Effects of an oral glucose tolerance test on the myogenic response in healthy individuals

Mary E. J. Lott,1 Cynthia Hogeman,1 Michael Herr,1 Robert Gabbay,2 and Lawrence I. Sinoway1  
Divisions of 1Cardiology and 2Endocrinology, Pennsylvania State University  
College of Medicine, Milton S. Hershey Medical Center, Hershey, Pennsylvania

Submitted 1 September 2005; accepted in final form 21 August 2006


The myogenic response, the inherent ability of blood vessels to rapidly respond to changes in transmural pressure, is involved in local blood flow autoregulation. Animal studies suggest that both acute hyperglycemia and hyperinsulinemia may impair myogenic vasoconstriction. The purpose of this study was to examine the effects of an oral glucose load on brachial artery MBV during increases in forearm transmural pressure in humans. Eight healthy men and women (38 ± 5 yr) underwent an oral glucose tolerance test (OGTT). MBV (in cm/s; Doppler ultrasound) responses to a rise in forearm transmural pressure (arm tank suction, −50 mmHg) were studied before and every 30 min for 120 min during the OGTT. Before the start of the OGTT, MBV was lower than baseline values 30 and 60 s after the application of negative pressure. This suggests that myogenic constriction was present. During the OGTT, blood glucose rose from 88 ± 2 to 120 ± 6 mg/dl (P < 0.05) and insulin rose from 14 ± 1 to 101 ± 32 μU/ml (P < 0.05). Glucose loading attenuated the reduction in MBV with arm suction (Δ−0.73 ± 0.14 vs. Δ−1.67 ± 0.43 cm/s and Δ−1.07 ± 0.14 vs. Δ−2.38 ± 0.54 cm/s, respectively, during 30 and 60 s of suction postglucose compared with preglucose values; all P < 0.05). We observed no such time effect for myogenic responses during a sham OGTT. In an additional 5 subjects, glucose loading had no effect on brachial diameters with the application of negative pressure. Oral glucose loading leads to attenuated myogenic vasoconstriction in healthy individuals. The role that this diminished postglucose reactivity plays in mediating postprandial hypotension and/or orthostasis needs to be further explored.

METHODS

Study Subjects

Eight healthy nonobese subjects (5 men and 3 women; age, 38 ± 5 yr, mean ± SE) participated in the primary study (Table 1). Subjects were nonsmokers, normotensive, and not on any medications. All subjects were free of symptoms and/or history of cardiac, vascular, pulmonary, metabolic, or neurological disease or diabetes. The women were tested 18.0 ± 4.7 days into their menstrual cycle, and none were on oral contraceptives. All subjects were recreationally active, but none were involved in a regular exercise program >4 days/wk. The Institutional Review Board of the Milton S. Hershey Medical Center approved the experimental protocol. Each person had the purposes and risks of the protocol explained to them before written consent was obtained.

Experimental Design

An arm tank, employing air suction (~–50 mmHg), was used to evoke a sustained increase in forearm transmural pressure. MBV responses were measured as glucose concentrations were changed during a 2-h oral glucose tolerance test (OGTT; experiment 1, n = 8). To exclude that time alone leads to MBV changes during the OGTT, six of the original eight subjects returned within 6 mo of their initial OGTT for a control trial (i.e., sham OGTT), examining MBV responses every 30 min over a 2-h time period but without any ingestion of an oral glucose load.
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/women</td>
</tr>
<tr>
<td>Age, yr</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>Weight, kg</td>
</tr>
<tr>
<td>Body-mass index kg/m²</td>
</tr>
<tr>
<td>Forearm circumference, cm</td>
</tr>
<tr>
<td>Forearm volume, ml</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
</tr>
</tbody>
</table>

Values are means ± SE.

of a glucose drink (see Fig. 1; experiment 2). In an additional five subjects, brachial diameter responses to arm suction were measured during the OGTT (experiment 3).

Experimental Measurements

Measurement of heart rate, blood pressure, and limb circumference. A standard electrocardiogram was used to monitor heart rate (HR). Systolic and diastolic blood pressures were continuously measured using the volume-clamp method (Finapres, Ohmeda, Madison, WI) with mean arterial pressure (MAP) calculated from the Finapres waveform. Before the testing, Finapres pressure was confirmed by an automated sphygmomanometer (Dinamap, Critikon, Tampa, FL). HR and MAP were recorded continuously and collected online at 200 Hz using a PowerLab system (AD Instruments, Castle Hill, Australia). Anthropometric measurements taken included forearm volume by water displacement technique and lower forearm circumferences (26).

Measurement of brachial MBV. Brachial artery MBV were measured on a beat-by-beat basis using a 4-MHz pulsed-wave Doppler ultrasound probe (500 M, Multigon, Yonkers, NY; n = 8). This flat probe was securely taped into a fixed position to the skin over the brachial artery ~8–10 cm proximal to the antecubital fossa. Since the brachial artery is approximately parallel to the skin surface, the insonation angle with the brachial artery was ~45°. The gate for ultrasound was also set to insonate the total width of the artery diameter. Maximal Doppler frequency shift was obtained with slight manual adjustments of the Doppler probe. MBV was measured continuously and collected online as noted above. The coefficient of variability for MBV measurements was 8.44 ± 1.55%.

Measurement of brachial diameters. Resting brachial arterial diameters were measured using Doppler ultrasound (n = 5; 12–5 MHz; model HDI 5000 CV, Advanced Technology, Bothell, WA). The coefficient of variability for the diameter measurements was 1.97 ± 0.06%.

Experimental Interventions

Pressurized forearm tank. The lower aspect of the nondominant arm, including the hand, was sealed above the elbow in a forearm boxlike tank. Two neoprene cuffs were fitted around the arm creating a snug nonconstricting seal. Negative pressures were obtained from an external vacuum source and directed to a holding pressure tank. From this pressurized holding tank, a manual switch opened the system to provide the appropriate negative air pressure (i.e., suction) to the forearm tank within 0.2–0.4 s. A primer trial was performed to ensure that the appropriate pressure (~50 mmHg) was present within the forearm tank.

Oral glucose tolerance testing. A 2-h OGTT was performed after a 12-h fast. All subjects had consumed a 300-g carbohydrate diet for 3 days before testing. Blood samples were drawn in the fasting state and after ingestion of the glucose drink (75 g) and every 30 min thereafter at 30, 60, 90, and 120 min for later analysis of glucose and insulin. All blood samples were immediately placed on ice and then centrifuged. Resting plasma samples were frozen at −80°C for later analysis. Glucose was measured in duplicate by the glucose oxidase method using a glucose analyzer (Stat Plus Model 2300, Yellow Springs Instrument). Insulin assays were done by using antibody radioimmunoassay (Linco Research, St. Charles, MO) in duplicate. In the General Clinical Research Center’s laboratory, the coefficient of variability for glucose and insulin assays were ~2.8% and ~7.1%, respectively.

Experimental Testing

All studies were performed in a quiet dimly lit and temperature-controlled room (21° to 24°C). Subjects were instructed to abstain from products containing caffeine and alcohol, as well as to abstain from any exercise 24 h before testing. Subjects were studied in the morning after a 12-h fast. Subjects were instrumented with ECG electrodes. The subject’s nondominant forearm was inserted into the forearm tank. A 20-gauge Teflon catheter was inserted into a brachial vein of the dominant arm, and a Finapres blood pressure device was attached to the middle finger of this hand.

Experiment 1. After a 20-min rest period, baseline blood samples (glucose, insulin, and cholesterol) and baseline measurements MBV measurements were made (n = 8). Negative pressure (~50 mmHg of forearm suction) was then applied for 1 min before returning back to ambient forearm pressure. After a rest period of 2 to 3 min, a second trial was performed and the responses were averaged. The subject then ingested the glucose drink. Blood samples (glucose and insulin) and the arm tank protocol were performed at 30-min intervals after glucose ingestion (30, 60, 90, and 120 min).

Experiment 2. Six subjects returned for a control time trial (sham OGTT) repeating experiment 1 but without glucose consumption.

Experiment 3. In an additional five subjects, brachial diameters were measured before and during a 2-h OGTT using the same forearm tank protocol as in Experiment 1. Three men and two women with a body-mass index of 23.5 ± 1 kg/m² were studied.

Data analysis. The following variables were measured on a beat-by-beat basis: HR, MAP, and MBV. Trials for each pressure time period were averaged for each individual. Raw values [i.e., additive (absolute delta change from baseline), not percent change] were used to analyze our data. For each outcome measurement, a linear mixed-effects model was fit to the data to assess responses to arm tank suction (0, 30, and 60 s) at the following time periods during the OGTT: 0, 30, 60, 90, and 120 min (21). The linear mixed-effects model is an extension of an analysis of variance model that accounts for the within-subject variability inherent in longitudinal experiments. In the event that modeling assumptions, such as normality, were not met, a transformation using the natural logarithm was applied to the response and used as the outcome measurement. To account for multiple comparisons testing in the post hoc analysis, Tukey’s procedure was used. For simplicity of data presentation and ease of

Fig. 1. Blood samples (glucose and insulin) and the pressurized arm tank protocol performed preglucose (0 min) and then at 30, 60, 90, and 120 min of postglucose consumption (75 g glucose) during the oral glucose tolerance test (OGTT). On a different day, the sham OGTT measured the same variables over the identical time frame but without the consumption of glucose.
Subjects had normal fasting glucose and insulin levels. After oral glucose loading, increases in transmural pressure evoked similar attenuated vasoconstrictor effects at 30 through 120 min times periods compared with the vasoconstrictor effect seen before glucose consumption (Fig. 2B). Because of the lack of MBV differences between the postglucose time periods, these times periods were averaged. At 30 s of underarm tank suction, sustained increases in transmural pressure evoked an attenuated vasoconstrictor effect compared with the vasoconstrictor effect seen before glucose consumption (Δ = 1.67 ± 0.43 vs. Δ = 0.73 ± 0.14 cm/s before and after glucose loading, respectively; P < .05; Fig. 2C). Similar findings were noted at 60 s (Δ = 2.38 ± 0.54 vs. Δ = 1.07 ± 0.14 cm/s before and after glucose loading, respectively; P < .05). There were no correlations between glucose and insulin on any of the flow variables (data not shown). HR and MAP before or during the OGTT remained constant during the application of negative pressure (Table 3).

Experiment 2: Control Time Trial (sham OGTT)
Six of the original subjects (4 men and 2 women) returned between 1 to 6 mo after their initial OGTT for a control time trial (0 to 120 min) without any glucose consumption (sham OGTT). Subjects followed all the same pretesting setup as with the OGTT. There was no significant difference in glucose values over time (Table 4). Average post-sham OGTT insulin levels actually decreased slightly over time (P < 0.05). There was no significant difference in resting HR, MAP, or MBV pre- versus post-sham OGTT. There was no difference in the initial peak MBV (i.e., initial rise in MBV with the application of suction) pre- and post-sham glucose (Δ13.89 ± 1.54 vs. Δ12.20 ± 1.00 cm/s, P > 0.05, pre- vs. postglucose). Flow responses to a rise in transmural pressure were the same in the pre- and post-sham OGTT conditions (Fig. 3). When comparing OGTT vs. sham OGTT, we observed that the average postsham MBV from 30 to 60 s under suction was lower than the sham values (after OGTT, Δ = 0.79 ± 0.15 vs. after sham OGTT, Δ = 1.37 ± 0.29 cm/s, P < 0.05; Fig. 4).

Table 3. Hemodynamic responses with negative pressure before and during the OGTT

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>30 s</th>
<th>60 s</th>
<th>Increased Forearm Suction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Preglucose</td>
<td>56 ± 1</td>
<td>55 ± 1</td>
<td>55 ± 1</td>
<td></td>
</tr>
<tr>
<td>Postglucose</td>
<td>57 ± 1</td>
<td>57 ± 1</td>
<td>57 ± 1</td>
<td></td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Preglucose</td>
<td>98 ± 4</td>
<td>97 ± 4</td>
<td>99 ± 4</td>
<td></td>
</tr>
<tr>
<td>Postglucose</td>
<td>98 ± 4</td>
<td>98 ± 4</td>
<td>99 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.
30, 60, 90, and 120 min of OGTT, respectively. Resting diameters were not significantly different before and at each time point during the OGTT (Table 5). In addition, brachial diameter responses did not significantly change with the application of negative pressure during any of the OGTT time periods.

**DISCUSSION**

In these studies, we examined myogenic constrictor responses during an OGTT in healthy individuals. The main finding of this study was that after glucose loading, steady-state vasoconstrictor responses to increases in transmural pressure were attenuated. This attenuation occurred at the 30-min time period during OGTT and remained attenuated over the 2-h OGTT period. The physiological significance of this finding is that the regulation of skeletal muscle vascular resistance may be impaired after the dietary intake of glucose. The mechanisms for these findings may be mediated by the rise in blood glucose and/or the rise in insulin.

**Effects of Glucose Loading on Vascular Function**

Chronic hyperglycemia has been linked to impairments in vascular function (i.e., vasodilation, vasoconstriction, and stiffness) (7, 20, 25, 37); however, acute hyperglycemia may also alter vascular function.

The effects of acute hyperglycemia on endothelial function have been extensively examined in both healthy and diabetic populations (3, 14, 27, 34, 38). Most reports (3, 34, 38), although not all (14, 27), suggest that acute hyperglycemia is associated with impaired endothelial dilation in healthy subjects. Differences in results may be due to the route of glucose administration, the blood glucose concentration [high, 300 mg/dl (16.7 mmol/l); moderate, 200 mg/dl (11.1 mmol/l); or low, 126 mg/dl (7.0 mmol/l)] and whether or not other hormones were controlled. Beckman et al. (3) “clamped” glucose concentrations at 300 mg/dl in healthy subjects. Under these elevated glucose conditions, endothelial vasodilation was impaired. On the other hand, Reed et al. (27), controlling for other hormonal effects, elevated blood glucose in the low to moderate range (95, 126, and 200 mg/dl) and found that endothelial vasodilation was not impaired.

**Effects of Glucose Loading on Myogenic Response**

The effects of acute hyperglycemia on vascular smooth muscle are less clear. Prior animal studies suggest that myogenic vasoconstriction may be enhanced in diabetic rat skeletal muscle arterioles (35) but attenuated in healthy rat cerebral arterioles (8). However, Blum et al. (5) have recently shown that retinal arterioles diameter responses were attenuated after an oral glucose load (100 g). This latter finding agrees with our study in which reductions in MBV were attenuated in the brachial artery postglucose loading.

Fig. 2. Delta mean blood velocity (MBV) responses at rest in the brachial artery and during the first 60 s after transmural pressure was raised (~50 mmHg) pre- and average postglucose (A). Solid vertical line denotes the point of pressure change. Comparison of the changes in mean blood velocity (ΔMBV) responses in the brachial artery during the first 60 s after transmural pressure was raised (~50 mmHg) at 0, 30, 60, 90, and 120 min OGTT (B). Vasoconstriction was attenuated postglucose at all OGTT time periods compared with that at preglucose consumption (P < 0.05). Thirty and sixty seconds after application of negative pressure, attenuated reductions in MBV from baseline values were noted postglucose (C). Values are means ± SE of n = 8 subjects. *Significant difference pre- vs. average postglucose loading.
If glucose alters myogenic responsiveness, it may do so by altering the production of some endothelial substance that acts on the smooth muscle and impairs its ability to contract in response to stretch. For example, Cipolla et al. (8) found that a high-glucose [44 mmol/l (792 mg/dl)] compared with a low-glucose [5 mmol/l (90 mg/dl)] environment impaired healthy rat cerebral artery myogenic responsiveness. Cell culture data suggest that high levels of glucose exposure augment the endothelial production of nitric oxide (NO) (12). Thus one mechanism for the impaired myogenic constrictor response to increased transmural pressure after glucose loading in healthy subjects could be a rise in glucose concentrations. However, it must be emphasized that the changes in glucose in our report were quite modest, and it is unclear whether these changes in and of themselves are sufficient to attenuate myogenic vasoconstriction.

As expected, insulin levels increased in response to the oral glucose challenge. Euglycemic-hyperinsulinemic clamp experiments using supraphysiological insulin levels demonstrate a dose-dependent increase in skeletal muscle blood flow in the arm and leg. Insulin-induced changes in blood flow are also evident at physiological plasma concentrations of insulin (36, 39). Thus, insulin can vasodilate blood vessels (28, 30). This vasodilatory effect in part is related to an effect of insulin on the endothelial isoform of NO synthase (32). Since the endothelium can be a modulator of the myogenic response (31), it is certainly possible that an increase in insulin concentration may be at least in part responsible for the reduced myogenic constriction seen after oral glucose loading in the present study. Insulin may also have a more direct affect on myogenic constrictor response to stretch. For example, Cipolla et al. (8) found that a negative increase in intracellular free Ca²⁺ is evident at physiological plasma concentrations of insulin (36, 39). Thus, insulin can vasodilate blood vessels (28, 30).

![Delta MBV responses at rest in the brachial artery and during the first 60 s after transmural pressure was raised (−50 mmHg) pre- and average post-sham OGTT. Solid vertical line denotes the point of pressure change. Values are means ± SE of n = 6 subjects.](http://ajpheart.physiology.org/download)

### Table 4. Resting variables pre- and post-sham OGTT

<table>
<thead>
<tr>
<th>Variable</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>90 min</th>
<th>120 min</th>
<th>Average Post-Sham Glc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>86.17±3.27</td>
<td>85.50±2.49</td>
<td>87.33±2.64</td>
<td>87.33±2.67</td>
<td>86.17±2.87</td>
<td>86.58±2.54</td>
</tr>
<tr>
<td>Insulin, μU/ml</td>
<td>13.67±0.49</td>
<td>11.50±0.96</td>
<td>12.33±0.71</td>
<td>11.83±0.65</td>
<td>10.83±0.60</td>
<td>11.63±0.65*</td>
</tr>
<tr>
<td>Resting heart rate, beats/min</td>
<td>54±1</td>
<td>52±1</td>
<td>54±1</td>
<td>55±1</td>
<td>55±2</td>
<td>54±1</td>
</tr>
<tr>
<td>Resting mean artery pressure, mmHg</td>
<td>93±5</td>
<td>93±5</td>
<td>93±5</td>
<td>94±5</td>
<td>94±5</td>
<td>93±5</td>
</tr>
<tr>
<td>Resting mean blood velocity, cm/s</td>
<td>5.97±0.84</td>
<td>5.42±1.11</td>
<td>4.95±0.59</td>
<td>5.31±0.62</td>
<td>5.36±0.73</td>
<td>5.26±0.73</td>
</tr>
<tr>
<td>Resting resistance, U</td>
<td>16.71±1.76</td>
<td>19.61±2.47</td>
<td>19.7±1.56</td>
<td>18.5±1.51</td>
<td>18.89±2.17</td>
<td>19.17±1.82</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 subjects. Average post-sham GLC = averaged sham glucose time periods 30 to 120 min. *P < 0.05 for averaged values vs. baseline (0 min OGTT) by paired t-tests.

![Brachial diameter responses with negative pressures before and during the OGTT](http://ajpheart.physiology.org/download)

### Table 5. Brachial diameter responses with negative pressures before and during the OGTT

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Increased Forearm Suction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 min OGTT, cm</td>
<td>0.43±0.04</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>30 min OGTT, cm</td>
<td>0.42±0.04</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>60 min OGTT, cm</td>
<td>0.43±0.04</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>90 min OGTT, cm</td>
<td>0.43±0.04</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td>120 min OGTT, cm</td>
<td>0.44±0.04</td>
<td>0.44±0.04</td>
</tr>
<tr>
<td>Average post-GLC, cm</td>
<td>0.43±0.04</td>
<td>0.43±0.04</td>
</tr>
</tbody>
</table>

Values are means ± SE. Average post-GLC = averaged OGTT time periods from 30 to 120-min postglucose loading.
smooth muscle cells (16). Thus it is possible that elevated insulin levels may be associated with impaired myogenic vascular constriction.

Study Limitations

Previous arm tank studies (23, 24) have demonstrated a lack of change in brachial diameters with changes in tank pressure. Other hyperglycemic studies (17, 34) have also shown no changes in baseline diameters with high elevations of glucose. In this report we used MBV as an index of flow. This can be problematic if glucose or suction altered the diameter of the vessels. However, we found no effect of glucose and transmural pressure on the diameter and thus feel comfortable using MBV as our index.

In this study, resting MBV decreased after the ingestion of glucose. To exclude the possibility that time alone influenced resting and vasoconstriction MBV responses to negative tank suction, six of the original eight subjects were able to return to the laboratory within 6 mo after their initial testing. Although there was a time lag in their return, fasting glucose and insulin levels were similar to their pre-OGTT fasting values. Therefore, we feel that the control sham emphasizes that the MBV changes we observed with acute hyperglycemia were attenuated.

In our arm tank model, we were interested in increasing transmural pressure to the entire lower arm, including the hand and measuring brachial conduit MBV. Room temperature was maintained during the experiments so as not to alter skin blood flow. Although we cannot totally exclude the influence of hand circulation on the absolute brachial MBV responses, we feel that since the same technique was used during all OGTT time periods, any hand circulation effects were minimized.

The rise in glucose could not explain the lower baseline forearm flow velocities observed in this report (no correlations). The mechanism leading to the reduction in resting MBV postglucose is unclear. Giugliano et al. (11) noted a reduction in basal leg blood flow during a hyperglycemic clamp. This study suggested that hyperglycemia reduced the NO bioavailability. Other studies (4, 6, 33) have suggested that elevated glucose may promote basal vasoconstriction through an increase in diacylglycerol and protein kinase C. Of note, a rise in insulin levels can also lead to sympathoexcitation (2, 13, 29). Finally, it is possible that glucose could have actually augmented myogenic tone and evoked the rise in resting tone.

Conclusion and Clinical Implications

In conclusion, our results suggest that oral glucose loading leads to an impaired myogenic constrictor response in healthy individuals. We would speculate that the impaired myogenic response is due to a rise in NO that is secondary to either the rise in glucose, insulin, or both. Future experiments will be necessary to try and separate the effects of insulin from those of glucose. Since blood pressure homeostasis during standing may be affected by dietary intake (19), we propose that diminished postoral glucose myogenic vasoconstriction may play a role in mediating postprandial orthostasis.

ACKNOWLEDGMENTS

We are grateful to Jennifer Stoner for expert typing and manuscript preparation, Kristen Gray for figures, Allen Kunselman for statistical support, and the staff of Penn State General Clinical Research Center (GCRC).

GRANTS

This study was supported by the Penn State GCRC Feasibility Grant (to M. E. J. Lott), National Heart, Lung, and Blood Institute Grants R01-HL-070222 and P01-HL-077670 (to L. I. Sinoway), and National Center for Research Resources Grants M01-RR-010732 (GCRC grant) and C06-RR-016499 (construction grant).

REFERENCES