Effect of liver disease and transplantation on urea synthesis in humans: relationship to acid-base status

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Shangraw, Robert E., and Farook J. Ahoor. Effect of liver disease and transplantation on urea synthesis in humans: relationship to acid-base status. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G1145–G1152, 1999.—It has been suggested that hepatic urea synthesis, which consumes HCO₃⁻, plays an important role in acid-base homeostasis. This study measured urea synthesis rate (Ra urea) directly to assess its role in determining the acid-base status in patients with end-stage cirrhosis and after orthotopic liver transplantation (OLT). Cirrhotic patients were studied before surgery (n = 7) and on the second postoperative day (n = 11), using a 5-h primed-constant infusion of [¹⁵N₂]urea. Six healthy volunteers served as controls. Ra urea was 5.05 ± 0.40 (SE) and 3.11 ± 0.51 µmol·kg⁻¹·min⁻¹, respectively, in controls and patients with cirrhosis (P < 0.05). Arterial base excess was 0.6 ± 0.3 meq/l in controls and −1.1 ± 1.3 meq/l in cirrhotic patients (not different). After OLT, Ra urea was 15.05 ± 1.73 µmol·kg⁻¹·min⁻¹, which accompanied an arterial base excess of 7.0 ± 0.3 meq/l (P < 0.001). We conclude that impaired Ra urea in cirrhotic patients does not produce metabolic alkalosis. Concurrent postoperative metabolic alkalosis and increased Ra urea indicate that the alkalosis is not caused by impaired Ra urea. It is consistent with, but does not prove, the concept that the graft liver responds to metabolic alkalosis by augmenting Ra urea, thus increasing HCO₃⁻ consumption and moderating the severity of metabolic alkalosis produced elsewhere.

LIVER IS THE PREDOMINANT source of urea production in the body. Urea synthesis consumes HCO₃⁻ produced from substrate metabolism, with a stoichiometric relationship described as

\[ 2 \text{NH}_4^+ + 2 \text{HCO}_3^- \rightarrow \text{urea} + \text{CO}_2 + 3 \text{H}_2\text{O} \]

The relationship between urea synthesis and acid-base balance remains controversial. Atkinson and Bourke (1) proposed that hepatic urea synthesis plays an important role in acid-base homeostasis, comparable to that of the lungs and the kidneys. The experimental basis to support this proposal is based, in part, on data from cirrhotic patients. It is well documented that urea synthetic capacity is impaired in the patient with end-stage liver disease. Severe liver disease causes hyperammonemia because hepatic capacity to synthesize urea via the urea cycle is impaired (25, 28, 34, 41). Haussinger et al. (21) correlated the degree of urea synthetic impairment in vitro with the magnitude of metabolic alkalosis in 28 patients with cirrhosis. This led to the implication of decreased urea synthesis in vivo as a cause of metabolic alkalosis in cirrhotic patients (7, 21). However, metabolic alkalosis is not consistently observed in patients with end-stage liver disease. Moreau et al. (33) demonstrated a respiratory, but not metabolic, alkalosis in 105 patients with a spectrum of liver disease severity from mild to end stage. Furthermore, we found no evidence of metabolic alkalosis in 66 consecutive patients with end-stage liver disease presenting for orthotopic liver transplantation (OLT; see Ref. 44).

Others, using indirect methods, have not detected a decrease in basal urea synthesis by cirrhotic patients (13). Fujii et al. (16) reported no decrease in urea synthesis from [¹⁵N₄]Cl in cirrhotic patients until the disease had reached end stage. One problem with the approach used by Fujii et al., however, is that their calculation of urea synthesis depends on uncompromised urinary excretion of urea (16). Hence, the calculated urea synthesis rate can be inaccurate if renal function is compromised, a distinct possibility in cirrhotic patients (6). The results can also be affected by changes in amino acid metabolism, since almost 80% of [¹⁵N] from intravenously administered ammonium is first incorporated into synthesis of nonessential amino acids (37). An association whereby alterations in urea synthesis in vivo affects acid-base balance in humans has not been reported.

Conversely, primary alterations in acid-base balance may affect urea synthesis. Intravenous NaHCO₃ administration in rats increases, and HCl decreases, urinary urea excretion (4). Several investigators (2, 20, 26) reported that NaHCO₃ stimulates urea synthesis by incubated liver, although this has not been uniformly observed (18). A marked metabolic alkalosis occurs early after OLT, with a peak magnitude on the second postoperative day, and persists for several days (44). Its etiology is unclear but does not appear to be related to clearance of plasma lactic acid or citrate from blood transfusion because these substrates return to baseline concentrations by the first postoperative day (10, 44). Metabolic alkalosis occurs despite evidence of good graft liver function, as assessed by plasma concentrations of coagulation factors and prealbumin, as well as hepatic substrate clearance. The present treatment is to inhibit carbonic anhydrase with acetazolamide and,
rarely, by HCl infusion to maintain a stable arterial pH. This therapy, however, may not address the underlying problem and may even exacerbate it, as both acetazolamide and HCl decrease urea synthesis (4, 20, 40). If the defect in OLT patients is compromised urea synthesis, steps could instead be taken to stimulate ornithine cycle activity.

The relationship between amino acid metabolism and urea synthesis has been reviewed (31). However, there have been no reports of the relationship between urea synthesis in vivo and acid-base balance in the presence of either end-stage liver disease or a graft liver. The purpose of this study was to measure urea synthesis directly in humans to determine the relationship between urea synthesis and acid-base balance in patients with end-stage liver disease and in patients after OLT with an apparently well-functioning graft liver.

MATERIALS AND METHODS

Subjects. This study was approved by the Institutional Review Boards at Oregon Health Sciences University (OHSU) and the Portland, Oregon, Veterans Affairs Medical Center (VAMC). Eighteen patients with stable end-stage cirrhosis were enrolled after written informed consent. Seven of these patients were studied while awaiting OLT. Inclusion criteria for cirrhotic patients were end-stage liver disease, hemodynamic stability, no history of insulin-dependent diabetes mellitus, and adequate renal function as determined by plasma creatinine concentration <1.4 mg/dl. No subject had gross evidence of ascites, required recent paracentesis, or had other gastrointestinal disturbances such as nausea, vomiting, or bleeding. All patients were receiving lactulose and diuretic therapy consisting of furosemide and spironolactone. None was receiving steroids or α-adrenergic agonists or antagonists. Severity of liver disease was assessed by the Pugh-Childs scoring system (38). The remaining 11 patients were studied on the second day after OLT. The second postoperative day was chosen because it is the time of peak metabolic alkalosis, and patients generally were exhuasted, hemodynamically unstable, required no blood product transfusion, and were ready for transfer from the intensive care unit to the ward. Additional criteria for postoperative patients, beside those for cirrhotic preoperative patients, were evidence of good graft liver function as indicated by decreasing plasma aspartate aminotransferase, alanine aminotransferase, and bilirubin concentrations, and no blood product transfusion requirement for 24 h. Six healthy individuals without clinical or laboratory evidence of liver disease were also enrolled as controls. Postoperative OLT subjects were studied in the VAMC Surgical Intensive Care Unit, whereas healthy control and preoperative cirrhotic subjects were studied in the OHSU Clinical Research Center. On the evening before the isotope infusion protocol, food intake was 18.58 ± 2.13 (SE) cal/kg for controls and 18.33 ± 1.88 cal/kg for cirrhotic subjects, of which the protein intake was 0.81 ± 0.09 and 0.71 ± 0.11 g/kg, respectively (not different). All subjects were awake, alert, and postabsorptive for at least 8 h, during which time they received no intravenous glucose or other sources of calories. Screening arterial and venous blood samples were drawn before the start of the stable isotope infusion protocol.

Arterial blood for gas analysis was collected in tubes containing heparin and was analyzed immediately. Blood for assessment of coagulation status, collected in tubes containing sodium citrate, was also analyzed immediately. Samples for NH₃ analysis, collected in chilled tubes containing EDTA, were analyzed within 15 min of collection. Samples for determination of plasma concentrations of urea and glucose, and plasma isotopic enrichment of urea and ketoisocaproic acid (KIC), were collected in chilled tubes containing lithium heparin. Samples for analysis of electrolytes, total CO₂ content, and creatinine concentrations were collected in 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. For postoperative patients, an indwelling central venous catheter was used for isotope infusion, and an indwelling radial arterial catheter was used for blood sampling.

After baseline blood sampling, a 5-h-primed constant infusion of stable isotope tracers was begun. [15N₂]urea (99% 15N; Cambridge Isotope Laboratories, Woburn, MA) was infused in all subjects at 0.135 µmol·kg⁻¹·min⁻¹. An 81 µmol/kg [15N₂]urea prime was used for control and postoperative OLT subjects. Preliminary studies in seven subjects indicated that a [15N₂]urea prime of 81 µmol/kg was inadequate in cirrhotic patients because it failed to obtain a plateau in plasma isotopic enrichment in any of the seven subjects (Fig. 1). Increasing the [15N₂]urea prime for cirrhotic subjects to 162 µmol/kg produced a static-state plateau for urea isotopic enrichment without altering plasma urea concentration. Control and postoperative subjects were also infused with [1-13C]leucine (99% 13C; Cambridge Isotope Laboratories) at 0.12 µmol·kg⁻¹·min⁻¹ with a prime of 7.2 µmol/kg. Blood was collected at 210, 240, 270, and 300 min of tracer infusion and centrifuged immediately, and the plasma was stored at −70°C until isotope analysis.

Analytical procedures. Arterial blood gases were determined using an automated analyzer (model 1312 blood gas manager; Instrumentation Laboratories, Lexington, MA). Whole blood base excess was calculated using the Siggaard-Andersen alignment nomogram (Radiometer, Copenhagen, Denmark). Plasma NH₃ concentration was assayed by glutatione dehydrogenase using an autoanalyzer (model 917; Boehringer Mannheim/Toshiba, Indianapolis, IN; see Ref. 8).

![Fig. 1. Plasma isotopic enrichment in patients with end-stage cirrhosis during a 5-h-primed constant infusion of [15N₂]urea at 0.135 µmol·kg⁻¹·min⁻¹. Patients received a [15N₂]urea prime of either 81 µmol/kg (○) or 162 µmol/kg (●) at time 0. Isotopic enrichment was determined by gas chromatography-mass spectrometry. Each value is a mean of 7 patients ± SE.](http://ajpgi.physiology.org/doi/10.1210/jc.1998-0819)
Venous plasma concentrations of electrolytes, total CO₂, glucose, urea, and creatinine were measured using autoan-
ylyzers (model CX3 and glucose analyzer 2; Beckman Instruments, Brea, CA).

Plasma for urea isotopic enrichment was deproteinized with 1 N acetic acid, and the urea was isolated from plasma
by ion exchange chromatography (Dowex 50W-X8, 100–200
mesh; Bio-Rad Laboratories, Richmond, CA) and dried. Se-
parated urea was esterified by reacting it with a mixture of
acetyl chloride and distilled propanol (1:5 vol/vol) at 110°C
for 20 min. The propyl ester was cooled, evaporated, and reacted
with heptafluorobutyric anhydride (Sigma Chemical, St.
Louis, MO) at 60°C for 20 min to form the n-propyl ester
heptafluorobutyramide derivative. This derivative was dried
under N₂ and reconstituted with 400 µl ethyl acetate. The
heptafluorobutyramide derivative was measured by negative chemical ionization gas
chromatography-mass spectroscopy (NCI-GC/MS, models
5890e/5989; Hewlett-Packard, Fullerton, CA), with selective
monitoring of ions at mass-to-charge ratios (m/z) 236, 237,
and 238. "Intracellular" leucine isotope ratio was determined by
measuring the isotope ratio of plasma α-KIC (the transami-
nation product of leucine) by NCI-GC/MS, on the pentafluoro-
benzyl derivative of KIC, with selective ion monitoring at m/z
129 and 130 (17).

Calculations. Urea production rate (Rₐ urea) was calcu-
lated as

\[ Rₐ \text{ urea} = \frac{IE_{\text{infusate}}}{IE_{\text{plateau}}} \times F \]

where F is the infusion rate of tracer urea (µmol·kg⁻¹·min⁻¹),
IEₐ is the isotopic enrichment of the infused tracer urea
(mole % excess), and IE₂ is the isotopic enrichment of
plasma urea (mole % excess) at isotopic steady state (24).
A similar calculation was used for Rₐ leucine, using plasma
KIC, rather than leucine, isotopic enrichment (19).

To correct for the possible confounding effect of hypoalbu-
mimia on acid-base balance (29), we also evaluated acid-
base balance using the Stewart physicochemical approach, as
modified by Fencl and Leith (14). Apparent strong ion differ-
ence (SIDapparent) was calculated as the sum of electrolyte
charges (14)

\[ \text{SIDapparent (meq/l)} = [\text{Na}^+] + [\text{K}^+] + [\text{Ca}^{2+}] + [\text{Mg}^{2+}] - [\text{Cl}^-] \]

Effective strong ion difference (SIDeffective; see Ref. 14) was
calculated as the sum of charges on buffers, i.e., HCO₃⁻ and
weak nonvolatile acids (albumin and phosphate), using a net
charge of −2.6 meq/g for albumin (15) and −1.8 mmol/mmol
for inorganic phosphorus based on the equilibrium between
HPO₄²⁻ and H₂PO₄⁻ (46). SIDeffective was determined according
to the equation (14)

\[ \text{SIDeffective (meq/l)} = [\text{HCO}_3^-] + [\text{albumin (g/l } \cdot 2.6)] \\
+ [\text{inorganic phosphate (mmol/l } \cdot 1.8)] \]

Unidentified anions were calculated as the charge difference
between SIDapparent and SIDeffective (14).

Statistical analysis. Data are expressed as means ± SE.
One-way ANOVA with Tukey’s post hoc test was used to
compare Rₐ urea and base excess among groups. Student’s
t-test was used to compare postoperative Rₐ leucine values
with those of controls. Statistical tests were performed using
a specialized software program (Crunch 4; Crunch Software,
Oakland, CA). Differences were considered statistically signifi-
cant at P < 0.05. Six subjects per group for the comparison of
Rₐ urea values provided statistical power (1 − β) of 0.8 to
detect a difference of 2.0 µmol·kg⁻¹·min⁻¹ between groups,
with α = 0.05, given mean and SD values of 4.7 and 1.1
µmol·kg⁻¹·min⁻¹, respectively, in healthy human volunteers
(9, 19, 24).

RESULTS

Baseline characteristics, electrolytes, and acid-base
balance. The control group, consisting of four men and
one woman, averaged 32 ± 3 yr, weighed 68.9 ± 4.4 kg,

Table 1. Subject demographic characteristics

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Liver Pathology</th>
<th>Pugh-Childs Score</th>
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<tbody>
<tr>
<td>1</td>
<td>48</td>
<td>M</td>
<td>89.0</td>
<td>27.3</td>
<td>ETOH cirrhosis</td>
<td>10</td>
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<tr>
<td>2</td>
<td>41</td>
<td>M</td>
<td>89.1</td>
<td>30.1</td>
<td>ETOH/HCV cirrhosis</td>
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<tr>
<td>3</td>
<td>53</td>
<td>M</td>
<td>88.4</td>
<td>29.5</td>
<td>HCV cirrhosis</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>M</td>
<td>59.6</td>
<td>19.8</td>
<td>ETOH/HCV cirrhosis</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>31</td>
<td>M</td>
<td>94.0</td>
<td>29.7</td>
<td>ETOH/HCV cirrhosis</td>
<td>7</td>
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<tr>
<td>6</td>
<td>57</td>
<td>F</td>
<td>56.4</td>
<td>18.8</td>
<td>ETOH cirrhosis</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>47</td>
<td>F</td>
<td>63.6</td>
<td>23.3</td>
<td>Cryptogenic cirrhosis</td>
<td>8</td>
</tr>
<tr>
<td>Average</td>
<td>47 ± 3*</td>
<td>5/2</td>
<td>77.1 ± 5.2</td>
<td>25.5 ± 1.7</td>
<td>Cryptogenic cirrhosis</td>
<td>9 ± 1</td>
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</table>

OLT, postoperative

<table>
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<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Liver Pathology</th>
<th>Pugh-Childs Score</th>
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<tr>
<td>1</td>
<td>48</td>
<td>F</td>
<td>56.4</td>
<td>20.7</td>
<td>Primary biliary cirrhosis</td>
<td>10</td>
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<tr>
<td>2</td>
<td>45</td>
<td>M</td>
<td>90.9</td>
<td>30.4</td>
<td>ETOH cirrhosis</td>
<td>8</td>
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<tr>
<td>3</td>
<td>35</td>
<td>M</td>
<td>79.6</td>
<td>26.6</td>
<td>Primary sclerosing cholangitis</td>
<td>7</td>
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<tr>
<td>4</td>
<td>43</td>
<td>F</td>
<td>75.0</td>
<td>28.2</td>
<td>Primary biliary cirrhosis</td>
<td>9</td>
</tr>
<tr>
<td>5</td>
<td>51</td>
<td>F</td>
<td>48.7</td>
<td>19.8</td>
<td>Cryptogenic cirrhosis</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>F</td>
<td>64.9</td>
<td>18.3</td>
<td>Cryptogenic cirrhosis</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>M</td>
<td>54.8</td>
<td>19.1</td>
<td>HBV cirrhosis</td>
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</tr>
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<td>8</td>
<td>53</td>
<td>M</td>
<td>58.5</td>
<td>22.0</td>
<td>ETOH/HCV cirrhosis</td>
<td>12</td>
</tr>
<tr>
<td>9</td>
<td>45</td>
<td>M</td>
<td>67.5</td>
<td>28.2</td>
<td>ETOH/HCV cirrhosis</td>
<td>12</td>
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<td>10</td>
<td>44</td>
<td>M</td>
<td>80.6</td>
<td>27.2</td>
<td>Cryptogenic cirrhosis</td>
<td>10</td>
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<tr>
<td>11</td>
<td>46</td>
<td>M</td>
<td>86.0</td>
<td>27.8</td>
<td>ETOH/HCV cirrhosis</td>
<td>11</td>
</tr>
<tr>
<td>Average</td>
<td>45 ± 2*</td>
<td>7/4</td>
<td>71.5 ± 4.0</td>
<td>24.6 ± 1.1</td>
<td>ETOH/HCV cirrhosis</td>
<td>10 ± 1</td>
</tr>
</tbody>
</table>

Averages are means ± SE. M, male; F, female; BMI, body mass index; ETOH, ethanol; HCV, hepatitis C viral infection; HBV, hepatitis B viral
infection; OLT, orthotopic liver transplantation. Pugh-Childs score (38) was from time of preoperative evaluation. *P < 0.001 vs. controls.
Table 2. Biochemical parameters

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 6)</th>
<th>Cirrhosis (n = 7)</th>
<th>OLT (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin, g/dl</td>
<td>4.5 ± 0.1</td>
<td>3.3 ± 0.2*</td>
<td>3.2 ± 0.1*</td>
</tr>
<tr>
<td>Bilirubin, mmol/l</td>
<td>12.0 ± 1.7</td>
<td>68.4 ± 25.6*</td>
<td>136.8 ± 27.4*</td>
</tr>
<tr>
<td>Prothrombin time, s</td>
<td>12.2 ± 0.3</td>
<td>14.8 ± 0.3*</td>
<td>15.9 ± 0.5*</td>
</tr>
<tr>
<td>Na⁺, meq/l</td>
<td>139 ± 1</td>
<td>136 ± 1*</td>
<td>142 ± 1†</td>
</tr>
<tr>
<td>K⁺, meq/l</td>
<td>4.1 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>3.8 ± 0.1†</td>
</tr>
<tr>
<td>Ca²⁺, mmol/l</td>
<td>2.35 ± 0.02</td>
<td>2.26 ± 0.07</td>
<td>2.13 ± 0.05*</td>
</tr>
<tr>
<td>Mg²⁺, meq/l</td>
<td>2.01 ± 0.02</td>
<td>1.84 ± 0.09*</td>
<td>1.81 ± 0.05*</td>
</tr>
<tr>
<td>Cl⁻, meq/l</td>
<td>105 ± 1</td>
<td>106 ± 2</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>Phosphate, mmol/l</td>
<td>6.8 ± 0.1</td>
<td>6.3 ± 0.1</td>
<td>6.3 ± 0.1†</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>4.73 ± 0.17</td>
<td>5.28 ± 0.55</td>
<td>9.35 ± 0.50*</td>
</tr>
<tr>
<td>Creatinine, mmol/l</td>
<td>49.7 ± 1.0</td>
<td>49.7 ± 1.0</td>
<td>49.7 ± 1.0*</td>
</tr>
<tr>
<td>Total CO₂, mmol/l</td>
<td>59.6 ± 1.5</td>
<td>59.6 ± 1.5</td>
<td>59.6 ± 1.5</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3†</td>
</tr>
<tr>
<td>Arterial blood gases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.41 ± 0.01</td>
<td>7.43 ± 0.02</td>
<td>7.48 ± 0.01†</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>38.6 ± 2.0</td>
<td>37.2 ± 2.1</td>
<td>42.2 ± 1.3†</td>
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<tr>
<td>HCO₃⁻, mmol/l</td>
<td>24.4 ± 0.7</td>
<td>22.4 ± 0.4</td>
<td>31.6 ± 0.3*</td>
</tr>
<tr>
<td>Base excess, meq/l</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3</td>
<td>0.6 ± 0.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. SID, strong ion difference; UA, unidentified anions. SID and UA definitions are from Ref. 15. *P < 0.05 vs. controls; †P < 0.05 vs. cirrhosis.

and had a body mass index of 22.8 ± 1.2 kg/m². Table 1 shows demographic characteristics of the two patient groups. The most common etiology for cirrhosis in the 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 body mass index. Cirrhotic patients did not differ from OLT patients in any demographic characteristic, including the severity of liver disease immediately before OLT.

Table 2 lists screening laboratory and arterial blood gas values. Compared with controls, cirrhotic patients were hyponatremic, hypoalbuminemic, hyperbilirubinemic, and coagulopathic (as evidenced by prolonged prothrombin time). There was no indication of an acid-base disturbance in cirrhotic patients, as indexed by either arterial blood base excess or total venous CO₂ content. Postoperative OLT patients also exhibited hypoalbuminemia, hyperbilirubinemia, and coagulopathy compared with controls. OLT patients exhibited a marked metabolic alkalosis, as indexed by both arterial blood base excess and total venous CO₂ content (P < 0.01). When OLT patients were compared with preoperative cirrhotics, they exhibited a similar coagulopathy and hyperbilirubinemia, but arterial base excess was markedly higher in the OLT patients. Immediate preoperative values for OLT patients (plasma albumin 3.1 ± 0.1 g/dl, arterial pH 7.43 ± 0.01, arterial PCO₂ 34.9 ± 1.4 mmHg, arterial HCO₃⁻ 22.8 ± 0.8 mmol/l, and arterial base excess -0.6 ± 0.7 meq/l) were comparable to those for cirrhotic subjects. Thus metabolic alkalosis in postoperative OLT subjects was not present preoperatively.

Strong ion difference in cirrhotic and postoperative OLT patients. To determine whether arterial acid-base balance was artificially affected by hypoalbuminemia in both cirrhotic and OLT patients, the SID was compared with that in controls (Table 2). Compared with controls, the apparent SID was decreased in cirrhotic patients and increased in OLT patients (P < 0.02). Effective SID, composed of the buffers HCO₃⁻, albumin, and phosphate, was decreased in cirrhotic patients (P < 0.001) but not different from controls in the OLT group. Compared with preoperative cirrhotics, OLT patients showed a marked increase in apparent SID and an increased effective SID (P < 0.001). There was no difference in unidentified anion concentration among the three subject groups. No physicochemical evidence was found for an acid-base disturbance in cirrhotic patients vs. controls. In contrast, the metabolic alkalosis in postoperative OLT patients exceeded that which can be accounted for on the basis of hypoalbuminemia.

Effect of cirrhosis and liver transplantation on urea synthesis and plasma concentration. Plasma urea isotopic enrichment reached a steady-state plateau in all three groups. Rₜ urea and plasma urea concentrations in controls and cirrhotic and OLT patients are shown in Fig. 2. Rₜ urea in cirrhotic patients was 38% lower than that of controls (P < 0.05, Fig. 2A). Simultaneously, plasma urea concentration in cirrhotic patients, a proxy for urea pool size, did not differ from the control.
value (5.71 ± 1.07 vs. 4.64 ± 0.36 mmol/l, Fig. 2B). OLT patients exhibited an almost threefold greater Ra urea and plasma urea concentration (12.14 ± 1.43 mmol/l) than respective control values (P < 0.001, Fig. 2). When OLT patients were compared with the cirrhotic group, the OLT Ra urea was sixfold greater whereas the plasma urea concentration was only doubled (P < 0.01, Fig. 2). Plasma urea concentration of the OLT subjects taken immediately before surgery (5.7 ± 0.7 mmol/l) did not differ from that of the cirrhotic group.

Effect of cirrhosis and liver transplantation on NH3 concentration and leucine flux. We measured plasma NH3 concentration, using it as a proxy for NH3 supply, to evaluate its possible role in the regulation of Ra urea. Plasma NH3 concentration in cirrhotic patients was twofold higher than the control value (P < 0.02, Fig. 3). Plasma NH3 concentration in the postoperative group did not differ from that of controls and was 42% lower than that in preoperative cirrhotics (P < 0.02). Plasma NH3 concentration in OLT patients taken immediately before surgery (38.7 ± 3.5 mmol/l) did not differ from that in the cirrhotic group.

We measured leucine flux (Ra leucine) in postoperative OLT patients to test whether amino-nitrogen flux was increased despite no change in plasma NH3 concentration. Plasma α-KIC reached an isotopic steady state in both postoperative OLT patients and controls. Ra leucine in postoperative OLT patients was modestly greater than that of controls (Fig. 4).

Fig. 2. Plasma urea concentration (mean ± SE) in controls (n = 6) and in patients 2 days after OLT (n = 11). Subjects received a primed, constant infusion of [1-13C]leucine for 5 h, and Ra leucine was determined by isotope dilution as an index of proteolysis. Values are expressed as means ± SE. *Difference from control value (P < 0.01).

DISCUSSION

Our study showed that urea synthesis is markedly lower in the presence of end-stage cirrhosis and is stimulated early after OLT. In cirrhosis, the degree of urea synthetic impairment is not reflected by the plasma urea concentration. Compared with the control value, the almost 40% slower Ra urea was not associated with a decreased plasma urea concentration. Furthermore, the marked impairment of urea synthesis, which was accompanied by a doubling of plasma NH3 concentration, did not lead to any appreciable disturbance of acid-base balance. It thus appears that Ra urea does not play a major role in regulation of acid-base balance in cirrhotic patients. On the other hand, the higher Ra urea and concomitant metabolic alkalosis of the OLT group indicate that metabolic alkalosis after OLT is not caused by inhibited urea production.

Fencl and others (14, 29) have argued that an accurate description of acid-base balance must account for the plasma concentration of strong electrolytes and weak acids other than HCO3-, i.e., albumin and phosphate. We therefore evaluated acid-base balance using the physicochemical SID, as well as the conventional approach of calculated base excess, to assess acid-base balance. McAuliffe and associates (29) showed that hypoalbuminemia produces an apparent metabolic alkalosis even in the setting of normal HCO3- concentration, due to loss of the buffering capacity of albumin, such that a decrease of 1 g of plasma albumin/dl produces a calculated base excess equivalent to +3.7 meq/l (29). Applying the physicochemical correction to our cirrhotic patient data yields a hypoalbuminemia-induced difference in base excess of 3.7 meq/l × 1.2 g/dl = 4.44 meq (see Table 2). Adding this to our calculated base excess of −1.1 meq yields an effective metabolic acidosis, with a base deficit of 5.5 meq/l, concomitant with a marked increase in urea synthesis. Because our cirrhotic patients did not exhibit metabolic alkalosis regardless of the acid-base approach utilized despite direct evidence of decreased urea synthesis, our data refute the concept that impaired urea synthesis precipitates metabolic alkalosis in cirrhosis.

Metabolic alkalosis consistently occurred in our postoperative OLT patients. When the calculated arterial base excess, 7.0 meq/l, was corrected for hypoalbuminemia using the physicochemical correction factor (29), the result is 7.0 – 4.8 (= 3.7 meq/l × 1.3 g/dl), or +2.2 meq/l. The hypoalbuminemic correction actually over-corrects for the observed change in base excess in our OLT patients because they increased the effective SID to a normal value by increased plasma phosphate and HCO3- concentrations (see Table 2). Our OLT patients nevertheless exhibited metabolic alkalosis regardless of the method used to assess acid-base balance. Urea
synthesis was markedly increased at this time to a rate consistent with maximal stimulation, such as seen after extensive burn injury (23). Metabolic alkalosis in our OLT subjects was not caused by an occult decrease in urea synthesis.

Metabolic alkalosis could be due to increased production or decreased elimination of HCO3-. Increased HCO3- production could occur secondary to increased metabolism of citrate or lactate. Plasma concentrations of both citrate and lactate are increased during OLT, largely due to their high concentrations in transfused blood products and decreased utilization during the preanhepatic and anhepatic stages of the operation (10, 44). Plasma citrate concentration increases 10-fold during the anhepatic period of OLT but decreases to within normal limits by 24 h postoperatively (10). Plasma lactate concentration increases sevenfold during OLT but is also within normal limits by 24 h postoperatively (44). Thus the potential effect of citrate and lactate on postoperative acid-base balance would be even smaller. Stimulated urea synthesis by cirrhotics is refractory to alanine or glucagon infusion. On the other hand, our data are consistent with the results of Fabbri et al. (13), who detected no decrease in urea synthesis by cirrhotics using an indirect method. Their cirrhotic patients, however, had milder disease as a group, only half of whom had end-stage disease (13). Moreover, their indirect method measured the increase in urea concentration in a defined “urea space” that is sensitive to errors in determination of the urea space. This method also assumes that renal urea excretion is unchanged by cirrhosis. Fabbri et al. (13) did find that urea synthesis in cirrhotic patients is refractory to stimulation by alanine or glucagon infusion. On the other hand, our data are consistent with the results of Fujii et al. (16) and Rypins et al. (42). Fujii et al. (16) demonstrated a 60% decrease in urea synthesis from 15NH4Cl in patients with end-stage liver disease. However, assessment of urea synthesis by measuring incorporation of 15NH4Cl into urea is inaccurate for reasons already stated in the introduction. Rypins et al. (42) measured urea synthesis by an unprimed constant infusion of [14C]urea and reported a 30% decrease in urea synthesis in OLT patients (Fig. 1).

Urea pool size is determined by the difference between the rate of urea synthesis and its rate of disposal via renal excretion plus colonic hydrolysis and salvage of urea (30). A plasma urea concentration in our cirrhotics that did not differ from that of healthy controls, despite a slower synthetic rate, could be explained by either a decreased volume of distribution or impaired disposal of urea. Our failure to adequately label the body urea pool in cirrhotics using a standard priming dose indicates that the volume of distribution for urea in cirrhotics is expanded rather than contracted. This suggests a marked decrease in urea disposal by cirrhot-
ics, coincident with decreased synthesis. Meakins and Jackson (30) evaluated the relationship between diet-related changes in urea synthesis and urea disposal. When they decreased dietary protein intake from adequate, 0.90 g·kg$^{-1}$·day$^{-1}$, to inadequate, 0.39 g·kg$^{-1}$·day$^{-1}$, the consequent ~50% decrease in urea production (from 5.2 to 2.9 µmol·kg$^{-1}$·min$^{-1}$) in turn decreased plasma urea concentration and urea pool size by only ~10% (30). Urea pool size and plasma concentration were conserved secondary to a 33% decrease in renal urea excretion during the protein-deficient diet (30). Urea production rates and corresponding plasma urea concentrations reported by Meakins and Jackson (30) for the healthy subjects on the adequate and inadequate protein intakes are almost identical to those of the controls and cirrhotic patients in the present study (see Fig. 2). Together these findings suggest that a physiological decrease in renal urea excretion in response to a marked decrease in urea production occurs to maintain urea pool size. That is, renal urea clearance may be decreased despite adequate renal function in the cirrhotic patients.

It is tempting to speculate that the adaptation to maintain urea pool size may be necessary to facilitate movement of urea into the colon for hydrolysis and salvage. However, such a notion was ruled out by Meakins and Jackson (30), who showed that the rate of urea hydrolysis decreased by 60% on the inadequate protein diet, despite maintenance of urea pool and plasma concentration near control values. There is also the possibility that renal urea elimination is pathologically compromised in cirrhotic subjects despite their normal plasma creatinine concentration. Caregaro et al. (6) found a markedly decreased creatinine clearance in cirrhotics despite a normal plasma creatinine concentration. Even creatinine clearance greatly underestimated true renal dysfunction, compared with inulin clearance, in cirrhotic patients (6). Subclinical renal dysfunction may mask the loss of urea production by limiting renal elimination and maintaining an unchanged plasma urea concentration. A limitation of the present study is that we did not undertake a more precise measure of renal function.

Slower urea synthesis could result from either a reduction in dietary protein intake or a defect in hepatic synthetic capacity. It is unlikely that the slower urea synthetic rate in our cirrhotic patients was due to a lower intake of protein as the intake of the cirrhotic patients on the evening before the study, 0.71 g/kg, did not differ from the 0.81 g/kg of healthy controls. Langran et al. (27) showed that, in healthy adults on a similar protein intake, 1 g·kg$^{-1}$·day$^{-1}$, the rate of urea production, 4.81 µmol·kg$^{-1}$·min$^{-1}$, was almost identical to the 5.0 µmol·kg$^{-1}$·min$^{-1}$ of our controls. Furthermore, there was only a modest decrease (~10%) in urea production when dietary protein intake was decreased from 1 to 0.5 g·kg$^{-1}$·day$^{-1}$ (27). In a similar study, Meakins and Jackson (30) showed that urea production decreased significantly only when dietary protein intake fell to 0.39 g·kg$^{-1}$·day$^{-1}$. Hence it is unlikely that the 40% lower urea production rate by cirrhotic patients was due to less dietary protein intake. Protein catabolism, as indexed by leucine flux, is either unchanged (32, 43) or modestly increased (45) in cirrhotic subjects. On the other hand, the concomitant elevation in plasma NH$_3$ concentration indicates that urea synthesis was likely compromised due to an ornithine cycle defect. Our finding of a twofold increased plasma NH$_3$ concentration in cirrhosis is comparable to that previously reported in patients with stable end-stage liver disease (32, 36).

Increased leucine flux in our postoperative OLT patients is comparable to that exhibited by patients with sepsis or after severe burn injury (23). However, our OLT patients showed no concomitant increase in plasma NH$_3$ concentration. Plasma NH$_3$ concentration is reported to normalize within 24 h after OLT, possibly even before the patient leaves the operating room, in the setting of good graft liver function (11, 36, 39). It is important to distinguish the meaning of increased leucine flux (or proteolysis) in the postoperative patient, because there are other routes for liberated amino acids besides oxidation and release of the NH$_3$ group to aspartate or glutamine and, ultimately, urea. One important pathway for the extra amino acids released by stress-induced proteolysis is reincorporation into newly synthesized protein, e.g., the acute-phase proteins, a pathway not quantitated in the present study.

To summarize, first, there is a marked impairment of urea synthesis in patients with cirrhosis that is not reflected by a parallel decrease in plasma urea nitrogen concentration. This defect does not appear to affect acid-base balance. Second, urea synthesis by the well-functioning graft liver is not inhibited. Instead, stimulated urea production early after OLT appears to be a physiological response to increased HCO$_3^-$ supply, possibly attenuating the severity of the alkalosis.

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