Cutaneous vascular function during acute hyperglycemia in healthy young adults

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Charkoudian, N., A. Vella, A. S. Reed, C. T. Minson, P. Shah, R. A. Rizza, and M. J. Joyner. Cutaneous vascular function during acute hyperglycemia in healthy young adults. J Appl Physiol 93: 1243–1250, 2002.—Although it is well established that severe chronic hyperglycemia is associated with microvascular disease, it is not known whether transient hyperglycemia similar to that observed with impaired glucose tolerance or early Type 2 diabetes contributes to this pathology by altering microvascular function. To test the hypothesis that acute hyperglycemia decreases microvascular vasodilator responsiveness in human skin, we measured the cutaneous vasodilator response to local warming. This response can be divided into two phases, an initial peak that relies predominantly on local sensory nerves and a second slower phase that is largely dependent on endothelial nitric oxide. We reasoned that a change in one or both phases would indicate a change in the corresponding mechanism(s) with hyperglycemia. Twenty-eight healthy volunteers (14 women, 14 men) were randomly divided into three groups, corresponding to 6 h of euglycemia (n = 8), 6 h when glucose was clamped at ~7 mmol/l (n = 10), or 6 h when glucose was varied to mimic a postprandial pattern (i.e., peak glucose ~11.1 mmol/l) commonly observed in individuals with impaired glucose tolerance (n = 10). Insulin concentrations in all instances were maintained at ~65 pmol/l by means of continuous infusions of somatostatin and insulin. Glucagon and growth hormone were also continuously infused to maintain their basal concentrations. Despite substantial differences in both the level and pattern of glucose concentrations, neither maximum cutaneous vasodilation nor the pattern of the vasodilator response to local warming differed over the 6 h of study. We conclude that acute hyperglycemia similar to levels commonly observed in people with either early Type 2 diabetes or impaired glucose tolerance does not alter the vasodilator response to local warming of the skin in humans.

skin blood flow; laser Doppler; diabetes; vasodilation; endothelium

ALTERATIONS IN THE MICROCIRCULATION contribute to the vascular and cutaneous complications of Type 2 diabetes mellitus (2, 17, 22, 29). The slow onset of Type 2 diabetes mellitus, with gradual development of abnormal glucose regulation over several years, often goes undiagnosed because of the lack of overt symptoms (24). Importantly, microvascular complications are frequently present at the time of diagnosis, indicating that microvascular pathology is often undetected during the period of undiagnosed hyperglycemia (11).

Abnormal glucose regulation during the development of Type 2 diabetes mellitus is characterized by hyperglycemia, which is usually most marked after meals. Thus a person with impaired glucose tolerance is likely to experience intermittent episodes of acute hyperglycemia interspersed among periods of relative normoglycemia. Furthermore, the risk of microvascular complications rises dramatically when fasting blood glucose exceeds 7 mmol/l (126 mg/dl) (8, 20, 24). Many therapies used in the treatment of Type 2 diabetes are more effective in lowering fasting than postprandial glucose levels (9, 10). In recent years, the relative importance of postprandial vs. fasting hyperglycemia in the pathogenesis of vascular complications has become a matter of debate (7).

It is presently not known whether either a single transient postprandial rise in glucose or a single episode of sustained but modest hyperglycemia alters vascular function. Some evidence suggests that such acute hyperglycemia may do so. In coronary arteries of healthy dogs (18) and in mesenteric resistance vessels of healthy rats (26), vasodilator responsiveness to acetylcholine was diminished during acute exposure to hyperglycemia. In healthy humans, brachial artery flow-mediated dilation was diminished by acute systemic hyperglycemia induced by oral glucose loading (28). Also in healthy subjects, 6 h of “local” hyperglycemia [16.7 mmol/l (300 mg/dl)] glucose infusions into the brachial artery] decreased endothelium-dependent forearm vasodilation to intra-arterial methacholine (30). This impairment was reversed by inhibition of protein kinase C-β, suggesting that hyperglycemia might impair vascular function by increasing the activity of this enzyme (3). Whether lower levels of hyperglycemia, in patterns seen early in diabetes, have similar effects is unknown.

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Clearly, the skin is a site of substantial pathology in diabetes. However, whether the acute effects of hyperglycemia noted above are true for the cutaneous microcirculation and could thus contribute to the development of pathology in this vascular bed is not clear. In the present study, we tested whether, in humans, systemic hyperglycemia per se (i.e., in the absence of the insulin response) at levels corresponding to those shown to increase risk for vascular disease [i.e., fasting glucose of 7 mmol/l (126 mg/dl) or peak postprandial glucose of 11.1 mmol/l (200 mg/dl)] has the effects in the skin previously shown for higher levels of hyperglycemia in other species and/or circulations.

In humans, direct local warming of the skin causes substantial vasodilation of the area being warmed. The cutaneous vasodilator response to prolonged local warming is biphasic, including an initial peak and modest decrease followed by a slower second phase that attains a plateau around 30 min of warming (13, 15, 21). The slower phase of this vasodilation was recently identified to be largely (65–75%) dependent on nitric oxide (15, 21), whereas the initial peak is dependent on axon reflex activity of cutaneous sensory nerves (21). Thus this local warming paradigm can be used as a tool to assess nitric oxide-dependent and axon-reflex vasodilation in the cutaneous microvasculature (15, 21).

With this information as a background, we hypothesized that acute hyperglycemia would diminish the second, largely nitric oxide-dependent phase of this vasodilator response but have no effect on the initial, neurally mediated phase. We tested this hypothesis by observing cutaneous vasodilator responses to local warming of the skin before, during, and after elevated plasma glucose concentrations mimicking those observed after a meal in an individual with Type 2 diabetes mellitus and compared these with the cutaneous vasodilator responses to local warming during sustained hyperglycemia and euglycemia.

METHODS

Subjects

The protocol for this study was approved by the Institutional Review Board of the Mayo Clinic. Subjects were normal healthy nonsmokers with no history of cardiovascular disease or diabetes and were taking no medications (with the exception of oral contraceptives). None of the subjects had first-degree relatives with a history of diabetes. All female subjects were taking oral contraceptives and all were studied at the General Clinical Research Center and fasted overnight.

At 0600, a forearm vein in the dominant arm was cannulated with an 18-gauge intravenous catheter for the systemic infusions (see below). At 0700, the brachial arterial catheter of the nondominant arm was cannulated with an 18-gauge catheter for blood sampling and measurement of arterial blood pressure. From this time until the end of the study (1600 h), the subject remained supine and did not consume any food or beverage.

Procedures

Heart rate was monitored by electrocardiogram, and arterial blood pressure was measured directly from the brachial arterial catheter that was connected to a pressure transducer positioned at the level of the heart. The brachial artery catheter was also used for periodic infusions of small amounts of acetylcholine and sodium nitroprusside as part of a separate protocol.

Skin blood flow was measured by laser-Doppler flowmetry (LDF) (Perimed Periflux System 5000, Stockholm, Sweden) on the lateral aspect of the lower leg, approximately halfway between the knee and the ankle. The laser-Doppler probe, in the center of a 12-cm² local warming unit, was attached to the measurement site at the beginning of the study day and was not moved or removed until the end of the study day.

Protocols

Subjects were randomly assigned to one of three groups corresponding to one of three plasma glucose protocols: protocol 1, the glucose profile group (n = 10; 5 men, 5 women); protocol 2, the mild hyperglycemia group (n = 10; 5 men, 5 women); and protocol 3, the euglycemia group (n = 8; 4 men, 4 women). The timelines for these protocols are shown schematically in Fig. 1.

Protocol 1 (glucose profile). This protocol was designed to mimic the plasma glucose concentrations likely to occur in individuals with glucose intolerance after carbohydrate ingestion. Starting at 0730, somatostatin was infused (60 ng·kg⁻¹·min⁻¹) to inhibit endogenous insulin secretion; growth hormone (3 ng·kg⁻¹·min⁻¹) and glucagon (0.65 ng·kg⁻¹·min⁻¹) were also infused to maintain systemic fasting concentrations. Insulin was infused at 0.20 μU·kg⁻¹·min⁻¹ to achieve high physiological concentrations for the duration of the experiment. A variable glucose infusion was begun at 0730 to maintain plasma glucose concentrations at 5 mmol/l (95 mg/dl) until 0900. At 0900 (time 0 in Fig. 1), plasma glucose concentrations were increased in such a fashion that a peak plasma glucose concentration of ~11.1 mmol/l (~200 mg/dl) was achieved at ~60 min (1000 h) followed by a return to baseline levels as shown in Fig. 1. Trace amounts of [3-¹⁴C]glucose were also infused as part of a separate protocol. Blood glucose was measured every 10 min by use of a Beckman glucose analyzer to facilitate clamping of glucose at target levels.

Protocol 2 (constant hyperglycemia). This protocol was designed to reproduce the modest elevations in fasting glucose

<table>
<thead>
<tr>
<th>n</th>
<th>Glucose Profile</th>
<th>Constant Hyperglycemia</th>
<th>Euglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25 (5 F, 5 M)</td>
<td>27 (5 F, 5 M)</td>
<td>27 (4 F, 4 M)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.73 ± 0.03</td>
<td>1.71 ± 0.05</td>
<td>1.75 ± 0.05</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.43 ± 3.93</td>
<td>71.74 ± 5.56</td>
<td>77.00 ± 6.16</td>
</tr>
<tr>
<td>Fasting glucose, mmol/l</td>
<td>5.04 ± 0.08</td>
<td>5.02 ± 0.11</td>
<td>4.91 ± 0.07</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. F, female; M, male.
that commonly occur in individuals with early Type 2 diabetes mellitus. This protocol was identical to protocol 1, except that from 0900 sufficient exogenous glucose was infused to clamp plasma glucose at ~7.0 mmol/l (126 mg/dl) for the 6 h of study. Protocols 1 and 2 were designed such that the total glycemic excursions (areas under the curve for glucose from minute 0 to minute 390) did not differ.

Protocol 3 (euglycemia). Protocol 3 was the same as the other two protocols except that sufficient glucose was infused to maintain glucose concentrations at ~5.3 mmol/l.

Local Warming of the Skin

The LDF probe was placed in a specialized holder that can both measure and control local temperature over 12 cm² at the measurement site (Perimed). As mentioned above, the probe-heater unit was fixed to the skin of the lower leg, halfway between the knee and the ankle, for the duration of the study day. Subjects were instructed to remain relaxed and keep the leg very still during all measurements of skin blood flow. Each local warming procedure was preceded by a 10-min period during which local temperature held constant at 33°C. Local temperature was then increased to 42°C over 1.5 min. Care was taken to increase temperature at this slow rate every time, because faster increases in local temperature have been shown to engender a pain response that can alter the local vasodilator response (19). Local temperature was held at 42°C for 30 min, after which temperature was allowed to decrease to normal. Local temperature always decreased back to 33°C within 10–15 min of each local warming trial. LDF was measured continuously during the baseline and local warming periods. LDF and arterial pressure were sampled at 250 Hz and subsequently analyzed off-line (Windaq, Dataq Instruments, Akron, OH).

There were four local warming trials during each study. Figure 1 shows the timing of the four local warming trials as related to plasma glucose levels during the three protocols. A baseline local warming trial was conducted at ~30 min, such that all individuals were at euglycemia; in this way each individual served as his or her own control, such that data during hyperglycemia were compared with data from the baseline period. The second trial, at 60 min, corresponded to peak plasma glucose in protocol 1. The third trial (minute 180) corresponded to the time point at which plasma glucose levels for protocols 1 and 2 were similar. The fourth trial (minute 360) corresponded to the time point at which the areas under the curves for glucose were the same between protocols 1 and 2. Importantly, no subject experienced pain at any time during any local warming trial.

Blood Samples

Blood samples were drawn from the arterial catheter for the measurement of plasma glucose, insulin, C peptide, glucagon, and growth hormone. During the study, blood glucose was measured every 10 min by use of a Beckman glucose analyzer to facilitate clamping of glucose at target levels.

A separate set of samples for poststudy glucose and hormone analysis was placed on ice, centrifuged at 4°C, separated, and stored at ~20°C until assay. Plasma C-peptide and glucagon concentrations were measured in the Mayo Immunochemical Core Laboratory by using reagents purchased from Linco Research (St. Louis, MO). Insulin and growth hormone were measured by chemiluminescence (Access Assay, Beckman, Chaska, MN). Glucose was measured by using a Yellow Springs glucose analyzer.

Data Analysis

LDF was divided by mean arterial pressure (MAP) to derive an index of cutaneous vascular conductance (CVC). Maximum CVC was defined as the average CVC over the last 3 min of local warming. To analyze the pattern of the cutaneous vasodilator response to local warming, the response was divided into two phases corresponding to the two mechanisms previously described (21). The initial peak was quantified as the average of the 30 s during which CVC reached a peak in the 2–3 min after the onset of local warming. It was quantified as both absolute units and as a percentage of maximum. The nadir after the initial peak was defined as the 30 s after the initial peak at which CVC was at its lowest level before increasing again in the second phase of the vasodilation (5, 21).

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 minute 0 and minute 390. Total glycemic excursion was compared between protocols 1 and 2 by unpaired t-test.

For the remaining statistical analysis, each subject served as his or her own control, such that data during hyperglycemia were compared with data from the baseline period. Baseline CVC and CVC response to local warming were compared across local warming trials by ANOVA with repeated measures. To test whether hyperglycemia altered the nadir after the initial peak, peak and nadir values were compared by t-test at each trial time point. Plasma glucose and insulin concentrations, as well as heart rate and blood pressure during each trial, were analyzed by ANOVA with repeated-measures ANOVA. When a significant effect was detected, Tukey’s post hoc test was used to identify individual differences. Statistical significance was accepted for P < 0.05.
RESULTS

Plasma Glucose, Insulin, and C-Peptide Concentrations

Plasma glucose concentrations were similar among the three groups before the start of the variable glucose infusions. Plasma glucose and insulin levels during each of the four local warming trials for each of the three protocols are shown in Fig. 2. During protocol 1 (glucose profile), plasma glucose concentrations rose to a peak of $11.2 \pm 0.5$ mmol/l at 60 min. During protocols 2 and 3, plasma glucose concentrations were kept constant at $7.2 \pm 0.1$ and $5.3 \pm 0.1$ mmol/l, respectively. Although there was a slight decrease in plasma glucose at 180 min in protocol 3, values remained well within the euglycemic range.

As designed, the total glycemic excursion during protocols 1 and 2 did not differ ($2,942.3 \pm 56.1$ vs. $2,823.2 \pm 27.3$ mmol/h, $P > 0.05$). Insulin concentrations did not differ among the three protocols. Somatostatin resulted in prompt and comparable suppression of C peptide during all three protocols; glucagon and growth hormone remained constant. These values are shown in Table 2.

Maximum CVC

Values for maximum CVC during local warming are shown in Table 3. There was no influence of acute hyperglycemia on the maximum values for CVC during local warming. After demonstration that maximum CVC was not affected by hyperglycemia, data were subsequently expressed and analyzed as percentage of maximum to allow for intra- and interindividual comparison (6, 13).

CVC at 33°C

CVC at 33°C, before each local warming trial, was not significantly affected by either type of acute hyperglycemia (see Table 4). As can be seen in the table, there was a tendency for this CVC value to increase at the 360-min trial (including the euglycemia protocol). This may have been due to a slight increase in ambient temperature over the course of the protocol, although local temperature around the site of measurement was always controlled at 33°C during the baseline period. Alternatively, this slight increase could have been due to a lingering effect of the previous local warming trials.

Table 2. Plasma C-peptide, glucagon, and growth hormone concentrations during the 4 local warming trials by protocol

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>60 min</th>
<th>180 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protocol 1: glucose profile</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C peptide, nmol/l</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>148 ± 10</td>
<td>134 ± 6</td>
<td>133 ± 4</td>
<td>138 ± 8</td>
</tr>
<tr>
<td>Growth hormone, µg/l</td>
<td>1.0 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td><strong>Protocol 2: constant hyperglycemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C peptide, nmol/l</td>
<td>0.09 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>139 ± 6</td>
<td>134 ± 3</td>
<td>123 ± 7</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>Growth hormone, µg/l</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td><strong>Protocol 3: euglycemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C peptide, nmol/l</td>
<td>0.10 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Glucagon, ng/l</td>
<td>155 ± 17</td>
<td>140 ± 13</td>
<td>130 ± 6</td>
<td>129 ± 10</td>
</tr>
<tr>
<td>Growth hormone, µg/l</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.0 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SE.

Fig. 2. Plasma glucose (A) and insulin (B) concentrations during the 4 local warming trials for each of the 3 protocols.
Table 3. Maximum cutaneous vascular conductance during local warming

<table>
<thead>
<tr>
<th>Trial</th>
<th>Glucose Profile</th>
<th>Constant Hyperglycemia</th>
<th>Euglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>2.16 ± 0.17</td>
<td>1.96 ± 0.14</td>
<td>1.89 ± 0.16</td>
</tr>
<tr>
<td>60 min</td>
<td>2.10 ± 0.13</td>
<td>2.01 ± 0.13</td>
<td>1.87 ± 0.13</td>
</tr>
<tr>
<td>180 min</td>
<td>2.15 ± 0.15</td>
<td>1.94 ± 0.13</td>
<td>1.90 ± 0.13</td>
</tr>
<tr>
<td>360 min</td>
<td>2.05 ± 0.15</td>
<td>2.07 ± 0.14</td>
<td>1.96 ± 0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE in Laser-Doppler units/mmHg.

Dynamic Response to Local Warming

Figure 3 shows the responses of a representative individual from the glucose profile group (protocol 1) to local warming during the four trials. As can be seen from this figure, the initial peak and slower second phase of the response remained unaltered throughout the hyperglycemia protocol. Interestingly, the nadir after the initial peak was absent at the 360-min trial. This was true in all subjects for this trial; that is, comparison of peak and nadir revealed that these values were not different at 360 min (P > 0.05). However, it did not appear to be an influence of hyperglycemia because it occurred in the euglycemia group as well. The reason for the disappearance of this nadir in the last local warming trial is unknown but may relate to sensitization of skin or sensory nerves to the local warming stimulus over the course of the four trials.

Figure 4 shows average values for the initial peak phase of the vasodilator response. For all trials, the initial peak in CVC attained ~80% of maximum and was thus consistent with previously reported values for this peak (5, 21). This initial peak value was unaffected by acute hyperglycemia. There was a slight, but significant, increase in initial peak CVC during the 180- and 360-min trials; as with the above, however, this did not appear to be an effect of hyperglycemia because it was seen in all groups, including those maintained at euglycemia. As mentioned above, this slight augmentation of the initial peak may have been due to a sensitization of the skin to the local heating; this possibility requires further investigation.

Systemic Hemodynamics

MAP did not change significantly over time during any of the protocols (P > 0.05). Overall average MAP values were 85.8 ± 0.4 (glucose profile); 84.2 ± 0.4 (constant hyperglycemia); and 85.9 ± 0.4 mmHg (euglycemia). Heart rate also remained constant over time (overall averages: 62 ± 1 (glucose profile); 62 ± 1 (constant hyperglycemia); and 61 ± 1 beats/min (euglycemia)) with the exception of a slight but statistically significant increase (to 65 ± 4 beats/min) during the 60-min trial in the glucose profile group (P = 0.03).

DISCUSSION

The main new finding of the present study is that acute changes in systemic plasma glucose concentrations of a magnitude that presumably commonly occur after eating in individuals with impaired glucose tol-erance or during the night in individuals with mild Type 2 diabetes mellitus do not alter the vasodilator response to local warming of the skin in healthy humans. This is of interest because the degrees of hyperglycemia created in the present study correspond to those shown to represent significantly increased risk for vascular disease in population-based studies (8, 20). The present data indicate that up to 6 h of sustained mild hyperglycemia or 4–5 h of postprandial hyperglycemia do not modify baseline skin blood flow or the ability to vasodilate during local warming.

We do not, however, exclude the possibility that longer episodes of hyperglycemia, or repeated episodes of similar duration, could have an effect on the cutaneous microcirculation over time. In the forearm circulation, higher levels of “local” hyperglycemia caused decreased endothelium-dependent vasodilation (3, 30). It is possible that higher levels of hyperglycemia in the present study would have resulted in impairment of the cutaneous vasodilator response to local warming.

Recent studies of the cutaneous microvascular response to local warming in humans provide important information regarding the mechanisms of this response. Regarding the second, prolonged phase of the response, Kellogg et al. (15), as well as Minson et al. (21), demonstrated that local administration of the nitric oxide synthase inhibitor nitro-L-arginine methyl ester via microdialysis causes a 65–75% reduction. Although the remaining 25–35% of the vasodilation remains unexplained, the findings of these investigators make local warming of the skin a useful tool to study the endothelial nitric oxide system in the human cutaneous microcirculation. The present experimental design did not allow us to distinguish between the nitric oxide-mediated and non-nitric oxide-mediated portions of the vasodilation; however, the fact that the response was unaltered suggests that both portions were unaffected. The lack of effect of hyperglycemia on the vasodilator response as a whole also indicates, more generally, that blood vessel function “downstream” from the endothelium remained intact as well.

It is clear that the cutaneous microvascular dysfunction seen in diabetes is due in large part to endothelial dysfunction. For example, individuals with established diabetes have decreased cutaneous microvascular responses to local iontophoresis of acetylcholine (2, 17, 22, 29). This decrease in endothelium-dependent vasodilation is associated with decreased expression of en-

Table 4. Cutaneous vascular conductance at 33°C before each local heating trial during each of the 3 glucose clamp protocols

<table>
<thead>
<tr>
<th>Trial</th>
<th>Glucose Profile</th>
<th>Constant Hyperglycemia</th>
<th>Euglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>baseline</td>
<td>12.72 ± 1.37</td>
<td>16.97 ± 2.67</td>
<td>14.05 ± 1.26</td>
</tr>
<tr>
<td>60 min</td>
<td>13.10 ± 2.30</td>
<td>13.98 ± 2.47</td>
<td>11.68 ± 1.03</td>
</tr>
<tr>
<td>180 min</td>
<td>13.10 ± 1.99</td>
<td>15.48 ± 2.24</td>
<td>12.49 ± 1.07</td>
</tr>
<tr>
<td>360 min</td>
<td>17.33 ± 2.58</td>
<td>21.24 ± 2.70</td>
<td>18.32 ± 1.02</td>
</tr>
</tbody>
</table>

Values are means ± SE, given as % of maximum. *P < 0.05 vs. baseline, 60-min, and 180-min trials.
dothelial nitric oxide synthase in the skin of diabetic subjects (4, 29). Epidemiological data show a clear relationship between hyperglycemia and microvascular disease, when assessed as either fasting plasma glucose or response to an oral glucose tolerance test (8, 20). In this context, we questioned whether acute hyperglycemia over several hours has deleterious effects that could contribute to the development of this pathology. We addressed this question using transient hyperglycemia such as that seen after a carbohydrate meal in an individual with impaired glucose tolerance or in an individual with impaired fasting glucose or early Type 2 diabetes. We hypothesized that acute hyperglycemia would decrease the prolonged, largely nitric oxide-dependent vasodilator response to local warming of the skin. The present results argue against our hypothesis.

Our results contrast with the conclusions of Akbari et al. (1), who measured the cutaneous microvascular vasodilator response to iontophoresis of acetylcholine in healthy subjects before and 1 h after ingestion of 75 g of glucose. They concluded that the vasodilator response was diminished during acute hyperglycemia because the percent increase from baseline was less after glucose ingestion than in the fasting state. However, their data are open to several interpretations (27). First, the baseline level of skin blood flow was increased after glucose ingestion. Therefore, even though they report the same absolute level of vasodilation between fasting and postprandial measure-
ments, this value was necessarily a lower percent of the higher baseline in the postprandial state. Second, these investigators apparently did not control (or report measurements of) insulin levels between fasting and postprandial states. Insulin, itself a nitric oxide-port measurements of insulin levels between fasting these investigators apparently did not control (or re-
ments, this value was necessarily a lower percent of the higher baseline in the postprandial state. Second, these investigators apparently did not control (or report measurements of) insulin levels between fasting and postprandial states. Insulin, itself a nitric oxide-port measurements of insulin levels between fasting these investigators apparently did not control (or re-
study by Houben et al. likely means that sufficient acetylcholine did not reach the skin area being measured. To avoid any such potentially confounding factors in the present study, we utilized a stimulus specific to the skin and measurement of skin blood flow directly at the site of that stimulus.

The initial peak phase of the vasodilator response to local warming was recently shown by Minson et al. (21) to be dependent on local sensory nerves. This is most likely via an axon reflex mechanism, because the vasodilation does not require intact connection to the central nervous system (21, 23) but was abolished by local application of anesthetic (EMLA cream) (21). Axon reflex mechanisms probably also account for the indirect vasodilation in response to iontophoresis of acetylcholine; this response has been shown to be diminished in diabetes (2, 17). Our results suggest that sensory nerve-dependent vasodilation in the skin was not immediately affected by the hyperglycemia protocols of the present study.

We observed a time-dependent alteration in the vasodilator response that was not a function of hyperglycemia, because it was also seen in the euglycemia control group. That is, the initial peak was slightly augmented and the nadir was absent in the fourth trial of each protocol compared with the first three. This phenomenon may be related to sensitization of the skin or sensory nerves to local warming over several trials; however, the mechanisms for these changes are unknown.

In the present experiments, we studied the effect of transient hyperglycemia per se in healthy volunteers. Although we did not observe an effect on cutaneous microvascular function, it is important to note that individuals with impaired glucose tolerance and diabetes are exposed daily to rising and falling glucose concentrations, as well as to transient changes in insulin concentrations (absent in the present study). Potential influences of these repeated transient changes in glucose and insulin levels on microvascular control and the mechanisms involved in such effects remain unclear at this time. Additionally, the manner in which acute and chronic hyperglycemia interact with other factors (e.g., hypertension, hyperlipidemia) likely to be present in diabetics or in those at risk for diabetes is presently unknown.

In summary, neither the sensory nerve-dependent initial peak nor the largely nitric oxide-dependent second phase of the cutaneous vasodilator response to local warming was altered by either an acute increase in glucose to ~11.1 mmol/l or by 6 h of sustained modest hyperglycemia. These data indicate that a transient increase in glucose to concentrations com-
monly observed in people with either impaired glucose tolerance or impaired fasting glucose do not alter nitric oxide-dependant or axon reflex vasodilation in the skin. However, it remains to be determined whether repeated similar rises or less frequent more marked increases in glucose contribute to the cutaneous microvascular endothelial dysfunction typical of Type 2 diabetes mellitus.

We are grateful to the subjects for their patient participation. These studies were supported by National Institutes of Health (NIH) Grants DK-29953, HL-63328, and AR-08610 and NIH General Clinical Research Center Grant RR-00585 (to the Mayo Clinic).

REFERENCES


