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

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Abstract

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 therefore studied on two occasions. First, an oral glucose tolerance test (OGTT) was performed. Second, insulin secretion was inhibited and glucose infused in a pattern and amount mimicking the systemic delivery rate of glucose following a carbohydrate meal. Insulin was concurrently infused to mimic a healthy postprandial insulin profile. Post-absorptive 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 FFA (P=0.05) concentrations in the cirrhotic than in the control subjects. Endogenous glucose release (EGR) (11.50±0.50 vs. 11.73±1.00 µmol/kg/min; P=0.84) and the contribution of gluconeogenesis to EGR were unaltered by cirrhosis (6.60±0.47 vs. 6.28±0.64 µmol/kg/min; P=0.70). A minimal model recently developed for the OGTT demonstrated an impaired insulin sensitivity index (P<0.05), while the β-cell responsivity to glucose was unaltered (P=0.72). During the prandial glucose and insulin infusions, the integrated glycemic response was greater in the cirrhotic than in the 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 [3-3H] glucose infusion produced higher tracer concentrations in the cirrhotic than in the control subjects (P<0.05) implying a defect in glucose uptake. In conclusion, carbohydrate intolerance in liver cirrhosis is determined by both insulin resistance and the ability of glucose to stimulate insulin secretion. During the 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 to abnormalities in glucose production or β-cell function. While 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.

**Introduction**

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, insufficient insulin secretion or a combination hereof. In healthy man, 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 that. 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 following 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 glucose 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 physiologic analyses of glucose metabolism 1) a modified
minimal model analysis developed for OGTT data and 2) a prandial glucose and insulin
infusion protocol developed to estimate glucose production and utilization during
conditions resembling those that occur after ingestion of a carbohydrate meal.

Based on minimal model analyses of glucose and C-peptide concentrations,
indexes of β-cell function and insulin sensitivity were calculated using two independent
analytical models (6; 10; 25). In a second set of experiments in the same subjects,
endogenous insulin secretion was inhibited and insulin and glucose 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 following ingestion of 50 g
glucose. Identical prandial insulin concentrations were achieved in the 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 differing insulin concentrations observed during the OGTT. The effect of liver
cirrhosis on gluconeogenesis was assessed by combining the isotope dilution method with
the $^{2}$H$_{2}$O technique (24). Measurements of glucose turnover were performed using the
“hot-GINF” technique to ensure accurate estimates of glucose production and utilization during the infusions.

**Methods**

**Subjects.** After approval from the Research Ethics Committee, the County of Aarhus, 7 Caucasian males with biopsy-verified liver cirrhosis and 7 healthy males matched for age (54.4±3.2 vs. 54.6±4.4 years), BMI (25.3±1.2 vs. 25.9±1.3 kg/m²) 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 weight was measured using bioelectric impedance (Animeter, HTS-Engenering, APS, Odense, Denmark). Study subjects receiving diuretics or other medications known to affect carbohydrate metabolism were instructed to discontinue the medication at least 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 three days prior to study, subjects were instructed not to engage in vigorous exercise.

**Experimental design.** All subjects underwent both a standardized OGTT and participated in a prandial glucose and insulin infusion study (Fig. 1). These studies were performed in random order and separated by at least two weeks. Liver function was assessed using a galactose elimination capacity analysis (GEC) (9; 44; 46).
Oral glucose tolerance test (OGTT). Following an overnight fast, study subjects were admitted to the general clinical research centre at 0800 h. An intravenous cannula was inserted into an antecubital vein for blood sampling and 75 g glucose was ingested over 1 min. Blood samples for determination of plasma glucose, insulin, C-peptide and free fatty acids concentrations were drawn at 0, 15, 30, 45, 60, 90, 120, 150 and 180 min after glucose ingestion. Glucose intolerance was defined according to the American Diabetes Association (ADA) criteria as plasma glucose concentrations at 2-h between 7.8 and 11.1 mmol/l (17).

Galactose elimination capacity (GEC) analysis. The GEC was used as a quantitative measure of liver function. Following an overnight fast, 80 ml of a 50% galactose solution was injected and blood samples were obtained at 5-min intervals from 25 to 60 min after injection. The GEC was determined from blood concentration decay curves corrected for urinary excretion as described by Tygstrup (45). The percentage GEC of the expected normal value per kg total body weight was estimated and used as an index of liver function (Table 1).

Fasting gluconeogenesis, glucose production and prandial glucose and insulin infusion study. Subjects were admitted to the clinical research centre at 2200 h on the evening prior to study. An 18-gauge catheter was inserted into a forearm vein. At 0500, 0600 and 0700 h the subjects drank 1.7 ml of $^{2}$H$_2$O (99.9% H, Cambridge Isotopes Laboratories, Andover, MA, USA) per kg of body water. Body water was calculated to be 60% of total body weight. Water ingested *ad libitum* thereafter was enriched to 0.5% $^{2}$H$_2$O to maintain
isotopic steady state. At 0700 h (-180 min) a prime-continuous infusion of $[3^{-3}\text{H}]$ glucose (17 µCi prime, 0.17 µCi/min continuous) was started and it was continued until study termination. At 0800 h an additional cannula was inserted into a dorsal hand vein for arterialised venous blood sampling. The hand was then placed in a heated Plexiglas box and maintained at a temperature of $\sim 55^0\text{C}$. Post-absorptive glucose turnover and gluconeogenesis were determined from 0930 to 1000 h (-30 to 0 min).

At 1000 h (0 min), an infusion of somatostatin (60 ng/kg/min) was started to inhibit endogenous insulin secretion. Growth hormone (2 ng/kg/min) and glucagon (0.65 ng/kg/min for the first two hours and thereafter 1.3 ng/kg/min) were also infused to maintain basal hormone levels. The glucagon infusion rate was doubled at 120 min following the start of the prandial glucose and insulin infusions in an attempt to simulate the normal prandial rise in glucagon secretion following a carbohydrate meal (8; 14). At time 0, a 5-h pre-programmed variable glucose and insulin infusion was started using separate infusion pumps (model PhD 2000, Harvard Apparatus, South Natick, MA, USA) driven by a PC 2/30 265 computer (IBM, Rochester, MN, USA). Glucose was infused in an amount and pattern mimicking the systemic rate of delivery of glucose following 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 keep constant specific activities, the rate of basal $[3^{-3}\text{H}]$ glucose was adjusted according to the anticipated suppression of EGR during the variable glucose and insulin infusions (-180–0 min, 100% of basal; 0-15 min, 70% of basal; 15-75 min, 30% of basal; 75-120 min, 45% of basal; 120-300 min, 50% of basal). Furthermore, all glucose
infused contained [3-3H] glucose. Hence, glucose-specific activities remained constant in the study groups (Fig 7).

To avoid the confounding effects of counter-regulation, the experiments were terminated when plasma glucose concentrations fell below 4 mmol/l (~70 mg/dl), which happened in 5 control and 2 cirrhotic subjects.

Analysis. Arterialized plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA, USA). Samples for hormone analysis were placed on ice, centrifuged at 4°C, separated and stored at -20°C until assay. Serum insulin was determined by a two-site enzyme-linked immunosorbent assay (2). Plasma glucagon and C-peptide concentrations were measured by radioimmunoassay. Serum-free fatty acids (FFA) were measured enzymatically using a Wako NEFA (non-esterified fatty acid) Test Kit (Wako Chemicals, Neuss, Germany).

The measurement of deuterium enrichment on carbon 2 and 5 in glucose was performed as previously described (24). Briefly, 15 ml blood was diluted with 30 ml demineralised water and deproteinized using 15 ml of 0.3 N ZnSO₄ and 15 ml 0.3 N Ba(OH)₂. The samples were centrifuged at 2000 rpm for 15 min and the pellet diluted in 15 ml demineralised 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 carbon 5 (C5), the glucose was converted to xylose. C5 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 carbon 2 (C2) 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 \([3\text{-}^3\text{H}]\) glucose (dpm/ml) divided by plasma glucose concentration (\(\mu\text{mol/ml}\)), was smoothed according the OOPSEG program as suggested by Bradley et al. (5). Glucose production and utilization were calculated using Steele’s equations for non-steady state (41). The pool correction factor was assumed to be 0.65 ml/kg, the glucose volume of distribution 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 between glucose utilization (Rd) and the corresponding plasma glucose concentration. Post-absorptive gluconeogenesis rates were calculated as the ratio between deuterium bound to C5 and C2 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 post-absorptive state and from 120 to 150 min during the prandial glucose and insulin infusions were calculated using the equations of Frayn et al. (18).

**Assessment of insulin sensitivity**

Insulin sensitivity was calculated using two different approaches, one relying on the post-absorptive measurements of insulin, glucose and FFA concentrations, the other on the
dynamic insulin and glucose data measured during the OGTT. Insulin, glucose and FFA concentrations measured in the post-absorptive 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 (denoted as $SI_{oral}$) from the areas under the measured glucose and insulin concentration curves (12; 13).

Assessment of $\beta$-cell function

$\beta$-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 responsivity (denoted $\Phi$) that measures the ability of the $\beta$-cell to secrete insulin in response to glucose ingestion. It is emphasized that the $\Phi$ index does not reflect insulin secretion $t$out $c$ourt, but rather insulin secretion normalized to the glucose stimulus. $\Phi$ has been shown to be well-approximated by the ratio between the area under the incremental (above basal) C-peptide concentration and the area under the incremental glucose concentration during the OGTT (6).
**Statistical analysis.** Data in the text and figures are expressed as means ±SEM. Glucose appearance, disappearance and clearance rates were 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. Post-absorptive measures were defined as the mean of the values present during the 30 min before the prandial glucose infusion, i.e. from 0930 to 1000 (-30 to 0 min). A non-paired Student’s 2-tailed t-test was used to test for statistical difference. A non-parametric two-sample analysis was used for data not fulfilling the criteria for normal distribution (27). A P-value <0.05 was considered statistically significant.

**Results**

*Galactose elimination capacity 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*

Post-absorptive 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. Following 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 highest in the cirrhotic subjects (Fig. 2 and 3).
Impaired glucose tolerance was present in 3 of the 7 cirrhotic subjects, while 3 were marginally intolerant with 2-h glucose concentrations between 7.1 and 7.4 mmol/l. The cirrhotic subjects were on average glucose intolerant with a 2-h plasma glucose concentration of 8.47±1.03 mmol/l vs. 6.74±0.89 mmol/l in the control subjects.

Post-absorptive FFA concentrations were higher in subjects with liver cirrhosis than in the controls (0.78±0.13 vs. 0.47±0.06 mmol/l; P=0.05), but were suppressed to control levels following 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 the SIoral insulin sensitivity index 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 X 10⁻⁴ dL/kgEmin/µUEmL; P<0.05).

Index of β-cell function
The β-cell responsitivity index to glucose, Φ, was not different in control and cirrhotic subjects (53±22 vs. 44±10 min⁻¹Epmol/L; P=0.72), suggesting that - when corrected for differences in glucose concentration - the β-cell response is unaltered in cirrhotic subjects.
Post-absorptive endogenous glucose release and gluconeogenesis

Post-absorptive glucose production (11.50±0.50 vs. 11.73±1.00 µmol/kg/min; P=0.84) and gluconeogenesis (6.60±0.47 vs. 6.28±0.64 µmol/kg/min; P=0.70) did not differ in controls and cirrhotic subjects although the latter had higher insulin concentrations (Fig. 4). Gluconeogenesis accounted for 57.4±2.9% of production in controls and 54.0±3.9% in cirrhotic subjects.

Substrate and hormone concentrations during prandial glucose and insulin infusions

Before initiation of the prandial glucose and insulin profile, C-peptide concentrations (804±132 vs. 511±63 mmol/l; P=0.06) were higher in cirrhotic than in control subjects (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).

Post-absorptive 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/min; P=0.53) and lipid oxidation (5.77±0.74 vs. 6.34±0.69 µmol/kg/min; P=0.58) did not differ in the two groups following an overnight fast.

During glucose and insulin infusion, FFA concentrations decreased in the cirrhotic subjects to control levels, implying an unaltered suppression of lipolysis. During the prandial glucose and insulin infusions, carbohydrate oxidation was equally increased (18.18±1.48 vs. 14.33±2.18 µmol/kg/min; P=0.21) and lipid oxidation (2.04±0.29 vs. 4.52±1.41 mmol/kg/min; P=0.18) equally decreased in the two groups.
Plasma glucose and insulin concentrations during prandial glucose and insulin infusion

Prior to somatostatin infusion (time 0), post-absorptive 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 followed by 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 post-absorptive insulin concentrations, basal glucose concentrations did not differ in the 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 concentrations 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 level at approximately 3 hours following initiation of the prandial glucose and insulin infusions, they did not reach baseline levels even after 5 hours in five of the cirrhotic subjects.

Glucose turnover during prandial glucose and insulin infusions

Despite higher post-absorptive insulin concentrations, EGR did not differ in controls and cirrhotic subjects (11.50±0.50 vs. 11.73±1.00 µmol/kg/min; P=0.84). During the prandial glucose and insulin infusions, EGR was promptly and comparably suppressed in the two groups (793±184 vs 1265±273 µmol/kg per 5 h; P=0.18). Moreover, the ratio between the area below basal of EGR and the area above basal of glucose concentration did not differ
(5.6±1.0 vs 4.0±1.8 ml/kg/min; P=0.45), which lends further support to an unaltered ability of glucose and insulin to suppress EGR (Table 2).

Post-absorptive glucose utilization rates (Fig. 8) also did not differ in controls and cirrhotic subjects (11.50±0.50 vs 11.72±1.00 µmol/kg/min; P=0.85). During the prandial glucose and insulin infusions, glucose uptake rose comparably in the two groups (2528±140 vs 2667±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 both from the calculated glucose clearance rates (4.23±0.43 vs 2.88±0.19 ml/kg/min; 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 controls and the cirrhotic subjects. The higher [3-³H] glucose concentrations 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. While impaired insulin action has previously 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 physiologic 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, since 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 both the hepatic and extrahepatic response may differ in the presence or absence of physiologic glucose and insulin concentrations.

Among the recruited cirrhotic subjects approximately half were glucose intolerant and three were marginally intolerant, 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, both the post-absorptive and the 2-h insulin and C-peptide concentrations were 2-3-fold greater in the former than in the latter. Finally, insulin action was impaired, while glucose production and gluconeogenesis were
unaltered. This was evident both 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. Since this occurred in the presence of higher glucose levels, this finding did not permit conclusions concerning the β-cell responsivity 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 responsivity to glucose, Φ, that can be considered a measure of the insulin secretory response normalized to the glucose stimulus. No difference was found between the Φ values in the two groups. In conclusion, cirrhotic subjects secrete excessive amounts of insulin due to a prolonged stimulation by hyperglycemia induced by insulin resistance. The present results do not provide evidence for an enhanced β-cell responsivity to glucose in liver cirrhosis, nor do they suggest an exhaustion of β-cell function.

Unaltered rates of post-absorptive glucose output have been reported earlier (7; 11; 34; 36), but the equal rates of post-absorptive 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, since equal numbers of patients were recruited both in these and in our studies. In our opinion it is more likely that the discrepancy is rooted in the difference in the length of the fast. We
measured gluconeogenesis following a 12-h fast, whereas these other studies used a 15-18-h fast. The number of cirrhotic patients having had to rely on gluconeogenesis in order to uphold their glucose production will presumably have risen with the length of the fast, since cirrhotic patients have lower amounts of glycogen than non-cirrhotic subjects (32). Alternatively, the stage of cirrhosis and consequently the metabolic derangement may have been different in our study than in earlier studies. In addition, a greater increment in insulin secretion may have suppressed gluconeogenesis, thereby obscuring a potential difference between the two groups.

While 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. Kruszynska et al. (23) studied patients with biopsy-proven liver cirrhosis using the dual isotope method. As in the present study, these subjects were glucose intolerant despite unaltered suppression of EGR suggesting a normal hepatic response to glucose and insulin. Using the glucose clamp technique impaired insulin action has also been reported in several papers (11; 34; 36). In these studies, basal glucose production rates were unaltered and suppressed comparably in the control and cirrhotic subjects. However, non-oxidative 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 non-oxidative glucose uptake in skeletal muscle has been demonstrated (40), and Cavallo-Perin et al. (11) and Taylor et al. (42) both reported receptor and post-receptor defects in cirrhotic subjects. Noteworthy, a four-day octreotide infusion triggered a 30-40% reduction of plasma insulin coincident with a normalization
of insulin action owing to increased muscle glycogen synthesis (35). Altogether, 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 both 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 between the area below basal of EGR and the area above basal of glucose concentration (Table 2). This estimate did not differ in the two groups. While 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. Fig. 7 suggests that whereas EGR suppression appears unaltered during the early part of the prandial glucose and insulin infusions, the rate of suppression seems to be inadequate from 90 min and onward, implying an abnormal ability of glucose to suppress EGR. This potential defect in hepatic glucose effectiveness has not previously been reported. It will require further experiments 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. Since 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 post-absorptvine 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 post-absorptive 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 suppression of lipolysis. Moreover, post-absorptive lipid and carbohydrate oxidation levels did not differ in the two groups and were comparably suppressed/increased during the prandial glucose and insulin infusions. Noteworthy, if FFA oxidation induced insulin resistance, a decrease in carbohydrate oxidation would have been expected. An increase in Randle cycle activity is therefore unlikely to contribute to defects in insulin action in cirrhotic subjects.

In conclusion, the present study demonstrates that while 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 uptake 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, and the data therefore 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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**Legends to figures.**

Table 1.
Laboratory characteristics of cirrhotic patients.

Table 2.
Mean rates of glucose clearance and rate of endogenous glucose release divided by plasma glucose concentration in controls and subjects with liver cirrhosis during prandial glucose and insulin infusions.
Fig. 1.
Experimental design as described in Materials and Methods.

Fig. 2.
Glucose and insulin concentrations in controls (υ) and subjects with liver cirrhosis (Ο) during oral glucose tolerance test. Glucose was ingested at time 0.

Fig. 3.
Free fatty acid and C-peptide concentrations in controls (υ) and subjects with liver cirrhosis (Ο) during oral glucose tolerance test. Glucose was ingested at time 0.

Fig. 4.
Post-absorptive 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 carbon 5 to that at carbon 2 in glucose by the mean rate of fasting endogenous glucose release.

Fig 5.
Free fatty acid (upper panel) and C-peptide (lower panel) concentrations in controls (υ) and subjects with liver cirrhosis (Ο) during prandial glucose and insulin infusions. The prandial glucose and insulin infusion was started at time 0.

Fig. 6.
Glucose (upper panel), insulin (middle panel) and glucagon (lower panel) concentrations in controls (υ) and subjects with liver cirrhosis (Ο) during prandial glucose and insulin infusions. The prandial glucose and insulin infusion was started at time 0.
Fig 7.
Endogenous glucose release (upper panel) and [3-3H] glucose specific activity (lower panel) in controls (•) and subjects with liver cirrhosis (O) during prandial glucose and insulin infusions. The prandial glucose and insulin infusion was started at time 0.

Fig. 8.
Rate of disappearance (upper panel), glucose clearance (middle panel) and [3-3H] glucose (lower panel) concentrations in controls (•) and subjects with liver cirrhosis (O) during prandial glucose and insulin infusions. The prandial glucose and insulin infusion was started at time 0.


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Quantification of gluconeogenesis in cirrhosis: Response to glucagon.

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Table 1

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hgb</th>
<th>ALAT</th>
<th>Alk.Phos</th>
<th>Bilirubin</th>
<th>Urea</th>
<th>Creatinine</th>
<th>APTT</th>
<th>GEC</th>
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<tbody>
<tr>
<td></td>
<td>mmol/l (8.4-10.8)</td>
<td>U/l (10-40)</td>
<td>U/l (80-270)</td>
<td>µmol/l (&lt;22)</td>
<td>Mmol/l (2.5-7.8)</td>
<td>µmol/l (62-133)</td>
<td>Sec (25-38)</td>
<td>µmol/kg/min</td>
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<tr>
<td>1*</td>
<td>9.0</td>
<td>16</td>
<td>152</td>
<td>10</td>
<td>4.8</td>
<td>92</td>
<td>32</td>
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<tr>
<td>2*</td>
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<td>176</td>
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<td>47</td>
<td>6.8</td>
<td>102</td>
<td>30</td>
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<tr>
<td>3*</td>
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<td>28</td>
<td>434</td>
<td>28</td>
<td>1.6</td>
<td>69</td>
<td>38</td>
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<tr>
<td>4*</td>
<td>7.1</td>
<td>12</td>
<td>141</td>
<td>21</td>
<td>7.8</td>
<td>154</td>
<td>33</td>
<td>15.8</td>
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<tr>
<td>5*</td>
<td>8.7</td>
<td>44</td>
<td>126</td>
<td>21</td>
<td>2.1</td>
<td>74</td>
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<td>22.5</td>
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<tr>
<td>6*</td>
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<td>32</td>
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<td>61</td>
<td>35</td>
<td>19.1</td>
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<tr>
<td>7*</td>
<td>9.1</td>
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<td>186</td>
<td>18</td>
<td>4.3</td>
<td>63</td>
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<tr>
<td>Mean±SEM</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>
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#: Alcoholic cirrhosis

*: Primary biliary cirrhosis
**Table 2**

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

* Mean rate of glucose clearance (ml/kg/min) from 0 to 300 min (Rd / plasma glucose)

** Mean rate (ml/kg/min) from 0 to 300 min calculated as the ratio between the area below basal of EGR divided by the area above basal of glucose concentration.
EXPERIMENTAL DESIGN

7 Healthy subjects
7 Subjects with liver cirrhosis  Suplemental Glucagon

Prandial Glucose Infusion
Prandial Insulin Infusion

$\textsuperscript{2}$H$_2$O: 1.7 ml/kg
Somatostatin
Glucagon, Growth Hormone

$[3-\textsuperscript{3}H]$ glucose

0500 0600 0700 1000 1200 1500
(0 min) (120 min) (300 min)

Fig 1.
Fig 2.
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 8