacomp.2005.08.010 [published Online First: 2006/10/31]
36. Zoungas S, Patel A, Chalmers J, et al. Severe hypoglycemia and risks of vascular events and death. *The New England journal of medicine* 2010;363(15):1410-8. doi: 10.1056/NEJMoa1003795 [published Online First: 2010/10/12]
37. Mellbin LG, Ryden L, Riddle MC, et al. Does hypoglycaemia increase the risk of cardiovascular events? A report from the ORIGIN trial. *Eur Heart J* 2013;34(40):3137-44. doi: 10.1093/eurheartj/eht332 [published Online First: 2013/09/04]
38. Fisher BM, Gillen G, Hepburn DA, et al. Cardiac responses to acute insulin-induced hypoglycemia in humans. *Am J Physiol* 1990;258(6 Pt 2):H1775-9. doi: 10.1152/ajpheart.1990.258.6.H1775 [published Online First: 1990/06/01]

39. Wright RJ, Frier BM. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes Metab Res Rev* 2008;24(5):353-63. doi: 10.1002/dmrr.865 [published Online First: 2008/05/08]

40. Sasaki S, Higashi Y, Nakagawa K, et al. A low-calorie diet improves endothelium-dependent vasodilation in obese patients with essential hypertension. *Am J Hypertens* 2002;15(4 Pt 1):302-9. doi: 10.1016/s0895-7061(01)02322-6 [published Online First: 2002/05/07]

41. Higashi Y, Sasaki S, Sasaki N, et al. Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension. *Hypertension (Dallas, Tex : 1979)* 1999;33(1 Pt 2):591-7. doi: 10.1161/01.hyp.33.1.591 [published Online First: 1999/02/04]

42. Goto C, Higashi Y, Kimura M, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. *Circulation* 2003;108(5):530-5. doi: 10.1161/01.Cir.0000080893.55729.28 [published Online First: 2003/07/23]

Figure Legends

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

Figure 2. 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.

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, 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, 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
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 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, yr	61±10	66±10	60±10	61±9	57±10	<0.001
Gender, men/women	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, mmHg	133±17	128±18	133±16	136±16	138±19	0.004
Diastolic blood pressure, mmHg	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
Hemoglobin A1c, %	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
Dyslipidemia	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)					

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 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 (1.80-5.14)	<0.001	1.33 (0.54-3.31)	0.53
Model 2	2.49 (1.44-4.33)	0.001	1.20 (0.46-3.13)	0.71
Model 3	2.57 (1.45-4.54)	0.001	1.29 (0.43-3.91)	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, creatine, presence of hypertension, dyslipidemia and CVD
FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation; OR, odds ratio; CI, confidence interval; CVD, cardiovascular disease.
Low quartile of FMD indicates less than 2.1%. Low quartile of NID indicates less than 6.2%.

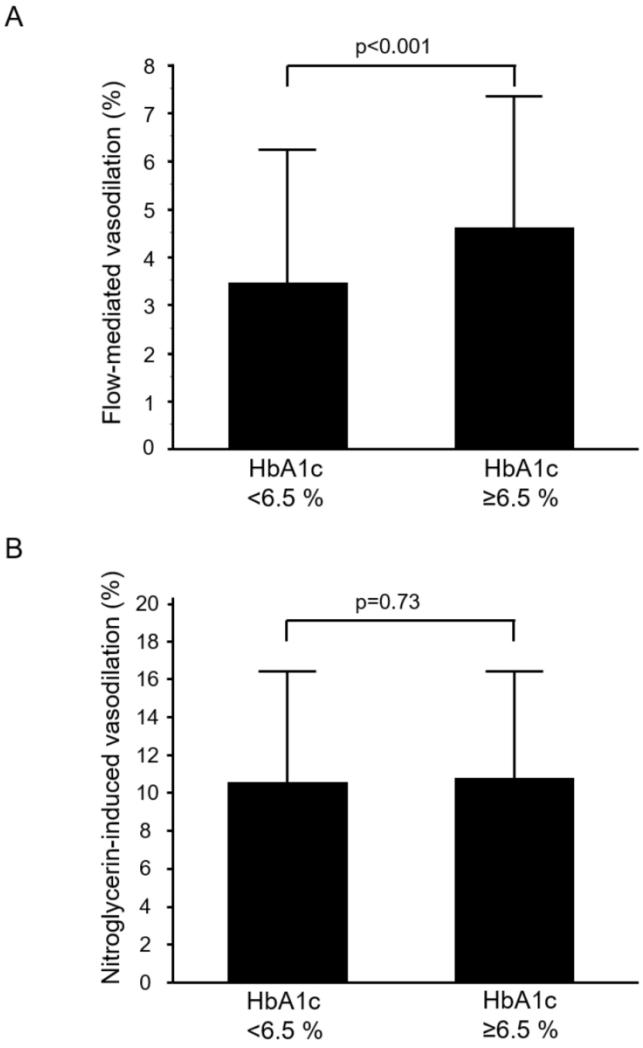

Figure 1

127x203mm (300 x 300 DPI)

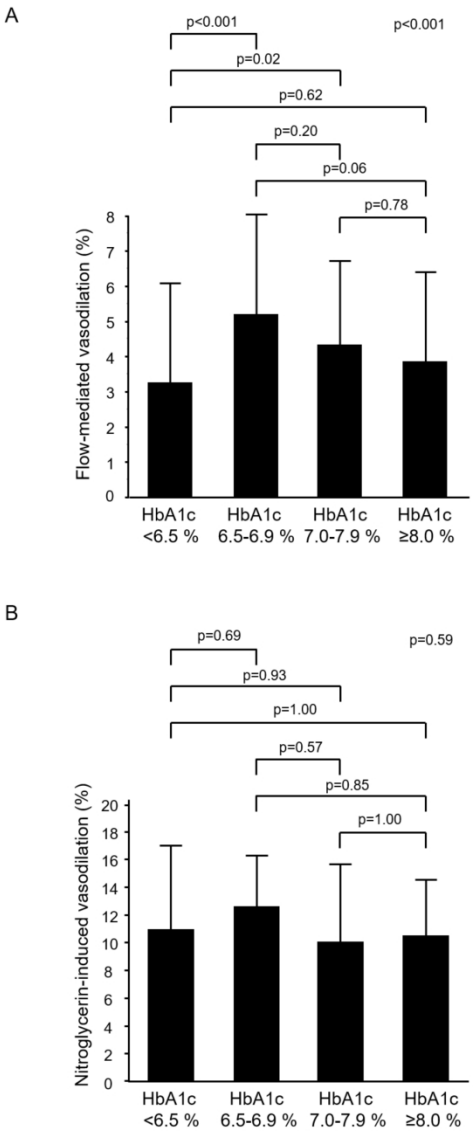

Figure 2

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Supplementary Material

An Inconvenient Relationship of Hemoglobin A1c Level with Endothelial Function in Type 2 Diabetes in A Cross-sectional Study

Short title: Low HbA1c and endothelial function

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Supplementary Methods

Measurement of FMD and NID

Subjects fasted the previous night and abstained from alcohol, smoking, caffeine, antioxidant vitamins, and all medications including hypertensive drugs, lipid-lowering drugs and antidiabetic drugs on the day of the FMD and NID examinations, and only drinking water was given to the subjects. The subjects were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature of 23°C to 26°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the left deep antecubital vein to obtain blood samples. At least 20 min after maintaining the supine position, baseline brachial artery diameter was measured. Then FMD and NID were measured. The observers were blind to the form of examination.

A blood pressure cuff was placed around the forearm of each subjects. The brachial artery was scanned longitudinally 5 to 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 seconds after cuff deflation. 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 seconds, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 minutes. 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 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. After acquiring baseline rest images for 30 seconds, a sublingual tablet (nitroglycerine, 75 μg) was given and imaging of the artery was done continuously for 5 minutes. NID was automatically calculated as a percentage change in peak vessel diameter from the baseline. Percentage of NID [(Peak diameter-Baseline diameter)/Baseline diameter] was used for analysis.

Online Supplementary Tables

Table 1. Clinical Characteristics of Patients with Type 2 Diabetes in Four Groups on the Basis on HbA1c Level

Variables	Total (n=1215)	HbA1c <6.5% (n=474)	HbA1c 6.5-6.9% (n=333)	HbA1c 7.0-7.9% (n=272)	HbA1c ≥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, mg/dL	188±37	180±33	194±35	186±38	202±46	<0.001
Triglycerides, mg/dL	148±109	130±81	147±111	164±130	177±133	<0.001
HDL-C, mg/dL	54±15	57±16	55±15	51±14	51±14	<0.001
LDL-C, mg/dL	107±32	101±29	112±31	106±32	118±39	<0.001
Creatinine, mg/dL	0.84±0.29	0.86±0.31	0.82±0.24	0.84±0.30	0.83±0.31	0.26
Uric acid, mg/dL	5.7±1.4	5.8±1.4	5.8±1.4	5.6±1.4	5.3±1.4	<0.001
Glucose, mg/dL	138±46	119±27	130±26	149±37	202±78	<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 2. Univariate Analysis of Relationships among FMD, HbA1c Level and Variables

Variables	FMD		HbA1c	
	r	P value	r	P value
Age, yr	-0.30	<0.001	-0.21	<0.001
Body mass index, kg/m^2	0.01	0.63	0.07	0.01
Heart rate, bpm	-0.03	0.27	-0.01	0.73
Systolic blood pressure, mmHg	0.04	0.13	0.13	<0.001
Diastolic blood pressure, mmHg	0.17	<0.001	0.14	<0.001
Total cholesterol, mg/dL	0.03	0.33	0.18	<0.001
Triglycerides, mg/dL	-0.04	0.15	0.18	<0.001
HDL-C, mg/dL	-0.01	0.6	-0.14	<0.001
LDL-C, mg/dL	0.04	0.2	0.16	<0.001
Creatinine, mg/dL	-0.09	0.002	0.004	0.88
Uric acid, mg/dL	-0.01	0.62	-0.11	<0.001
Glucose, mg/dL	-0.02	0.44	0.57	<0.001
HbA1c, %	0.08	0.004		
FMD, %			0.08	0.004
NID, %	0.33	<0.001	-0.02	0.70

HDL-C indicates high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FMD, flow-mediated vasodilation; NID, nitroglycerine-induced vasodilation.
Univariate analysis of the relations among FMD, HbA1c levels and variables (Pearson's correlation analysis).

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 Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 6.5-6.9%

Variables	HbA1c <6.5% (n=238)	HbA1c 6.5-6.9% (n=238)	P value
Age, yr	63±10	63±10	0.94
Gender, men/women	171/67	162/76	0.37
Body mass index, kg/m^2	25.2±4.2	25.3±4.1	0.75
Heart rate, bpm	67±11	68±11	0.6
Systolic blood pressure, mmHg	132±17	132±16	0.92
Diastolic blood pressure, mmHg	78±10	78±11	0.96
Total cholesterol, mg/dL	188±33	189±34	0.63
Triglycerides, mg/dL	126±60	130±69	0.51
HDL-C, mg/dL	56±14	56±16	0.84
LDL-C, mg/dL	108±29	109±30	0.94
Creatinine, mg/dL	0.82±0.21	0.82±0.26	0.58
Uric acid, mg/dL	5.8±1.3	5.8±1.4	0.95
Glucose, mg/dL	119±26	130±26	<0.001
Hemoglobin A1c, %	5.9±0.4	6.7±0.1	<0.001
Medical history, n (%)			
Hypertension	191 (80.3)	192 (80.7)	0.91
Dyslipidemia	174 (73.1)	183 (76.9)	0.34
CVD, n (%)	76 (31.9)	76 (31.9)	1.00
Current Smoking, n (%)	53 (22.3)	56 (23.5)	0.74
Medication, n (%)			
Antihypertensive drugs	39 (79.6)	36 (73.4)	0.47
Lipid-lowering drugs	131 (55.0)	135 (56.7)	0.71
Antidiabetic drugs	186 (78.2)	140 (58.8)	<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 5. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 7.0-7.9%

Variables	HbA1c <6.5% (n=192)	HbA1c 7.0-7.9% (n=192)	P value
Age, yr	63±10	63±9	0.96
Gender, men/women	163/29	151/41	0.11
Body mass index, kg/m^2	25.3±3.9	25.4±4.2	0.80
Heart rate, bpm	67±11	67±12	0.99
Systolic blood pressure, mmHg	133±18	133±16	0.94
Diastolic blood pressure, mmHg	79±11	79±10	0.74
Total cholesterol, mg/dL	178±33	181±37	0.36
Triglycerides, mg/dL	135±76	132±84	0.72
HDL-C, mg/dL	52±13	53±15	0.54
LDL-C, mg/dL	101±29	104±32	0.29
Creatinine, mg/dL	0.88±0.30	0.84±0.29	0.16
Uric acid, mg/dL	5.8±1.4	5.7±1.4	0.32
Glucose, mg/dL	118±21	146±36	<0.001
Hemoglobin A1c, %	5.9±0.4	7.3±0.3	<0.001
Medical history, n (%)			
Hypertension	162 (84.4)	158 (82.3)	0.58
Dyslipidemia	150 (78.1)	157 (81.8)	0.37
CVD, n (%)	75 (39.1)	77 (40.1)	0.84
Current Smoking, n (%)	49 (25.5)	45 (23.4)	0.64
Medication, n (%)			
Antihypertensive drugs	138 (71.9)	135 (70.3)	0.74
Lipid-lowering drugs	111 (57.8)	117 (60.9)	0.53
Antidiabetic drugs	159 (82.8)	146 (76.0)	0.10

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 6. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of ≥8.0 %

Variables	HbA1c <6.5% (n=93)	HbA1c ≥8.0% (n=93)	P value
Age, yr	58±11	58±10	0.78
Gender, men/women	68/25	71/22	0.61
Body mass index, kg/m^2	24.9±4.9	25.1±4.1	0.78
Heart rate, bpm	68±12	67±12	0.76
Systolic blood pressure, mmHg	136±18	134±17	0.61
Diastolic blood pressure, mmHg	80±11	80±13	0.86
Total cholesterol, mg/dL	193±40	190±39	0.58
Triglycerides, mg/dL	129±59	139±72	0.28
HDL-C, mg/dL	54±14	53±15	0.67
LDL-C, mg/dL	114±34	110±35	0.41
Creatinine, mg/dL	0.85±0.36	0.85±0.33	1.00
Uric acid, mg/dL	5.3±1.2	5.4±1.1	0.88
Glucose, mg/dL	118±22	189±72	<0.001
Hemoglobin A1c, %	5.9±0.4	9.0±1.2	<0.001
Medical history, n (%)			
Hypertension	62 (66.7)	65 (69.9)	0.64
Dyslipidemia	65 (69.9)	67 (72.0)	0.75
CVD, n (%)	33 (35.5)	39 (41.9)	0.37
Current Smoking, n(%)	25 (26.9)	27 (29.0)	0.74
Medication, n (%)			
Antihypertensive drugs	54 (58.1)	59 (63.4)	0.45
Lipid-lowering drugs	43 (46.2)	43 (46.2)	1.00
Antidiabetic drugs	80 (86.0)	73 (78.5)	0.18

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

Variables	Total (n=349)	HbA1c <6.5% (n=101)	HbA1c ≥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, mg/dL	199±39	186±33	204±40	<0.001
Triglycerides, mg/dL	169±139	133±82	183±154	0.002
HDL-C, mg/dL	54±15	57±15	52±15	0.005
LDL-C, mg/dL	116±32	110±30	119±32	0.02
Creatinine, mg/dL	0.8±0.3	0.85±0.37	0.79±0.20	0.07
Uric acid, mg/dL	5.8±1.5	6.0±1.7	5.7±1.5	0.10
Glucose, mg/dL	137±46	119±28	143±50	<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.

Table 8. Univariate Analysis of Relationships among FMD, HbA1c Level and Variables in Patients with Type 2 Diabetes Not Taking Antidiabetic Drugs

Variables	FMD		HbA1c	
	r	P value	r	P value
Age, yr	-0.24	<0.001	-0.2	<0.001
Body mass index, kg/m^2	0.09	0.08	0.06	0.28
Heart rate, bpm	0.05	0.33	-0.01	0.82
Systolic blood pressure, mmHg	0.10	0.048	0.17	0.001
Diastolic blood pressure, mmHg	0.19	<0.001	0.12	0.02
Total cholesterol, mg/dL	0.02	0.66	0.22	<0.001
Triglycerides, mg/dL	-0.02	0.64	0.23	<0.001
HDL-C, mg/dL	-0.05	0.30	-0.19	<0.001
LDL-C, mg/dL	0.03	0.59	0.14	0.01
Creatinine, mg/dL	-0.03	0.62	-0.07	0.20
Uric acid, mg/dL	0.08	0.15	-0.10	0.07
Glucose, mg/dL	-0.08	0.12	0.70	<0.001
HbA1c, %	0.05	0.40		
FMD, %			0.05	0.40
NID, %	0.36	<0.0001	-0.02	0.78

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

Table 9. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 6.5-6.9%

Variables	HbA1c <6.5% (n=49)	HbA1c 6.5-6.9% (n=49)	P value
Age, yr	64±9	66±7	0.29
Gender, men/women	29/20	26/23	0.54
Body mass index, kg/m^2	25.3±3.7	24.8±3.6	0.51
Heart rate, bpm	69±9	68±10	0.5
Systolic blood pressure, mmHg	131±17	129±16	0.46
Diastolic blood pressure, mmHg	78±11	76±9	0.30
Total cholesterol, mg/dL	189±35	190±28	0.95
Triglycerides, mg/dL	126±48	132±86	0.66
HDL-C, mg/dL	56±13	58±16	0.47
LDL-C, mg/dL	112±30	108±24	0.52
Creatinine, mg/dL	0.79±0.22	0.76±0.18	0.49
Uric acid, mg/dL	5.6±1.2	5.3±1.3	0.32
Glucose, mg/dL	117±18	124±22	0.14
Hemoglobin A1c, %	5.8±0.4	6.7±0.2	<0.001
Medical history, n (%)			
Hypertension	39 (79.6)	39 (79.6)	1.00
Dyslipidemia	37 (75.5)	40 (81.6)	0.46
CVD, n (%)	12 (24.5)	12 (24.5)	1.00
Medication, n (%)			
Antihypertensive drugs	39 (79.6)	36 (73.4)	0.47
Lipid-lowering drugs	29 (59.2)	30 (61.2)	0.84
Antidiabetic drugs	0 (0)	0 (0)	
Current Smoking, n (%)	12 (24.5)	10 (20.4)	0.63

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 10. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of 7.0-7.9%

Variables	HbA1c <6.5% (n=29)	HbA1c 7.0-7.9% (n=29)	P value
Age, yr	63±10	65±8	0.37
Gender, men/women	19/10	20/9	0.78
Body mass index, kg/m^2	24.9±3.8	24.8±3.9	0.91
Heart rate, bpm	70±10	68±11	0.4
Systolic blood pressure, mmHg	133±17	134±14	0.74
Diastolic blood pressure, mmHg	80±12	81±8	0.78
Total cholesterol, mg/dL	189±31	183±39	0.51
Triglycerides, mg/dL	168±116	146±143	0.51
HDL-C, mg/dL	49±12	51±15	0.47
LDL-C, mg/dL	114±28	108±32	0.48
Creatinine, mg/dL	0.80±0.24	0.78±0.22	0.68
Uric acid, mg/dL	5.7±1.4	5.4±1.5	0.40
Glucose, mg/dL	119±23	136±32	0.02
Hemoglobin A1c, %	5.8±0.4	7.3±0.3	<0.001
Medical history, n (%)			
Hypertension	25 (86.2)	27 (93.1)	0.39
Dyslipidemia	23 (79.3)	23 (79.3)	1.00
CVD, n (%)	8 (27.6)	9 (31.0)	0.77
Current Smoking, n (%)	8 (27.6)	5 (17.2)	0.34
Medication, n (%)			
Antihypertensive drugs	24 (82.7)	19 (65.5)	0.13
Lipid-lowering drugs	18 (62.1)	15 (51.7)	0.43
Antidiabetic drugs	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.

Table 11. Clinical Characteristics of Type 2 Diabetes Patients with HbA1c of <6.5% and with HbA1c of ≥8.0%

Variables	HbA1c <6.5% (n=20)	HbA1c ≥8.0% (n=20)	P value
Age, yr	59±11	58±11	0.82
Gender, men/women	15/5	16/4	0.71
Body mass index, kg/m^2	24.9±4.5	25.4±4.6	0.71
Heart rate, bpm	72±10	67±8	0.11
Systolic blood pressure, mmHg	138±20	136±21	0.80
Diastolic blood pressure, mmHg	84±14	82±11	0.65
Total cholesterol, mg/dL	199±40	213±40	0.31
Triglycerides, mg/dL	149±73	203±142	0.13
HDL-C, mg/dL	56±16	48±14	0.10
LDL-C, mg/dL	126±38	125±32	0.89
Creatinine, mg/dL	0.76±0.16	0.85±0.34	0.30
Uric acid, mg/dL	5.8±1.4	6.0±1.7	0.67
Glucose, mg/dL	116±17	212±63	<0.001
Hemoglobin A1c, %	6.0±0.4	9.4±1.2	<0.001
Medical history, n (%)			
Hypertension	14 (70.0)	15 (75.0)	0.72
Dyslipidemia	13 (65.0)	16 (80.0)	0.29
CVD, n (%)	5 (25.0)	3 (15.0)	0.43
Medication, n (%)			
Antihypertensive drugs	12 (60.0)	11 (55.0)	0.75
Lipid-lowering drugs	5 (25.0)	4 (20.0)	0.71
Antidiabetic drugs	0 (0)	0 (0)	
Current Smoking, n (%)	6 (30.0)	4 (20.0)	0.47

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

Online Supplementary Figures

Figure 1

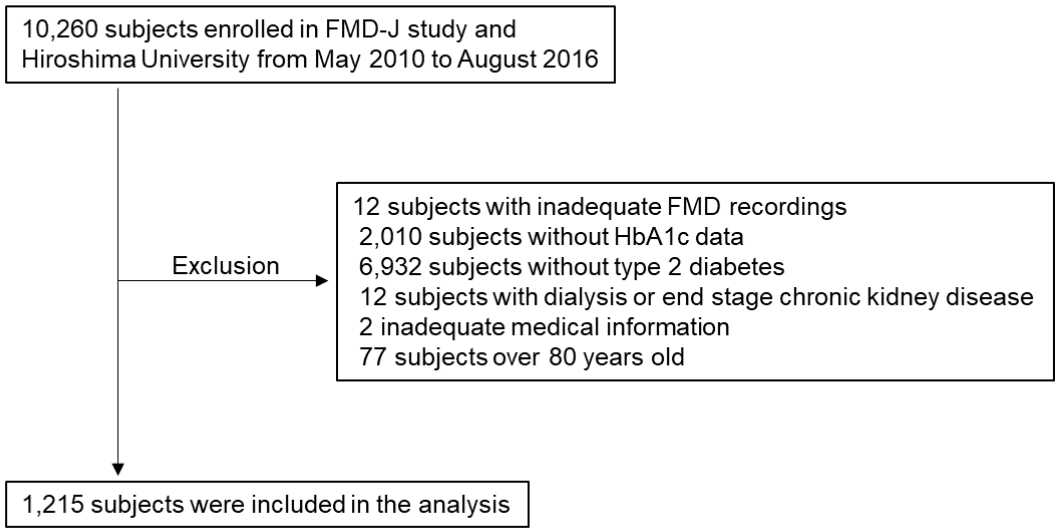

Figure 1. Flow chart of the study design.

Figure 2

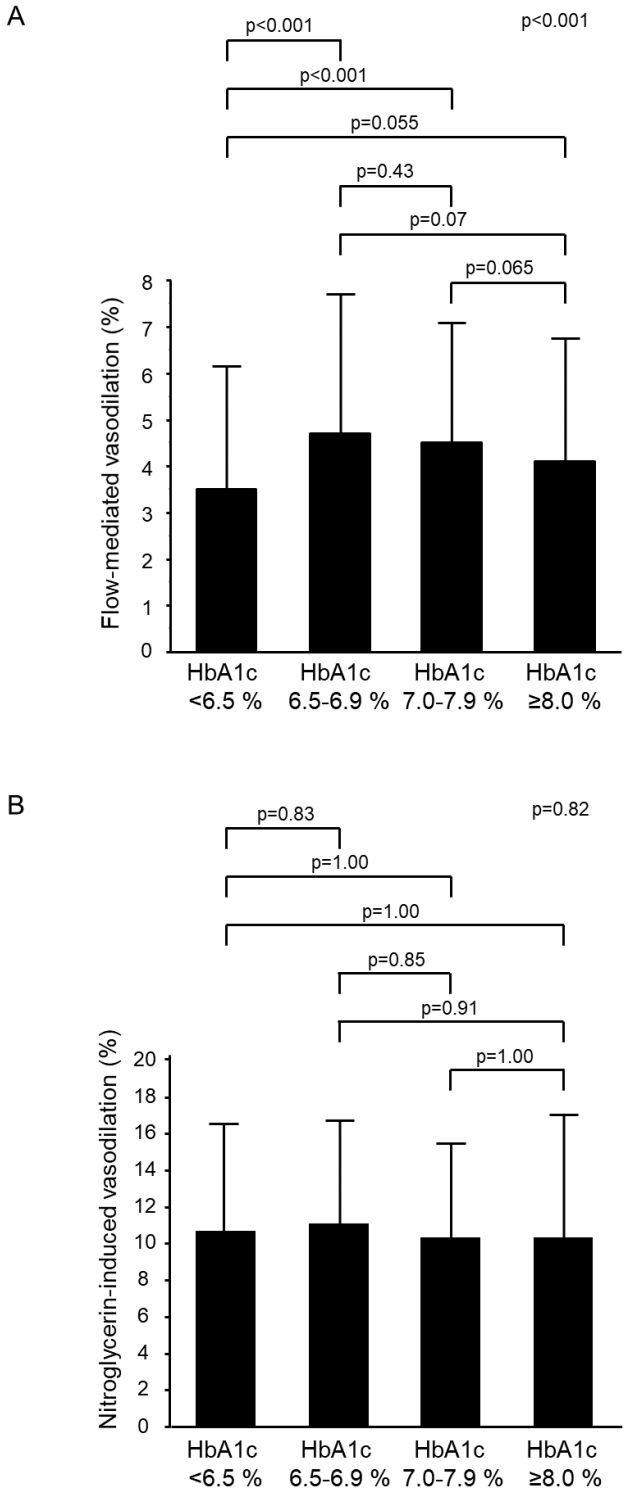

Figure 2. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in 4 groups according to HbA1c levels.

Figure 3

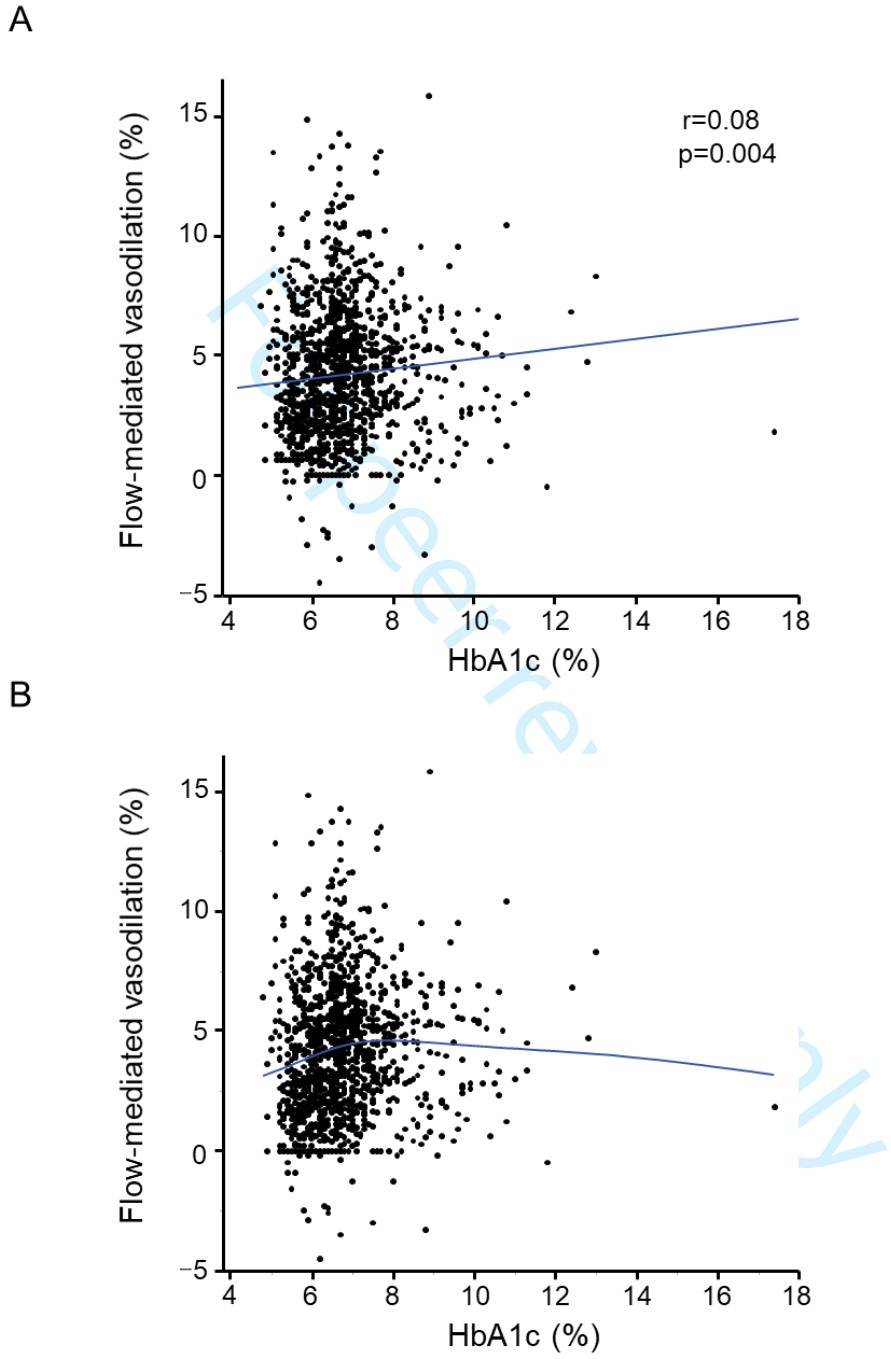

Figure 3. Scatter plots show the relationship between flow-mediated vasodilation and serum HbA1c levels in all patients (A) and locally weighted regression smoothing (Lowess) plot (B)

Figure 4

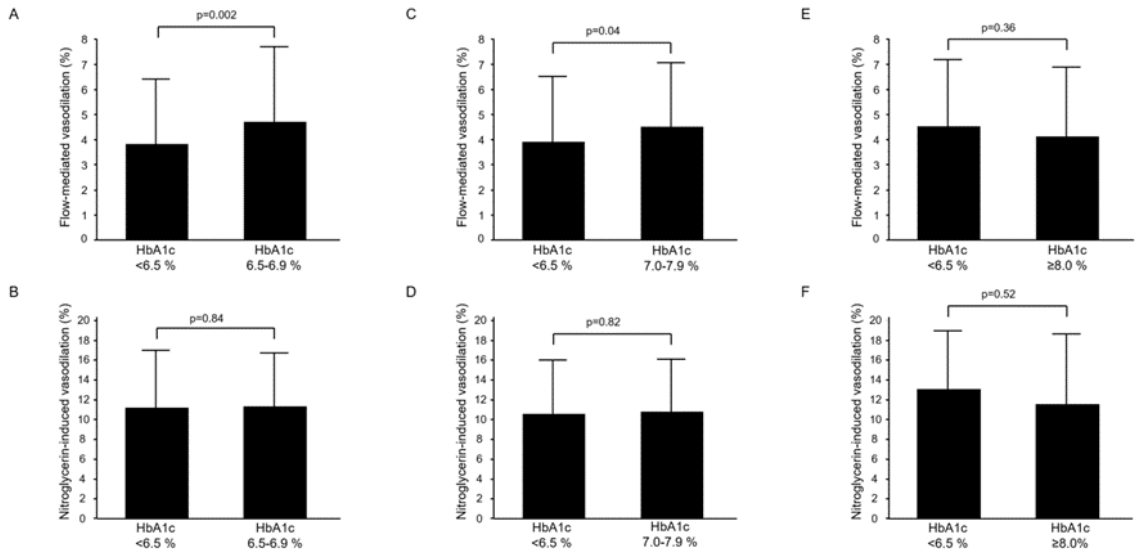

Figure 4. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of 6.5%-6.9%, flow-mediated vasodilation (C) and nitroglycerine-induced vasodilation (D) in patients with HbA1c of <6.5% and patients with HbA1c of 7.0%-7.9%, and flow-mediated vasodilation (E) and nitroglycerine-induced vasodilation (F) in patients with HbA1c of <6.5% and patients with HbA1c of ≥8.0% in a propensity score-matched population.

Figure 5

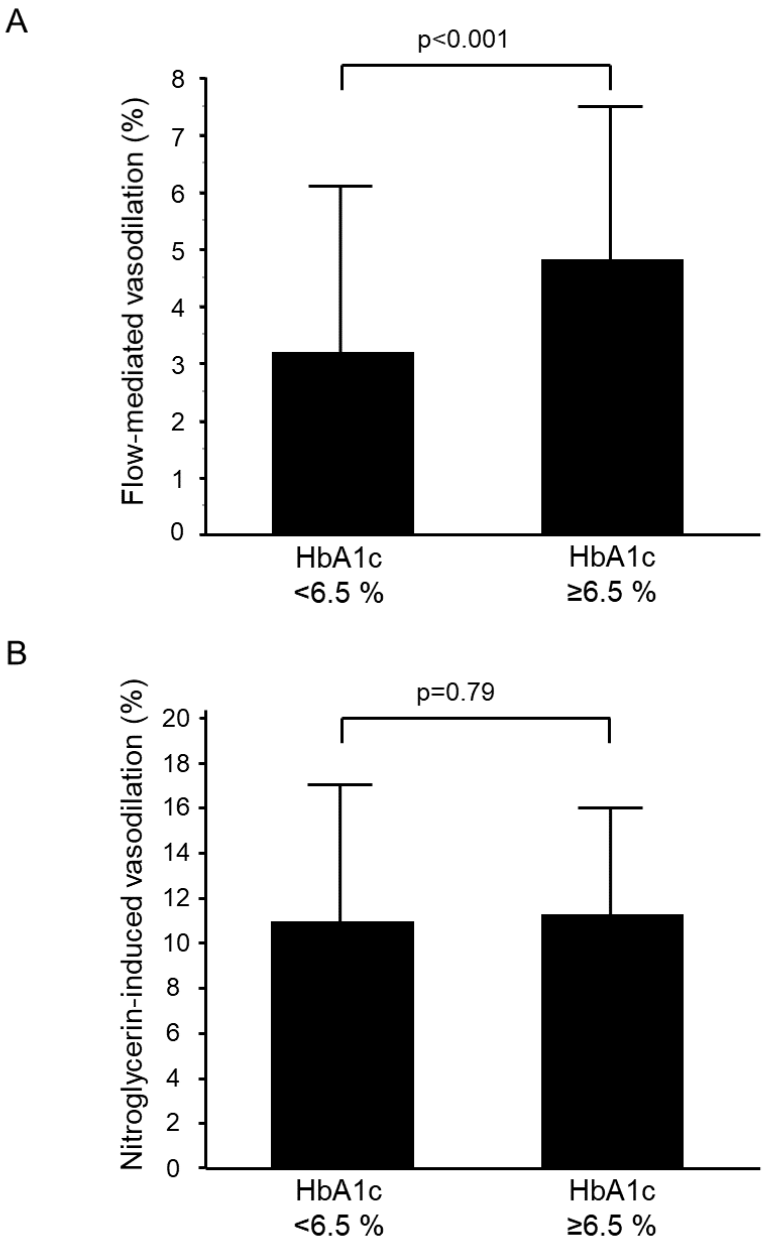

Figure 5. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of ≥6.5% who were not receiving antidiabetic drug treatment.

Figure 6

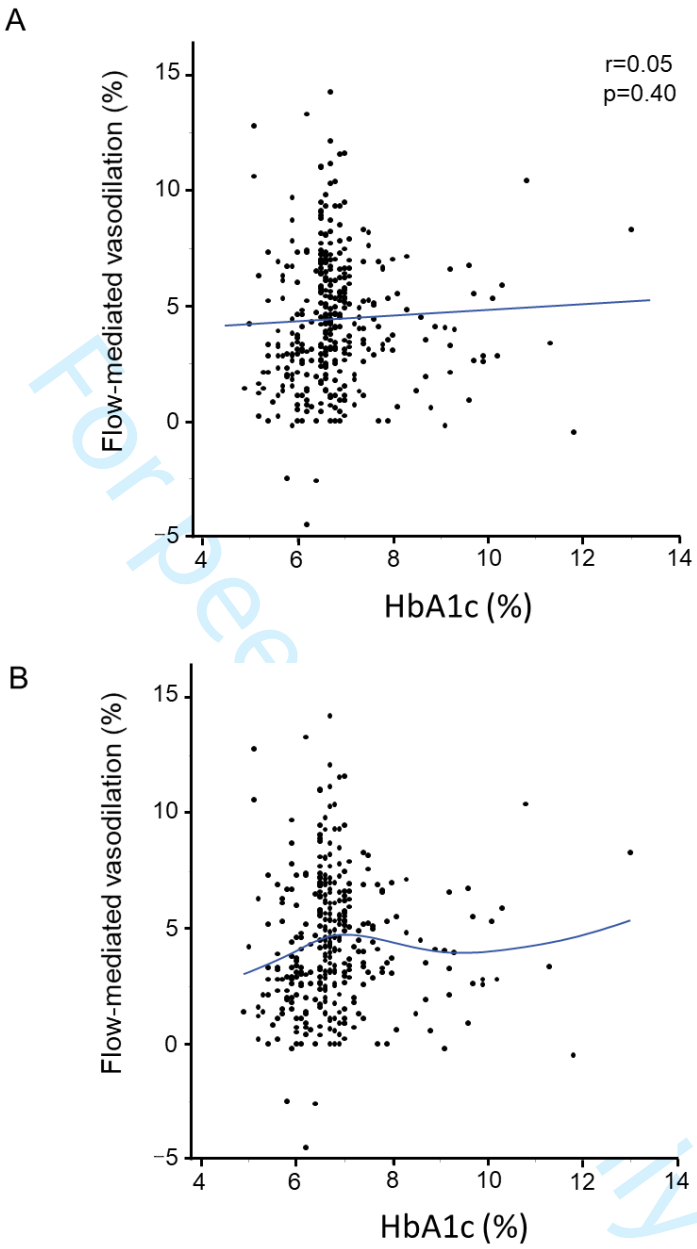

Figure 6. Scatter plots show the relationship between flow-mediated vasodilation and serum HbA1c levels in patients not receiving antidiabetic drug treatment (A) and locally weighted regression smoothing (Lowess) plot (B）.

Figure 7

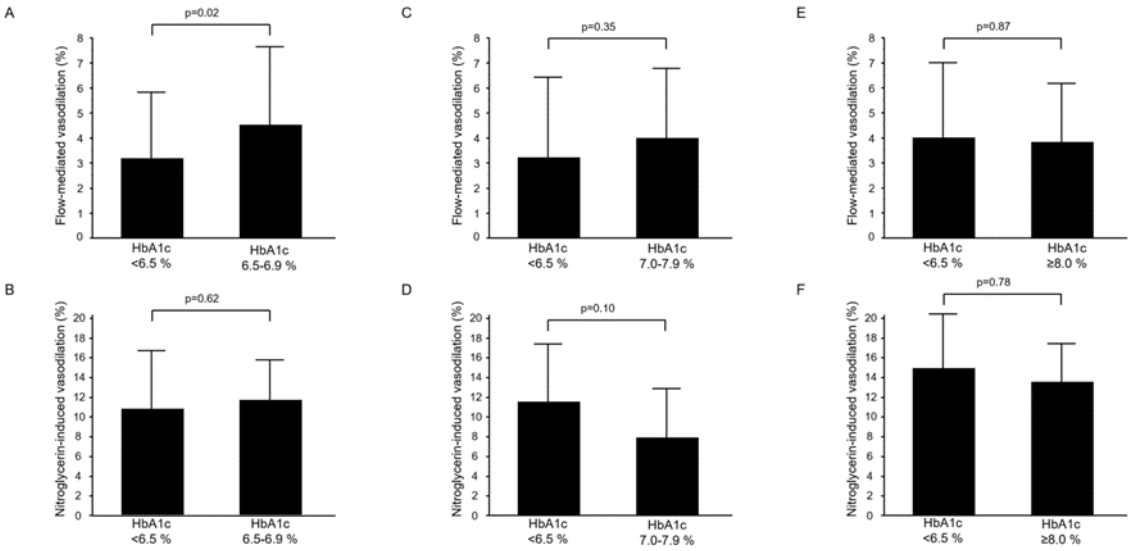

Figure 7. Bar graphs show flow-mediated vasodilation (A) and nitroglycerine-induced vasodilation (B) in patients with HbA1c of <6.5% and patients with HbA1c of 6.5%-6.9% not receiving antidiabetic drug treatment, flow-mediated vasodilation (C) and nitroglycerine-induced vasodilation (D) in patients with HbA1c of <6.5% and patients with HbA1c of 7.0%-7.9% not receiving antidiabetic drug treatment, and flow-mediated vasodilation (E) and nitroglycerine-induced vasodilation (F) in patients with HbA1c of <6.5% and patients with HbA1c of ≥8.0% not receiving antidiabetic drug treatment antidiabetic drugs treatment in a propensity score-matched population.

STROBE Statement—Checklist of items that should be included in reports of ***cross-sectional studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3
Objectives	3	State specific objectives, including any prespecified hypotheses	3-4
Methods			
Study design	4	Present key elements of study design early in the paper	3-4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants	4
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4
Bias	9	Describe any efforts to address potential sources of bias	4
Study size	10	Explain how the study size was arrived at	4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4
		(*b*) Describe any methods used to examine subgroups and interactions	4
		(*c*) Explain how missing data were addressed	4
		(*d*) If applicable, describe analytical methods taking account of sampling strategy	4
		(*e*) Describe any sensitivity analyses	4-5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5-6
		(b) Give reasons for non-participation at each stage	5-6
		(c) Consider use of a flow diagram	5-6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5-7
		(b) Indicate number of participants with missing data for each variable of interest	5-7
Outcome data	15*	Report numbers of outcome events or summary measures	5-8
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	5-8

		(*b*) Report category boundaries when continuous variables were categorized	6-8
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6-8
Discussion			
Key results	18	Summarise key results with reference to study objectives	8-9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9-10
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	9-10
Generalisability	21	Discuss the generalisability (external validity) of the study results	9-10
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	11

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.