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

Takayuki Yamaji, Takahiro Harada, Yu Hashimoto, Yuji Takaeko, Masato Kajikawa, Yiming Han, Tatsuya Maruhashi, Shinji Kishimoto, Haruki Hashimoto, Yasuki Kihara, Eisuke Hida, Kazuaki Chayama, Chikara Goto, Farina Mohamad Yusoff, Ayumu Nakashima, Yukihito Higashi

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

Design Cross-sectional study.

Setting 22 university hospitals and affiliated clinics in Japan.

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

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

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

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

Trial registration number UMIN000012950, UMIN000012951, UMIN000012952 and UMIN000003409.

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

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

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

Diabetes is associated with endothelial dysfunction.\textsuperscript{15,16} Chronic hyperglycaemia is a major contributor to increased oxidative stress and causes endothelial dysfunction through inactivation of nitric oxide.\textsuperscript{17} Several studies have shown that endothelial function is improved by antidiabetic therapy, including use of antidiabetic drugs.\textsuperscript{13,15,16} 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 10,260 subjects (7,385 patients from the Flow-mediated Dilation-Japan (FMD-J) study and 2,875 patients who underwent a health check-up at Hiroshima University Hospital between August 2007 and August 2016) were recruited in this study. The FMD-J study was a prospective multicentre registry. The design of the FMD-J study has been described in detail previously.\textsuperscript{20} The protocol used for measurement of FMD was the same as in the FMD-J study and at Hiroshima University Hospital. Exclusion criteria are shown in online supplemental figure 1. Finally, we enrolled 12,151 subjects in this study. Hypertension was defined as use of antihypertensive drugs or systolic blood pressure of more than 140 mm Hg or diastolic blood pressure of more than 90 mm Hg measured in a sitting position on at least three occasions. Dyslipidaemia was defined according to the third report of the National Cholesterol Education Program.\textsuperscript{21} Diabetes was defined according to the American Diabetes Association recommendation.\textsuperscript{22} Smokers were defined as those who were current smokers. CVD was defined as coronary heart disease and cerebrovascular disease. Coronary heart disease included angina pectoris, prior myocardial infarction and unstable angina. Cerebrovascular disease included ischaemic stroke, haemorrhagic stroke and transient ischaemic attack. Written informed consent for participation in this study was obtained from all participants. All methods were performed in accordance with the relevant guidelines and regulations.

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

In study 1, we assessed the relationships between HbA1c level and vascular function as assessed by measurement of FMD, an index of endothelium-dependent vasodilation, and by measurement of nitroglycerine-induced vasodilation (NID), an index of endothelium-independent vasodilation, in 1,215 patients with type 2 diabetes. First, we divided the patients into two groups based on their HbA1c level: <6.5% and ≥6.5%. Multivariate regression analysis was performed to identify independent variables associated with vascular function. Next, we divided the patients into four groups according to HbA1c level: <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%. We next assessed the relationships of HbA1c levels with FMD and NID using propensity score matching.

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

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

**Measurements of FMD and NID**

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

**Statistical analysis**

Results are presented as mean±SD. All reported probability values were two-sided and a probability value of <0.05 was considered statistically significant. An association between FMD and HbA1c level was explored visually using a locally weighted regression smoothing (Lowess) plot. Categorical values were compared by means of $\chi^2$ test. Continuous variables were compared using analysis of variance (ANOVA) multiple groups. Comparisons between the groups categorised according to HbA1c level were carried out using repeated measures ANOVA with Tukey’s post-hoc test. Univariate linear regression analyses were performed to assess the relationships among the variables. Multivariate logistic regression analysis was performed to identify independent variables associated with lower quartiles of FMD (<2.1%) and NID (<0.2%). Age, gender, body mass index (BMI), creatinine levels, current smoking, and the presence of hypertension, dyslipidaemia and CVD were entered into the multivariate logistic regression analysis. As a sensitivity analysis, propensity score analysis was used to minimise the selection bias for evaluation of the relationship between HbA1c level and vascular function. The propensity score was calculated for each patient on the basis of logistic regression analysis of the probability of not taking antidiabetic drugs within groups stratified by HbA1c level (<6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%) using clinical
variables including age, sex, BMI, systolic blood pressure, diastolic blood pressure, heart rate, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, uric acid levels, current smoking (yes or no), medication with antihypertensive drugs (yes or no), medication with lipid-lowering drugs (yes or no) and presence of CVD (yes or no). With these propensity scores using a calliper width of 0.25 SD of the logit of the propensity score, two well-matched groups based on clinical characteristics were created for comparison of the prevalence of endothelial dysfunction defined as FMD of <2.1%, the division point for the lowest quartile of FMD in all participants. All data were processed using JMP Pro V.14.0 software (SAS Institute, Cary, North Carolina, USA).

RESULTS

Study 1

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

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

Next, the patients were divided into four groups based on HbA1c level: <6.5%, 6.5%–6.9%, 7.0%–7.9% and ≥8.0%. The baseline characteristics are summarised in online supplemental table 1. FMD values were 3.5%±2.7% in the HbA1c <6.5% group, 4.8%±2.9% in the HbA1c 6.5%–6.9% group, 4.5%±2.6% in the HbA1c 7.0%–7.9% group, and 4.2%±2.7% in the HbA1c ≥8.0% group (p<0.001). FMD was significantly smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% group and HbA1c 7.0%–7.9% group (p<0.001 and p<0.001, respectively; online supplemental figure 2A). There was no significant difference in FMD between the HbA1c <6.5% group and HbA1c ≥8.0% group (p=0.055; online supplemental figure 2A). NID values were 10.6%±5.9% in the
HbA1c <6.5% group, 11.2±5.4% in the HbA1c 6.5%–6.9% group, 10.4±5.2% in the HbA1c 7.0%–7.9% group, and 10.4±6.8% in the HbA1c ≥8.0% group. There were no significant differences in NID values among the four groups (p=0.82; online supplemental figure 2B).

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

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

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

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

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

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

Study 2

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

Next, we evaluated the relationship between HbA1c level and FMD in patients with type 2 diabetes who were not taking antidiabetic drugs in order to eliminate possible effects of antidiabetic drugs and antidiabetic drug-induced hypoglycaemia on vascular function. The
baseline characteristics of those patients are summarised in table 2. The mean FMD value was 4.2%±2.8% and the mean NID value was 10.6%±5.8%.

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

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

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

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

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

| Variables | Total (n=349) | HbA1c <6.5% (n=101) | HbA1c 6.5%–6.9% (n=149) | HbA1c 7.0%–7.9% (n=67) | HbA1c ≥8.0% (n=32) | P value |
|-----------|--------------|---------------------|--------------------------|-------------------------|---------------------|--------|
| Age, years | 61±10        | 66±10               | 60±10                    | 61±9                    | 57±10               | <0.001 |
| Gender, male/female | 245/104 | 59/42               | 108/41                   | 52/15                   | 26/6                | 0.01   |
| Body mass index, kg/m² | 25.4±4.2 | 24.6±4.1            | 25.5±4.2                 | 26.0±4.6                | 25.8±4.2            | 0.1    |
| Heart rate, bpm | 69±11 | 70±11               | 68±11                    | 68±10                   | 69±11               | 0.21   |
| Systolic blood pressure, mm Hg | 133±17 | 128±18              | 133±16                   | 136±16                  | 138±19              | 0.004  |
| Diastolic blood pressure, mm Hg | 80±11 | 77±12               | 81±10                    | 82±10                   | 83±10               | 0.002  |
| Total cholesterol, mg/dL | 199±39 | 186±33              | 205±36                   | 197±45                  | 216±45              | <0.001 |
| Triglycerides, mg/dL | 169±139 | 133±82              | 169±143                  | 205±173                 | 206±162             | 0.003  |
| HDL-C, mg/dL | 54±15 | 57±15               | 55±16                    | 48±12                   | 49±12               | <0.001 |
| LDL-C, mg/dL | 116±32 | 110±30              | 119±31                   | 115±36                  | 127±30              | 0.04   |
| Creatinine, mg/dL | 0.8±0.3 | 0.8±0.4             | 0.8±0.2                  | 0.8±0.2                 | 0.8±0.3             | 0.33   |
| Uric acid, mg/dL | 5.8±1.5 | 6.0±1.7             | 5.8±1.5                  | 5.5±1.4                 | 5.5±1.7             | 0.23   |
| Glucose, mg/dL | 137±46 | 119±28              | 125±22                   | 145±36                  | 224±87              | <0.001 |
| HbA1c, % | 6.8±1.0 | 5.9±0.4             | 6.7±0.1                  | 7.3±0.3                 | 9.4±1.2             | <0.001 |
| Medical history, n (%) | | | | | | |
| Hypertension | 266 (76.2) | 75 (74.3) | 112 (75.2) | 56 (83.6) | 23 (71.9) | 0.45   |
| Dyslipidaemia | 275 (78.8) | 79 (78.2) | 116 (77.9) | 57 (85.1) | 23 (71.9) | 0.46   |
| CVD, n (%) | 79 (22.6) | 27 (26.7) | 29 (19.5) | 17 (25.4) | 6 (18.8) | 0.50   |
| Current smoking, n (%) | 79 (22.6) | 20 (19.8) | 34 (23.3) | 17 (25.4) | 8 (26.7) | 0.79   |
| Medication, n (%) | | | | | | |
| Antihypertensive drugs | 217 (62.2) | 78 (77.2) | 85 (57.1) | 41 (61.2) | 13 (40.6) | <0.001 |
| Lipid-lowering drugs | 144 (41.3) | 59 (58.4) | 57 (38.3) | 24 (35.8) | 4 (12.5) | <0.001 |
| Antidiabetic drugs | 0 (0) | | | | | |

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

Yamaji T, et al. BMJ Open 2021;11:e045415. doi:10.1136/bmjopen-2020-045415
HbA1c level was significantly correlated with age ($r=-0.2, p<0.001$), systolic blood pressure ($r=0.17, p=0.001$), diastolic blood pressure ($r=0.12, p=0.02$), total cholesterol ($r=0.22, p<0.001$), triglycerides ($r=0.23, p<0.001$), HDL cholesterol ($r=-0.19, p<0.001$), LDL cholesterol ($r=0.14, p=0.01$) and glucose level ($r=0.70, p<0.001$). Linear regression analysis revealed that HbA1c level was not significantly correlated with FMD ($r=0.05, p=0.40$; online supplemental figure 6A). Scatter plots between FMD and HbA1c with a Lowess smoothed curve are shown in online supplemental figure 6B. FMD gradually increased with increase in HbA1c level to about 6.5%–6.9% and then decreased with increase in HbA1c level above 7.0%.

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

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

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

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

DISCUSSION

In the present study, we demonstrated that a low HbA1c level of $<6.5\%$ was independently associated with small FMD in patients with type 2 diabetes. After adjustments for confounding factors, FMD was significantly smaller in the HbA1c $<6.5\%$ group than in the HbA1c 6.5%–6.9% group and HbA1c 7.0%–7.9% group. In patients who were not taking antidiabetic drugs, FMD was also significantly
smaller in the HbA1c <6.5% group than in the HbA1c 6.5%–6.9% group and HbA1c 7.0%–7.9% group. We also confirmed by using propensity score matching analysis that FMD was significantly smaller in the low HbA1c group than in the HbA1c 6.5%–6.9% group. To our knowledge, the present study is the first study showing detailed relationships between HbA1c level and endothelial function in patients with type 2 diabetes, including patients not taking antidiabetic drugs.

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

It is controversial whether endothelium-independent vasodilation assessed by NID as well as endothelium-dependent vasodilation assessed by FMD are impaired in individuals with cardiovascular risk factors and patients with CVD.24 25 In the present study, although we found that there was an inverted U-shaped pattern of association between FMD and HbA1c, there was no significant relationship between NID and HbA1c. In a previous study, we showed that both NID and FMD were maintained in subjects without cardiovascular risk factors and that FMD was significantly smaller in subjects with cardiovascular risk factors than in subjects without cardiovascular risk factors, but that NID was significantly smaller in patients with CVD than in both subjects with and those without cardiovascular risk factors, whereas there was no significant difference in NID between subjects with and those without cardiovascular risk factors, suggesting that FMD values and NID values are different in relation to the grade of atherosclerosis.23 The Hoorn Study showed that although FMD was significantly smaller in patients with type 2 diabetes than in subjects with normal glucose metabolism, NID values were similar in the two groups.24 Kubota et al25 showed that NID did not alter after treatment with sitagliptin in patients with type 2 diabetes and that changes in NID did not correlate with changes in HbA1c, while FMD improved in relation to decrease in HbA1c. These previous studies support our results showing that NID is not associated with HbA1c levels in patients with type 2 diabetes.

It is well known that the incidence of myocardial infarction increases in relation to HbA1c level.26 It is thought that FMD, an index of endothelial function, decreases with increase in HbA1c level. However, in the present study, a low HbA1c level of <6.5% was found to be independently associated with endothelial dysfunction in patients with type 2 diabetes. To avoid the effects of antidiabetic drugs on HbA1c levels and to minimise the effect of hypoglycaemia, we evaluated the relationship between HbA1c level and FMD in patients with type 2 diabetes who were not taking antidiabetic drugs, and we found that the results were similar for patients taking and those not taking antidiabetic drugs.

The key finding of this study was that an inverted U-shaped pattern of association between HbA1c and FMD was observed at the peak of HbA1c of about 7% in patients with type 2 diabetes. This result may reflect the existence of a J-curve pattern of association between HbA1c and all causes of mortality. Diabetes is well known as a risk factor for endothelial dysfunction as well as for CVD.13 26 29 However, the effect of intensive glucose control therapy on all causes of mortality is still controversial. Previous studies focused on the relationship between HbA1c and all causes of mortality. Some studies showed a positive linear relationship between HbA1c and all causes of mortality,30 31 while other studies showed a J-shaped relationship between HbA1c and all causes of mortality.32 33 The effects of intensive glucose control therapy on morbidity and mortality of cardiovascular events are also controversial.34 35 The United Kingdom Prospective Diabetes Study (UKPDS) 73 study showed that the frequency of hypoglycaemia in patients not taking antidiabetic drugs was 0.1%.35 Hypoglycaemia during intensive glucose control is probably a predictor of morbidity and mortality of cardiovascular events. It has been shown that the HRs for all causes of mortality including cardiovascular events in patients with severe hypoglycaemia episodes are between 1.74 and 3.27.36 37 It has been postulated that hypoglycaemia activates the sympathetic nervous system, resulting in release of catecholamines that cause increases in heart

---

### 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

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

Model 1: unadjusted model.
Model 2: adjusted for age, gender and body mass index.
Model 3: adjusted for age, gender, body mass index, current smoking, creatinine, presence of hypertension, dyslipidaemia and CVD.

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

CVD, cardiovascular disease; FMD, flow-mediated vasodilation; HbA1c, haemoglobin A1c; NID, nitroglycerine-induced vasodilation.

---

Yamaji T, et al. BMJ Open 2021;11:e045415. doi:10.1136/bmjopen-2020-045415
rate and myocardial contractility, and activates platelet aggregation, leading to acute coronary syndrome and fatal arrhythmia. 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 hyperglycaemia contribute to low HbA1c-induced endothelial dysfunction.

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

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

Author affiliations
1Department of Cardiovascular Medicine, Hiroshima University Faculty of Medicine
2Graduate School of Biomedical and Health Sciences, Hiroshima, Japan
3Department of Cardiovascular Regeneration and Medicine, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan; Yutaka Ishibashi, MD, PhD (Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Kana Kanai, PhD; Haruka Morimoto, PhD (Department of Cardiovascular Regeneration and Medicine, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan); Tomohisa Sakashita, MD, PhD; Yoshiki Kudo, MD, PhD (Department of Obstetrics and Gynecology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Taijiro Sueda, MD, PhD (Department of Surgery, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan); Hirofumi Tomiyama, MD, PhD, FAHA; Akira Yamashina, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Sonei Takase, MD, PhD, FAHA (Division of Biomedical Engineering, National Defense Medical College Research Institute, Tokorozawa, Japan); Takahide Kohro, MD, PhD (Department of Cardiology, Tokyo Medical University, Tokyo, Japan); Toru Suzuki, MD, PhD (Cardiovascular Medicine, University of Leicester, Leicester, UK); Tomoko Ishizu, MD, PhD (Cardiovascular Division, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan); Shinichiro Ueda, MD, PhD (Department of Clinical Pharmacology and Therapeutics, University of the Ryukyus School of Medicine, Okinawa, Japan); Tsutomu Yamazaki, MD, PhD (Clinical Research Support Center, Faculty of Medicine, University of Tokyo, Tokyo, Japan); Tomoe Furumoto, MD, PhD (Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Hokkaido, Japan); Kazuo Kario, MD, PhD (Division of Cardiovascular Medicine, Jichi Medical University School of Medicine, Tochigi, Japan); Teruo Inoue, MD, PhD (Department of Cardiovascular Medicine, Dokkyo Medical University, Mibu, Tochigi, Japan); Shinji Koba, MD, PhD (Department of Medicine, Division of Cardiology, Showa University School of Medicine, Tokyo, Japan); Kentaro Watanabe, MD, PhD (Department of Neurology, Hematology, Metabolism, Endocrinology and Diabetology (ONHAMED), Yamagata University School of Medicine, Yamagata, Japan); Yasuhiko Takemoto, MD, PhD (Department of Internal Medicine and Cardiology, Osaka City University Graduate School of Medicine, Osaka, Japan); Takuzo Haneda, MD, PhD (Department of Medical Education and Population-based Medicine, Postgraduate School of Medicine, Wakayama Medical University, Wakayama, Japan); Masatoshi Sata, MD, PhD (Department of Cardiovascular Medicine, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan); Yutaka Ishibashi, MD, PhD (Department of General Medicine, Shimane University Faculty of Medicine,Association between FMD and HbA1c and a low HbA1c level (<6.5%) is associated with endothelial dysfunction in patients with type 2 diabetes, even in patients with type 2 diabetes who are not taking antidiabetic drugs.
izumo, Japan); Koichi Node, MD, PhD (Department of Cardiovascular and Renal Medicine, Saga University, Saga, Japan); Koji Maemura, MD, PhD (Department of Cardiovascular Medicine, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan); Yusuke Ohyama, MD, PhD (Third Department of Internal Medicine, University of the Ryukyus, Okinawa, Japan); Taij Furukawa, MD, PhD (Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan); Hiroshi Ito, MD, PhD (Department of Cardiovascular Medicine, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Japan); and Hisao Ikeda, MD, PhD (Faculty of Fukukura Medical Technology, Teikyo University, Omita, Japan).

Contributors TH and YHi: drafting the article and conception of the study. TH, YHi, TK, SY, YK, and YRo: revising the article critically for important intellectual content. YHi 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.

Funding Funding was received from Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (18590815 and 21590898 to YHi) and Grant-in-Aid of Japanese Arteriosclerosis Prevention Fund to (YHi).

Competing interests None declared.

Patient consent for publication Not required.

Ethics approval 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.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. There is no additional information.

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iD Yukihito Higashi http://orcid.org/0000-0001-5813-3672

REFERENCES
1. Standl E, Ballestaro B, Dahl B, et al. Predictors of 10-year macrovascular and overall mortality in patients with NIDDM: the Munich general practitioner project. Diabetes 
   1996;39:1540–5.
2. Khaw K-T, Wareham N, Bingham S, et al. Association of hyperglycemia A1c with cardiovascular disease and mortality in adults: the European prospective investigation into cancer in Norfolk. Ann Intern Med 
   2004;141:413–20.
3. Stamler J, Vaccaro AR, Neaton JD, et al. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the multiple risk factor intervention trial. Diabetologia 
   1986;29:45–58.
4. ACCORD Study Group, ACCORD Eye Study Group, Chew EY, et al. Effects of medical therapies on retinopathy progression in type 2 diabetes. N Engl J Med 
   2010;363:33–44.
5. ADVANCE Collaborative Group, Patel A, MacMahon S, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 
   2008;358:2560–72.
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;28:103–17.
7. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 
   2009;360:129–39.
8. Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 
   2008;358:2545–59.
9. Higashi Y, Noma K, Yoshizumi M, et al. Endothelial function and oxidative stress in cardiovascular diseases. Circ J 
   2009;73:411–8.
10. Soga J, Noma K, Hata T, et al. Rho-associated kinase activity, endothelial function, and cardiovascular risk factors. Arterioscler Thromb Vasc Biol 
    2011;31:2533–9.
11. Yeojo B, 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;120:902–9.
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;125:823–30.
13. Martens FMAC, 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;27:1605–9.
14. Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelin-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. Pharmacol Ther 
    2004;102:87–96.
15. Maruhashi T, Soga J, Fujimura N, et al. Relationship between flow-mediated vasodilatation and cardiovascular risk factors in a large community-based study. Heart 
    2013;99:1837–42.
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;61:542–9.
17. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 
    2001;414:812–20.
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.
19. Moreno B, de Faria AR, Ritter AM, et al. Glycated hemoglobin correlates with arterial stiffness and endothelial dysfunction in patients with resistant hypertension and uncontrolled diabetes mellitus. J Clin Hypertens 
    2018;20:910–7.
20. Tomyiama 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;53:170–5.
21. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 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;285:2486–97.
22. American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2018. Diabetes 
    2018;67:S1–27.
23. Maruhashi T, Soga J, Fujimura N, et al. Nitroglycerine-induced vasodilation for assessment of vascular function: a comparison with flow-mediated vasodilatation. Arterioscler Thromb Vasc Biol 
    2013;33:1401–8.
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.
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;234:72–4.
26. Henry RMA, Ferreira I, Kostense PJ, et al. Type 2 diabetes is associated with impaired endothelial-dependent, flow-mediated dilation, but impaired glucose metabolism is not; the Hoorn study. Atherosclerosis 
    2004;174:49–56.
27. Kubota Y, Miyamoto M, Takagi G, et al. The depeptidyl peptidase-4 inhibitor sitagliptin improves vascular endothelial function in type 2 diabetes. J Korean Med Sci 
    2012;27:1364–70.
28. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 33): prospective observational study. BMJ 
    2000;321:405–12.
29. Williams SB, Cusco JA, Roddy MA, et al. Impaired nitric oxide-mediated vasodilation in patients with non-insulin-dependent diabetes mellitus. J Am Coll Cardiol 
    1996;27:586–74.
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 
    2017;64:453–60.
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:e38877.

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;11:138–52.

33 Wan EYF, Fung CSC, Wong CKH, et al. Association of hemoglobin A1c levels with cardiovascular disease and mortality in Chinese patients with diabetes. *J Am Coll Cardiol* 2016;67:456–8.

34 Rawshani A, Rawshani A, Fränsson S, et al. Risk factors, mortality, and cardiovascular outcomes in patients with type 2 diabetes. *N Engl J Med* 2018;379:633–44.

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;20:395–401.

36 Zoungas S, Patel A, Chalmers J, et al. Severe hypoglycemia and risks of vascular events and death. *N Engl J Med* 2010;363:1410–8.

37 ORIGIN Trial Investigators, Mellbin LG, Rydén L, et al. Does hypoglycaemia increase the risk of cardiovascular events? A report from the origin trial. *Eur Heart J* 2013;34:3137–44.

38 Fisher BM, Gillen G, Hepburn DA, et al. Cardiac responses to acute insulin-induced hypoglycemia in humans. *Am J Physiol* 1990;258:H1775–9.

39 Wright RJ, Frier BM. Vascular disease and diabetes: is hypoglycaemia an aggravating factor? *Diabetes Metab Res Rev* 2008;24:353–63.

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;15:302–9.

41 Higashi Y, Sasaki S, Sasaki N, et al. Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension. *Hypertension* 1999;33:591–7.

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;108:530–5.