Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis

KITT FALK PETERSEN,1 MARTIN KRSSAK,1 VICTOR NAVARRO,1 VISVANATHAN CHANDRAMOULI,1 RIPUDAMAN HUNDAL,1 WILLIAM C. SCHUMANN,2 BERNARD R. LANDAU,2 AND GERALD I. SHULMAN1,3
1Department of Internal Medicine, and the 2Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut 06520-8020; and 2Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, Ohio 44106-4951

Petersen, Kitt Falk, Martin Krssak, Victor Navarro, Visvanathan Chandramouli, Ripudaman Hundal, William C. Schumann, Bernard R. Landau, and Gerald I. Shulman. Contributions of net hepatic glycogenolysis and gluconeogenesis to glucose production in cirrhosis. Am. J. Physiol. 276 (Endocrinol. Metab. 39): E529–E535, 1999.—Net hepatic glycogenolysis and gluconeogenesis were examined in normal (n = 4) and cirrhotic (n = 8) subjects using two independent methods [13C nuclear magnetic resonance spectroscopy (NMR) and a 2H2O method]. Rates of net hepatic glycogenolysis were calculated by the change in hepatic glycogen content before (−11:00 PM) and after (−7:00 AM) an overnight fast using 13C NMR and magnetic resonance imaging. Gluconeogenesis was calculated as the difference between the rates of glucose production determined with an infusion of [6,6-2H2]glucose and net hepatic glycogenolysis. In addition, the contribution of glycogenolysis to glucose production was determined by the 2H enrichment in C-5/C-2 of blood glucose after intake of 2H2O (5 ml/kg body weight). Plasma levels of total and free insulin-like growth factor I (IGF-I) and IGF-I binding proteins-1 and -3 were significantly increased in the cirrhotic subjects (P < 0.01 vs. controls). Postprandial hepatic glycogen concentrations were 34% lower in the cirrhotic subjects (P = 0.007). Rates of glucose production were similar between the cirrhotic and healthy subjects [9.0 ± 0.9 and 10.0 ± 0.8 µmol·kg body wt−1·min−1, respectively]. Net hepatic glycogenolysis was 3.5-fold lower in the cirrhotic subjects (P = 0.01) and accounted for only 13 ± 6% of glucose production compared with 40 ± 10% (P = 0.03) in the control subjects. Gluconeogenesis was markedly increased in the cirrhotic subjects and accounted for 87 ± 6% of glucose production vs. controls: 60 ± 10% (P = 0.03). Net hepatic glycogenolysis was 3.5-fold lower in the cirrhotic subjects (P = 0.01) and accounted for only 13 ± 6% of glucose production compared with 40 ± 10% (P = 0.03) in the control subjects. Gluconeogenesis was markedly increased in the cirrhotic subjects and accounted for 87 ± 6% of glucose production vs. controls: 60 ± 10% (P = 0.03). Gluconeogenesis was calculated by multiplying those rates of net hepatic glycogenolysis from the rates of glucose production determined using [6,6-2H2]glucose. From the ratios of the 2H enrichments of the hydrogen bound to C-5 and C-2 of blood glucose after oral intake of 2H2O (21), the fractional contributions of gluconeogenesis to rates of glucose production were determined. Rates of gluconeogenesis were calculated by multiplying those ratios by the rates of glucose production.

METHODS

Subjects. Eight subjects with biopsy-confirmed cirrhosis of the liver (5 men and 3 women, age: 43 ± 1 yr, wt: 86 ± 6 kg, body mass index: 27 ± 1 kg/m2) were studied. All patients had hepatitis C [except 1 patient who had cryptogenic (micronodular) cirrhosis]. All were in stable condition, without signs of encephalopathy or peripheral edema; two had minimal ascites as confirmed by the abdominal magnetic resonance imaging (MRI) examination. Two patients had experienced esophageal variceal bleeding episodes more than three years before the study. The control group consisted of four lean, healthy volunteers matched for age and body weight (3 men and 1 woman, age: 38 ± 4 yr, wt: 82 ± 6 kg, body mass index: 25 ± 1 kg/m2). All study participants were instructed to eat a diet containing ~150 g carbohydrate/day and to abstain from glucose production have been reported to be both normal (5, 32, 33, 45) and decreased (13, 26, 29, 36) in patients with liver cirrhosis. Glycogen metabolism has also been suggested to be altered in patients with liver cirrhosis, but, due to the invasiveness of the liver biopsy technique, this conclusion is based on limited biopsy data. Splanchnic balance studies have found that amino acid and lactate uptake is increased in cirrhotic subjects, accounting for ~90% of splanchnic glucose production, which suggests gluconeogenesis is increased in patients with liver cirrhosis, but this approach is indirect and relies on assumptions in regard to the intrahepatic fate of the C-3 precursors. In contrast, recent studies using [14C]glycerol found no differences in 14C incorporation into glucose, suggesting that gluconeogenesis is not altered in patients with liver cirrhosis. However, this method is also indirect and only quantifies gluconeogenesis from glycerol, which under most circumstances is a minor gluconeogenic precursor (18).

To address these questions, we applied two independent methods to assess gluconeogenesis in normal and cirrhotic subjects after an overnight fast. 13C nuclear magnetic resonance (NMR) spectroscopy was used to measure directly hepatic glycogen content and hence rates of net hepatic glycogenolysis (40). Rates of gluconeogenesis were estimated by subtracting the rates of net hepatic glycogenolysis from the rates of glucose production determined using [6,6-2H2]glucose. From the ratios of the 2H enrichments of the hydrogen bound to C-5 and C-2 of blood glucose after oral intake of 2H2O (21), the fractional contributions of gluconeogenesis to rates of glucose production were determined. Rates of gluconeogenesis were calculated by multiplying those ratios by the rates of glucose production.

ALTHOUGH HEPATIC GLUCOSE metabolism is generally agreed to be altered in patients with chronic liver failure, the specific alterations remain unclear. Rates of

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alcohol, caffeine, and exercise except for normal daily activities for 3 days before the study. The protocol was approved by the Yale University Human Investigation Committee, and informed consent was obtained from each subject.

Study protocol. On the day of the study, the subjects were admitted to the Yale University/New Haven Hospital General Clinical Research Center (GCRC) between 3:00 and 4:00 PM. At 5:00 PM (time (t) = 0 h), dinner in the form of a liquid meal (450 ml) consisting of 1,000 kcal [60% carbohydrate (80% of which was in the form of free glucose), 20% protein, and 20% fat] was ingested over a 10-min period. At 9:00 PM, the subjects were brought to the Magnetic Resonance Center (MRC) and were placed in a 2.1-T NMR spectrometer (Bruker Biospec Spectrometer, Billerica, MA), and liver glycogen concentration was measured continuously with 13C NMR spectroscopy from 10:00 to 11:00 PM (t = 5–6 h). Hereafter, the subjects were brought back to the GCRC and given a total of 5 ml/kg body water of 2H2O (99.9% 2H; Isotec, Miamisburg, OH) to drink. The volume was divided into three portions of equal size and was given at 11:45 PM (t = 6 h and 45 min), 12:45 AM (t = 7 h and 45 min), and 1:30 AM (t = 8 h and 30 min) to minimize discomfort such as light dizziness or nausea, which has been reported (20). Water ingested ad libitum during the fast was enriched 0.5% with 2H2O to maintain isotopic steady state. From 6:00 AM to 7:00 AM (t = 13–14 h) the 13C NMR measurements of hepatic glycogen concentration were repeated, whereafter the subjects were brought back to the GCRC. To avoid physical activity during the study, the subjects were transported in a wheeled chair between the MRC and the GCRC. In the GCRC, all subjects remained in bed at all times. A catheter was placed retrogradely in a hand vein for blood collection. This hand was kept in a hot box at 70°C to arterIALIZ the blood samples, and basal blood samples were collected. After placement of an antecubital intravenous catheter in the contralateral arm for infusion, a primed (275 μmol/m2) continuous (4.89 μmol/(m2·min)−1) infusion of [6,6-2H2]glucose (Cambridge Isotopes Laboratories, Andover, MA) was begun and continued for 240 min (7:30 to 11:30 AM) for determination of rates of glucose production. After 200 min of infusion, blood samples were taken every 10 min for the next 40 min of infusion for determination of [6,6-2H2]glucose (m + 2) enrichment. Blood was collected at 0 min (14.5-h fast), 60 min (15.5-h fast), 180 min (17.5-h fast), and 240 min (18.5-h fast) for determination of 2H enrichment of the hydrogens bound to C-2 and C-5 of blood glucose and at 240 min for determination of 2H enrichment in body water. After the last blood sample, the intravenous lines were removed, and the subjects were given a regular meal.

The volume of the liver was measured upon admission after ingestion of the meal by clinical imaging on a 1.5-T magnet (Signa II; General Electric, Milwaukee, WI) using multiecho axial scanning (TE 20/80, TR 2000). In two healthy subjects, MRI of liver volume was performed 2 days after the study. The data were transferred to an independent workstation (IGS, Toronto, Canada) and processed with the CAMRA S200 Program (IGS) that provided three-dimensional image reconstruction and volume determinations. Accuracy of the measurement was assessed with water-filled phantoms of known volume and was determined to be ±5% with a coefficient of variation of ±2.5%.

To assess glucose tolerance to the mixed meal, the cirrhotic subjects were admitted to the GCRC on a separate day after an overnight fast. A liquid mixed meal identical to the one given during the initial study (450 ml, 1,000 kcal [60% carbohydrate (80% in the form of free glucose), 20% protein, and 20% fat]) was given, and blood was collected every 30 min (t = 0–300 min) for determination of plasma glucose concentrations.

13C NMR spectroscopy. The subjects were placed in the supine position in a 2.1-T Bruker Biospec Spectrometer, and, from 10:00 PM to 11:00 PM (5–6 h into the fast) and from 6:00 AM to 7:00 AM (13–14 h into the fast), 13C NMR spectra were obtained with a 9-cm circular 13C observation coil, and a 12 × 14-cm coplanar butterfly 1H decoupler coil was placed rigidly over the lateral aspect of the abdomen as previously described (9). In brief, initial coil placement was determined by percussion of the borders of the liver, and the position of the coil over the liver was confirmed by imaging the liver from the surface coil with a multislice gradient echo image. The magnet was shimmed with the water signal obtained from the decoupling coil. Localized 13C NMR liver spectra were obtained with a modified one-dimensional inversion-based sequence for surface suppression. On the basis of 1H density profiles of the sequence, <15% of the glycogen signal could arise from tissues within 2.5 cm of the surface of the subject, assuming equal glycogen concentration throughout the volume of observation. In each subject, all skin and muscle was within 2.5 cm of the surface as assessed by the image. Heating from decoupling radio frequency calculated with a magnetic vector potential model was <4 watts/kg of tissue, which is below Food and Drug Administration limits for local heating. Spectra were processed with a mild 30-Hz Lorentzian to Gaussian filter and a 500-Hz convolution difference. After processing, the glycogen line width was 70–90 Hz. Resonance intensity was measured by integrating over a bandwidth of ±120 Hz. The glycogen concentration of liver was determined by comparing the signal intensity obtained with the localized spectroscopy sequence in vivo with the signal intensity obtained from a volume-selected rectangular phantom containing a solution of 50 mmol/l KCl and 150 mmol/l glycogen at the same coordinates relative to the coil. The phantom was raised 2.5 cm above the surface of the coil to simulate liver depth. Spectrometer sensitivity was normalized between phantom and in vivo measurements with the signal from the formate sphere as a calibration standard. Resonances from the C-1 glycosyl units in liver glycogen have been shown to be ±100% visible by this method in vivo (9). Reproducibility of the glycogen concentration measurement was assessed in an earlier study with a coefficient of variation between the pairs of measurements of 7%. The standard deviation in the 13C NMR glycogen concentration measurement due to spectral noise was ±5% (40).

2H2O. Body water was assumed to be 50% of body weight in women and 60% in men (46).

Analyses. Plasma glucose concentrations were measured by the glucose oxidase method (Glucose Analyzer II; Beckman Instruments, Palo Alto, CA). Plasma lactate concentrations were measured using commercial double-antibody radiommunoassay kits (insulin: Diagnostic Systems Laboratories, Webster, TX; glucagon: Linco Research, St. Charles, MO; cortisol: Diagnostic Product, Los Angeles, CA). Plasma triglyceride and free fatty acid (FFA) concentrations were determined using a microfluorometric method (27).

Plasma concentrations of total and free insulin-like growth factor (IGF)-1 as well as binding proteins 1 and 3 were measured using commercial kits (Nichol’s Diagnostic Institute, San Juan Capistrano, CA).

Enrichments of the hydrogens at C-2 and C-5 of blood glucose were determined as previously described (21). Protein was precipitated from the blood by the addition of 0.3 N
ZnSO₄ and 0.3 N Ba(OH)₂, and the mixture was centrifuged. The supernatant was deionized by passage through a column of anion and cation exchange resins, and glucose in the effluent was isolated using HPLC. An aliquot of the glucose was converted to xylose, and the xylose was oxidized with periodate to yield C-5 of glucose with its hydrogen in formaldehyde. Hexamethylenetetramine (HMT) was prepared from the formaldehyde and was assayed for mass m + 1 (14, 20, 21). Another aliquot of the glucose was converted to ribulose-5-phosphate (P), which was reduced to a mixture of ribitol-5-P and arabitol-5-P. They were also oxidized with periodate, yielding formaldehyde containing C-2 with its hydrogen. Again, the formaldehyde was condensed to form an HMT, which was assayed for mass (m + 1). In each subject, enrichments of C-2 and C-5 of glucose from blood were determined four times (14.5, 15.5, 17.5, and 18.5 h). The enrichment at C-2 and C-5 at 18.5 h of fasting was used in the calculations. Enrichments in body water was 100 ± 2% in the control and 94 ± 2% in the cirrhotic subjects of the enrichments at C-2 of glucose from blood collected at 18.5 h of fasting. Enrichments of plasma were determined using an isotope ratio mass spectrometer at Metabolic Solutions (Merrimack, NH). Plasma water weight was assumed to be 94% of plasma volume (6).

Plasma glucose collected at 10-min intervals beginning 200 min after the start of the [6,6-2H₂]glucose infusion was also oxidized with periodate. The formaldehyde formed, which contained C-6 of the glucose with its hydrogen, was converted into HMT, which was assayed for mass m + 2. Masses were five or more times natural abundance. HMTs from [6,6-2H₂]glucose of 0.25, 0.50, 0.75, 1.00, 1.50, 2, and 3% enrichments provided a standard curve. When subjects were given [6,6-2H₂]glucose, but not 2H₂O, no detectable increase above natural abundance was found in m + 1 of HMTs from C-2 and C-5 of blood glucose (2).

Calculations. Rates of glucose production (µmol·kg body wt⁻¹·min⁻¹) were calculated by dividing the tracer infusion rate times the tracer enrichment by the average percent enrichment of plasma [6,6-2H₂]glucose and subtracting the tracer infusion rate. The m + 2 enrichment was stable during the sampling period with a mean coefficient of variance of 3% (2).

¹³C NMR spectroscopy. Rates of net hepatic glycogenolysis were calculated by the slope of the decrease in the liver glycogen concentration mmol/l liver from the maximum liver glycogen content ~5–6 h after ingestion of the meal to the concentration measured in the morning after ~14 h of fast. This slope was multiplied by the liver volume (liter) and was divided by body weight (kg). The NMR-determined rate of glycogenolysis was calculated as the difference between the rates of glucose production and net glycogenolysis.

²H₂O method. The fraction of glucose production from gluconeogenesis was set equal to the ratio of the enrichment of the hydrogens bound to C-5 and to C-2 of blood glucose after oral ²H₂O (5 ml/kg body water). Percent gluconeogenesis equals 100 times that fraction. The rate of gluconeogenesis was calculated by multiplying that ratio by the rate of glucose production.

Statistics. Data are given as means ± SE. Differences between groups were assessed with the nonparametric Mann-Whitney test.

RESULTS

Fasting plasma concentrations of glucose, insulin, glucagon, cortisol, and FFA are shown in Table 1. Plasma concentrations of glucose, lactate, and cortisol were similar between the two groups, whereas plasma FFA (P < 0.01), insulin (P < 0.01), and glucagon (P < 0.01) concentrations were two- to threefold higher in the cirrhotic subjects. Plasma concentrations of total and free IGF-I were fourfold lower (25%) in the cirrhotic subjects compared with the concentrations in the control subjects (P < 0.01; Table 2). The plasma concentrations of IGF-I binding protein-3 in the cirrhotic subjects were ~50% lower than in the control subjects (P < 0.01), whereas the concentrations of binding protein-1 were twofold increased in the cirrhotic subjects compared with the control subjects (P = 0.02; Table 2).

On a separate occasion, blood was collected before and after meal ingestion of the meal in six of the cirrhotic subjects to test their glucose tolerance to this large liquid meal. Plasma glucose concentrations before and after the liquid meal were 5.4 ± 0.2 mmol/l; 30 min: 8.9 ± 0.4; 1 h: 7.2 ± 0.7; 2 h: 7.7 ± 0.7; 3 h: 7.7 ± 0.8; 4 h: 7.3 ± 0.9; and 5 h: 6.0 ± 0.6 mmol/l (n = 6).

The liver glycogen concentration 5–6 h after the evening meal (10:00–11:00 PM) was significantly reduced in the cirrhotic subjects (226 ± 16 mmol/l liver compared with the healthy control subjects (343 ± 17 mmol/l liver; P = 0.007; Fig. 1.A). The mean liver volume as measured by MRI was similar in the two groups of subjects (cirrhotic subjects: 1.62 ± 0.21 liters vs. healthy subjects: 1.62 ± 0.21 liters), although there was a wider range of liver volumes in the cirrhotic subjects.

Table 1. Plasma hormone and metabolite concentrations in normal and cirrhotic subjects

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Glucose, mmol/l</th>
<th>Lactate, mmol/l</th>
<th>Insulin, pmol/l</th>
<th>Glucagon, pg/ml</th>
<th>Cortisol, nmol/l</th>
<th>Free Fatty Acids, µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>4</td>
<td>5.21 ± 0.17</td>
<td>0.60 ± 0.08</td>
<td>48 ± 6</td>
<td>42 ± 2</td>
<td>545 ± 52</td>
<td>433 ± 25</td>
</tr>
<tr>
<td>Cirrhotic subjects</td>
<td>8</td>
<td>5.04 ± 0.28</td>
<td>0.67 ± 0.10</td>
<td>120 ± 24</td>
<td>112 ± 16</td>
<td>557 ± 97</td>
<td>850 ± 24</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>NS</td>
<td>NS</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>NS</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. NS, not significant.

Table 2. Plasma concentrations of total and free (unbound) IGF-I as well as the IGF-I binding proteins 1 and 3 of subjects with liver cirrhosis and healthy control subjects after an overnight fast

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total IGF-I, ng/ml</th>
<th>Free IGF-I, ng/ml</th>
<th>BP-1, ng/ml</th>
<th>BP-3, ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>4</td>
<td>242 ± 13</td>
<td>0.79 ± 0.11</td>
<td>30 ± 6</td>
<td>3,072 ± 201</td>
</tr>
<tr>
<td>Cirrhotic subjects</td>
<td>8</td>
<td>60 ± 16</td>
<td>0.20 ± 0.03</td>
<td>62 ± 6</td>
<td>1,429 ± 280</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. IGF-I, insulin-like growth factor 1; BP, binding protein.
(0.95–2.79 liters) than in the control group (1.23–2.28 liters). Total hepatic glycogen content, calculated as the product of hepatic glycogen concentration and the MRI-determined liver volume, was significantly higher in the control group (548 ± 55 mmol) than in the cirrhotic group (364 ± 47 mmol; P = 0.06).

During the overnight fast, the rate of net hepatic glycogenolysis, calculated as the product of the net hepatic glycogen content during the overnight fast (mmol/l liver) and the MRI-determined liver volume (liter), was only 0.058 ± 0.020 mmol·l liver⁻¹·min⁻¹ in the cirrhotic subjects, which was approximately one-third the rate in the control subjects (0.193 ± 0.027 mmol·l liver⁻¹·min⁻¹; P = 0.01). Hepatic glycogen content 13–14 h after the meal (Fig. 1A) was 246 ± 29 mmol/l in the control and 198 ± 15 mmol/l in the cirrhotic subjects (P = 0.17).

Glucose production as determined by [6,6-2H₂]glucose was similar in the two groups of subjects as recorded in Table 3. The rate of gluconeogenesis for each group of subjects as determined by the difference between rates of glucose production and the 13C NMR-determined rates of net hepatic glycogenolysis expressed per kilogram body weight are shown in Table 3. The relative contribution of gluconeogenesis to overall rates of glucose production was significantly increased compared with the healthy control subjects, whereas the absolute rates of gluconeogenesis tended to be higher in the cirrhotic than in the control subjects.

As determined by the 3H₂O method from the 2H₂O enrichment at C-5 to C-2 (Fig. 1B), the percent contribution of gluconeogenesis to glucose production was also significantly higher at 18.5 h of fast (11:30 AM) in the cirrhotic (68 ± 3%) than in the control subjects (54 ± 2%; P = 0.02). There tended to be a small, gradual increase in the contribution over the 4 h of blood collection (from 7:30 to 11:30 AM; Fig. 1B). The absolute rates of gluconeogenesis, calculated by multiplying the percent gluconeogenesis at 18.5 h by the rate of glucose production, tended to be higher in the cirrhotic subjects (6.2 ± 0.8 µmol·kg body wt⁻¹·min⁻¹) compared with the control subjects (5.3 ± 0.4 µmol·kg body wt⁻¹·min⁻¹) but did not reach statistical significance (P = 0.19). The percent contribution of gluconeogenesis calculated from the ratio of the enrichment at C-5 to that in body water (2, 21) was 72 ± 4% in the cirrhotic subjects and again 54 ± 2% in the control subjects (P = 0.008). The absolute rate of gluconeogenesis was then 6.5 ± 0.7 µmol·kg⁻¹·min⁻¹ in the cirrhotic subjects and again 5.3 ± 0.3 µmol·kg⁻¹·min⁻¹ in the control subjects (P = 0.19).

**DISCUSSION**

There was a marked difference between the hepatic glycogen concentrations in the cirrhotic subjects and the healthy control subjects, despite following a similar
dietary regimen for 3 days before the study and an identical evening meal on the day of the study. Hepatic glycogen concentrations in the cirrhotic subjects were 34% lower than in the control subjects at 11:00 PM when glycogen stores typically reach the maximum concentration (12, 31). The low hepatic glycogen content may be due to a net loss of functioning hepatocytes that are replaced by fibrosis and malfunctioning immature hepatocytes, and/or it may be due to portosystemic shunting so that absorbed carbohydrates from the gut are disposed of peripherally. Alternatively, low hepatic glycogen content could also be due to the chronically elevated glucagon levels that were observed in the cirrhotic subjects and that have also been observed previously (44). After the overnight fast, the hepatic glycogen concentrations in the cirrhotic subjects were similar to the fasting glycogen levels in the normal subjects. There are no earlier reports of liver glycogen content in cirrhotic subjects in the fed state. However, hepatic glycogen concentrations were found to be lower in liver biopsies from eight subjects with liver cirrhosis (143 ± 20 mmol/l liver) compared with two overnight-fasted healthy subjects (178 and 267 mmol/l liver; see Ref. 29). Handling of the biopsy tissue requires rapid sampling and freezing to minimize glycogen degradation that occurs during and after removing the liver tissue. Furthermore, sampling error due to the small size of the liver biopsy is likely to be substantial in situations with unequal distribution of glycogen, which is likely in liver cirrhosis. These sources of error may explain why the fasting glycogen content in the healthy and cirrhotic subjects was lower in the previous study than in the present study. After the overnight fast, hepatic glycogen stores changed minimally in the cirrhotic subjects, resulting in rates of net hepatic glycogenolysis that were 66% lower than in the control subjects.

Glucose production measured after 18.5 h of fasting was similar in the control and cirrhotic subjects, which is consistent with most (5, 16, 17, 32–34, 39, 45) but not all previous studies in which glucose production was found to be decreased (13, 26, 29, 36). Cirrhosis is a heterogenous disease with many causes (alcoholic, viral, biliary, etc.), and it is possible that the type and staging of chronic liver failure may contribute to these differences.

We found the relative contribution of gluconeogenesis to glucose production during the overnight fast to be similar by both methods in the control subjects (2H2O method: 54 ± 2%, 13C NMR: 60 ± 10%) and increased in the cirrhotic subjects (2H2O method: 68 ± 3%, 13C NMR: 87 ± 5%). Although the relative contribution of gluconeogenesis to glucose production was higher in the cirrhotic subjects by the 13C NMR method compared with the 2H2O method, these methods measure different processes.

The 13C NMR technique provides a direct measure of hepatic glycogen content and rate of net hepatic glycogenolysis during the fast and demonstrated a markedly lower rate of net hepatic glycogenolysis in the cirrhotic subjects. The rate of glycogenolysis estimated using 13C NMR is the average rate over the 8-h period. The calculated rate of gluconeogenesis is also the average then for that period. However, that calculation depends on the measurement of glucose production hours later. Glucose production may remain the same or decrease during those 4 h (2, 31). Therefore, the contribution of gluconeogenesis would be expected to have been the same or somewhat more, if glucose production had been measured during the 8-h period between the first and the second 13C NMR measurement.

On the other hand, the C-5/C-2 ratio in blood glucose provides a direct measure of the contribution of gluconeogenesis to glucose production not only at the time of blood collection but also glucose formed at earlier times. Percent gluconeogenesis may be underestimated to the extent that the relative contribution of gluconeogenesis to glucose production increases during the study. However, because the increase was only a few percent per hour, at the rate of glucose production, this underestimate is likely to be small. Gluconeogenesis using the C-5/C-2 ratio will be overestimated to the extent glucose-6-P before its conversion to glucose 1) undergoes triose phosphate cycling (glycogen → glucose-6-P → triose phosphate → glucose-4-P → glucose), 2) is metabolized in the pentose cycle (23), or 3) experiences the transaldolase exchange reactions (22). Triose phosphate cycling results in an overestimation of 2–3% as evidenced by the 3H bound to C-1 and C-6 of blood glucose produced in normal subjects fasted overnight and infused with [1-3H]galactose (Landau, unpublished observation). A similar percent overestimate may be due to pentose cycle activity (23, 25). The contribution of the transaldolase reaction is also likely to be small but is yet to be quantitated. Evidence that the sum of these contributions is small is the similar estimates by the two methods of the contributions to glucose production of gluconeogenesis in the normal subjects. Triose phosphate cycling, pentose cycle, and transaldolase reactions do not affect estimates by the NMR method.

With the use of the C-5/C-2 ratio, equilibration between glucose-6-P and fructose-6-P is assumed to be so rapid that the hydrogen bound to C-2 of glucose released into the blood has the enrichment of body water (24). Similar enrichments at C-2 and in body water at 18.5 h of fasting supports that assumption. Enrichment at C-2 has also been found to be 90% or more of that in body water in other studies in humans (4, 13, 24). In accord with that: 1) when hepatocytes from fasted rats were incubated with lactate, pyruvate, and 3H2O, label of the hydrogen C-2 of the glucose formed was ~100% of that in the water (38) and 2) when hepatocytes were incubated with glucose in the presence of 3H2O or 3H2O and when rats were given 3H2O and glucose, the hydrogen at C-2 of glucose from the glycogen that formed was ~90% labeled (19, 35, 49).

In normal subjects fasted overnight, only 80% of 3H of [2-3H]galactose was removed in its conversion to glucose (48). However, there is an isotope effect in the deamination of [2-3H]glucose-6-P (15, 49).

The reason for the apparently greater contribution of gluconeogenesis to glucose production, estimated by...
the $^{13}$C NMR rather than the $^2$H$_2$O method, most clearly evident in the cirrhotic subjects, is uncertain. To the extent glycogen cycling occurs (10, 24, 37), the $^2$H$_2$O method gives an underestimate of the contribution. That is because [2-$^2$H]glucose would be released from glycogen while [2,5-$^2$H]glycogen would be formed. However, estimates were made only beginning 6 h after the end of the $^2$H$_2$O ingestion. With time after ingestion, the effect of glycogen cycling diminishes, because glucose released from the glycogen then becomes increasingly 2,5-$^2$H$_2$ labeled. Glycogen cycling has no effect on the $^{13}$C NMR measurements, which measure net changes in hepatic glycogen content.

Hepatic glycogen cycling can be extensive under certain hyperinsulinenic (24, 30) and hyperglucagonemic conditions (37). Although cycling is undetectable in healthy humans after an overnight fast (30), the hyperglucagonemic conditions in the cirrhotic subjects possibly promoted cycling. This could explain the better agreement in the estimates of the contribution of gluconeogenesis between the two methods in the control than in the cirrhotic subjects. Glucose production by both liver and kidney is measured using [6,6-$^2$H$_2$]glucose, and the total glycogen content of the kidney is minimal compared with the liver. Therefore, differences in estimates by the two methods are unlikely to be due to renal metabolism.

The contribution of gluconeogenesis as determined by $^{13}$C NMR spectroscopy assumes that the carbohydrate of the meal was absorbed into the blood between the ingestion of the meal and the first $^{13}$C NMR measurement of hepatic glycogen content; therefore, a liquid meal was given to favor rapid absorption (31). Also, there was no significant difference between plasma glucose concentration in the cirrhotic subjects at the time of meal intake and the time the measurement of hepatic glycogen content was initiated, i.e., 5 h. Still, if only 1.3 g glucose from the meal were absorbed per hour during the 8-h period, the estimate of gluconeogenesis in the cirrhotic subjects would decrease from a mean of $\sim$87 to 71%. That rate of absorption would not be expected to be increasing the plasma glucose concentration above the fasting level. A plasma concentration 1 mmol/l higher at 5 h than before the meal, assuming a distribution of glucose in body water of 20 liters, would mean the "storing" of 3.6 g of glucose from the meal in that space. Return to the premeal glucose concentration during the 8-h period would reduce the estimate of the contribution of gluconeogenesis by $\sim$5%.

Chronically elevated plasma concentrations of both glucagon and FFA might contribute to the relative increase in gluconeogenesis that was observed in the cirrhotic subjects. Consistent with this possibility are the data obtained by Käller et al. (17), who demonstrated a greater reduction in net splanchnic glucose production in cirrhotic subjects than in control subjects when somatostatin was used to suppress plasma glucagon concentrations. Similarly increased concentrations of FFA have been reported to increase hepatic gluconeogenesis in human subjects (3, 47), possibly by increasing intracellular acetyl-CoA concentrations and thereby activating pyruvate carboxylase (1).

Another factor that might contribute to the higher relative gluconeogenesis in the cirrhotic subjects is the lower plasma concentration of IGF-I (total and free IGF-I) in subjects with liver cirrhosis. The lower concentration of IGF-I can be attributed to a diminished hepatic synthesis, consistent with earlier observations (28, 42, 43). Although the implications of IGF-I deficiency are not fully understood, possibly lower levels of free IGF-I could contribute to the catabolic state in cirrhotic subjects by promoting muscle proteolysis, which in turn might provide more amino acids to the liver for gluconeogenesis (7, 8, 41).

In summary, we examined hepatic glycogenolysis and gluconeogenesis using two independent methods and found that cirrhotic subjects have increased contributions of gluconeogenesis and decreased contributions of net hepatic glycogenolysis to glucose production compared with control subjects. These alterations are likely important contributing factors to their altered carbohydrate metabolism and may at least in part be attributed to chronically increased plasma glucagon and FFA concentrations and/or decreased plasma IGF-I levels.

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Address for correspondence and reprint requests: K. Falk Petersen, Yale Univ. School of Medicine, Dept. of Internal Medicine, PO Box 208020, New Haven, CT 06520-8020 (E-mail: Kitti.Petersen@Yale.edu).

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