Effect of simulated postprandial hyperglycemia on coronary blood flow in cardiac transplant recipients

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McNulty PH, Tulli MA, Robertson BJ, Lendel V, Harach LA, Scott S, Boehmer JP. Effect of simulated postprandial hyperglycemia on coronary blood flow in cardiac transplant recipients. Am J Physiol Heart Circ Physiol 293: H103–H108, 2007. First published March 16, 2007; doi:10.1152/ajpheart.00779.2006.—Patients with diabetes mellitus exhibit postprandial hyperglycemia, systemic oxidative stress, impaired endothelium-dependent, nitric oxide (NO)-mediated coronary artery dilatation, and an increased incidence of coronary events. Whether hyperglycemia causally mediates these associations is unknown. To test the hypothesis that postprandial hyperglycemia acutely impairs coronary endothelial function in humans, we compared the ability of the endothelium-dependent vasodilator acetylsalicylic acid to increase conduit coronary diameter (the macrovascular response) and coronary blood flow velocity (the microvascular response) in 12 cardiac transplant recipients without diabetes before and after blood glucose was raised from 6.7 ± 1.3 mmol/l (121 ± 24 mg/dl) to 17.8 ± 1.5 mmol/l (321 ± 27 mg/dl) for 1 h. Hyperglycemia acutely doubled circulating levels of the oxidation product malondialdehyde, indicating systemic oxidative stress, but did not affect the ability of acetylcholine to dilate conduit coronary segments or accelerate coronary blood flow. We conclude that the oxidative stress associated with a single acute episode of hyperglycemia affects neither acetylcholine-mediated coronary endothelial NO release nor the subsequent bioavailability, metabolism, or action of NO within the coronary circulation of cardiac transplant recipients. These observations imply that the relationship among hyperglycemia, oxidative stress, and coronary endothelial dysfunction is presumably mediated by mechanisms operating over longer periods of time.

diabetes mellitus; coronary artery; coronary endothelial dysfunction

ONE OF THE EARLIEST ABNORMALITIES identified in both animal models of diabetes mellitus (29, 34) and humans with the disease (31, 32, 37) is impairment in the ability of the coronary endothelium to effect nitric oxide (NO)-mediated arterial dilatation. Several lines of evidence suggest this may be mediated by hyperglycemia. Hyperglycemia is the hallmark of diabetes and one of its earliest features. In vitro, hyperglycemia accelerates glucose consumption by endothelial cells resulting in cytosolic accumulation of diacylglycerol, activation of protein kinase C, and inhibition of endothelial NO synthase (eNOS) (12, 43). Hyperglycemia also fuels the formation of reactive oxygen species (ROS) and of advanced glycosylation end products of endovascular proteins, both of which quench NO in vitro (5, 26). Perfusioning the isolated rat heart with hyperglycemic solution for 1 h accelerates venous washout of nitrotyrosine (a byproduct of the reaction of ROS with NO) and increases coronary vascular resistance (9). Hyperglycemia may also stimulate the elaboration of substances [e.g., endothelin-1 (24, 35), tumor necrosis factor-α (11, 30)], which indirectly oppose endothelium-dependent vasodilatation (46).

Both high dietary glucose consumption (25) and postprandial hyperglycemia (7, 8, 17) are recognized to increase the risk of adverse clinical coronary events. Whether hyperglycemia itself mediates this risk, by episodically impairing endothelium-dependent coronary dilatation and thereby interfering with minute-to-minute control of coronary tone, is a question of considerable clinical importance. We used selective coronary angiography and intracoronary Doppler ultrasonography to test the hypothesis that acutely raising circulating glucose concentration from a fasting to a supraphysiological level acutely reduces the capacity for endothelium-dependent coronary arterial dilatation in human subjects.

RESEARCH DESIGN AND METHODS

Study subjects. The study was approved by the Institutional Review Board of The Pennsylvania State University College of Medicine, and all subjects gave written consent. Experiments were performed in cardiac transplant patients undergoing annual coronary angiography to screen for graft coronary artery disease. Transplant patients are frequently glucose intolerant, and they develop an accelerated form of coronary atherosclerosis of which the pathogenesis is incompletely understood. However, because they usually carry the hearts of healthy young organ donors, transplant patients typically exhibit robust endothelium-dependent coronary responses, especially early after transplantation (18, 23). They are consequently a uniquely convenient population in which to test the study hypothesis.

Twelve heart transplant patients with no history of diabetes were studied in the morning after an overnight fast. All subjects were being treated with cyclosporine or tacrolimus and a statin drug, but none were receiving corticosteroids. Clinical and demographic characteristics are listed in Table 1.

Study protocol. Transfemoral right and left heart catheterization and selective coronary angiography were performed in standard fashion using a low-osmolality radiocontrast agent. After we waited 15 min for radiocontrast to clear the coronary circulation, heparin sodium was administered by intravenous bolus in a dose of 60 U/kg body mass to prevent coronary thrombosis during the research protocol. Blood-activated coagulation time (ACT) was measured at intervals during each study and was maintained >200 s by administration of additional heparin if needed. A size 6-Fr guiding catheter was positioned in the left main coronary artery and used to introduce a 0.014-in. diameter Doppler flow wire (FloMap system, Joned, Rancho Cordova, CA) into the proximal left anterior descending (LAD) coronary artery. This device incorporates a 20-MHz Doppler transducer, which generates a continuous, high-fidelity recording of the velocity of red blood cells traversing a 0.5-cm³ volume immediately distal to the wire.

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tip within the coronary artery lumen. The wire’s position was adjusted to give an optimal Doppler spectral signal, and this position was recorded by digital angiography to allow confirmation of stable wire position throughout each study.

After a sample of femoral arterial blood was removed for chemical analyses, LAD coronary vasodilator reserve was measured as previously reported (28). Briefly, endothelium-dependent microvascular dilatation was measured by recording the coronary flow velocity response to sequential 3-min infusions of a 20 µg/ml solution of the endothelium-dependent dilator acetylcholine (Clinalfa) administered through the coronary guiding catheter at rates of 0.5 and 1.0 ml/min (10 and 20 µg/min) calculated to achieve estimated concentrations in coronary capillary blood of 100 and 200 µg/l (0.7 and 1.4 × 10⁻⁶ M), respectively. At the end of the 20 µg/min infusion, selective left coronary angiography was repeated, and endothelium-dependent macrovascular dilatation was calculated as the change in diameter of the LAD coronary segment containing the Doppler wire in response to acetylcholine. After 3 min was allowed for the acetylcholine effect to dissipate, endothelium-independent vasodilatation was determined by measuring the LAD coronary flow velocity response to a 40-µg intracoronary injection of adenosine (Fujisawa) calculated to achieve an estimated concentration in coronary capillary blood of 400 µg/l (1.5 × 10⁻⁶ M). Subjects then received a primed (12.5 g over 1 min), continuous (0.8 g/min) 60-min intravenous infusion of glucose, and measurements were repeated in the hyperglycemic state.

Chemical analyses. Glucose concentration was measured in whole blood using an automated glucose oxidase analyzer (Analox Instruments). Insulin concentration was measured in plasma by radioimmunoassay. Plasma free fatty acids (FFA) were measured using a colorimetric assay kit (Fisher Scientific). Oxidative stress is known to form malondialdehyde [detectable in plasma as a thiobarbituric acid-reactive substance (TBARS)] and rise in plasma insulin concentration (19). Because glucose infusion affected neither systemic arterial blood pressure nor heart rate, we used the known data for glucose infusion.

LAD coronary blood flow. To calculate a surrogate of actual volumetric LAD coronary blood flow (cm³/s) under each of the experimental conditions examined, measured peak diastolic blood flow velocity (cm/s) was multiplied by the calculated cross-sectional area (cm² = π × coronary radius²) of the coronary artery segment containing the tip of the Doppler. For this analysis it was assumed that the LAD coronary has a circular cross section. It is recognized that the quantity so calculated will be an overestimation of true LAD coronary blood flow volume, the calculation of which would require multiplying LAD cross-sectional area by a factor integrating instantaneous blood flow velocity during systole and diastole throughout each cardiac cycle.

Calculations. Continuous variables were compared between basal and hyperglycemic conditions using analysis of variance. Average flow velocities during each 10-min interval were compared using repeated measures analysis of variance with the Bonferroni test for repeated measures. Differences are considered significant if P < 0.05.

RESULTS

Angiographic and hemodynamic measurements. Demographic and metabolic data are shown in Table 1 and hemodynamic data are shown in Table 2. No subject’s coronary angiogram demonstrated significant arteriopathy. Baseline systemic arterial, pulmonary arterial, and pulmonary capillary wedge pressures were within normal limits in all subjects, and glucose infusion affected neither systemic arterial blood pressure nor heart rate.

Metabolic parameters. Individual metabolic data are shown in Table 2. Blood glucose concentration averaged 6.7 ± 1.3 mmol/l (121 ± 24 mg/dl) in the basal fasting state (normal range: 4.0–6.5 mmol/l) and rose to 17.8 ± 1.5 mmol/l (321 ± 27 mg/dl) during the 60-min glucose infusion (Fig. 2). This was accompanied by a rise in plasma insulin concentration from 151 ± 100 pmol/l (19 ± 12 µU/ml) to 520 ± 200 pmol/l (65 ± 25 µU/ml) and a corresponding fall in plasma FFA concentration from 0.91 ± 0.18 to 0.42 ± 0.13 mmol/l. Serum TBARS concentration rose more than twofold during glucose infusion from 0.44 ± 0.10 to 1.12 ± 0.13 mmol/l.
Coronary flow velocity. LAD coronary flow velocity averaged $27 \pm 6$ cm/s in the fasting state (Fig. 3). Average flow velocity did not change significantly during the first 40 min of glucose infusion, but the 50-min value exceeded the basal value for LAD flow velocity in 10 of 12 subjects ($27 \pm 6$ cm/s, $P = 0.25$) suggesting a possible mild coronary vasodilator effect of hyperglycemia and/or hyperinsulinemia.

Endothelium-independent and -dependent microvascular coronary dilatation. In the basal state, intracoronary acetylcholine infusion was associated with a $44 \pm 17\%$ increase in LAD coronary flow velocity at the $10 \mu g/min$ dose and a $100 \pm 28\%$ increase at the $20 \mu g/min$ dose, whereas infusion of adenosine was associated with a $288 \pm 23\%$ increase. When subjects were separated into two groups on the basis of time elapsed since transplantation, the more recently transplanted group ($n = 6$, elapsed time = $2 \pm 1$ y) had a greater ($P < 0.05$) average acetylcholine-mediated flow velocity response ($140 \pm 24\%$ increase at the $20 \mu g/min$ dose) than the more remotely transplanted group ($n = 6$, elapsed time = $4 \pm 2$ y, $60 \pm 15\%$ increase at the $20 \mu g/min$ dose). Time elapsed since transplantation had no effect on the coronary flow response to adenosine. Coronary flow responses to acetylcholine and adenosine in the hyperglycemic state were nearly identical to those observed in the basal state for the 12 subjects, whether considered together (Fig. 4) or analyzed on the basis of time elapsed since transplantation (data not shown).

Endothelium-dependent macrovascular coronary dilatation. In the basal state, the $20 \mu g/min$ acetylcholine infusion increased LAD coronary diameter by $6 \pm 4\%$ from $3.3 \pm 0.3$ to $3.5 \pm 0.4$ mm ($P < 0.01$), whereas in the hyperglycemic state LAD diameter was $3.3 \pm 0.4$ mm before and $3.5 \pm 0.3$ mm after acetylcholine ($P = 0.01$) ($P = $ not significant vs. basal postacetylcholine diameter).

DISCUSSION

Hyperglycemic patients with diabetes mellitus exhibit impaired endothelium-dependent vasodilatation in both the pe-
ripheral (21, 44) and coronary (20, 37, 43) circulation. Longitudinal studies have demonstrated this impairment to be associated with a substantially increased risk of adverse coronary events (2). Previous studies testing whether hyperglycemia per se confers this impairment have been limited to the limb circulation and have yielded mixed results. Thus, whereas some investigators have reported that hyperglycemic exposures ranging in duration from 1 to 6 h impair endothelium-dependent vasodilation in the forearm circulation of healthy subjects (1, 4, 22, 42, 45), others have observed no such impairment (19, 38) even after hyperglycemic exposure for 24 h (19).

In the present study, which we believe is the first to examine this question directly in the human coronary circulation, we sought to mimic the physiological state produced by ingestion of a high-glucose meal by glucose-intolerant patients (i.e., temporary systemic hyperglycemia, endogenous insulin secretion, fall in circulating FFA levels) and measured both macrovascular and microvascular responses to the potent endothelium-dependent vasodilator acetylcholine. Study subjects exhibited moderate fasting elevations in blood levels of glucose and insulin, consistent with systemic metabolic insulin resistance. Despite the known association of insulin resistance with impaired endothelium-dependent vasodilation (29, 31–32, 34, 37), they nevertheless demonstrated robust basal coronary vasodilator responses to acetylcholine. This is consistent with previous observations that cardiac grafts obtained from healthy young donors retain relatively normal coronary endothelial function for several years after transplantation, even in middle-aged recipients who exhibit conditions (e.g., systemic insulin resistance) normally associated with vascular endothelial dysfunction and who demonstrate endothelial dysfunction in non-coronary vascular beds (18, 23). We note that some observers have described substantial coronary endothelial dysfunction, associated with elevated circulating levels of oxidized low-density lipoprotein cholesterol, in cardiac transplant recipients examined early after transplantation (13). The more normal endothelium-dependent coronary responses we observed may reflect the universal use of statins by our subjects (40) and the more complete recovery of their cardiac grafts from perioperative ischemic injury (36).

In our subjects, hyperglycemic glucose infusion more than doubled serum concentrations of TBARS, suggesting increased vascular ROS formation (14) but did not acutely impair the ability of acetylcholine to dilate either capacitance coronary arteries or the coronary microcirculation. This implies that, in nondiabetic subjects, a single episode of acute hyperglycemia affects neither acetylcholine-mediated coronary endothelial NO release nor the subsequent bioavailability, metabolism, or action of endothelium-derived NO within the coronary circulation.

Insulin, like acetylcholine, has been reported to stimulate endothelial NO secretion and produce endothelium-dependent vasodilation in several tissues, including the heart (3, 27, 41). We previously observed that physiological hyperinsulinemia

![Fig. 3. Coronary flow velocity measured by Doppler ultrasonography in the left anterior descending (LAD) coronary artery during hyperglycemic glucose infusion. Data represent means ± SD in 12 subjects. *P < 0.05 vs. basal.](http://ajpheart.physiology.org/)

![Fig. 4. Effect of acetylcholine (10 μg/min (ACh-10) or 20 μg/min (ACh-20) coronary infusion] or adenosine (40 μg coronary bolus) on LAD coronary flow velocity (A) and LAD coronary blood flow (B). Open circles are observations made in the basal state at blood glucose = 6.7 ± 1.3 mmol/l; closed circles are observations made during the final 5 min of a 60-min hyperglycemic glucose infusion, at blood glucose = 17.8 ± 1.5 mmol/l. *P < 0.05 vs. value at basal glucose level.](http://ajpheart.physiology.org/)
modestly increased coronary blood flow in subjects without diabetes (43), but that this effect was absent in patients with Type 2 diabetes who were studied under hyperglycemic conditions (20). In the present study, a 1-h glucose infusion induced endogenous insulin secretion. Coincident with this, we observed a modest increase in LAD coronary flow velocity in 10 of 12 subjects (Fig. 3), despite the concomitant presence of hyperglycemia. This suggests that the impairment in coronary vasodilator response to insulin we previously observed in hyperglycemic patients with diabetes (20) likewise cannot be entirely attributed to hyperglycemia.

FFA have been hypothesized to affect both endothelium-dependent and endothelium-independent vasodilator responses by reducing eNOS activity (12) and increasing blood viscosity (39) respectively. Whereas little information is available about the influence of blood FFA concentration on coronary blood flow in vivo in humans, this hypothesis has potentially important clinical implications. For example, it would predict that the diurnal variation in FFA levels associated with normal feeding and fasting cycles might produce a corresponding diurnal variation in coronary vasodilator reserve. Our failure to observe an amplification of the coronary flow velocity response to acetylcholine or adenosine associated with the fall in plasma FFA level from 0.9 to 0.4 mmol/l in our experiment suggests that changes in circulating FFA level within the physiological range probably do not exert a clinically significant influence on coronary vasodilator reserve in humans.

Several limitations must be acknowledged. Safety considerations related to performing invasive studies within the coronary circulation of volunteer subjects limited the duration of the experiments to about 1 h. The possibility that longer duration exposure to hyperglycemia, or repetitive exposure, might impair acetylcholine-mediated coronary dilatation cannot be excluded. Likewise, we cannot exclude that hyperglycemia might have impaired the coronary dilator response to endothelial stimulants (e.g., shear stress) other than acetylcholine. Whereas the choice of cardiac transplant recipients as study subjects ensured a robust basal endothelium-dependent coronary flow response against which to measure potential effects of hyperglycemia, we acknowledge that because the transplanted heart is de-energized, our study could not have detected neurally mediated effects of hyperglycemia on coronary flow. Our subjects had no evidence of coronary artery disease as judged by selective coronary angiography. We acknowledge that they may nevertheless have had subclinical coronary atherosclerosis and that this could have affected their responsiveness to vasoactive stimuli. Likewise, we studied subjects at only one point in time along the continuum of coronary endothelial dysfunction and cannot exclude that abnormal coronary responses to hyperglycemia might be present at earlier or later time points.

In contrast to the present observations in nondiabetic subjects, studies in the peripheral circulation have suggested that hyperglycemia may impair endothelium-dependent vasodilatation in patients with diabetes (6, 10). If this suggestion is correct, the negative findings of the present study would logically suggest that one or more diabetes-specific factors may play a permissive role in mediating the effects of hyperglycemia on the coronary endothelium. Additional studies testing the effect of hyperglycemia on endothelium-dependent coronary vasodilatation in patients with diabetes will be necessary to test this hypothesis.

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