

# Acute Hyperglycemia Attenuates Endothelium-Dependent Vasodilation in Humans In Vivo

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**Background**—Endothelial function is impaired in patients with diabetes mellitus. However, the factors contributing to this defect are currently unknown. Hyperglycemia attenuates endothelium-dependent relaxation in normal rabbit arteries in vitro and rat arterioles in vivo. Accordingly, this study examined the effect of acute hyperglycemia on endothelium-dependent vasodilation in nondiabetic humans in vivo.

**Methods and Results**—Endothelium-dependent vasodilation was assessed through brachial artery infusion of methacholine chloride both before and during 6 hours of local hyperglycemia (300 mg/dL) achieved by intra-arterial infusion of 50% dextrose. Forearm blood flow was determined by plethysmography. In a group of 10 subjects, there was a trend toward attenuated methacholine-mediated vasodilation during hyperglycemia compared with euglycemia ( $P=.07$  by ANOVA; maximal response,  $13.3\pm 2.8$  versus  $14.7\pm 1.5$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$ , respectively). In these subjects, the systemic serum insulin levels increased significantly during the dextrose infusion ( $P<.001$ ). To eliminate the confounding vasoactive effects of insulin, the protocol was repeated during systemic infusion of octreotide (30 ng  $\cdot$  kg $^{-1}$   $\cdot$  min $^{-1}$ ) to inhibit pancreatic secretion of insulin. In these subjects ( $n=10$ ), hyperglycemia significantly attenuated the forearm blood flow response to methacholine ( $P<.01$  by ANOVA; maximal response,  $16.9\pm 2.5$  before versus  $12.7\pm 1.8$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  during hyperglycemia). Methacholine-mediated vasodilation was not attenuated by an equimolar infusion of mannitol ( $P>.40$ ), nor did hyperglycemia reduce endothelium-independent vasodilation to verapamil ( $P>.50$ ).

**Conclusions**—Acute hyperglycemia impairs endothelium-dependent vasodilation in healthy humans in vivo. This finding suggests that elevated glucose may contribute to the endothelial dysfunction observed in patients with diabetes mellitus. (*Circulation*. 1998;97:1695-1701.)

**Key Words:** diabetes mellitus ■ endothelium-derived factors ■ vasodilation ■ glucose ■ nitric oxide

Diabetes mellitus is associated with accelerated atherosclerosis and increased prevalence of cardiovascular disease.<sup>1</sup> Both macrovascular disease (resulting in myocardial infarction, stroke, and claudication) and microvascular disease (resulting in diabetic nephropathy and retinopathy) are more prevalent in diabetic than in nondiabetic populations and contribute importantly to the morbidity associated with this disease.<sup>1-3</sup>

There is substantial evidence that vasodilation mediated by endothelium-derived nitric oxide is impaired in animal models of diabetes<sup>4-8</sup> and in patients both with insulin-dependent<sup>9-11</sup> and non-insulin-dependent<sup>12-14</sup> diabetes mellitus. Nitric oxide possesses a variety of antiatherogenic properties, including inhibition of leukocyte adhesion,<sup>15</sup> platelet aggregation,<sup>16</sup> and vascular smooth muscle proliferation.<sup>17</sup> Thus, the pathogenesis of diabetic vascular disease may involve an abnormality in the bioavailability of endothelium-derived nitric oxide, contributing to the development and pathological consequences of atherosclerosis through loss of these protective properties.

The factors that contribute to endothelial dysfunction in diabetes are currently unknown. Hyperglycemia is the hallmark of diabetes mellitus, and recent large-scale clinical trials have correlated poor glycemic control with an increased incidence of both microvascular<sup>18</sup> and macrovascular<sup>19,20</sup> disease. Furthermore, acute hyperglycemia attenuates endothelium-dependent vasodilation in normal rabbit aortas in vitro<sup>21</sup> and in normal rat arterioles in vivo,<sup>22</sup> suggesting high glucose levels may mediate the abnormality. Accordingly, the objective of this study was to test the hypothesis that acute hyperglycemia impairs endothelium-dependent vasodilation in nondiabetic humans in vivo.

## Methods

### Subjects

Protocols were approved by Brigham and Women's Hospital Human Research Committee, and written informed consent was obtained from healthy volunteers recruited from the Boston area through newspaper advertisements. All subjects underwent screening medical history, physical examination, and laboratory analyses, which

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included complete blood count, serum electrolytes, blood urea nitrogen, creatinine, fasting glucose, and transaminases. Exclusion criteria included tobacco use; hypertension; elevated LDL cholesterol ( $\geq 75$ th percentile for age and sex); cardiac or pulmonary disease; or use of any antihypertensive, cardiac, or vasoactive medication.

### Protocol

Subjects were studied in the morning in the postabsorptive state. Cyclooxygenase inhibitors were prohibited for 72 hours and alcohol and caffeine for 12 hours before the study. Under local anesthesia and sterile conditions, a 20-gauge Teflon catheter was inserted into the brachial artery for determination of blood pressure and infusion of drugs. Arterial blood samples were obtained at baseline for serum insulin, glucose, and glycosylated hemoglobin. Venous catheters were inserted into the antecubital fossa of both the study arm and the contralateral control arm for measurement of local and systemic glucose and insulin concentrations. The vascular research laboratory was quiet, the lights were dimmed, and the room temperature was 23°C. Subjects rested for 30 minutes after catheter placement to establish a stable baseline before data acquisition.

The effect of acute hyperglycemia on endothelium-dependent vasodilation was examined in 10 healthy, nondiabetic subjects. First, during fasting euglycemia, methacholine chloride was administered through the brachial artery in increasing concentrations (0.3, 1.0, 3.0, and 10.0  $\mu\text{g}/\text{min}$ ) to assess vasodilation to endothelium-derived nitric oxide. Basal conditions were reestablished after waiting  $\geq 30$  minutes. Then, the forearm glucose concentration was raised and clamped at 300 mg/dL (16.7 mmol/L) through intra-arterial infusion of 50% dextrose at a rate determined by a glucose mass balance (described below). Venous glucose samples in the infused study arm were monitored to document that the desired level of hyperglycemia was achieved and maintained. Systemic glucose and insulin samples were obtained via the contralateral venous cannula. After 6 hours of local hyperglycemia (based on the *in vitro* investigations by Tesfamariam and colleagues<sup>21</sup>), the methacholine dose-response was reevaluated. The dextrose infusion rate was proportionally increased at each dose of methacholine to continue to maintain the forearm glucose at 300 mg/dL (16.7 mmol/L) despite methacholine-mediated increases in forearm blood flow. The methacholine dose-response curves obtained before and during hyperglycemia were compared to determine the effect of acute hyperglycemia on endothelium-dependent vasodilation.

### Effect of Hyperglycemia on the Response to Methacholine During Concomitant Infusion of Octreotide

Although the primary objective was to increase forearm glucose concentration, systemic insulin levels increased significantly during the dextrose infusion in the initial protocol (see "Results"). Insulin is a known vasodilator,<sup>23,24</sup> and several lines of evidence suggest that insulin-mediated vasodilation is achieved via endothelial release of nitric oxide; insulin-mediated vasodilation is inhibited by the nitric oxide synthase antagonist, *N*<sup>G</sup>-monomethyl-L-arginine (L-NMMA),<sup>25,26</sup> and concomitant insulin infusion augments local vasodilation to methacholine.<sup>25</sup> To eliminate potential confounding vasoactive effects of insulin, a similar protocol was performed in 10 additional healthy subjects during concomitant inhibition of pancreatic insulin secretion with octreotide. The octreotide infusion (30 ng  $\cdot$  kg<sup>-1</sup>  $\cdot$  min<sup>-1</sup>) was begun 30 minutes before the baseline methacholine dose-response measurement and was continued at a constant rate through the duration of the protocol to ensure identical systemic concentrations of octreotide during both euglycemia and hyperglycemia. This design eliminates possible confounding from vasoactive effects of octreotide. Importantly, however, no vasoactive effects of octreotide were identified in recent studies using identical doses of the drug.<sup>27</sup> The methacholine dose-response curves obtained during euglycemia and hyperglycemia were compared to determine the effect of acute hyperglycemia on endothelium-dependent vasodilation.

### Effect of Hyperglycemia on the Response to Verapamil

To examine the possibility that the attenuated vasodilation during acute hyperglycemia is not confined to the endothelium, a subset of 7 of the 10 subjects was reevaluated on a separate day with the calcium channel blocker verapamil administered at doses of 10, 30, 100, and 300  $\mu\text{g}/\text{min}$  before and after 6 hours of hyperglycemia as described, along with octreotide. The verapamil dose-response curves obtained during euglycemia and hyperglycemia were compared to determine the effect of acute hyperglycemia on endothelium-independent vasodilation.

### Effect of Hyperosmolality on the Response to Methacholine

As a time and osmolality control, the octreotide protocol was again repeated in a subset of 7 of the 10 participants by replacing the dextrose infusion with an equimolar infusion of 25% mannitol. Venous samples from the infused study arm were obtained throughout the protocol to document the achieved level of hyperosmolality. The methacholine dose-response curves obtained before and during the intra-arterial mannitol infusion were compared to determine the effect of acute hyperosmolality on endothelium-dependent vasodilation.

### Techniques

#### Forearm Hyperglycemic Clamp Method

A forearm hyperglycemic clamp was used to raise and maintain the forearm glucose concentration at 300 mg/dL (16.7 mmol/L). The dextrose infusion rate was calculated from a mass balance equation by multiplying the measured forearm blood flow by the difference of the desired (300 mg/dL) and systemic glucose concentration measured in the contralateral arm. The infusion rate determined by this method proved slightly lower than required because of variable uptake and utilization of glucose by forearm tissues. Therefore, the calculated infusion rate was used as the starting infusion rate, and the forearm glucose concentration was monitored and infusion rate was adjusted empirically every 10 to 30 minutes until steady state was achieved.

#### Biochemical Analyses

Whole-blood glucose concentration was measured at the bedside by the glucose oxidase method with a glucose reflectometer; however, the reported values represent analyses performed on plasma with a Glucose Analyzer II (Beckman Instruments Inc) at the conclusion of each study. Insulin was measured by a radioimmunoassay technique.<sup>28</sup> Osmolality was determined by measurement of freezing point depression. All samples for glucose, insulin, and osmolality were performed in duplicate.

#### Hemodynamic Measurements

Bilateral forearm blood flow was determined by venous occlusion mercury-in-Silastic strain-gauge plethysmography by the methods described previously.<sup>12</sup> Arterial blood pressure was measured via the arterial cannula, which was attached to a pressure transducer aligned to an amplifier on a physiological recorder. Forearm vascular resistance was calculated as the ratio of mean blood pressure to forearm blood flow and is expressed in arbitrary units (AU). The heart rate was determined from a simultaneously obtained ECG and was calculated from the RR interval.

#### Statistical Analyses

Data are presented as mean  $\pm$  SE. Comparisons of baseline forearm blood flow, osmolality, and glucose and insulin concentrations before and during hyperglycemia were made by use of paired two-tailed *t* tests. Statistical analyses on the dose-response curves for each drug (methacholine chloride or verapamil) were conducted on the absolute increase and the percentage increase in forearm blood flow from baseline. Two-way repeated-measures ANOVA was performed to compare the dose-response curves obtained before and during the hyperglycemic clamp. Statistical significance was accepted at the 95% confidence level ( $P \leq .05$ ).

TABLE 1. Characteristics of the Study Populations

	Hyperglycemia/Methacholine Without Octreotide	Hyperglycemia/Methacholine With Octreotide
n	10	10
Sex, F/M	3/7	4/6
Age, y	33±2	32±3
Mean blood pressure, mm Hg	83±3	80±4
Total cholesterol, mg/dL	161±9	158±5
HDL cholesterol, mg/dL	42±2	46±3
LDL cholesterol, mg/dL	101±8	94±5
Triglycerides, mg/dL	92±10	94±15
Insulin, $\mu$ U/mL	3.5±0.6	1.9±0.6
Glucose, mg/dL	73±3	71±7
Glycosylated hemoglobin, %	3.6±0.2	3.8±0.2

## Results

### Baseline Values of the Study Populations

Baseline characteristics of the healthy, nondiabetic study subjects are summarized in Table 1. The two methacholine chloride protocols, without and with octreotide, represent distinct populations. The 7 study subjects in the verapamil and mannitol protocols represent a random subset of the 10 volunteers from the octreotide protocol. Serum insulin, glucose, glycohemoglobin, mean arterial pressure, and lipid profiles of all subjects were within normal limits.

### Effect of Hyperglycemia on the Response to Methacholine (Without Octreotide)

The initial protocol was designed to evaluate the independent effect of acute, isolated forearm hyperglycemia on endothelium-dependent vasodilation. In fact, the forearm hyperglycemic clamp increased and maintained the glucose concentration in the study arm as desired, and the corresponding osmolality increased proportionally (Table 2). The baseline forearm blood flow in the study arm increased significantly during hyperglycemia (Table 2); similarly, the baseline forearm vascular resistance fell from  $47\pm 5$  AU during euglycemia to  $32\pm 4$  AU during hyperglycemia ( $P<.05$ ). The heart rate, mean arterial pressure, and contralateral forearm blood flow were all unaffected by the intra-arterial dextrose infusion.

Intra-arterial infusion of methacholine chloride increased forearm blood flow during both euglycemia and hyperglycemia (Figure 1). There was a trend toward attenuated methacholine-induced vasodilation during hyperglycemia compared with euglycemia. At the highest dose of methacholine ( $10\ \mu\text{g}/\text{min}$ ), forearm blood flow increased by only  $13.3\pm 2.8\ \text{mL}\cdot\text{min}^{-1}\cdot 100\ \text{mL}^{-1}$  during hyperglycemia compared with  $14.7\pm 1.5\ \text{mL}\cdot\text{min}^{-1}\cdot 100\ \text{mL}^{-1}$  during euglycemia ( $P=.07$ , ANOVA). When analyzed as percent change from baseline to control for the augmented baseline blood flow observed during the dextrose infusion, a significant attenuation of methacholine-mediated vasodilation was demonstrated during hyperglycemia ( $575\pm 80\%$  during hyperglycemia versus  $901\pm 180\%$  during euglycemia;  $P<.05$ , ANOVA).

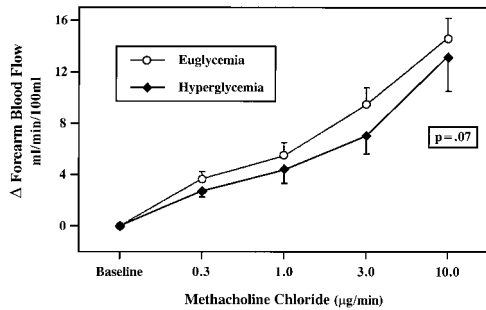
However, systemic insulin levels were found to increase significantly during the forearm glucose infusion (Table 2) ( $P<.001$ , paired  $t$  test). Furthermore, the increase in systemic insulin levels correlated inversely with the severity of attenuated endothelium-dependent vasodilation ( $P<.01$ ) and therefore suggested possible confounding by insulin-mediated vasodilation.

There were no systemic effects of the methacholine infusion either before or during forearm hyperglycemia as measured by contralateral forearm blood flow, change in mean arterial pressure, or heart rate during drug administration.

TABLE 2. Baseline Parameters Before and During Hyperglycemic Clamping

	Euglycemia	Hyperglycemia
Effect of hyperglycemia on the response to methacholine (without octreotide)		
Forearm glucose concentration, mg/dL	73±3	322±21†
Forearm osmolality, mosm/kg	283±1	296±2†
Systemic insulin concentration, $\mu$ U/mL	3.5±0.6	8.4±1.0†
Baseline forearm blood flow, $\text{mL}\cdot\text{min}^{-1}\cdot\text{dL}^{-1}$	1.9±0.2	2.7±0.3*
Effect of hyperglycemia on the response to methacholine (with octreotide)		
Forearm glucose concentration, mg/dL	71±7	326±27†
Forearm osmolality, mosm/kg	281±2	303±5†
Systemic insulin concentration, $\mu$ U/mL	1.9±0.6	2.6±0.7
Baseline forearm blood flow, $\text{mL}\cdot\text{min}^{-1}\cdot\text{dL}^{-1}$	2.0±0.2	2.9±0.3†
Effect of hyperglycemia on the response to verapamil (with octreotide)		
Forearm glucose concentration, mg/dL	51±5	317±48†
Forearm osmolality, mosm/kg	281±2	302±7*
Systemic insulin concentration, $\mu$ U/mL	2.2±2.0	3.9±2.0
Baseline forearm blood flow, $\text{mL}\cdot\text{min}^{-1}\cdot\text{dL}^{-1}$	1.9±0.2	2.9±0.4*
Effect of hyperosmolality on the response to methacholine (with octreotide)		
Forearm glucose concentration, mg/dL	52±8	90±8*
Forearm osmolality, mosm/kg	282±1	301±3*
Systemic insulin concentration, $\mu$ U/mL	1.3±0.6	1.0±0.5
Baseline forearm blood flow, $\text{mL}\cdot\text{min}^{-1}\cdot\text{dL}^{-1}$	1.8±0.2	3.2±0.4*

\* $P<.05$ ; † $P<.001$ .



**Figure 1.** Absolute increase in forearm blood flow from baseline during graded intra-arterial infusion of methacholine chloride both before and during hyperglycemic clamping (without octreotide). There was a trend toward attenuated vasodilation during hyperglycemia compared with euglycemia as analyzed by repeated-measures ANOVA ( $P = .07$ ).

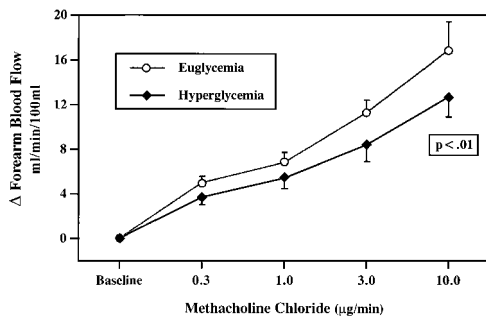
### Effect of Hyperglycemia on the Response to Methacholine (With Octreotide)

In the series of experiments including the octreotide infusion, the forearm glucose concentration achieved by the hyperglycemic clamping method again was close to the target of 300 mg/dL (16.7 mmol/L), whereas the systemic insulin concentration remained unchanged (Table 2). Baseline forearm blood flow again increased significantly during hyperglycemia (Table 2), and baseline forearm vascular resistance fell proportionally ( $47 \pm 4$  before versus  $30 \pm 4$  AU during hyperglycemia,  $P < .001$ ).

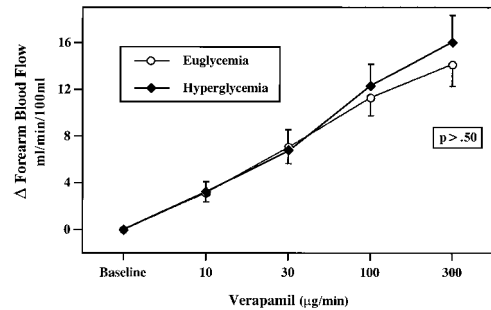
The vasodilative response to methacholine was attenuated significantly during hyperglycemia compared with euglycemia (Figure 2). At the highest dose of methacholine, the forearm blood flow increased by  $16.9 \pm 2.5$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  during euglycemia but only by  $12.7 \pm 1.8$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  during hyperglycemia ( $P < .01$ , ANOVA). Similarly, the methacholine-mediated percent increase in forearm blood flow was attenuated during hyperglycemia ( $911 \pm 150\%$  versus  $456 \pm 70\%$ , respectively;  $P < .001$ , ANOVA).

### Effect of Hyperglycemia on the Response to Verapamil

The effects of hyperglycemia on the baseline forearm blood flow, osmolality, and systemic insulin concentration in the 7



**Figure 2.** Absolute increase in forearm blood flow from baseline during graded intra-arterial infusion of methacholine chloride and concomitant pancreatic inhibition with octreotide both before and during hyperglycemic clamping. The response to methacholine chloride was significantly attenuated during hyperglycemia compared with euglycemia as analyzed by repeated-measures ANOVA ( $P < .01$ ).

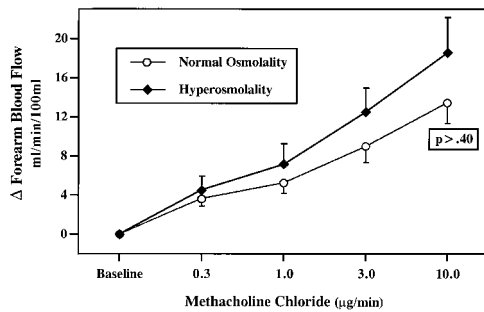


**Figure 3.** Absolute increase in forearm blood flow from baseline during graded intra-arterial infusion of verapamil both before and during hyperglycemic clamping. No significant difference in the response to verapamil was seen between euglycemic and hyperglycemic states as analyzed by repeated-measures ANOVA ( $P > .50$ ).

subjects participating in the verapamil protocol (Table 2) were similar to those observed during the methacholine series. The verapamil infusion increased forearm blood flow both before and during hyperglycemia (Figure 3). However, in contrast to the findings with methacholine, neither the vasodilator response ( $14.2 \pm 1.9$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  euglycemia versus  $16.1 \pm 2.3$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  hyperglycemia;  $P > .50$ , ANOVA) nor the percent increase in forearm blood flow ( $776 \pm 100\%$  versus  $712 \pm 100\%$ , respectively;  $P > .30$ , ANOVA) to verapamil was attenuated during hyperglycemia. There was no change in forearm blood flow in the contralateral arm, nor was there a change in blood pressure or heart rate during verapamil either before or during hyperglycemia.

### Effect of Hyperosmolality on the Response to Methacholine

As a time and osmolality control, the octreotide protocol was repeated in a subset of 7 of the 10 subjects by replacing the dextrose infusion with an equimolar mannitol infusion. The increase in osmolality with mannitol was comparable to that observed overall in the hyperglycemia protocols (Table 2) and in the subset of 7 patients who received both dextrose and mannitol ( $280 \pm 3$  to  $303 \pm 5$  mosm/kg and  $282 \pm 1$  to  $301 \pm 3$  mosm/kg, respectively;  $P > .60$ , ANOVA). Neither the serum glucose nor insulin concentrations were significantly affected by the mannitol infusion (Table 2). Baseline forearm blood flow increased significantly during the infusion of mannitol (Table 2), and the corresponding baseline forearm vascular resistance decreased from  $48 \pm 5$  to  $27 \pm 3$  AU during hyperosmolality ( $P < .001$ ). Forearm blood flow increased significantly in response to methacholine chloride before and during the mannitol infusion (Figure 4). However, in contrast to hyperglycemia, there was a trend toward augmented (rather than attenuated) methacholine-mediated vasodilation during hyperosmolality. At the maximal dose of methacholine, forearm blood flow increased by  $13.5 \pm 2.1$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  before mannitol and by  $18.6 \pm 3.6$  mL  $\cdot$  min $^{-1}$   $\cdot$  100 mL $^{-1}$  during the mannitol infusion ( $P > .40$ , ANOVA), and the percent increase in blood flow to methacholine was unchanged during hyperosmolality ( $702 \pm 90\%$  versus  $611 \pm 90\%$ ,  $P > .20$ ).



**Figure 4.** Absolute increase in forearm blood flow from baseline during graded intra-arterial infusion of methacholine chloride both before and during hyperosmolar clamping. There was no significant effect of osmolality on the response to methacholine chloride as analyzed by repeated-measures ANOVA ( $P > .40$ ).

## Discussion

These experiments demonstrate that endothelium-dependent vasodilation is attenuated by acute hyperglycemia in healthy, nondiabetic humans in vivo. The attenuated response to methacholine chloride but not to the calcium channel blocker verapamil indicates that the deficit involves endothelium-derived nitric oxide. Control studies with equimolar infusions of mannitol indicate that the attenuated response observed during hyperglycemia cannot be attributed either to hyperosmolality or to protocol duration.

### Evidence of Endothelial Dysfunction in Diabetes Mellitus

Extensive evidence exists for endothelial dysfunction in diabetes mellitus. Multiple investigators have demonstrated nitric oxide-mediated vasomotor dysfunction in animal models of diabetes<sup>4-8</sup> and in humans in vivo. We and others have demonstrated that nitric oxide-mediated vasodilation is blunted in patients both with non-insulin-dependent<sup>12-14</sup> and insulin-dependent diabetes mellitus.<sup>9-11</sup>

The mechanism(s) of endothelial dysfunction in diabetes are unknown. Although the deficit may be secondary to comorbid conditions, including hypertension and dyslipidemia, many clinical studies demonstrating endothelial dysfunction in diabetes were control-matched for these parameters. Recent clinical trials have demonstrated that glycemic control predicts the incidence of not only microvascular complications<sup>18</sup> but also coronary artery disease<sup>19</sup> and peripheral arterial disease.<sup>20</sup> The effect of acute hyperglycemia on endothelial function has been examined in animals in vitro and in vivo. Tesfamariam et al<sup>21</sup> reported that rings of rabbit aorta incubated in 44 mmol/L (790 mg/dL) glucose showed significantly decreased endothelium-dependent relaxation to acetylcholine compared with rings incubated in glucose solutions of 5.5 mmol/L (99 mg/dL) and 11 mmol/L (198 mg/dL). Furthermore, relaxation in response to the endothelium-independent agent sodium nitroprusside was not different between rings exposed to control and elevated glucose, indicating that the hyperglycemia-mediated deficit is limited to the endothelium. Similarly, Bohlen and Lash<sup>22</sup> demonstrated in vivo that glucose concentrations of 300 mg/dL (16.7 mmol/L) and 500 mg/dL (27.8 mmol/L) significantly suppressed the vasodilatory response to acetylcholine but not

nitroprusside. Our study is the first to demonstrate defective nitric oxide-mediated vasodilation during acute hyperglycemia in humans in vivo. Of relevant interest is the recent report by Giugliano et al,<sup>29</sup> who found that the hemodynamic and rheological disturbances induced by systemic hyperglycemia were replicated by the nitric oxide synthase antagonist L-NMMA and reversed by L-arginine, implicating reduced availability of nitric oxide during hyperglycemia. These data, however, contrast with those of Houben et al,<sup>30</sup> who failed to demonstrate impaired nitric oxide-mediated vasodilation in the human forearm during acute hyperglycemia. Several possibilities exist for the discrepant results. Houben and colleagues<sup>30</sup> did not clamp insulin, and elevated insulin levels observed during hyperglycemia could have resulted in insulin-mediated vasodilation; furthermore, the subject's vasodilatory response was compared on separate days with potential error resulting from inherent physiological variability as manifest by the large confidence intervals.

### Mechanisms of Hyperglycemia-Mediated Endothelial Dysfunction

Investigations in animal models are beginning to elucidate the biochemical mechanism(s) by which hyperglycemia induces endothelial dysfunction. Potential mechanisms include hyperglycemia-mediated formation of oxygen-derived free radicals, activation of protein kinase C, and formation of advanced glycosylation end products. Free radicals inactivate endothelium-derived nitric oxide,<sup>31,32</sup> interfere with endothelium-dependent vasodilation,<sup>33</sup> and are produced during prostaglandin formation.<sup>34-36</sup> Increased prostaglandin synthesis has been demonstrated in models of both acute<sup>21,37</sup> and chronic<sup>7,38</sup> hyperglycemia. Moreover, nitric oxide-mediated vasodilation is restored by blockade of prostaglandin synthesis,<sup>6,7,21,22</sup> consistent with prostanoid-mediated free radical formation. Alternatively, hyperglycemia may lead directly to formation of free radicals by glucose auto-oxidation.<sup>39,40</sup> Studies with antioxidants support hyperglycemia-induced free radical formation. Endothelial dysfunction resulting from both acute hyperglycemia<sup>22,41</sup> and chronic states of hyperglycemia in experimental diabetes<sup>42-44</sup> can be reversed by pretreatment with free radical scavengers. Furthermore, we have previously demonstrated that endothelium-dependent vasodilation is restored both in patients with non-insulin-dependent diabetes<sup>13</sup> and insulin-dependent diabetes<sup>45</sup> by the short-term administration of the antioxidant vitamin C.

Hyperglycemia-mediated activation of protein kinase C has also been postulated to contribute to the vascular dysfunction in diabetes mellitus.<sup>46</sup> Cultured vascular cells exposed to elevated glucose concentrations in vitro exhibit increased synthesis of diacylglycerol which results in activation of protein kinase C.<sup>47</sup> Protein kinase C activators reproduce the abnormalities in vascular function observed during hyperglycemia,<sup>38,47,48</sup> and protein kinase C inhibitors restore vascular function in both acute hyperglycemia<sup>48</sup> and diabetic animal models.<sup>47,49</sup> Several mechanisms have been proposed to account for the effect of activated protein kinase C on endothelium-dependent vasodilation, including increased generation of vasoconstrictor prostanoids<sup>46</sup> and phosphorylation of endothelial cell muscarinic receptors.<sup>50</sup> However,

activation of protein kinase C has also been linked to nitric oxide synthesis and activity.<sup>51,52</sup> Inhibition of protein kinase C results in upregulation of endothelial nitric oxide synthase,<sup>52</sup> and the brain isoform of nitric oxide synthase has regulatory sites for protein kinase C, which, when phosphorylated, effect significantly decreased nitric oxide activity.<sup>51</sup> Furthermore, protein kinase C has been shown to contribute to the formation of oxygen-derived free radicals<sup>53,54</sup> with possible resultant inactivation of nitric oxide.

Advanced glycosylation end products, which form during hyperglycemia via nonenzymatic protein glycosylation and cross-linking reactions, also could contribute to the endothelial dysfunction observed in diabetes. Advanced glycosylation end products inactivate nitric oxide in vitro and inhibit nitric oxide-mediated vasodilation.<sup>8</sup> Furthermore, incubation of rat aortic rings with glycosylated human hemoglobin inhibits endothelium-dependent relaxation in vitro.<sup>55</sup> Although our data do not exclude this theory as a contributing factor to the endothelial dysfunction in clinical diabetes, the deficit demonstrated in this study in nondiabetic patients occurs after a time interval of hyperglycemia too brief for significant formation of advanced glycosylation end products.

### Study Limitations

Octreotide has well-documented systemic effects (including suppression of multiple pancreatic, adrenocortical, and pituitary hormones) and therefore had the potential to confound our analysis of the independent effect of hyperglycemia on endothelial function. However, the fact that methacholine-induced vasodilation was blunted during hyperglycemia with or without octreotide, combined with the fact that methacholine-induced vasodilation was attenuated during octreotide/glucose but not octreotide/mannitol infusions, makes it unlikely that the results of this study were attributable either directly or indirectly to the octreotide. Furthermore, the octreotide protocols were designed so that the systemic octreotide levels were identical during both euglycemia and hyperglycemia.

Baseline forearm blood flow of the healthy nondiabetic study subjects increased significantly during acute local hyperglycemia. Insulin-mediated vasodilation could not account for this increase in flow because it could be reproduced during insulin suppression with octreotide. Furthermore, studies that demonstrate insulin-mediated vasodilation require insulin levels in the range of 50 to 70  $\mu\text{U/mL}$  and do not occur at levels of  $<10 \mu\text{U/mL}$ .<sup>23,24</sup> (Despite this fact, insulin has been shown to potentiate the vasodilator response to methacholine,<sup>25</sup> rendering suppression of pancreatic insulin secretion with octreotide requisite to eliminate confounding.) The augmented baseline forearm blood flow during hyperglycemia may be secondary to the hyperosmolality associated with hyperglycemia rather than to a biochemical effect of elevated glucose per se, as demonstrated by the increase in baseline blood flow observed in the mannitol control studies. However, because endothelium-dependent vasodilation remained intact during mannitol infusion despite a similar increase in baseline blood flow, the acute hyperperfusion during hyperglycemia/hyperosmolality could not account for the endothelial dysfunction observed during hyperglycemia.

### Conclusions

These studies demonstrate that acute hyperglycemia impairs endothelium-dependent vasodilation in nondiabetic humans in vivo, implicating elevated glucose as a cause of the endothelial dysfunction in patients with diabetes. Our data may provide insight into the vasculoprotective effects of strict glycemic control that have been observed in clinical trials. However, blood glucose concentrations in diabetic patients cannot be completely normalized even under optimal conditions, and most patients eventually develop vascular complications. Understanding the mechanism(s) by which acute hyperglycemia impairs endothelial function in diabetes mellitus may lead to secondary preventive strategies to reduce cardiovascular morbidity and mortality in this highly prevalent disease.

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