Hemodynamic Effects of Acute Hyperglycemia in Type 2 Diabetic Patients

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OBJECTIVE — The aim of the present study was to evaluate the hemodynamic effects of acute hyperglycemia in type 2 diabetic patients and to see whether these effects are related to changes in nitric oxide (NO) availability.

RESEARCH DESIGN AND METHODS — Twenty newly diagnosed complication-free diet-treated type 2 diabetic patients participated in the study. All patients underwent 3 hyperglycemic glucose clamps in random order: 1) the control study was performed with plasma glucose clamped at 18 mmol/l for 2 h; 2) the octreotide study with plasma insulin blocked at basal levels during the clamp; and 3) the L-arginine study with L-arginine (1 g/min) infused during the last 30 min of the clamp. A group of 8 patients also underwent a glutathione infusion (600 mg as an intravenous bolus followed by 5 mg/min infusion) during the clamp.

RESULTS — During hyperglycemia, there were significant increments of systolic (sBP) (from 115.5 ± 9.1 to 120.3 ± 8.2 mmHg, P < 0.01) and diastolic (dBP) (from 70.3 ± 7.8 to 79.7 ± 5.3 mmHg, P < 0.01) blood pressure, as well as heart rate (from 75.2 ± 7.8 to 80.8 ± 5.4 beats/min, P < 0.01) and plasma catecholamines (P < 0.05). Squatting ratios, a measure of the baroreflex activity, significantly deteriorated after hyperglycemia (P < 0.01). The infusion of octreotide, used to avoid the possible confounding influence of insulin, did not change the hemodynamic effects of hyperglycemia. Glutathione, a free radical scavenger, completely prevented the vascular effects of hyperglycemia. L-Arginine produced a fall in sBP and dBP to baseline values and normalized squatting ratios.

CONCLUSIONS — Acute hyperglycemia in newly diagnosed type 2 diabetic patients causes significant hemodynamic changes that are independent of endogenous insulin and are prevented by glutathione and reversed by L-arginine, suggesting an interference with endogenous NO availability. These observations could help explain the adverse cardiovascular effects of hyperglycemic spikes.

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Glucosic control of diabetes is an important predictor of both microvascular disease (1) and macrovascular complications (2). Among the many mechanisms proposed to explain the toxic effect of hyperglycemia on the vascular tree, impaired endothelium-dependent vascular relaxation, also known as endothelial dysfunction, has recently emerged as an important one. In vitro, endothelial dysfunction induced by high glucose develops quickly (within minutes or hours) (3,4), which makes the contribution of advanced glycosylation end products in quenching and deactivating nitric oxide (NO) unlikely (5). Hyperglycemia-mediated rapid generation of free radicals from endothelial cells seems more likely to come into play. This interpretation is strengthened by the observation that acute hyperglycemia produces relevant systemic hemodynamic changes and alters baroreflex activity in healthy subjects, via a glutathione-sensitive, presumably free-radical-mediated pathway (6). Furthermore, the effects of acute hyperglycemia are mimicked by N^G^-monomethyl-L-arginine, an inhibitor of NO synthesis, and reversed by L-arginine, the natural precursor of NO formation (7). The whole of such data seems to suggest that acute hyperglycemia may reduce NO availability in healthy people.

Several studies (8) have demonstrated impaired endothelium-dependent relaxation in diabetes. However, the hemodynamic consequences of acute hyperglycemia are poorly characterized in the diabetic patient (9). Also, it is not known whether endogenous NO is implicated. In the present study, we evaluated the effect of acute elevations of plasma glucose levels on some hemodynamic parameters in newly diagnosed, complication-free type 2 diabetic patients. In particular, we investigated whether acute sustained hyperglycemia affects blood pressure and heart rate (HR), as well as noradrenergic and baroreflex activity. Because insulin may have vasodilatory activity (10), our second goal was to eliminate this confounding insulin effect with octreotide, a potent inhibitor of insulin secretion (11). Finally, the hypothesis that changes in NO availability could mediate the hemodynamic effects of acute hyperglycemia was tested by giving the diabetic patients either exogenous glutathione, a free-radical scavenger, or L-arginine, the precursor of NO.

RESEARCH DESIGN AND METHODS

Patients
Twenty newly diagnosed (within 6 months of diagnosis) type 2 diabetic patients took part in the study. The diagnosis of diabetes was made according to the World Health Organization criteria on the basis of fasting
Table 1—Characteristics of study patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>47 ± 4</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>9/11</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26 ± 1</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>8.0 ± 1.0</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>7.8 ± 0.8</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td>4.9 ± 0.9</td>
</tr>
<tr>
<td>Plasma triglyceride (mmol/l)</td>
<td>1.8 ± 0.6</td>
</tr>
<tr>
<td>sBP (mmHg)</td>
<td>115 ± 9.0</td>
</tr>
<tr>
<td>dBP (mmHg)</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>SqTv (R/R)</td>
<td>0.68 ± 0.04</td>
</tr>
<tr>
<td>SqTs (R/R)</td>
<td>1.33 ± 0.07</td>
</tr>
</tbody>
</table>

Data are n or means ± SD.

(≥7.8 mmol/l) or postload (≥11.1 mmol/l) plasma glucose. To enter the study, the diabetic patients had to be normotensive, have cholesterol levels within normal ranges, and be free from diabetic complications, both microvascular and macrovascular. In particular, the presence of autonomic neuropathy was excluded with the cardiovascular reflex tests (deep breathing, lying-to-standing, and Valsalva ratio). Moreover, the diabetic patients had to have normal values of clotting ratios. All patients were treated only by diet. Their clinical characteristics are reported in Table 1. All patients gave their informed written consent and the protocol was approved by the ethics committee of our institution.

Study design
After a 12-h overnight fast, the diabetic patients were placed in a supine comfortable position with the room temperature kept between 20 and 24°C. All patients were instructed to refrain from smoking and from drinking coffee or alcoholic beverages from the night before the study. Intravenous lines were inserted in a large antecubital vein of one arm for infusions and in a dorsal vein of the contralateral arm for blood sampling. Patency was preserved by a slow saline infusion (0.9% NaCl). The patients were then instrumented for automatic measurements of blood pressure and HR. The study began after the patients had rested for 30 min.

All patients underwent the following tests in random order and with a 7-day interval between each test: 1) One test consisted of a hyperglycemic glucose clamp in which plasma glucose concentrations were acutely raised with a bolus injection of 0.20 g/kg glucose (50% solution). This was followed by a continuous variable 20% glucose infusion to achieve steady-state plasma glucose levels at ~18 mmol/l for 120 min. To prevent hypokalemia, 0.26 mmol/l KCl added to the glucose solution. 2) Another test consisted of a hyperglycemic clamp, as described above, plus octreotide infusion (Longastatina; Italfarmaco, Milan, Italy) (25 µg as intravenous bolus followed by a 0.5-µg/min infusion). The infusion was started 5 min before the priming glucose pulse and was interrupted at the end of the clamp, 125 min later. 3) In the last test, L-arginine (30 g infused at the rate of 1 g/min) was infused during the last 30 min of the hyperglycemic clamp from 90 to 120 min. L-Arginine monohydrochloride was purchased from Damor Pharmaceuticals (Naples, Italy) as a ready-to-use 30% solution dissolved in saline. The hyperglycemic clamp was also repeated in 8 diabetic patients along with an infusion of glutathione (Tationil; Roche, Milan, Italy, 600 mg as an intravenous bolus followed by a 5-mg/min infusion). Glutathione infusion started 5 min before the priming glucose pulse and was interrupted at the end of the clamp, 125 min later. To ascertain whether volume infusion could have altered hemodynamic parameters, control experiments were performed in 4 diabetic patients in whom an identical volume of saline (~300 ml) was infused over 2 h.

Procedures
HR and finger arterial pressure were recorded with a noninvasive technique (Finapres; Ohmeda 2300, Englewood, CA) that uses the unloaded artery principle (12). R-R intervals (distance between 2 R-waves in 2 consecutive QRS complexes) were recorded with a standard electrocardiogram. Baroreflex activity was investigated with the squating test (SqT), which has both vagal and sympathetic functions (13). In brief, each subject stood still for 1 min (phase I), then squated for 1 min (phase II), and finally stood up again (phase III), remaining in this position for another minute. The ratio between the baseline R-R interval and the longest R-R interval during phase II is called the squating vagal ratio (SqTv) and represents the bradycardia secondary to baroreflex activation triggered by the raised arterial pressure after squating. The ratio between the baseline R-R interval and the shortest R-R interval during phase III is called the sympathetic ratio (SqTs) and is a measure of the tachycardia after the increased sympathetic drive brought about by the fall in blood pressure during phase III. During the squatting maneuver, the hand connected to the Finapres was placed at heart level and was supported by a band during postural changes. Data were analyzed by software allowing systolic blood pressure (sBP), diastolic blood pressure (dBP), mean blood pressure (MBP), and HR to be expressed in graphs. The baseline SqT was performed at the end of the equilibration period and was repeated at 90 and 120 min, with the subject still under hyperglycemic conditions.

Measurements
Plasma glucose and insulin concentrations were determined every 10 min. Samples for plasma glucose were collected in tubes containing a trace of sodium fluoride and the samples for insulin in tubes containing a mixture (0.1 ml/ml blood) of EDTA-Trasylol solution (5,000 U/ml Trasylol, 1.2 g/L disodiu EDTA). Plasma glucose was determined with the glucose oxidase method on an autoanalyzer (Beckman, Fullerton, CA). Plasma insulin levels were determined by radioimmunoassay, as previously described (14). Plasma catecholamines were measured at 0, 90, and 120 min of the studies. Samples were collected in iced heparanized tubes, and the plasma was separated within 120 min of collection and stored at −70°C as perchloric acid extracts until assayed. Plasma catecholamines were measured with high-performance liquid chromatography. In our laboratory, the assay has a detection limit of 20 ng/l; the intra-assay and interassay coefficients of variation are 8.0 and 8.6%, respectively.

Statistical analysis
The 3-min data for blood pressure and HR collected every 10 min were averaged to a single value. Data were analyzed by analysis of variance for repeated measures. Post hoc comparisons were made with the Scheffé test. P < 0.05 was considered statistically significant. All data are presented as means ± SD.

RESULTS

Hyperglycemic clamp (control study)
Basal plasma glucose levels averaged 8.0 ± 1.0 mmol/l. During the clamp, plasma glucose stabilized at ~8 mmol/l and remained around this level throughout the study. Basal plasma insulin levels were 57 ± 18 pmol/l. The pattern of insulin release during...
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The clamp was characterized by a progressive increase of insulin levels (no biphasic pattern of secretion was observed), which reached its maximum at the end of glucose infusion (Fig. 1). SBP, DBP, and HR increased during the clamp starting from 30 min and remained significantly elevated above baseline until the end of the clamp (Fig. 2). Plasma norepinephrine rose from a basal value of 194 ± 39 to 237 ± 42 (P < 0.05) and 248 ± 50 ng/l (P < 0.02) after 90 and 120 min of the clamp, respectively. Plasma epinephrine rose from a basal value of 49 ± 14 to 67 ± 19 (P < 0.05) and 78 ± 20 ng/l (P < 0.05), respectively. HR variations during the SqT were significantly reduced after hyperglycemia (P < 0.01). Both SqTv and SqTs ratios showed a significant deterioration (Table 2).

Hyperglycemic clamp plus octreotide (octreotide study)

Basal and clamped plasma glucose levels in the octreotide study were not significantly different from those of the control study. Despite similar basal plasma insulin concentrations, glucose-stimulated insulin responses were markedly and significantly reduced by octreotide. Starting from 30 min, plasma insulin levels reached baseline values and remained unchanged despite ongoing glucose infusion (Fig. 1). Hemodynamic parameters rose significantly during the clamp; these changes did not differ from those recorded in the control clamp (Fig. 2). Basal plasma norepinephrine and epinephrine levels did not differ between control and octreotide studies (Table 2), nor was there a difference in plasma catecholamine concentrations attained during the clamps. The squatting ratios showed a response that was similar to that obtained during the control clamp (Table 2).

Hyperglycemic clamp plus L-arginine (L-arginine study)

Basal plasma glucose and insulin levels were similar to those of the previous studies. During the first 90 min of the clamp, there was no difference in the steady-state plasma glucose concentrations or in the plasma insulin responses to hyperglycemia (Fig. 1). L-Arginine infusion, in the last 30 min of the study, produced a significant increase in plasma-insulin levels (Fig. 1). Hemodynamic parameters (SBP, DBP, and HR) rose significantly during the first 90 min of the clamp; these changes did not differ from those of previous studies. When L-arginine was superimposed to the ongoing glucose infusion, there was a fall in blood pressure levels persisting throughout the entire infusion period (Fig. 2). HR and plasma catecholamine levels were not significantly modified by L-arginine infusion (Fig. 2, Table 2). The SqT ratios deteriorated during hyperglycemia; however, HR variations during the SqT increased after L-arginine infusion. At the end of infusion (120 min), the SqT ratios were not significantly different from baseline values.

Hyperglycemic clamp plus glutathione (glutathione study)

Basal plasma glucose and insulin levels were similar to those recorded in previous studies, and there was no difference in the steady-state plasma glucose and insulin concentrations attained during the clamp. Similarly, plasma catecholamine concentrations and squatting ratios remained unchanged at the end of the clamp study (Table 3).

In the saline study, 4 patients were infused with saline to determine the effects of volume expansion on hemodynamic variables. No significant change was observed in any of the variables (both metabolic and hemodynamic) investigated during the saline infusion (data not shown).

The increase of plasma glucose concentrations correlated with the changes of MBP (r = 0.66, P < 0.001), HR (r = 0.69, P < 0.005), and HR variations during the...
SqT (SqTv, \( r = 0.51, P < 0.02; \) SqTs, \( r = 0.56, P < 0.02 \)). All of the changes were still significant after adjusting for age, sex, and BMI (P < 0.03 for all correlations). The changes in plasma insulin concentrations did not correlate with all of the variables.

**CONCLUSIONS** — The results of the present study show that acute hyperglycemia (up to 6 mmol/l above fasting values) in newly diagnosed, complication-free type 2 diabetic patients produces significant increase of blood pressure and alterations in baroreflex activity, which are prevented by glutathione and reversed by L-arginine infusion.

The increase in arterial blood pressure, HR, and plasma catecholamine concentrations seen during the hyperglycemic clamp in diabetic patients suggests vasoconstriction and might have been implicated in the alteration of baroreflex activity. To the best of our knowledge, there are no reported studies that specifically addressed the effects of acute short-term hyperglycemia on systemic hemodynamics in type 2 diabetic patients like those evaluated in the present investigation. However, some studies have addressed this topic in nondiabetic people and therefore seem pertinent to the present results. We have previously reported that clamping plasma glucose concentrations to 15 mmol/l in normal subjects pro-

**Table 2** — Norepinephrine and epinephrine levels and squatting ratios (SqTv, SqTs) in 20 type 2 diabetic patients during the 3 different hyperglycemic clamps of the study

<table>
<thead>
<tr>
<th>Control study</th>
<th>Octreotide study</th>
<th>L-Arginine study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>90 min</td>
</tr>
<tr>
<td>Norepinephrine (ng/l)</td>
<td>194 ± 49</td>
<td>237 ± 40*</td>
</tr>
<tr>
<td>Epinephrine (ng/l)</td>
<td>49 ± 14</td>
<td>67 ± 19*</td>
</tr>
<tr>
<td>SqTv (R/R)</td>
<td>0.66 ± 0.06</td>
<td>0.76 ± 0.08†</td>
</tr>
<tr>
<td>SqTs (R/R)</td>
<td>1.33 ± 0.07</td>
<td>1.25 ± 0.12†</td>
</tr>
</tbody>
</table>

Data are means ± SD. *P < 0.05; †P < 0.001 vs. basal values; ‡P < 0.001 vs. 90 min values. The infusion of L-arginine (1 g/min) was superimposed during the last 30 min of the L-arginine study.
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Table 3—sBP and dBP, HR, norepinephrine and epinephrine levels, and squatting ratios (SqTv, SqTs) in 8 type 2 diabetic patients during hyperglycemic clamp plus glutathione (glutathione study)

<table>
<thead>
<tr>
<th></th>
<th>0 min</th>
<th>90 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>sBP (mmHg)</td>
<td>114 ± 7.8</td>
<td>116 ± 6.9</td>
<td>115 ± 8.1</td>
</tr>
<tr>
<td>dBP (mmHg)</td>
<td>70 ± 5.4</td>
<td>71 ± 7.5</td>
<td>73 ± 6.6</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>73 ± 4.3</td>
<td>74 ± 5.1</td>
<td>76 ± 6.3</td>
</tr>
<tr>
<td>Norepinephrine (ng/l)</td>
<td>189 ± 52</td>
<td>207 ± 45</td>
<td>214 ± 49</td>
</tr>
<tr>
<td>Epinephrine (ng/l)</td>
<td>45 ± 19</td>
<td>47 ± 12</td>
<td>58 ± 29</td>
</tr>
<tr>
<td>SqTv (R/R)</td>
<td>0.66 ± 0.06</td>
<td>0.68 ± 0.07</td>
<td>0.65 ± 0.04</td>
</tr>
<tr>
<td>SqTs (R/R)</td>
<td>1.30 ± 0.06</td>
<td>1.28 ± 0.11</td>
<td>1.29 ± 0.13</td>
</tr>
</tbody>
</table>

Data are means ± SD.

duced significant increases in sBP and dBP, HR, and plasma catecholamine levels and decreased leg blood flow. These hemodynamic changes persisted during suppression of insulin with octreotide and were completely reversed by simultaneous infusion of glutathione (6), a free-radical scavenger that lowers oxidative stress in people (15). As we have shown, similar effects have been observed in newly diagnosed, complication-free type 2 diabetic patients. These results led us to hypothesize that, as occurs in animal studies (3,16), acute hyperglycemia may increase the production of free radicals that mediate its hemodynamic effects.

Local hyperglycemia may also affect systemic hemodynamics. Houben et al. (17) found that local intra-arterial forearm hyperglycemia (16 mmol/l for 7 h) did not affect micro- or macrovascular blood flow or vascular reactivity to norepinephrine in normal people. In spite of the modest increase in systemic glycemia (0.6 mmol/l), there was a small but significant increase in the MBP range (1–6 mmHg) as well as a diminished increase in absolute forearm blood flow, suggesting relative vasoconstriction. Williams et al. (18) reported that local forearm hyperglycemia (17 mmol/l for 6 h) attenuated endothelium-dependent vasodilation in healthy people that was not affected by inhibition of insulin release with octreotide.

Acute physiologic increments in plasma insulin concentrations stimulate the sympathetic noradrenergic activity, which is offset by its vasodilatory partly NO-mediated action on skeletal muscle blood flow (10). These opposing hemodynamic effects of insulin may help explain why acute hyperinsulinemia does not elevate blood pressure in normal humans (10). In our diabetic patients, blockade of insulin secretion with octreotide did not significantly modify the hemodynamic responses seen during the hyperglycemic clamp. On the other hand, there was no significant correlation between plasma insulin concentrations attained during the clamp and increases of blood pressure, HR, and HR variations during squatting, whereas positive significant relations were obtained for plasma glucose values. All this suggests a minor, if any role for plasma insulin in the mediation of systemic hemodynamic changes brought about by acute hyperglycemia in diabetic patients.

Acute hyperglycemia may increase free-radical formation that may quench and deactivate NO, reducing its availability for target cells. Among free radicals, the superoxide anion appears the more likely candidate in mediating the hemodynamic changes of acute hyperglycemia; because it is rapidly generated in hyperglycemic conditions, it rapidly reacts with NO, and its serum concentrations correlate with indexes of glycemic control, such as plasma glucose and glycated proteins (19). Endothelium-derived NO is now recognized as the most potent vasodilating and anti-atherogenic endogenous substance and is formed from the semi-essential amino acid L-arginine by the constitutive form of NO synthase (20). If the hemodynamic effects of acute hyperglycemia were related to reduced availability of NO, we reasoned that either a free-radical scavenger (glutathione) or the natural precursor of NO synthesis, L-arginine. These observations could help explain why hyperglycemic spikes, which occur in the postprandial state, may contribute to the poor cardiovascular outlook of the diabetic patient (9).

References