Does glutamine act as a substrate for transamination reactions in the liver of fed and fasted sheep?

S. O. Hoskin[1]*, S. Gavet[2], E. Milne[1] and G. E. Lobley[1]†

[1]*Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK*
[2]*Ecole Nationale Superiore d'Agronomie de Rennes, 35042 Rennes Cedex, France*

The present study investigated the relative importance of glutamine as a transamination source in the ovine liver by examination of the labelling of amino acids (AA) in the hepatic free pool, mixed liver and plasma proteins of fed and fasted sheep, following infusion of isotopically-labelled glutamine. In a cross-over design four sheep were either fasted for 3 d or fed to $1.2 \times$ energy maintenance and finally euthanased. At each intake, the sheep were infused for 6 h with [2-^{15}N]glutamine (150 μmol/h) and samples of total plasma protein isolated. Following the terminal infusion, liver tissue total proteins were prepared and hydrolysed and ^{15}N-enrichments in seventeen AA were determined by GC–combustion–isotope-ratio mass spectrometry. All AA were enriched (relative to natural abundance) except lysine and threonine, with the lowest enrichments in phenylalanine and histidine. There was no effect of the fed *v.* fasted state, except for leucine and isoleucine in liver protein ($P < 0.05$). Enrichments in liver protein were greater than in plasma protein ($P < 0.01$, except proline) and probably reflect the faster turnover rate of hepatic constitutive proteins compared with export proteins. Amination to methionine was greater than that to phenylalanine ($P < 0.01$), suggesting a mechanism for preferentially protecting the former. This factor could be important for ruminant production, as methionine is often considered to be the first limiting AA for animals offered certain silages and conserved forages. Enrichments in all AA (except for glutamine, alanine and aspartate) were less than that for glutamate ($P < 0.01$), and thus transaminations may have occurred with glutamine directly or via glutamate, following the action of hepatic glutaminase.

Glutamine: Transamination: Liver: Amino acids: Enrichment

The ability of glutamine to be concentrated within cells by Na-dependent transporters (for example, see Le Boucher *et al.* 1997) provides a potential source of amido and NH_2 groups for metabolic interconversions. Although the role of the amido-N in a range of key reactions is well established (Welbourne *et al.* 1986; Haussinger *et al.* 1994; Calder, 1995), less attention has focused on the metabolism of the NH_2-N. In particular, the presence of glutamine aminotransferase has been demonstrated in a number of tissues, across a range of species (for example, see Cooper & Meister, 1972; Costa *et al.* 1986; Wu & Thompson, 1989) and, *in vitro*, this enzyme can use a variety of amino acids (AA) as NH_2 group donors and oxo-acids as acceptors (Blarzino *et al.* 1994). The predominant donor *in vivo* in the liver is probably glutamine, owing to the high intracellular concentration, while phenylpyruvate (to yield phenylalanine) and 2-oxo-4-methylthiobutyrate (OMTB; to yield methionine) are the main acceptors in rodent hepatocytes *in vitro* (Meister, 1979; Haussinger *et al.* 1985).

For ruminants offered many forages or silages, methionine is often considered as the first limiting AA (Storm & Ørskov, 1984). Similarly, a high percentage (>90) of absorbed phenylalanine is removed across the ovine liver (Lobley & Milano, 1997), reducing its availability to peripheral tissues. Furthermore, demands for phenylalanine may be increased substantially during the acute phase of infection (Reeds *et al.* 1994). Thus, mechanisms that protect these, and other, AA from hepatic catabolism may have important consequences for both animal production and health. The glutamine transaminase present in the

---

bovine liver has similar substrate specificity *in vitro* to the rodent enzyme (Costa *et al.* 1986), but whether action of this enzyme represents an important mechanism by which AA may be protected from oxidation *in vivo* in ruminants is unknown. This question has been addressed in the current study through investigation of the transfer of the NH_2 group of [2-^{15}N]glutamine into various AA isolated from the hepatic free pool, plus both mixed liver and plasma proteins, in fed and fasted sheep. Part of the present study has been presented previously in abstract form (Hoskin *et al.* 1999).

Materials and methods

Protocol

Four Suffolk-cross sheep (age 12 months, live weight mean 40·8 (range 38–43) kg) were prepared with a polyvinyl chloride catheter (Lobley *et al.* 1990) in each external jugular vein: one at a distance of 20 cm for sampling and the other into the right ventricle of the heart (approximately 35–40 cm) for infusion. These catheters were filled with dextrose (26 g/l) plus sodium citrate (38 g/l, pH 7·4) as anticoagulant. Animals were accustomed to metabolism cages with hourly feeding by means of automated feeders and received 1000 g of a mixed diet (w/w:50 % hay, 30 % barley, 10 % molasses, 9 % fishmeal, 1 % salts and vitamins; metabolisable energy 11·5 MJ/kg DM, N 21·4 g/kg DM).

A cross-over balanced design was adopted with the treatments as either fed (1000 g mixed diet) or fasted for 3 d. Measurements on the two intake treatments were made 7 d apart.

On infusion days, a pre-sample of plasma (10 ml) was taken for background (initially natural abundance) enrichments for both free and protein-bound AA. A 6 h continuous infusion (15 g/h) of [2-^{15}N]glutamine (34 mM, 15 g/h; Mass Trace, Woburn, MA, USA) in sterile 0·15 M-NaCl was then initiated. Blood samples (6 ml) were taken every 30 min during the last 3 h. Immediately following the second infusion, the sheep were euthanased and a portion of the liver was removed and frozen in liquid N_2, followed by storage at −80°C.

Analyses

Plasma samples (1 ml) were deproteinised with sulfosalicylic acid (final concentration 70 g/l plasma), desalted with Dowex-50 H$^+$ and the free glutamine eluted, along with the other AA, with 2 M-NH_4OH as described previously (Lobley *et al.* 1990). Liver samples (5 g) were homogenised in water (15 ml). To 3 ml of this homogenate was added 0·5 ml sulfosalicylic acid (48 % w/v), and the free AA in the supernatant were extracted as for plasma. The remainder of the liver homogenate, as well as plasma samples, were dialysed against 0·05 M-sodium phosphate buffer, pH 7·4, for 48 h with three changes of a 100-fold liquid excess to remove free AA. To portions of the dialysates (each equivalent to approximately 15 mg protein) was added pronase E (1 mg; Merck Ltd, Lutterworth,

Leics, UK) plus proteinase K (1 mg; Merck Ltd) and the mixture was incubated for 48 h at 25°C. The reaction was stopped by addition of sulfosalicylic acid (to final concentration 7 %, w/v) and the liberated AA were isolated as described for the free-pool extracts.

The enrichment of free [2-^{15}N]glutamine in plasma and liver was determined by electron-impact GC–mass spectrometry (MS; HP 5889A MS Engine Engine, Hewlett Packard, Avondale, PA, USA) as the *t*-butyldimethylsilyl derivative, as described by Calder & Smith (1988). Fragment ions m:z of 258 and 259 were monitored. Analyses were also performed to examine the contribution of [5-^{15}N]glutamine. For plasma free glutamine this was <3 % of the enrichment, in line with the low contribution that glutamine-N makes to whole-body NH_3 kinetics in sheep (Gate *et al.* 1999). The corresponding value in the liver free pool was <8 %, lower than observed in non-ruminants (Jahoor *et al.* 1988). The AA liberated from plasma and liver protein plus those from the liver free pool were also converted to their *t*-butyldimethylsilyl derivatives and then analysed by GC–combustion (C)–isotope-ratio (IR) MS. Sample concentrations were adjusted to 1·6 mM with regard to leucine, and 1 μl was injected in splitless mode onto a Hewlett Packard 5890 series II gas chromatograph (Hewlett Packard Ltd) interfaced to an Orchid module (Europa Scientific, Crewe, UK). The latter oxidises the individual AA separated on the GC by passage through a quartz tube containing platinumised Cu wire (at 830°C). The resulting gases, including CO_2, N_2, nitrogen oxides and water, pass through a Nafion membrane to remove water; nitrogen oxides are reduced to N_2 in a second quartz tube containing Cu metal (at 600°C). Finally, liquid N_2 was used to remove CO_2 and the resulting pure N_2 gas was admitted to a SIRA Series II mass spectrometer (VG Isotech, Middlewich, Cheshire, UK). The m:z 29:28 was measured and compared with reference, O_2-free N_2 gas. Plasma proteins were compared with samples taken just before the appropriate infusion. Both liver free and protein-bound AA were compared with those in plasma protein just before the first infusion. Glutamine was found to yield two derivatives, glutamine and pyroglutamine (see Fig. 1). These derivatives gave similar enrichments, but their relative peak magnitudes varied with analytical conditions. For consistency, the pyroglutamine peak was used for comparative purposes.

Calculations and statistical analysis

The free AA were measured by GC–MS and expressed as atom percent excess (ape) as described by Campbell (1979). All GC–C–IRMS enrichments were determined as atom percent and then calculated as ape relative to appropriate background samples. In order to adjust the GC–C–IRMS data for comparison with GC–MS values, the enrichments of free glutamine were adjusted for the number of N atoms present in the molecule, i.e. only the N-2 was assumed to be labelled. In order to calculate the contribution of the NH_2-N of glutamine to the other AA it was necessary to adjust the enrichment values for the number of N atoms in each molecule. The assumption
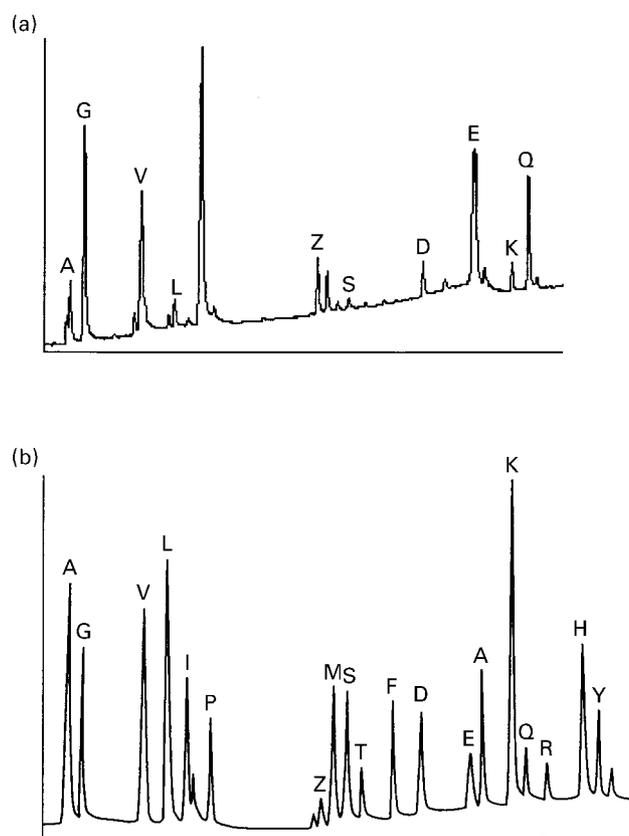
Fig. 1. Combustion–isotope-ratio mass spectrometry of ^{15}N-labelled amino acids isolated from (a) the liver free pool and (b) liver mixed proteins. A, alanine; G, glycine; V, valine; L, leucine; I, isoleucine; P, proline; Z, pyroglutamine; M, methionine; S, serine; T, threonine; F, phenylalanine; D, aspartate; E, glutamate; K, lysine; Q, glutamine; R, arginine; H, histidine; Y, tyrosine.

was made that ^{15}N transferred only between the NH_2 groups and that all other N atoms were at natural abundance. These values were then scaled against that for glutamine in the appropriate pool.

The irreversible loss rate (ILR; mmol/h) of glutamine was calculated from plasma free AA enrichments (ape) by the conventional formula:

$$ILR = [(99{\cdot}4/plasma\ ape) - 1]$$

$$\times\ glutamine\ infusion\ rate\ (mmol/h),$$

where 99·4 is the enrichment of the infused [2-^{15}N]glutamine.

To determine increased enrichment above background as a result of [2-^{15}N]glutamine infusion, samples were analysed by one-way ANOVA with animals treated as blocks. The effects of the fed or fasted condition on enrichments were analysed by two-way ANOVA with animals treated as blocks and intake × period as factors. In practice, period was not significant, and was removed from the analysis. All analyses were performed with Genstat for Windows (version 3.2; Lawes Educational Trust, Rothamsted, Herts., UK).

Results

Glutamine plasma irreversible loss rate was not influenced by whether the animals were fasted or fed (13·05 and 13·95 mmol/h respectively, SED 1·19). Liver free [2-^{15}N]glutamine enrichments averaged 0·71 those of the plasma glutamine and gave similar values by both GC–MS and GC–C–IRMS methods (means 2·68, 2·61 and 2·61 ape for measurements by GC–MS and by GC–C–IRMS for glutamine and pyroglutamine respectively, SED 0·256; NS). Only a limited number of free AA in the liver cytosol gave peak areas of sufficient magnitude for confident values on the GC–C–IRMS system (Fig. 1a). All these AA gave enrichments that were lower than that for the [2-^{15}N]glutamine (Table 1), with the non-essential AA, in general, more enriched than the essential AA. The enrichment in hepatic free lysine, although low, was significantly different ($P<0{\cdot}05$) at the end of the second infusion from that of the initial plasma protein-bound lysine. This difference may represent lysine synthesised within the digestive tract by micro-organisms following the first infusion, followed by absorption and retention within the

Table 1. ^{15}N-enrichments (atom percent excess (ape) × 1000) of free amino acids in liver homogenates from fed and fasted sheep following a second 6 h infusion of [2-^{15}N]glutamine*

(Mean values for two sheep at each intake)

	Enrichment (ape × 1000)			Statistical significance of difference:
	Fed	Fasted	SED	P†
Alanine	279	258	38·3	NS
Glycine	160	200	30·0	NS
Valine	197	284	18·4	0·042
Leucine	111	86	77·4	NS
Glutamine	1096	1513	195·7	NS
Serine	145	151	20·7	NS
Aspartate	445	755	37·0	0·014
Glutamate	390	778	25·8	0·004
Lysine	31	73	6·6	0·024

* Each sheep previously infused (7 d earlier) with [2-^{15}N]glutamine for 6 h during the alternative intake; for details of procedures, see p. 592.
† Effect of intake, 2 residual df.

Table 2. ^{15}N-enrichments (atom percent excess (ape) $\times$ 1000) of amino acids in liver proteins at the end of a second 6 h infusion of [2-^{15}N]glutamine in fed and fasted sheep*

(Mean values of two sheep at each intake)

	Enrichment (ape × 1000)†			Statistical significance:	
	Fed	Fasted	SED‡	P‡	P§
Alanine	22·8	25·5	1·08	NS	<0·001
Glycine	13·6	14·5	1·68	NS	<0·001
Valine	9·2	10·4	0·60	NS	<0·001
Leucine	12·8	16·9	0·72	0·030	<0·001
Isoleucine	16·4	19·6	0·68	0·044	<0·001
Proline	3·9	5·6	3·34	NS	0·030
Glutamine	86·6	100·1	5·5	NS	<0·001
Methionine	9·6	11·2	2·08	NS	<0·001
Serine	18·7	23·5	2·93	NS	<0·001
Threonine	0·3	6·0	3·25	NS	NS
Phenylalanine	6·6	7·2	1·92	NS	<0·001
Aspartate	30·1	48·4	4·09	NS	<0·001
Glutamate	23·7	20·9	8·29	NS	<0·001
Lysine	2·9	1·6	1·68	NS	NS
Arginine	13·2	19·1	2·09	NS	<0·001
Histidine	5·8	2·8	2·26	NS	0·006
Tyrosine	12·7	15·0	0·85	NS	<0·001

* Each sheep previously infused (7 d earlier) with [2-^{15}N]glutamine for 6 h during the alternative intake; for details of procedures, see p. 592.
† Natural abundance 'background' assumed the same as for plasma protein-bound amino acids before any infusion of [2-^{15}N]glutamine.
‡ Effect of intake, 2 residual df.
§ Difference from pre-infusion plasma protein enrichments, 7 residual df.

body proteins (Torrallardona *et al.* 1996). For fasting compared with the fed state, higher enrichments were obtained for glutamate ($P<0.01$), aspartate, valine and lysine ($P<0.05$), although the amount of label in lysine was very low.

Most AA were present in sufficient quantities from liver mixed proteins for reliable values to be obtained (Fig. 1(b); Table 2). For the protein-bound AA, the highest enrichment was for glutamine and the descending order of enrichment was: glutamine > aspartate > alanine = glutamate = serine > glycine = arginine = leucine = tyrosine > valine = methionine > phenylalanine > histidine > proline. Compared with the abundance of pre-infusion samples, there was no significant increase in enrichment for threonine and lysine. For most protein-bound hepatic AA, enrichment values were greater in the fasted state, but the difference was significant only for leucine and isoleucine ($P<0.05$). These enrichments were determined only after the second infusion. In the absence of a suitable background sample and because of the limited number of samples, other differences may have been masked.

Enrichments of AA from plasma protein were lower than those from liver protein ($P<0.01$, except for proline), although the order of enrichments was similar (Table 3). Again, there was no enrichment above background for lysine, threonine and histidine. Background enrichments were greater for most protein-bound AA from plasma proteins before the second, compared with the first, infusion ($P<0.01$; except proline, phenylalanine, lysine and arginine). Intake had no effect on the incorporation of ^{15}N from glutamine into the various AA (Table 3). Natural abundance values for AA, before the first infusion, extended over a relatively wide range, with threonine and, to a lesser extent, lysine, tyrosine and histidine, 'depleted' compared with the other AA. This finding is in agreement with those of other studies (Metges & Petzke, 1997).

The contribution of the NH$_2$-N of glutamine to the other AA ranged from 4 to 23 % (Table 4), but were not different

Table 3. ^{15}N-enrichments (atom percent excess (ape) $\times$ 1000) of amino acids in plasma protein at the end of a 6 h infusion of [2-^{15}N]glutamine in fed and fasted sheep*

(Mean values of four sheep at each intake)

	Natural abundance (atom percent)	Enrichment (ape × 1000)†			Statistical significance:		
		Fed	Fasted	SED	P‡	P§	P‖
Alanine	0·3733	4·9	5·0	0·74	<0·001	<0·001	<0·001
Glycine	0·3714	3·1	2·1	0·88	<0·001	0·001	<0·001
Valine	0·3730	1·1	1·3	0·40	<0·001	<0·001	0·011
Leucine	0·3719	1·5	2·7	0·56	<0·001	<0·001	0·005
Isoleucine	0·3724	3·5	4·3	0·45	<0·001	<0·001	<0·001
Proline	0·3758	1·0	2·0	0·44	0·003	0·095	NS
Glutamine	0·3716	22·6	38·6	8·24	<0·001	0·002	<0·001
Methionine	0·3714	2·7	5·0	2·7	0·007	0·003	0·063
Serine	0·3704	4·0	4·1	1·47	<0·001	0·002	0·003
Threonine	0·3652	−0·7	−0·6	0·40	NS	NS	0·044
Phenylalanine	0·3708	0·5	1·7	0·65	0·027	0·003	NS
Aspartate	0·3714	9·3	15·1	4·31	<0·001	0·002	0·002
Tyrosine	0·3692	3·2	2·7	0·76	<0·001	0·003	NS
Glutamate	0·3702	4·2	4·5	1·24	<0·001	0·013	0·009
Lysine	0·3696	0·1	0·5	0·86	NS	0·041	NS
Arginine	0·3718	2·3	4·5	1·66	0·002	0·001	0·001
Histidine	0·3692	0·1	−0·1	0·2	NS	0·036	NS

* For details of procedures, see p. 592.
† Values corrected for enrichments immediately before infusion.
‡ Significance of difference from pre-infusion enrichment, 7 residual df.
§ Comparison of enrichments at end of terminal (second) infusion for liver *v.* plasma protein, by one-way ANOVA, sheep as blocks, 3 residual df.
‖ Comparison of background enrichments for protein-bound amino acids in plasma protein before first and second infusions.

Table 4. Relative contribution of [2-^{15}N]glutamine to free (F) and protein-bound (LP) amino acids (AA) of liver and plasma protein (PP) from four sheep, fed and fasted combined

AA*	F	LP	PP	Stat F v. LP	P† LP v. PP	F v. PP
Alanine	0·107	0·130	0·032	NS	<0·001	0·017
Glycine	0·071	0·076	0·023	NS	<0·001	0·005
Valine	0·094	0·053	0·011	0·010	<0·001	0·002
Leucine	0·040	0·079	0·017	0·082	<0·001	NS
Isoleucine		0·096	0·028		<0·001	
Proline		0·025	0·014		NS	
Methionine		0·056	0·042		NS	
Serine	0·059	0·113	0·034	0·030	0·001	NS
Threonine		0·016	−0·003		NS	
Phenylalanine		0·037	0·009		0·004	
Aspartate	0·228	0·208	0·116	0·092	0·002	0·003
Glutamate	0·220	0·121	0·024	0·063	0·025	0·002
Lysine	0·039	0·025	0·005	NS	0·043	0·018
Arginine		0·343	0·122		<0·001	
Histidine		0·071	0·000		0·053	
Tyrosine		0·074	0·025		0·001	

* Data corrected for N content of AA, i.e. atom percent excess (Tables 2, 3 and 4) × 2 for glutamine and lysine, ×3 for histidine and ×4 for arginine.
† Only data for second infusion, by one-way ANOVA, 3 residual df, with animals treated as blocks.

between the fed and fasted states, so the values were combined. Although the enrichments of AA isolated from total liver proteins were lower than those for the free form, the ratios with regard to glutamine (the transamination ratio) were not different, except for valine and serine. For most AA isolated from total plasma protein, the transamination ratio was significantly lower than those from hepatic proteins (except proline, threonine and methionine; Table 4) and from the liver free pool (except leucine and serine).

Discussion

The finding that plasma glutamine irreversible loss rate was not different between the fed or fasted states is in line with earlier observations based on ^{14}C kinetics in sheep (Heitmann & Bergman, 1978). Furthermore, the whole-body flux through 2-(amino)-N glutamine is equal to, or greater than, that through the 5-(amido)-N moiety (post-absorptive human subjects, Darmaun et al. 1986; fed sheep, GE Lobley, unpublished results). This is despite the many reactions with which the latter is associated, and suggests that glutamine transaminations have considerable importance in overall body metabolism.

Extensive transfer of the NH$_2$-N of glutamine to most other free AA occurred within the liver cytosol. This transfer may be indirect, following conversion of glutamine by mitochondrial glutaminase to glutamate and subsequent glutamate transaminase activity. Indeed up to 22 % of glutamate NH$_2$-N arose from glutamine. Label ^{15}N transfer from glutamine occurs in both the rodent liver (Aqvist, 1951; Cooper et al. 1987) and ovine hepatocytes (Luo et al. 1995). Nonetheless, the ^{15}N-labelling ratios of individual AA:glutamate in liver protein were higher (by 50–700 %) in the current study than those reported for fed rodents following [^{15}N]glutamate injection (Aqvist, 1951) and for

ovine hepatocytes incubated with ^{15}NH$_3$ (Luo et al. 1995; GE Lobley and QJ Luo, unpublished results), e.g. glycine 0·57, 019 and 0·31; serine 0·79, 0·46 and 0·40; leucine 0·54, 0·30 and 0·05; phenylalanine 0·28, 0·14 and 0·04 for the current study, rodent values and ovine hepatocyte data respectively.

These differential labellings suggest that part of the NH$_2$ group transfers occurs directly from glutamine through the action of glutamine transaminase. Although the reaction catalysed is reversible, the high concentration of glutamine in the liver, coupled with the rapid removal of 2-oxoglutaramate, means that the reaction probably proceeds in the direction of transfer of NH$_2$-N from glutamine to oxo-acids (Cooper & Meister, 1972; Meister, 1979). The enzyme exists in two forms that are characterised by their affinities for different oxo-acids; L form has a high affinity for OMTB (the oxo-acid of methionine), while K form has similar affinities for both OMTB and phenylpyruvate (the oxo-acid of phenylalanine; see Cooper & Meister, 1981). Although purified glutamine transaminase from bovine liver has greater K_m values for OMTB than phenylpyruvate (0·2 and 0·1 mM respectively; Costa et al. 1986), a reduced catalytic constant:K_m indicated that phenylpyruvate was a less-favoured amino-acceptor than OMTB (Blarzino et al. 1994). These observations in vitro are supported by the lower rates of relative transamination from glutamine to phenylalanine, compared with methionine ($P<0·01$), observed for the current study in vivo.

Under a number of dietary situations, e.g. silages and conserved forages, methionine is considered to be the first limiting AA in rumen microbial protein (Storm & Ørskov, 1984). Hence, conservation of methionine by re-amination of the oxo-acid would favour protection against further catabolism. Based on comparison of glutamine and methionine enrichments, perhaps <6 % of the methionine is 'protected' by this mechanism. This may in part be due to the fact that methionine has two 'catabolic' pathways, involving trans-sulfuration and transamination. The latter is the minor route in most circumstances (Benevenga et al. 1983), but may become important in certain situations, such as a lowered availability of methyl donors to maintain the re-methylation of homocysteine to methionine (Storch et al. 1988). Nonetheless, even prevention from oxidation of 6 % of the methionine may have important beneficial effects.

A number of reasons might explain the higher apparent incorporation of tracer into liver constitutive proteins compared with plasma proteins. First, no allowance could be made for residual label in the hepatic proteins following the first infusion. Second, liver proteins in sheep in both the fed and fasted states have more rapid half-lives (3·5 d; Connell et al. 1997) than do plasma proteins (12–18 d; Abbott et al. 1985; Connell et al. 1997). Indeed, the four-fold difference in labelling observed in the current study is compatible with these reported values. Third, hepatic constitutive and export proteins are probably synthesised from different precursor pools; one involves the cell milieu while the other comprises AA that have just entered the cell from the extracellular matrix (see Fern & Garlick, 1976; Connell et al. 1997; Stoll et al. 1998). These pools will have different relative enrichments between individual AA and glutamine. This interpretation requires some caution,

however, because not all the plasma proteins are produced within the liver. A focus on albumin, which is synthesised exclusively by the liver, would have provided a more direct comparison.

When expressed relative to free glutamine enrichments in the hepatic cytosol, the proportion of NH_2-N derived from glutamine did not appear to alter between the fed and fasted states. These data need to be interpreted with caution because, in the present study, the hepatic free AA were limited to just two measurements for each nutritional state and without an appropriate background sample. Furthermore, changes in the flux of the free AA within the liver cytosol should be considered. These measurements are not available, but whole-body flux does increase with intake, as does AA oxidation (Lobley *et al.* 1987; Harris *et al.* 1992). In consequence, net transamination from glutamine, whether direct or indirect, would probably be elevated at the higher intakes. For many AA, whole-body flux is usually two to three times absorption (Reeds & Lobley, 1980), while oxidation ranges between 5 and 20 % of flux (for example, see Reeds *et al.* 1980; Lobley *et al.* 1987; Harris *et al.* 1992). Thus, a 5 % re-amination of flux may represent a net sparing in oxidation of 30–100 %, equivalent to 12–15 % of intake, which represents an important mechanism in maintaining the AA economy of the animal.

In conclusion, glutamine NH_2-N is transferred to other AA within the ovine liver *in vivo*. Although these transfers are dominated by the non-essential AA, there are significant movements for most of the essential AA. Of the latter, methionine appears to be a preferred acceptor, and this would agree with the substrate specificities of glutamine aminotransferase *in vitro*. This might provide a mechanism to protect dietary methionine from catabolism, an important requirement for ruminants offered certain forms of silage and conserved forages.

Acknowledgements

References

Abbott EM, Parkins JJ & Holmes PH (1985) Influence of dietary protein on the pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Blackface lambs given a single moderate infection. *Research in Veterinary Science* **38**, 54–60.

Aqvist SEG (1951) Metabolic interrelationships among amino acids studied with isotopic nitrogen. *Acta Chimica Scandinavica* **5**, 1046–1064.

Benevenga NJ, Radcliffe BC & Egan AR (1983) Tissue metabolism of methionine in sheep. *Australian Journal of Biological Sciences* **36**, 475–485.

Blarzino C, Coccia R, Pensa B, Cini C & De Marco C (1994) Selenomethionine as a substrate for glutamine transaminase. *Biochemistry and Molecular Biology International* **32**, 79–86.

Calder AG & Smith A (1988) Stable isotope ratio analysis of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* **2**, 14–16.

Calder PC (1995) Glutamine action in cells of the immune system. In *Pharmacological Nutrition – Immune Nutrition*, pp. 10–20 [L Cynober, P Fürst and PW Lawin, editors]. Munich, Germany: Zuckschwerdt Verlag.

Campbell IM (1979) Incorporation and dilution values – their calculation in mass spectrally assayed stable isotope labelling experiments. *Bioorganic Chemistry* **3**, 386–397.

Connell A, Calder AG, Anderson SE & Lobley GE (1997) Hepatic protein synthesis in the sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. *British Journal of Nutrition* **77**, 255–271.

Cooper AJL & Meister A (1972) Isolation and properties of highly purified glutamine transaminase. *Biochemistry* **11**, 661–671.

Cooper AJL & Meister A (1981) Comparative studies of glutamine transaminases from rat tissues. *Comparative Biochemistry and Physiology* **69**, 137–145.

Cooper AJL, Nieves E, Coleman AE, Filc-De Ricco S & Gelbard AS (1987) Short term metabolic fate of [^{13}N] ammonia in rat liver *in vivo*. *Journal of Biological Chemistry* **262**, 1073–1080.

Costa M, Pensa B, Fontana M, Foppoli C & Cavallini D (1986) Transamination of L-cystathione and related compounds by a bovine liver enzyme. Possible identification with glutamine transaminase. *Biochimica et Biophysica Acta* **881**, 314–320.

Darmaun D, Matthews DE & Bier DM (1986) Glutamine and glutamate kinetics in humans. *American Journal of Physiology* **251**, E117–E126.

Fern EB & Garlick PJ (1976) Compartmentation of albumin and ferritin synthesis in rat liver *in vivo*. *Biochemical Journal* **156**, 189–192.

Gate JJ, Parker DS & Lobley GE (1999) The metabolic fate of the amido-N group of glutamine in the tissues of the gastrointestinal tract in 24 h-fasted sheep. *British Journal of Nutrition* **81**, 297–306.

Harris PM, Skene PA, Buchan V, Milne E, Calder AG, Anderson SE, Connell A & Lobley GE (1992) Effect of food intake on hind-limb and whole-body protein metabolism in growing lambs: chronic studies based on arterio–venous techniques. *British Journal of Nutrition* **68**, 389–407.

Haussinger D, Lang F & Gerok W (1994) Regulation of cell function by the cellular hydration state. *American Journal of Physiology* **267**, E343–E355.

Haussinger D, Steele T & Gerok W (1985) Glutamine metabolism in isolated perfused rat liver. The transamination pathway. *Biological Chemistry Hoppe-Seyler* **366**, 527–536.

Heitmann RN & Bergman EN (1978) Glutamine metabolism, inter-organ transport and glucogenicity in the sheep. *American Journal of Physiology* **234**, E197–E203.

Hoskin SO, Gavet S, Milne E & Lobley GE (1999) Transamination of ovine hepatic oxo-acids *in vivo* by glutamine. *Book of abstracts, VIIIth International Symposium on Protein Metabolism and Nutrition*, Aberdeen, p. 7 [GE Lobley, A White and JC MacRae, editors]. Wageningen, The Netherlands: Wageningen Pers.

Jahoor F, Jackson AA & Golden NHN (1988) *In vivo* metabolism of nitrogen precursors for urea synthesis in the post prandial rat. *Annals of Nutrition and Metabolism* **32**, 240–244.

Le Boucher J, Coudray LC, Lasnier E, Jardel A, Ekindjian OG & Cynober LA (1997) Enteral administration of ornithine alpha-ketoglutarate or arginine alpha-ketoglutarate: A comparative study of their effects on gluatmine pools in burn-injured rats. *Critical Care Medicine* **25**, 293–298.

Lobley GE, Connell A & Buchan V (1987) Effect of food intake

on protein and energy metabolism in finishing beef steers. *British Journal of Nutrition* **57**, 457–465.

Lobley GE, Connell A, Milne E, Buchan V, Calder AG, Anderson SE & Vint H (1990) Muscle protein synthesis in response to testosterone administration in weather lambs. *British Journal of Nutrition* **64**, 691–704.

Lobley GE & Milano GD (1997) Regulation of hepatic nitrogen metabolism in ruminants. *Proceedings of the Nutrition Society* **46**, 547–563.

Luo QJ, Maltby SA, Lobley GE, Calder G & Lomax MA (1995) The effect of amino acids on the metabolic fate of $^{15}NH_4Cl$ in isolated sheep hepatocytes. *European Journal of Biochemistry* **228**, 912–917.

Meister A (1979) Biochemistry of glutamate:glutamine and glutathione. In *Glutamic Acid: Advances in Biochemistry and Physiology*, pp. 69–84 [LJ Filer Jr, editor]. New York: Raven Press.

Metges CC & Petzke KJ (1997) Measurement of N-15/N-14 isotopic composition in individual plasma free amino acids of human adults at natural abundance by gas chromatography combustion isotope ratio mass spectrometry. *Analytical Biochemistry* **247**, 158–164.

Reeds PJ, Cadenhead A, Fuller MF, Lobley GE & McDonald JD (1980) Protein turnover in growing pigs. Effects of age and food intake. *British Journal of Nutrition* **43**, 445–455.

Reeds PJ, Fjeld CR & Jahoor F (1994) Do the differences between the amino acid compositions of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *Journal of Nutrition* **124**, 906–910.

Reeds PJ & Lobley GE (1980) Protein synthesis: are there real species differences? *Proceedings of the Nutrition Society* **39**, 43–52.

Stoll B, Burrin DG, Henry J, Yu H, Jahoor F & Reeds PJ (1998) Dietary amino acids are the preferential source of hepatic protein synthesis in piglets. *Journal of Nutrition* **128**, 1517–1524.

Storch KJ, Wagner DA, Burke JF & Young VR (1988) Quantitative study *in vivo* of methionine cycle in humans using [methyl-^{2}H3] and [1-^{13}C]methionine. *American Journal of Physiology* **255**, E322–E331.

Storm E & Ørskov ER (1984) The nutritive value of rumen microorganisms in ruminants. 4. The limiting amino acids of microbial protein in growing sheep determined by a new approach. *British Journal of Nutrition* **57**, 613–622.

Torrallardona D, Harris CI, Coates ME & Fuller MF (1996) The contribution of intestinal microflora to amino acid requirements in pigs. In *Proceedings of VIth International Symposium on Digestive Physiology in Pigs. European Association for Animal Production Publication* no. 80, pp. 245–248 [WB Souffrant and H Hagemeister, editors]. Dummerstorf, Germany: Forschungsinstitut für die Biologie landwirtschaftlicher Nutztiere.

Welbourne TC, Childress D & Givens G (1986) Renal regulation of interorgan glutamine flow in metabolic acidosis. *American Journal of Physiology* **226**, R858–R866.

Wu G & Thompson JR (1989) Methionine transamination and glutamine transaminases in skeletal muscle. *Biochemical Journal* **262**, 690–691.