Reduced glucose clearance as the major determinant of postabsorptive hyperglycemia in diabetic rats

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Wi, Jae K., Jason K. Kim, and Jang H. Youn. Reduced glucose clearance as the major determinant of postabsorptive hyperglycemia in diabetic rats. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E257–E264, 1998.—The relationships between postabsorptive glucose concentration and hepatic glucose output (HGO) and glucose clearance were studied in rats one day after treatment with various doses of streptozotocin (STZ; 0, 15, 30, 40, 50, or 75 mg/kg, n = 6 per dose; study 1). Glucose fluxes were estimated using a prolonged (6-h) infusion of [3-3H]glucose to ensure complete tracer equilibration at hyperglycemia. Postabsorptive glucose was significantly increased at the high doses of STZ (50 and 75 mg/kg; P < 0.01) and was strongly correlated with glucose clearance across all doses (r = −0.85, P < 0.001) but less strongly with HGO (r = 0.46, P < 0.01). In the group treated with 50 mg/kg STZ, postabsorptive glucose was increased twofold compared with the control (i.e., zero dose) group, with no change in HGO and a 45% decrease in glucose clearance, indicating that the hyperglycemia was due to a decrease in glucose clearance. To understand the cellular mechanisms of decreased glucose clearance in STZ diabetic rats, skeletal muscle glucose clearance and intracellular glucose and glucose 6-phosphate (G-6-P) concentrations were determined in normal and STZ (50 mg/kg) diabetic rats at their postabsorptive glucose levels as well as at matched hyperglycemia (12 mM; study 2). Glucose clearance was significantly decreased in soleus (P < 0.05) muscles of the diabetic rats, and this was associated with significantly decreased intracellular glucose and G-6-P levels at matched hyperglycemia (P < 0.05), suggestive of decreased glucose transport. In conclusion, postabsorptive hyperglycemia in STZ diabetic rats was largely due to decreased glucose clearance rather than to increased HGO and to investigate the cellular mechanisms of decreased glucose clearance in diabetic rats. For these goals, we examined the relationships between postabsorptive glucose level and HGO and glucose clearance in rats treated with different doses of STZ to induce various degrees of hyperglycemia. Animals were studied one day after STZ injection to avoid chronic (i.e., several days of) exposure to hyperglycemia and/or hypoinsulinemia, which may result in glucose toxicity (24) and/or insulin resistance (31). Finally, to understand the cellular mechanisms of decreased glucose clearance in diabetic rats, glucose clearance and intracellular substrate levels were compared in skeletal muscles of normal and diabetic rats at their ambient glucose concentrations and at matched hyperglycemia.

MATERIALS AND METHODS

Animals

Male Wistar rats weighing 250–300 g were obtained from Charles River (Wilmington, MA) and housed under controlled temperature (22 ± 2°C) and lighting (12-h light, 0600–1800; 12-h dark, 1800–0600) and with free access to water and standard rat chow. At least 4 days before the experiments, animals were placed into individual cages with wire floors, and the distal one-third of each animal’s tail was drawn through a hole placed low on the side of the cage and secured there with a rubber stopper. This arrangement was required for access to tail blood vessels during experimental procedures (2, 3); it allowed animals to move about freely and provided unrestricted access to food and water. Two tail-vein infusion catheters were implanted on the evening before the experiment, and one tail artery blood sampling catheter was implanted 5 h before the start of tracer infusion. Catheters were implanted percutaneously during local anesthesia with lidocaine while animals were restrained in a towel. Animals were returned to their cages after catheter placement with tails secured as described above. Patency of the arterial catheter was maintained by a slow (0.017 ml/min) infusion of heparinized saline (10 U/ml).

Experimental Procedures

Relationships between postabsorptive glucose concentration and production and clearance (study 1). In the fed state, and at ~1 PM, animals were injected with various doses of STZ (0, 15, 30, 40, 50, or 75 mg/kg ip; n = 6 per dose) dissolved in citrate buffer, pH 4.5. Experiments were conducted on the following day. Experiments were started at ~12 PM (~5 h

Fasting hyperglycemia is a hallmark of diabetes mellitus. It has been postulated, although still debated, that fasting hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM) arises from hepatic overproduction of glucose (6, 7, 11, 13). In addition, postabsorptive hepatic glucose output (HGO) has been reported to be elevated in insulin-deficient diabetic humans (8) and animals (1, 4, 17, 22). However, recent studies with prolonged (4–8 h (5, 12, 14, 26)) or “adjusted primed” tracer infusion (12, 13) showed that fasting HGO was not elevated in NIDDM subjects with fasting glucose concentrations of <12 mM. In addition, our previous study showed that postabsorptive HGO was similar between normal and streptozotocin (STZ) diabetic rats when estimated after a 6-h tracer infusion (28). Thus these recent data have challenged the classical view that increased HGO is responsible for fasting hyperglycemia in diabetes.

The present study was designed to further substantiate our previous suggestion (28) that postabsorptive hyperglycemia in STZ diabetic rats was largely due to decreased glucose clearance rather than to increased HGO and to investigate the cellular mechanisms of decreased glucose clearance in diabetic rats. For these goals, we examined the relationships between postabsorptive glucose level and HGO and glucose clearance in rats treated with different doses of STZ to induce various degrees of hyperglycemia. Animals were studied one day after STZ injection to avoid chronic (i.e., several days of) exposure to hyperglycemia and/or hypoinsulinemia, which may result in glucose toxicity (24) and/or insulin resistance (31). Finally, to understand the cellular mechanisms of decreased glucose clearance in diabetic rats, glucose clearance and intracellular substrate levels were compared in skeletal muscles of normal and diabetic rats at their ambient glucose concentrations and at matched hyperglycemia.
after food removal) with infusion of d-[3-3H]glucose (high-performance liquid chromatography purified; Du Pont, Boston, MA) at a rate of 0.05 µCi/min. The tracer infusion was continued for 6 h to ensure complete tracer equilibration in hyperglycemic states (28). Blood samples (60 µl each) were collected at 10- to 30-min intervals throughout the 6-h tracer infusion period for the determination of plasma glucose concentration and specific activity. In addition, blood samples (150 µl each) were collected at 2-h intervals for the determination of plasma insulin and free fatty acids. At the end of the experiment, a final blood sample was obtained for the determination of plasma glucagon, and rats were killed by pentobarbital sodium injection. Urine was collected from the urinary bladder with a syringe, and the bottom of the cage was rinsed to collect and measure the amount of excreted tracer in urine.

Cellular mechanisms of decreased glucose clearance in diabetic rats (study 2). Postabsorptive conditions (study 2A). In this study, glucose clearance and the concentrations of intracellular glucose and glucose 6-phosphate (G-6-P) were determined in soleus and extensor digitorum longus (EDL) muscles of normal and diabetic (treated with 50 mg/kg STZ as in study 1) rats in the postabsorptive state (i.e., at their ambient glucose concentrations; n = 7 per group). Experiments were started at ~12 PM (~5 h after food removal). L-[3H]glucose (Du Pont), an extracellular marker, was infused at a rate of 0.2 µCi/min for 170 min to estimate the extracellular glucose space in muscles. To estimate muscle glucose uptake, 2-deoxy-d-[1-14C]glucose (2-[14C]DG; Du Pont) was administered as a bolus (30 µCi) at 120 min, and blood samples (50 µl) were taken at 0, 120, 122, 125, 130, 135, 140, 145, 150, 155, 160, 165, and 170 min to measure plasma L-[3H]glucose and/or 2-[14C]DG concentrations. Additional blood samples (100 µl) were taken at 0, 120, and 170 min to determine plasma insulin concentration. At the end of the experiments (170 min), rats were anesthetized by pentobarbital sodium injection and soleus and EDL muscles were taken from each hindlimb. Muscle samples were always taken in the order of soleus and EDL from the left hindlimb, followed by the right hindlimb. Once exposed, each muscle sample was taken within 2 s, frozen immediately using liquid N2-cooled aluminum blocks, and stored at −70°C for later analysis. The concentrations of intracellular glucose and G-6-P concentration were determined from the first muscle taken of each muscle group (i.e., from the left hindlimb). Accurate determination of intracellular glucose and G-6-P concentrations requires rapid sampling and freezing of muscles. The soleus and EDL muscles were chosen to meet these requirements. Sampling was limited to these muscles so that muscle sampling could be completed within a short time period during which extracellular glucose would not vary significantly.

Matched hyperglycemic conditions (study 2B). In this study the concentrations of intracellular glucose and G-6-P were determined in soleus and EDL muscles of normal and diabetic (50 mg/kg STZ) rats during matched hyperglycemic (12 mM) clamps (n = 7 for each group). Plasma glucose was clamped at 12 mM rather than at the ambient glucose level of STZ rats to avoid potential saturation of the glucose transport system and/or a breakthrough of inhibition of insulin secretion with somatostatin (see below) at severe hyperglycemia.

Phlorizin (50 µg·kg⁻¹·min⁻¹) was infused to lower plasma glucose in the diabetic rats to the target glucose concentration. Phlorizin was also infused in the normal group. In normal rats, glucose was raised and clamped at the target glucose concentration (12 mM), with insulin response suppressed by use of somatostatin infusion (Bachem, Torrance, CA; 5 µg·kg⁻¹·min⁻¹ after a bolus injection of 60 µg/kg).

Starting at ~12 PM (~5 h after food removal), phlorizin and somatostatin were infused intravenously for 5 h. Blood samples (40 µl) were collected at 5- or 10-min intervals for the immediate measurement of plasma glucose. Glucose (20%, wt/vol) was infused at adjusted rates to maintain plasma glucose at 12 mM. At the end of the experiment, rats were anesthetized and muscle samples were taken as in study 2A. Because muscle G-6-P concentrations may be sensitive to plasma glucose, glucose infusion was continued during the muscle sampling procedure to prevent significant perturbation of plasma glucose.

Analysis of Plasma Samples

For the determination of glucose and [3H]glucose (study 1), plasma was deproteinized with ZnSO₄ and Ba(OH)₂. The supernatant was dried to remove H₂O, resuspended in water, and counted in scintillation fluid (Ready Safe; Beckman, Fullerton, CA). In addition, the supernatant was analyzed for glucose with a glucose reagent from Sigma Chemical (HK-10). In study 2A, plasma samples (20 µl) were directly counted in scintillation fluid for the determination of plasma L-[3H]glucose and 2-[14C]DG on dual channels that allow separate counting of 3H and 14C. During the hyperglycemic clamps (study 2B), plasma glucose was analyzed by a glucose oxidase method utilizing 10 µl of plasma on a Beckman Glucose Analyzer II. Plasma insulin was measured by radioimmunoassay with a kit from Linco Research (St. Charles, MO). Plasma glucagon was measured by radioimmunoassay using a kit obtained from Novo-Nordisk. This assay uses antisera K5563 and incorporates a step for ethanolic extraction of plasma. Plasma free fatty acids (FFA) were determined using an acyl-CoA oxidase-based colorimetric kit (Wako Pure Chemical Industries, Osaka, Japan).

Analysis of Muscle Samples

For the determination of muscle 2-[14C]DG-6-phosphate (2-[14C]DG-6-P) content, muscle samples were homogenized in distilled water, and the supernatants were subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG, as previously described (30). The muscle supernatant was also counted for L-[3H]glucose for the estimation of extracellular glucose space in muscles. The concentrations of total muscle glucose and G-6-P were determined by using G-6-P dehydrogenase, as described by Michal (21). Hexokinase (HK) activities were determined (study 2B) as described by Mandarino et al. (19) with minor modifications. Briefly, muscle samples were homogenized using a Tekmar homogenizer (Tekmar; Cincinnati, OH) in a buffer consisting of 50 mM potassium phosphate (pH 7.4), 2 mM dithiothreitol, 2 mM EDTA, and protease inhibitors (i.e., leupeptin, 1 µg/ml; antipain, 2 µg/ml; benzamidine, 10 µg/ml; chelostatin, 1 µg/ml; papstatin, 1 µg/ml; phenylmethylsulfonyl fluoride, 0.1 mM). Homogenates were centrifuged at 16,000 g for 15 min, and the pellets were resuspended in the homogenization buffer containing 0.1% of Triton X-100 [the supernatants and pellets represent cytosolic and mitochondrial fractions, respectively (18)]. HK I and HK II activities were separately taken of the different temperature sensitivities of these enzymes (19).

Calculations

Endogenous glucose production (study 1) was calculated from the data during the final 30 min of the experiments by use of Steel's steady-state equation (i.e., endogenous glucose production = tracer infusion rate ÷ specific activity of plasma.
glucose); HGO was assumed to equal endogenous glucose production, although we are aware that a fraction of endogenous glucose production is accounted for by renal glucose output. Whole body glucose uptake was calculated by subtracting urinary glucose loss from the rate of glucose appearance. Urinary glucose loss was calculated as the amount of tracer recovered in urine divided by the area under the curve of specific activity of plasma glucose (28); urinary glucose loss was calculated using the tracer data rather than by determining urinary glucose concentration because of the difficulty of emptying the bladder in rats at the beginning of the experiment, a necessary procedure for the latter method. The two methods should give similar results, because plasma glucose (and thus urinary glucose clearance) is reasonably constant during the experiments. Whole body glucose clearance was calculated as whole body glucose uptake divided by plasma glucose concentration. Glucose uptake and clearance in individual muscles (study 2A) were calculated from the muscle 2-[14C]DG-6-P content and plasma 2-[14C]DG profile, as previously described (30). The extracellular glucose space in muscle was calculated as muscle l-[14C]glucose content divided by plasma l-[14C]glucose concentration. Extracellular glucose content in muscle was then calculated as plasma glucose concentration times muscle extracellular glucose space. In study 2B, extracellular glucose was calculated using the extracellular glucose space estimated in study 2A (i.e., 15%). Intracellular glucose was calculated as total muscle glucose minus extracellular glucose.

Statistical Analysis

Values are reported as means ± SE. The significance of differences between mean values was assessed using Student's t-test. For multiple comparisons, significance was evaluated by analysis of variance (ANOVA). When the ANOVA indicated significant differences, Dunnett's test was used for post hoc analysis.

RESULTS

Relationships Between Postabsorptive Glucose Concentration and Production and Clearance (Study 1)

STZ treatment at low doses (i.e., 15, 30, and 40 mg/kg) did not significantly alter postabsorptive glucose concentrations on the following day (Table 1 and Fig. 1A). In contrast, high doses of STZ (i.e., 50 and 75 mg/kg) caused substantial increases in postabsorptive glucose concentrations. During the 6-h tracer infusion period, plasma glucose was constant in control (i.e., zero dose) and low-dose STZ groups (P > 0.05; Fig. 1A) but decreased steadily in the groups with high STZ doses (P < 0.01 vs. time 0). Postabsorptive plasma insulin concentrations were similar in all groups, with a tendency toward a decrease in the 75 mg/kg STZ group (Table 1; P > 0.05). Plasma glucagon and FFA concentrations were similar in all groups (Table 1; P > 0.05).

During the constant 6-h [3H]glucose infusion, the time course of [3H]glucose radioactivity in plasma was very similar in control and low-dose STZ groups (Fig. 1B). In contrast, the radioactivity of [3H]glucose rose to substantially higher levels in the high-dose (i.e., 50 and 75 mg/kg) STZ groups, indicating decreased glucose clearance. The time course of [3H]glucose specific activity was also similar in control and low-dose STZ groups (Fig. 1C), whereas it was apparently slower in high-dose STZ groups. Although the time course of specific activity was slower in the group with 50 mg/kg STZ, the specific activity reached levels similar to those in control and low-dose STZ groups at the end of experiment.

Postabsorptive HGO and glucose clearance were calculated from the data during the final 30 min of the experiments (see MATERIALS AND METHODS). Glucose clearance was markedly reduced with high doses (50 or 75 mg/kg) of STZ, and these changes were inversely proportional to changes in postabsorptive glucose concentration (Fig. 2, A and B). Postabsorptive HGO was not significantly different among groups (P > 0.05 by ANOVA), with the exception of a significant increase in HGO with 75 mg/kg STZ, compared with the control (i.e., 0 dose) group (P < 0.05 by Dunnett's test; Fig. 2C). Glucose clearance was strongly correlated with postabsorptive glucose concentration (Fig. 3A; r = −0.85, P < 0.001). Although postabsorptive HGO was also correlated with postabsorptive glucose concentration (Fig. 3B; r = 0.46, P < 0.01), the correlation was notably weaker than that for glucose clearance.

In the group with 50 mg/kg STZ, postabsorptive glucose concentration was increased about twofold (12.8 ± 1.4 vs. 6.8 ± 0.3 mM in control group; P < 0.01). The increased postabsorptive glucose was accompanied by normal HGO (58 ± 3.7 vs. 55.7 ± 4.1 µmol·kg⁻¹·min⁻¹ in control group; P > 0.05) but an ~45% reduction in glucose clearance (4.6 ± 0.5 vs. 8.2 ± 0.5 ml·kg⁻¹·min⁻¹; P < 0.01). Therefore, it was

Table 1. Metabolic parameters in postabsorptive state in rats injected with different doses of STZ (study 1)

<table>
<thead>
<tr>
<th>STZ Doses (mg/kg)</th>
<th>Plasma Glucose (mM)</th>
<th>Insulin (pM)</th>
<th>Glucagon (pg/ml)</th>
<th>Free Fatty Acids (µM)</th>
<th>HGO (µmol·kg⁻¹·min⁻¹)</th>
<th>Glucose Uptake (µmol·kg⁻¹·min⁻¹)</th>
<th>Glucose Clearance (ml·kg⁻¹·min⁻¹)</th>
<th>Urinary Glucose Loss (µmol·kg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.8 ± 0.3</td>
<td>247 ± 34</td>
<td>111 ± 15 (6)</td>
<td>0.60 ± 0.04</td>
<td>55.7 ± 4.1</td>
<td>55.2 ± 4.1</td>
<td>8.2 ± 0.5</td>
<td>0.5 ± 0.04</td>
</tr>
<tr>
<td>15</td>
<td>7.7 ± 0.3</td>
<td>292 ± 30</td>
<td>132 ± 25 (6)</td>
<td>0.49 ± 0.03</td>
<td>59.3 ± 4.4</td>
<td>58.7 ± 4.4</td>
<td>7.5 ± 0.4</td>
<td>0.6 ± 0.04</td>
</tr>
<tr>
<td>30</td>
<td>8.1 ± 0.3</td>
<td>207 ± 13</td>
<td>120 ± 8 (5)</td>
<td>0.45 ± 0.11</td>
<td>64.5 ± 4.1</td>
<td>64.0 ± 4.1</td>
<td>8.2 ± 0.8</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>40</td>
<td>7.8 ± 0.5</td>
<td>177 ± 20</td>
<td>173 ± 19 (4)</td>
<td>0.60 ± 0.08</td>
<td>59.4 ± 7.7</td>
<td>58.8 ± 7.6</td>
<td>7.5 ± 0.9</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>50</td>
<td>12.8 ± 1.4</td>
<td>250 ± 36</td>
<td>134 ± 16 (4)</td>
<td>0.57 ± 0.05</td>
<td>58.8 ± 3.7</td>
<td>55.8 ± 3.6</td>
<td>4.6 ± 0.5</td>
<td>3.0 ± 1.2</td>
</tr>
<tr>
<td>75</td>
<td>20.7 ± 0.6†</td>
<td>155 ± 8</td>
<td>132 ± 6 (9)</td>
<td>0.67 ± 0.09</td>
<td>76.4 ± 5.3†</td>
<td>46.4 ± 4.2</td>
<td>2.2 ± 0.2†</td>
<td>30.1 ± 6.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 for all measurements except glucagon, for which n values are provided in parentheses. STZ, streptozotocin; HGO, hepatic glucose output. *P < 0.05; †P < 0.01 vs. control (i.e., 0 dose).
apparent that postabsorptive hyperglycemia in this group was linked to decreased glucose clearance and not to increased HGO. It should be noted that postabsorptive insulin in this group was normal (Table 1), indicating that the decrease in glucose clearance was not due to low basal insulin concentrations.

Cellular Mechanisms of Decreased Glucose Clearance in Diabetic Rats (Study 2)

Muscle glucose clearance and substrate concentrations in the postabsorptive state (study 2A). In this study we examined whether the decreased whole body glucose clearance in STZ (50 mg/kg) diabetic rats was associated with similar decreases in skeletal muscle glucose clearance. As in study 1, postabsorptive plasma glucose was substantially higher in STZ than in control

Fig. 2. STZ dose responses of postabsorptive glucose (A), glucose clearance (B), and hepatic glucose output (HGO, C). Data with low STZ doses (15, 30, and 40 mg/kg) are similar (see Table 1 for individual group data) and therefore pooled for simplicity. Values are means ± SE for 6 (0, 15-40, and 50 mg/kg) doses or 18 experiments (15-to 40 mg/kg doses). *P < 0.05; **P < 0.01 vs. control (i.e., 0 dose).
this may be consistent with decreased glucose clearance in soleus but not in EDL muscles of STZ diabetic rats, and thus glucose transport appeared to be impaired in higher in STZ than in normal rats (Table 2). In the presence of tendency for intracellular glucose and G-6-P concentrations to be lower in STZ than in control rats. The fact, intracellular glucose content showed a tendency to be lower (Table 2). In contrast to the findings in soleus muscles, both intracellular glucose and G-6-P contents in EDL muscles showed a tendency to be higher in STZ than in normal rats (Table 2; P > 0.05). Thus glucose transport appeared to be impaired in soleus but not in EDL muscles of STZ diabetic rats, and this may be consistent with decreased glucose clearance in soleus but not in EDL muscles of STZ diabetic rats.

Muscle intracellular glucose and G-6-P levels at matched hyperglycemic conditions (study 2B). To further examine the apparent defect at the step of glucose transport in soleus muscles of diabetic rats, we compared muscle intracellular glucose and G-6-P concentrations between normal and diabetic rats at matched hyperglycemia. Plasma glucose was clamped at 12 mM in both groups (Fig. 4), and plasma insulin concentration was not statistically different in the two groups (161 ± 15 in normal vs. 134 ± 18 µM in diabetic rats, P > 0.05). Intracellular glucose content was significantly lower in soleus (1.26 ± 0.01 vs. 1.86 ± 0.02 µmol/g; P < 0.001) but not in EDL muscles (0.98 ± 0.02 vs. 1.07 ± 0.01 µmol/g; P > 0.05). Intracellular glucose content was significantly lower in soleus (1.26 ± 0.18 vs. 1.86 ± 0.26 µmol/g; P < 0.05 by 1-tailed t-test) but not in EDL muscles (0.98 ± 0.17 vs. 0.69 ± 0.07 µmol/g; P > 0.05) of diabetic rats compared with normal rats (Fig. 5). Similarly, muscle G-6-P was significantly lower in soleus (0.07 ± 0.01 vs. 0.19 ± 0.01 µmol/g; P < 0.001) but not in EDL muscles (0.24 ± 0.01 vs. 0.24 ± 0.02 µmol/g; P > 0.05) of diabetic rats compared with normal rats (Fig. 5). Although data are presented here as group averages, regression was performed on individual data (n = 36).

### Table 2. Glucose clearance and substrate concentrations in soleus and EDL muscles of normal and STZ (50 mg/kg) diabetic rats (study 2A) in the postabsorptive state.

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Diabetic</th>
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<tbody>
<tr>
<td><strong>Soleus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose clearance, ml·kg⁻¹·min⁻¹</td>
<td>21.0 ± 3.5 (7)</td>
<td>10.3 ± 1.5* (7)</td>
</tr>
<tr>
<td>Extracellular glucose space, µl/g</td>
<td>146 ± 27 (7)</td>
<td>152 ± 9 (7)</td>
</tr>
<tr>
<td>Total muscle glucose, µmol/g</td>
<td>3.86 ± 0.22 (7)</td>
<td>3.67 ± 0.25† (6)</td>
</tr>
<tr>
<td>Intracellular glucose, µmol/g</td>
<td>0.86 ± 0.31 (6)</td>
<td>0.74 ± 0.17 (5)</td>
</tr>
<tr>
<td>Glucose 6-phosphate, µmol/g</td>
<td>0.21 ± 0.04 (6)</td>
<td>0.13 ± 0.04 (6)</td>
</tr>
<tr>
<td><strong>EDL</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose clearance, ml·kg⁻¹·min⁻¹</td>
<td>3.0 ± 0.6 (6)</td>
<td>2.3 ± 0.3 (7)</td>
</tr>
<tr>
<td>Extracellular glucose space, µl/g</td>
<td>148 ± 15 (7)</td>
<td>120 ± 11 (7)</td>
</tr>
<tr>
<td>Total muscle glucose, µmol/g</td>
<td>1.27 ± 0.08 (7)</td>
<td>3.08 ± 0.22† (7)</td>
</tr>
<tr>
<td>Intracellular glucose, µmol/g</td>
<td>0.25 ± 0.14 (7)</td>
<td>0.64 ± 0.29 (7)</td>
</tr>
<tr>
<td>Glucose 6-phosphate, µmol/g</td>
<td>0.29 ± 0.06 (7)</td>
<td>0.46 ± 0.09 (7)</td>
</tr>
</tbody>
</table>

Values are means ± SE; numbers in parentheses represent no. of muscles used for each measurement. EDL, extensor digitorum longus. Rats were made diabetic by injections of 50 mg/kg STZ. *P < 0.05; †P < 0.001 vs. normal.
absorptive hyperglycemia in STZ diabetic rats was largely due to decreased peripheral glucose clearance rather than to increased HGO, consistent with our previous suggestion (28). The present study also demonstrates that reduced glucose clearance in STZ diabetic rats was associated with a similar decrease in glucose clearance in soleus (type 1) muscles, which appears to arise from a defect in glucose transport into muscle cells.

The present study is distinguished from many previous studies (1, 4, 17, 22), including our own (28), by the following two experimental strategies: first, we examined the relationship between postabsorptive glucose and various metabolic parameters 1 day after STZ treatment, and, second, animals were treated with various doses of STZ to produce various degrees of hyperglycemia. One day after STZ treatment is presumably the earliest time point at which steady-state glucose turnover can be studied, when we consider that glucose and insulin concentrations fluctuate at least during the initial 10 h after STZ injection (15). The purpose of this design was to avoid confounding metabolic changes that would occur as a consequence (rather than a cause) of chronic hyperglycemia/hypoinsulinemia. Our results reveal that certain metabolic changes observed in previous studies of STZ diabetes, such as basal hypoinsulinemia (1, 4, 17, 22, 28), elevated FFA (27), and decreased muscle HK activities (16), did not occur one day after STZ and therefore could be excluded from the list of candidate mechanisms for postabsorptive hyperglycemia.

The second strategy of utilizing STZ dose responses allowed us to delineate the relative roles of various metabolic factors (i.e., HGO, glucose clearance, insulin, glucagon, and FFA) in causing postabsorptive hyperglycemia in STZ diabetic rats. Our data indicate that there was a threshold in the effect of STZ to increase postabsorptive glucose; STZ doses up to 40 mg/kg did not alter postabsorptive glucose 1 day after STZ, but the higher doses (i.e., 50 and 75 mg/kg) caused a substantial increase in postabsorptive glucose concentration. The significant change in postabsorptive glucose at the breakoff dose (i.e., 50 mg/kg) was accompanied by a change only in glucose clearance among the metabolic parameters examined. In addition, the STZ dose-dependent changes in postabsorptive glucose were inversely proportional to those in glucose clearance. Therefore, these data convincingly demonstrate that peripheral glucose clearance was the major determinant of postabsorptive glucose in STZ diabetic rats, in contrast to the previous view that excessive glucose production is responsible for hyperglycemia in STZ diabetic rats (4).

HGO was elevated at the highest STZ dose (75 mg/kg) used in the study. The 37% increase in HGO in these rats compared with control rats would have increased plasma glucose by 37% (from 6.8 to 9.3 mM) if glucose clearance had been normal. In contrast, with normal HGO, the decrease in glucose clearance alone (from 8.2 to 2.2 ml·kg⁻¹·min⁻¹; Table 1) would have increased plasma glucose by 360% (from 6.8 to 24.5

![Graph](https://example.com/graph.png)

**Fig. 6.** Hexokinase (HK I and II) activities in cytosolic and mitochondrial fractions of soleus muscles from normal (open bars) and STZ diabetic (filled bars) rats (study 2B). Values are means ± SE of 6 muscles.

![Graph](https://example.com/graph.png)

**Fig. 5.** Intracellular (Intracell.) glucose and glucose 6-phosphate (G-6-P) concentrations in soleus and extensor digitorum longus (EDL) muscles of normal (open bars) and STZ diabetic (filled bars) rats at matched plasma or extracellular (Extracell.) glucose concentrations (study 2B). Extracellular glucose was calculated with assumption that extracellular glucose space is 15% in all muscles, on basis of estimations in study 2A (Table 2). Values are means ± SE of 4–7 muscles. *P < 0.05; **P < 0.001 vs. normal by 1-tailed t-test.

![Graph](https://example.com/graph.png)

**DISCUSSION**

The present study demonstrates that postabsorptive glucose concentration was inversely correlated with peripheral glucose clearance ($r = -0.85$) in rats treated with various doses of STZ. In addition, in rats treated with 50 mg/kg STZ, postabsorptive glucose concentration was increased about twofold, and this increase was accompanied by no change in HGO and a 45% reduction in glucose clearance. These data indicate that postabsorptive hyperglycemia in STZ diabetic rats was largely due to decreased peripheral glucose clearance rather than to increased HGO, consistent with our previous suggestion (28). The present study also demonstrates that reduced glucose clearance in STZ diabetic rats was associated with a similar decrease in glucose clearance in soleus (type 1) muscles, which appears to arise from a defect in glucose transport into muscle cells.

The present study is distinguished from many previous studies (1, 4, 17, 22), including our own (28), by the following two experimental strategies: first, we examined the relationship between postabsorptive glucose and various metabolic parameters 1 day after STZ treatment, and, second, animals were treated with various doses of STZ to produce various degrees of hyperglycemia. One day after STZ treatment is presumably the earliest time point at which steady-state glucose turnover can be studied, when we consider that glucose and insulin concentrations fluctuate at least during the initial 10 h after STZ injection (15). The purpose of this design was to avoid confounding metabolic changes that would occur as a consequence (rather than a cause) of chronic hyperglycemia/hypoinsulinemia. Our results reveal that certain metabolic changes observed in previous studies of STZ diabetes, such as basal hypoinsulinemia (1, 4, 17, 22, 28), elevated FFA (27), and decreased muscle HK activities (16), did not occur one day after STZ and therefore could be excluded from the list of candidate mechanisms for postabsorptive hyperglycemia.

The second strategy of utilizing STZ dose responses allowed us to delineate the relative roles of various metabolic factors (i.e., HGO, glucose clearance, insulin, glucagon, and FFA) in causing postabsorptive hyperglycemia in STZ diabetic rats. Our data indicate that there was a threshold in the effect of STZ to increase postabsorptive glucose; STZ doses up to 40 mg/kg did not alter postabsorptive glucose 1 day after STZ, but the higher doses (i.e., 50 and 75 mg/kg) caused a substantial increase in postabsorptive glucose concentration. The significant change in postabsorptive glucose at the breakoff dose (i.e., 50 mg/kg) was accompanied by a change only in glucose clearance among the metabolic parameters examined. In addition, the STZ dose-dependent changes in postabsorptive glucose were inversely proportional to those in glucose clearance. Therefore, these data convincingly demonstrate that peripheral glucose clearance was the major determinant of postabsorptive glucose in STZ diabetic rats, in contrast to the previous view that excessive glucose production is responsible for hyperglycemia in STZ diabetic rats (4).

HGO was elevated at the highest STZ dose (75 mg/kg) used in the study. The 37% increase in HGO in these rats compared with control rats would have increased plasma glucose by 37% (from 6.8 to 9.3 mM) if glucose clearance had been normal. In contrast, with normal HGO, the decrease in glucose clearance alone (from 8.2 to 2.2 ml·kg⁻¹·min⁻¹; Table 1) would have increased plasma glucose by 360% (from 6.8 to 24.5
mM) without consideration for urinary glucose clearance. Thus the change in glucose clearance appears to be quantitatively more important than that in HGO in causing postabsorptive hyperglycemia in the STZ rats.

One may argue that decreased glucose clearance in hyperglycemic STZ rats is the result (rather than a cause) of postabsorptive hyperglycemia. However, this proposal may be questioned on the basis of the following considerations: first, in normal rats, glucose uptake has been shown to increase in proportion to plasma glucose concentrations, even without an insulin response, such that glucose clearance was not decreased at hyperglycemia (15 mM) compared with normoglycemia (5.6 mM) (9). In addition, our previous study showed a normalization of postabsorptive glucose in STZ diabetic rats (with phlorizin) did not normalize glucose clearance (28). Finally, in the present study HGO was normal in the 50 mg/kg STZ group (study 1); thus it may be difficult to explain the postabsorptive hyperglycemia without acknowledging a primary defect in peripheral glucose metabolism. Taken together, these data indicate that there is a defect(s) in peripheral glucose clearance in rats treated with high doses of STZ.

To study the cellular mechanisms underlying reduced peripheral glucose clearance in STZ diabetic rats, we examined glucose clearance and substrate concentrations in skeletal muscles. In rats, but not in humans, glucose uptake by the brain accounts for only a small fraction [\(\sim 5\%\) (10)] of postabsorptive glucose uptake. In contrast, skeletal muscle constitutes \(\sim 40\%\) of the body mass and contributes significantly \(\sim 36\%\) (10) to basal glucose uptake in rats. Therefore, we reasoned that reduced glucose clearance in diabetic rats may arise from decreased glucose utilization in skeletal muscle. Indeed, the present results indicate that decreased glucose clearance in diabetic (50 mg/kg STZ) rats was accompanied by a similar decrease in glucose clearance in soleus (type I) muscles. Although glucose clearance was not significantly reduced in EDL muscles of diabetic rats, the contribution of this type II muscle to postabsorptive glucose clearance was much smaller than that of the type I soleus muscles (see Table 2). Thus decreased glucose clearance in diabetic rats was accompanied by a selective or primary decrease in glucose clearance in type I muscles.

The decrease in glucose clearance in soleus muscles of diabetic rats was associated with a significant decrease in intracellular glucose and G-6-P levels, compared with normal rats, at matched hyperglycemia (see Fig. 5). The decrease in G-6-P level indicates that decreased muscle clearance was due to a defect in glucose transport and/or phosphorylation rather than in metabolism beyond G-6-P. HK activities in soleus muscles (both cytosolic and mitochondrial fractions) were similar between normal and diabetic rats. Therefore, it is likely that the decrease in G-6-P level was a result of decreased intracellular glucose, which would indicate a defect in glucose transport into muscle cells. The apparent defect observed in soleus muscles was not observed in EDL muscles of diabetic rats, and this may be consistent with decreased glucose clearance in soleus but not in EDL muscles of diabetic rats. Thus these data indicate that decreased glucose clearance in soleus muscles of STZ diabetic rats was associated with an apparent defect in glucose transport.

It is unclear in the present study how the defect in glucose transport in soleus muscles was brought about as early as 1 day after STZ treatment. Postabsorptive plasma insulin was not significantly altered 1 day after STZ at various doses up to 75 mg/kg, consistent with previous studies (15), and therefore the defect was not due to basal hyperinsulinemia. On the other hand, insulin response to a rise of plasma glucose was markedly impaired in STZ-treated rats (Fig. 7), and postprandial hyperglycemia would be expected in these animals. Therefore, the glucose transport defect may be a consequence of an overnight postprandial hyperglycemia after STZ treatment. In humans, an overnight hyperglycemia has been shown to decrease insulin-stimulated glucose disposal (29), possibly through the hexosamine biosynthesis pathway that has been shown to regulate insulin’s action on the glucose transport system in insulin-sensitive cells (20, 22, 25). However, the effect of overnight hyperglycemia on fasting glucose clearance has not been studied. The exact mechanisms of the
early defect in glucose transport in soleus muscle after STZ treatment remain to be studied.

In conclusion, the present data suggest that postabsorptive hyperglycemia in STZ diabetic rats was largely due to decreased peripheral glucose clearance, although increased HGO might also be a contributing factor at a very high STZ dose. In addition, decreased glucose clearance in STZ diabetic rats was associated with a decrease in glucose clearance in soleus muscles, apparently arising from a defect in glucose transport.

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REFERENCES


