

## Conversion of [<sup>15</sup>N]ammonia into urea and amino acids in humans and the effect of nutritional status

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Hepatic NH<sub>3</sub> detoxification by ureagenesis requires an input of aspartate-N, originating either from amino acid-N or NH<sub>3</sub>-N. The relative importance of these two routes may depend on the nutritional state. To test this, four volunteers were given a liquid diet for 2 d and then on day 3 were either fed every 20 min or fasted. Doses of <sup>15</sup>NH<sub>4</sub>Cl were taken orally every 20 min for 6 h (total 1.5 g) and blood was sampled hourly. Urea-N elimination under fasted conditions was only 0.75 of that for the fed state. Considering the increase in body urea pool during feeding, ureagenesis during fasting was probably closer to 0.6 of that during feeding. Since the [<sup>14</sup>N<sup>15</sup>N]urea enrichment was not different between the fed and fasted states, the proportion of the <sup>15</sup>NH<sub>3</sub> dose converted to urea during fasting was also 0.6 of that during the fed condition. No change in [<sup>14</sup>N<sup>15</sup>N]urea and [amide-<sup>15</sup>N]glutamine enrichment suggested that NH<sub>3</sub> enrichment was also not affected by nutritional state. Enrichment of [<sup>15</sup>N<sup>15</sup>N]urea was approximately 0.05 that of [<sup>14</sup>N<sup>15</sup>N]urea which indicates that <sup>15</sup>NH<sub>3</sub> can also enter the aspartate route, the importance of which is yet unknown. Both [<sup>15</sup>N<sup>15</sup>N]urea and [amino-<sup>15</sup>N]glutamine enrichment in the fasted state were approximately 1.7 times that in the fed state, indicating increased labelling of precursors and/or increased NH<sub>3</sub> flux through the aspartate route. Glutamate, valine, leucine and isoleucine showed comparable increases in enrichment during fasting. Arginine enrichment was unaltered by nutritional state, but was lower than [<sup>14</sup>N<sup>15</sup>N]urea, indicating incomplete equilibration with the arginine pool in periportal hepatocytes. The present study indicates that hepatic NH<sub>3</sub> detoxification may use the aspartate route, gaining importance in the fasted state. The majority of urea was supplied with only one N atom from NH<sub>3</sub>, thus provision of the other may have consequences for alternative substrates, in particular amino acids.

### Ammonia detoxification: Ureagenesis: Glutamine: Fasting

NH<sub>3</sub> absorption and production by the gastrointestinal tract is considerable in both ruminants and non-ruminants, and comparable hepatic portal vein NH<sub>3</sub> concentrations are observed between the species (e.g. Cooper *et al.* 1987; Loblely *et al.* 1995). Hepatic detoxification of NH<sub>3</sub> is necessary to prevent peripheral hyperammonaemia and brain damage (Summerskill & Wolpert, 1970), and this process has been suggested as a possible contributor to the low amino acid utilization observed for the ruminant (see Reynolds, 1992). Whether a similar low amino acid utilization might occur in humans has not been investigated.

Hepatic ureagenesis occurs in the periportal hepatocytes (Häussinger, 1990) with one atom of N derived from NH<sub>3</sub> used for mitochondrial carbamoyl-phosphate (CP) synthesis, while the other is obtained from cytosolic aspartate. Hepatic detoxification of NH<sub>3</sub>-N thus involves at least one equivalent of amino acid-N, but whether this represents a net amino acid-N availability for anabolic purposes depends on whether NH<sub>3</sub>-N can also readily enter the aspartate-N pool. If this occurs then the introduction of <sup>15</sup>NH<sub>3</sub> should result in the formation of [<sup>15</sup>N<sup>15</sup>N]urea; if not then [<sup>14</sup>N<sup>15</sup>N]urea would be the only labelled species.

\* For reprints.

Recently, Patterson *et al.* (1993) showed that oral intake of  $^{15}\text{NH}_4\text{Cl}$  in a single fasted human subject resulted apparently in a large proportion of  $[\text{}^{15}\text{N}^{15}\text{N}]$ urea appearance. The data of Patterson *et al.* (1993) are uncorrected for the spectral overlap between  $m+1$  and  $m+2$   $m/z$  ions but do raise the intriguing possibility of preferential channelling of ammonia-N into both the CP and aspartate pools which would lead to efficient removal in that no net drain would occur on other N sources. These data contrast with recent observations in fed sheep in which infusion of  $^{15}\text{NH}_3$  into the mesenteric vein yielded only 3% of labelled urea as the  $[\text{}^{15}\text{N}^{15}\text{N}]$ urea form (Lobley *et al.* 1995) although with isolated hepatocytes from fasted sheep the predominant species was  $[\text{}^{15}\text{N}^{15}\text{N}]$ urea (Luo *et al.* 1994). Based on these reports it was hypothesized that nutritional state might, in part, regulate the channelling of  $\text{NH}_3$ -N into CP and aspartate. In consequence, the approach of Patterson *et al.* (1993) was extended to a balanced study on the effect of feeding and fasting on the synthesis of labelled urea and amino acids following oral ingestion of  $^{15}\text{NH}_3$ . Preliminary accounts of this study have been reported elsewhere (Weijs *et al.* 1994a, b).

#### MATERIALS AND METHODS

Four volunteers gave informed consent to participate in the study. All subjects were healthy and performed their normal daily activities throughout the experimental periods. Characteristics of the subjects are shown in Table 1. A balanced crossover design was adopted with dietary intake regulated for the first 2 d and then isotopic measurement performed on day 3 in either the fed (portions every 20 min) or fasted condition (Fig. 1). Treatment periods were at 7 d intervals.

The diet was a flavoured liquid feed (Fortisip; Cow & Gate Nutricia, Trowbridge, Wilts.), containing approximately 50 g protein and 6.3 MJ/l, supplied to provide 1 g protein/kg body weight per d. Subjects were advised to spread the feeds over the day, in order to avoid gastrointestinal problems. The first subject had slight diarrhoea on day 1, which was overcome by increased meal frequency. The feed was supplemented daily with 1 litre orange juice (6 g protein and 1.78 MJ/l). A 24 h urine collection into 100 ml glacial acetic acid was started after morning voiding on day 2 and finished with morning voiding on day 3. At 10.00 hours on day 3, urine and blood samples were obtained to provide background (natural abundance) measurements.

The  $^{15}\text{NH}_4\text{Cl}$  (99 atom %; Europa Scientific, Crewe, Ches.) was taken orally at 20 min intervals between 10.00 and 16.00 hours (total dose 1.5 g). Doses were weighed individually and dissolved in approximately 20 ml deionized water immediately before ingestion. The flasks were rinsed with an equal volume of water; this ensured complete intake, alleviated the salty taste and assisted the hourly voiding required. Blood samples (each approximately 7 ml) were taken from a superficial arm vein into evacuated, heparinized tubes at 3, 4, 5 and 6 h after the start of  $^{15}\text{NH}_4\text{Cl}$  ingestion. Duplicate 0.6 ml blood samples were haemolysed with an equal volume of deionized water. From the remainder, plasma was prepared by centrifugation and stored frozen ( $-20^\circ$ ). The hourly urine samples were stored at  $4^\circ$  after acidification with acetic acid.

#### *Chemical analyses*

From the hourly urine samples representative fractions (0.1) were combined to give a composite urine sample for the experimental period. Both the 24 h collection and composite urine samples were analysed for N concentration by the Dumas reaction (Macro-N; Foss Heraeus, Weldrake, York) as were the feeds. Urea concentrations in all urine samples and the 0 h and 6 h haemolysed blood samples were determined by an autoanalyser method. The blood was assayed to allow correction of body urea-pool changes.

Table 1. *Characteristics of subjects*  
(Mean values and standard deviations for four subjects)

Variable	Mean	SD
Age (years)	33	12.1
Height (m)	1.80	0.085
Weight (kg)	81.5	11.1
Haemoglobin (g/l)	156	2.1
Packed cell volume (%)	45.8	3.4
Blood pH	7.378	0.017

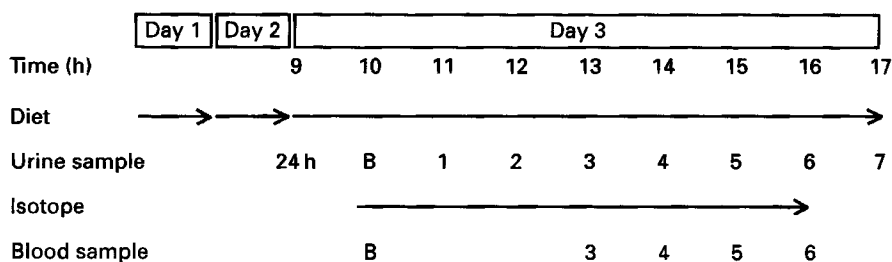


Fig. 1. Experimental design.

#### <sup>15</sup>N determinations

Plasma and haemolysed blood samples (1.2 ml) were thawed and deproteinized with sulphosalicylic acid (SSA; 480 g/l) to a final concentration of 70 g SSA/l. After centrifugation (13 000 rev./min for 5 min) amino acids and urea were isolated from the supernatant fraction by ion-exchange chromatography (Lobley *et al.* 1995) and then converted to the *t*-butyldimethylsilyl (TBDMS) derivatives (Calder & Smith, 1988). For arginine analysis the *N*-butyl-heptafluorobutyrate (HFB) derivative was prepared (Lobley *et al.* 1995). Enrichments were determined on a gas chromatograph-mass spectrometer (GCMS; HP 5989A, Hewlett Packard, Manchester) in the electron impact mode.

Urea from blood and plasma was analysed for the amounts of unlabelled, singly and doubly-labelled molecules (*m/z* of 273, 274 and 275 respectively). Tri- and tetra-TBDMS derivatives of glutamine were formed; the tri-form was scanned at *m/z* of 431 and 432 (the latter corresponds to fragments containing either amide- or amine-N) while the tetra-derivative was scanned for fragments of *m/z* 545, 546 and 547 (unlabelled, singly and doubly-labelled) and at 258 and 259. These last two gave the enrichment of the amine-N and, by difference from total singly-labelled species, the value for amide-N enrichment could be calculated. Doubly-labelled urea and glutamine values were calculated with appropriate corrections for spectral overlap from singly-labelled species. Of the other amino acids monitored, glutamate, alanine, glycine, serine, valine, leucine, isoleucine and arginine were analysed as the M-57 fragment (Calder & Smith, 1988). Data were calculated as mole % excess (MPE) with respect to appropriate natural abundance samples (Campbell, 1974).

#### Statistical analysis

N data were analysed by Student's *t* test for paired samples and enrichment data were analysed by ANOVA with treatment effects for nutritional state or fluid type.

## RESULTS

*Nitrogen and urea elimination*

For both periods the total urinary-N elimination at day 2 matched total-N intake (faecal losses were minimal with the digestible feed), of which 83% was as urea-N (Table 2). During the 7 h urine collection on day 3 both total N and urea-N elimination were significantly lower than N intake for the fed period ( $P < 0.001$ ).

*Urea concentration and enrichments*

The blood urea concentration increased significantly ( $P < 0.005$ ) during feeding (between 0 and 6 h), and showed a tendency to decrease during fasting ( $P = 0.056$ ; Table 2).

Between 3 and 6 h there was a linear increase in plasma [ $^{14}\text{N}^{15}\text{N}$ ]urea enrichment (Fig. 2). There were no differences between fasted and fed values nor between plasma and blood (Table 3).

Temporal changes in [ $^{15}\text{N}^{15}\text{N}$ ]urea enrichment were less pronounced (Fig. 3) and the level of enrichment was much lower (approximately 0.05) compared with the [ $^{14}\text{N}^{15}\text{N}$ ] species. Fasting, however, significantly increased ( $P = 0.002$ ) the [ $^{15}\text{N}^{15}\text{N}$ ]urea enrichment to approximately 1.7 times the fed level, for both blood and plasma.

*Glutamate and glutamine amino-nitrogen and amide-nitrogen*

Amino-N-glutamine followed the same trend as [ $^{15}\text{N}^{15}\text{N}$ ]urea (Fig. 3). The enrichments during fasting were higher ( $P = 0.003$ ) than for the fed state and continued to increase throughout the period of  $^{15}\text{NH}_4\text{Cl}$  dosing (Fig. 3) in contrast to amide-N-glutamine which reached a plateau by 3 h (Fig. 2). For both types of labelled glutamine there was no significant difference between blood and plasma. The amounts of doubly-labelled glutamine formed were less than the detection limits of the GCMS ( $< 0.05$  MPE).

The enrichment of glutamate was greater in plasma than blood ( $P < 0.001$ ), and was significantly ( $P = 0.046$ ) higher during the fasted compared with the fed state (Table 3).

*Other amino acids*

Arginine enrichment, as with glutamate, was significantly lower in blood than in plasma ( $P < 0.001$ ), but was not different between the fed and fasted states (Table 3).

Alanine ( $P = 0.015$ ) and serine ( $P = 0.091$ ) enrichments were lower during fasting and blood serine enrichments were significantly higher than plasma values ( $P < 0.001$ ; Table 3). Glycine had a higher enrichment in the fasted state ( $P < 0.001$ ), especially the plasma value which exceeded those of alanine and serine ( $P < 0.01$ ; Table 3). Similarly, the enrichments of the branched-chain amino acids were greater during fasting ( $P < 0.05$ ; see Table 3).

## DISCUSSION

The present investigation was instigated to examine the various fates of  $\text{NH}_3$  absorbed across the gut and, in particular, to determine whether the  $\text{NH}_3$  could be efficiently detoxified through ureagenesis by channelling of the N into both the CP and aspartate pathways. Formation of the [ $^{15}\text{N}^{15}\text{N}$ ]urea species from orally ingested  $^{15}\text{NH}_3$  would substantiate this efficient detoxification. On the other hand, failure to detect [ $^{15}\text{N}^{15}\text{N}$ ]urea might imply the preferential inflow of other unlabelled N sources (most probably amino acids) into either the CP or aspartate pools where the resultant dilution in enrichment would lower the likelihood of two  $^{15}\text{N}$  atoms being incorporated into the same urea molecule.

Direct evidence of  $\text{NH}_3$  channelling requires measurement of the enrichment of both

Table 2. Nitrogen intake, urinary nitrogen excretion and urea-nitrogen excretion by human subjects receiving a liquid diet for 2 d and either fed or fasted on day 3, together with blood urea-nitrogen (BUN) concentrations before ( $t_0$ ) and after ( $t_6$ ) 6 h of ammonium chloride ingestion on day 3†

(Mean values with their standard errors for four subjects)

	Fed		Fasted	
	Mean	SE	Mean	SE
Day 2 (g/24 h)				
N intake	13.04	1.03§	13.04	1.03
N excretion	13.17	0.84	13.33	0.69
Urea-N excretion	10.88	0.68	11.04	0.57
Day 3 (g/7 h)				
N intake	8.05		0.38	
N excretion	5.29	0.19	4.00**	0.15
Urea-N excretion	4.41	0.13	3.34**	0.11
BUN $t_0$ (mmol/l)	4.75	0.58	4.92	0.46
BUN $t_6$ (mmol/l)	5.48††	0.62	4.72**	0.42

\*\* Mean values were significantly different from those for fed subjects,  $P < 0.005$ .

†† Mean value was significantly different from that for  $t_0$ ,  $P < 0.005$ .

‡ For details of subjects and procedures, see Table 1, Fig. 1 and pp. 492–493.

§ Intakes were constant within an individual.

|| Intake as [ $^{15}\text{N}$ ]ammonium chloride.

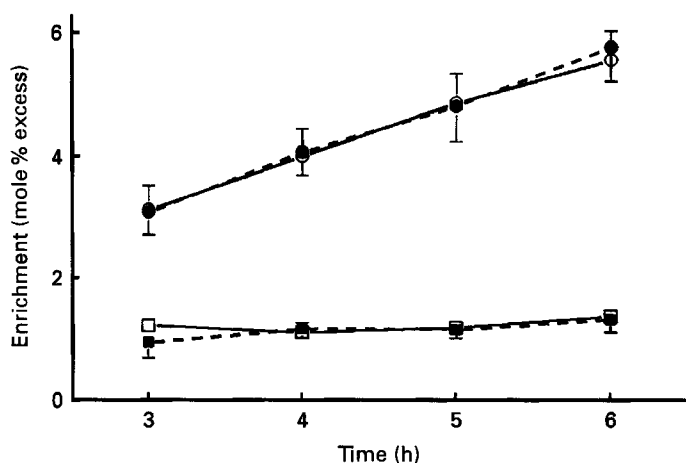


Fig. 2. Temporal plasma enrichments of [ $^{14}\text{N}^{15}\text{N}$ ]urea (○, ●) and [amide- $^{15}\text{N}$ ]glutamine (□, ■) in fed (○, □) and fasted (●, ■) human subjects ingesting [ $^{15}\text{N}$ ]ammonium chloride every 20 min for 6 h. Values are means for four subjects, with their standard errors represented by vertical bars.

$\text{NH}_3$  and aspartate (or the relevant intermediate metabolites) at the site of ureagenesis, the hepatic mitochondria and cytosol. Such determinations are impractical with normal human volunteers and thus it is necessary to resort to examination of product labelling in easily sampled pools.

In the present study the plasma aspartate enrichments were below the level of detection by the GCMS procedure. Studies *in vivo* with  $^{13}\text{NH}_3$  injection into the hepatic portal vein

Table 3. Mean urea and amino acid enrichments (mole % excess) in plasma and blood of human subjects ingesting [ $^{15}\text{N}$ ]ammonium chloride every 20 min for 6 h in either the fed or the fasted state\*

(Mean 6 h values with their standard errors for four subjects)

	Plasma				Blood				Significance level (ANOVA): <i>P</i>	
	Fed		Fasted		Fed		Fasted			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Nutritional state	Fluid type
Urea M + 1†	5.58	0.47	5.78	0.56	5.55	0.45	5.71	0.52	—	—
Urea M + 2‡	0.29	0.02	0.49	0.06	0.27	0.03	0.46	0.06	0.002	—
Amide-N-glutamine	1.38	0.08	1.33	0.22	1.11	0.17	1.27	0.04	—	—
Amino-N-glutamine	0.55	0.13	1.08	0.13	0.57	0.12	0.79	0.08	0.003	—
Glutamate	1.43	0.25	1.94	0.10	0.32	0.05	0.45	0.09	0.046	< 0.001
Arginine	4.03	0.63	4.29	0.53	0.93	0.10	1.10	0.40	—	< 0.001
Alanine	0.58	0.06	0.38	0.06	0.47	0.07	0.32	0.05	0.015	—
Serine	0.28	0.01	0.22	0.02	0.52	0.05	0.41	0.08	—	< 0.001
Glycine	0.18	0.06	0.82	0.10	0.17	0.05	0.32	0.04	< 0.001	< 0.001
Valine	0.19	0.02	0.29	0.05	0.11	0.02	0.25	0.04	0.005	—
Leucine	0.21	0.02	0.32	0.05	0.18	0.05	0.36	0.03	0.007	—
Isoleucine	0.29	0.02	0.45	0.09	0.25	0.02	0.41	0.10	0.034	—

\* For details of subjects and procedures see Table 1, Fig. 1 and pp. 492–493.

† [ $^{14}\text{N}^{15}\text{N}$ ]urea.

‡ [ $^{15}\text{N}^{15}\text{N}$ ]urea.

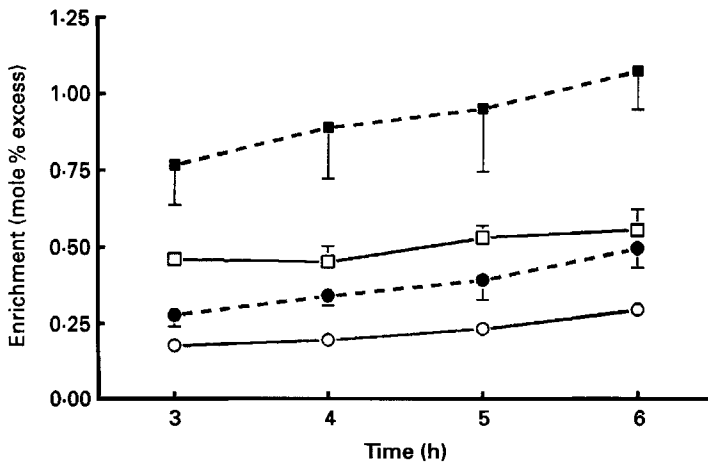


Fig. 3. Temporal plasma enrichments of [ $^{15}\text{N}^{15}\text{N}$ ]urea (○, ●) and [amino- $^{15}\text{N}$ ]glutamine (□, ■) in fed (○, □) and fasted (●, ■) human subjects ingesting [ $^{15}\text{N}$ ]ammonium chloride every 20 min for 6 h. Values are means for four subjects, with their standard errors represented by vertical bars.

of rats showed that within 5 s much of the label was transferred into glutamate by mitochondrial glutamate dehydrogenase (*EC* 1.4.1.2; GDH), and by 60 s 93% of the labelled glutamate was in the cytosol (Cooper *et al.* 1987). This exchange was probably mediated through mitochondrial aspartate aminotransferase (*EC* 2.6.1.1), with aspartate entering the cytosol by the glutamate–aspartate anti-porter (Meijer *et al.* 1990) and the

subsequent action of aspartate aminotransferase giving rise to labelled glutamate. The incorporation of  $^{15}\text{NH}_3$  into glutamate by mitochondrial GDH should, therefore, also readily label the cytosolic aspartate pool which provides substrate for ureagenesis. Unfortunately, this labelling is not reflected in the plasma glutamate enrichment which remains low due to a combination of circumstances including the large differences in hepatic cytosol:plasma glutamate concentrations (275:1), which result in a poor exchange between tissue and plasma glutamate (Darmaun *et al.* 1986), and the high flux for plasma glutamate (Bässler, 1993), much of which arises from extra-hepatic sources.

An alternative approach to the problem is to examine the predicted and observed frequencies with which  $^{14}\text{N}$  and  $^{15}\text{N}$  atoms enter the ornithine cycle from  $\text{NH}_3$ . If, in an extreme case, all the  $\text{NH}_3$  flows to CP then only [ $^{14}\text{N}^{14}\text{N}$ ]- and [ $^{14}\text{N}^{15}\text{N}$ ]urea would be synthesized. Alternatively, if there was rapid and complete equilibration of  $\text{NH}_3$ -N between CP and aspartate pools (i.e. they have equal enrichments) then the probabilities of  $^{14}\text{N}$  and  $^{15}\text{N}$  entering urea would be in proportion to their population frequencies, i.e.  $p^2$ ,  $2pq$  and  $q^2$ , where  $p$  and  $q$  are the respective fractional distributions of  $^{14}\text{N}$  and  $^{15}\text{N}$  and where  $p = (1 - q)$ . Under such circumstances the difference in [ $^{14}\text{N}^{15}\text{N}$ ]:[ $^{15}\text{N}^{15}\text{N}$ ]urea ratios between the fed and fasted conditions can be explained in two ways. First, the precursor enrichments ( $q$ ) are different and these can be estimated from the probability relationship given previously, i.e.

$$\begin{aligned} [\text{}^{14}\text{N}^{15}\text{N}]:[\text{}^{15}\text{N}^{15}\text{N}] &= 2q(1 - q)/q^2, \\ &= (2/q) - 2, \end{aligned}$$

$$\text{enrichment } (q) = 2/([\text{}^{14}\text{N}^{15}\text{N}]:[\text{}^{15}\text{N}^{15}\text{N}] + 2) \quad (\text{Milano } \textit{et al.} \text{ 1996}),$$

which yields estimates of 9.4 and 14.5 MPE for the fed and fasted states respectively, after correction for the natural abundances of the two urea species. Such values would then yield, at plateau, predicted enrichments for [ $^{14}\text{N}^{15}\text{N}$ ]urea of 17 and 25 MPE, much lower than the cumulative value observed over the 6 h of isotope administration. The alternative explanation for the nutritional influences on [ $^{14}\text{N}^{15}\text{N}$ ]:[ $^{15}\text{N}^{15}\text{N}$ ]urea enrichments involves non-equilibrium conditions and specific channelling through one route, probably CP.

The current data do not allow resolution of these two possibilities. The equilibrium scenario would lead to the conclusion that  $\text{NH}_3$  production across the gut is lower in the fasted compared with the fed state as the predicted enrichment was higher at the same isotope input. This would agree with observations in other species (Seal & Reynolds, 1993). The non-equilibrium option would relate to the demands of the ornithine cycle for equal inputs of N via CP and aspartate. Thus, under conditions where amino acid oxidation is dominant and supply of free  $\text{NH}_3$  limiting, as may occur during fasting, N has to be transferred from other sources into the  $\text{NH}_3$  pool and thus any exogenous  $\text{NH}_3$  may be preferentially channelled into CP. The existence of doubly-labelled urea does allow the statement that some of the  $\text{NH}_3$ -N is transferred to both aspartate and CP, but without further information on the extent of equilibration which occurs it is not possible to quantify how the net inflows of N are altered with nutritional status and thus whether detoxification of  $\text{NH}_3$  might lead, for example, to increased amino acid oxidation under certain conditions.

Another fate of  $\text{NH}_3$  is in the synthesis of glutamine, which may occur either in the perivenous hepatocytes or peripheral tissues. In other studies, infusion of  $^{15}\text{NH}_4\text{Cl}$  into the peripheral (post-hepatic) circulation, at doses comparable to the current study, resulted in higher enrichments of [amide- $^{15}\text{N}$ ]glutamine (Stein *et al.* 1976; Nissim *et al.* 1984), probably arising from synthesis in muscle. Probably little of the ingested  $^{15}\text{NH}_3$  entered the peripheral circulation because hepatic extraction can be as great as 99% (Nieto *et al.* 1996)

and thus the [amide- $^{15}\text{N}$ ]glutamine produced was from the perivenous hepatocytes. That the enrichment of this was lower than [ $^{14}\text{N}^{15}\text{N}$ ]urea, synthesized in the periportal hepatocytes, supports the concept of both extraction of labelled and release of unlabelled  $\text{NH}_3$  occurring during transit across the hepatic bed, with the enrichment in the perivenous cells lower than in the periportal region (Nieto *et al.* 1996). The similarity in plateau enrichment for [amide- $^{15}\text{N}$ ]glutamine between the fed and fasted states thus only reflects isotopic activities in the perivenous cells and cannot be taken as representative of  $\text{NH}_3$  enrichment in the hepatic portal vein.

Production of [amino- $^{15}\text{N}$ ]glutamine through amination of [ $^{15}\text{N}$ ]glutamate might also occur in both perivenous hepatocytes and peripheral tissues. Glutamate is not readily transported across membranes, however, as illustrated by the considerable differences in enrichment values between blood and plasma (Table 3). It is probable, therefore, that [ $^{15}\text{N}$ ]glutamate is taken up by the high-affinity glutamate transport system in the perivenous hepatocytes (Häussinger, 1990; Meijer *et al.* 1990), where it acts as a precursor for [amino- $^{15}\text{N}$ ]glutamine synthesis. Substantial differences existed in the labelling pattern of [amino- $^{15}\text{N}$ ]glutamine between nutritional states but, as with [ $^{15}\text{N}^{15}\text{N}$ ]urea, this may have been due to either greater enrichments of precursor  $\text{NH}_3$  for the GDH reaction or increased channelling of  $\text{NH}_3$  through aspartate.

Enrichments of glutamate and the branched-chain amino acids (BCAA; valine, leucine and isoleucine) were higher in the fasted state. This may have resulted from a decrease of the unlabelled substrate (amino acid concentrations) and/or an increase in precursor  $^{15}\text{N}$  enrichment, either as  $\text{NH}_3$  (for glutamate) or amino acids (for transamination with the BCAA). The amino acid concentrations were not measured, but the total amount of  $^{15}\text{N}$  transferred to non-urea fates can be estimated to be greater in the fasted state. Thus, urea excretion in the fed state was 1.3-fold that during fasting and, when alterations in body urea pool size are taken into account (based on changes in plasma urea concentration and estimates of urea space; Long *et al.* 1978), urea synthesis was probably nearer 1.65-fold that during the fasted state. Consequently, during food deprivation only 0.6 of  $^{15}\text{N}$  label was transferred, in relative terms, to urea, with a greater proportion thus available to enrich other metabolites. The substantial increase in glycine enrichment during fasting may be linked to increased action of the glycine synthase complex (*EC* 1.4.4.2 and *EC* 2.1.2.10) and less emphasis on transhydroxymethylation with serine. The lower enrichment of alanine is probably explained by a change in net flux towards deamination and provision of C skeletons for energy and glucose synthesis during fasting.

Although arginine is directly involved in the ornithine cycle, the plasma enrichments, although substantial, were lower than for urea. Hepatic arginine is, however, continuously subjected to the considerable arginase (*EC* 3.5.3.1) activity in the liver (Barbul, 1985) and equilibration with the plasma pool is therefore unlikely. Plasma arginine flux has been reported to be determined primarily by arginine intake and synthesis from citrulline in the kidney (Castillo *et al.* 1993). Citrulline can also be synthesized from ornithine and  $^{15}\text{NH}_3$  in gut tissue (Barbul, 1985) but, for technical reasons, the enrichment could not be determined in the current study. Citrulline is an attractive candidate for estimation of precursor CP enrichment although interpretation would be confounded by the existence of several sites of synthesis within the body and the half-life of the citrulline pool.

The current study confirms that transfer of  $\text{NH}_3\text{-N}$  to both CP and aspartate occurs in the human liver in both the fed and fasted conditions. It is not yet possible to decide the extent of these transfers although the rates are less than those calculated based on the uncorrected data of Patterson *et al.* (1993) and probably match more closely those observed in other large species (Lobley *et al.* 1995). The evidence also supports a rapid decrease in  $\text{NH}_3$  enrichment across the liver such that hepatic syntheses of urea and glutamine, the



major end-products of  $\text{NH}_3$  metabolism, arise from cellular compartments of very different isotopic activity.

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