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Pre-impaired fasting glucose state is a risk factor for endothelial dysfunction: Flow-mediated Dilation Japan (FMD-J) study

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ABSTRACT

Introduction Diabetes mellitus is associated with endothelial dysfunction. However, there is little information on the relationships of fasting blood glucose (FBG), including high normal blood glucose and impaired fasting glucose (IFG) with endothelial function. The purpose of this study was to evaluate the relationship between FBG level and flow-mediated vasodilation (FMD) using a large sample size.

Research design and methods This study was a cross-sectional study. We measured FMD in 7265 subjects at 31 general hospitals. The subjects were divided into four groups based on FBG levels: <100, 100–109, 110–125, and ≥126 mg/dL or known diabetes. The subjects were also divided into six groups based on FBG levels: <90, 90–94, 95–99, 100–109, 110–125, and ≥126 mg/dL or known diabetes.

Results FMD decreased in relation to increase in FBG level. There was a significant difference in FMD between the FBG of <100 mg/dL group and the other three groups (6.7±3.1% vs 5.9±2.8%, 5.7±3.1%, and 5.1±2.6%, respectively; p<0.001). After adjustment for confounding factors, the odds of having the lowest quartile of FMD were significantly higher in the FBG of 95–99, 100–104, 105–109, 110–125, and ≥126 mg/dL or known diabetes groups than in the FBG of the <90 mg/dL group.

Conclusions These findings suggest that FBG of 100–109 mg/dL and FBG of 110–125 mg/dL are similarly associated with endothelial dysfunction and that a pre-IFG state (FBG of 95–99 mg/dL) is also a risk for endothelial dysfunction compared with FBG of <90 mg/dL.

Trial registration number UMIN000012950, UMIN000012951, UMIN000012952, and UMIN000003409.

Significance of this study

What is already known about this subject?

- Diabetes is associated with endothelial dysfunction.
- The relationship between impaired fasting glucose and endothelial function is still controversial.

What are the new findings?

- A preimpaired fasting glucose (IFG) state (fasting blood glucose (FBG) of 95–99 mg/dL) is a risk for endothelial dysfunction compared with an FBG of <90 mg/dL.

How might these results change the focus of research or clinical practice?

- Intensive lifestyle modification is needed in subjects with a pre-IFG state (FBG of 95–99 mg/dL).

INTRODUCTION

Diabetes mellitus (DM) is a well-established risk factor for cardiovascular events.[1] It is well known that cardiovascular risk is increased before the onset of DM as a state of pre-diabetes.[2] In the American Diabetes Association (ADA) classification, a fasting blood glucose (FBG) range of 100–125 mg/dL is categorized as impaired fasting glucose (IFG).[3] However, before 2003, an FBG range of 110–125 mg/dL was categorized as IFG in the ADA classification. In WHO criteria, an FBG range of 110–125 mg/dL is categorized as IFG. In the Japan Diabetes Society criteria, an FBG range of 110–125 mg/dL is categorized as pre-diabetes, and a range of 100–109 mg/dL is categorized as high normal blood glucose.[4] The definition of IFG differs between countries and eras.

Endothelial dysfunction is known as the first step in the pathogenesis of atherosclerosis and is also known as a marker of cardiovascular events.[5 6] Measurement of flow-mediated vasodilation (FMD) in the brachial artery is widely used for assessment of endothelial function. FMD is also well known as an independent predictor of cardiovascular events.[7–11] In addition, measurement of FMD is an independent predictor of cardiovascular events in the general population, including individuals with DM. It has been established that DM is associated with endothelial dysfunction.[12–14]

However, the relationship between IFG and endothelial function is still controversial. Some investigators have shown that IFG is associated with endothelial dysfunction.[15–18] On the other hand, Henry *et al* reported that IFG was not associated with endothelial dysfunction.[13]

There has been no study on the detailed relationships of FBG, including a normal range of glucose, pre-IFG range, and IFG range with endothelial function. First, we divided subjects into four groups according to the ADA classification, the WHO criteria and the Japan Diabetes Society criteria, FBG of <100, 100–109, 110–125, and ≥126 mg/dL or known DM, and assessed the FMD values. Furthermore, there is no information on from what level of FBG adversely affects endothelial function. Therefore, we divided FBG of <100 mg/dL, which was previously classified as normal blood glucose, into three groups, FBG of <90 md/dL, FBG of 90–94 mg/dL and FBG of 95–99 mg/dL, and evaluated the relationships of FBG levels of <90, 90–94, 95–99, 100–109, 110–125 and ≥126 mg/dL or known DM with FMD in multiple centers using a large sample size.

RESEARCH DESIGN AND METHODS

Study subjects

The Flow-mediated Dilation Japan (FMD-J) Registry was a prospective multicenter registry that was established between April 1, 2010, and August 31, 2018, at 31 institutes in Japan with the aim of determining the usefulness of FMD measurement. All of the subjects had an obligation to undergo health screening every year under the regulations of the society-managed health insurance union in Japan. The design of the FMD-J study has been described in detail.[19] Subjects with severe chronic heart failure (New York Heart Association level of more than III), subjects with severe valvular disease, arrhythmia for which they were receiving treatment, dialysis, end-stage chronic kidney disease or malignancy, subjects taking steroids, non-steroidal anti-inflammatory drugs or immunosuppressive drugs, subjects over 80 years of age, and subjects without information on FBG or with unclear images of the brachial artery interfaces were excluded. Finally, we enrolled 7265 subjects in this study. Hypertension was defined as the 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. Dyslipidemia was defined according to the third report of the National Cholesterol Education Program.[20] DM was defined according to the ADA recommendation.[21] Smokers were defined as those who were current smokers. Cardiovascular disease was defined as coronary heart disease and cerebrovascular disease. Coronary heart disease included angina pectoris, prior myocardial infarction, and unstable angina. Cerebrovascular disease included ischemic stroke, hemorrhagic stroke, and transient ischemic attack.

Study protocol

We measured the vascular response to reactive hyperemia in the brachial artery for assessment of endothelium-dependent FMD. The patients fasted overnight and abstained from alcohol, smoking, caffeine and antioxidant vitamins for at least 12 hours before the study. The participants were kept in the supine position in a quiet, dark, air-conditioned room (constant temperature from 22°C to 25°C) throughout the study. A 23-gauge polyethylene catheter was inserted into the deep antecubital vein to obtain blood samples for measurements, including measurement of FBG. After maintaining the supine position for 30 min, FMD was measured. The observers were blind to the form of examination.

Measurement of FMD

A high-resolution linear artery transducer (resolution of 10MHz and an H-shaped probe capturing two short-axis images and one long-axis image) was coupled to computer-assisted analysis software (UNEXEF18G; UNEX Corporation, Nagoya, Japan) that used an automated edge detection system for measurement of the brachial artery diameter.[22] Online supplemental figure S1A shows the system for measurement of FMD. A blood pressure cuff was placed around the forearm of each subjects. The brachial artery was scanned longitudinally 5–10 cm above the elbow. When the clearest B-mode image of the anterior and posterior intimal interfaces between the lumen and the vessel wall was obtained, the transducer was held at the same point throughout the scan by using a special probe holder (UNEX Corporation) to ensure consistency of the imaging (online supplemental figure S1B). 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 were displayed in real time using the FMD mode of the tracking system. This allowed the ultrasound images to be optimized at the start of the scan and the transducer position to be adjusted immediately for optimal tracking performance throughout the scan. Pulsed Doppler flow was assessed at baseline and during peak hyperemic flow, which was confirmed to occur within 15 s after cuff deflation. Blood flow velocity was calculated from the color Doppler data and was displayed as a waveform in real time (online supplemental figure S1B,C). Baseline longitudinal images of the artery were acquired for 30 s, and then the blood pressure cuff was inflated to 50 mm Hg above systolic pressure for 5 min. Blood flow volume was calculated by multiplying the Doppler flow velocity (corrected for the angle) by heart rate and vessel cross-sectional area ($-r^2$). The longitudinal image of the artery was recorded continuously until 3 min after cuff deflation. Changes in brachial artery diameter were immediately expressed as percentage changes relative to the vessel diameter before cuff inflation. FMD was automatically calculated as the percentage change in peak vessel diameter from the baseline value. Percentage

of FMD [(peak diameter-baseline diameter)/baseline diameter] was used for analysis. All of the sonographers specialized in FMD measurement at the participating institutions underwent training for a standard protocol of FMD measurement and training for scanning and analysis of the record at the core laboratory in the FMD-J study. All recordings of brachial artery scans obtained during the measurements of FMD were sent from the participant institutions to the core laboratory by universal serial bus flash drives and were individually analyzed by a well-experienced reader at the core laboratory without any information about the patients in the FMD-J study. We set outliers as FMD <−10% and FMD >25%. 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 intraobserver variabilities (coefficients of variation) were 10.1%–11.2%.[23] Measurements for FMD were performed according to the guideline.[24]

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. Categorical values were compared by means of the χ^2 test. Continuous variables were compared by using analysis of variance (ANOVA) on multiple groups. Comparisons between the groups categorized according to FBG 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 regression analyses using ordinary least squares were performed to identify independent variables associated with FMD from the following covariates with p<0.05 for inclusion: age, body mass index, systolic blood pressure, heart rate, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, FBG, uric acid, and creatinine. Multivariate logistic regression analysis was performed to identify independent variables associated with lower quartiles of FMD (<4.2%). Age over 65 years old, gender, obesity (body mass index of 30 or higher), current smoking, presence of hypertension and presence of dyslipidemia were entered into the multivariate logistic regression analysis. All data were processed using JMP Pro V.14.0 software (SAS Institute, Cary, North Carolina, USA)

RESULTS

Baseline characteristics of the subjects

The baseline characteristics of the 7265 subjects are summarized in table 1. The mean age of the subjects was 51±10 years. The 7265 subjects included 5804 men (79.9%) and 1461 women (20.1%). The mean FBG level in the subjects was 101±20mg/dL, and the mean HbA1c level was 5.7±0.7%. Among the subjects, 3188 (43.9%) had hypertension; 3760 (51.8%) had dyslipidemia; 685 (9.4%) had DM; 689 (9.5%) had previous cardiovascular disease; and 2189 (30.3%) were current smokers. The mean FMD value was 6.3±3.1%.

Relationships among FMD, FBG and variables

Online supplemental table S1 shows univariate relationships among FMD, FBG level and variables. FMD was significantly correlated with age (r=−0.31, p<0.001), body mass index (r=−0.19, p<0.001), heart rate (r=0.06, p<0.001), systolic blood pressure (r=−0.15, p<0.001), diastolic blood pressure (r=−0.08, p<0.001), total cholesterol (r=−0.03, p=0.03), triglycerides (r=−0.11, p<0.001), high-density lipoprotein cholesterol (r=0.08, p<0.001), creatinine (r=−0.07, p<0.001), uric acid (r=−0.13, p<0.001), FBG level (r=−0.14, p<0.001) (online supplemental figure S2A) and HbA1c level (r=−0.16, p<0.001). FBG level was significantly correlated with age (r=−0.26, p<0.001), body mass index (r=0.23, p<0.001), heart rate (r=0.13, p<0.001), systolic blood pressure (r=0.19, p<0.001), diastolic blood pressure (r=0.11, p<0.001), triglyceride (r=0.18, p<0.001), high-density lipoprotein cholesterol (r=−0.15, p<0.001), uric acid (r=0.06, p<0.001), HbA1c level (r=0.77, p<0.001) and FMD (r=−0.14, p<0.001).

Online supplemental table S2 shows multivariate linear relationships among FMD, FBG level and variables. Multiple linear regression analysis revealed that age (β=−0.23, p<0.001), body mass index (β=−0.10, p<0.001), heart rate (β=0.09, p<0.001), systolic blood pressure (β=−0.04, p=0.001), triglycerides (β=−0.04, p=0.001), high-density lipoprotein cholesterol (β=−0.03, p=0.02), uric acid (β=−0.07, p<0.001), FBG (β=−0.03, p=0.02), antihypertensive drug treatment (β=0.13, p<0.001), and current smoking (β=0.07, p<0.001) were independent predictors of FMD.

FMD values in four groups of FBG levels

The baseline characteristics of subjects with FBG levels of <100, 100–109, 110–125 and ≥126 mg/dL or known DM are also summarized in table 1. There were significant differences in age, gender, body mass index, heart rate, systolic blood pressure, diastolic blood pressure, levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, uric acid, FBG, and HbA1c, prevalences of hypertension, dyslipidemia, and cardiovascular disease, and use of antihypertensive drugs and lipid-lowering drugs among the four groups. FMD was significantly correlated with FBG level of 90–125 mg/dL (r=−0.12, p<0.001) (online supplemental figure S2B). Online supplemental figure S3 shows FMD values in the four groups. FMD values were 6.7±3.1% in the FBG of the <100 mg/dL group, 5.9±2.8% in the 100–109 mg/dL group, 5.7±3.1% in the 110–125 mg/dL group, and 5.1±2.6% in the ≥126 mg/dL or known DM group (p<0.001). FMD values in the FBG 100–109, 110–125 and ≥126 mg/dL or known DM groups were significantly smaller than the values in the FBG<100 mg/dL group (p<0.001, respectively). FMD

Table 1 Clinical characteristics of patients in four groups of FBG levels

Variables	Total (n=7265)	FBG <100 mg/dL (n=4357)	FBG 100–109 mg/dL (n=1540)	FBG 110–125 mg/dL (n=551)	FBG ≥126 mg/dL or DM (n=817)	P value
Age (years)	51±10	48±10	53±9	54±8	59±9	<0.001
Gender, men (%)	5804 (79.9)	3287 (75.4)	1356 (88.1)	492 (89.3)	669 (81.9)	<0.001
Body mass index (kg/m^2)	23.5±3.3	22.8±3.1	24.1±3.0	25.0±3.4	25.3±3.9	<0.001
Heart rate (beats/min)	64±10	63±10	64±11	65±10	66±11	<0.001
Systolic blood pressure (mm Hg)	127±16	124±16	130±16	132±16	134±17	<0.001
Diastolic blood pressure (mm Hg)	80±12	78±12	82±11	83±12	81±11	<0.001
Total cholesterol (mg/dL)	201±34	200±32	207±34	207±36	192±37	<0.001
Triglycerides (mg/dL)	128±92	116±81	139±92	153±98	154±127	<0.001
HDL-C (mg/dL)	59±16	61±16	58±15	56±15	53±15	<0.001
LDL-C (mg/dL)	117±30	116±29	122±30	122±33	109±32	<0.001
Creatinine (mg/dL)	0.82±0.17	0.81±0.16	0.84±0.17	0.83±0.16	0.82±0.21	<0.001
Uric acid (mg/dL)	5.8±1.4	5.6±1.4	6.1±1.3	6.2±1.4	5.8±1.4	<0.001
FBG (mg/dL)	101±20	91±6	104±3	115±4	139±36	<0.001
Hemoglobin A1c (%)	5.7±0.7	5.4±0.3	5.6±0.3	5.8±0.3	6.9±1.0	<0.001
Medical history, n (%)						
Hypertension	3188 (43.9)	1488 (34.2)	763 (49.6)	306 (55.6)	631 (77.3)	<0.001
Dyslipidemia	3760 (51.8)	1867 (42.9)	912 (59.3)	374 (68.0)	607 (74.4)	<0.001
DM	685 (9.4)	0	0	0	685 (83.8)	<0.001
CVD, n (%)	689 (9.5)	214 (4.9)	137 (8.9)	71 (12.9)	267 (32.7)	<0.001
Current smoking, n (%)	2189 (30.3)	1355 (31.2)	456 (29.7)	161 (29.5)	217 (27.2)	0.12
Medication, n (%)						
Antihypertensive drugs	2009 (27.7)	829 (19.0)	480 (31.2)	200 (36.4)	500 (61.3)	<0.001
Lipid-lowering drugs	1116 (15.4)	402 (9.2)	238 (15.5)	106 (19.3)	370 (45.3)	<0.001
Antidiabetic drugs	489 (6.7)	0 (0)	0 (0)	0 (0)	489 (60.0)	<0.001

CVD, cardiovascular disease; DM, diabetes mellitus; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

values were similar in the FBG of the 100–109 group and 110–125 mg/dL group (p=0.80).

The division points for the lowest quartile and second quartile were FMD of 4.2%. Therefore, we defined small FMD as FMD of <4.2%. We took the FBG of <100 mg/dL group as a reference for deriving the low quartiles of FMD in the other groups. After adjustments for age over 65 years, gender, presence of hypertension, presence of dyslipidemia, presence of obesity (body mass index ≥30 m^2/kg), and current smoking, the odds of having the lowest quartile of FMD were significantly higher in the FBG of 100–109 mg/dL group, 110–125 mg/dL group and ≥126 mg/dL or known DM group than in the reference group: 100–109 mg/dL (OR 1.26, 95% CI 1.10 to 1.45), 110–125 mg/dL (OR 1.42, 95% CI 1.16 to 1.74), and ≥126 mg/dL or known DM (OR 1.37, 95% CI 1.15 to 1.64) (online supplemental table S3).

FMD values in six groups of FBG levels

Finally, we divided the patients into six groups based on their FBG levels: <100 mg/dL (<90, 90–94, and 95–99 mg/dL), 100–109, 110–125 and ≥126 mg/dL or known DM, and assessed their endothelial function. There were significant differences in age, gender, body mass index, heart rate, systolic blood pressure, diastolic blood pressure, levels of total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, creatinine, uric acid, FBG, and HbA1c, prevalences of hypertension, dyslipidemia, and cardiovascular disease, and use of antihypertensive drugs and lipid-lowering drugs among the six groups (table 2). FMD values were 6.9±3.1% in the FBG of the <90 mg/dL group, 6.7±3.1% in the 90–94 mg/dL group, 6.3±3.1% in the 95–99 mg/dL group, 5.9±2.8% in the 100–109 mg/dL group, 5.7±3.1% in the 110–125 mg/dL group, and 5.1±2.6% in the ≥126 mg/dL or known DM group (p<0.001, figure 1). FMD values were similar in the FBG of <90 mg/dL group, 90–94 mg/dL group, 100–109 mg/dL group and 110–125 mg/dL group (figure 1).

We took the FBG of the <90 mg/dL group as a reference and for deriving for the low quartiles of FMD in

Table 2 Clinical characteristics of patients in six groups of FBG levels

Variables	Total (n=7265)	FBG <90 mg/dL (n=1630)	FBG 90–94 mg/dL (n=1419)	FBG 95–99 mg/dL (n=1308)	FBG 100–109 mg/dL (n=1540)	FBG 110–125 mg/dL (n=551)	FBG ≥126 mg/dL or DM (n=817)	P value
Age (years)	51±10	46±11	49±10	50±10	53±9	54±8	59±9	<0.001
Gender, men (%)	5804 79.9)	1105 (67.8)	1092 (77.0)	1090 (83.3)	1356 (88.1)	492 (89.3)	669 (81.9)	<0.001
Body mass index (kg/m^2)	23.5±3.3	22.2±3.1	22.9±3.1	23.6±3.0	24.1±3.0	25.0±3.4	25.3±3.9	<0.001
Heart rate (beats/min)	64±10	62±9	63±10	63±10	64±11	65±10	66±11	<0.001
Systolic blood pressure (mm Hg)	127±16	122±16	125±15	127±15	130±16	132±16	134±17	<0.001
Diastolic blood pressure (mm Hg)	80±12	77±12	79±12	80±11	82±11	83±12	81±11	<0.001
Total cholesterol (mg/dL)	201±34	197±33	201±32	202±32	207±34	207±36	192±37	<0.001
Triglycerides (mg/dL)	128±92	107±81	118±83	126±77	139±92	153±98	154±127	<0.001
HDL-C (mg/dL)	59±16	62±16	61±16	59±15	58±15	56±15	53±15	<0.001
LDL-C (mg/dL)	117±30	114±29	118±28	123±30	122±30	122±33	109±32	<0.001
Creatinine (mg/dL)	0.82±0.17	0.79±0.16	0.81±0.16	0.83±0.16	0.84±0.17	0.83±0.16	0.82±0.21	<0.001
Uric acid (mg/dL)	5.8±1.4	5.4±1.4	5.7±1.4	5.9±1.3	6.1±1.3	6.2±1.4	5.8±1.4	<0.001
FBG (mg/dL)	101±20	85±4	92±1	97±1	104±3	115±4	139±36	<0.001
Hemoglobin A1c (%)	5.7±0.7	5.4±0.3	5.4±0.3	5.5±0.3	5.6±0.3	5.8±0.3	6.9±1.0	<0.001
Medical history, n (%)								
Hypertension	3188 (43.9)	434 (26.6)	501 (35.3)	553 (42.3)	763 (49.6)	306 (55.6)	631 (77.3)	<0.001
Dyslipidemia	3760 (51.8)	585 (35.9)	624 (44.0)	658 (50.3)	912 (59.3)	374 (68.0)	607 (74.4)	<0.001
DM	685 (9.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	685 (83.8)	<0.001
CVD, n (%)	689 (9.5)	62 (3.8)	68 (4.8)	84 (6.4)	137 (8.9)	71 (12.9)	267 (32.7)	<0.001
Current smoking, n (%)	2189 (30.3)	523 (32.1)	431 (30.5)	401 (30.8)	456 (29.7)	161 (29.5)	217 (27.2)	0.22
Medication, n (%)								
Antihypertensive drugs	2009 (27.7)	217 (13.3)	294 (20.7)	318 (24.3)	480 (31.2)	200 (36.4)	500 (61.3)	<0.001
Lipid-lowering drugs	1116 (15.4)	110 (6.8)	134 (9.5)	158 (12.1)	238 (15.5)	106 (19.3)	370 (45.3)	<0.001
Antidiabetic drugs	489 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	489 (60.0)	<0.001

CVD, cardiovascular disease; DM, diabetes mellitus; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

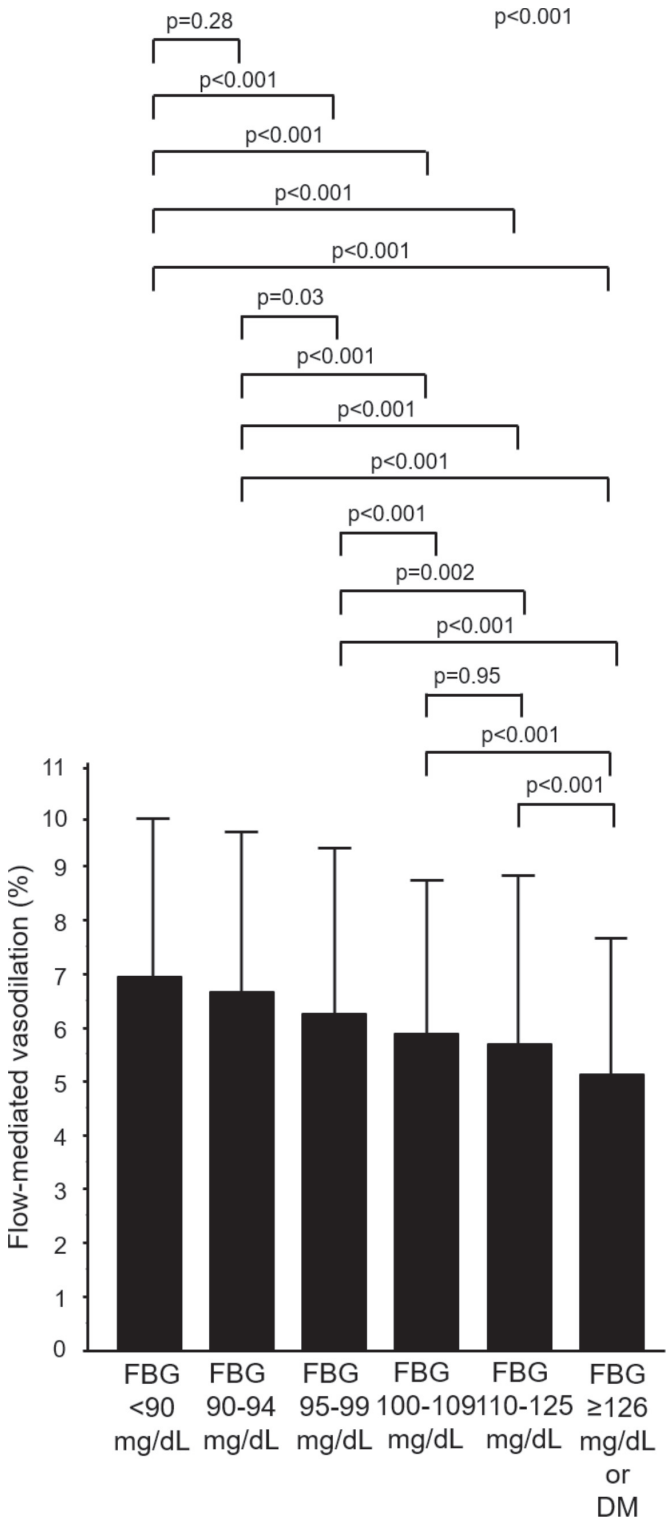

Figure 1 Bar graphs show flow-mediated vasodilation in subjects with FBG of <90, 90–94, 95–99, 100–109, 110–125, and ≥126 mg/dL or known diabetes. DM, diabetes mellitus; FBG, fasting blood glucose.

the other groups. After adjustments for confounding factors, the odds of having lowest quartile of FMD were significantly higher in FBG of the 95–99 mg/dL group, the 100–109 mg/dL group, the 110–125 mg/dL group and the ≥126 mg/dL or known DM group than in the reference group: 95–99 mg/dL group (OR 1.28, 95% CI 1.07 to 1.55), 100–109 mg/dL group (OR 1.41, 95% CI 1.18 to 1.68), 110–125 mg/dL group (OR 1.59, 95% CI 1.26 to 2.01), and ≥126 mg/dL or known DM group (OR 1.54, 95% CI 1.25 to 1.91). The odds of having the lowest quartile of FMD in subjects with FBG of <90 md/dL and subjects with FBG of 90–94 mg/dL were similar (OR 1.07, 95% CI 0.89 to 1.29) (table 3).

DISCUSSION

In the present study, we demonstrated that FBG was an independent predictor of FMD, that FMD was similarly impaired in the FBG of 100–109 and 110–125 mg/dL groups compared with that in the FBG of the <100 mg/dL group, and that after adjustments for confounding factors, FMD values in the FBG of the 100–109 mg/dL group, the 110–125 mg/dL group, and the ≥126 mg/dL or known DM group were significantly smaller than the value in the FBG of the <100 mg/dL group. We also demonstrated for the first time that FMD was significantly smaller in the FBG of the 95–99 mg/dL group than in the FBG of the <90 mg/dL group.

First, to evaluate the relationships between FBG levels and endothelial function in more detail, we divided the subjects into four groups of FBG levels. Endothelial function was similarly impaired in the FBG of the 100–109 mg/dL group and in the FBG of the 110–125 mg/dL group compared with that in the FBG of the <100 mg/dL group. Previous studies showing that subjects in the FBG of the 100–125 mg/dL group and FBG of 110–125 mg/dL group have endothelial dysfunction support our findings.[15–18 25] These finding suggest that FBG of ≥100 mg/dL is a risk factor of endothelial dysfunction. In 2003, ADA proposed that the lower limit of IFG should be lowered from 110 mg/dL to 100 mg/dL since an IFG cut-off value of 100 mg/dL is better than an IFG cut-off value of 110 mg/dL for predicting future type 2 DM onset.[26] From the aspect of vascular function, endothelial function in subjects with FBG of 100–109 mg/dL was similarly impaired in the FBG of 110–125 mg/dL group.

Next, there is no information on from what level of FBG adversely affects endothelial function. Therefore, we divided FBG of <100 mg/dL, which was previously classified as normal blood glucose, into the three groups, FBG of <90 md/dL, FBG of 90–94 mg/dL and FBG of 95–99 mg/dL, and evaluated the relationships between FBG levels of <90, 90–94, 95–99, 100–109, 110–125 and ≥126 mg/dL or known DM and FMD. In the present study, the odds of having the lowest quartile of FMD was significantly higher in the FBG of 95–99 mg/dL group than in the FBG of <90 mg/dL group. A pre-IFG state, FBG of 95–99 mg/dL, was also associated with endothelial dysfunction. It is likely that endothelial function is almost simultaneously impaired when FBG level increases over 95 mg/dL, while it is unclear whether the association of elevation of FBG with the existence of endothelial dysfunction is a cause or consequence.

Table 3 Multivariate analysis of relationships among low quartiles of FMD and FBG levels

	OR (95% CI), P value		
FBG (mg/dL)	Unadjusted	Model 1	Model 2
FBG <90	1 (reference)	1 (reference)	1 (reference)
FBG 90–94	1.19 (0.99 to 1.43), 0.06	1.15 (0.96 to 1.39), 0.13	1.07 (0.89 to 1.29), 0.46
FBG 95–99	1.52 (1.27 to 1.82), <0.001	1.43 (1.19 to 1.72), <0.001	1.28 (1.07 to 1.55), 0.01
FBG 100–109	1.82 (1.54 to 2.16), <0.001	1.66 (1.39 to 1.97), <0.001	1.41 (1.18 to 1.68), <0.001
FBG 110–125	2.25 (1.81 to 2.81), <0.001	2.04 (1.63 to 2.56), <0.001	1.59 (1.26 to 2.01), <0.001
FBG ≥126 or DM	2.82 (2.32 to 3.41), <0.001	2.19 (1.79 to 2.67), <0.001	1.54 (1.25 to 1.91), <0.001

Model 1; adjusted for age ≥65 years old, gender.
Model 2; adjusted for age ≥65 years old, gender, hypertension, dyslipidemia, obesity, and current smoking.
Low quartile of FMD indicates less than 4.2%.
DM, diabetes mellitus; FBG, fasting blood glucose; FMD, flow-mediated vasodilation.

It is thought that the mechanisms by which hyperglycemia impairs endothelial function are increases in oxidative stress and inflammation. Hyperglycemia-induced increase in reactive oxygen species plays a critical role in oxidative stress through several pathways, including overproduction of mitochondria-derived superoxide anion, polyol pathway, advanced glycation end products/receptor for advanced glycation end-product pathway, protein kinase C pathway and hexosamine pathway, leading to a decrease in nitric oxide bioavailability and resulting in endothelial dysfunction.[27–31] Hyperglycemia involves not only a high blood glucose state but also metabolic disorders, including obesity, dyslipidemia, insulin resistance and hypertension. Under the condition of hyperglycemia, obesity-induced hypertrophic adipocytes produce inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6, and reduce anti-inflammatory cytokine adiponectin, leading to an increase in inflammation and resulting in endothelial dysfunction. It is well known that hypertension and dyslipidemia induce increases in oxidative stress and inflammation.[32–34] Oxidative stress and inflammation make a vicious circle and contribute to endothelial dysfunction.[35] In the present study, we found associations of FBS with obesity and with increases in blood pressure and low-density lipoprotein cholesterol level. FMD decreased in relation to an increase in FBG, and FBG was an independent predictor of FMD.

This study has some limitations. First, although this study was conducted by multiple centers (31 institutes) and used a large sample size, this study was a cross-sectional study. Therefore, we were able to evaluate the association but not causality between FBG level and FMD. Second, we did not have information on results of 75 g oral glucose tolerance tests. Therefore, we could not obtain data on impaired glucose tolerance (IGT). Previous studies have shown that IGT is a predictor of cardiovascular events and that it is potentially a better predictor than IFG of cardiovascular events,[36] while it is controversial whether IFG is an independent predictor of cardiovascular events.[36 37] There have been a few studies showing a relationship between high-normal blood glucose (pre-IFG) and diabetic vascular complications. Shaye *et al*[38] showed that subjects with an FBG of 95–99 mg/dL had a higher incidence of cardiovascular events than subjects with an FBG of <80 mg/dL. On the other hand, although Pereg *et al*[39] showed the relationships between FBG levels and coronary revascularization in subjects with an FBG of <100 mg/dL and with previous percutaneous coronary intervention or coronary artery bypass graft, after adjustment for coronary risk factors, there were no significant associations of FBG of 92–99 and ≤80 mg/dL with cardiovascular events. It is still controversial whether pre-IFG is associated with cardiovascular events. In the present study, we clearly showed that endothelial function is impaired not only in subjects with IFG but also even in subjects with pre-IFG. Third, this study was conducted in Japan. It is well known that insulin sensitivity is lower in Asians, including Japanese, than in Caucasians and that Asians are more likely to have an increased risk of DM.[40] We cannot deny the possibility that our results are not applicable to other races. Fourth, we have no information on serum insulin levels. Therefore, we could not discuss the relationships among insulin levels, insulin resistance and endothelial function. Fifth, it is well known that oxidative stress and inflammation contribute to endothelial dysfunction and make a vicious circle between oxidative stress and inflammation and endothelial dysfunction.[35] In addition, previous studies have shown that lifestyle (eg, physical activity and diet) other than smoking status influences endothelial function.[41–44] Unfortunately, in the present study, the FMD-J database did not include data on chemical biomarkers of oxidative stress and inflammation and physical activity and diet. Assessment of oxidative stress and inflammatory markers and assessment of physical activity and diet would enable more specific conclusions concerning the role of FBG in endothelial function to be drawn. Finally, the latest guideline recommends measurement of shear rate.[45] Unfortunately, we had no information on shear rate. Assessment of shear rate would enable more specific conclusions concerning the role of FBG in endothelial function to be drawn.

In conclusion, high normal blood glucose of 95–99 mg/dL as well as an FBG of 110–125 mg/dL was associated with endothelial dysfunction. Endothelial dysfunction may begin from an FBG of 95–99 mg/dL, a so-called pre-IFG state. It is thought that intensive lifestyle modification or pharmacological intervention is needed to decrease FBG in individuals with pre-IFG who have an FBG of more than 95 mg/dL.

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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;39:1540–5.
2 Haffner SM*et al*. Cardiovascular risk factors in confirmed prediabetic individuals. *JAMA* 1990;263:2893–8.
3 American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2019. *Diabetes Care* 2019;42:S13–28.
4 Haneda M, Noda M, Origasa H, *et al*. Japanese clinical practice guideline for diabetes 2016. *Diabetol Int* 2018;9:1–45.
5 Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med* 1999;340:115–26.
6 Higashi Y, Noma K, Yoshizumi M, *et al*. Endothelial function and oxidative stress in cardiovascular diseases. *Circ J* 2009;73:411–8.
7 Modena MG, Bonetti L, Coppi F, *et al*. Prognostic role of reversible endothelial dysfunction in hypertensive postmenopausal women. *J Am Coll Cardiol* 2002;40:505–10.
8 Gokce N, Keaney JF, Hunter LM, *et al*. Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. *Circulation* 2002;105:1567–72.
9 Brevetti G, Silvestro A, Schiano V, *et al*. Endothelial dysfunction and cardiovascular risk prediction in peripheral arterial disease: additive value of flow-mediated dilation to Ankle-brachial pressure index. *Circulation* 2003;108:2093–8.
10 Lerman A, Zeiher AM. Endothelial function: cardiac events. *Circulation* 2005;111:363–8.

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;120:502–9.
12 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;99:1837–42.
13 Henry RMA, 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;174:49–56.
14 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;27:567–74.
15 Vehkavaara S, Seppälä-Lindroos A, Westerbacka J, *et al*. In vivo endothelial dysfunction characterizes patients with impaired fasting glucose. *Diabetes Care* 1999;22:2055–60.
16 Xiang G-da, Wang Y-lin. Regular aerobic exercise training improves endothelium-dependent arterial dilation in patients with impaired fasting glucose. *Diabetes Care* 2004;27:801–2.
17 Rodriguez CJ, Miyake Y, Grahame-Clarke C, *et al*. Relation of plasma glucose and endothelial function in a population-based multiethnic sample of subjects without diabetes mellitus. *Am J Cardiol* 2005;96:1273–7.
18 Su Y, Liu X-M, Sun Y-M, *et al*. Endothelial dysfunction in impaired fasting glycemia, impaired glucose tolerance, and type 2 diabetes mellitus. *Am J Cardiol* 2008;102:497–8.
19 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;53:170–5.
20 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.
21 American diabetes association: clinical practice recommendations 1999. *Diabetes Care* 1999;22(Suppl 1):S1–114.
22 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;33:1401–8.
23 Tomiyama H, Kohro T, Higashi Y, *et al*. Reliability of measurement of endothelial function across multiple institutions and establishment of reference values in Japanese. *Atherosclerosis* 2015;242:433–42.
24 Inoue T, Matsuoka H, Higashi Y, *et al*. Flow-mediated vasodilation as a diagnostic modality for vascular failure. *Hypertens Res* 2008;31:2105–13.
25 Diehl KJ, Templeton DL, Ma J, *et al*. Impaired fasting blood glucose is associated with increased endothelin-1 vasoconstrictor tone. *Atherosclerosis* 2013;229:130–3.
26 Genuth S, Alberti KGMM, Bennett P, *et al*. Follow-up report on the diagnosis of diabetes mellitus. *Diabetes Care* 2003;26:3160–7.
27 Brownlee M. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813–20.
28 Du XL, Edelstein D, Rossetti L, *et al*. Hyperglycemia-induced mitochondrial superoxide overproduction activates the hexosamine pathway and induces plasminogen activator inhibitor-1 expression by increasing Sp1 glycosylation. *Proc Natl Acad Sci U S A* 2000;97:12222–6.
29 Koya D, King GL. Protein kinase C activation and the development of diabetic complications. *Diabetes* 1998;47:859–66.
30 Kolm-Litty V, Sauer U, Nerlich A, *et al*. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. *J Clin Invest* 1998;101:160–9.
31 Stirban A, Negrean M, Stratmann B, *et al*. Benfotiamine prevents macro- and microvascular endothelial dysfunction and oxidative stress following a meal rich in advanced glycation end products in individuals with type 2 diabetes. *Diabetes Care* 2006;29:2064–71.
32 Aroor AR, McKarns S, Demarco VG, *et al*. Maladaptive immune and inflammatory pathways lead to cardiovascular insulin resistance. *Metabolism* 2013;62:1543–52.
33 Supriya R, Tam BT, Yu AP, *et al*. Adipokines demonstrate the interacting influence of central obesity with other cardiometabolic risk factors of metabolic syndrome in Hong Kong Chinese adults. *PLoS One* 2018;13:e0201585.
34 Després J-P, Lemieux I. Abdominal obesity and metabolic syndrome. *Nature* 2006;444:881–7.
35 Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circ Res* 2000;87:840–4.
36 Tominaga M, Eguchi H, Manaka H, *et al*. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata diabetes study. *Diabetes Care* 1999;22:920–4.
37 Doi Y, Ninomiya T, Hata J, *et al*. Impact of glucose tolerance status on development of ischemic stroke and coronary heart disease in a general Japanese population: the Hisayama study. *Stroke* 2010;41:203–9.
38 Shaye K, Amir T, Shlomo S, *et al*. Fasting glucose levels within the high normal range predict cardiovascular outcome. *Am Heart J* 2012;164:111–6.
39 Pereg D, Elis A, Neuman Y, *et al*. Cardiovascular risk in patients with fasting blood glucose levels within normal range. *Am J Cardiol* 2010;106:1602–5.
40 Kodama K, Tojjar D, Yamada S, *et al*. Ethnic differences in the relationship between insulin sensitivity and insulin response: a systematic review and meta-analysis. *Diabetes Care* 2013;36:1789–96.
41 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.
42 Higashi Y, Sasaki S, Sasaki N, *et al*. Daily aerobic exercise improves reactive hyperemia in patients with essential hypertension. *Hypertension* 1999;33:591–7.
43 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.
44 Schreuder THA, Green DJ, Nyakayiru J, *et al*. Time-course of vascular adaptations during 8 weeks of exercise training in subjects with type 2 diabetes and middle-aged controls. *Eur J Appl Physiol* 2015;115:187–96.
45 Thijssen DHJ, Bruno RM, van Mil ACCM, *et al*. Expert consensus and evidence-based recommendations for the assessment of flow-mediated dilation in humans. *Eur Heart J* 2019;40:2534–47.