Effects of insulin-like growth factor I on glucose metabolism in rats with liver cirrhosis

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Petersen, Kitt Falk, Ralph J. Jacob, A. Brian West, Robert S. Sherwin, and Gerald I. Shulman. Effects of insulin-like growth factor I on glucose metabolism in rats with liver cirrhosis. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1189–E1193, 1997.—To determine the effect of insulin-like growth factor I (IGF-I) on glucose metabolism in cirrhosis, a 2-h euglycemic clamp with IGF-I (0.65 nmol·kg⁻¹·min⁻¹) or insulin (12 pmol·kg⁻¹·min⁻¹) was performed in awake rats with carbon tetrachloride-induced liver cirrhosis. Rates of [³-¹⁴C]glucose-determined whole body glucose turnover were similar in the fasting state in cirrhotic and control rats (36.4 ± 2.6 and 37.7 ± 2.8 µmol·kg⁻¹·min⁻¹, respectively). In the control group, IGF-I and insulin had similar effects on turnover (81.6 ± 27.0 and 76.1 ± 9.9 µmol·kg⁻¹·min⁻¹), muscle glycogen synthesis (47.5 ± 12.3 and 37.5 ± 2.5 mmol·g muscle⁻¹·min⁻¹), and suppression of endogenous glucose production (EGP; −54 ± 14 and −60 ± 12%). Cirrhotic rats were markedly insulin resistant, reflected by a 43% reduction of turnover (43.8 ± 9.4 µmol·g muscle⁻¹·min⁻¹; P = 0.03), a 73% reduction in muscle glycogen synthesis (10.2 ± 3.4 nmol·g muscle⁻¹·min⁻¹; P < 0.0001), and a diminished suppression of EGP (~32 ± 17% vs. control: −56 ± 14%; P < 0.05). In contrast, during the IGF-I clamps, turnover increased threefold in the cirrhotic rats (P = 0.001), rates of muscle glycogen synthesis were 7.4 times higher than during the insulin stimulation (P < 0.0001), and EGP was suppressed by 80 ± 12% (P < 0.05). In conclusion, insulin resistance in cirrhotic rats is mostly due to defects in insulin-stimulated muscle glycogen synthesis, and the ability of IGF-I to stimulate muscle glycogen synthesis as well as suppress EGP is maintained in cirrhotic rats. These findings suggest that alterations in both hepatic and peripheral glucose metabolism in patients with cirrhosis might be amenable to IGF-I therapy.

carbon tetrachloride-induced liver cirrhosis; muscle glycogen synthesis
treatment was continued for 14 wk before definite clinical signs of liver damage were noticed. Postmortem examination revealed no or minimal amounts (<0.5 ml) of ascites. The control rats were given a weekly dose of 2 ml of corn oil by gavage for 12 wk. The protocol was approved by the Yale Animal Care and Use Committee.

Euglycemic clamp with IGF-I or insulin. On the morning of the experiment, the catheters were opened and flushed with heparinized saline. The venous line was kept open for blood collection by a constant infusion of saline at a rate of 0.02 ml/min. Through the arterial line, a primed (18 µCi) constant (0.24 µCi/min) infusion of high-performance liquid chromatography-purified [3-3H]glucose (Amersham, Arlington Heights, IL) was begun at time 0 (1:5). One hundred microliters of the tissue homogenate were determined by homogenizing 0.3–0.4 g tissue in 0.03 M HCl to follow the hormone infusions, the rates of hepatic glucose production could be estimated as the difference between rates of whole body glucose uptake and the amount of glucose infused to maintain euglycemia.

The incorporation of [3H] into muscle glycogen during the basal equilibration period was determined in a separate set of experiments. Twenty-four-hour-fasted normal rats (n = 4) were given a 180-min infusion of [3H]glucose at a rate of 0.24 µCi/min. Because there was no net incorporation of [3H]glucose into muscle glycogen under these conditions, [3H] incorporation during the equilibration period of the clamp (0–125 min) was assumed to be negligible. During the hormone infusions, the rates of muscle glycogen synthesis could be estimated as the [3H] activity in muscle glycogen divided by the time-weighted [3H] specific activity of plasma glucose, multiplied by a factor F, which is the correction factor for the time lag for plasma [3H]glucose specific activity to reach steady state (25).

Statistical methods. Results are expressed as means ± SE. Comparisons between groups were performed with analysis of variance and Student-Newman-Keuls post hoc testing. Comparisons within groups were performed using the paired t-test.

RESULTS

Figure 1 shows typical histological sections of liver biopsies after hematoxylin-eosin staining obtained from a normal rat (Fig. 1A) and a cirrhotic rat (Fig. 1B).

Basal state. The concentrations of plasma glucose and insulin were similar in the cirrhotic and control groups (Table 1), whereas the mean fasting plasma concentration of glucagon was 70% higher in the cirrhotic than in the control rats (P = 0.004) (Table 1).

Euglycemic-hyperinsulinemic clamp. In the healthy age-matched rats, the infusion of insulin caused a twofold increase in rates of whole body glucose turnover (76.1 ± 9.9 µmol·kg⁻¹·min⁻¹; P = 0.016 vs. basal; Table 2) and stimulated rates of muscle glycogen synthesis to 37.5 ± 2.5 nmol·g·muscle⁻¹·min⁻¹ (Fig. 2). Rates of hepatic glucose production decreased by 60 ± 12% to 13.9 ± 3.0 µmol·kg⁻¹·min⁻¹ (P = 0.009 vs.
IGF-I EFFECTS ON GLUCOSE METABOLISM

During the insulin infusion, rates of muscle glycogen synthesis (60.03 vs. control) and rates of muscle glycogen synthesis (43.8 ± 9.4 µmol·kg⁻¹·min⁻¹; P = 0.03 vs. control) and rates of muscle glycogen synthesis (10.2 ± 3.4 nmol·g muscle⁻¹·min⁻¹; P < 0.0001 vs. control; Fig. 2). During the insulin infusion, rates of hepatic glucose production decreased by 32 ± 17% (from 36.4 ± 2.6 µmol·kg⁻¹·min⁻¹ in the basal state to 25.0 ± 6.9 µmol·kg⁻¹·min⁻¹; P < 0.05 vs. control).

IGF-I clamp. IGF-I at 0.65 nmol·kg⁻¹·min⁻¹ resulted in responses similar to insulin infusion at 12 pmol·kg⁻¹·min⁻¹ in the control rats (Table 2). Rates of whole body glucose turnover increased twofold during IGF-I stimulation to 81.6 ± 27.0 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. basal), and rates of muscle glycogen synthesis were 47.5 ± 12.3 nmol·g muscle⁻¹·min⁻¹, values indistinguishable from those caused by insulin stimulation (Fig. 2). The IGF-I infusion induced a suppression of rates of hepatic glucose production of 56 ± 14% to 15.6 ± 8.9 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. basal).

In contrast to insulin, the IGF-I infusion increased rates of whole body glucose turnover in the cirrhotic rats threefold to 106.4 ± 41.5 µmol·kg⁻¹·min⁻¹ (P = 0.0002 vs. basal; P = 0.232 vs. control) and markedly decreased rates of hepatic glucose production by 80 ± 12% to 8.0 ± 8.5 µmol·kg⁻¹·min⁻¹ (P < 0.05 vs. basal and P = 0.292 vs. control). During IGF-I stimulation, rates of muscle glycogen synthesis were ~55% higher in the cirrhotic rats than in the untreated control rats (cirrhotic: 75.4 ± 28.0 nmol·g muscle⁻¹·min⁻¹; control: 47.5 ± 12.3 nmol·g muscle⁻¹·min⁻¹; P = 0.08) and more than eightfold higher than the rates of muscle glycogen synthesis during insulin stimulation (P < 0.0001 vs. insulin). During the IGF-I infusion, the

Table 1. Levels of plasma glucose, insulin, and glucagon concentration

<table>
<thead>
<tr>
<th></th>
<th>Plasma Glucose, mmol/l</th>
<th>Plasma Insulin, pmol/l</th>
<th>Plasma Glucagon, ng/l</th>
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<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
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<tr>
<td>Control</td>
<td>14</td>
<td>6.09 ± 0.06</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Cirrhotic</td>
<td>16</td>
<td>6.10 ± 0.06</td>
<td>27 ± 3</td>
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<tr>
<td>Insulin clamp</td>
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<td></td>
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<tr>
<td>Control</td>
<td>6</td>
<td>6.10 ± 0.10</td>
<td>232 ± 22†</td>
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<tr>
<td>Cirrhotic</td>
<td>7</td>
<td>6.08 ± 0.19</td>
<td>271 ± 8†</td>
</tr>
<tr>
<td>IGF-I clamp</td>
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<td></td>
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<tr>
<td>Control</td>
<td>8</td>
<td>6.15 ± 0.11</td>
<td>12 ± 3§</td>
</tr>
<tr>
<td>Cirrhotic</td>
<td>8</td>
<td>6.07 ± 0.19</td>
<td>17 ± 4§</td>
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</table>

Values are means ± SE for nos. (n) of animals/group. IGF-I, insulin-like growth factor I. *P < 0.004 vs. control; †P < 0.0001 vs. basal; ‡P < 0.05 vs. control; §P < 0.05 vs. basal.

Table 2. Rates of whole body glucose turnover and hepatic glucose production

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cirrhotic</th>
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<tbody>
<tr>
<td>Rates of whole body glucose turnover</td>
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<tr>
<td>Basal</td>
<td>37.7 ± 2.8 (n = 14)</td>
<td>36.4 ± 2.6 (n = 16)</td>
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<tr>
<td>Insulin clamp</td>
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<tr>
<td>Whole body glucose turnover</td>
<td>76.1 ± 9.9 (n = 8)</td>
<td>43.8 ± 9.4 (n = 7)</td>
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<tr>
<td>Hepatic glucose production</td>
<td>13.9 ± 3.0 (n = 5)</td>
<td>25.0 ± 6.3 (n = 5)</td>
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<tr>
<td>IGF-I clamp</td>
<td></td>
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<tr>
<td>Whole body glucose turnover</td>
<td>81.6 ± 27.0 (n = 6)</td>
<td>106.4 ± 41.5 (n = 9)</td>
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<tr>
<td>Hepatic glucose production</td>
<td>15.6 ± 8.9 (n = 5)</td>
<td>8.0 ± 8.5 (n = 5)</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in µmol·kg⁻¹·min⁻¹; n, no. of animals/group. aP < 0.05 vs. basal; bP < 0.05 vs. control; cP < 0.009 vs. basal; dP = 0.0002 vs. basal; eP = 0.292 vs. control.
plasma insulin concentrations decreased by 35–45% (P < 0.05 vs. basal), whereas plasma glucagon concentrations were unchanged and remained higher in the cirrhotic than in the control group throughout the IGF-I infusion (P = 0.003 cirrhotic vs. control; Table 1).

**DISCUSSION**

Liver cirrhosis in humans is not a uniform disease, and in rats it has been very difficult to reproduce an irreversible form of liver cirrhosis resembling the most common human form of micronodular cirrhosis. CCl₄ treatment has been reported to induce a ‘human-like’ liver cirrhosis in rats, in which the hepatotoxicity is irreversible and recognized as the micronodular cirrhosis that closely resembles the human disease (5, 21). In the present study, body weight during the posttreatment period increased to reach the final weights of the normal age-matched control group. The fasting liver and muscle glycogen concentrations were similar to those of the control group. Other investigators have found that liver glycogen content was decreased in both the fed and the fasted states in the CCl₄ cirrhotic rat and that total hepatic glycogen synthase activity was decreased by 45% compared with age-matched littermates (11). However, it is unclear whether a posttreatment recovery was allowed and whether the acute toxic effects of the CCl₄ treatment had disappeared at the time of the study. In the present study, the pathological changes in the livers of the cirrhotic rats were graded histologically as micronodular cirrhosis. No histological signs of damage to other organs were found, and a small amount of ascites was present in most animals. Immediately after cessation of the CCl₄ treatment, the body weights were ~70% of the control value (P < 0.01), and during the recovery period normal body weights were achieved. The rates of whole body glucose uptake, glucose infusion, hepatic glucose production, and muscle glycogen synthesis were similar to those obtained by other investigators during the euglycemic-hyperinsulinemic clamp in CCl₄ cirrhotic rats (11, 15).

This study demonstrates that the rodent model of liver cirrhosis induced by CCl₄ treatment is associated with marked insulin resistance but a normal response to IGF-I. The stimulatory effects of IGF-I on glucose uptake and muscle glycogen synthesis in the cirrhotic rats were comparable to or greater than those seen in the age-matched control rats. When extrapolated to the whole body (with the assumption of similar whole body muscle mass in normal control and cirrhotic rats) (14), these rates of muscle glycogen synthesis could account for ~65% of the increase in rates of whole body glucose uptake in both normal and cirrhotic rats, indicating that IGF-I stimulates whole body glucose uptake mainly by increasing rates of muscle glycogen synthesis rather than through stimulation of glucose oxidation. In contrast, insulin resistance in the cirrhotic rats could be accounted for by reduced rates of muscle glycogen synthesis. This is in agreement with an earlier study in rats with liver cirrhosis, where rates of muscle glycogen synthesis were increased during euglycemic-hyperinsulinemic clamp conditions (15). Glucose intolerance and insulin resistance in cirrhotic patients are well described, and several recent euglycemic-insulin clamp studies indicate that the major defect is in nonoxidative muscle glucose metabolism, which under most conditions can be attributed to muscle glycogen synthesis (16, 18, 27, 29, 30). Together these data suggest that IGF-I could be the liver factor facilitating nonoxidative glucose metabolism, as suggested in earlier studies (12, 17).

In the control group, insulin and IGF-I suppressed rates of hepatic glucose production similarly by 50–55%, which is in accordance with previous studies in humans (18). In an earlier study in rats, IGF-I had a more limited effect to suppress hepatic glucose production (8). The reason for this discrepancy is unclear but might possibly be due to 1) the duration of the study, which in that of Jacob et al. (8) was only a 90-min baseline followed by a 90-min study period, or 2) differences in the IGF-I preparation. In the present study, as in the human studies (2), rhIGF-I was used, whereas rhIGF-I (Thr 59) was used in the previous animal study (8). It should be mentioned that, although Jacob et al. found no significant effects of IGF-I on net hepatic glucose production, rates of hepatic glycogen synthesis were significantly higher in the IGF-I studies than in the insulin studies. Other studies of IGF-I’s effects on hepatic glucose production have shown that suppression of hepatic glucose production is achieved only with high levels of IGF-I, which may be due to the relatively smaller number of IGF-I receptors present in liver than in skeletal muscle (20, 35). In the cirrhotic rats, IGF-I suppressed hepatic glucose production by ~80%. Together, these data are consistent with the hypothesis that IGF-I at this dose is working through its own receptors (6, 8, 9). If IGF-I had been acting through the same cellular mechanisms as insulin, one might have expected the insulin-resistant cirrhotic rats to have diminished responses to IGF-I as well.

The pathogenesis of insulin resistance in liver cirrhosis is still unknown, and several factors may be involved, including lower plasma concentrations of IGF-I, increased plasma concentrations of free fatty acids, growth hormone, glucagon, catecholamines, and possibly altered membrane lipid composition (1). Plasma concentrations of glucagon were ~70% higher in the cirrhotic rats than in the normal controls in the basal, fasting state and were likely a contributing factor to the insulin resistance-protein catabolic state in the cirrhotic rats (Table 1). However, changes in plasma glucagon concentrations cannot account for the observed difference in glucose metabolism between the insulin- and IGF-I-infused animals, because the glucagon concentrations remained equally elevated during the insulin and IGF-I clamp studies (Table 1). Regardless of the mechanisms, the current data suggest that the insulin-resistant catabolic state associated with hepatic cirrhosis may be amenable to IGF-I therapy. The role of IGF-I-binding proteins and other factors in modulating peripheral sensitivity to IGF-I will have to be clarified before the mechanisms behind the therapeutic effects of IGF-I can be understood fully.

In summary, this study shows that insulin resistance is present in rats with CCl₄-induced liver cirrhosis and that IGF-I is more effective than insulin in stimulating
rates of whole body glucose uptake and suppressing rates of hepatic glucose production in these insulin-resistant cirrhotic rats. The sensitivity of the cirrhotic rates of whole body glucose uptake and suppressing CT 06520–8020.

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