We used isotope dilution techniques (constant intravenous [IV] infusion of 2-3H-glycerol and 1-14C-palmitate) and indirect calorimetry to measure lipid kinetics and substrate oxidation rates during IV fructose administration at 200 and then 500 mg/kg/h in eight cirrhotic patients and seven normal control subjects. Fasting plasma glucose, glycerol, and glycerol appearance rate (Ra) were similar in both groups, but insulin levels were fourfold higher in cirrhotics (P < .01). Fasting serum nonesterified fatty acid (NEFA) levels (cirrhotics, 869 ± 124, controls, 717 ± 90 μmol/L) and NEFA Ra (7.1 ± 0.8 v 5.5 ± 0.9 μmol/min/kg) were higher in cirrhosis, but the differences were not significant. Plasma fructose was similar in both groups at both fructose infusion rates. Fructose appeared to stimulate insulin secretion. With IV fructose, serum NEFA levels decreased, reaching similar low levels when 500 mg/kg/h was infused, due to a reduction in NEFA Ra and an increase in the NEFA metabolic clearance rate (MCR). Glycerol levels showed little change. As glycerol Ra decreased by less than 20% in both groups, the decrease in serum NEFA was primarily due to enhanced reesterification of fatty acids both within adipose tissue (preventing their release) and in other tissues (enhancing their removal from plasma). Although total fructose utilization was normal in cirrhosis, they oxidized more of the infused fructose; nonoxidative disposal was reduced (first step, 242 ± 12 v 318 ± 16 mg/kg in 2 hours, P < .002; second step, 657 ± 32 v 786 ± 21 mg/kg in 2 hours, P < .005). Although tissue fructose uptake is insulin-independent, insulin resistance in cirrhosis may influence the intracellular metabolism of fructose.

SUBJECTS AND METHODS

Eight patients (four men, four women) with biopsy-proven alcoholic cirrhosis and seven normal subjects (four men, three women) were studied. The women were postmenopausal apart from one cirrhotic patient. The cirrhotics had abstained from alcohol for at least 2 months before study (six for more than 1 year). All were outpatients in good nutritional state who undertook normal physical activity. Their clinical details are presented in Table 1. Seven patients were Pugh's grade A, and one was grade B (Pugh's score of 7): two were taking spironolactone, but none had ascites at the time of study. Six patients had esophageal varices on endoscopy. Four of these had bled from varices, but not in the 12 months preceding the study. Four cirrhotics had participated in a previous study of insulin sensitivity by the euglycemic clamp technique and were found to be markedly insulin-insensitive. All subjects had a normal fasting blood glucose concentration, and all consumed a diet containing at least 200 g carbohydrate/d. Each subject gave informed consent before participating in the study, which was approved by the local Ethical Committee.
and energy expenditure were calculated from standard equations. During the infusion of fructose, particularly at the higher concentration of the study were obtained to determine urinary N excretion rates. Carbohydrate and lipid oxidation were measured at minute intervals for 15 minutes for calculations. A 1-hour basal urine sample and a sample at the time of the infusion. At 8 minutes and 45 mg/kg in 10 minutes, respectively. Blood samples for %NEFA and 2-H-glycerol concentrations and specific activities and for fructose, glucose, insulin, TG, lactate, and 3-hydroxybutyrate levels were taken every 15 minutes until +240 minutes. During the last 15 minutes of each fructose step (from +105 to +120 minutes and from +225 to +240 minutes), samples were taken every 5 minutes for measurement of steady-state levels of glycerol and NEFA specific activities.

Protocol

Glycerol and NEFA kinetics and substrate oxidation were measured basally and during a 4-hour stepped-dose IV fructose infusion. Studies commenced at 8 AM after a 14-hour overnight fast. For blood sampling, a venous cannula was inserted retrogradely into the vein of a hand, which was placed in a hand warmer at 65°C. After sampling, the cannula was flushed with 0.15 mol/L NaCl. Another cannula was inserted into an antecubital vein for infusions. At 8 AM, a 6-hour infusion of 1-14C-palmitate (5 μCi prime in 1 minute, 0.25 μCi/min) complexed to 2%-wt/vol human albumin (Sigma, Poole, Dorset, UK) in 0.15 mol/L NaCl and 2-H-glycerol (8 μCi prime, 0.4 μCi/min) (Amersham International, Amersham, UK) was begun. During the last 15 minutes of the first 2 hours of tracer infusion, four basal blood samples were taken (t = −15 minutes to t = 0 minutes) for measurement of basal palmitate and glycerol turnover rates and substrate concentrations. Then (t = 0 minutes) a primed IV infusion of 10% wt/vol fructose in water was started at 200 mg/kg/h for 2 hours, followed by 500 mg/kg/h for a further 2 hours using primes of 30 mg/kg in 10 minutes and 45 mg/kg in 10 minutes, respectively. Blood samples for 14C-NEFA and 2-H-glycerol concentrations and specific activities and for fructose, glucose, insulin, TG, lactate, and 3-hydroxybutyrate levels were taken every 15 minutes until +240 minutes. During the last 15 minutes of each fructose step (from +105 to +120 minutes and from +225 to +240 minutes), samples were taken every 5 minutes for measurement of steady-state levels of glycerol and NEFA specific activities.

Whole-Body Carbohydrate and Lipid Oxidation

Substrate oxidation was determined by indirect calorimetry (Deltatrac Metabolic Monitor, Datex Instruments, Helsinki, Finland). A clear plastic ventilated hood was placed over the head; room air was drawn through the hood at 40 L/min. Oxygen and CO2 production were measured at minute intervals for 15 minutes before t = 0 minutes and during the second half of each 30-minute period after starting the IV fructose infusion. The means of values during the last 10 minutes of each measurement interval were used for calculations. A 2-hour basal urine sample and a sample at the conclusion of the study were obtained to determine urinary nitrogen and fructose excretion rates and during the infusion of fructose. The nonprotein respiratory quotient was calculated from O2 consumption, CO2 production (VCO2), and urinary N excretion rates. During the infusion of fructose, particularly at the higher rate, negative rates of lipid oxidation were observed during one or more measurement intervals. These negative values are thought to be due to lipid synthesis when the nonprotein respiratory quotient exceeds 1.0, there is no net lipid oxidation. When negative rates of lipid oxidation were obtained, the following equation was used to calculate carbohydrate oxidation: carbohydrate oxidized = 1.34(1.00 VCO2 − 4.88 N). Since fructose availability has only a small effect on glucose kinetics and plasma glucose levels, an increase in glucose oxidation during IV fructose is unlikely—an inhibition of glucose oxidation would be more likely. The increase in carbohydrate oxidation above basal rates is thus likely to be a minimal estimate of the rate of fructose oxidation during IV fructose. A maximal estimate of the quantity of fructose stored as glycogen or TG was calculated by subtracting the amount of carbohydrate oxidized above that in the basal state (over the same time interval) plus that remaining in the "fructose space." A fructose distribution space similar to that of glucose (0.2 L/kg) was assumed. To determine the thermogenic response to IV fructose, administration, basal energy expenditure (kcal/min) was subtracted from the energy expenditure calculated for the last 15 minutes of each fructose step.

Analytical Procedures

Plasma glucose levels were determined by a glucose oxidase method (Yellow Springs Glucose Analyser, Clandon Scientific, London, UK). Plasma glycerol levels were determined by an enzymic fluorimetric method in an LS50 luminescence spectrometer (Perkin Elmer, Beaconsfield, UK) with an intraassay coefficient of variation (CV) of 2.3%. Serum NEFA levels were determined using a WAKO NEFA-C kit (Wako Chemicals, Neuss, Germany), and serum TG levels, a GPO-PAP kit (Boehringer, Mannheim, Germany). Blood for estimation of lactate and 3-hydroxybutyrate levels was deproteinized with perchloric acid (0.6 mol/L), and the extract was assayed by enzymic fluorimetric methods. Plasma and urine fructose concentrations were measured by the resorcinol/thiouria method; plasma fructose values were corrected for the 6% cross-reactivity with glucose. Urinary N levels were measured by the method of Kjeldahl. Serum insulin levels were measured by a double-antibody technique; intraassay and interassay CVs were 5.7% and 7.6%, respectively.

To determine 2-3H-glycerol specific activity, 1.0 mL plasma was deproteinized with Ba(OH)2-ZnSO4. The supernatant after centrifugation was evaporated, and the residue was dissolved in 1 mL water. After adding 10 mL scintillation fluid (Cocktail T, BDH, Poole, UK), 3 H dpm were counted in a PW4700 liquid scintillation counter (NV Philips' Gloeilampenfabrieken, Lelyweg, Holland) by dual-channel scintillation spectrometry using an external standard to correct for efficiency and overlapping. Quadruplicate aliquots of the infusate were added to nonradioactive plasma and processed in parallel with the plasma samples to allow calculation of the 2-3H-glycerol infusion rate. The 14C-radioactivity in serum NEFA and TG was identified following their separation by column chromatography. One milliliter of serum was extracted with 4 mL 2:1 vol/vol chloroform:methanol. The extracted lipids were backwashed with water and then passed through a 3-mL amipropyl bonded phase column (Bond Elut, Alpha Laboratories, Eastleigh, UK). Glycerides were eluted with 4 mL 2:1 vol/vol chloroform:isopropanol and NEFA with 4 mL 98.2 vol/vol diethyl ether:acetic acid. The eluate was dried under N2, and the radioactivity was determined by dual-channel scintillation spectrometry. Aliquots of the infusate added to nonradioactive serum were processed in parallel to allow calculation of the 1-14C-palmitate infusion rate. To determine the percentage contribution of palmitate to plasma NEFA, fatty acids were converted to their methyl esters and then analyzed by gas-liquid chromatography.
Calculations

Plasma concentrations and specific activities of NEFA and glycerol were constant during the last 15 minutes of the basal period and the last 15 minutes of each fructose infusion step. The rates of glycerol and NEFA appearance (R,) and disappearance during the last 15 minutes of each of these three periods were therefore calculated using steady-state equations, substrate R (μmol/min) = tracer infusion rate (dpm/min)/plasma substrate specific activity (dpm/μmol). Glycerol R was during fructose infusion was also calculated using the non-steady-state equations of Steele.30 A glycerol distribution volume of 0.65 L/kg has been used by others.1' However, this may be excessive, since steady-state glycerol specific activities are reached within approximately 30 minutes when labeled glycerol is infused IV.31 and glycerol does not equilibrate across the hepatocyte membrane.5: We assumed a glycerol pool size of 0.2 L/kg (similar to that for glucose) and a pool fraction of 0.5.31 Nonetheless, since the rate of change of glycerol concentration and specific activity was slow, the estimated pool size had relatively little effect on calculated glycerol R. The fractional turnover rate of palmitate is representative of the total NEFA pool.32 Plasma NEFA R was therefore calculated by dividing the rate of infusion of 1-14C-palmitate (dpm/min) by the steady-state plasma NEFA specific activity (dpm/μmol). Glycerol and NEFA metabolic clearance rates (MCRs) were calculated by dividing their turnover rates by their respective plasma concentrations. Assuming no reutilization of glycerol in human adipose tissue, intraadipocyte recycling of fatty acids was calculated using the equation fatty acid recycling = (3 × γ1 × glycerol R)/(NEFA R x 3 × γ1 × glycerol R), x 100.

Statistical Analysis

Results are expressed as the mean ± SEM unless otherwise indicated. Correlations were determined by Pearson's least-squares method. The significance of differences was tested by Student's paired or unpaired t test as appropriate. A P value less than .05 was considered statistically significant.

RESULTS

Plasma Substrate and Insulin Levels

Plasma glucose levels after a 16-hour fast were similar, but insulin levels were four times higher in cirrhotics than in controls (Table 2, P < .01). Plasma fructose levels increased promptly following the prime administered at the beginning of the low- and high-dose fructose infusions, and then plateaued at similar levels in cirrhotics and controls (Fig 1, Table 2). Fructose MCR was not influenced by the fructose infusion rate and did not differ between cirrhotics and controls (Table 2). The total quantity of fructose infused was similar in the two groups (cirrhotics, 102.5 ± 5.4 g; controls, 110.9 ± 8.9 g). With the low-dose fructose infusion, there was in both groups an initial small increase in plasma glucose to a peak approximately 0.3 mmol/L above basal at +30 minutes followed by a slow decline (Fig 1). In response to the higher fructose infusion rate, the control subjects had a further small increase in plasma glucose level. This was due to the results in one subject in whom glucose levels increased by 1.5 mmol/L during the second fructose step. The remaining six control subjects and the cirrhotics showed no increase in plasma glucose during the second fructose step compared with levels at +120 minutes.

There was a small increase in serum insulin levels in response to the low-dose fructose infusion (Fig 1). In both groups, insulin peaked at +15 minutes after the start of IV fructose; at this time, levels were significantly higher than basal in both groups (cirrhotics, P < .05; controls, P < .005). When the fructose infusion rate was increased at +120 minutes, there was a further increase in insulin levels in both groups. Again, the peak insulin response occurred 15 minutes after increasing the fructose infusion rate (P < .05 v 120-minute levels in both groups). The increase was approximately three times greater in the cirrhotics. In both groups, insulin levels remained significantly higher than in the basal state (Table 2, Fig 1). The blood lactate response to IV fructose was similar in cirrhotics and controls (Fig 1). In both groups, lactate levels increased to a plateau of approximately 1.5 mmol/L during the last 60 minutes of the low-dose fructose infusion. With the higher fructose infusion rate, there was a further increase in blood lactate levels to a plateau of approximately 2.5 mmol/L in both groups (Fig 1, Table 2). Fructose infusion had no effect on serum phosphate, uric acid, or bicarbonate levels in either group. There was a small but significant decrease in plasma urica by the end of the study compared with the basal state (cirrhotics, 4.2 ± 0.5 v 4.7 ± 0.6 mmol/L, P < .05; controls, 4.9 ± 0.6 v 5.7 ± 0.6 mmol/L, P < .002). Plasma albumin

<table>
<thead>
<tr>
<th>Table 2. Plasma Substrate and Insulin Levels (mean ± SEM) in Eight Cirrhotic Patients and Seven Normal Control Subjects After an Overnight Fast and During a Stepped IV Fructose Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basal</strong></td>
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<tr>
<td>Controls</td>
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<tr>
<td>n</td>
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<tr>
<td>Plasma fructose (mmol/L)</td>
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<tr>
<td>Fructose MCR (mL/min/kg)</td>
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<tr>
<td>Plasma glucose (mmol/L)</td>
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<tr>
<td>Serum insulin (pmol/L)</td>
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<tr>
<td>Blood lactate (mmol/L)</td>
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<tr>
<td>Blood 3-hydroxybutyrate (μmol/L)</td>
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<td>Serum TG (mmol/L)</td>
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</table>

*P < .05, †P < .02, ‡P < .01; compared with normal control subjects
decreased by 9.1% ± 1.6% in cirrhotics ($P < .005$) and by 7.9% ± 1.7% in controls ($P < .01$).

Serum NEFA decreased to 0.315 ± 0.045 mmol/L in cirrhotics and to 0.423 ± 0.105 mmol/L in controls during the last 15 minutes at the low fructose dose; the extent of NEFA suppression from basal levels was greater in cirrhotics (0.534 ± 0.085 mmol/L decrease) than in controls (0.294 ± 0.024 mmol/L decrease; $P < .02$). With the higher fructose infusion rate, there was a further decrease in serum NEFA in both groups (Fig 2). During the last 15 minutes at the high fructose dose, serum NEFA levels were lower in cirrhotics than in controls (Table 3), but the difference did not reach statistical significance ($t = 1.54, .1 < P < .2$). Plasma glycerol levels in control subjects did not change during the IV fructose infusion at either dose (Fig 2). In cirrhotics, plasma glycerol levels increased during the first fructose step, and at the end of this period were 22% higher than in the basal state ($P < .05$). With the higher fructose infusion rate, plasma glycerol levels decreased slightly and then plateaued at a level not significantly different from basal levels. Plasma glycerol levels did not differ between cirrhotics and controls during IV fructose administration. Blood 3-hydroxybutyrate concentrations decreased promptly with IV fructose in both groups (Fig 2). There was no change in serum TG levels with IV fructose in either group (Fig 2, Table 2).

**NEFA and Glycerol Kinetics**

Basal serum NEFA levels and NEFA turnover rates were higher in cirrhotics than in controls (Table 3), but the differences did not reach statistical significance. Basal NEFA MCR was similar in the two groups (Table 3). There was a small increase in serum NEFA specific activity in both groups in response to the low fructose infusion rate, but a more marked increase was seen with the higher fructose infusion rate (Fig 3). Palmitate accounted for 25.0% ± 2.4% ($±SD$) of serum NEFA in cirrhotics and for 25.5% ± 2.7% in controls. The contribution of palmitate to serum NEFA did not change with either fructose infusion rate (data not shown). In both groups, NEFA turnover calculated for the last 15 minutes of the first fructose step was lower than in the basal state, and there was no difference between cirrhotics and controls (Table 3). There was a further decrease in NEFA turnover during the second fructose step, but again the turnover rates were similar in the two groups (Table 3). In both groups, there was a significant increase in NEFA MCR with IV fructose infusion (Table 3). By the end of the first fructose step, NEFA MCR had increased by 48% in cirrhotics and by 29% in controls. There was a further increase in NEFA MCR with the higher fructose infusion rate, so that NEFA MCR during the last 15 minutes of the second fructose step was
FRUCTOSE METABOLISM IN CIRRHOSIS

Fig 2. Serum NEFA and triglycerides, plasma glycerol, and blood 3-hydroxybutyrate levels (mean ± SEM) after an overnight fast and in response to a primed IV fructose infusion at 200 mg/kg/h (0 to 120 minutes) and 500 mg/kg/h (120 to 240 minutes) in eight cirrhotics (○) and seven normal controls (●).

After an overnight fast, glycerol turnover and glycerol MCR were not significantly different in cirrhotics and controls (Table 3). The profiles of glycerol specific activity and R₃₆₀ are shown in Fig 3. In both groups, there was an increase in glycerol specific activity in response to IV fructose. In controls, there was a small decrease in glycerol R₃₆₀ during the first fructose step (Fig 3), and from +105 to +120 minutes it was 19% lower than in the basal state (Table 3, P < .02); there was little further change during the second fructose step. In cirrhotics, there was a small but transient decrease in glycerol R₃₆₀ during the first fructose step, so that at the end of the first fructose step it was not significantly different from basal glycerol R₃₆₀ (Table 3). With the higher fructose infusion rate, there was a further small decrease in glycerol R₃₆₀ in cirrhotics (Fig 3). From +225 to +240 minutes, it was 17% lower than in the basal state (Table 3, P < .05). In both groups, there was a significant decrease in glycerol MCR with IV fructose (Table 3). The reduction in glycerol MCR was seen with the low rate of fructose infusion in both groups; there was little further change with the higher rate of fructose infusion. Glycerol turnover and glycerol MCR did not differ between cirrhotics and controls during IV fructose.

In the basal state, intraadipocyte recycling of fatty acids was lower in cirrhotics than in controls (Table 3, P < .05). In both groups, fatty acid recycling increased during IV fructose infusion and was not different between the two groups at the end of the first or second fructose steps (Table 3).

Substrate Oxidation Rates and Energy Expenditure

After an overnight fast, the respiratory quotient (cirrhotics, 0.83 ± 0.02; controls, 0.84 ± 0.01), protein oxidation (cirrhotics, 0.57 ± 0.07; controls, 0.63 ± 0.09 mg/kg/min; NS), and the rate of carbohydrate oxidation (cirrhotics, 1.41 ± 0.20; controls, 1.49 ± 0.12 mg/kg/min) were similar (Fig 4). Basal lipid oxidation did not differ significantly in cirrhotics (0.74 ± 0.15 mg/kg/min) and controls (0.48 ± 0.06 mg/kg/min; NS). Basal serum NEFA levels correlated with basal rates of lipid oxidation (n = 15, r = .63, P < .02) and inversely with rates of carbohydrate oxidation (n = 15, r = -.67, P < .01). Resting energy expenditure after the overnight fast was similar in cirrhotics (1.10 ± 0.09 kcal/min) and controls (1.04 ± 0.07 kcal/min).

In response to IV fructose, there was an increase in the respiratory quotient, a decrease in whole-body lipid oxidation, and an increase in whole-body carbohydrate oxidation (Fig 4). These changes were more marked with the higher rate of fructose infusion. From 105 to 120 minutes at the low fructose dose and throughout the second fructose step, carbohydrate oxidation rates were significantly higher in the cirrhosis (Fig 4). At both fructose infusion rates, cirrhotics oxidized more of the infused fructose and stored less of it as either glycogen or TG (Table 4), assuming that glucose oxidation remained the same as in the basal state. Even if our assumption concerning unchanged glucose
oxidation rates is incorrect, the higher rates of carbohydrate oxidation found in cirrhotics means that their net deposition of glycogen would have been lower in response to IV fructose. The total quantity of lipid oxidized during the first fructose step was similar in cirrhatics (50.80 ± 13.36 mg/kg in 2 hours) and controls (50.14 ± 10.66 mg/kg in 2 hours). With the higher fructose infusion rate, lipid oxidation rates showed a further decrease in both groups and tended to be lower in cirrhotics than in controls (Fig 4). Mean lipid oxidation rates in cirrhotics were negative during the second fructose step, implying net lipid synthesis. However, total net lipid oxidation during the second fructose step was not statistically significantly different in cirrhotics and controls, or (2) reduced uptake of fructose by the cirrhotic liver is associated with a greater uptake of fructose by peripheral tissues. The first of these explanations would mean that it is shunting rather than impaired hepatocyte uptake that would be the major factor in oral fructose intolerance of cirrhosis. This is in keeping with the marked intolerance to oral fructose that follows a surgical portocaval shunt.

Phytoinsulinemia in cirrhosis is due to increased secretion of insulin by the liver and peripheral tissues, with splanchnic tissues accounting for about 40% to 50% of fructose removal during systemic IV fructose administration. The similarity of fructose levels after IV fructose can only be explained if (1) the liver and peripheral tissues take up a similar amount of fructose in cirrhosis and controls, or (2) reduced uptake of fructose by the cirrhotic liver is associated with a greater uptake of fructose by peripheral tissues. The first of these explanations would mean that it is shunting rather than impaired hepatocyte uptake that would be the major factor in oral fructose intolerance of cirrhosis. This is in keeping with the marked intolerance to oral fructose that follows a surgical portocaval shunt. Fructose MCR was the same during the low and high fructose infusion steps, confirming that fructose utilization is proportional to its plasma concentration.

Like others, we found only a small (25 mmol/L) increase in plasma glucose levels with IV fructose (Fig 1). We have shown previously in cirrhotics and controls that the increase in plasma glucose after oral fructose is primarily due to a small and transient increase in glucose R, with a reduction in the rate of disappearance being quantitatively less important. Thus, the much smaller increase in plasma glucose with IV than with oral fructose in both groups suggests either that the intestinal conversion of fructose to glucose during fructose absorption makes a significant contribution to glucose R, or that less fructose is taken up by the liver with the IV route. However, the extent of intestinal conversion of fructose to glucose in man is unclear. Reduced hepatic fructose delivery with the IV route does not adequately explain the difference in glucose responses to IV and oral fructose, since one would expect a greater glucose increase with higher rates of fructose infusion; others using a fructose infusion rate twofold greater than our high rate found a glucose increment of only 0.2 mmol/L.

Table 3. NEFA and Glycerol Kinetics in Cirrhotics and Controls in the Basal State and During a Stepped IV Fructose Infusion

<table>
<thead>
<tr>
<th></th>
<th>Basal Controls</th>
<th>Cirrhotics</th>
<th>IV Fructose Step I Controls</th>
<th>Cirrhotics</th>
<th>Controls</th>
<th>Cirrhotics</th>
<th>IV Fructose Step II Controls</th>
<th>Cirrhotics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum NEFA (µmol/L)</strong></td>
<td>7.0 ± 0.9</td>
<td>6.9 ± 0.8</td>
<td>4.1 ± 0.9</td>
<td>3.9 ± 0.8</td>
<td>6.7 ± 0.8</td>
<td>6.6 ± 0.7</td>
<td>4.2 ± 0.8</td>
<td>3.8 ± 0.7</td>
</tr>
<tr>
<td><strong>NEFA turnover (µmol/min/kg)</strong></td>
<td>5.5 ± 0.8</td>
<td>5.3 ± 0.7</td>
<td>2.6 ± 0.7</td>
<td>2.4 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>3.0 ± 0.5</td>
<td>2.7 ± 0.6</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td><strong>NEFA MCR (mL/min/kg)</strong></td>
<td>7.9 ± 0.7</td>
<td>8.2 ± 0.6</td>
<td>10.5 ± 0.9</td>
<td>12.2 ± 1.1</td>
<td>8.5 ± 0.8</td>
<td>8.7 ± 0.7</td>
<td>11.4 ± 1.2</td>
<td>12.6 ± 1.3</td>
</tr>
<tr>
<td><strong>Plasma glycogen (µmol/L)</strong></td>
<td>186 ± 10</td>
<td>183 ± 12</td>
<td>186 ± 12</td>
<td>182 ± 12</td>
<td>188 ± 12</td>
<td>186 ± 12</td>
<td>190 ± 12</td>
<td>188 ± 12</td>
</tr>
<tr>
<td><strong>Glycerol turnover (~mol/min/kg)</strong></td>
<td>5.5 ± 0.7</td>
<td>5.0 ± 0.6</td>
<td>2.9 ± 0.7</td>
<td>2.8 ± 0.6</td>
<td>3.0 ± 0.6</td>
<td>2.9 ± 0.5</td>
<td>3.1 ± 0.6</td>
<td>2.9 ± 0.5</td>
</tr>
<tr>
<td><strong>Glycerol MCR (mL/min/kg)</strong></td>
<td>19.5 ± 1.4</td>
<td>18.7 ± 1.5</td>
<td>15.7 ± 0.5</td>
<td>14.1 ± 1.1</td>
<td>16.1 ± 0.4</td>
<td>14.9 ± 1.4</td>
<td>16.1 ± 0.4</td>
<td>14.9 ± 1.4</td>
</tr>
<tr>
<td><strong>Intrahepatocyte NEFA recycling %</strong></td>
<td>9.24 ± 1.10</td>
<td>9.9 ± 0.99</td>
<td>4.8 ± 0.89</td>
<td>4.5 ± 0.87</td>
<td>0.89 ± 0.78</td>
<td>0.9 ± 0.89</td>
<td>0.89 ± 0.78</td>
<td>0.9 ± 0.89</td>
</tr>
<tr>
<td></td>
<td>47.2 ± 7.6</td>
<td>20.1 ± 9.9</td>
<td>53.2 ± 10.8</td>
<td>53.8 ± 4.8</td>
<td>80.9 ± 4.6</td>
<td>81.7 ± 3.6</td>
<td>80.9 ± 4.6</td>
<td>81.7 ± 3.6</td>
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</tbody>
</table>

**NOTE.** Values are the means of concentrations and kinetic data during the last 15 minutes at 200 mg/kg/h (step 1) and 500 mg/kg/h (step 2) IV fructose.

*P < .05 compared with normal controls.
 tP < .01, §P < .001: compared with basal.
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5000
3700
E
g
6000
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5400
iz
1a
3000
Fig 3. Specific activities (SA) of serum NEFA and plasma glycerol and glycerol R, (mean ± SEM) in the basal state and during the primed IV fructose infusion at 200 mg/kg/h (0 to 120 minutes) and 500 mg/kg/h (120 to 240 minutes) in eight cirrhotics (○) and seven normal controls (●).

tion and decreased clearance, with the latter being the more important.47 Both mechanisms could explain the greater insulin response to IV fructose in cirrhotics (Fig 1). The small insulin response to oral or IV fructose is usually attributed to the increase in plasma glucose levels. However, the further increase in insulin levels during the second step (Fig 1) in the absence of an increase in plasma glucose levels (with the exception of one normal subject) suggests that fructose may enhance insulin secretion independently of an increase in plasma glucose concentration. This conclusion is consistent with studies in isolated islets showing that fructose can augment insulin secretion in the presence of low concentrations of glucose.48 Islet β cells contain fructokinase, this suggests that, like hepatocytes, they have the capacity for rapid uptake of fructose and its phosphorylation to fructose-1-phosphate, which can stimulate β-cell glucokinase activity and hence insulin secretion.50

Mean serum NEFA levels and NEFA R, were higher in
but decreased reesterification of fatty acids, which ac-

Our cirrhotics had normal rates of lipolysis (glycerol R,)
this ratio implies intraadipocyte fatty acid recsterification.

glycerol produced by lipolysis cannot be reutilized because
In the absence of any adipocyte recycling of fatty acids,
a good measure of the activity of adipose tissue TG lipase.
NEFA R, would be three times glycerol R,; a decrease in
necessary for reesterification is produced by glycolysis;
reesterified within the adipocyte.3" Glycerol-3-phosphate
those in the present study. Not all fatty acids produced by
increase in adiposity with age, the tendency for a more
related to the small number of subjects studied and the
large range of values found in both groups. Another factor
could be better matching of cirrhotic and controls for age
found by others in middle-aged, slightly overweight normal
subjects." Age as an explanation is plausible given the
increase in insulin during the

our cirrhotics, but the differences did not reach signifi-
cance. Since most53152 but not all53 previous studies have
shown increased NEFA levels and NEFA R, in overnight-
fasted cirrhotics, this may represent a type 2 statistical error
related to the small number of subjects studied and the
wide range of values found in both groups. Another factor
could be better matching of cirrhotic and controls for age
and nutritional status than in some of the studies reporting
large differences in serum NEFA levels.5154 Indeed, serum
NEFA levels in our cirrhocyte were similar to those found by
others,5315254 whereas our controls had higher fasting
NEFA levels than those found in studies using a younger
control group.5154 although they were similar to levels
found by others in middle-aged, slightly overweight normal
subjects.5 Age as an explanation is plausible given the
increase in adiposity with age, the tendency for a more
central distribution of adipose tissue with age, and the
higher lipolytic rates associated with a central distribution
of body fat.55

Surprisingly, basal glycerol levels and glycerol R, were
not increased in our cirrhotics (Table 3). A similar associa-
tion between glycerol and NEFA levels in cirrhotics was
reported by Muller et al.,53 although previously13 we found
elevated glycerol levels in a group of cirrhotics similar to
those in the present study. Not all fatty acids produced by
the action of TG lipase in adipocytes are released; some are
reesterified within the adipocyte.36 Glycerol-3-phosphate
necessary for reesterification is produced by glycolysis;
glycerol produced by lipolysis cannot be reutilized because
human adipocytes lack glycerokinase.36 Thus, glycerol R, is
a good measure of the activity of adipose tissue TG lipase.
In the absence of any adipocyte recycling of fatty acids.
NEFA R, would be three times glycerol R,; a decrease in
this ratio implies intradipocyte fatty acid reesterification.
Our cirrhotics had normal rates of lipolysis (glycerol R,)
but decreased reesterification of fatty acids, which ac-
counted for their somewhat higher serum NEFA and
NEFA R, (Table 3). Impaired reesterification despite
increased insulin levels could be due to a lack of glycerol-3-
phosphatase secondary to decreased adipocyte glucose
transport55; adipocyte glycerol-3-phosphate availability may
be rate-limiting for reesterification.57

Suppression of NEFA was incomplete with the lower
fructose dose, but a further decrease to very low levels was
seen with the higher dose (Fig 2), and NEFA levels did not
differ between the two groups. The decrease in serum
NEFA was largely due to the reduction in NEFA R, (Table
3). However, in both groups fructose (particularly at the
higher infusion rate) also induced an increase in NEFA
MCR, implying more efficient removal of NEFA from
plasma. It has generally been assumed that plasma NEFA
levels are determined by their rate of release from adipose
tissue and that factors affecting plasma NEFA levels do so
by acting on adipocytes rather than by influencing the
removal mechanisms. Our finding of an increase in NEFA
MCR with IV fructose supports the finding by Bonadonna
et al.58 of an increase in NEFA MCR with insulin, and
suggests that NEFA uptake is also subject to regulation and
can thus influence serum NEFA levels. The most likely
explanation for the improvement in NEFA clearance from
plasma is increased availability of glycerol-3-phosphate in
liver and other tissues that can use both fructose and fatty
acids; increased glycerol-3-phosphate levels would promote
reesterification of serum fatty acids.

Surprisingly, glycerol levels did not change with IV
fructose in the controls; indeed, in the cirrhotics an increase
was found at the end of the first fructose step (Table 3).
Bjorkman et al.59 also reported unchanged glycerol levels
but a decrease in serum NEFA in normal subjects adminis-
tered IV fructose, whereas in their type I diabetic patients,
glycerol levels were increased by IV fructose. The stable
glycerol levels with IV fructose in controls are due to the
small decrease in glycerol R, being accompanied by a
quantitatively similar decrease in glycerol MCR (Table 3);
the two factors have opposite effects on plasma glycerol
levels. In the cirrhotics glycerol R, at the end of the first
fructose step was not different from that in the basal state,
but glycerol MCR decreased, hence the initial increase in
glycerol levels. The liver is the main site of glycerol
clearance, with a glycerol extraction ratio of approximately
0.6.62,59 Glycerol clearance is influenced by hepatic glycolytic/
gluconeogenic flux rates. Thus, clearance is greater when
gluconeogenesis is enhanced, as in diabetes.60 The reduc-
tion of glycerol clearance with fructose administration is
likely to be due to increase in hepatic glycerol-3-phosphate
levels,62 consequent upon the rapid uptake and phosphory-
lization of fructose via fructokinase and the subsequent
cleavage of fructose-1-phosphate by aldolase B; a reduction
of hepatic adenosine triphosphate levels with IV fruc-
tose61.62 might also impair the activity of glycerokinase. Our
study shows that in both normal and cirrhotic subjects,
increased reesterification of fatty acids is the main mecha-
nism by which fructose leads to a reduction in adipocyte
NEFA release. Glycerol R, decreased by less than 20% in
both groups, despite the increase in insulin during the
second fructose step to levels greater than those shown to

<table>
<thead>
<tr>
<th>Table 4. Carbohydrate Oxidation and Storage (mean ± SEM) During IV Fructose Administration at 200 mg/kg/h (first step) and 500 mg/kg/h (second step) in Eight Cirrhotic Patients and Seven Normal Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IV fructose step 1</strong></td>
</tr>
<tr>
<td>Carbohydrate oxidized in 2 hours</td>
</tr>
<tr>
<td>(mg/kg)</td>
</tr>
<tr>
<td>Total</td>
</tr>
<tr>
<td>307 ± 16†</td>
</tr>
<tr>
<td>Above basal</td>
</tr>
<tr>
<td>130 ± 15†</td>
</tr>
<tr>
<td>Fructose stored in 2 hours</td>
</tr>
<tr>
<td>mg/kg</td>
</tr>
<tr>
<td>242 ± 12†</td>
</tr>
<tr>
<td>% of fructose infused</td>
</tr>
<tr>
<td>56 ± 3†</td>
</tr>
<tr>
<td>NEFA R,</td>
</tr>
<tr>
<td>238 ± 19</td>
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<tr>
<td>50 ± 13</td>
</tr>
<tr>
<td>318 ± 16</td>
</tr>
<tr>
<td>74 ± 4</td>
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</tbody>
</table>

**NOTE.** Estimates of fructose storage are maximal values, since their
calculation assumes no change in the rate of glucone oxidation during
IV fructose administration.

**P < .01, †P < .005, ‡P < .001: compared with normal control sub-
jects.**
be half-maximal for inhibition of NEFA release (~77 pmol/L [13 mU/L]). Thus, contrary to popular belief, inhibition of TG lipase is not the main mechanism for suppression of NEFA release by fructose either in cirrhotic or normal subjects.

Basal rates of whole-body lipid oxidation were higher in our cirrhotics, but the differences were not statistically significant (Fig 4). Our findings agree with those of Petrides et al,5,10 and Muller et al.,11 but contrast with those of others who found that overnight-fasted cirrhotics derive more of their energy requirements from the oxidation of fat and less from carbohydrate.5,12 Different subject characteristics may explain the conflicting reports. We studied outpatients in good nutritional state who had abstained from alcohol for at least 2 months and had no complications of their liver disease at the time of study. By contrast, in the studies reporting increased basal fat oxidation,5,12 cirrhotics were inpatients with more marked abnormalities of liver function; some were cachectic, and abstinence from alcohol was not a criterion for inclusion in the studies. Muller et al.11,12 found that basal fat oxidation rates were increased in cirrhotics with advanced liver disease (Child's class C), but not in well-compensated Child's class A cirrhotics.

With IV fructose, fat oxidation rates decreased and carbohydrate oxidation increased in both groups (Fig 4). With the higher fructose dose, mean lipid oxidation rates in cirrhotics were negative and negative rates were observed in many of the controls, suggesting net lipid synthesis.21,22 Our finding that serum TG levels were constant during IV fructose despite the marked decrease in serum NEFA and NEFA R, is consistent with de novo lipogenesis from fructose. However, the more negative fat oxidation rates in cirrhotics (Fig 4) were not accompanied by an increase in serum TG. It is possible that cirrhotics have a relative impairment of incorporation of TG into very-low-density lipoprotein, as suggested by the frequent finding of fat in liver biopsies from alcoholic cirrhotics, or that extrahepatic tissues contribute to de novo lipogenesis.

Although overall fructose utilization was similar in the two groups, cirrhotics oxidized more of the infused fructose at both infusion rates (Table 4). Carbohydrate oxidation rates in cirrhotics during the second fructose step (~4 mg/kg/min) were similar to those found in normal subjects during glucose clamps at supraphysiological insulin levels.16 This supports the view that the capacity for carbohydrate oxidation is unimpaired in cirrhosis,5,16 and that when exogenous carbohydrate is made available it is the preferred substrate for maintenance of fuel homeostasis. Nonoxidative fructose disposal includes fructose used for glycogen and TG synthesis and that converted to lactate. As with oral fructose,13 and in agreement with others,3 the blood lactate response was similar in cirrhotics and controls (Fig 1). Glycogen deposition in skeletal muscle and liver is the major determinant of nonoxidative disposal with IV fructose, with fructose oxidation and deposition as muscle and liver glycogen accounting for approximately 90% of infused fructose.5,13 It seems likely therefore that reduced glycogen deposition in liver and/or skeletal muscle is responsible for the lower nonoxidative fructose disposal in our cirrhotics (Table 4). Several studies have shown decreased nonoxidative glucose disposal in cirrhotics during a hyperinsulenic euglycemic clamp14,15 and after oral glucose,16 which accounts for lower rates of glucose utilization. However, in the current study, nonoxidative fructose disposal was impaired when overall carbohydrate utilization was normal. This is important because it indicates that a defect at the level of glucose transport cannot be the only explanation for impaired glycogen synthesis from glucose;12,17 intracellular defects must also be present. Impaired muscle glycogen synthase activation by insulin in cirrhotics15 could clearly be relevant.

Thus, in contrast to the response to oral fructose,13 compensated cirrhotics have normal IV fructose tolerance and a glycemic response similar to that of matched normal controls. In cirrhotics and controls, the decrease in serum NEFA is primarily due to enhanced reesterification of fatty acids both within adipose tissue preventing their release and in other tissues enhancing their removal from plasma. Overall glycerol and NEFA utilization during IV fructose are normal in cirrhotics. However, cirrhotics oxidize a greater proportion of infused fructose and store less. Thus, although tissue fructose uptake is insulin-independent,13 insulin resistance may influence the intracellular metabolism of fructose.

We used the IV route in this study to enable us to compare fructose metabolism in cirrhotics and controls at similar systemic fructose administration rates and plasma levels. We would not advocate its use intravenously in a clinical setting, because of the risks of lactic acidosis when fructose is rapidly infused.10,11 However, it is clear from this and our previous study13 that in compensated cirrhotics fructose is a good energy substrate, is oxidized in preference to lipid, and does not produce excessive blood lactate levels. A moderate intake of fructose in the diet may be beneficial in these patients.

ACKNOWLEDGMENT

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REFERENCES


