Contribution of defects in glucose uptake to carbohydrate intolerance in liver cirrhosis: assessment during physiological glucose and insulin concentrations

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Nielsen, Michael F., Andrea Caumo, Niels Kristian Aagaard, Visvanathan Chandramouli, William C. Schumann, Bernard R. Landau, Ole Schmitz, and Hendrik Vilstrup. Contribution of defects in glucose uptake to carbohydrate intolerance in liver cirrhosis: assessment during physiological glucose and insulin concentrations. Am J Physiol Gastrointest Liver Physiol 288: G1135–G1143, 2005. First published January 6, 2005; doi:10.1152/ajpgi.00278.2004.—It is well established that subjects with liver cirrhosis are insulin resistant, but the contribution of defects in insulin secretion and/or action to glucose intolerance remains unresolved. Healthy individuals and subjects with liver cirrhosis were studied on two occasions: 1) an oral glucose tolerance test was performed, and 2) insulin secretion was inhibited and glucose was infused in a pattern and amount mimicking the systemic delivery rate of glucose after a carbohydrate meal. Insulin was concurrently infused to mimic a healthy postprandial insulin profile. Postabsorptive glucose concentrations were equal (5.36 ± 0.12 vs. 5.40 ± 0.25 mmol/L, P = 0.89), despite higher insulin (P < 0.01), C-peptide (P < 0.01), and free fatty acid (P = 0.05) concentrations in cirrhotic than in control subjects. Endogenous glucose release (EGR; 11.50 ± 0.50 vs. 11.73 ± 1.00 μmol·kg−1·min−1, P = 0.84) and the contribution of gluconeogenesis to EGR (6.60 ± 0.47 vs. 6.28 ± 0.64 μmol·kg−1·min−1, P = 0.70) were unaltered by cirrhosis. A minimal model recently developed for the oral glucose tolerance test demonstrated an impaired insulin sensitivity index (P < 0.05), whereas the β-cell response to glucose was unaltered (P = 0.72). During prandial glucose and insulin infusions, the integrated glycemic response was greater in cirrhotic than in control subjects (P < 0.05). EGR decreased promptly and comparably in both groups, but glucose disappearance was insufficient at the prevailing glucose concentration (P < 0.05). Moreover, identical rates of [3-13C]glucose infusion produced higher tracer concentrations in cirrhotic than in control subjects (P < 0.05), implying a defect in glucose uptake. In conclusion, carbohydrate intolerance in liver cirrhosis is determined by insulin resistance and the ability of glucose to stimulate insulin secretion. During prandial glucose and insulin concentrations, EGR suppression was unaltered, but glucose uptake was impaired, which demonstrates that intolerance can be ascribed to a defect in glucose uptake, rather than abnormalities in glucose production or β-cell function. Although insulin secretion ameliorates glucose intolerance, impaired glucose uptake during physiologic glucose and insulin concentrations produces marked and sustained hyperglycemia, despite concurrent abnormalities in glucose production or insulin secretion.

insulin action; minimal model; gluconeogenesis

Impaired glucose tolerance is a well-established feature of liver cirrhosis (23, 33, 39, 42) that may result from excess glucose production, impaired glucose utilization, and/or insufficient insulin secretion. In healthy humans, glucose concentration rarely exceeds 8 mM (8, 15), because the coordinated rise in glucose and insulin suppresses glucose production and stimulates its utilization. In liver cirrhosis, carbohydrate ingestion produces marked and sustained hyperglycemia (22, 42). Several disturbances may account for this hyperglycemia. Insulin secretion may be decreased and delayed. Moreover, cirrhosis is characterized by insulin resistance. Either of these abnormalities may alter glucose tolerance. Several studies have demonstrated impaired insulin-mediated glucose uptake (11, 21, 35, 36, 40), but it remains unclear whether this can fully account for carbohydrate intolerance or whether defects in endogenous glucose release (EGR) also contribute during conditions characteristic of daily living, i.e., in the presence of variable glucose and insulin concentrations.

Hepatic insulin action has been demonstrated to be normal in liver cirrhosis (11, 34, 36). However, after meal ingestion, EGR depends both on the ability of insulin to suppress glucose production and on the mass action effect of glucose per se (28, 48). A defect in suppression of EGR during prandial insulin and insulin concentrations may, therefore, contribute to glucose intolerance, irrespective of an unaltered hepatic insulin sensitivity.

The present study was designed to address this question by combining two mutually independent, complementary physiological analyses of glucose metabolism: 1) a modified minimal model analysis developed for oral glucose tolerance test (OGTT) data and 2) a prandial glucose and insulin infusion protocol developed to estimate glucose production and utilization during conditions resembling those following ingestion of a carbohydrate meal.

On the basis of minimal model analyses of glucose and C-peptide concentrations, indexes of β-cell function and insulin sensitivity were calculated using two independent analytic models (6, 10, 25). In a second set of experiments in the same subjects, endogenous insulin secretion was inhibited, and insulin and glucose were infused using a computerized infusion system, delivering insulin at a rate mimicking the prandial insulin secretion profile at concentrations recorded during the OGTT. Glucose was infused at a rate mimicking the systemic rate of glucose appearance after ingestion of 50 g of glucose. Identical prandial insulin concentrations were achieved in the

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two groups, allowing determination of the effects of physiologic changes in glucose and insulin concentrations on glucose turnover while taking into account the confounding effects of the different insulin concentrations observed during the OGTT. The effect of liver cirrhosis on gluconeogenesis was assessed by combining the isotopic dilution method with the \(^{2}H\text{O}\) technique (24). Glucose turnover was measured using the radioisotopic-infused glucose (“hot-GINF”) technique to ensure accurate estimates of glucose production and utilization during the infusions.

**METHODS**

**Subjects.** After approval from the Research Ethics Committee (County of Aarhus), seven Caucasian men with biopsy-verified liver cirrhosis and seven healthy men matched for age (54.4 ± 3.2 vs. 54.6 ± 4.4 yr), body mass index (25.3 ± 1.2 vs. 25.9 ± 1.3 kg/m\(^2\)), and lean body mass (64.6 ± 3.3 vs. 67.2 ± 3.2 kg) gave written informed consent to participate. Six were diagnosed with alcoholic and one with primary biliary cirrhosis. All cirrhotic subjects were class B according to the Child-Pugh classification. Liver biopsies were performed in all cirrhotic subjects. The biochemical characteristics are shown in Table 1. Lean body mass was measured using bioelectric impedance (Anitmetro, HTS Engenberg, APS, Odense, Denmark). Study subjects receiving diuretics or other medications known to affect carbohydrate metabolism were instructed to discontinue the medication ≥48 h before the study. All were in good health, had normal blood pressure, and were at stable weight. None had a family history of diabetes among first-degree relatives. At least 3 days before the study, subjects were instructed not to engage in vigorous exercise.

**Experimental design.** All subjects underwent a standardized OGTT and participated in a prandial glucose and insulin infusion study (Fig. 1). The studies were performed in random order and separated by ≥2 wk. Liver function was assessed using a galactose elimination capacity (GEC) analysis (9, 44, 46).

**OGTT.** After an overnight fast, study subjects were admitted to the General Clinical Research Centre at 0800. An intravenous cannula was inserted into an antecubital vein for blood sampling. The hand was then placed in a heated Plexiglas box and maintained at ~55°C. Postabsorptive glucose turnover and gluconeogenesis were determined from 0930 to 1000 (from −30 to 0 min).

At 1000 (0 min), an infusion of somatostatin (60 ng·kg\(^{-1}\)·min\(^{-1}\)) was started to inhibit endogenous insulin secretion. Growth hormone (2 ng·kg\(^{-1}\)·min\(^{-1}\)) and glucagon (0.65 ng·kg\(^{-1}\)·min\(^{-1}\) for the first 2 h and 1.3 ng·kg\(^{-1}\)·min\(^{-1}\), thereafter) were also infused to maintain basal hormone levels. The glucagon infusion rate was doubled at 120 min after the start of the prandial glucose and insulin infusions in an attempt to simulate the normal prandial rise in glucagon secretion after a carbohydrate meal (8, 14). At time 0, a 5-h preprogrammed variable glucose and insulin infusion was started using separate infusion pumps (model PhD 2000, Harvard Apparatus, South Natick, MA) driven by a personal computer (model PC 2/30 265, IBM, Rochester, MN). Glucose was infused in an amount and pattern mimicking the systemic rate of delivery of glucose after ingestion of a meal containing 50 g of carbohydrate, as previously described (1). Insulin was infused simultaneously to reproduce serum insulin concentrations in non diabetic subjects (3).

In an effort to maintain constant specific activities, the rate of basal [\(^{3}H\)glucose] was adjusted according to the anticipated suppression of EGR during the variable glucose and insulin infusions: from −180 to 0 min for 100% of basal, from 0 to 15 min for 70% of basal, from 15 to 75 min for 30% of basal, from 75 to 120 min for 45% of basal, and from 120 to 300 min for 50% of basal. Furthermore, all infused glucose contained [\(^{3}H\)glucose]. Hence, glucose-specific activities remained constant in the study groups (see Fig. 7). To avoid the confounding effects of counterregulation, the experiments were terminated when plasma glucose concentrations fell below 4 mmol/l (~70 mg/dl), which occurred in five control and two cirrhotic subjects.

**Analysis.** Arterialized plasma glucose was measured in duplicate immediately after samples were obtained (Beckman Instruments, Palo Alto, CA). Samples for hormone analysis were placed on ice, centrifuged at 4°C, separated, and stored at −20°C until assay. Serum

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**Table 1. Biochemical characteristics of cirrhotic subjects**

<table>
<thead>
<tr>
<th>Subj No.</th>
<th>Hb, mmol/l (8.4–10.8)</th>
<th>ALAT, U/l (10–40)</th>
<th>AKP, U/l (80–270)</th>
<th>Bilirubin, μmol/l (&lt;22)</th>
<th>Urea, mmol/l (2.5–7.8)</th>
<th>Creatinine, μmol/l (62–133)</th>
<th>APTT, s (25–38)</th>
<th>GEC μmol/kg·h(^{-1})·min(^{-1})</th>
<th>% Normal</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>9.0</td>
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<td>10</td>
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<td>186</td>
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<td>4.3</td>
<td>63</td>
<td>36</td>
<td>21.6</td>
<td>58</td>
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<tr>
<td>Mean ± SE</td>
<td>8.3 ± 0.3</td>
<td>47 ± 22</td>
<td>315 ± 105</td>
<td>26 ± 5</td>
<td>4.2 ± 0.9</td>
<td>88 ± 12</td>
<td>34 ± 1</td>
<td>23.0 ± 2.8</td>
<td>62 ± 7</td>
</tr>
</tbody>
</table>

Subjects 1, and 3–7 had alcoholic cirrhosis; subject 2 had primary biliary cirrhosis; ALAT alanine aminotransferase; AKP, alkaline phosphatase; APTT, activated partial thromboplastin time; GEC, galactose elimination capacity.

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insulin was determined by a two-site enzyme-linked immunosorbent assay (2). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay. Serum FFA were measured enzymatically using a nonesterified fatty acid test kit (Wako Chemicals, Neuss, Germany).

The deuterium enrichment on C-2 and C-5 of glucose was measured as previously described (24). Briefly, 15 ml of blood were diluted with 30 ml of demineralized water and deproteinized using 15 ml of 0.3 N ZnSO4 and 15 ml of 0.3 N Ba(OH)2. The samples were centrifuged at 2,000 rpm for 15 min, and the pellet was diluted in 15 ml of demineralized water to wash out the remaining glucose. Glucose was isolated by successive ion-exchange chromatography and high-performance liquid chromatography. For determination of 2H of the hydrogen bound to C-5, the glucose was converted to xylose. C-5 of glucose with its hydrogens was cleaved by periodate oxidation, yielding formaldehyde, which was condensed with ammonium hydroxide to form hexamethylenetetramine (HMT). The 2H bound to C-2 of glucose was isolated in formaldehyde after conversion of the glucose to ribitol-5-phosphate and arabitol-5-phosphate and was also treated to form HMT. The HMTs were analyzed in a Hewlett-Packard mass spectrometry system. Standard solutions of glucose of known enrichment were run along with the unknown samples.

**Calculation of glucose turnover.** Glucose specific activity, calculated as [3-3H]glucose (dpm/ml) divided by plasma glucose concentration (μmol/ml), was smoothed according to the OOPSEG program as suggested by Bradley et al. (5). Glucose production and utilization were calculated using Steele’s equations for nonsteady state (41). The pool correction factor was assumed to be 0.65 ml/kg, and the glucose volume of distribution was assumed to be 200 ml/kg. Glucose production and utilization at time 0 were calculated using the average specific activity from −30 to 0 min. Glucose clearance was calculated as the ratio of glucose utilization to the corresponding plasma glucose concentration. Postabsorptive gluconeogenesis rates were calculated as the ratio of deuterium bound to C-5 to deuterium bound to C-2 of glucose multiplied by the mean rate of glucose production from −30 to 0 min. During glucose infusion, EGR was determined by subtracting the glucose infusion rate from the tracer-determined rate of glucose appearance. Carbohydrate and lipid oxidation during the postabsorptive state and from 120 to 150 min during the prandial glucose and insulin infusions were calculated using the equations of Frayn (18).

**Assessment of insulin sensitivity.** Insulin sensitivity was calculated using two different approaches: one relied on the postabsorptive measurements of insulin, glucose, and FFA concentrations and the other on the dynamic insulin and glucose data measured during the OGTT. Insulin, glucose, and FFA concentrations measured in the postabsorptive state were used to calculate the revised Quicki index as suggested by Perseghin et al. (31). This index was adopted, because it takes into account the difference in FFA concentrations in control and cirrhotic subjects. Insulin sensitivity was also assessed from the glucose and insulin concentrations measured during the OGTT according to Caumo et al. (10). This novel method extends the minimal model approach originally developed for analysis of the intravenous glucose tolerance test (4) to the OGTT and provides a measure of insulin sensitivity (SImean) from the areas under the measured glucose and insulin concentration curves (12, 13).

**Assessment of β-cell function.** β-Cell function was determined from the C-peptide and glucose concentrations measured during the OGTT using the minimal model developed by Toffolo et al. (43) and recently applied to the OGTT by Breda et al. (6). The model has been reported to successfully assess insulin secretion in normal subjects (43) and has been validated in individuals with various degrees of glucose intolerance (6). The model yields a global index of pancreatic response (Φ), which measures the ability of the β-cell to secrete insulin in response to glucose ingestion. It is emphasized that Φ does not reflect insulin secretion per se but, rather, insulin secretion normalized to the glucose stimulus. Φ has been shown to be well approximated by the ratio of the area under the incremental (above basal) C-peptide concentration to the area under the incremental glucose concentration during the OGTT (6).

**Statistical analysis.** Values are means ± SE. Glucose appearance, disappearance, and clearance rates are expressed per kilogram of lean body mass. Integrated responses and responses above basal were calculated using the trapezoidal rule. Integrated response was defined as total area above zero. Postabsorptive measures were defined as the mean of the values during the 30 min before the prandial glucose infusion, i.e., from 0930 to 1000 (−30 to 0 min). A nonpaired Student’s two-tailed t-test was used to test for statistical difference. A nonparametric two-sample analysis was used for data not fulfilling the criteria for normal distribution (27). P < 0.05 was considered statistically significant.

**RESULTS**

**GEC and liver enzymes.** The patient characteristics are shown in Table 1. GEC was, on average, 62 ± 7% of the expected value in healthy subjects, which suggests impaired liver function in the cirrhotic subjects.
Glucose, insulin, C-peptide, and FFA concentrations during the OGTT. Postabsorptive plasma glucose concentrations (5.40 ± 0.25 vs. 5.36 ± 0.12 mM, P = 0.89) were equal in the two groups, despite higher insulin (70.6 ± 7.3 vs. 38.4 ± 4.8 pmol/l, P < 0.01) and C-peptide (848 ± 138 vs. 411 ± 21 pmol/l, P < 0.01) concentrations in the cirrhotic subjects. After glucose ingestion, insulin (39.7 ± 9.3 vs. 73.9 ± 11.1 nmol/l per 5 h, P < 0.05) and C-peptide (479.7 ± 86.3 vs. 224.1 ± 33.5 mmol/l per 5 h, P < 0.05) concentrations were higher in the cirrhotic subjects (Figs. 2 and 3).

Glucose tolerance was impaired in three of the seven cirrhotic subjects, whereas three subjects were marginally intolerant, with 2-h glucose concentrations between 7.1 and 7.4 mM. The cirrhotic subjects were, on average, glucose intolerant, with a 2-h plasma glucose concentration of 8.47 ± 1.03 vs. 6.74 ± 0.89 mM in the control subjects.

Postabsorptive FFA concentrations were higher in subjects with liver cirrhosis than in controls (0.78 ± 0.13 vs. 0.47 ± 0.06 mmol/l, P = 0.05) but were suppressed to control levels after glucose ingestion, which suggests an unaltered suppression of lipolysis (Fig. 3). Glucagon concentrations did not differ during the OGTT in the two groups (41 ± 7 vs. 45 ± 11 pg/ml, P = 0.76).

Insulin sensitivity. Insulin sensitivity as determined by the revised Quicki method (31) was lower in subjects with liver cirrhosis than in control subjects (0.35 ± 0.01 vs. 0.42 ± 0.02 units, P < 0.01), implying impaired insulin sensitivity. Also, SIoral, calculated from glucose and insulin concentrations during the OGTT, was lower in cirrhotic than in control subjects (4.41 ± 1.35 vs. 12.54 ± 3.54 × 10⁻⁴ dl⁻¹·kg⁻¹·min⁻¹·μU⁻¹·ml⁻¹, P < 0.05).

Index of β-cell function. Φ was not different in control and cirrhotic subjects (53 ± 22 vs. 44 ± 10 min⁻¹·pmol⁻¹·l⁻¹, P = 0.72), suggesting that, when corrected for differences in glucose concentration, the β-cell response is unaltered in cirrhotic subjects.

Postabsorptive endogenous glucose release and gluconeogenesis. Postabsorptive glucose production (11.50 ± 0.50 vs. 11.73 ± 1.00 μmol·kg⁻¹·min⁻¹, P = 0.84) and gluconeogenesis (6.60 ± 0.47 vs. 6.28 ± 0.64 μmol·kg⁻¹·min⁻¹, P = 0.70) did not differ in controls and cirrhotic subjects, although insulin concentrations were higher in cirrhotic subjects (Fig. 4). Gluconeogenesis accounted for 57.4 ± 2.9% and 54.0 ± 3.9% of production in control and cirrhotic subjects, respectively.

![Fig. 2. Glucose and insulin concentrations in control subjects and subjects with liver cirrhosis during oral glucose tolerance test. Glucose was ingested at time 0.](http://ajpgi.physiology.org/)  
![Fig. 3. Free fatty acid and C-peptide concentrations in controls and subjects with liver cirrhosis during oral glucose tolerance test. Glucose was ingested at time 0.](http://ajpgi.physiology.org/)  
![Fig. 4. Postabsorptive rates of endogenous glucose release and gluconeogenesis in controls and subjects with liver cirrhosis. Gluconeogenesis was calculated by multiplying the mean ratio of deuterium enrichment at C-5 to that at C-2 of glucose by the mean rate of fasting endogenous glucose release.](http://ajpgi.physiology.org/)
Substrate and hormone concentrations during prandial glucose and insulin infusions. Before initiation of the prandial glucose and insulin profile, C-peptide concentrations were higher in cirrhotic than in control subjects (804 ± 132 vs. 511 ± 63 μmol/l, P = 0.06; Fig. 5). Somatostatin infusion promptly inhibited insulin secretion in both groups, producing C-peptide concentrations almost suppressed to control levels in the cirrhotic subjects (Fig. 5). Glucagon concentrations also did not differ in the two groups during the prandial glucose and insulin infusions (Fig. 6).

Postabsorptive FFA concentrations were higher in cirrhotic than in control subjects (0.944 ± 0.087 vs. 0.619 ± 0.054 mmol/l, P < 0.01). Carbohydrate oxidation (9.00 ± 1.52 vs. 10.14 ± 0.94 μmol·kg⁻¹·min⁻¹, P = 0.53) and lipid oxidation (5.77 ± 0.74 vs. 6.34 ± 0.69 μmol·kg⁻¹·min⁻¹, P = 0.58) did not differ in the two groups after an overnight fast. During glucose and insulin infusion, FFA concentrations decreased to control levels in the cirrhotic subjects, implying an unaltered suppression of lipolysis. During the prandial glucose and insulin infusions, carbohydrate oxidation was equally increased (804 ± 132 vs. 511 ± 63 μmol/l, P = 0.06; Fig. 5). Glucagon concentrations also did not differ in the two groups after an overnight fast.

Plasma glucose and insulin concentrations during prandial glucose and insulin infusion. Before somatostatin infusion (time 0), postabsorptive serum insulin concentrations were higher in cirrhotic than in control subjects (52 ± 8 vs. 30 ± 4 pmol/l, P < 0.05; Fig. 6). During the prandial insulin infusion, insulin concentrations increased promptly and equally in both groups and reached a smaller second peak at 120 min, simulating a second-phase insulin response. During the 300-min insulin and glucose infusion, peak insulin concentrations and the integrated response above basal did not differ in the two groups.

Despite higher postabsorptive insulin concentrations, basal glucose concentrations did not differ in control and cirrhotic subjects (5.44 ± 0.08 vs. 5.39 ± 0.26 mmol/l, P = 0.86; Fig. 6). During the prandial glucose and insulin infusions, glucose concentration, measured as the integrated glycemic response above basal, was higher in the cirrhotic than in the control subjects (886 ± 227 vs. 275 ± 70 μmol/kg, P < 0.05). Whereas glucose concentrations in the latter returned to baseline levels at ~3 h after initiation of the prandial glucose and insulin infusions, they did not reach baseline levels even after 5 h in five of the cirrhotic subjects.

Glucose turnover during prandial glucose and insulin infusions. Despite higher postabsorptive insulin concentrations, EGR did not differ in control and cirrhotic subjects (11.50 ± 0.50 vs. 11.73 ± 1.00 μmol·kg⁻¹·min⁻¹, P = 0.84; Fig. 7). During the prandial glucose and insulin infusions, EGR was promptly and comparably suppressed in the two groups (793 ± 184 vs. 1,265 ± 273 μmol/kg per 5 h, P = 0.18). Moreover, the ratio of the area below basal of EGR to the area above basal of glucose concentration did not differ (5.6 ± 1.0 vs. 4.0 ± 1.8 ml·kg⁻¹·min⁻¹, P = 0.45), which lends further support to an unaltered ability of glucose and insulin to suppress EGR (Table 2).

Postabsorptive glucose utilization rates (Fig. 8) also did not differ in control and cirrhotic subjects (11.50 ± 0.50 vs. 11.72 ± 1.00 μmol·kg⁻¹·min⁻¹, P = 0.85). During the prandial glucose and insulin infusions, glucose uptake rose comparably in the two groups (2,528 ± 140 vs. 2,667 ± 290
μmol/kg per 5 h, \( P = 0.67 \)). However, the rate of glucose uptake was insufficient at the prevailing glucose concentration in the cirrhotic subjects. This was evident from the calculated glucose clearance rates (4.23 ± 0.43 vs. 2.88 ± 0.19 ml·kg\(^{-1}\)·min\(^{-1}\), \( P < 0.01 \)), which were lower in the cirrhotic subjects, and from the \([3-3H]\)glucose concentrations.

Identical amounts of \([3-3H]\)glucose were infused in the control and cirrhotic subjects. The higher \([3-3H]\)glucose concentration in the latter (666,300 ± 75,500 vs. 303,200 ± 70,900 dpm/ml per 5 h, \( P < 0.01 \)) implies a lower glucose clearance and confirms the presence of an impairment in glucose uptake (Fig. 8).

**DISCUSSION**

The present study demonstrates that a defect in glucose uptake produces marked and sustained hyperglycemia in cirrhotic subjects. Although impaired insulin action has been reported in such subjects (34, 36, 42), the present experiment is, to our knowledge, the first to demonstrate that glucose utilization is abnormal also during physiological glucose and insulin concentrations and that this defect may cause glucose intolerance, despite unaltered rates of glucose production and insulin secretion.

It is well established that cirrhotic subjects are glucose intolerant (23, 29, 36, 42): 60–80% display impaired glucose tolerance, and 10–30% are anticipated to develop diabetes (36). Moreover, cirrhotic patients are insulin resistant (22, 23, 36, 40, 47), which has been demonstrated mainly by the euglycemic and hyperglycemic clamp technique.

Under conditions of daily living, a rise in plasma glucose concentration entails an increase in insulin secretion. The euglycemic glucose clamp was developed to circumvent this feedback loop by assessing insulin action and/or secretion in the presence of fixed, constant glucose concentrations. However, this technique suffers from methodological limitations. It allows assessment of the severity of defects in insulin action and secretion but does not permit determination of the impact of these defects on glucose tolerance. This is not a trivial problem, because insulin resistance may alter the ability of glucose to regulate its own metabolism (19). Moreover, hyperglycemia can compensate for an insulin secretion defect, and its effects on glucose production and utilization in response to a change in glucose and insulin may differ from the response obtained during clamp conditions (16). In particular, the dynamic interaction between insulin secretion and action may be abnormal in cirrhosis, and the hepatic and extrahepatic responses may differ in the presence or absence of physiological glucose and insulin concentrations.

Among the recruited cirrhotic subjects, approximately one-half were glucose intolerant and three were marginally intol-

![Endogenous Glucose Release](image)

![Specific Activity](image)

**Fig. 7.** Endogenous glucose release and \([3-3H]\)glucose specific activity in controls and subjects with liver cirrhosis during prandial glucose and insulin infusions. Prandial glucose and insulin infusion was started at \( t = 0 \).

![Rate of Disappearance](image)

![Glucose Clearance](image)

![[3-3H] Glucose](image)

**Fig. 8.** Rate of disappearance, glucose clearance, and \([3-3H]\)glucose concentration in controls and subjects with liver cirrhosis during prandial glucose and insulin infusions. Prandial glucose and insulin infusion was started at \( t = 0 \).

**Table 2.** Clearance and EGP-to-glucose concentration ratio from 0 to 300 min in control and cirrhotic subjects

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<th></th>
<th>Controls</th>
<th>Cirrhosis</th>
<th>( P )</th>
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<tr>
<td>Clearance</td>
<td>4.23±0.43</td>
<td>2.88±0.19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>EGP/glucose</td>
<td>5.6±1.0</td>
<td>4.0±1.8</td>
<td>0.45</td>
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</table>

Values are means ± SE in ml·kg\(^{-1}\)·min\(^{-1}\). Clearance, rate of glucose clearance from 0 to 300 min i.e. (rate of disappearance ÷ plasma glucose), EGP/glucose, rate from 0 to 300 min calculated as ratio of area below basal of endogenous glucose release (EGR) divided by area above basal of glucose concentration.
erant, reflecting the metabolic heterogeneity of this disease. All individuals had abnormal glucose metabolism: First, FFA concentrations were higher in the cirrhotic than in the control subjects. Second, the postabsorptive and the 2-h insulin and C-peptide concentrations were two- to threefold greater in the former than in the latter. Third, insulin action was impaired, whereas glucose production and gluconeogenesis were unaltered. This was evident with the revised Quicki method (31) and a newly revised minimal model for analyzing OGTT data (6, 12, 13), implying that the presence or absence of glucose intolerance in liver cirrhosis relies on a compensatory rise in β-cell function.

The cirrhotic subjects secreted higher amounts of insulin than the control subjects. Because this occurred in the presence of higher glucose levels, this finding did not permit conclusions concerning the β-cell response to glucose. Hence, a measure of β-cell function capable of accounting for the different time courses of glucose concentration experienced by the two groups was obtained by using the minimal model of β-cell secretion proposed by Breda et al. (6) for OGTT analysis. This model provides an estimate of β-cell response to glucose, Φ, which can be considered a measure of the insulin secretory response normalized to the glucose stimulus. No difference was found between Φ values in the two groups. In conclusion, cirrhotic subjects secrete excessive amounts of insulin because of a prolonged stimulation by hyperglycemia induced by insulin resistance. The present results do not provide evidence for an enhanced β-cell response to glucose in liver cirrhosis, nor do they suggest an exhaustion of β-cell function.

Unaltered rates of postabsorptive glucose output have been reported (7, 11, 34, 36), but the equal rates of postabsorptive gluconeogenesis observed in the present study contrast with previous findings in similar subjects (7, 30, 32). This discrepancy might be attributed to a type 2 error, but this explanation seems unlikely, because equal numbers of patients were recruited in these earlier studies and in our study. In our opinion, it is more likely that the discrepancy is rooted in the difference in the length of the fast. We measured gluconeogenesis after a 12-h fast, whereas the previous studies used a 15- to 18-h fast. The number of cirrhotic patients having had to rely on gluconeogenesis to maintain their glucose production will presumably have risen with the length of the fast, because glycogen levels are lower in cirrhotic than in noncirrhotic subjects (32). Alternatively, the stage of cirrhosis and, consequently, the metabolic derangement in our study may have been different from conditions in earlier studies. In addition, a greater increment in insulin secretion may have suppressed gluconeogenesis, thereby obscuring a potential difference between the two groups.

Although glucose uptake has been shown to be reduced by 40–50% in cirrhotic subjects (11, 20, 37, 42), several studies have demonstrated a normal insulin-induced suppression of hepatic glucose production. Using the dual-isotope method, Kruszynska et al. (23) studied patients with biopsy-proven liver cirrhosis. As in the present study, these subjects were glucose intolerant, despite unaltered suppression of EGR, suggesting a normal hepatic response to glucose and insulin. With use of the glucose clamp technique, impaired insulin action has also been reported in several studies (11, 34, 36). In these studies, basal glucose production rates were unaltered and suppressed comparably in the control and cirrhotic subjects. However, nonoxidative glucose disposal was reduced, thus implying that insulin resistance is attributable to a defect in extrahepatic glycogen synthesis, presumably due to lower muscular glycogen synthase activity (21). Moreover, decreased glucose transport and decreased nonoxidative glucose uptake in skeletal muscle have been demonstrated (40), and Cavallo-Peiri et al. (11) and Taylor et al. (42) reported receptor and postreceptor defects in cirrhotic subjects. A 4-day octreotide infusion triggered a 30–40% reduction in plasma insulin coincident with a normalization of insulin action owing to increased muscle glycogen synthesis (35). Together, these studies suggest that skeletal muscle, not the liver, is the primary site of insulin resistance and that the impaired insulin action may be secondary to a decrease in hepatic insulin extraction (22). This hypothesis, however, remains controversial and needs to be further investigated (26).

In the present experiments, the variable glucose and insulin infusions suppressed EGR promptly and comparably in cirrhotic and control subjects, demonstrating an unaltered response to glucose and insulin (Fig. 7). Of particular interest is that this suppression was achieved in the presence of higher glucose concentrations in the cirrhotic subjects. To correct for this confounder, the suppression rate was assessed by calculating the ratio of the area below basal of EGR to the area above basal of glucose concentration (Table 2). This estimate did not differ in the two groups. Although the present data thus demonstrate that abnormal suppression of EGR during prandial glucose and insulin concentrations is unlikely to contribute to glucose intolerance, an insufficient suppression of EGR by glucose per se, i.e., impaired hepatic glucose effectiveness, cannot be dismissed. Figure 7 suggests that although EGR suppression appears unaltered during the early part of the prandial glucose and insulin infusions, the rate of suppression seems to be inadequate beginning at 90 min, implying an abnormal ability of glucose to suppress EGR. This potential defect in hepatic glucose effectiveness has not previously been reported. Further experiments are required to determine whether the ability of glucose to suppress EGR is abnormal in liver cirrhosis.

During the variable glucose and insulin infusions, glucose uptake also did not differ. However, in the cirrhotic subjects, glucose utilization was insufficient at the prevailing glucose concentration. This defect, demonstrated by a lower glucose clearance in the cirrhotic subjects, was also confirmed by a difference in tracer concentration. Because all subjects were infused with identical amounts of [3-3H]glucose, the tracer concentration will constitute a model-independent assessment of glucose uptake. The presence of higher [3-3H]glucose concentrations is therefore in agreement with the impairment in glucose clearance and confirms a defect in glucose uptake.

Despite higher postabsorptive insulin concentrations, FFA concentrations were higher in the cirrhotic than in the control subjects. According to the “glucose-fatty acid cycle,” FFA may inhibit intracellular glucose oxidation, thereby promoting insulin resistance (38). The presence of elevated postabsorptive FFA concentrations may thus suggest an intrinsic reduction in the antilipolytic effects of insulin. However, during the OGTT, as well as during the prandial glucose and insulin infusions, the decline in FFA was comparable, suggesting a normal suppres-
sion of lipolysis. Moreover, postabsorptive lipid and carbohy-
drate oxidation levels did not differ in the two groups and were 
comparably suppressed/increased during the prandial glucose and insulin infusions. If FFA oxidation induced insulin resis-
tance, a decrease in carbohydrate oxidation would have been 
expected. An increase in Randle cycle activity is therefore un-
likely to contribute to defects in insulin action in cirrhotic subjects.

In conclusion, the present study demonstrates that although insulin resistance is a consistent defect in liver cirrhosis, the severity and pattern of hyperglycemia may differ according to the compensatory increase in β-cell function. Moreover, carbohydrate intolerance is attributable to impaired glucose up-
take and not to an insufficient suppression of EGR by glucose and insulin. This defect was evident, despite the presence of unaltered rates of glucose production and gluconeogenesis; therefore, the data suggest that defects in glucose tolerance can be attributed to impaired glucose utilization without coincident abnormalities in glucose production or insulin secretion. If normoglycemia is desirable, our results suggest that agents improving glucose uptake are likely to produce better glucose tolerance than agents stimulating insulin secretion. They also suggest that agents aiming to improve the hepatic response to insulin are unlikely to improve the glycemic profile in cirrhotic subjects.

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