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

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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, we studied the effects of IGF-I on glucose metabolism in rats with carbon tetrachloride-induced liver 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-H]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 pmol·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 pmol·kg⁻¹·min⁻¹), muscle glycogen synthesis (47.5 ± 12.3 and 37.5 ± 2.5 nmol·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 pmol·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; that the abnormality 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.

CIRRHOSIS is often associated with glucose intolerance and insulin resistance (7, 22, 23, 33). Recent studies in patients with liver cirrhosis have shown that the failure of insulin to stimulate glucose uptake can be attributed to defects in nonoxidative glucose metabolism (19, 27), but the underlying mechanisms remain unknown. Lang et al. (12) suggested that the normal liver produces a “factor” that facilitates glucose uptake in extrahepatic tissues and that lack of this factor may be the cause of the insulin resistance in chronic liver failure. In support of this hypothesis, liver extracts were found to stimulate muscle glucose uptake when added to the perfusate of a perfused rat hindquarter system (17). Insulin-like growth factor I (IGF-I) is a possible candidate for such a liver factor, because it is to a large extent produced in the liver and it has been reported to be reduced in patients with liver cirrhosis (28). IGF-I has been shown to lower plasma glucose and stimulate rates of whole body glucose uptake similarly to the effects of insulin in normal (8) and diabetic rats (9, 24) and in humans (2). The chemical structure of IGF-I is similar to that of insulin, and the biological action of IGF-I, although mediated predominantly via its own receptor, is to a lesser extent exerted through the insulin receptor (35). Most of the circulating IGF-I is bound to specific carrier proteins, and some of these IGF-I-binding proteins may modulate the biological effects of the hormone (4). In the present study, we compared the effects of IGF-I and insulin on rates of whole body glucose uptake and muscle glycogen synthesis in rats with carbon tetrachloride (CCl₄)-induced liver cirrhosis by using doses of the hormones that produce similar effects on glucose metabolism in normal age- and weight-matched control rats.

METHODS

Eighteen-week-old male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) with CCl₄-induced liver cirrhosis were studied. The control group consisted of normal male Sprague-Dawley rats matched for age and weight. Animals were housed in the Yale Animal Care Facility, kept on a 12:12-h day-night cycle, and fed a standard rat chow diet consisting of 51% carbohydrate, 22% protein, and 5% fat (with remaining weight supplied by moisture and nonmetabolizable solids). Four to six days before the study, the rats were anesthetized with pentobarbital sodium (50 mg/kg) and catheters were implanted into the left carotid artery for infusion and into the right jugular vein for blood sampling; catheters were externalized at the back of the neck and sealed with a polyvinylpyrrolidone solution, as previously described (32). All rats were fasted for 24 h before the study to deplete liver glycogen, as earlier described (31). The final body weight of the animals was 447 ± 19 g (cirrhotic, n = 16) and 460 ± 21 g (control, n = 14).

Induction of liver cirrhosis. Liver cirrhosis was induced by twelve weekly doses of CCl₄ dissolved in corn oil and given by gavage. The volume of the solution was 2 ml, and the amount of CCl₄ was adjusted according to the changes in body weight. The initial dose was 40 µl (initial body weight 150–160 g), increasing to 200 µl by week 12 (5, 21). Phenobarbital (350 mg/kg) was added to the drinking water 1 wk before start of the CCl₄ treatment and throughout the 12 wk of treatment to induce the hepatic microsomal P-450 system, thereby increasing the metabolism of CCl₄ and the production of the toxic metabolites –CCl₃ and Cl⁻COO⁻ (34). After cessation of treatment, the rats were allowed to recover from the acute toxic effects of CCl₄ and its metabolites for 2 wk (13), with free access to regular drinking water and food, before they were studied at the age of 18–20 wk. Rats were selected for the study after physical examination for liver damage based on decreased weight gain and lethargy. In three rats, the CCl₄
treatment was continued for 14 wk before definite clinical signs of liver damage were noticed. Postmortem examinations 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.

A 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 and continued throughout the study for determination of rates of whole body glucose turnover and tissue glycogen synthesis. After 125 min of equilibration (t = 125 min), recombinant human IGF-I (rhlIGF-I, Genentech, San Francisco, CA) was administered as a prime dose of 33 nmol rhIGF-I/kg followed by a constant infusion of 0.65 nmol rhIGF-I·kg⁻¹·min⁻¹ for 120 min (8). In the insulin studies, a prime dose of 360 pmol U/kg was given at t = 125 min, followed by a constant infusion of 12 pmol·kg⁻¹·min⁻¹. This dose of insulin has been shown in previous studies to be equal in effectiveness to the IGF-I dose (8). The plasma glucose concentration was maintained at 6.0 ± 0.1 mmol/l during the infusions of IGF-I and insulin by a variable infusion of 25% dextrose (32). Plasma samples were taken every 5 min for determination of plasma glucose concentration and every 10 min during the last 40 min of both the equilibration and experimental periods for determination of [3H]glucose specific activity. Additional samples of plasma were collected for insulin and glucagon concentrations immediately before the start of the hormone infusion (basal samples) and again at the end of the study (t = 245 min). The total blood loss of ~4 ml was replaced throughout with a transfusion of rat donor blood, as previously described (26). At the end of the study, the rats were anesthetized with an intravenous injection of pentobarbital (40 mg/kg); the abdomen was quickly opened, and a liver biopsy (~0.5 g) was taken and preserved in Carnoy’s buffer for histological analysis. Thereafter, the rats were killed by an additional dose of pentobarbital sodium, and a tissue sample of ~2 g of the quadriiceps muscles was freeze-clamped between thongs prechilled in liquid nitrogen. The muscle tissues were sampled in <1.5 min from the opening of the abdomen and stored at −70°C until analyzed. A urine sample was taken from the bladder for estimation of urinary loss of glucose (which in all studies were negligible). Fasting concentrations of glycogen in quadriceps muscle were measured in a separate group of cirrhotic (n = 6) and normal age-matched rats (n = 7) after a 24-h fast.

Analyses. Plasma glucose concentration was measured on a Beckman (Fullerton, CA) glucose analyzer. Plasma immunoreactive insulin and glucagon concentrations were measured with double antibody radioimmunoassays (RIA) (insulin: Diagnostic System Laboratories, Webster, TX; glucagon: Linco Research, St. Charles, MO). Plasma concentrations of total (free plus protein-bound) IGF-I were measured using an RIA assay (Nichols Diagnostic Institute, San Juan Capistrano, CA). The concentrations of glycogen in liver and quadriceps muscle were measured by the glucose oxidase method after extraction and hydrolysis to glucose, as described (10). Plasma [3H]glucose activity was measured in duplicate after precipitation with Ba(OH)₂/Zn(SO₄)₂ and evaporated to dryness to eliminate [3H]₂O. The [3H]glucose activity in muscle was determined by homogenizing 0.3–0.4 g tissue in 0.03 M HCl (1:5). One hundred microliters of the tissue homogenate were applied to filter paper (Whatman 3D), dried under a heat lamp, and washed for 40 min in 33% ethanol under gentle stirring. This washing was performed three times to eliminate free [3H]glucose. The [3H] specific activity in muscle glycogen was determined in duplicate by scintillation counting of the dried filter papers (3).

Liver histology. Within 2 wk of sampling, the liver biopsies were analyzed by microscopic examination after hematoxylin-eosin staining. Only studies in which the histological examination of the livers showed established cirrhosis (with tissue necrosis, regeneration nodules, extensive bridging fibrosis, and no signs of acute inflammation) were included in the study (success rate ~70%). All samples from kidney, esophagus, and small and large intestines were normal except in one rat with advanced liver cirrhosis, in which there was focal chronic inflammation of the small bowel.

Calculations. The rates of whole body glucose turnover were calculated by dividing the infusion rate of [3-3H]glucose (disintegrations·min⁻¹·dpm) by the plasma [3H]glucose specific activity (dpm/mmol) (32). During the hormone infusions, the rates of hepatic glucose production were calculated 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 injection of [3-3H]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). Despite these differences in plasma glucagon levels, rates of basal hepatic glucose production were similar in the two groups (cirrhotic 36.4 ± 2.6 µmol·kg⁻¹·min⁻¹; control: 37.7 ± 2.8 µmol·kg⁻¹·min⁻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 µmol·min⁻¹·kg⁻¹ (Fig. 2). Rates of hepatic glucose production decreased by 60 ± 12% to 13.9 ± 3.0 µmol·min⁻¹·kg⁻¹ (P = 0.009 vs.
IGF-I effects on glucose metabolism

During the insulin infusion, rates of muscle glycogen synthesis (10.2 ± 0.03 vs. control) and 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) 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). During IGF-I stimulation, 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 whole body glucose turnover increased twofold during IGF-I stimulation to 81.6 ± 27.0 µmol·kg⁻¹·min⁻¹ (P < 0.0001 vs. insulin). During IGF-I stimulation, the 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

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<tr>
<td>Plasma glucose, mmol/l</td>
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<td>Plasma insulin, pmol/l</td>
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<td>27 ± 3</td>
<td>232 ± 22†</td>
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<td>Plasma glucagon, ng/l</td>
<td>40 ± 2</td>
<td>67 ± 8*</td>
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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.

Fig. 1. Histological sections of liver (formalin fixed, stained with hematoxylin and eosin, final magnification ×60). A: normal liver of control animal; B: liver of cirrhotic animal.

Fig. 2. Rates of muscle glycogen synthesis during euglycemic clamps with insulin-like growth factor I (IGF-I) and insulin. *P < 0.001 vs. control+insulin; **P < 0.0001 vs. cirrhotic+insulin.
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 measured 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 rats to IGF-I normalizes glucose metabolism, and the mechanism behind this is mainly the stimulating effect of IGF-I on muscle glycogen synthesis.

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