Association Between Insulin Resistance, Oxidative Stress, Sympathetic Activity and Coronary Microvascular Function in Patients With Early Stage Impaired Glucose Metabolism

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Background: Although acute hyperglycemia and insulin resistance (IR) are risk factors for atherosclerosis development through oxidative stress and sympathetic activation in diabetes mellitus, the association of these factors with coronary microvascular function in the early diabetic stage remains controversial.

Methods and Results: Using transthoracic echocardiography, coronary flow velocity (CFV) and its reserve (CFVR) as parameters of coronary microvascular function were measured before and 1 h after an oral glucose tolerance test (OGTT) in 40 patients (aged 59 ± 12 years) without diagnosed diabetes mellitus or coronary artery disease. Plasma glucose, insulin and thiobarbituric acid reactive substance (TBARS; an oxidative stress marker) were measured during the OGTT. IR was evaluated as homeostasis model assessment of IR (HOMA-R). Sympathetic activity was evaluated by using plasma catecholamines after OGTT. CFVR decreased after an OGTT ($P < 0.0001$) mainly because of an increased baseline CFV ($P < 0.0001$). Although the change in CFVR was not associated with the change in TBARS and catecholamines, it was independently associated with HOMA-R on the multivariate regression analysis ($\beta = -0.40$, $P = 0.01$). Another multivariate regression analysis revealed that change in baseline CFV was independently associated with HOMA-R ($\beta = 0.35$, $P = 0.03$).

Conclusions: IR, rather than oxidative stress and sympathetic activity, was associated with an increase in baseline CFV and a decline in CFVR during acute hyperglycemia. IR might play an important role in increased myocardial oxygen demand and coronary microvascular dysfunction.

Key Words: Coronary microvascular function; Insulin resistance; Oxidative stress; Sympathetic activity

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from a decrease in insulin secretion, insulin action, or both.¹ Decrease in insulin action is considered on the background of insulin resistance (IR), which is the basis of metabolic syndrome, and it begins in the early stage of impaired glucose metabolism.^{2,3} The main risk factors affecting large vessel arteriosclerosis in this stage are considered to be IR and glucose fluctuations, represented by postprandial hyperglycemia. Prediabetes is characterized by IR and postprandial hyperglycemia. Prediabetes, including impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) diagnosed by a 75-g oral glucose tolerance test (OGTT), are established as risk factors for arteriosclerotic cardiovascular disease.⁴ Both increased levels of

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blood glucose and insulin cause vascular damage directly or indirectly, and induces stress on the cardiovascular system through sympathetic activation and increased oxygen requirements. In the stage of IGT, acute hyperglycemia induces peripheral vascular endothelial dysfunction,⁵ and this phenomenon is affected by factors resulting from acute hyperglycemia and/or hyperinsulinemia, including elevated oxidative stress and reduced reactivity of vasodilators such as nitric oxide (NO).⁶⁻⁹ In the study using continuous glucose sensors in patients with type 2 DM, even in those with excellent hemoglobin A1c (HbA1c) values, glucose variability was found to have predictive value for the develop-

Received June 29, 2021; revised manuscript received October 2, 2021; accepted October 5, 2021; J-STAGE Advance Publication released online November 18, 2021 Time for primary review: 15 days

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ISSN-1346-9843

ment of coronary artery disease.¹⁰ Acute glucose fluctuations were found to exhibit a more specific triggering effect on oxidative stress than sustained hyperglycemia.¹¹ Furthermore, acute glucose fluctuations also activate sympathetic tone directly and/or via induction of hyperinsulinemia and increase of oxidative stress.^{12–14} Therefore, the association of various factors induced by acute glucose fluctuations and IR with diabetic vascular diseases in the early stage of impaired glucose metabolism gets more clinical attention; however, there are few reports regarding the association of factors caused by acute glucose fluctuations and IR with impairment of coronary microvascular function in this early stage.

The high frequency probe and harmonic imaging have enabled the measurement of coronary flow velocity (CFV) using transthoracic Doppler echocardiography. Measurements of CFV reserve (CFVR) obtained using transthoracic Doppler echocardiography is one of the methods used for evaluation of coronary microvascular function.¹⁵ Compared with cardiac magnetic resonance, positron emission tomography or invasive methods using Doppler guide wires, the advantages of transthoracic Doppler echocardiography are that it can be performed inexpensively, repeatedly, and non-invasively without radiation exposure. This method has already been applied clinically, and it is considered to be the most suitable for measuring immediate changes of coronary blood flow during acute hyperglycemia.

Therefore, the aim of this study was to investigate the association of levels of IR, oxidative stress, and sympathetic activity with coronary microvascular dysfunction, which was assessed by echocardiographic CFV and CFVR, induced by acute hyperglycemia in response to an OGTT in patients in the early stage of impaired glucose metabolism.

Methods

Study Subjects

Forty patients with no evidence of myocardial ischemia and no previously diagnosed DM were included in this study (mean age, 59±12 years; 32 men and 8 women). Fasting blood glucose levels of all patients were ≤126 mg/dL and their glycosylated HbA1c levels were ≤6.5% (evaluated by using the National glycosylated hemoglobin standardization program: NGSP).¹⁶ Patients with abnormal left ventricular wall motion, left ventricular hypertrophy, or clinically significant valvular disease detected by echocardiography, as well as patients without sinus rhythm, were excluded from this study. To exclude patients with myocardial ischemia, all patients underwent stress myocardial scintigraphy or stress electrocardiography. Before performing the study, written informed consent concerning decisions about the protection of human rights by Tokyo Medical University, as well as the study protocol, was obtained from all patients.

Measurements

Echocardiographic Evaluation and Measurement of CFVR

Transthoracic Doppler echocardiography was performed with an ultrasound machine (Sequoia 512; SIEMENS AG, Mountain View, CA, USA) equipped with a 3.5–7.0 MHz broadband probe. Left ventricular (LV) end-diastolic diameter (LVDD), LV end-systolic diameter, intraventricular septum (IVS) thickness, and end-diastolic posterior wall (PW) thickness were measured. LV mass was calculated using the American Society of Echocardiography

method,¹⁷ as follows:

$$\text{LV mass} = 0.8 (1.04[(\text{IVS} + \text{LVDD} + \text{PW})^3 - \text{LVDD}^3] + 0.6)$$

LV mass index was defined as LV mass divided by body surface area. Cardiac output was calculated as the product of heart rate and stroke volume, which was calculated from the diameter of the LV outflow tract and the time velocity integral at the LV outflow tract.¹⁸ Stroke volume index (SI) and cardiac index (CI) were defined as stroke volume and cardiac output divided by body surface area, respectively. LV systolic and diastolic function were measured according to the guidelines of the American Society of Echocardiography.^{19,20} After fundamental examinations of LV function, the subject assumed a left semi-lateral position, and the probe was placed at the 4th to 5th intercostal space in the left mid-clavicular line to detect blood flow in the distal portion of the left anterior descending coronary artery from the apical approach, guided by color Doppler imaging. The color Doppler velocity range was set to 7–32 cm/s. After confirming a coronary blood flow signal, CFV was measured by the pulsed-wave Doppler method to determine the mean diastolic CFV. After measuring baseline CFV, CFV on hyperemia (hyperemia CFV) induced by continuous intravenous administration of adenosine triphosphate (0.15 mg/kg/min) was measured. Mean diastolic CFV was calculated by averaging the values of 3 consecutive heart beats, and then CFVR was calculated by dividing hyperemia CFV by baseline CFV.¹⁵ The percentage change in CFVR was determined by dividing the difference in CFVR between before and 1 h after an OGTT by CFVR before an OGTT. Changes in baseline CFV and in hyperemia CFV were also determined in the same way. Recorded waveforms were saved digitally on a DVD, and off-line analyses were performed. The reproducibility of each of the measurements at our hospital was as follows: in 45 volunteers, there was good agreement between 2 observers' measurements for baseline CFV, hyperemia CFV, and CFVR ($r=0.99$, $r=0.96$, and $r=0.96$, respectively). Interobserver and intraobserver variabilities for the measurement of the Doppler velocity were 4.8% and 4.1%, respectively.

Blood Measurements Blood samples were collected to measure plasma blood glucose and insulin levels before and at 1 h and at 2 h after an OGTT. Plasma levels of thiobarbituric acid reactive substances (TBARS) before and 1 h after an OGTT were also measured as a marker of lipid peroxidation. Additionally, change in TBARS was calculated by dividing the percent change in plasma TBARS between before and 1 h after an OGTT, by plasma TBARS before an OGTT. TBARS was measured by a spectrophotometric assay that quantifies a chromogen produced by the reaction of thiobarbituric acid with malondialdehyde.²¹ For this measurement, plasma was mixed with 2% butylated hydroxytoluene and the Quintanilla reagent (26 mmol/L thiobarbituric acid and 918 mmol/L trichloroacetic acid). The mixture was boiled for 15 min to facilitate the reactions, then the reaction mixture was cooled and centrifuged at 3,000 g for 10 min. The soluble phase was measured by spectrophotometry at a wavelength of 535 nm. Fasting blood was also collected to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and creatinine. As an indicator of sympathetic activity, plasma concentrations of catecholamines including epinephrine, norepinephrine and dopamine that were obtained 1 h after an OGTT were measured by using high-performance liquid

chromatography. Serum creatinine and age were used to calculate estimated glomerular filtration rate (eGFR; mL/min/1.73 m²). The following estimation formula modified for Japanese patients with respect to the modification of diet in renal disease (MDRD) was used to calculate eGFR: $eGFR = 0.808 \times 175 \times Cr^{-1.154} \times age^{-0.203} (\times 0.742 \text{ for females})$.²² Homeostasis model assessment of IR (HOMA-R), an indirect index of IR, was calculated as: fasting serum insulin (μ U/mL) $\times$ fasting plasma glucose (mg/dL) / 405.²³ In accordance with the World Health Organization (WHO) guidelines, and depending on the plasma glucose levels 2 h after an OGTT, subjects were assigned to the following 2 groups: normal glucose tolerance (N group: blood glucose <140 mg/dL, n=16), and IGT/DM group (n=24) including IGT (140 $\leq$ blood glucose <200 mg/dL) and DM (blood glucose $\geq$ 200 mg/dL).²⁴

Study Protocol

All patients did not take any type of medication from at least 2 days before the study to exclude any possible effects on CFVR. All examinations were started at 09:00 h after fasting for at least 8 h from the previous night. Oral administration of 75 g glucose (Trelan G; Takeda Pharmaceutical Co. Ltd., Tokyo, Japan) was used to induce acute hyperglycemia. Before glucose administration, left ventricular function, cardiac output, CFV and CFVR were measured. While measuring CFV and CFVR, blood pressure and heart rate were monitored. One hour after glucose administration, echocardiographic CFVR measurement was performed again. All procedures in the study were approved by the Institutional Review Board of the Center for Research Administration at the Tokyo Medical University (reference number: 206 and T2020-0333). The study protocol conformed to the principles of the Declaration of Helsinki.

Statistical Analysis

All data are expressed as mean $\pm$ SD. Changes in variables of hemodynamics, TBARS, baseline CFV, hyperemia CFV and CFVR before and 1 h after an OGTT were assessed by using the paired t-test. Correlations between blood sampling data or hemodynamic data and CFVR before or after an OGTT, change in CFVR, change in baseline CFV, and change in hyperemia CFV were also analyzed using simple linear regression analysis. Multivariate linear regression analysis, using demographic and clinical variables, was used to build models for the association between CFVR before

or after an OGTT, change in CFVR, change in baseline CFV, change in hyperemia CFV and other parameters. Comparisons of data among the patients divided into 3 groups by HOMA-R data were performed by using 1-way analysis of variance followed by Scheffé's multiple comparison test. All analyses were conducted using SPSS soft-

Table 1. Characteristics of All Study Participants

Age, years	59 $\pm$ 12
Male, n (%)	32 (80)
Current smoker, n (%)	24 (60)
Body mass index, kg/m ²	24 $\pm$ 3
Calcium channel blocker use, n (%)	17 (43)
ACEI/ARB use, n (%)	6 (15)
HMG coA RI use, n (%)	12 (30)
Hypertension, n (%)	25 (63)
IGT and DM, n (%)	24 (60)
Hemoglobin A1c, %	5.3 $\pm$ 0.4
HOMA-R	2.4 $\pm$ 1.5
Total cholesterol, mg/dL	196 $\pm$ 32
Triglyceride, mg/dL	142 $\pm$ 64
LDL cholesterol, mg/dL	115 $\pm$ 27
HDL cholesterol, mg/dL	51 $\pm$ 15
Creatinine, mg/dL	0.84 $\pm$ 0.22
eGFR, mL/min/1.73 m ²	74 $\pm$ 19
Epinephrine, pg/mL	20 $\pm$ 11
Norepinephrine, pg/mL	349 $\pm$ 133
Dopamine, pg/mL	12 $\pm$ 6
Left ventricular ejection fraction, %	66 $\pm$ 7
E/A ratio	1.0 $\pm$ 0.3
Deceleration time, ms	201 $\pm$ 46
Left ventricular mass index, g/m ²	108 $\pm$ 20

Data are presented as the mean $\pm$ standard deviation or as n (%). ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; DM, diabetes mellitus; E/A ratio, early diastolic filling velocity/atrial contraction filling velocity ratio; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HMG coA RI, hydroxymethylglutaryl CoA reductase inhibitor; HOMA-R, homeostasis model assessment of insulin resistance; IGT, impaired glucose tolerance; LDL, low-density lipoprotein; TBARS, thiobarbituric acid reactive substance. Hypertension defined as >140 mmHg of systolic blood pressure or 90 mmHg of diastolic blood pressure or anti-hypertensive medication use.

Table 2. Comparison of Parameters Before and 1 h After an OGTT

Parameter	Before	After	P value
Plasma glucose, mg/dL	98 $\pm$ 10	190 $\pm$ 43	<0.0001
TBARS, nmol/mL	2.9 $\pm$ 1.1	3.2 $\pm$ 1.2	<0.0001
Heart rate, beats/min	59 $\pm$ 10	61 $\pm$ 11	0.001
Stroke volume index, mL/m ²	52 $\pm$ 11	53 $\pm$ 13	0.39
Cardiac index, L/min/m ²	3.0 $\pm$ 0.7	3.2 $\pm$ 0.8	0.03
Systolic blood pressure, mmHg	132 $\pm$ 14	132 $\pm$ 13	0.99
Diastolic blood pressure, mmHg	77 $\pm$ 10	78 $\pm$ 9	0.55
CFV on baseline, cm/s	22.5 $\pm$ 6.6	25.7 $\pm$ 7.7	<0.0001
CFV on hyperemia, cm/s	68.5 $\pm$ 21.8	63.6 $\pm$ 21.4	0.005
CFVR	3.1 $\pm$ 0.8	2.5 $\pm$ 0.6	<0.0001

Data are presented as the mean $\pm$ standard deviation. CFV, coronary flow velocity; CFVR, coronary flow velocity reserve; OGTT, oral glucose tolerance test; TBARS, thiobarbituric acid reactive substance.

Table 3. Results of Univariate and Multivariate Analyses to Assess the Association of Change in CFVR With Demographic Data, Blood Sampling Data and Hemodynamic Data

Variables	For change in CFVR			
	Univariate analysis		Multivariate analysis ($R^2=0.305$)	
	r	P value	β	P value
Age	-0.09	0.60	-0.02	0.90
Gender	-0.15	0.37	-0.07	0.61
Current smoking	-0.05	0.76		
ACEI/ARB use	-0.26	0.10		
Calcium channel blocker use	-0.27	0.09		
HMG coA RI use	0.17	0.29		
Hypertension	-0.27	0.09		
IGT and DM	-0.28	0.08		
Body mass index	-0.31	0.06		
Hemoglobin A1c	-0.22	0.18		
HOMA-R	-0.48	0.002	-0.40	0.01
Plasma glucose 1 h after OGTT	-0.21	0.19		
Change in TBARS	-0.20	0.20		
Total cholesterol	0.23	0.15		
Triglyceride	-0.02	0.93		
HDL cholesterol	0.16	0.31		
LDL cholesterol	0.24	0.15		
Epinephrine	0.23	0.15		
Norepinephrine	0.05	0.76		
Dopamine	-0.01	0.95		
Creatinine	0.12	0.50		
eGFR	-0.12	0.48		
E/A ratio	0.25	0.12		
Deceleration time	-0.14	0.38		
Left ventricular mass index	-0.24	0.13		
Change in systolic blood pressure	-0.22	0.18		
Change in diastolic blood pressure	-0.22	0.18		
Change in heart rate	-0.40	0.01	-0.26	0.12
Change in stroke volume index	-0.05	0.76		

β , standardized coefficient; r, correlation coefficient. Other abbreviations are as per Tables 1,2. Change in each variable means the percent change in each variable before and 1 h after an oral glucose tolerance test.

ware for Windows, version 27.0J (SPSS, Inc., Chicago, IL, USA) and P values of <0.05 were considered to indicate a statistically significant difference between groups.

Results

Baseline Characteristics

Table 1 shows the patients' characteristics, including their demographic data, blood sampling data and LV function data. Discontinuation of anti-hypertensive medicine in all study patients during 2 days did not cause any adverse events under the careful monitoring of their blood pressure. The mean value of HbA1c was $5.3 \pm 0.4\%$, and HOMA-R was 2.4 ± 1.5 . HOMA-R was significantly associated with the sum of all plasma insulin concentration data on fasting, 60 min, and 120 min after an OGTT, which suggested a hyperinsulinemia state ($r=0.56$, $P=0.001$). More than 60% of the patients had hypertension.

Changes in Parameters During an OGTT

Table 2 shows the comparisons of plasma level of TBARS, hemodynamic data, CFV and CFVR during an OGTT.

Plasma level of TBARS, heart rate and CI were significantly increased after an OGTT. Although baseline CFV was significantly increased, hyperemia CFV and CFVR were significantly decreased after an OGTT.

Association of Blood and Hemodynamic Data With CFVR, and Change in CFV and CVFR

No significant correlation between CFVR before an OGTT and demographic, clinical variables and blood, and hemodynamics data was observed on multivariate analysis (**Supplementary Table 1**). CFVR after an OGTT significantly correlated with CFVR before an OGTT ($\beta=0.70$, $P<0.0001$) and HOMA-R ($\beta=-0.52$, $P<0.0001$) on multivariate analysis (**Supplementary Table 2**). Change in CFVR significantly correlated with HOMA-R and change in heart rate during an OGTT on univariate analyses. After the multivariate analysis, HOMA-R was the only factor that showed a significant correlation with change in CFVR (**Table 3**). In the next step, we analyzed change in baseline CFV and change in hyperemia CFV using univariate and multivariate analyses. Change in baseline CFV correlated with change in heart rate and HOMA-R on univariate

Table 4. Results of Univariate and Multivariate Analyses to Assess the Association of Change in Baseline CFV With Demographic Data, Blood Sampling Data and Hemodynamic Data

Variables	For change in baseline CFV			
	Univariate analysis		Multivariate analysis (R ² =0.209)	
	r	P value	β	P value
Age	0.17	0.28	0.09	0.58
Gender	0.25	0.12	0.20	0.17
Current smoking	0.17	0.30		
ACEI/ARB use	0.25	0.13		
Calcium channel blocker use	0.16	0.32		
HMG coA RI use	0.09	0.58		
Hypertension	0.31	0.051	0.15	0.35
IGT and DM	−0.04	0.83		
Body mass index	−0.05	0.76		
Hemoglobin A1c	0.05	0.76		
HOMA-R	0.44	0.004	0.35	0.03
Change in TBARS	−0.07	0.66		
Total cholesterol	−0.19	0.24		
Triglyceride	−0.11	0.49		
HDL cholesterol	0.07	0.66		
LDL cholesterol	−0.22	0.17		
Epinephrine	0.02	0.89		
Norepinephrine	0.13	0.42		
Dopamine	0.17	0.28		
Creatinine	0.06	0.71		
eGFR	−0.14	0.38		
E/A ratio	−0.28	0.09		
Deceleration time	0.15	0.36		
Left ventricular mass index	−0.01	0.93		
Change in systolic blood pressure	0.13	0.44		
Change in diastolic blood pressure	0.06	0.71		
Change in heart rate	0.34	0.03	0.13	0.43
Change in stroke volume index	−0.27	0.10		

Abbreviations are as per Tables 1–3.

analyses. After multivariate analysis, HOMA-R was the only factor that showed a significant correlation with change in baseline CFV (Table 4). In contrast, there was no significant correlation between the change in hyperemia CFV and any parameters on multivariate analysis.

Differences in CFVR Changes Among the Patients Grouped by HOMA-R

IR was defined as a HOMA-R value of >2.5. Figure shows differences in CFVR changes among the patients divided into 3 groups by the tertiles of the HOMA-R value (Left panel) and between the 2 groups with/without IR (Right panel). In the left panel, significant differences in changes in CFVR between the groups 1 and 2 ($-7\pm 14\%$ vs. $-22\pm 9\%$, $P=0.01$), and groups 1 and 3 ($-7\pm 14\%$ vs. $-25\pm 14\%$, $P=0.002$) were observed; however, there was not a significant difference between the groups 2 and 3. The right panel shows that changes in CFVR in patients with IR were significantly lower than those in patients without IR ($-26\pm 13\%$ vs. $-12\pm 12\%$, $P=0.002$).

Discussion

In this study, we assessed CFVR during an OGTT non-

invasively in patients without any myocardial ischemia and without a history of DM. Our present study demonstrated that: (1) acute hyperglycemia in response to an OGTT rapidly suppressed CFVR owing to increased baseline CFV and decreased hyperemia CFV; (2) IR, rather than oxidative stress or sympathetic activation, was independently associated with both the changes in CFVR and baseline CFV; and (3) the decline in CFVR was associated with the severity of IR.

The prediabetes state, including IFG and IGT, is characterized by postprandial hyperglycemia, which is associated with the development of specific vascular complications, and is a strong predictor for adverse outcomes in patients with coronary artery diseases.²⁵ Exposure to hyperglycemia can be described as a function of 2 components: the duration and magnitude of chronic sustained hyperglycemia and the acute fluctuations of glucose over a daily period.^{26,27} Monnier et al demonstrated a significant relationship between acute glucose fluctuations evaluated by the mean amplitude of glycemic excursions, and activation of oxidative stress, estimated from the 24-h urinary excretion rate of free 8-iso prostaglandin F₂.¹¹ Endothelium-dependent vasodilation is known to be mediated by the endothelium-derived relaxing factor, NO. Previous studies

have demonstrated that oxygen-derived free radicals interfere with endothelium-dependent vasodilation by inactivating NO in the normal coronary arteries of animal models.^{7,28} Impairment of endothelium-dependent vasodilation of the human brachial artery was associated with an increase in plasma levels of TBARS after acute hyperglycemia, and the degree of the decrease was greater in prediabetes than in normal glucose tolerance.⁵ These phenomena may moderate coronary microvascular dysfunction, which fails to reach maximal vasodilation, to the decline of CFVR after acute hyperglycemia.

In contrast, hyperinsulinemia and IR are established risk factors for atherosclerosis.²⁹ Insulin causes endothelium-dependent vasodilation by releasing NO,^{30,31} and this action is affected by defective myocardial cellular metabolism under the reduction of insulin sensitivity and glucose uptake. Picchi et al demonstrated that a reduction in coronary flow reserve was mainly caused by increased baseline coronary blood flow using an intracoronary pressure/temperature sensor-tipped guidewire in patients in the early stage of diabetes without coronary artery stenosis, and both baseline coronary blood flow and coronary flow reserve significantly correlated with IR assessed by using the HOMA index.³² They described that the relationship between increased baseline coronary blood flow and IR suggested an underlying compensatory mechanism to meet the increased oxygen demands of the early stage diabetic myocardium. The effects of acute hyperglycemia and hyperinsulinemia on myocardium or coronary flow have not been fully understood. Previous studies have demonstrated that acute hyperglycemia combined with hyperinsulinemia is associated with myocardial damage after successful percutaneous coronary intervention in patients with acute coronary syndrome.³³ Various factors such as increased pro-inflammatory cytokines and endogenous sympathetic activation can be the reasons. Subsequently, the protective effects of insulin are abolished under the

state of IR, and the membrane glucose transportation and myocardial glucose uptake can be reduced.³⁴ Acute hyperglycemia and hyperinsulinemia can cause various synergistic effects for myocardium demand ischemia. Our present study demonstrated that both changes in CFV and changes in CFVR directly evaluated by transthoracic Doppler echocardiography during an OGTT were useful for elucidation of the association between acute hyperglycemia, hyperinsulinemia and coronary microvascular dysfunction.

CFVR impairment is driven either by a reduced hyperemia CFV or by an increased baseline CFV. The contribution of these 2 mechanisms to CFVR impairment in early stage diabetic patients is still controversial. Our present study demonstrated that CFVR impairment after acute hyperglycemia was caused by an increased baseline CFV and decreased hyperemia CFV, probably through increased myocardial oxygen demands and reduced reactivity of coronary vasodilation. The degree of CFVR impairment was related to severity of IR, but was not significantly related to endogenous sympathetic activation or rapidly increased oxidative products induced by acute hyperglycemia. In addition, IR was independently related to increased baseline CFV after acute hyperglycemia. These findings suggest that factors caused by IR under repeated postprandial hyperglycemia might play an important role in the development of relative myocardial ischemia and progression of coronary microvascular dysfunction, even in the early stage of impaired glucose metabolism.

Study Limitations

In the present study, all patients were registered at a single institution. There were more subjects with either IGT or DM compared with the general population. The present study analysis was conducted on a limited number of patients, which may induce some analytic drawbacks. The principal findings of the present study were the changes in baseline CFV, hyperemia CFV and CFVR after glucose

loading. Because coronary blood flow is affected by coronary arterial diameter, CFV measured by transthoracic Doppler echocardiography might not be appropriate for measuring the absolute coronary blood flow and, therefore, confirmation of the present findings by direct measurements of the coronary blood flow would be the next logical step. Measuring CFV and CFVR of the right coronary artery and/or left circumflex coronary artery is also important in the complete evaluation of coronary microvascular function. However, because of the detection rate of CFV of these 2 vessels, the measurements of these 2 vessels were not suitable for this study protocol. Sympathetic activation induced by an OGTT could not be completely evaluated in this study. The plasma concentration of catecholamines before an OGTT could not be obtained in the protocol because of the lack of medical insurance coverage. Therefore, we placed greater importance on the plasma concentration of catecholamines after an OGTT than before an OGTT. The measurements of CFVR are affected by smoking, food, hormonal status and drugs.^{35–37} To exclude their effects, we studied men and postmenopausal women after 8 h of fasting, all of whom did not smoke and did not take any type of medication from at least 2 days before the study.

Conclusions

This study performed on a limited number of patients suggests that the decline of CFVR after acute hyperglycemia could be associated with the severity of IR, probably through increased myocardial demands and reduced reactivity of vasodilation, in patients with early stage of impaired glucose metabolism. The association of both oxidative stress and sympathetic activity with the decline of CFVR was not significant. The appropriate intervention for IR, even in the early diabetic stage, might lead to novel strategies for the prevention of diabetes-related myocardial damage.

Acknowledgments

The authors gratefully acknowledge the medical editors from the Department of International Medical Communications of the Tokyo Medical University for their English-language editing of the manuscript.

Disclosures

The authors declare no conflicts of interest and no funding grants were received for this study.

IRB Information

This study was approved by the Institutional Review Board of the Center for Research Administration at the Tokyo Medical University (Reference number: 206 and T2020-0333).

Data Availability

The deidentified participant data will not be shared.

References

- American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2021. *Diabetes Care* 2021; **44**: S15–S33.
- Jandeleit-Dahm KA, Gray SP, Insulin and cardiovascular disease: Biomarker or association? *Diabetologia* 2012; **55**: 3145–3151.
- Nakamura K, Sakurai M, Miura K, Morikawa Y, Ishizaki M, Yoshita K, et al. Homeostasis model assessment of insulin resistance and the risk of cardiovascular events in middle-aged non-diabetic Japanese men. *Diabetologia* 2010; **53**: 1894–1902.
- Cai X, Zhang Y, Li M, Wu JH, Mai L, Li J, et al. Association between prediabetes and risk of all cause mortality and cardiovascular disease: Updated meta-analysis. *BMJ* 2020; **370**: m2297.
- Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, et al. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. *J Am Coll Cardiol* 1999; **34**: 146–154.
- Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997; **96**: 25–28.
- Rubanyi GM, Vanhoutte PM. Oxygen-derived free radicals, endothelium, and responsiveness of vascular smooth muscle. *Am J Physiol* 1986; **250**: H815–H821.
- King GL, Kunisaki M, Nishio Y, Inoguchi T, Shiba T, Xia P. Biochemical and molecular mechanisms in the development of diabetic vascular complications. *Diabetes* 1996; **45**: S105–S108.
- Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. *Diabetic Care* 1996; **19**: 257–267.
- Su G, Mi S, Tao H, Li Z, Yang H, Zheng H, et al. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol* 2011; **10**: 19.
- Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; **295**: 1681–1687.
- Hoffman RP, Hausberg M, Sinkey CA, Anderson EA. Hyperglycemia without hyperinsulinemia produces both sympathetic neural activation and vasodilation in normal humans. *J Diabetes Complications* 1999; **13**: 17–22.
- Van de Borne P, Hausberg M, Hoffman RP, Mark AL, Anderson EA. Hyperinsulinemia produces cardiac vagal withdrawal and nonuniform sympathetic activation in normal subjects. *Am J Physiol* 1999; **276**: R178–R183.
- Ye S, Zhong H, Yanamadala S, Campese VM. Oxidative stress mediates the stimulation of sympathetic nerve activity in the phenol renal injury model of hypertension. *Hypertension* 2006; **48**: 309–315.
- Chen C, Wei J, AlBadri A, Zarrini P, Bairey Merz CN. Coronary microvascular dysfunction: Epidemiology, pathogenesis, prognosis, diagnosis, risk factors and therapy. *Circ J* 2016; **81**: 3–11.
- American Diabetes Association; European Association for the Study of Diabetes; International Federation of Clinical Chemistry and Laboratory Medicine; International Diabetes Federation. Consensus statement on the worldwide standardisation of the HbA1c measurement. *Diabetologia* 2007; **50**: 2042–2043.
- Devereux RB, Alonso DR, Lutas EM, Gottlieb GJ, Campo E, Sachs I, et al. Echocardiographic assessment of left ventricular hypertrophy: Comparison to necropsy findings. *Am J Cardiol* 1986; **57**: 450–458.
- Zoghbi WA, Quinones MA. Determination of cardiac output by Doppler echocardiography: A critical appraisal. *Herz* 1986; **11**: 258–268.
- Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2015; **28**: 1–39.
- Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, et al. Recommendations for evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016; **29**: 277–314.
- Cherubini A, Ruggiero C, Polidori MC, Mecocci P. Potential markers of oxidative stress in stroke. *Free Radic Biol Med* 2005; **39**: 841–852.
- Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982–992.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–419.
- World Health Organization. Report of a WHO study group. Prevention of diabetes mellitus. Geneva; World Health Organization; 1994. Tech Rep. Ser., No 844.

25. Bartnik M, Malmberg K, Norhammar A, Tenerz A, Ohrvik J, Ryden L. Newly detected abnormal glucose tolerance: An important predictor of long-term outcome after myocardial infarction. *Eur Heart J* 2004; **25**: 1990–1997.
26. Ceriello A. The possible role of postprandial hyperglycaemia in the pathogenesis of diabetic complications. *Diabetologia* 2003; **46**: M9–M16.
27. Ceriello A, Hanefeld M, Leiter L, Monnier L, Moses A, Owens D, et al. International Prandial Glucose Regulation (PGR) Study Group: Postprandial glucose regulation and diabetic complications. *Arch Intern Med* 2004; **164**: 2090–2095.
28. Gryglewski RJ, Palmer RMJ, Moncada S. Superoxide anion is involved in the breakdown of endothelium-dependent vascular relaxing factor. *Nature* 1986; **320**: 454–456.
29. Després JP, Lamarche B, Mauriège P, Cantin B, Dagenais GR, Moorjani S, et al. Hyperinsulinemia as an independent risk factor for ischemic heart disease. *N Engl J Med* 1996; **334**: 952–957.
30. Baron AD. Hemodynamic actions of insulin. *Am J Physiol* 1994; **267**: E187–E202.
31. Scherrer U, Randin D, Vollenweider P. Nitric oxide release accounts for insulin's vascular effects in humans. *J Clin Invest* 1994; **94**: 2511–2515.
32. Picchi A, Limbruno U, Focardi M, Cortese B, Micheli A, Boschi L, et al. Increased basal coronary blood flow as a cause of reduced coronary flow reserve in diabetic patients. *Am J Physiol Heart Circ Physiol* 2011; **301**: H2279–H2284.
33. Ishihara M. Acute hyperglycemia in patients with acute myocardial infarction. *Circ J* 2012; **76**: 563–571.
34. Wang Z, Wang Y, Han Y, Yin Q, Hu S, Zhao T, et al. Akt is a critical node of acute myocardial insulin resistance and cardiac dysfunction after cardiopulmonary bypass. *Life Sci* 2019; **234**: 116734.
35. Otsuka R, Watanabe H, Hirata K, Tokai K, Muro T, Yoshiyama M, et al. Acute effects of passive smoking on the coronary circulation in healthy young adults. *JAMA* 2001; **286**: 436–441.
36. Hozumi T, Eisenberg M, Sugioka K, Kokkirala AR, Watanabe H, Teragaki M, et al. Change in coronary flow reserve on trans-thoracic Doppler echocardiography after a single high-fat meal in young healthy men. *Ann Intern Med* 2002; **136**: 523–528.
37. Hirata K, Shimada K, Watanabe H, Muro T, Yoshiyama M, Takeuchi K, et al. Modulation of coronary flow velocity reserve by gender, menstrual cycle and hormone replacement therapy. *J Am Coll Cardiol* 2001; **38**: 1879–1884.

Supplementary Files

Please find supplementary file(s);
<http://dx.doi.org/10.1253/circj.CJ-21-0549>