Forearm vascular control during acute hyperglycemia in healthy humans

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The vascular endothelium is a site of pathological changes in patients with diabetes mellitus that may be related to severe chronic hyperglycemia. However, it is unclear whether transient hyperglycemia alters vascular function in an otherwise healthy human forearm. To test the hypothesis that acute, moderate hyperglycemia impairs endothelium-dependent forearm vasodilation, we measured vasodilator responses in 25 healthy volunteers (11 F, 14 M) assigned to one of three protocols. In protocol 1, glucose was varied to mimic a postprandial pattern (i.e., peak glucose ~11.1 mmol/l) commonly observed in individuals with impaired glucose tolerance. Protocol 2 involved 6 h of mild hyperglycemia (~7 mmol/l). Protocol 3 involved 6 h of euglycemia. Glucose concentration was maintained with a variable systemic glucose infusion. Insulin concentrations were maintained at ~65 pmol/l by means of a somatostatin and “basal” insulin infusion. Glucagon and growth hormone were replaced at basal concentrations. Forearm blood flow (FBF) was calculated from Doppler ultrasound measurements at the brachial artery. In each protocol, FBF dose responses to intrabrachial acetylcholine (ACh) and sodium nitroprusside (NTP) were assessed at basal concentrations. Forearm blood flow (FBF) during episodes of reactive hyperemia and found impaired flow-mediated dilation (FMD) during acute hyperglycemia compared with controls. Additionally, the degree of impaired dilation appears to correlate with duration of disease (7). These data suggest that, with underlying endothelial damage, vasodilation is impaired during acute hyperglycemia.

In healthy humans, there is less consensus. One series of studies performed by Creager and colleagues (2, 3, 30) showed that, by infusing concentrated glucose into the brachial artery of study subjects to achieve regional concentrations of 300 mg/dl, forearm blood flow was impaired during local methacholine infusion but was restored by a variety of antioxidants. In these studies, octreotide was infused to suppress insulin secretion during hyperglycemia, but other pancreatic hormones were not replaced. In another study, Title et al. (27) gave healthy participants a 75-g oral glucose load and measured FBF during episodes of reactive hyperemia and found impaired Doppler ultrasound FMD during moderate hyperglycemia. However, insulin and hormone concentrations were unregulated in this study, and insulin in particular rose 10-fold over baseline during peak glucose levels. In contrast to the studies of Creager and colleagues, Houben et al. (18) found that similar “local” glucose infusions did not decrease FBF responses to repeated acetylcholine infusions.

These discrepant results in healthy humans can, at least in part, be explained by methodology. First, high levels of glucose infused directly into the brachial artery, as used in the series of articles by Creager and colleagues (2, 3, 30) and the studies of Houben et al. (18), is a different paradigm from whole body hyperglycemia and could have a different effect on vasodilator mechanisms. Indeed, Cardillo et al. (5a) found that systemic, but not local, hyperinsulinemia induced forearm vasodilation. This indicates that local and systemic hormone and possibly glycemic conditions may produce different physiological effects. In addition, uncontrolled hormone levels, such as those in previous studies (2, 3, 18, 27, 30), have known effects on sympathetic function (17, 26). Finally, severe hyperglycemia (300 mg/dl) may have different effects from acute hyperglycemia on vascular function in the healthy human forearm.

endothelial function; vasodilation; postprandial hyperglycemia

IN THE LAST 20 YEARS, the endothelium has emerged as a major site of vascular regulation and disease (20, 22). Endothelial dysfunction is a recognized consequence of exposure to most traditional cardiovascular risk factors, including hyperglycemia due to diabetes mellitus (15). In diabetes mellitus, micro- and macrovascular disease are common complications (13a, 21, 22); indeed, macrovascular disease is the most common cause of death in affected patients.

The effects of acute episodes of hyperglycemia on vascular function, independent of the chronic changes seen in the diabetic endothelium, are less well defined. Numerous studies to date have attempted to determine whether a single exposure to acute hyperglycemia can impair vasodilation in diabetic and healthy humans. In diabetics and those at risk for developing diabetes mellitus, there appears to be an adverse effect of acute hyperglycemia on forearm blood flow (FBF) (14, 19). Studies performed by Clarkson et al. (7), Anastasiou et al. (1), and Caballero et al. (4) all conclude that diabetics, or those at risk for diabetes, including individuals with impaired glucose tolerance or a history of gestational diabetes, have impaired flow-mediated dilation (FMD) during acute hyperglycemia compared with controls. The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
mildly elevated glucose achieved during a 75-g glucose load. These factors have likely contributed to the inconsistencies in the existing literature on this topic.

Another important methodological consideration in both diabetic and nondiabetic studies to date is the technique used to assess endothelial function. Although FMD has emerged as a noninvasive surrogate marker for endothelial function, it may not be an accurate index of endothelial nitric oxide (NO)-mediated vasodilation. For example, Doshi et al. (11) recently showed that FMD is only 30–40% blocked by infusion of the nitric oxide synthase inhibitor L-NAME, indicating that FMD is only partially mediated by NO release from the vascular endothelium. Furthermore, Eskurza et al. (13) recently compared FMD with direct measures of endothelial function (drug infusions) in 44 subjects and found that the two tests of endothelial function do not produce similar results.

The foregoing physiological and technical issues limit the comparisons and conclusions that can be drawn from studies to date. Our goal in the present study was to evaluate the effects of acute, moderate hyperglycemia on forearm vasodilator mechanisms while addressing issues that may have limited the interpretation of previous studies. We sought to determine whether a transient increase in glucose to ~11 mmol/l or a sustained 7-h increase to ~7 mmol impairs vascular endothelial function in nondiabetic humans under conditions in which insulin, glucagon, and growth hormone concentrations were maintained constant. The former pattern was chosen to mimic the glucose concentrations commonly observed in people with impaired glucose tolerance following carbohydrate ingestion, whereas the latter pattern was chosen to mimic the fasting glucose levels commonly observed in people with fasting hyperglycemia or early type 2 diabetes. We hypothesized that, in the presence of identical insulin concentrations and glycemic excursion, an acute increase to 200 mg/dl (intended to mimic the postprandial state in type 2 diabetes) would impair endothelial function to a greater degree than maintaining hyperglycemia at 126 mg/dl and that both patterns of hyperglycemia would result in greater impairment of endothelial function than euglycemia (95 mg/dl).

### MATERIALS AND METHODS

#### Subjects

Twenty-five subjects, 11 females and 14 males between ages 18 and 40 yr, were studied. All protocols were approved by the Mayo Institutional Review Board, and written informed consent was obtained before participation. All females had a negative pregnancy test and were also infused with [3-3 H]glucose (29), and skin blood flow was measured using laser Doppler flowmetry as previously reported (6). Subjects were also infused with [3-3 H]glucose (29), and skin blood flow was measured using laser Doppler flowmetry as previously reported (6).

#### Protocol 1

On this arm, the pulsed Doppler probe and echo Doppler probe holder were moved to the dominant arm, and the same vessel was used. A constant angle of insonation allows for intrasubject analysis of change in velocity. The exact location on each subject was chosen by directing the probe at the center of the artery and adjusting the depth to a signal of maximal visual and audio intensity. The gate (width) was kept constant across all subjects (10 mm) to ensure insonation of the entire arterial diameter.

On the study day, forearm volume in the nondominant arm was measured by water volume displacement, and a forearm vein in the dominant arm was cannulated with a 18-gauge catheter. This was used for glucose and hormone infusions during the study day. An 8-cm double-lumen Teflon arterial catheter (Arrow International, Reading, PA) was placed in the nondominant brachial artery by aseptic technique under local 1% lidocaine anesthesia. The catheter was connected to a pressure transducer and flushed with normal saline at 2 ml/h to maintain patency (9, 12).

#### Protocol 2

On the study day, forearm volume in the nondominant arm was measured by water volume displacement, and a forearm vein in the dominant arm was cannulated with an 18-gauge catheter. This was used for glucose and hormone infusions during the study day. An 8-cm double-lumen Teflon arterial catheter (Arrow International, Reading, PA) was placed in the nondominant brachial artery by aseptic technique under local 1% lidocaine anesthesia. The catheter was connected to a pressure transducer and flushed with normal saline at 2 ml/h to maintain patency (9, 12).

#### Protocol 3

On this arm, the pulse Doppler probe and echo Doppler probe holder were moved to the dominant arm, and the same vessel was used. A constant angle of insonation allows for intrasubject analysis of change in velocity. The exact location on each subject was chosen by directing the probe at the center of the artery and adjusting the depth to a signal of maximal visual and audio intensity. The gate (width) was kept constant across all subjects (10 mm) to ensure insonation of the entire arterial diameter.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Protocol 1 (Profile)</th>
<th>Protocol 2 (Hyperglycemia)</th>
<th>Protocol 3 (Euglycemia)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>9 (4F, 5M)</td>
<td>9 (4F, 5M)</td>
<td>7 (3F, 4M)</td>
</tr>
<tr>
<td>Age, yr</td>
<td>25.9 ± 0.8</td>
<td>27.0 ± 1.2</td>
<td>27.3 ± 1.56</td>
</tr>
<tr>
<td>Height, cm</td>
<td>1.74 ± 0.03</td>
<td>1.71 ± 0.03</td>
<td>1.77 ± 0.04</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>76.79 ± 4.12</td>
<td>72.71 ± 6.13</td>
<td>79.43 ± 6.54</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25.23 ± 0.77</td>
<td>24.58 ± 1.32</td>
<td>25.11 ± 1.38</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.08 ± 0.10</td>
<td>5.07 ± 0.13</td>
<td>4.92 ± 0.08</td>
</tr>
<tr>
<td>Glycosylated Hb, %</td>
<td>4.90 ± 0.13</td>
<td>4.78 ± 0.22</td>
<td>4.93 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE. BMI, body mass index; NA, not applicable.

### Measurement of FBF

FBF was calculated from Doppler ultrasound measurements of the brachial artery, as previously described (28). Briefly, to obtain brachial artery mean blood velocity (MBV), a Doppler in pulsed-wave mode with a flat 4-MHz probe was used (model 500V; Multigon Industries, Mt. Vernon, NY). The probe was placed over the brachial artery ~2 cm proximal to the antecubital fossa and fastened with adhesive tape for the entire duration of the study to ensure constant angle of insonation (~60°) and position through the entire study day. A constant angle of insonation allows for intrasubject analysis of change in velocity. The exact location on each subject was chosen by directing the probe at the center of the artery and adjusting the depth to a signal of maximal visual and audio intensity. The gate (width) was kept constant across all subjects (10 mm) to ensure insonation of the entire arterial diameter.

Brachial artery radius was determined with an imaging Doppler (HDI 5000; ATL Ultrasound, Bothell, WA) in B-mode with a 6.0-MHz probe. This probe was placed immediately proximal to the pulsed Doppler probe after optimal position was determined visually. A probe holder was fastened at this site so that the same vessel segment was imaged throughout the study.

### Experimental Protocols and Data Analysis

Subjects were admitted to the Mayo General Clinical Research Center at 1700 the evening before the study and were given a standard mixed meal. After completion of the meal, participants fasted overnight.

**Monitoring/instrumentation.** On the study day, forearm volume in the nondominant arm was measured by water volume displacement, and a forearm vein in the dominant arm was cannulated with an 18-gauge catheter. This was used for glucose and hormone infusions during the study day. An 8-cm double-lumen Teflon arterial catheter (Arrow International, Reading, PA) was placed in the nondominant brachial artery by aseptic technique under local 1% lidocaine anesthesia. The catheter was connected to a pressure transducer and flushed with normal saline at 2 ml/h to maintain patency (9, 12). Blood draws and arterial pressure (BP) monitoring was accomplished using one port, and the second port was used for infusions of acetylcholine (ACh) and sodium nitroprusside (NTP). Subjects were also infused with [3-3 H]glucose (29), and skin blood flow was measured using laser Doppler flowmetry as previously reported (6). Subjects were asked to lie supine with the nondominant arm comfortably resting in a specially constructed arm rest at the level of the heart. On this arm, the pulsed Doppler probe and echo Doppler probe holder were moved to the dominant arm, and the same vessel was used. A constant angle of insonation allows for intrasubject analysis of change in velocity. The exact location on each subject was chosen by directing the probe at the center of the artery and adjusting the depth to a signal of maximal visual and audio intensity. The gate (width) was kept constant across all subjects (10 mm) to ensure insonation of the entire arterial diameter.
were attached as described above. A pediatric blood pressure cuff was positioned at the wrist. This cuff was inflated to suprasystolic pressure to occlude blood flow to the hand during FBF measurements. Heart rate (HR) was monitored with a five-lead electrocardiogram. Ambient temperature was maintained at 23–25°C throughout all protocols.

To assess endothelium-dependent vasodilation, dose-response curves to ACh (1, 2, and 4 μg·100 ml forearm volume−1·min−1 each for 3 min) were performed four times over the course of the study day. Dose responses to NTP (0.25, 0.5, and 2.0 μg·100 ml forearm volume−1·min−1 each for 3 min) were similarly performed to assess endothelium-independent vasodilation (Fig. 1). The doses of ACh and NTP were chosen on the basis of previous experience in this laboratory and pilot data obtained before this study to stimulate substantial progressive forearm vasodilation while avoiding systemic effects (9, 12).

Data acquisition and analysis. MBV, HR, and BP were each sampled at 250 Hz and subsequently analyzed offline (Windaq; Datalog, Akron, OH). Echo Doppler data were stored on VHS videotape. A single observer unfamiliar with the protocol measured vessel diameters at 15-s intervals over the final minute of each trial. FBF was then calculated using the formula MBV (brachial artery cross-sectional area) × HR² (25).

All blood samples were obtained from the brachial artery catheter. Specimens were collected for glucose, hormone, and catecholamine concentrations. Glucose samples were centrifuged immediately and assayed with a Beckman monitor (Beckman, Chaska, MN) to facilitate experimental control of blood glucose. Additional glucose samples, as well as hormone and catecholamine samples, were immediately placed on ice, centrifuged at 4°C, and stored at −20°C. Stored glucose samples were processed with a Yellow Springs (YSI) glucose analyzer. C-peptide, cortisone, and glucagon were measured in the Mayo immunochemical core laboratory using reagents purchased from Linco Research (St. Louis, MO). Insulin and growth hormone were measured using a chemiluminescence assay with Beckman reagents (Access Assay; Beckman).

Protocol randomization. Before hospital admission, subjects were randomized to one of three protocols (Fig. 1).

Protocol 1 (Profile). At 0730, a continuous infusion of glucagon (0.65 ng·kg−1·min−1), somatostatin (60 ng·kg−1·min−1), and growth hormone (3 ng·kg−1·min−1) was started and continued for the duration of the experiment. This had previously been shown to effectively inhibit endogeneous hormone secretion and replace glucagon and growth hormone in such a way as to ensure maintenance of basal concentrations of these hormones (29). Insulin was also started at this time and was infused at 0.20 mU·kg−1·min−1. A variable glucose infusion was started at 0730 to clamp blood glucose at 95 mg/dl. At 0900, an infusion of glucose mimicking the prandial state was begun (24). The prandial curve was designed to create a peak glucose concentration of ~200 mg/dl at 1000 followed by a fall to 126 mg by 1200 and to 95 mg/dl by 1300. This curve was designed so that Doppler recordings and blood samples were obtained at baseline (0830), at peak glucose (1000), at the point where blood glucose levels in protocols 1 and 2 were identical (1200), and just before completion of the study (1500), when blood glucose levels in protocols 1 and 3 were identical. The curve was also designed so that the glycemic excursions in protocols 1 and 2 were identical (Fig. 1).

Four FBF trials were performed during the study. These trials took place starting at 0830, 1000, 1200, and 1500 and lasted 30 min each.

First, the wrist cuff was inflated to 220 mmHg. One minute later, Doppler measurements of brachial arterial diameter and blood velocity commenced with 2 min of baseline recording followed by an ACh dose-response determination. Doppler recordings were continuous throughout this 11-min period. The cuff was then deflated, and MBV and arterial diameter were allowed to return to baseline over 5 min. The wrist cuff was then reinflated 1 min before measurements, and the 11-min cycle was repeated with NTP.

Blood samples were obtained at baseline (0600) and before (0830, 1000, 1200, 1500) and after (0900, 1030, 1230, 1530) each blood flow trial and also at 0930, 1100, 1130, 1300, 1400, and 1430 for hormone and catecholamine content. Glucose concentration was measured every 10 min with a Beckman analyzer to facilitate clamping.

Protocol 2 (Hyperglycemia). This protocol was identical to protocol 1 except that, at 0900, glucose was clamped at 126 mg/dl for the duration of the study.

Protocol 3 (Euglycemia). This protocol was identical to protocol 1 except that, at 0900, the variable glucose infusion was used to continue clamping blood glucose at 95 mg/dl for the duration of the study.

Data Analysis

Data are reported as means ± SE. One-way ANOVA was used to compare baseline subject traits across protocols. All other data were analyzed separately for each protocol by use of generalized linear mixed models (SAS/STAT; SAS Institute, Cary, NC), taking into account the correlation of repeated measures within individuals (10).

For FBF, dose (of ACh or NTP) and time were independent classification variables, and the dose-by-time interaction term was included to assess whether the dose response changed over the course of the study day. Post hoc comparisons between time periods were performed using the Tukey-Kramer adjustment for multiple comparisons.

To compare total glycemic excursion between protocols 1 and 2, the area under the curve for glucose was calculated for each subject by taking the sum of time-weighted averages of plasma glucose values between minutes 0 and 390. Total glycemic excursion was compared between protocols 1 and 2 by unpaired t-test. In all cases, P values <0.05 were considered statistically significant. Plasma glucose and hormone concentrations have been previously reported as parts of separate studies examining the effects of glucose on glucose production (29) and skin blood flow (6).

RESULTS

Plasma insulin levels were similar during all four trials in each protocol. Somatostatin successfully suppressed endoge-
nous insulin production as seen by C-peptide levels in all three protocols (Fig. 2 and Table 2). Growth hormone and glucagon levels were similar across trials, as were HR and BP (Table 2).

Plasma glucose levels are shown in Fig. 2. At -30 min (trial 1), plasma glucose values were similar in all three trials (Table 2). Peak glucose in protocol 1 (profile) was 11.26 ± 0.54 mmol/l at 60 min. As designed, glucose levels were similar between protocol 1 and protocol 2 (hyperglycemia) at 180 min (7.41 ± 0.26 vs. 7.15 ± 0.13 mmol/l). By trial 4, protocol 1 glucose values had returned to baseline (5.26 ± 0.13 mmol/l). Constant hyperglycemia was maintained during the final three trials in protocol 2, and euglycemia was achieved in protocol 3 during all four trials. As designed, glycemic excursion did not differ in protocols 1 and 2 (2.945.72 ± 58.83 vs. 2.820.67 ± 31.84 mmol/390 min, P > 0.05).

Baseline FBF before ACh infusion did not differ significantly across the four trials in any protocol, although there was a slight trend upward during the course of the experiment in all three protocols (Table 3).

FBF dose-response curves to ACh infusion are shown in Fig. 3. Peak responses to ACh in all three protocols are listed in Table 4. In contrast to our hypothesis, there was a trend toward increasing FBF in all three protocols. This trend reached statistical significance in protocol 1 (P = 0.03) and protocol 3.
The main conclusion of the present study is that acute hyperglycemia similar to that experienced by an individual with glucose intolerance following a meal does not impair endothelial function (11). We chose to study healthy humans to determine the effect of acute hyperglycemia on an otherwise normal endothelium. Diabetes mellitus is characterized by profound vascular disease and endothelial dysfunction (5, 13a, 16, 22). In healthy humans, the vascular smooth muscle is regulated by the endothelium via release of NO and other substances. Increased release of NO by the endothelial cell acts via cGMP to induce relaxation of the vascular smooth muscle and, as a result, vasodilation (5, 16). However, the endothelial cells are markedly aberrant in diabetics (5, 13a, 16). Additionally, in type 2 diabetics, other associated pathologies, such as hypertension, obesity, and hyperlipidemia (13a), may also accelerate vascular damage. Because hyperglycemia per se is often implicated in the vascular dysfunction seen in diabetics, we thought it was important to study the effects of acute hyperglycemia alone on endothelium not already compromised by changes due to chronic disease.

As noted above, an important aspect of the present experimental design was our ability to isolate the effects of acute hyperglycemia per se by controlling all other hormonal variables that could have influenced our results. For example, glucagon and growth hormone suppress insulin action (2) and also affect sympathetic nerve function (17, 26), and uncontrolled levels of these hormones might alter other physiological influences of these hormones as well as decreasing the risk of microvascular complications in individuals with type 2 diabetes (13a). Third, we assessed total FBF, since assessments relying only on brachial artery diameter may be a weaker index of vascular endothelial function (11).

Values are means ± SE. GH, growth hormone; BP, blood pressure; HR, heart rate.

Table 3. FBF baseline before ACh infusion

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Trial 1 (30 min)</th>
<th>Trial 2 (60 min)</th>
<th>Trial 3 (180 min)</th>
<th>Trial 4 (360 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Profile)</td>
<td>32 ± 5</td>
<td>32 ± 4</td>
<td>37 ± 5</td>
<td>39 ± 7</td>
</tr>
<tr>
<td>2 (Hyperglycemia)</td>
<td>38 ± 6</td>
<td>38 ± 5</td>
<td>42 ± 6</td>
<td>41 ± 8</td>
</tr>
<tr>
<td>3 (Euglycemia)</td>
<td>35 ± 4</td>
<td>35 ± 4</td>
<td>41 ± 5</td>
<td>45 ± 6</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml/min. FBF, forearm blood flow.
variables. We maintained identical hormone concentrations among study days by administering somatostatin to suppress endogenous insulin (and other hormone) production and then replaced growth hormone, glucagon, and insulin to ensure constant levels throughout the study day and across subjects and protocols (Table 2). Thus previous studies that did not control for these variables may have missed such confounding influences.

In addition, many of the existing data on forearm vasodilation during acute hyperglycemia were obtained using a "local" glucose clamp (2, 3, 18, 30), wherein glucose was infused directly into the brachial artery of study. Although previous previous

### Table 4. Peak FBF to ACh and NTP

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Trial 1 (−30 min)</th>
<th>Trial 2 (60 min)</th>
<th>Trial 3 (180 min)</th>
<th>Trial 4 (360 min)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACh</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Profile)</td>
<td>265 ± 75</td>
<td>285 ± 68</td>
<td>329 ± 101</td>
<td>336 ± 100</td>
<td>0.03</td>
</tr>
<tr>
<td>2 (Hyperglycemia)</td>
<td>307 ± 47</td>
<td>325 ± 52</td>
<td>353 ± 65</td>
<td>370 ± 70</td>
<td>0.46</td>
</tr>
<tr>
<td>3 (Euglycemia)</td>
<td>183 ± 30</td>
<td>210 ± 28</td>
<td>237 ± 36</td>
<td>266 ± 39</td>
<td>0.02</td>
</tr>
<tr>
<td>NTP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 (Profile)</td>
<td>230 ± 46</td>
<td>239 ± 51</td>
<td>260 ± 47</td>
<td>215 ± 31</td>
<td>0.55</td>
</tr>
<tr>
<td>2 (Hyperglycemia)</td>
<td>284 ± 47</td>
<td>288 ± 42</td>
<td>279 ± 39</td>
<td>283 ± 38</td>
<td>0.93</td>
</tr>
<tr>
<td>3 (Euglycemia)</td>
<td>232 ± 35</td>
<td>222 ± 38</td>
<td>225 ± 46</td>
<td>227 ± 43</td>
<td>0.60</td>
</tr>
</tbody>
</table>

Values are means ± SE in ml/min. NTP, sodium nitroprusside.
authors (2, 3, 30) using this local model have controlled for potential effects of osmolality by administering mannitol intraarterially in a control group, other effects of glucose administered in high concentrations directly into the artery of study are unknown. In addition, the different glucose concentrations [e.g., 300 mg/dl intra-arterially vs. 121 mg/dl systemically (2)] have uncertain effects on hormone action and regulation. In our protocols, a systemic glucose clamp was used. We infused glucose into a forearm vein of the contralateral (nonstudied) arm, and systemic glucose levels, as determined by samples obtained from the study arm, were used to clamp glucose at the desired level.

Our results contrast with those reported by Title et al. (27). These investigators administered a 75-g oral glucose load to healthy nondiabetics and used shear stress created during reactive hyperemia to stimulate endothelium-dependent vasodilation. Flow-mediated dilation of the brachial artery was impaired both 2 and 3 h after glucose load but was restored by vitamin C and E administration. However, as noted previously, the relationship between flow-mediated dilation of the brachial artery and studies of blood flow control in forearm resistance vessels suggest that at least two different mechanisms are involved (13).

Our results also differ with a series of reports by Creager and colleagues (2, 3, 30). In their laboratory, FBF responses to methacholine infusion were significantly diminished during “local” hyperglycemia in healthy humans (local glucose infusion into the brachial artery). This attenuation was corrected by the antioxidant vitamin C (16) and also by administering a protein kinase C inhibitor (2). Although Creager and colleagues administered octreotide systemically to suppress endogenous insulin production to hyperglycemia, this somatostatin analog inhibits pancreatic function globally and therefore suppresses a number of hormone levels in addition to insulin. Because we replaced hormones affected by somatostatin, the hormonal milieu was clearly different between the two studies. Additionally, Creager and colleagues (2, 3, 30) infused glucose into the brachial artery to achieve a local concentration...
near 16.7 mmol/l (300 mg/dl). In our study, peak glucose concentration approached ~11 mmol/l in protocol 1 and ~7 mmol/l in protocol 2. It is possible that the higher glucose concentrations used in their studies are needed to elicit significant changes in forearm vasodilator responsiveness, at least in healthy humans. However, the glucose levels chosen in our study reflect the point identified by numerous population-based studies and the ADA as the point where microvascular risk increases (13a). Because of this, we thought it was relevant to evaluate endothelial function at these critical blood glucose levels.

Although our results differ from the data reported by Title et al. (27) and Creager and colleagues (2, 3, 30), our results are similar to those described by Houben et al. (18). Using a local glucose clamp at ~15 mmol/l, Houben et al. demonstrated that endothelium-dependent dilation to ACh did not differ compared with baseline at 6, 12, or 24 h in the healthy human forearm. In fact, there was a trend toward increasing FBF over the course of the study day, similar to our results. In the present study, systemic hyperglycemia was maintained at levels corresponding to increased risk for vascular disease, and other potentially confounding hormonal responses were prevented. We therefore conclude that a single episode of hyperglycemia at these levels does not cause endothelial damage or vascular dysfunction.

Caballero et al. (4) measured brachial artery diameter changes during reactive hyperemia in four groups: individuals with a first-degree relative with type 2 diabetes, those with impaired glucose tolerance, those with type 2 diabetes, and healthy controls. All groups besides the overt diabetics were normoglycemic during measurements. However, brachial artery dilation during reactive hyperemia was impaired in all three study groups compared with controls. In addition, serum markers of endothelial dysfunction, including endothelin-1, were elevated in all three study groups (4). Although our study was not designed to evaluate the diabetic endothelium, these findings suggest a permissive role for a baseline endothelial dysfunction as a result of advanced glycosylation end products, chronic oxidative stress, and other factors (16) in causing significantly impaired endothelium-dependent vasodilation during hyperglycemia.

In the present study, there was a trend for overall FBF (baseline and peak values) to increase over the course of the study day. However, this trend was seen in all three protocols (Fig. 4), was not consistent with any pattern of hyperglycemia, and was in the direction opposite to that of our hypothesis. Because the trend occurred in our euglycemia trials (protocol 3), we do not think that this tendency for FBF to increase was an effect of hyperglycemia.

In summary, we studied healthy humans to assess whether acute, mild hyperglycemia would impair presumably normal endothelial cell function in the absence of other confounding factors seen in a diabetic population. Our data indicate that a transient increase in blood glucose to ~11 mmol/l or sustained increase to ~7 mmol/l (i.e., the fasting and postglucose challenge concentrations used to diagnose diabetes and thought to be predictive of future microvascular complications) do not impair endothelium-dependent or endothelium-independent vasodilator responses in the human forearm. Our study design allowed us to rule out potentially confounding contributions of hormonal responses to changes in systemic glucose levels. We therefore conclude that a single episode of hyperglycemia at these levels does not impair these vasodilator mechanisms in the healthy human forearm. Further studies will be needed to determine how repeated transient and/or chronic sustained increases in glucose concentrations impair endothelium-dependent vasodilation in type 2 diabetes.

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