Amino Acid Kinetics During the Anhepatic Phase of Liver Transplantation

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Alanine and glutamine are interorgan nitrogen/carbon carriers for ureagenesis and gluconeogenesis, which are mainly but not necessarily only hepatic. The liver is central to alanine and glutamine metabolism, but most organs can produce and use them. We studied amino acid kinetics after liver removal to depict initial events of liver failure and to provide a model to study extrahepatic gluconeogenesis and nitrogen disposal in humans. We measured amino acid kinetics with [5,5,5-$^{2}H_3$] leucine and [3-^{13}C]alanine or [1,2-$^{13}C_2$]glutamine tracers in 21 subjects during and after the anhepatic phase of liver transplantation: 12 were at 7 months posttransplantation, and 7 were healthy control subjects. Anhepatic leucine kinetics, including proteolysis, was unchanged. Alanine plasma and whole-body contents increased 3× and 2×, with a halved metabolic clearance and a doubled production, 2% greater than disposal. Free whole-body glutamine decreased 25% but increased 50% in plasma. Glutamine clearance was halved, and the production decreased by 25%, still 2% greater than disposal. Liver replacement decreased alanine and glutamine concentrations, leaving leucine unchanged. Liver removal caused doubled alanine fluxes, minor changes in glutamine, and no changes in leucine. The initial events after liver removal are an accumulation of three-carbon compounds, an acceleration of alanine turnover, and limited nitrogen storage in alanine and glutamine. *Diabetes* 51:1690–1698, 2002

During liver transplantation, there is a time lag in which the recipient's liver is removed and the donor's liver has not yet replaced it. The anhepatic phase of liver transplantation offers a unique surgical model to investigate in humans complex physiological questions and to depict the initial events after liver failure. The liver is important to ureagenesis and gluconeogenesis. Alanine and glutamine are important carbon and nitrogen donors for these processes because in order to oxidize amino acids, the muscle transfers the amino-nitrogen to pyruvate, α-keto glutarate, and glutamate (1) and exports a load of alanine and glutamine that is disposed mainly but not exclusively in the liver to synthesize urea and glucose. The resulting alanine (or glutamine)-glucose cycle is in turn critical to provide with glucose carbons those tissues that need to oxidize amino acids but cannot dispose their nitrogen in a nontoxic form (2–4).

The interorgan transport is a first complication of glutamine and alanine metabolism. For example, glutamine may be converted to alanine in the gut before utilization in the liver (5,6). Because of its anatomic position, to measure the liver contribution to alanine and glutamine metabolism would require an invasive transhepatic balance, sampling blood and measuring the flow rates in the sovrahepatic veins, the portal vein, and an arterial vessel. When the sovrahepatic (but not portal) veins were catheterized, the contribution of the liver could not be dissected from that of the gut. Thus, the relative importance of the liver and the other organs in alanine and glutamine metabolism remains unclear in humans. A second complication is that the two amino acids can be simultaneously removed and produced in the same organ. Anatomic structures designed to couple glutamine/alanine catabolism and nitrogen disposal on one side and glutamine/alanine synthesis and nitrogen scavenging on the other were found in the liver with its periportal and perivenous hepatocytes (7), in the kidney (8), and in the brain, where neurons use glutamine and release alanine and astrocytes remove alanine and synthesize glutamine (9). In addition, several tissues can express to some degree glucose-6-phosphatase (10,11), and, in theory, they could release in the systemic circulation the glucose produced from alanine or glutamine gluconeogenesis.

Glutamine and alanine metabolism in humans is largely involved in vital functions such as the control of acid-base equilibrium in the kidney (8), the neurotransmitter metabolism in the brain (12), or gluconeogenesis (13). The clinical consequences of liver failure reflect this complexity. Glutamine accumulation in astrocytes causes part of the brain damage in hepatic encephalopathy (14). During acute liver failure, the glutamine concentration is much more increased than that of alanine, suggesting that the muscle produces glutamine from alanine to scavenge ammonia (15). Because the muscle normally releases alanine, it is unclear whether muscle alanine removal may represent an adaptive change in the glutamine synthetic pathway triggered by the alanine and the ammonia accumulation after liver failure. Despite the physiological and clinical importance of the topic, the differential effects of

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GCMS, gas chromatography-mass spectrometry; PCR, plasma clearance rate; R_a, rate of appearance; R_d, rate of disappearance.

TABLE 1
Characteristics of the subjects

	ANHEP subjects preintervention	POST subjects	CON subjects
n	21	12	7
Age (years)	52 ± 2	52 ± 2	35 ± 3
Weight (kg)	69.1 ± 1.9	69.6 ± 2.5	66.8 ± 5.2
Height (cm)	169 ± 1	169 ± 1	170 ± 2
Platelets (1,000/mm^3)	110 ± 30	—	—
Albumin (g/l)	3.7 ± 0.1	4.5 ± 0.3	5.1 ± 0.5
Bilirubin total (mg/100 ml)	1.9 ± 1.3	1.2 ± 0.3	0.6 ± 0.1
Prothrombin time (%)	74 ± 5	96 ± 6	100 ± 6
Child-Pugh classification			
No cirrhosis	34	—	—
Class A	40	—	—
Class B	13	—	—
Class C	13	—	—

Data are means ± SE, unless otherwise specified.

liver failure on alanine and glutamine metabolism are unknown.

The purpose of this study was to evaluate the initial changes in alanine and glutamine metabolism immediately after liver removal in subjects with a previously maintained liver function. To control for the effect of surgery and anesthesia per se, we also measured the changes in amino acid metabolism after the liver replacement, which rendered the metabolic picture similar to that of published studies of intraoperative amino acid metabolism. We found that without the liver, the body can produce and dispose an increased load of gluconeogenic amino acids in the absence of net changes in the proteolytic rate. The first event after liver removal was a marked increment in alanine production, whereas glutamine production and consumption were less affected. Immediately after the liver replacement, the alanine and the glutamine concentrations decreased, whereas the leucine concentrations remained unchanged.

RESEARCH DESIGN AND METHODS

Materials. L-[3-^{13}C]alanine, L-[1,2-$^{13}C_2$]glutamine, and L-[5,5,5-$^2H_3$]leucine were purchased from MassTrace (Woburn, MA). Chemical and isotopic purity of the tracers was determined by gas chromatography-mass spectrometry (GCMS). In many of the experiments described here, D-[6,6-$^2H_2$]glucose and L-[*phenyl*-$^2H_5$]phenylalanine were also infused. The results derived from these infusions are reported separately.

Subjects. Table 1 reports the subjects' characteristics. Twenty-one subjects (ANHEP) were studied during liver transplantation for localized hepatocarcinoma (single <5 cm or multiple <2 cm diameter, $n = 16$) (16), primary amyloidosis ($n = 1$), carcinoid ($n = 2$), and hemangioendothelioma ($n = 1$). The last subject underwent two sequential grafts spaced 1 month apart because of the failure of the first one, and he was studied on both occasions. It should be stressed that 75% of the patients had mild cirrhosis or no cirrhosis at all. Accordingly, the albumin and bilirubin concentrations, the prothrombin time, and the number of platelets reflected only a minor impairment in liver function. The isotopes bolus was repeated in 12 of the recipients 7 months after transplantation (POST subjects) and was also administered in 7 healthy control subjects (CON).

Surgical model of the anhepatic phase of liver transplantation. For the purpose of this work, the anhepatic phase began when the recipient's liver was removed and ended when the graft circulation was reestablished by portal unclamping (16). In using this model, the major difficulties are caused by the short duration (45–75 min) of the anhepatic phase and by the changed metabolic environment abruptly induced by the liver removal. We decided to measure the amino acid kinetics by a single tracer injection during the anhepatic phase and not by a primed-continuous infusion commencing before the anhepatic phase. First, with continuous infusions, the time required to reach a tracer steady state is several times greater than the duration of the anhepatic phase itself; thus, the experiment should have begun hours before the anhepatic phase. Considering the possible displacement of body fluids as a result of the surgical procedure (blood losses and replacement with hemoderivatives, and fluid compartmentalization as a result of portal vein and vena cava clamping and unclamping), we doubted that we could have excluded any carryover from the isotopic dilution that existed before the anhepatic phase. Thus, the surgical and the medical teams agreed to limit the duration of the experiment to the anhepatic phase and to warrant in that period a stable hemodynamic condition without blood losses and administration of hemoderivatives.

During the anhepatic phase, the subjects received saline and other electrolytes (Normosol R; Abbott Laboratories, Abbott Park, IL), sevoflurane anesthesia with remifentanil and pancuronium bromide, and, occasionally, calcium chloride, furosemide, and human albumin solutions.

Tracer injection and sampling protocol. At the beginning of the anhepatic phase, two basal samples of arterial blood were drawn 5 min apart. Immediately thereafter, one of the following tracer studies was performed to quantify the leucine, alanine, and glutamine kinetics. The tracer solutions were prepared as previously described (17). Study 1 ($n = 12$) used a bolus of L-[3-^{13}C]alanine and L-[5,5,5-$^2H_3$]leucine (90 and 20 μmol/kg). Study 2 ($n = 9$) used L-[1,2-$^{13}C_2$]glutamine and L-[5,5,5-$^2H_3$]leucine (30 and 20 μmol/kg). The bolus was delivered in a central vein in 20 s, immediately followed by a flush with 15 ml of saline. Arterial blood was drawn at 2, 3, 4, 6, 8, 10, 12, 15, 20, 25, and 30 min after the bolus and every 15 min thereafter until 15 min after the circulation through the grafted liver was reestablished.

The POST subjects (study 1, $n = 9$; study 2, $n = 4$) and CON subjects (study 1, $n = 4$; study 2, $n = 3$) received the same protocol, with the difference that "arterialized" venous blood was drawn up to 150 min after the bolus from a dorsal hand vein cannulated retrogradely and heated in a warming box (17), and that CON subjects received half the alanine dose (45 μmol/kg).

Aliquots of blood were placed in tubes that contained EDTA and stored on ice until the plasma was prepared by centrifugation at 4°C. A 0.5-ml aliquot was withdrawn and frozen at −60°C after the addition of [$^2H_4$]alanine, [$^2H_7$]leucine, and [$^2H_5$]glutamine internal standards. Aliquots for glucagon, catecholamines, insulin, cortisol, and growth hormone were processed as previously described (17). Blood aliquots for the determination of lactate, pyruvate, glycerol, and β-hydroxybutyrate were placed in tubes that contained perchloric acid. All blood samples were placed on ice until the plasma or serum was prepared by centrifugation at 4°C (within 1.5 h of drawing). All plasma and serum aliquots were frozen at −60°C until later analysis.

Analytical methods. Plasma amino acid concentrations and enrichments were measured by electron impact GCMS after derivatization to *tris-t*-butyldimethylsilyl derivative as previously described (17–19). For all measurements, the background corrected tracer enrichments in mole percent excess were calculated as previously defined.

Plasma hormone concentrations were measured by radioimmunoassay with commercial kits as previously described (20). The catecholamine concentrations were measured by a high-performance liquid chromatography method (21). The concentrations of whole-body lactate, pyruvate, glycerol, and β-hydroxybutyrate were performed as previously described (22).

Data analysis. Because the liver uses and produces alanine, glutamine, and leucine, it stands to reason that its removal provokes a brisk change in the volume of distribution, plasma clearance rate (PCR), and rate of appearance (R_a) of these amino acids. The abrupt change in such kinetic parameters forces the plasma concentration of these amino acids to change during the

TABLE 2
Arterial blood hematocrit, pH, hemogasanalysis, and electrolyte concentrations in transplantation patients

	Preintervention	Anhepatic phase	*P* vs. preintervention
Hematrocrit (%)	34.8 ± 1.6	36.6 ± 1.8	
pH	7.43 ± 0.02	7.46 ± 0.02	
P_{O_2} (mm/Hg)	219.0 ± 18.2	227.0 ± 8.3	
P_{CO_2} (mm/Hg)	35.3 ± 1.5	33.4 ± 1.4	
Sodium (mmol/l)	138.6 ± 0.6	140.8 ± 0.9	*
Potassium (mmol/l)	3.6 ± 0.1	3.5 ± 0.1	
Calcium (mmol/l)	0.993 ± 0.037	1.145 ± 0.037	†
Chloride (mmol/l)	107.1 ± 1.0	104.2 ± 0.9	†
Gap anion (mmol/l)	12.4 ± 1.3	16.8 ± 0.9	*

Data are means ± SE. *$P < 0.01$, †$P < 0.05$ vs. ANHEP subjects.

anhepatic phase toward a new steady-state level (dictated by the new ratio R_a/PCR); the rate of disappearance of the amino acid (R_d) changes accordingly. Thus, when the tracer bolus is injected during the anhepatic phase, it can be reasonably assumed that the kinetics and R_a are in a new steady state, whereas the plasma concentration of the amino acid (i.e., the tracee) and R_d are not. All in all, the following conditions take place: the volume of distribution and PCR (and, in more general terms, the kinetics) are constant (and unknown), R_a is constant (and unknown), the measured plasma concentration of the substance (i.e., the tracee) is changing, and R_d is changing (and unknown).

Given the above scenario, we first quantified the volume of distribution and PCR during the anhepatic phase from tracer data using standard noncompartmental techniques (23), then estimated R_a using deconvolution (i.e., input-estimation) techniques (24), and finally derived R_d from the mass-balance equation of the accessible pool (23). Briefly, a two-exponential function was found to be necessary and sufficient to describe satisfactorily the tracer disappearance curve (which, normalized to the administered tracer dose, is the unitary impulse response of the system). The measurement error was assumed white, gaussian, of zero mean, and with experimentally determined standard deviation. Parameter estimation was performed by weighted nonlinear least squares. Weights were chosen optimally, i.e., equal to the inverse of the variance of the experimental error (24). The kinetic parameters that were derived from the analysis of the impulse response were the initial distribution volume (V_1; ml/kg), the PCR ($ml \cdot kg^{-1} \cdot min^{-1}$), the total distribution volume (V_T; $ml \cdot kg^{-1} \cdot min^{-1}$), and the total body mass (Q_T; mmol/kg). R_a (mmol $\cdot$ $kg^{-1} \cdot h^{-1}$) was estimated from the impulse response and tracee data by deconvolution, and R_d was then derived from the equation of the accessible pool: $R_d(t) = R_a - V_1 dc/dt$, where c is the tracee concentration and dc/dt its rate of change during the anhepatic phase. The kinetic analysis of the impulse response of measured in normal and posttransplantation patients followed the same approach outlined above. Because in such groups the tracee was in steady state, calculation of R_a and R_d from the impulse response was straightforward: $R_a = PCR \cdot c$ and $R_d = R_a$. The R_a of alanine and glutamine has two components: *1*) from proteolysis and *2*) from de novo synthesis. The alanine and glutamine release from proteolysis was estimated using the leucine release from protein breakdown, corrected for the ratio of alanine to leucine and glutamine to leucine molar concentrations in body proteins (1.11 and 0.74, respectively). Then, the de novo component of the alanine and glutamine appearance was calculated by subtracting the amount released from protein breakdown from the total alanine and glutamine R_a (17).

Statistical analysis. We used *t* tests for paired data to compare subjects during the anhepatic phase of liver transplantation (ANHEP subjects) and POST subjects and *t* tests for independent data to compare ANHEP and CON subjects, and we applied to both tests the Bonferroni correction. Because we did not find differences among the two control groups (POST and CON subjects), we also presented *t* tests for independent samples comparing ANHEP subjects with the pooled control groups.

RESULTS

Arterial blood hematocrit, pH, blood gas analysis, and electrolyte concentrations. Compared with basal, during the anhepatic phase, there were no significant changes in hematocrit, pH, and oxygen and CO_2 pressures. The patients did not require the administration of bicarbonates to correct pH changes. However, small changes in electrolytes revealed a 30% increment in the anion gap (Table 2).

Hormone concentrations. Both the insulin and the counterregulatory hormone concentrations were increased during the anhepatic phase, as would be expected in a model of severe surgical stress (25). In POST subjects, the hormone concentrations were similar to CON subjects, except for modest hyperinsulinemia (Table 3).

Glucose, lactate, glycerol, free fatty acid, and pyruvate concentrations. During the anhepatic phase, the subjects were hyperglycemic and showed a three to five-fold increment in lactate, glycerol, free fatty acid, and pyruvate concentrations. No significant differences were apparent between POST and CON subjects (Table 4).

Leucine metabolism. Figure 1 shows the natural leucine (upper) and the normalized L-[5,5,5-$^2H_3$]leucine tracer (lower) concentrations during the anhepatic phase and in the control group. The leucine concentration remained stable during the anhepatic phase and similar to that of control group. The liver replacement did not change the leucine concentration within 15 min. During the anhepatic phase, the decay curve of the tracer concentration was similar to the control group. The leucine kinetics was consequently similar, as shown in Table 5. The leucine metabolic clearance was 25% reduced in the absence of

TABLE 3
Hormone concentrations

	ANHEP subjects	POST subjects	*P* vs. ANHEP subjects	CON subjects	*P* vs. ANHEP subjects
Insulin (pmol/l)	345 ± 90	55 ± 7	*	33 ± 5	*†
C-peptide (nmol/l)	1.16 ± 0.19	1.18 ± 0.13		0.72 ± 0.12	†‡
Glucagon (ng/l)	286.6 ± 58.7	91.3 ± 10.1	‡	72.3 ± 2.5	§
Growth hormone (μg/l)	13 ± 3	3 ± 1		1 ± 0	
Cortisol (pmol/l)	1,722 ± 279	162 ± 45	‡	166 ± 32	§
Epinephrine (pmol/l)	2,792 ± 1,070	240 ± 76	*	196 ± 60	*

Data are means ± SE. *$P < 0.01$ vs. ANHEP subjects; †$P < 0.05$ vs. POST subjects; ‡$P < 0.05$, §$P < 0.001$ vs. ANHEP subjects.

TABLE 4
Metabolites concentrations

	ANHEP subjects	POST subjects	*P* vs. ANHEP subjects	CON subjects	*P* vs. ANHEP subjects
Glucose (mmol/l)	7.1 ± 0.4	4.9 ± 0.2	*	4.7 ± 0.1	*
Lactate (μmol/l)	3,865 ± 418	579 ± 65	†	614 ± 129	†
Glycerol (μmol/l)	215.2 ± 25.1	42.3 ± 5.7	*	28.0 ± 3.1	†
Free fatty acids (mmol/l)	1.76 ± 0.10	0.54 ± 0.06	†	0.55 ± 0.11	†
Pyruvate (μmol/l)	235 ± 16	69 ± 13	†	68 ± 8	†

Data are means ± SE. *$P < 0.01$, †$P < 0.001$ vs. ANHEP subjects.

the liver, but this difference was significant only versus pooled control groups. The leucine R_a (endogenous leucine flux, an index of proteolysis) was equal to the disappearance, because the leucine concentration was not changing during the anhepatic phase. The leucine flux during the anhepatic phase was insignificantly reduced compared with the control group, indicating that there were no changes in the proteolytic rate.

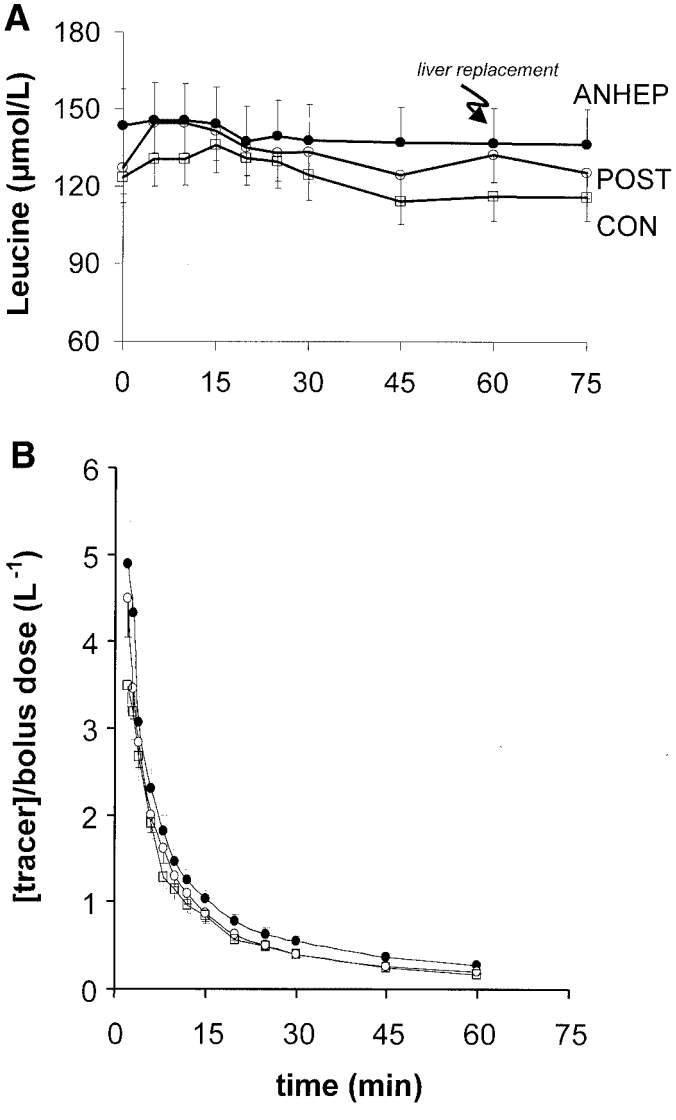

FIG. 1. ***A*: Concentration of unlabeled leucine. During the anhepatic phase, the concentration of leucine was similar to that of control subjects. The liver replacement did not change the leucine concentration.** ***B*: Concentration of the leucine tracer (normalized to the bolus dose). The profile of the tracer concentration during the anhepatic phase was similar to the control group, reflecting similar leucine kinetics.**

Alanine metabolism. Figure 2 shows the natural alanine (upper) and the normalized [3-^{13}C]alanine tracer concentrations (lower). In ANHEP, the alanine concentration was three times greater than in POST subjects and in CON subjects and increased by 104 ± 21 μmol · kg^{-1} · h^{-1}, indicating that the alanine R_a was greater than that of alanine R_d. The liver replacement reduced (−20 ± 4%; $P < 0.001$) the alanine concentration within 15 min. The tracer decay curve always remained above those measured in the control group, indicating a reduced clearance. Such remarks obtained from the visual inspection of tracee and tracer alanine data were confirmed by the results of the kinetic analysis reported in Table 5. In the absence of the liver, the tracer-miscible alanine mass was doubled and its clearance was halved. The alanine R_a was doubled. As a consequence of the increased alanine concentration, alanine uptake was also increased, but it remained ~10 μmol · kg^{-1} · h^{-1} smaller than the alanine R_d. The increment in alanine R_a was completely due to an increased alanine de novo synthesis and not to an increased release with proteolysis.

Glutamine metabolism. Figure 3 shows the natural glutamine (upper) and the normalized L-[1,2-$^{13}C_2$]glutamine tracer (lower) concentrations. In the absence of the liver, the glutamine concentration was 50% greater than in the control group and increased by 88 ± 25 μmol · kg^{-1} · h^{-1}, indicating that the glutamine R_a was greater than that of glutamine R_d. The liver replacement reduced (−22 ± 4%; $P < 0.001$) the glutamine concentration within 15 min. The tracer decay curve always remained above those in the control group, indicating a reduced clearance. The kinetic analysis (Table 5) confirmed the remarks obtained from the visual inspection of tracee and tracer glutamine data. Similar to alanine, the glutamine clearance was halved during the anhepatic phase, but the glutamine R_a was 25% reduced compared with the control groups because of a decreased de novo synthesis. In addition, different from alanine, the tracer-miscible glutamine mass was decreased (by ~60 μmol/kg), suggesting that this was the effect of the removal of the large hepatic glutamine pool. The glutamine R_d was 8 μmol · kg^{-1} · h^{-1} less than the glutamine R_a, and it was 30% less than R_d measured in the control groups.

In a single subject, the urinary losses of alanine, glutamine, and leucine were measured and resulted to be <2 μmol · kg^{-1} · h^{-1} for both alanine and glutamine and negligible for leucine.

TABLE 5
Amino acid concentrations and kinetics

	ANHEP subjects	POST subjects	*P* vs. ANHEP subjects	CON subjects	*P* vs. ANHEP subjects	*P* of pooled controls vs. ANHEP subjects
Leucine						
Concentration (μmol/l)	139 ± 8	133 ± 13		122 ± 7		
Total distribution volume (ml/kg)	354 ± 26	414 ± 38		407 ± 44		
Total mass (μmol/kg)	49 ± 5	54 ± 6		49 ± 3		
Clearance (ml · kg^{-1} · min^{-1})	13.3 ± 1.0	17.0 ± 1.8		18.4 ± 2.2		*
$R_a = R_d$ (μmol · kg^{-1} · h^{-1})	108 ± 9	128 ± 9		132 ± 9		
Alanine						
Concentration (μmol/l)	889 ± 68	253 ± 22	†	360 ± 75	†	†
Distribution volume (ml/kg)	287 ± 36	375 ± 54		385 ± 75		
Total mass (μmol/kg)	257 ± 37	91 ± 12	†	135 ± 36	*	†
Clearance (ml · kg^{-1} · min^{-1})	12.7 ± 1.3	24.3 ± 2.5	‡	20.0 ± 2.4	*	†
R_a (μmol · kg^{-1} · h^{-1})	713 ± 71	350 ± 21	†	411 ± 68	‡	†
De novo synthesis (μmol · kg^{-1} · h^{-1})	593 ± 63	208 ± 16	†	265 ± 57	‡	†
R_d (μmol · kg^{-1} · h^{-1})	704 ± 69	350 ± 21	†	411 ± 68	‡	†
Glutamine						
Concentration (μmol/l)	773 ± 89	530 ± 49	*	515 ± 16	*	*
Distribution volume (ml/kg)	220 ± 32	380 ± 59	*	377 ± 40		‡
Total mass (μmol/kg)	151 ± 12	205 ± 45		193 ± 16		*
Clearance (ml · kg^{-1} · min^{-1})	6.4 ± 1.1	12.8 ± 1.0	‡	12.9 ± 2.0	*	†
R_a (μmol · kg^{-1} · h^{-1})	284 ± 20	403 ± 25	*	396 ± 48		‡
De novo synthesis (μmol · kg^{-1} · h^{-1})	204 ± 14	308 ± 21	*	298 ± 41		‡
R_d (μmol · kg^{-1} · h^{-1})	276 ± 20	403 ± 25	‡	396 ± 48		†

Data are means ± SE. *$P < 0.05$, †$P < 0.001$, ‡$P < 0.01$ vs. ANHEP subjects. Total mass is the size of the tracer miscible pool.

DISCUSSION

Surgical and kinetic models. We used the anhepatic phase of liver transplantation as a model to demonstrate the potential role of extrahepatic tissues in amino acid metabolism and to study the initial events of liver failure. This model is not physiologic, but only in this condition it is ethical to perform such experiments in humans. It would be otherwise difficult to reach in animal models the same resources and quality of anesthesiology support. Most patients (75%) had absent or mild cirrhosis without liver failure, and most of their biochemical parameters were not different from healthy control subjects. We previously found that such patients do not have gross changes in amino acid metabolism before transplantation (22,26,27). Thus, a small effect of the antecedent cirrhosis cannot be excluded, but undoubtedly the metabolic events were maximally due to liver removal. The surgical stress increased the counterregulatory hormones that, in theory, could have stimulated protein catabolism and glutamine utilization (25). Glucagon increases glutamine uptake (17,19) almost exclusively in the liver. Epinephrine increases glutamine gluconeogenesis in the kidney (13) with a minimal effect on whole-body amino acid metabolism (28). Cortisol also increases the substrate availability (29), but it requires several hours to increase proteolysis and the glutamine and alanine de novo synthesis (30,31). Thus, neither of these hormones could have dramatically changed amino acid metabolism in the first hours of the surgical stress (17). In practice, studies of intraoperative amino acid metabolism during elective abdominal surgery support this view, as discussed in the following sections (32–36). The main aim of our study was to assess positively whether certain metabolic processes can take place outside the liver. Nonetheless, it was also important to evaluate the effect of surgery and anesthesia. We extended the sampling period after the liver replacement to provide an internal control to the absence of the liver during surgery and anesthesia. Our approach may have had limitations as to the assessment of amino acid kinetics and to the assumption that the liver is immediately functioning after replacement. We found that during the anhepatic phase, leucine concentration was similar to the other abdominal surgery models, and it did not change after the liver replacement. The alanine and glutamine concentrations were strikingly different from the other models and immediately tended to be normalized after the liver replacement. Thus, evidence both from our data and from other studies proved that what we observed was maximally the effect of liver removal and not that of anesthesia and surgery.

In previous studies, the single bolus and the primed continuous tracer infusion techniques provided comparable estimates of alanine, glutamine, and leucine kinetics. Our results in the control group were similar to these obtained by us and by others using both techniques (17,19,22,37–41). Nonetheless, for the short duration of the sampling period, we risked missing slower components (if any) of the amino acid kinetics and slightly overestimating their clearance. In the control group, we compared the clearances obtained analyzing the first hour of the experiment versus those obtained analyzing the full set of data (2.5 h). The clearances were never overestimated >10% when only the first-hour data were used. We reasoned that for proving our hypothesis, a <10% systematic error in the absolute alanine, glutamine, and leucine clearances (applied both to the study and to the control

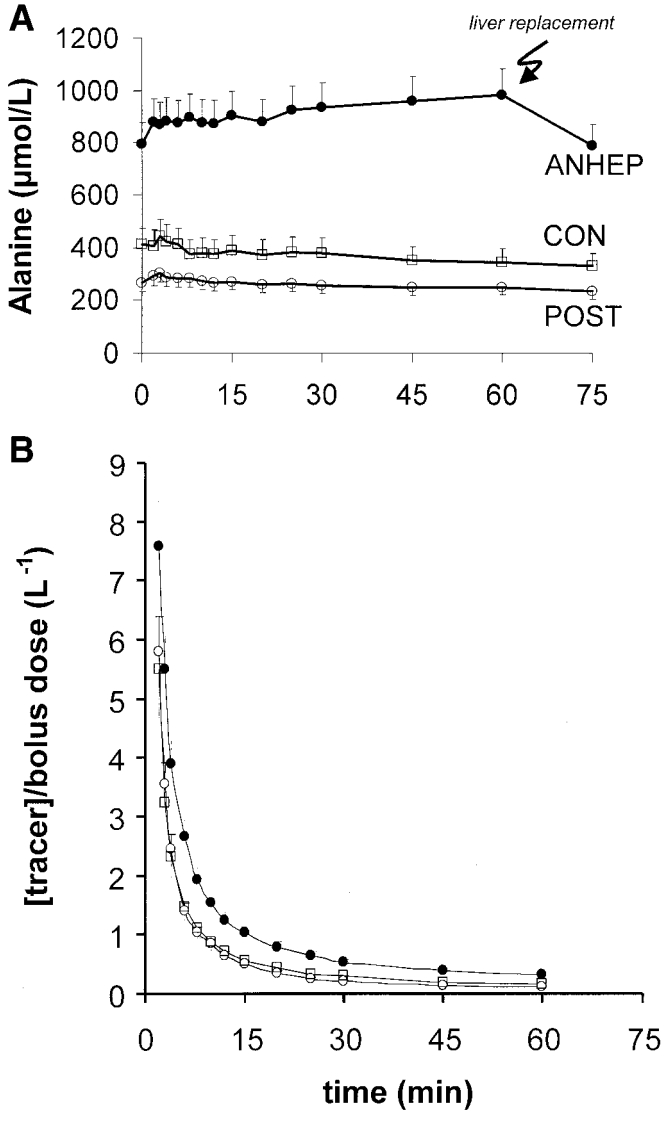

FIG. 2. *A*: **Concentration of unlabeled alanine. During the anhepatic phase, the concentration of alanine was increased threefold and continued to increase at the rate of 104 ± 21 μmol · kg^{-1} · h^{-1}, indicating that more alanine was produced than was consumed. The liver replacement immediately decreased the alanine concentration.** *B*: **Concentration of the alanine tracer (normalized to the bolus dose). The slower disappearance of the bolus during the anhepatic phase indicates a reduced alanine metabolic clearance. Despite the reduced clearance, the unlabeled alanine was so concentrated that the alanine uptake that resulted was double that in the control groups (see Table 5).**

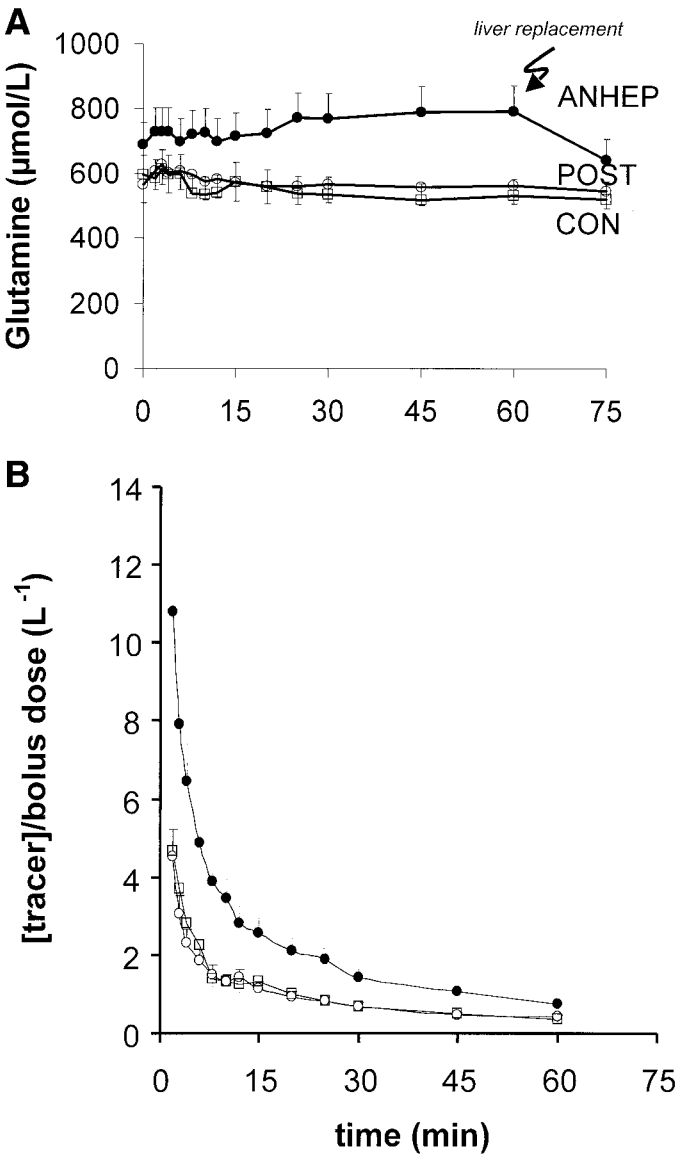

FIG. 3. *A*: **Concentration of unlabeled glutamine. During the anhepatic phase, the concentration of glutamine was 50% increased and continued to increase at the rate of 88 ± 25 μmol · kg^{-1} · h^{-1}, indicating that more glutamine was produced than was consumed. The liver replacement immediately decreased the glutamine concentration.** *B*: **Concentration of the glutamine tracer (normalized to the bolus dose). The higher initial tracer concentration indicates that, removing the liver, a part of the glutamine distribution volume disappeared. The slower disappearance of the bolus during the anhepatic phase indicates a reduced glutamine metabolic clearance. For glutamine, the net effect resulting from a reduced clearance and an increased concentration was a 30% reduction in uptake (see Table 5).**

groups) was not relevant, compared with the advantage of performing the study of the anhepatic metabolism excluding a carryover from the previous periods of the surgical operation.

Whole-body proteolysis and leucine kinetics. Proteolysis in the anhepatic phase was not different from control subjects. Excluding relevant effects of previous cirrhosis (27,42), the surgical stress could have increased the amino acid release from the peripheral tissues (25). Probably this was a minor effect because the stress was of short duration (3 h from skin incision), and in comparable abdominal surgery models proteolysis initially decreases (34,43). In contrast, as the liver is an important site for short-lived protein metabolism, its removal should have reduced proteolysis. The in vivo proteolytic rate of the liver was not defined in humans. On the basis of some estimates of the contribution of the splanchnic bed to the whole-body proteolysis (20–30%) (44,45), one could speculate that the liver contributes no more than this amount. Consequently, liver ablation should have reduced proteolysis and counterbalanced the presumable proteolytic effect of the surgical stress in the peripheral tissues. Provided that we could not exclude that we missed small changes in proteolysis, our data suggest that factors of small magnitude working in opposite directions left unchanged the proteolytic rate.

As regards leucine metabolism, the stable leucine con-

centration during the anhepatic phase proved that the rate of leucine release was exactly matched by uptake. In addition, the liver replacement left the leucine concentration unaffected in the short term. Likewise, in other surgical models, KIC and total branched-chain amino acids underwent only minor changes in the short term (32,33). In agreement with previous data (15,46), we suggest that the human liver is not a primary site of leucine uptake or release. It can be theoretically conceived that, being an essential amino acid, most of the dietary leucine passes through the liver to reach the peripheral tissues for local protein synthesis.

Alanine and glutamine production. Both the alanine and the glutamine concentrations were increasing during the anhepatic phase because their production was 2% greater than their uptake. This finding was strikingly different from the other surgical models, in which the concentration of alanine and glutamine are initially unchanged and then decreased (33,36,47). In absolute terms, only alanine, not glutamine, de novo synthesis was increased. Normally, the gut converts glutamine into alanine, which is immediately removed by the liver (40,48,49). Without the liver filter, the gut alanine production should be observed but could still not account for the doubling of alanine production. This finding must be interpreted as an increased nitrogen transfer to pyruvate. Circulating pyruvate and lactate were 3.5 and 7 times increased. The expanded pool of three-carbon compounds and ammonia probably stimulated the synthesis of alanine. This mechanism works in the brain as an alanine-glutamine cycle in which alanine serves as a nitrogen carrier from glutamatergic neurons to astrocytes (9). A related mechanism works in the kidney, which synthesizes alanine from glutamine during acidosis (8).

Different from alanine, the glutamine de novo synthesis was one-third reduced. The human liver has the machinery to remove and to synthesize simultaneously glutamine, but the fluxes are uncertain. Our data suggest that the liver is a net releaser of glutamine, in agreement with a former report in postabsorptive humans (50). Despite the increased glutamine concentration, the tracer-miscible glutamine pool was reduced by an amount similar to the hepatic glutamine content (~50 μmol/kg) (39). Thus, glutamine kinetics showed the removal of the large hepatic glutamine pool. The distribution volume of glutamine was reduced by 45% when the liver was removed. In contrast, the volumes of leucine and alanine were reduced by only 10 and 25%, suggesting that glutamine is much more concentrated than alanine or leucine in the liver. Overall, our data showed that the removal of the liver produced opposite changes in alanine and in glutamine production. We confirmed that the liver is not a site for alanine release, but we suggest that the liver is important for storing and releasing glutamine for other tissues.

Alanine and glutamine utilization. In the absence of the liver, the metabolic clearances of alanine and glutamine were almost halved and reflected a marked inefficiency in their metabolism. In absolute terms, alanine removal was doubled but glutamine uptake was 30% decreased. These rates reflected not only transamination but also disposal of their carbon-labeled skeletons. Given the importance of the kidney, brain, immune system, and gut in glutamine handling, it is not surprising that the whole-body glutamine uptake was almost maintained. Part of the huge alanine load could have been excreted in the urine, as alaninuria is commonly associated to hyperalaninemia (51), but this loss was negligible in the single subject who was tested.

Initial events after liver removal. Our study proved that the first event after liver removal is an accumulation of three-carbon compounds. The concentrations of lactate, glycerol, pyruvate, and alanine were increased 7, 6, 3.5, and 3 times, respectively. The increased alanine concentration was accompanied by increased fluxes and to an expansion of its pool. An increment in lactic acid concentration is a well-defined hallmark of the anhepatic phase of liver transplantation (52). Lactic acidosis, hyperalaninemia, and alaninuria also are clinical cornerstones of inborn defects in hepatic pyruvate dehydrogenase and pyruvate carboxylase (51), and, interestingly, liver ablation immediately reproduced this syndrome. We speculate that the block in hepatic disposal of three-carbon compounds (released from glycolysis, lipolysis, or proteolysis) immediately propagated backward, increasing the size of the pools rapidly exchanging with pyruvate, i.e., lactates and alanine. Thus, the liver is essential for the three-carbon compound's disposal, either by oxidation or recycling to compounds with a higher number of carbons (glucose, lipids, or glutamine). However, this does not suggest that the liver is required for the synthesis of glucose or glutamine. We found that during the anhepatic phase, the subjects were hyperglycemic, the glucose production was comparable to postabsorptive subjects, and only alanine gluconeogenesis was impaired, whereas glutamine gluconeogenesis was similar to control subjects (53). Our data suggest that the disposal of circulating three-carbon compounds, not gluconeogenesis, is a crucial function that cannot be replaced.

Concerning nitrogen metabolism, blood ammonia concentration reasonably increased immediately after the liver removal (54). An open question is whether nitrogen was disposed besides ammonia generation. During the anhepatic phase, alanine and glutamine accumulated by 10 and 8 $\mu mol \cdot kg^{-1} \cdot h^{-1}$, respectively. If urea is produced in the liver at a rate of 220 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ (scavenging 440 $\mu mol \cdot kg^{-1} \cdot h^{-1}$ of nitrogen) (55,56), then this accumulation removed only 6% (26 $\mu mol \cdot kg^{-1} \cdot h^{-1}$) of the nitrogen normally converted to urea each hour. A potential contribution of alanine and glutamine urinary excretion to nitrogen disposal is also unsupported by our preliminary data. It was suggested that in acute liver failure, the muscle scavenges nitrogen by producing glutamine from alanine (15), but we found that the absolute glutamine production was reduced in the first hour after the liver removal. The accumulation of three-carbon compounds and ammonia could increase glutamine synthesis in a longer term. Glutamine accumulation, however, could lead to astrocyte swelling, an important feature of hepatic encephalopathy (14). The relative sparing of glutamine metabolism in the first hour after liver removal could initially protect from cellular swelling and its pathologic consequences.

Final considerations relate to the ability of liver transplantation to normalize amino acid kinetics. As previously reported (22), postabsorptive proteolysis became normal

within the first year after transplantation. We also found that the leucine concentration was normalized by the 7th month, whereas in the previous series, characterized by more severe cirrhosis (Child class B-C), it was reduced for the first 2 years. This suggests that the status before transplantation may be important for the outcome of protein metabolism. Finally, we expanded on the former studies proving that also alanine and glutamine kinetics are normal after liver transplantation.

Conclusion. We studied the leucine, alanine, and glutamine kinetics in the first hour after liver removal in humans, and we showed that proteolysis and leucine metabolism are relatively unaffected. Alanine is increasingly produced and extensively disposed with other three-carbon compounds. The glutamine metabolism is relatively spared, but glutamine also accumulates over time. Our results prove that the hepatic disposal of three-carbon compounds is a function that cannot be replaced by other organs. The quantification of alanine and glutamine kinetics during the anhepatic phase now renders possible the study of the extent of nonhepatic gluconeogenesis and nitrogen disposal from circulating alanine and glutamine.

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REFERENCES

1. Matthews DE, Fong Y: Amino acid and protein metabolism. In *Clinical Nutrition: Parenteral Nutrition*. Rombeau JL, Caldwell MD, Eds. Philadelphia, WB Saunders, 1993, p. 75–112
2. Nurjhan N, Bucci A, Perriello G, Stumvoll M, Dailey G, Bier DM, Toft I, Jenssen TG, Gerich JE: Glutamine: a major gluconeogenic precursor and vehicle for interorgan carbon transport in man. *J Clin Invest* 95:272–277, 1995
3. Perriello G, Jorde R, Nurjhan N, Stumvoll M, Dailey G, Jenssen T, Bier DM, Gerich JE: Estimation of glucose-alanine-lactate-glutamine cycles in post-absorptive humans: role of skeletal muscle. *Am J Physiol* 269:E443–E450, 1995
4. Gelfand RA, Glickman MG, Jacob R, Sherwin RS, DeFronzo RA: Removal of infused amino acids by splanchnic and leg tissues in humans. *Am J Physiol* 250:E407–E413, 1986
5. Windmueller HG: Glutamine utilization by the small intestine. *Adv Enzymol* 53:201–237, 1982
6. Abumrad NN, Williams P, Frexes-Steed M, Geer R, Flakoll P, Cersosimo E, Brown LL, Melki I, Bulus N, Hourani H, Hubbard M, Ghishan F: Inter-organ metabolism of amino acids in vivo. *Diabetes Metab Rev* 5:213–226, 1989
7. Haussinger D: Nitrogen metabolism in liver: structural and functional organization and physiological relevance. *Biochem J* 267:281–290, 1990
8. Nissim I: Newer aspects of glutamine/glutamate metabolism: the role of acute pH changes. *Am J Physiol* 277:F493–F497, 1999
9. Waagepetersen HS, Sonnewald U, Larsson OM, Schousboe A: A possible role of alanine for ammonia transfer between astrocytes and glutamatergic neurons. *J Neurochem* 75:471–479, 2000
10. Rajas F, Bruni N, Montano S, Zitoun C, Mithieux G: The glucose-6 phosphatase gene is expressed in human and rat small intestine: regulation of expression in fasted and diabetic rats. *Gastroenterology* 117:132–139, 1999
11. Burchell A, Hume R: The glucose-6-phosphatase system in human development. *Histol Histopathol* 10:979–993, 1995
12. Shen J, Petersen KF, Behar KL, Brown P, Nixon TW, Mason GF, Petroff OA, Shulman GI, Shulman RG, Rothman DL: Determination of the rate of the glutamate/glutamine cycle in the human brain by in vivo 13C NMR. *Proc Natl Acad Sci U S A* 96:8235–8240, 1999
13. Stumvoll M, Meyer C, Perriello G, Kreider M, Welle S, Gerich J: Human kidney and liver gluconeogenesis: evidence for organ substrate selectivity. *Am J Physiol* 274:E817–E826, 1998
14. Haussinger D, Kircheis G, Fischer R, Schliess F, Vom DS: Hepatic encephalopathy in chronic liver disease: a clinical manifestation of astrocyte swelling and low-grade cerebral edema? *J Hepatol* 32:1035–1038, 2000
15. Clemmesen JO, Kondrup J, Ott P: Splanchnic and leg exchange of amino acids and ammonia in acute liver failure. *Gastroenterology* 118:1131–1139, 2000
16. Mazzaferro V, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L: Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 334:693–699, 1996
17. Battezzati A, Benedini S, Fattorini A, Piceni SL, Luzi L: Effect of hypoglycemia on amino acid and protein metabolism in healthy humans. *Diabetes* 49:1543–1551, 2000
18. Matthews DE, Pesola G, Campbell RG: Effect of epinephrine upon amino acid and energy metabolism in humans. *Am J Physiol* 258:E948–E956, 1990
19. Battezzati A, Simonson DC, Luzi L, Matthews DE: Glucagon increases glutamine uptake without affecting glutamine release in humans. *Metabolism* 47:713–723, 1998
20. Perseghin G, Scifo P, De Cobelli F, Pagliato E, Battezzati A, Arcelloni C, Vanzulli A, Testolin G, Pozza G, Del Maschio A, Luzi L: Intramyocellular triglyceride content is a determinant of in vivo insulin resistance in humans: a 1H–13C nuclear magnetic resonance spectroscopy assessment in offspring of type 2 diabetic parents. *Diabetes* 48:1600–1606, 1999
21. Grassi G, Seravalle G, Cattaneo BM, Lanfranchi A, Vailati S, Giannattasio C, Del Bo A, Sala C, Bolla GB, Pozzi M: Sympathetic activation and loss of reflex sympathetic control in mild congestive heart failure. *Circulation* 92:3206–3211, 1995
22. Luzi L, Perseghin G, Regalia E, Sereni LP, Battezzati A, Baratti D, Bianchi E, Terruzzi I, Hilden H, Groop LC, Pulvirenti A, Taskinen MR, Gennari L, Mazzaferro V: Metabolic effects of liver transplantation in cirrhotic patients. *J Clin Invest* 99:692–700, 1997
23. Cobelli C, Foster D, Toffolo G: Tracer kinetics in biomedical research. In *Data to Model*. Kluwer Academic/Plenum, London, 2001, p. 215–280
24. Cobelli C, Caumo A: Using what is accessible to measure that which is not: necessity of model of system. *Metabolism* 47:1009–1035, 1998
25. Matthews DE, Battezzati A: Substrate kinetics and catabolic hormones. In *Organ Metabolism and Nutrition: Ideas for Future Critical Care*. Kinney JM, Tucker HN, Eds. New York, Raven Press, 1994, p. 1–22
26. Perseghin G, Regalia E, Battezzati A, Vergani S, Pulvirenti A, Terruzzi I, Baratti D, Bozzetti F, Mazzaferro V, Luzi L: Regulation of glucose homeostasis in humans with denervated livers. *J Clin Invest* 100:931–941, 1997
27. Petrides AS, Luzi L, Reuben A, Riely C, DeFronzo RA: Effect of insulin and plasma amino acid concentration on leucine metabolism in cirrhosis. *Hepatology* 14:432–444, 11991
28. Matthews DE, Pesola G, Campbell RG: Effect of epinephrine upon amino acid and energy metabolism in humans. *Am J Physiol* 258:E948–E956, 1990
29. De Feo P, Perriello G, Torlone E, Ventura MM, Fanelli C, Santeusanio F, Brunetti P, Gerich JE, Bolli GB: Contribution of cortisol to glucose counterregulation in humans. *Am J Physiol* 257:E35–E42, 1989
30. Darmaun D, Matthews DE, Bier DM: Physiological hypercortisolemia increases proteolysis, glutamine and alanine production. *Am J Physiol* 255:E366–E373, 1988
31. Simmons PS, Miles JM, Gerich JE, Haymond MW: Increased proteolysis: an effect of increases in plasma cortisol within the physiologic range. *J Clin Invest* 73:412–420, 1984
32. Shaw JH, Humberstone DA, Douglas RG, Koea J: Leucine kinetics in patients with benign disease, non-weight-losing cancer, and cancer cachexia: studies at the whole-body and tissue level and the response to nutritional support. *Surgery* 109:37–50, 1991
33. Carli F, Ronzoni G, Webster J, Khan K, Elia M: The independent metabolic effects of halothane and isoflurane anaesthesia. *Acta Anaesthesiol Scand (Copenh)* 37:672–678, 1993
34. Carli F, Ramachandra V, Gandy J, Merritt H, Ford GC, Read M, Halliday D: Effect of general anaesthesia on whole body protein turnover in patients undergoing elective surgery. *Br J Anaesth* 65:373–379, 1990
35. Carli F, Elia M: The independent metabolic effects of enflurane anaesthesia and surgery. *Acta Anaesthesiol Scand (Copenh)* 35:329–332, 1991
36. Perseghin G, Corno A, Santoro F, Biagioli B, Paolini G, Battezzati A, Benedini S, Donatelli F, Pozza G, Grossi A, Luzi L: Myocardial metabolism studied during warm blood antero-retrograde reperfusion in ischaemic human hearts. *Acta Diabetol* 35:67–73, 1998
37. Hall SEH, Braaten JT, McKendry JBR, Bolton T, Foster D, Berman M:

Normal alanine-glucose relationships and their changes in diabetic patients before and after insulin treatment. *Diabetes* 28:737–745, 1979
38. Matthews DE, Battezzati A, Fürst P: Alanylglutamine kinetics in humans. *Clin Nutr* 12:57–58, 1993
39. Darmaun D, Matthews DE, Bier DM: Glutamine and glutamate kinetics in humans. *Am J Physiol* 251:E117–E126, 1986
40. Battezzati A, Haisch M, Brillon DJ, Matthews DE: Splanchnic utilization of enteral alanine in humans. *Metabolism* 48:915–921, 1999
41. Cobelli C, Saccomani MP, Tessari P, Biolo G, Luzi L, Matthews DE: A compartmental model of leucine kinetics in humans. *Am J Physiol* 261:E539–E550, 1991
42. Tessari P, Biolo G, Inchiostro S, Orlando R, Vettore M, Sergi G: Leucine and phenylalanine kinetics in compensated liver cirrhosis: effects of insulin. *Gastroenterology* 104:1712–1721, 1993
43. Essen P, McNurlan MA, Wernerman J, Vinnars E, Garlick PJ: Uncomplicated surgery, but not general anesthesia, decreases muscle protein synthesis. *Am J Physiol* 262:E253–E260, 1992
44. Meek SE, Persson M, Ford GC, Nair KS: Differential regulation of amino acid exchange and protein dynamics across splanchnic and skeletal muscle beds by insulin in healthy human subjects. *Diabetes* 47:1824–1835, 1998
45. Tessari P, Garibotto G, Inchiostro S, Robaudo C, Saffioti S, Vettore M, Zanetti M, Russo R, Deferrari G: Kidney, splanchnic, and leg protein turnover in humans: insight from leucine and phenylalanine kinetics. *J Clin Invest* 98:1481–1492, 1996
46. Matthews DE, Marano MA, Campbell RG: Splanchnic bed utilization of leucine and phenylalanine in humans. *Am J Physiol* 264:E109–E118, 1993
47. Jackson NC, Carroll PV, Russell-Jones DL, Sonksen PH, Treacher DF, Umpleby AM: The metabolic consequences of critical illness: acute effects on glutamine and protein metabolism. *Am J Physiol* 276:E163–E170, 1999
48. Lochs H, Roth E, Gasic S, Hubl W, Morse EL, Adibi SA: Splanchnic, renal, and muscle clearance of alanylglutamine in man and organ fluxes of alanine and glutamine when infused in free and peptide forms. *Metabolism* 39:833–836, 1990
49. Miller BM, Cersosimo E, McRae J, Williams PE, Lacy WW, Abumrad NN: Interorgan relationships of alanine and glutamine during fasting in the conscious dog. *J Surg Res* 35:310–318, 1983
50. Felig P, Wahren J, Karl I, Cerasi E, Luft R, Kipnis DM: Glutamine and glutamate metabolism in normal and diabetic subjects. *Diabetes* 22:573–576, 1973
51. Robinson BH: Lactic acidemia. In *The Metabolic Basis of Inherited Disease*. Scriver CR, Beaudet AL, Sly WS, Valle D, Eds. New York, McGraw-Hill, 1989, p. 869–888
52. Shangraw RE, Winter R, Hromco J, Robinson ST, Gallaher EJ: Amelioration of lactic acidosis with dichloroacetate during liver transplantation in humans. *Anesthesiology* 81:1127–1138, 1994
53. Battezzati A, Caumo A, Fattorini A, Piceni Sereni L, Regalia E, Romito R, Coppa J, Matthews DE, Mazzaferro V, Luzi L: Anhepatic glutamine metabolism and gluconeogenesis in humans (Abstract). *Diabetologia* 43:A153, 2000
54. Fukuzawa K, Shimada M, Itasaka H, Takenaka K, Sugimachi K: Ammonia elimination as a rapid index of viability in liver grafts in dogs. *J Surg Res* 50:88–92, 1991
55. Matthews DE, Downey RS: Measurement of urea kinetics in humans: a validation of stable isotope tracer methods. *Am J Physiol* 246:E519–E527, 1984
56. Jahoor F, Wolfe RR: Reassessment of primed constant-infusion tracer method to measure urea kinetics. *Am J Physiol* 252:E557–E564, 1987