Lipolysis and lipid oxidation in cirrhosis and after liver transplantation

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Shangraw, Robert E., and Farook J ahoor. Lipolysis and lipid oxidation in cirrhosis and after liver transplantation. Am J Physiol Gastrointest Liver Physiol 278: G967–G973, 2000.—On the basis of the finding that plasma glycerol concentration is not controlled by clearance in healthy humans, it has been proposed that elevated plasma free fatty acid (FFA) and glycerol concentrations in cirrhotic subjects are caused by accelerated lipolysis. This proposal has not been validated. We infused 10 volunteers, 10 cirrhotic subjects, and 10 patients after orthotopic liver transplantation (OLT) with [1-13C]palmitate and [2H5]glycerol to compare fluxes (Ra) and FFA oxidation. Cirrhotic subjects had higher plasma palmitate (52%) and glycerol (33%) concentrations than controls. Palmitate Ra was faster (1.45 ± 0.18 vs. 0.85 ± 0.17 µmol·kg⁻¹·min⁻¹) but glycerol Ra and clearance slower (1.20 ± 0.09 vs. 1.90 ± 0.24 µmol·kg⁻¹·min⁻¹ and 21.2 ± 1.2 vs. 44.7 ± 4.9 ml·kg⁻¹·h⁻¹, respectively) than in controls. After OLT, plasma palmitate and glycerol concentrations and palmitate Rₐ did not differ, but glycerol Rₐ (1.16 ± 0.11 µmol·kg⁻¹·min⁻¹) and clearance (26.7 ± 2.4 ml·kg⁻¹·h⁻¹) were slower than in controls. We conclude that 1) impaired reesterification, not accelerated lipolysis, elevates FFA in cirrhotic subjects; 2) normalized FFA after OLT masks impaired reesterification; and 3) plasma glycerol concentration poorly reflects lipolytic rate in cirrhosis and after OLT.

Materials and Methods

Subjects. This study was approved by the Institutional Review Boards at Oregon Health Sciences University (OHSU) and the Portland, OR Veterans Affairs Medical Center. Twenty patients with stable end-stage cirrhosis were enrolled after written informed consent. Of these, 10 patients were studied in the OHSU Clinical Research Center while awaiting OLT. Inclusion criteria for cirrhotic patients were end-stage liver disease; hemodynamic stability; no history of insulin-dependent diabetes mellitus; no recent history of cigarette smoking; medications that did not include β-adrenergic agonists, β-adrenergic antagonists, or steroids; and adequate renal function, as indexed by plasma creatinine concentration <1.4 mg/dl. Severity of liver disease was assessed by the Pugh-Childs scoring system (20). The remaining 10 patients

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were studied on day 7 after OLT. The seventh postoperative day was chosen because it is a time of stability when many patients are ready for hospital discharge, the prednisone steroid taper is almost complete, and chronic oral immunosuppression therapy has optimized. An inclusion criterion for postoperative subjects, in addition to those for preoperative subjects, was good liver graft function as indicated by circulating albumin, prealbumin, and bilirubin concentrations and coagulation status in the absence of blood product transfusion. Ten healthy volunteers without clinical or laboratory evidence of liver disease were enrolled as controls. Control subjects were studied in the OHSU Clinical Research Center. Dietary intake on the day before the study was measured for cirrhotic and control subjects but not postoperative OLT subjects. All three subject groups were awake and alert and postabsorptive for at least 8 h, during which time they received no intravenous glucose or other sources of calories. Screening venous blood samples were drawn before the start of the stable isotope infusion protocol.

Blood for assessment of coagulation status was collected into tubes containing sodium citrate and analyzed immediately. Samples for determination of plasma concentrations of palmitate, glycerol, total FFA, and glucose and plasma isotopic enrichment of palmitate and glycerol were collected into chilled tubes containing sodium EDTA. Blood for analysis of serum electrolytes, total CO2 content, creatinine, insulin, and glucagon concentrations was collected into chilled tubes without additives.

Infusion protocol. The protocol began at 0700, when one intravenous catheter was placed in an upper extremity for stable isotope tracer infusion and a second intravenous catheter was placed in the dorsum of the contralateral hand for sampling of “arterialized” blood. The sampling hand was warmed with a heating pad. An indwelling central venous catheter was used for isotope infusion in postoperative patients.

After baseline blood and breath sampling, each subject received NaH13CO3 (5 µmol/kg iv; 99% 13C, Cambridge Isotope Laboratories, Woburn, MA) followed by a 60-min constant infusion at 10 µmol·kg−1·h−1. At 2 h, the NaH13CO3 infusion was supplanted by a constant infusion of [1-13C]palmitate (99% 13C, Cambridge Isotope Laboratories) at 0.08 µmol·kg−1·min−1 and [1,2,3,3-2H5]glycerol (98% 2H, Cambridge Isotope Laboratories) at 0.15 µmol·kg−1·min−1 for 90 min. Each subject received a [2H5]glycerol prime of 2.25 µmol/kg (iv), equal to 15 min of infusion.

Blood was collected into prechilled tubes containing sodium EDTA at 60, 70, 80, and 90 min of tracer palmitate and glycerol infusion. Blood was immediately centrifuged, and plasma was stored at –70°C until isotope analysis.

Breath samples were collected by face mask through a one-way Phillips valve into sealed 3-l anesthesia bags and immediately aspirated into 10-ml vacuum tubes. Breath samples were taken at 30, 40, 50, and 60 min of NaH13CO3 infusion and at 60, 70, 80, and 90 min of tracer palmitate and glycerol infusion. Breath samples were stored at room temperature until analysis. Indirect calorimetry (Deltatrac, Sensormedics, Fullerton, CA) was performed before tracer infusion and during each tracer infusion period.

Analytical procedures. Plasma palmitate isotope ratio was determined by negative chemical ionization gas chromatography-mass spectroscopy (NCI-GC/MS) on a Hewlett-Packard 5989B GC/MS system (Hewlett Packard, Fullerton, CA) as described by Hachey et al. (6). The pentafluorobenzyl derivative was prepared and analyzed by selective ion monitoring at mass-to-charge ratios (m/z) 255 and 256. Plasma glycerol isotope ratio was also measured by NCI-GC/MS, on the pentafluorobenzyl derivative, with selective monitoring of ions at m/z 680–685 as described by Gilker et al. (4).

Breath 13CO2 content was determined by gas isotope ratio mass spectrometry (Europa Scientific, Crewe, UK). Plasma glucose concentration was assayed by glucose oxidase using an autoanalyzer (Glucose Analyzer 2, Beckman Instruments, Brea, CA). Serum total FFA content was determined by acyl-CoA synthetase and acyl-CoA oxidase using a commercial kit (Wako Chemicals, Richmond, VA) with each sample as its own control to overcome the effect of hyperbilirubinemia. Plasma palmitate and glycerol concentrations were determined by in vitro isotope dilution as described by Gilker et al. (4), using [2,2-2H5]palmitate (98% 2H) and [2,1-13C]glycerol (99% 13C, Cambridge Isotope Laboratories) as internal standards. Briefly, a known quantity of each internal standard was added to a 1.0-ml aliquot of the baseline plasma sample and the isotope ratios of palmitate and glycerol in the sample were measured as described above. For palmitate, the monitored ions were from m/z 255–257, and for glycerol the ions were m/z 680 and 681. These techniques theoretically provide a more accurate assay than corresponding colorimetric techniques because they are not affected by hyperbilirubinemia, which is characteristic of patients with end-stage cirrhosis.

Calculations. Palmitate flux (Ra, palmitate) was calculated by the equation

\[ \text{Ra} = \frac{\left| E_{\text{infusate}} \right| - 1}{E_{\text{plateau}}} \times F \]

where F is the infusion rate of tracer palmitate (µmol·kg−1·min−1), IEinfusate is the isotopic enrichment of the infused tracer palmitate (mole % excess), and IEplateau is the isotopic enrichment of plasma palmitate (mole % excess) at isotopic steady state. A similar calculation was used for glycerol flux (Rg, glycerol). Clearance of palmitate or glycerol (ml·kg−1·h−1) was calculated as substrate flux divided by its plasma concentration.

Palmitate oxidation rate was calculated as

\[ \text{Palmitate oxidation} = \frac{12\text{CO}_2 \text{ excretion}}{E_{\text{plateau}}} \]

where 12CO2 excretion (µmol·kg−1·min−1) is the product of total CO2 production (VCO2) and the 13C isotopic enrichment of CO2, R is the recovery of 12CO2, and IEplateau is the isotopic enrichment of plasma palmitate (mole % excess) at isotopic steady state (28). Two different methods were used to calculate the rate of palmitate oxidation. The first method was based on recovery of 12CO2 from NaH13CO3 infusion in each individual during the first phase of our study (28). The second method was based on the “acetate retention factor” described by Sidossis et al. (24) using an R value of 0.56. The acetate retention factor not only accounts for labeled CO2 losses into the body CO2 pool like NaHCO3 but also includes other important lipid-specific 13CO2 losses in the TCA cycle such as incorporation into oxaloacetate and glutamate (24). The two calculations provided a range of palmitate oxidation rates for each group.

Total FFA flux (Rf, FFA) was calculated as Rf palmitate divided by the plasma palmitate-to-FFA concentration ratio, and total FFA oxidation rate was calculated as palmitate oxidation divided by the plasma palmitate-to-FFA ratio.

Statistical analysis. Data are expressed as means ± SE. One-way ANOVA with Tukey’s post hoc test was used to compare plasma substrate and hormone concentrations, Ra values for palmitate, FFA, and glycerol, palmitate and FFA

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oxidation rates, and clearance of palmitate, FFA, and glycerol among groups. Statistical tests were performed using a specialized software program (Crunch 4, Crunch Software, Oakland, CA). Differences were considered statistically significant at P < 0.05.

Ten subjects per group for comparison of R_a palmitate provided statistical power (1 – β) of 0.8 to detect a difference of 0.46 µmol·kg⁻¹·min⁻¹ between groups, with α = 0.05, given mean and standard deviation values of 1.16 and 0.35 µmol·kg⁻¹·min⁻¹, respectively, in healthy volunteers (3, 27). Similarly, 10 subjects per group for comparison of R_g glycerol provided statistical power (1 – β) of 0.8 to detect a difference of 0.42 µmol·kg⁻¹·min⁻¹ between groups, with α = 0.05, given mean and standard deviation values of 1.62 and 0.32 µmol·kg⁻¹·min⁻¹, respectively, in healthy volunteers (3, 17).

RESULTS

Baseline characteristics. The control group, eight men and two women, were 28 ± 2 (SE) years old, weighed 70.3 ± 4.5 kg, and had a body mass index (BMI) of 23.4 ± 0.9 kg/m². Table 1 shows demographic characteristics of the two patient groups. The most common etiology for cirrhosis in preoperative patients was concomitant ethanol abuse and hepatitis C viral infection. A broad spectrum of initial presenting liver disease was represented in the postoperative OLT group. Compared with controls, the two patient groups were older (P < 0.001) but had similar body weight and BMI. Cirrhotic patients did not differ from postoperative patients in any demographic characteristic, including the severity of liver disease immediately before OLT. Mean time since last ethanol intake for the cirrhotic patients with a history of ethanol abuse was 41 mo, with median 42 mo and range 7–60 mo. For postoperative OLT subjects with a history of ethanol abuse, the mean time since last intake was 30 mo with median 24 mo and range 13–60 mo. Dietary intake on the day before the isotope study for the cirrhotic subjects was diminished compared with controls, in terms of carbohydrate (2.30 ± 0.30 vs. 3.09 ± 0.28 g/kg), protein (0.61 ± 0.08 vs. 0.95 ± 0.08 g/kg), fat (0.37 ± 0.04 vs. 0.66 ± 0.06 g/kg), and total calories (14.9 ± 1.6 vs. 21.8 ± 1.8 kcal/kg) (all P < 0.05).

Table 2 lists screening laboratory values. Compared with controls, cirrhotic patients were hyperglycemic, hyponatremic, hypoalbuminemic, hyperbilirubinemic, and coagulopathic as evidenced by prolonged prothrombin time. Postoperative OLT patients showed no coagulopathy but exhibited hyperglycemia, hypoalbuminemia, and hyperbilirubinemia compared with controls. OLT patients had higher circulating concentrations of glucose and urea than preoperative cirrhotic subjects and improved prothrombin time but otherwise did not differ in biochemical parameters.
Effect of cirrhosis and liver transplantation on bivariate kinetics and gas exchange. The primed-constant infusion of NaH\(^{13}\)CO\(_3\) led to a steady-state enrichment of expired CO\(_2\) in all groups. Recovery of NaH\(^{13}\)CO\(_3\) was not statistically different among controls (0.772 \(\pm\) 0.031%), cirrhotic patients (0.862 \(\pm\) 0.036%), and OLT patients (0.793 \(\pm\) 0.051%). Total CO\(_2\) production was 119 \(\pm\) 6, 114 \(\pm\) 3, and 125 \(\pm\) 4 \(\mu\)mol·kg\(^{-1}\)·min\(^{-1}\) for controls, cirrhotic patients and OLT patients, respectively (not different). Respiratory quotient in controls (0.83 \(\pm\) 0.02), cirrhotic patients (0.80 \(\pm\) 0.02), and OLT patients (0.84 \(\pm\) 0.02) also did not differ among groups.

Hormonal profile in cirrhosis and after OLT. Both cirrhotic patients and liver transplant patients exhibited a marked hyperinsulinemia, with serum insulin concentration two- and threefold higher, respectively, than in controls (Table 3). Hyperinsulinemia was paralleled by an elevated circulating C-peptide concentration. Concomitantly, serum glucagon concentration was higher than control value by more than threefold in cirrhotic patients and by almost fivefold in OLT patients. OLT patients showed higher serum concentrations of insulin, C-peptide, and glucagon compared with preoperative cirrhotic patients. Thus the hormonal profile did not correct toward corresponding control values after OLT. The isotope infusion protocol did not change the hormonal profile of any subject group.

Effect of cirrhosis on lipid flux and oxidation in vivo. Cirrhotic subjects had increased plasma concentrations of palmitate (by 52%), FFA (by 76%), and glycerol (by 33%) compared with corresponding control values (Table 4). Plasma palmitate and glycerol isotopic enrichment reached a steady state during the isotope infusion protocol in both subject groups. Ra palmitate in cirrhotic patients was 71% higher and total FFA flux was almost twofold higher, compared with their respective control values. FFA clearance in cirrhotic patients was unchanged compared with that of controls.

Glycerol flux and clearance in cirrhotic subjects were 37% and 53% lower than respective control values (P < 0.02). Cirrhotic subjects exhibited a ratio of palmitate flux to glycerol flux that was twofold higher and a ratio for total FFA flux to glycerol flux almost threefold higher than that in healthy controls. This finding was not reflected in the ratio of their plasma concentrations.

### Table 3. Hormonal profile in cirrhosis and after OLT

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cirrhosis</th>
<th>OLT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Insulin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial, ng/ml</td>
<td>0.71 (\pm) 0.06</td>
<td>1.71 (\pm) 0.20(^*)</td>
<td>2.83 (\pm) 0.30(^{†})</td>
</tr>
<tr>
<td>Final, ng/ml</td>
<td>0.68 (\pm) 0.05</td>
<td>1.99 (\pm) 0.19(^*)</td>
<td>2.57 (\pm) 0.16(^*)</td>
</tr>
<tr>
<td>C-peptide</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial, ng/ml</td>
<td>1.41 (\pm) 0.09</td>
<td>3.63 (\pm) 0.36(^*)</td>
<td>5.32 (\pm) 0.41(^{†})</td>
</tr>
<tr>
<td>Final, ng/ml</td>
<td>1.28 (\pm) 0.11</td>
<td>4.19 (\pm) 0.25(^*)</td>
<td>5.35 (\pm) 0.33(^{†})</td>
</tr>
<tr>
<td><strong>Glucagon</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial, pg/ml</td>
<td>63 (\pm) 3</td>
<td>218 (\pm) 41(^*)</td>
<td>299 (\pm) 38(^*)</td>
</tr>
<tr>
<td>Final, pg/ml</td>
<td>56 (\pm) 4</td>
<td>219 (\pm) 38(^*)</td>
<td>268 (\pm) 37(^*)</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE; n = 10 subjects/group. *P < 0.01 vs. controls; †P < 0.02 vs. cirrhosis.

### Table 4. Dynamics of lipid metabolism

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cirrhosis</th>
<th>OLT</th>
</tr>
</thead>
<tbody>
<tr>
<td>[palmitate], (\mu)mol/l</td>
<td>115 (\pm) 6</td>
<td>175 (\pm) 22(^*)</td>
<td>110 (\pm) 14(^†)</td>
</tr>
<tr>
<td>Ra palmitate, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>0.85 (\pm) 0.17</td>
<td>1.45 (\pm) 0.18(^*)</td>
<td>1.24 (\pm) 0.09</td>
</tr>
<tr>
<td>Cl palmitate, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>7.27 (\pm) 1.35</td>
<td>8.95 (\pm) 1.06</td>
<td>13.0 (\pm) 1.8(^*)</td>
</tr>
<tr>
<td>[FFA], (\mu)mol/l</td>
<td>354 (\pm) 30</td>
<td>622 (\pm) 87(^*)</td>
<td>304 (\pm) 41(^†)</td>
</tr>
<tr>
<td>Ra FFA, (\mu)mol·kg(^{-1})·min(^{-1})</td>
<td>2.56 (\pm) 0.51</td>
<td>5.09 (\pm) 0.56(^*)</td>
<td>3.54 (\pm) 0.43</td>
</tr>
<tr>
<td>Palmitate oxidation,(^b)</td>
<td>0.33 (\pm) 0.07</td>
<td>0.54 (\pm) 0.10</td>
<td>0.50 (\pm) 0.09</td>
</tr>
<tr>
<td>%Ra palmitate oxidized(^a)</td>
<td>41 (\pm) 3</td>
<td>35 (\pm) 3</td>
<td>39 (\pm) 6</td>
</tr>
<tr>
<td>Cl palmitate oxidation,(^b)</td>
<td>0.67 (\pm) 0.09</td>
<td>0.83 (\pm) 0.17</td>
<td>0.70 (\pm) 0.13</td>
</tr>
<tr>
<td>%Ra palmitate oxidized(^d)</td>
<td>55 (\pm) 3</td>
<td>53 (\pm) 4</td>
<td>55 (\pm) 8</td>
</tr>
<tr>
<td>FFA oxidation,(^a)</td>
<td>1.37 (\pm) 0.26</td>
<td>2.88 (\pm) 0.54</td>
<td>2.09 (\pm) 0.46</td>
</tr>
<tr>
<td>Ra glycerol, (\mu)mol·kg·min(^{-1})</td>
<td>1.90 (\pm) 0.24</td>
<td>1.20 (\pm) 0.09(^*)</td>
<td>1.16 (\pm) 0.11(^*)</td>
</tr>
<tr>
<td>FFA, (\mu)mol·kg·min(^{-1})</td>
<td>44.7 (\pm) 4.9</td>
<td>21.2 (\pm) 1.2(^*)</td>
<td>26.7 (\pm) 2.4(^*)</td>
</tr>
<tr>
<td>Ra palmitate/Ra glycerol</td>
<td>2.73 (\pm) 0.15</td>
<td>3.07 (\pm) 0.34</td>
<td>2.51 (\pm) 0.29</td>
</tr>
<tr>
<td>Ra FFA/Ra glycerol</td>
<td>0.47 (\pm) 0.10</td>
<td>1.20 (\pm) 0.09(^*)</td>
<td>1.14 (\pm) 0.11(^{†})</td>
</tr>
<tr>
<td>Ra FFA/Ra glycerol</td>
<td>1.42 (\pm) 0.29</td>
<td>4.17 (\pm) 0.26(^*)</td>
<td>3.32 (\pm) 0.46(^*)</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE; n = 10 subjects/group. Ra flux; Cl, clearance; FFA, free fatty acids. Palmitate\(^a\) and FFA oxidation\(^b\) are calculated using recovery of \(^{13}\)CO\(_2\) from NaH\(^{13}\)CO\(_3\) infusion; Palmitate\(^d\) and FFA oxidation\(^b\) are calculated using the "acetate recovery factor" for \(^{13}\)CO\(_2\) (24). %Ra palmitate oxidized\(^a\) and %Ra palmitate oxidized\(^b\) are based on palmitate oxidation\(^a\) and palmitate oxidation\(^b\), respectively. *P < 0.04 vs. control; †P < 0.03 vs. cirrhosis.

Palmitate and total FFA oxidation in cirrhotic subjects determined with either the NaH\(^{13}\)CO\(_3\) or the acetate correction factor did not differ from those of controls, although there was a trend toward increased lipid oxidation in cirrhotic subjects by both methods. Importantly, the fraction of palmitate flux that was oxidized to CO\(_2\) did not differ between cirrhotic and control subjects with either correction factor. The acetate correction factor produced a higher fractional oxidation rate than the NaH\(^{13}\)CO\(_3\) correction factor in both groups.

Effect of liver transplantation on lipid flux and oxidation in vivo. Seven days after OLT, patients had plasma concentrations of palmitate, total FFA, and glycerol that did not differ from those of controls, although there was a trend toward faster flux of both substrates. Clearance of fatty acids by OLT patients was 79% higher than in controls. Glycerol flux and clearance by OLT patients were ~40% slower than those in healthy controls (P < 0.01). The Ra palmitate-to-Ra glycerol ratio in OLT subjects was twofold higher, and the Ra FFA-to-Ra glycerol ratio almost threefold higher, than in controls. This flux relationship was not reflected in the ratio of plasma substrate concentra-
and Ra FFA did not differ from those in preoperative
the ratio of Ra palmitate to Ra glycerol, and the ratio of
oxidized to CO₂, which indicates the relationship be-
 tween fatty acid production and oxidation, did not differ
between groups.
OLT patients had lower plasma concentrations of
palmitate (by 37%), total FFA (by 51%), and glycerol (by
25%) than preoperative cirrhotic patients. R₃ palmitate
and R₃ FFA did not differ from those in preoperative
cirrhotic patients, with lipid flux by OLT patients being
intermediate between that by controls and cirrhotic
patients (Table 4). Clearance of fatty acid by OLT
subjects did not differ from that of preoperative cir-
 rhotic patients, although there was a trend toward
greater clearance in OLT patients. OLT patients oxi-
dized palmitate and total FFA at a rate comparable to
that of preoperative cirrhotic patients, with a similar
fraction of palmitate flux fated to oxidation. R₃ glycerol,
the ratio of R₃ palmitate to R₃ glycerol, and the ratio of
R₃ FFA to R₃ glycerol flux did not differ between OLT
patients and preoperative cirrhotic patients.

DISCUSSION
Our results showed that, although both FFA (palmit-
ate) and glycerol concentrations are elevated in pa-
 tients with end-stage cirrhosis, the kinetics underlying
these alterations differ. Whereas the flux of FFA is
faster, the flux of glycerol is slower than that of
controls. Glycerol flux is a more accurate index of
triglyceride breakdown rate (lipolysis) than palmitate
flux because glycerol, unlike palmitate, cannot be re-
cycled into triglyceride within the adipocyte. The slower
glycerol flux indicates that the rate of lipolysis is slower
in patients with end-stage cirrhosis than in healthy
volunteers; hence their hyperglycerolemia compared
with controls results from decreased glycerol clearance
rather than accelerated lipolysis. On the other hand,
the faster FFA flux in cirrhotic patients, despite a
slower rate of lipolysis, coupled with unimpaired FFA
oxidation indicates that there is a defect in the nonoxi-
dative disposal of FFA. Because nonoxidative disposal
of FFA is reincorporation into triglyceride (reesterifica-
tion), we propose that the cirrhosis-induced defect in
lipid metabolism is not accelerated lipolysis but prob-
ably an impairment of FFA reesterification into tri-
glyceride within the adipocyte. One week after OLT, nor-
malization of plasma palmitate and glycerol concentrations
masks a continued inhibition of lipolysis, as indicated
by the lower glycerol flux compared with controls, and
an apparent impairment of FFA reesterification into
triglyceride.

We measured palmitate flux and oxidation as indices
of FFA metabolism because palmitate kinetics reflect
those of total FFA in healthy volunteers (7) and the
higher plasma palmitate concentration in cirrhotic
subjects parallels that of total FFA (21). Our findings
of elevated plasma concentrations of palmitate and
glycerol in cirrhotic subjects agree with previous observ-
ations (9, 11, 21). Similarly, higher plasma total FFA
concentration in cirrhotic subjects has also been re-
ported previously (18, 21, 23). The somewhat less
marked increase in FFA and glycerol plasma concentra-
tions of our cirrhotic patients may reflect the exclusion
of cigarette smokers—a large percentage of patients
with end-stage cirrhosis—and patients receiving β-
agonist therapy, because nicotine and β-adrenergic stimu-
lation directly stimulate lipolysis (25).
Our flux values for lipid kinetics in healthy volun-
teers are in general agreement with previous reports
for R₃ palmitate (27), R₃ FFA (11), and R₃ glycerol (1, 11,
17). With regard to the kinetics underlying the elevated
plasma palmitate and FFA concentrations of cirrhotics,
the increased R₃ palmitate in the present study is
consistent with the findings of two previous isotope
dilution studies (11, 23). Romijn et al. (23) adminis-
tered a constant infusion of [¹³C]palmitate, and Kaye
et al. (11) used a [¹³C]palmitate infusion protocol similar
to ours. Our finding that glycerol flux is slower in
cirrhotic patients, however, contrasts with the faster
glycerol flux reported by Kaye et al. (11), despite similar
stable isotope tracer protocols. It is possible that the
qualitative difference in glycerol fluxes reflects more
severe liver disease in our patients. All 10 of our
patients had end-stage disease, whereas Kaye et al.
(11) studied five Pugh class A and three Pugh class B
patients. Alternatively, it is possible that either nico-
tine from cigarette smoking or β-adrenergic therapy in
the cirrhotic patients stimulated lipolysis independ-
ently of liver disease in the study by Kaye et al. (11,
25). It is noteworthy that our patients and those of
Kaye et al. exhibited a comparable hyperinsulinemia
relative to healthy controls. Our observation in cir-
rhotic patients that plasma glycerol concentration is
higher than that in controls despite slower glycerol flux
indicates that plasma glycerol concentration is in-
creased because of decreased clearance rather than
increased production. The concomitantly higher plasma
FFA concentration, FFA flux, and FFA oxidation despite
slower glycerol flux indicate that there is a reduced rate
of FFA reesterification within the adipocytes of cir-
rhotic patients.
One week after OLT, fatty acid flux and oxidation are
intermediate between that of patients with end-stage
cirrhosis and healthy controls. Lipolytic rate, as in-
dexed by glycerol flux, is concomitantly slower than
that of healthy volunteers. Together, these findings
indicate that the rate of FFA reesterification within the
adipocyte remains decreased, resulting in a faster FFA
flux. Because the absolute rate of FFA oxidation is not
increased to the same extent as FFA flux in the OLT
patients, more FFA will be available to the liver to be
reincorporated into triglyceride and released into the
circulation in the form of very low-density lipoprotein-
triglyceride. This may explain the hypertriglyceri-
demia reported in OLT patients by Jindal et al. (8) and
The underlying mechanism that impairs FFA reesteri-
fication within the adipocytes of cirrhotic and OLT
patients is unknown. It is possible that the supply of
glycerol phosphate is limited in adipocytes of cirrhotic
subjects, either by decreased availability of dihydroxyacetone secondary to impaired glucose uptake (11, 19) or consequent to a possible defect in glycerol-3-phosphate dehydrogenase activity. Alternatively, either acyl-CoA synthetase or any of the acyltransferase reactions, which catalyze fusion of acyl-CoA with glycerol-3-phosphate, may be impaired. We were surprised by the failure of OLT to reverse the effects of liver disease on lipid metabolism by the seventh postoperative day. Given the present results, it would be of interest to study patients further removed from their transplant surgery, perhaps at 6 or 12 mo postoperatively. It is possible that the immunosuppressive drugs used in OLT patients—cyclosporine, azathioprine, and prednisone—may contribute to the observed derangement in lipid metabolism. Glucocorticoid treatment increases plasma FFA concentration and lipid oxidation (26). Our OLT patients were receiving prednisone 1.5 mg·kg\(^{-1}\)·day\(^{-1}\) at the time of study, as part of a taper protocol that gradually decreased to 5–10 mg/day. The actions of cyclosporine and azathioprine on lipid metabolism is less well understood. After our study was completed, the introduction of tacrolimus for immunosuppression has increased the incidence of post-OLT hypertriglyceridemia relative to that observed with cyclosporine (8). As a practical matter, it is difficult to evaluate the effect of a transplanted liver without the potentially confounding effects of immunosuppressive therapy.

The influence of prior ethanol and other dietary intake on lipid metabolism deserves mention. It had been many months since the subjects in either liver disease group had consumed ethanol, and we feel that the effects of acute ethanol ingestion were not a factor in the present study. What remained in our patients in whom ethanol had been an etiologic factor in developing end-stage liver disease was the liver disease itself rather than an acute nutritional alteration. The previous dietary intake of our preoperative cirrhotic subjects was less than that of controls. Unfortunately, we were unable to document the dietary intake of post-OLT subjects, but we surmise that it too may have been decreased relative to that of controls. It is important to emphasize that all subjects were studied in the postabsorptive state. Nevertheless, it could be argued that the decreased dietary intake of our cirrhotic and post-OLT subjects would mimic a protracted fast that extended the effects of the overnight fasting. However, altered diet would not explain the decreased lipolysis and impaired reesterification observed in our cirrhotic and post-OLT subjects because protracted starvation stimulates rather than inhibits lipolysis, with parallel increases in glycerol and palmitate fluxes (29).

The role of hyperinsulinemia as a mediator of the changes in lipid metabolism observed in cirrhotic and OLT patients is subject to speculation. On the basis of their findings that accelerated lipolysis in cirrhotic patients coincided with hyperinsulinemia, Kaye et al. (11) concluded that cirrhotic patients must be resistant to the antilipolytic action of insulin, but they did not rule out other potential stimulators of lipolysis such as cigarette smoking or \(\beta\)-adrenergic therapy. Insulin inhibits glycerol flux in healthy humans in a dose-dependent manner, such that a modest increase in serum insulin concentration from 9.8 to 14–20 µU/ml suppresses \(R_0\) glycerol by 50% (16). Furthermore, the maximal restraining effect of insulin on lipolysis occurs well within physiological hyperinsulinemia (16). Hyperinsulinemia to the level exhibited by our cirrhotic and OLT patients could theoretically retard lipolysis in these patients relative to the rate in healthy controls. The ability of insulin to inhibit glycerol flux is preserved in obesity, when corrected for fat mass, despite “insulin resistance” of glucose metabolism (1, 22). In contrast, lipolysis is accelerated and peripheral tissue FFA uptake and oxidation are diminished in type 2 diabetic patients without liver disease, despite a well-documented hyperinsulinemia (12, 17). Both plasma concentrations and fluxes of FFA and glycerol decrease in cirrhotic patients during insulin infusion, indicating that at least some of the ability of insulin to inhibit lipolysis is retained (11). More extensive evaluation of the role of insulin as a modulator of lipolysis and FFA oxidation remains to be performed in patients with cirrhosis and after OLT.

Finally, it is clear that plasma glycerol concentration cannot be utilized as an index of lipolytic rate in the setting of liver disease. Glycerol utilization has been considered to occur predominantly in the liver (14). Although glycerol clearance is not the limiting factor in the relationship between its flux and plasma concentration in healthy or injured humans (2), the marked impairment of glycerol clearance secondary to cirrhosis causes an increase in its plasma concentration despite a decreased flux. The extent to which glycerol clearance must be impaired before plasma glycerol concentration no longer reflects glycerol flux remains to be determined. Johnston et al. (10) found a 35% decrease in glycerol clearance in 14 cirrhotic patients, most of whom had less marked liver disease than those in the present study. They further reported an inverse correlation between plasma glycerol concentration and glycerol clearance rate in their cirrhotic subjects. Nosadini et al. (15) reported decreased glycerol clearance in cirrhotic patients when hepatocellular perfusion was compromised either by marked fibrosis or portocaval shunting. It should be pointed out that all 10 of our cirrhotic patients had evidence of portal hypertension and that 2 of them had an indwelling portosystemic shunt at the time of study. The notion that 70–90% of glycerol clearance occurs across the liver has recently been challenged by data from Brunengraber and his associates (13), which indicates that the liver may directly account for only ~30% of whole body glycerol disposal. Further investigation is required to determine the mechanism for, including whether and to what extent extrahepatic tissue contributes to, the decreased whole body glycerol clearance in cirrhosis and after OLT.

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