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Hyperglycemia Rapidly Suppresses Flow-Mediated Endothelium-Dependent Vasodilation of Brachial Artery

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OBJECTIVES We examined whether endothelial dysfunction occurs when acute hyperglycemia is induced by oral glucose loading.

BACKGROUND Endothelial dysfunction has been shown to occur in patients with diabetes mellitus (DM), and chronic hyperglycemia is implicated as a cause of endothelial dysfunction. However, in many patients with Type 2 DM and in those with impaired glucose tolerance (IGT), fasting blood glucose may be within normal limits, and hyperglycemia occurred only post-prandially.

METHODS With ultrasound technique, we measured flow-mediated endothelium-dependent vasodilation during oral glucose tolerance test in 58 subjects: (17 patients with normal glucose tolerance [NGT], 24 with IGT, and 17 with type 2 DM). In addition, we measured the levels of thiobarbituric acid reactive substances (TBARS) and nitrite/nitrate.

RESULTS Flow-mediated vasodilation decreased after glucose loading (NGT: 7.53 ± 0.40, 4.24 ± 0.28 and 6.35 ± 0.40, in fasting, at 1- and 2-h, respectively, IGT: 6.50 ± 0.48, 1.40 ± 0.41** and 4.00 ± 0.47*, respectively; DM: 4.77 ± 0.37, 1.35 ± 0.38** and 1.29 ± 0.29%**, respectively; *$p < 0.01$ vs. fasting, **$p < 0.005$ vs. fasting). The TBARS concentration increased in parallel with plasma glucose level in each group (NGT: 1.43 ± 0.07, 2.03 ± 0.12 and 1.80 ± 0.12, respectively; IGT: 1.65 ± 0.11, 2.46 ± 0.12** and 1.94 ± 0.08*, respectively; DM: 1.73 ± 0.07, 2.34 ± 0.08** and 2.47 ± 0.09** nmol/ml, respectively; *$p < 0.05$ vs. fasting, **$p < 0.01$ vs. fasting). Glucose loading did not change nitrite/nitrate concentration in any of the groups.

CONCLUSIONS Hyperglycemia in response to oral glucose loading rapidly suppresses endothelium-dependent vasodilation, probably through increased production of oxygen-derived free radicals. These findings strongly suggest that prolonged and repeated post-prandial hyperglycemia may play an important role in the development and progression of atherosclerosis. (J Am Coll Cardiol 1999;34:146–54) © 1999 by the American College of Cardiology

Diabetes mellitus (DM) is one of the most common chronic diseases (1), and the predominant clinical form is Type 2 (non–insulin-dependent), which accounts for more than 90% of all cases (1,2). Most Type 2 diabetes is preceded by a symptom-free period of impaired glucose tolerance (IGT), which is characterized by a response to oral glucose load that is abnormal but does not satisfy the criteria for diabetes (1). Atherosclerosis, the main cause of coronary heart disease, occurs with higher than normal frequency in patients with diabetes and pre-diabetes, and is the major cause of morbidity and mortality in diabetic patients (1–4). However, the mechanism of the increased risk for atherosclerosis in diabetes is still controversial (2–6). The response-to-injury hypothesis of atherosclerosis states that the injurious responses initially occur to endothelium of the artery, leading to endothelial dysfunction (7). Indeed, endothelial dysfunction has been shown to occur in patients with diabetes (6–9), and chronic hyperglycemia is implicated as a cause of endothelial dysfunction (5,6,9–13). However, Type 2 diabetes and IGT are often associated with other risk factors, such as dyslipidemia, hypertension and obesity, each of which may cause endothelial dysfunction (1–3,5,14).

Furthermore, in many of these patients, fasting blood glucose may be within normal limits, and hyperglycemia occurs only post-prandially. Thus, the precise role of hyperglycemia in the genesis of endothelial dysfunction in Type 2 diabetes and IGT remains to be elucidated. Recently, noninvasive assessment of endothelial function of the brachial artery has been performed by measuring flow-mediated dilation (FMD) during reactive hyperemia with

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Abbreviations and Acronyms

ANOVA = analysis of variance
DM = diabetes mellitus
FMD = flow-mediated dilation
HDL = high-density lipoprotein
IGT = impaired glucose tolerance
LDL = low-density lipoprotein
NGT = normal glucose tolerance
NO = nitric oxide
OGTT = oral glucose tolerance test
TBARS = thiobarbituric acid reactive substances

ultrasound technique (15–19). The purpose of the present study was to examine whether endothelial dysfunction occurs when acute hyperglycemia is induced during oral glucose tolerance test (OGTT) in patients with Type 2 diabetes and in those with IGT by measuring FMD of the brachial artery. We also measured plasma levels of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxide (20), and the serum levels of nitrite/nitrate as metabolites of nitric oxide (NO) (21), the endothelium-derived relaxing factor. These levels were compared with the degree of the endothelium-dependent vasodilation in the patients.

METHODS

Study subjects. The study group comprised 58 patients (mean age, 57.8 ± 1.0 years) who were admitted in our hospital to examine coronary artery disease. All study subjects were under 70 years old, and none of them had been previously diagnosed with DM. All female subjects were postmenopausal. None had had congestive heart failure. None was receiving any kinds of drugs including hormones from at least seven days before the study. Fasting glucose levels of all the patients were <140 mg/dl, and a 75-g OGTT was performed to examine their risk factors for coronary artery disease. Both IGT and DM were diagnosed according to World Health Organization criteria (1). Twenty-four patients had IGT, 17 had Type 2 DM and 17 had normal glucose tolerance (NGT). The characteristics of the patients are shown in Table 1. Written informed consent was obtained from each patient before the study was commenced. The procedures used in the study were approved by the ethics committee at our institution.

Study design. We performed the present study during a 75-g OGTT to examine risk factors for coronary artery disease. In a fasting state, 1- and 2-h after the administration of a 75-g glucose equivalent load (Trelan-G, Takeda, Japan), FMD of the brachial artery was measured by two skillful examiners. Successively, the subjects lay quietly for 15 min. Then, sublingual nitroglycerin (0.3 mg) was administered, and 3 min later, the last measurements were performed. Response to nitroglycerin was used as a measure of endothelium-independent vasodilation. We excluded individuals with inadequate scan quality in either brachial artery before the start of the study. All studies were performed in a quiet and temperature-controlled room (22°C to 23°C).

Vasodilation responses of the brachial arteries were measured by the ultrasound technique. The validity of this method has been confirmed in our previous studies and by others (15–19). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 7.5-MHz linear array transducer (SSH-160A, Toshiba, Japan). Flow velocity in the brachial artery was measured using a pulsed Doppler signal at a 70° angle to the vessel, with the range gate (1.5 mm) in the center of the artery. The brachial artery was scanned in the antecubital fossa in a longitudinal fashion. Depth and gain settings were optimized at the beginning of the study and were kept constant throughout the recording period. When a satisfactory transducer position was found, the surface of the skin was marked, and the arm remained in the same position throughout the study. During the study, blood pressure was monitored in the other arm every 2 min by an automated blood pressure recorder.

Each subject lay quietly for 10 min before the first scan.

Table 1. Clinical Characteristics of Study Subjects

	NGT (n = 17)	IGT (n = 24)	DM (n = 17)
Age (yrs)	52.6 ± 1.8	58.5 ± 1.6	62.2 ± 1.2*
Men/Women	11/6	15/9	12/5
Body mass index (kg/m^2)	22.5 ± 0.3	23.9 ± 0.3	25.2 ± 0.4
Smoker	9 (52.9%)	15 (62.5%)	14 (82.4%)
Total cholesterol (mg/dl)	190.1 ± 5.8	206.4 ± 6.3*	216.8 ± 5.4†
HDL cholesterol (mg/dl)	47.8 ± 1.0	43.2 ± 1.1*	41.1 ± 1.2†
LDL cholesterol (mg/dl)	115.2 ± 5.7	132.8 ± 5.7*	142.2 ± 5.8†
Triglyceride (mg/dl)	135.6 ± 5.0	152.1 ± 4.7*	167.9 ± 4.4†
Increase in diameter after nitroglycerin (%)	13.9 ± 0.2	14.0 ± 0.3	14.2 ± 0.2

*$p < 0.05$ vs. NGT; †$p < 0.01$ vs. NGT. Data are expressed as mean ± SE.
DM = diabetes mellitus; HDL = high-density lipoprotein; IGT = impaired glucose tolerance; LDL = low-density lipoprotein; NGT = normal glucose tolerance.

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After baseline measurements of the diameter and the flow velocity in the brachial artery, a blood pressure cuff placed around the forearm was inflated with a pressure of 250 to 300 mm Hg and was released after 5 min. The measurements of the diameter and the flow velocity were continuously performed between the cuff inflation and after the cuff deflation. The ultrasound images were recorded on a super-VHS videocassette recorder (BR-S601M, Victor, Japan), and the arterial diameter was measured at a fixed distance from an anatomical marker with ultrasonic calipers by two independent observers. They were blinded as to not only whether the individual was in the NGT, IGT, or DM group, but also whether the images had been acquired with the patients in a fasting state, 1 or 2 h after the glucose loading.

Measurements were taken from the anterior to the posterior interface between the media and adventitia ("m" line) at the end diastole, incident with the R wave on a continuously recorded electrocardiogram (15–17,19,22). The diameters at four cardiac cycles were analyzed for each scan, and the measurements were averaged. Diameter measurements for the reactive hyperemia were taken 45 to 90 s after the cuff deflation to measure peak diameter (17). Responses of the vessel diameters to the reactive hyperemia and nitroglycerin were expressed as the percentage increase to the baseline value of the diameter. Blood flow was calculated by multiplying the velocity-time integral of the Doppler flow signal by heart rate and the vessel cross-sectional area. The increase in the blood flow was calculated by dividing the maximum flow within the first 15 s after the cuff deflation by the flow at baseline (15–17).

In our study, the inter-observer variability for the repeated measurements of resting arterial diameter was 0.05 ± 0.02 mm. The intra-observer variability for the repeated measurements of resting arterial diameter was 0.02 ± 0.02 mm. In a preliminary study, when these procedures were performed at the same time on two different days in 20 volunteers, the average intra-subject test-retest difference for the measurements of the arterial diameter during the reactive hyperemia was 0.05 ± 0.04 mm.

Blood sampling and assays. Blood specimens were obtained after a 12- to 14-h fast, and second and third specimens were obtained at 1- and 2-h after the glucose loading. The plasma glucose concentration was determined with an autoanalyzer using a glucose oxidase method. The serum insulin concentration was measured by immunoradiometric assay using an antihuman insulin antibody. The serum total cholesterol and triglyceride concentrations in the fasting state were measured enzymatically, and the serum high-density lipoprotein (HDL) cholesterol concentration was measured by heparin-Ca^{2+}/Ni^{2+} precipitation (15,23). Nitrite/nitrate, stable metabolites of NO, were measured using an autoanalyzer (TCI-NOX1000, Tokyo Kasei Kogyo, Japan), by a method based on the Griess reaction (15,21,24). Briefly, the samples were passed through a column containing copper-coated cadmium, which reduces all nitrate to nitrite. The nitrite was then detected when it reacted with Griess reagent (24). Absorbance was measured at 540 nm using a spectrophotometer (15). The plasma lipid peroxide content was determined using TBARS as markers (16,20). Briefly, 2.0 ml of trichloroacetic acid-thiobarbituric acid-HCl reagent was added to 1.0 ml of sample and vortexed. To minimize peroxidation during the assay procedure, butylated hydroxytoluene was added to the thiobarbituric acid reagent mixture. Results were expressed as malondialdehyde equivalent content (nmol MDA/ml plasma).

Statistical analysis. Comparisons of data among the three groups were performed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test. The chi-square test was used to compare the prevalence of smokers. The changes in variables were assessed by two-way ANOVA with repeated measures followed by post hoc testing with the Scheffe's test. Correlations between plasma glucose levels and FMD, between plasma glucose levels and plasma TBARS levels, between plasma TBARS levels and FMD, and between serum insulin levels and FMD were examined using linear regression analysis. Statistical significance was defined as $p < 0.05$.

RESULTS

The serum levels of total cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride were higher, and those of HDL cholesterol were lower in the IGT group and the DM group than in the NGT group (Table 1). Body mass index, mean age, and mean blood pressure tended to increase as glucose tolerance deteriorated from IGT to DM (Tables 1 and 2). The endothelium-independent vasodilation, as assessed by arterial dilation to nitroglycerin, was not different among the three groups (Table 1). The administration of a 75-g glucose equivalent load did not elicit any changes in the basal arterial diameter or the increase in blood flow during the reactive hyperemia in any group (Table 3). Heart rate, blood pressure and basal blood flow tended to increase after the oral glucose loading (Tables 2 and 3). However, the differences are not significant.

Table 2 shows the plasma glucose, the serum insulin and the serum triglyceride levels before and after oral glucose loading among the three groups. In the fasting state, both the plasma glucose and the serum insulin levels tended to increase as glucose tolerance deteriorated from IGT to DM, and there was a significant difference in the plasma glucose and the serum insulin levels between the DM group and the NGT group ($p < 0.01$). After the glucose loading, both the plasma glucose and the serum insulin levels increased markedly in each group. Plasma glucose levels were significantly higher in the IGT and the DM groups than in the NGT group at both 1 and 2 h. The levels were not different between the IGT group and the DM group at 1 h, but were significantly higher in the DM group than in the IGT group

Table 2. Hemodynamic Data and Blood Sampling Data During Oral Glucose Tolerance Test

	Fasting	1 h	2 h
NGT Group (n = 17)			
Heart rate (beats/min)	61.3 ± 1.5	62.4 ± 1.2	62.4 ± 0.9
Mean blood pressure (mm Hg)	97.8 ± 0.7	100.6 ± 1.0	101.2 ± 0.5
Plasma glucose levels (mg/dl)	90.5 ± 1.4	153.5 ± 6.3*	113.5 ± 3.4*†
Serum insulin levels (μU/ml)	8.6 ± 0.4	72.5 ± 12.9*	38.8 ± 5.1*†
Serum triglyceride levels (mg/dl)	135.6 ± 5.0	137.4 ± 4.4	134.1 ± 4.3
IGT Group (n = 24)			
Heart rate (beats/min)	60.9 ± 1.4	64.2 ± 1.3	66.0 ± 1.4
Mean blood pressure (mm Hg)	100.6 ± 0.8	104.1 ± 1.2	104.5 ± 1.1
Plasma glucose levels (mg/dl)	105.3 ± 2.8	220.2 ± 5.2*	173.3 ± 4.9*†
Serum insulin levels (μU/ml)	11.0 ± 0.4	77.6 ± 8.8*	81.4 ± 6.3*
Serum triglyceride levels (mg/dl)	152.1 ± 4.7	153.1 ± 4.6	152.6 ± 5.0
DM Group (n = 17)			
Heart rate (beats/min)	60.1 ± 1.7	63.6 ± 1.8	65.6 ± 1.1
Mean blood pressure (mm Hg)	102.7 ± 1.3	105.6 ± 1.3	106.4 ± 1.3
Plasma glucose levels (mg/dl)	128.4 ± 1.1	239.6 ± 8.8*	242.5 ± 7.7*
Serum insulin levels (μU/ml)	14.0 ± 0.9	79.4 ± 9.1*	79.2 ± 5.5*
Serum triglyceride levels (mg/dl)	167.9 ± 4.4	169.9 ± 4.2	167.5 ± 4.3

*$p < 0.005$, vs. Fasting; †$p < 0.01$, vs. 1 h. Data are expressed as mean ± SE.
DM = diabetes mellitus; IGT = impaired glucose tolerance; NGT = normal glucose tolerance.

at 2 h. Serum insulin levels were not significantly different among the three groups at 1 h, but they were significantly higher in the IGT group and the DM group than in the NGT group at 2 h. There was no difference in the levels between the IGT group and the DM group at 2 h. The administration of a 75-g glucose equivalent load did not elicit any changes in the serum triglyceride levels within each group.

Table 3. Brachial Arterial Data During Oral Glucose Tolerance Test

	Fasting	1 h	2 h
NGT Group (n = 17)			
Baseline arterial diameter (mm)	3.72 ± 0.11	3.73 ± 0.10	3.74 ± 0.10
Baseline blood flow (ml/min)	197.9 ± 6.4	200.5 ± 7.0	201.8 ± 5.3
Increase in peak blood flow during reactive hyperemia (%)	249.6 ± 9.9	250.6 ± 10.6	253.1 ± 10.7
Flow-mediated dilation of brachial artery (%)	7.53 ± 0.40	4.24 ± 0.28	6.35 ± 0.40
IGT group (n = 24)			
Baseline arterial diameter (mm)	3.66 ± 0.09	3.67 ± 0.09	3.69 ± 0.10
Baseline blood flow (ml/min)	198.8 ± 4.3	205.6 ± 5.3	206.9 ± 5.3
Increase in peak blood flow during reactive hyperemia (%)	257.0 ± 9.6	254.5 ± 10.5	256.5 ± 10.4
Flow-mediated dilation of brachial artery (%)	6.50 ± 0.48	1.40 ± 0.41†	4.00 ± 0.47*‡
DM Group (n = 17)			
Baseline arterial diameter (mm)	3.67 ± 0.11	3.74 ± 0.10	3.73 ± 0.11
Baseline blood flow (ml/min)	199.2 ± 4.6	204.6 ± 7.1	206.6 ± 5.2
Increase in peak blood flow during reactive hyperemia (%)	265.4 ± 8.8	249.9 ± 10.8	253.6 ± 10.6
Flow-mediated dilation of brachial artery (%)	4.77 ± 0.37	1.35 ± 0.38†	1.29 ± 0.29†

*$p < 0.01$, vs. Fasting; †$p < 0.005$, vs. Fasting; ‡$p < 0.01$, vs. 1 h. Data are expressed as mean ± SE.
DM = diabetes mellitus; IGT = impaired glucose tolerance; NGT = normal glucose tolerance.

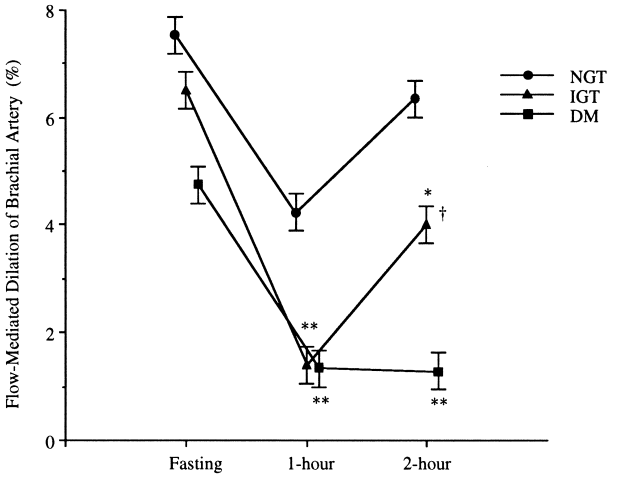

Figure 1. Effects of oral glucose loading on the flow-mediated dilation (FMD) of the brachial artery. There was a significant difference in FMD among the three groups ($p < 0.01$, by ANOVA). DM: diabetes mellitus; IGT: impaired glucose tolerance; NGT: normal glucose tolerance. Data are expressed as mean ± SE. *$p < 0.01$, versus fasting; **$p < 0.005$, versus fasting; †$p < 0.01$, versus 1 h.

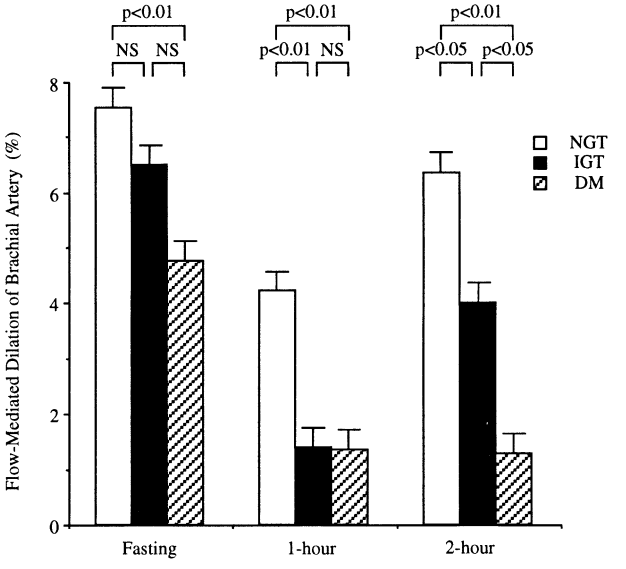

Figure 2. Comparison of the flow-mediated dilation of the brachial artery in the fasting state, 1 and 2 h after oral glucose loading among the three groups. DM: diabetes mellitus; IGT: impaired glucose tolerance; NGT: normal glucose tolerance. Data are expressed as mean ± SE.

Figure 1 and Table 3 show the flow-mediated endothelium-dependent vasodilation in response to oral glucose loading in the three groups. Flow-mediated dilation was diminished at 1 h in the IGT group, and it tended to increase toward baseline levels at 2 h but was still lower than the fasting levels. In the NGT group, FMD also tended to decrease at 1 h after the glucose loading and returned to the baseline levels at 2 h. Conversely, in the DM group, FMD was diminished at 1 h and remained diminished at similar levels at 2 h. There was a significant difference in the effect of the glucose loading on FMD among the three groups ($p < 0.01$, by ANOVA).

Figure 2 compares FMD in response to glucose loading among the three groups. In the fasting state, FMD tended to decrease as glucose tolerance deteriorated from IGT to DM, and there was a significant difference in the degree of FMD between the DM group and the NGT group ($p < 0.01$). At 1 h, FMD was more reduced in both the IGT and the DM group than in the NGT group ($p < 0.01$). There was no difference in the degree of reduction between the IGT group and the DM group. At 2 h, FMD was highest in the NGT group and was lowest in the DM group ($p < 0.05$, IGT vs. NGT and DM; $p < 0.01$, NGT vs. DM).

Figure 3 shows the plasma levels of TBARS in response to glucose loading in the three groups. In the NGT group, the plasma TBARS levels tended to increase at 1 h and to decrease toward the fasting levels at 2 h after the glucose loading. However, this was not significant (1.43 ± 0.07, 2.03 ± 0.12 and 1.80 ± 0.12 nmol/ml, in fasting, at 1 and 2 h, respectively). The levels were increased at 1 h and were decreased but still higher than the fasting levels at 2 h in the IGT group (1.65 ± 0.11, 2.46 ± 0.12**, and 1.94 ± 0.08* nmol/ml, respectively: *$p < 0.05$ vs. fasting, **$p < 0.01$, vs. fasting). In the DM group, the levels were increased at 1 h and remained increased at 2 h (1.73 ± 0.07, 2.34 ± 0.08*, and 2.47 ± 0.09* nmol/ml, respectively: *$p < 0.01$, vs. fasting). There was a significant difference in the effect of the glucose loading on the plasma TBARS levels among the three groups ($p < 0.01$ by ANOVA).

Figure 4 compares the plasma TBARS levels in response to glucose loading among the three groups. In the fasting

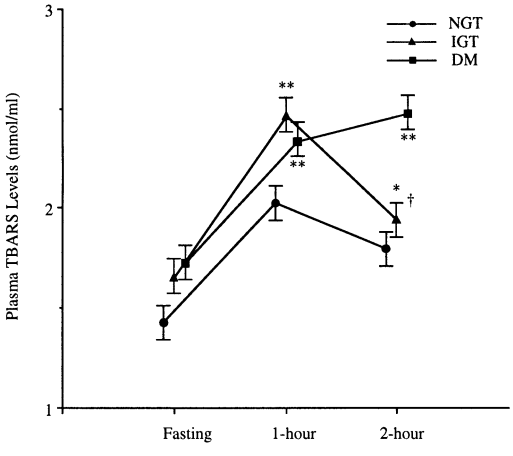

Figure 3. Effects of oral glucose loading on plasma TBARS levels. There was a significant difference in the plasma TBARS levels among the three groups ($p < 0.01$ by ANOVA). DM: diabetes mellitus; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; TBARS: thiobarbituric acid reactive substances. Data are expressed as mean ± SE. *$p < 0.05$, versus fasting; **$p < 0.01$, versus fasting; †$p < 0.05$, versus 1 h.

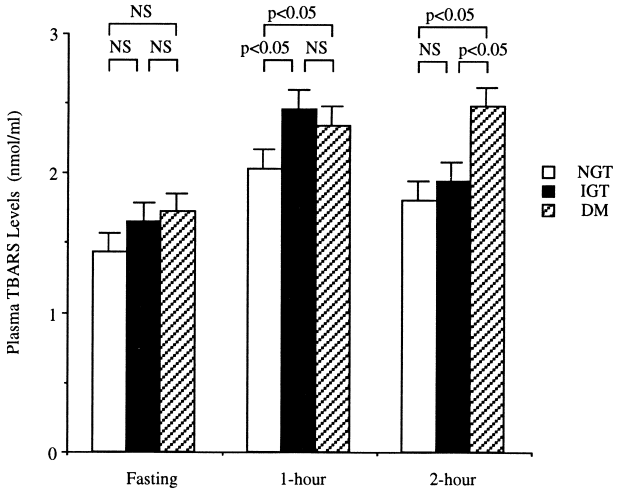

Figure 4. Comparison of plasma TBARS levels in the fasting state, 1 and 2 h after oral glucose loading among the three groups. DM: diabetes mellitus; IGT: impaired glucose tolerance; NGT: normal glucose tolerance; TBARS: thiobarbituric acid reactive substances. Data are expressed as mean ± SE.

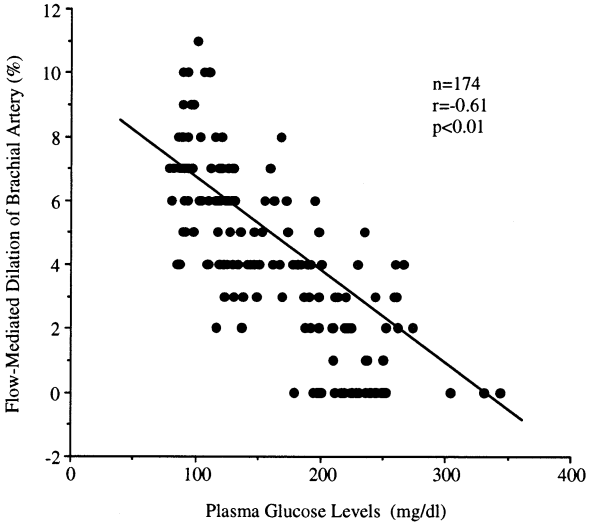

Figure 5. Correlation between the flow-mediated dilation of the brachial artery and the plasma glucose levels. There was a significant negative correlation between them.

state, no differences existed in the levels among the three groups. At 1 h, the levels were higher in both the IGT group and the DM group than in the NGT group ($p < 0.05$, NGT vs. IGT and DM), and no significant difference occurred in the levels between the IGT group and the DM group. In contrast, at 2 h, the levels in the IGT group were comparable to those in the NGT group, whereas the levels were higher in the DM group than in the NGT and the IGT groups ($p < 0.05$, DM vs. NGT and IGT).

No differences were seen in the serum levels of nitrite/nitrate among the three groups in the fasting state. The glucose loading did not significantly change the levels in any of the groups (NGT, 66.57 ± 5.52, 65.62 ± 3.40, and 62.55 ± 3.11 μmol/liter, in fasting, at 1 and 2 h, respectively: NS; IGT, 67.66 ± 4.73, 64.97 ± 4.37 and 62.67 ± 4.12 μmol/liter, respectively: NS; DM, 67.30 ± 5.57, 65.52 ± 5.44 and 64.25 ± 5.80 μmol/liter, respectively: NS).

The plasma glucose levels showed a significant negative correlation with FMD ($r = -0.61$, $p < 0.01$) and a significant positive correlation with plasma TBARS levels ($r = 0.62$, $p < 0.01$), (Fig. 5 and 6). There was also a significant correlation in FMD and plasma TBARS levels ($r = -0.58$, $p < 0.01$). In contrast, there was no significant correlation between FMD and serum insulin levels.

DISCUSSION

There is widespread agreement that diabetes increases the risk of coronary heart disease (1–4). However, the mechanism of this increased risk is controversial (2–6,25). Conversely, Type 2 diabetes and IGT are commonly associated with other risk factors, such as dyslipidemia, hypertension and obesity (1–5,14). This was also shown in this study: Total cholesterol, LDL cholesterol and triglyceride levels were higher, HDL cholesterol was lower and blood pressure, body mass index and prevalence of smokers tended to increase in the DM and the IGT groups compared with the NGT group. These risk factors may explain the reason why the flow-mediated endothelium-dependent vasodilation in the fasting state was reduced in the IGT group and the DM group as compared with the NGT group.

Hyperglycemia may have a specific role in the increased risk for atherosclerosis in patients with diabetes and IGT (12,26). Although hyperglycemia is clearly related to diabetic microvascular complications (12), its contribution to

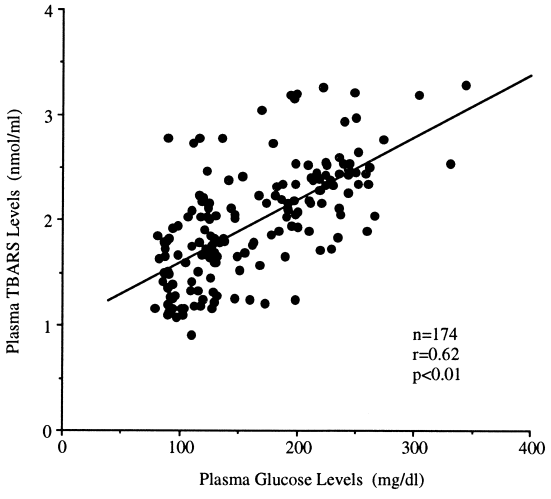

Figure 6. Correlation between the plasma TBARS levels and the plasma glucose levels. There was a significant positive correlation between them. TBARS: thiobarbituric acid reactive substances.

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increased risk for atherosclerosis in Type 2 diabetes remains controversial.

Despite the lack of data to support a relationship between the degree of hyperglycemia and the occurrence of coronary heart disease in Type 2 diabetes, there is evidence for an association between glycemia and coronary heart disease in populations in which most people are not diabetic (2,27). Nathan and co-workers suggested that risk for coronary heart disease in patients with glucose intolerance may be continuous and graded as other risk factors, such as lipids or blood pressure (2,3). The recent 20-year follow-up for the Whitehall Study, the Paris Study and the Helsinki Policemen Study also supports this concept (28).

The changes of endothelial function after glucose loading. The present study showed, for the first time, that the flow-mediated endothelium-dependent vasodilation of the brachial artery was rapidly reduced after glucose loading and that a significant negative correlation occurred between the flow-mediated endothelium-dependent vasodilation and the plasma glucose levels in humans. These results indicate that an elevated glucose concentration rapidly induces endothelial dysfunction and are in agreement with those of the previous in vitro and ex vivo studies in other species (29,30). It is worth noting that, even in the NGT subjects and the IGT patients whose fasting plasma glucose levels were within normal limits, the endothelium-dependent vasodilation was rapidly impaired in response to glucose loading. Endothelial function returned to the baseline levels after 2 h in the NGT group, but remained reduced in the IGT group and the DM group (in the latter more severely). Thus, it is possible that prolonged and repeated exposure to postprandial hyperglycemia may play an important role in the development and acceleration of atherosclerosis, even in those who have normal fasting plasma glucose levels. This supports the idea proposed by Nathan and his co-workers regarding the risk of glycemia for atherosclerosis (2,3).

Possible mechanisms of endothelial dysfunction after glucose loading. Endothelium-dependent vasodilation has been shown to be mediated by the endothelium-derived relaxing factors, which are now identified as NO (21). Previous studies have established that oxygen-derived free radicals interfere with or destroy endothelium-dependent vasodilation by inactivating NO in normal vessels (31,32). In the present study, the endothelium-dependent vasodilation of the brachial conduit artery decreased in association with an increase in plasma levels of TBARS, a marker of oxygen-derived free radicals. In contrast, the serum levels of nitrite/nitrate, the metabolites and the marker for production of NO (21) did not change in any of the groups after the glucose loading. Because the nitrite/nitrate concentration includes the oxidative products of NO (33), hyperglycemia rapidly suppresses the endothelium-dependent vasodilation, probably through an increase of oxygen-derived free radicals, and results in a quenching of NO, and not through a decrease of production/release of NO. This is in agreement with the results in vitro studies by previous workers (9,29,30,34). Changes of the serum triglyceride levels after a meal also affect FMD of the brachial artery (19). However, there were no changes in the serum triglyceride levels throughout OGTT in any of the groups in the present study.

The sources of oxygen-derived free radicals during acute hyperglycemia may include a variety of mechanisms (35–37). These include autoxidation, the nonenzymatic glycation of proteins due to extended exposure to hyperglycemia, the metabolism of glucose via aldose reductase with changes in sorbitol-myoinositol concentrations and the increased de novo synthesis of diacylglycerol from glycolytic intermediates and subsequent activation of the protein kinase C pathway (36,37). However, nonenzymatic glycation processes do not account for the rapid increase of oxidative products in response to acute hyperglycemia because the glycation processes occur slowly over days to weeks (11).

Hyperinsulinemia and insulin resistance have been shown to be risk factors for atherosclerosis (38,39), and the serum levels of insulin increased in response to glucose loading in this study. However, the increased insulin after the glucose loading probably may not play an important role in the acute induction of the endothelial dysfunction in the present study; this is because there was no significant relationship between FMD and the serum insulin levels. Insulin causes endothelium-dependent vasodilation by releasing NO (40,41), and this action may be impaired in the presence of hyperglycemia.

Clinical implications. Endothelium-derived NO inhibits platelet aggregation and development of atherosclerosis (21). Impairment of the endothelium-dependent vasodilation has been shown to be related to the pathophysiology of vascular diseases (42). The increased concentrations of oxygen-derived free radicals associated with elevated glucose level indicate failure in protective mechanisms. A close relationship was recognized between the magnitude of the endothelium-dependent vasodilation in the coronary arteries and that in the brachial arteries (16,18). The present results suggest that the increased oxidative stress during exposure to elevated glucose plays an important role in the morbidity/mortality of the cardiovascular disease as any other known risk factors for atherosclerosis (7).

Reactive hyperemia after temporary interruption of the blood flow may result from an interplay between physical (myogenic) and local metabolic factors, including prostaglandins and adenosine (43,44). Increased wall shear stress owing to an increase in the blood flow results in the production of the endothelium-derived NO (45,46). We have demonstrated that after transient arterial occlusion, NO plays a major role in the duration of hyperemia or flow debt repayment but not in the peak reactive hyperemia (47). In fact, local administration of the NO synthase inhibitor N^G-monomethyl-L-arginine did not affect the peak increase in blood flow during the reactive hyperemia in human

peripheral conduit arteries (48). This may explain why the comparable increases were observed in the peak blood flow among the three groups, irrespective of the differences in the FMD during OGTT.

Study limitations. In the present study, patients were hospitalized at our institution so we could examine coronary artery disease. Thus, there were more patients with IGT and those with DM as compared with the general population.

The principal finding of the present study was the decrease in the flow-mediated endothelium-dependent vasodilation after glucose loading. Because FMD was affected by the size of the baseline arterial diameter, each patient rested on a bed during the study to exclude its effect. In fact, in each group, the size of the baseline arterial diameter did not change before and after the glucose loading. In contrast, a slight increase occurred in the heart rate, blood pressure and baseline blood flow after the oral glucose loading. This may be due to increase of cardiac output and its redistribution to other tissue regions such as splanchnic bed.

The measurements of nitrite/nitrate and TBARS are influenced by smoke, food, hormonal status and drugs (15,16,21,49). To exclude their effects, we studied men and postmenopausal women after 12- to 14-h fast, all of whom did not smoke or receive any type of drugs from at least seven days before the study.

Conclusions. Finally, hyperglycemia in response to oral glucose loading rapidly suppresses endothelium-dependent vasodilation, probably through an increased production of oxygen-derived free radicals in humans. These findings strongly suggest that prolonged and repeated post-prandial hyperglycemia may play an important role in the development and progression of atherosclerosis.

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