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Glutamine metabolism in ovine splanchnic tissues: effects of infusion of ammonium bicarbonate or amino acids into the abomasum

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This study investigates the effects of increased NH_3 or amino acid supply on glutamine utilisation and production by the splanchnic tissues of fed sheep. Six sheep, prepared with vascular catheters in the aorta, mesenteric, portal and hepatic veins, were fed grass pellets to 1·1 × energy maintenance requirements. Each treatment involved a 4 d abomasal infusion, of either ammonium bicarbonate (AMM; 23·4 μmol/$kg^{0·75}$ per min), water (CONT), or a mixture of amino acids that excluded glutamine and glutamate (AA; 46·8 μmol amino acid-N/$kg^{0·75}$ per min). The treatments simulated nutritional extremes in terms of the balance of absorbed N. Kinetics across the whole gut and the liver were monitored during an intra-jugular infusion of [5-^{15}N]glutamine. Blood flow across the whole gut or liver were unaffected by treatment. Both AMM and AA infusions doubled the hepatic release of urea-N compared with CONT ($P<0·02$). AA infusion decreased arterial glutamine concentration by 26 % ($P<0·01$) and 23 % ($P<0·05$) compared with AMM and CONT respectively. Despite this, whole-body glutamine flux was not affected by treatment. In contrast, AMM infusion increased hepatic glutamine production by 40 % compared with CONT ($P<0·02$). This provided a mechanism to ensure NH_3 supply to the periphery was maintained within the normal low physiological levels. Hepatic glutamine utilisation tended to increase during AA infusion, probably to ensure equal inflows of N to the ornithine cycle. Between 6 and 10 % of NH_3 absorbed across the digestive tract was derived from the amido-N of glutamine. Overall, splanchnic glutamine utilisation accounted for 45–70 % of whole-body glutamine flux.

Glutamine: ^{15}N kinetics: Ureagenesis: Ammonia: Amino acids: Portal-drained viscera: Liver: Sheep

Glutamine is now recognised as an extremely important amino acid, with key roles as a N precursor for synthesis of nucleic acids and amino sugars (Garber, 1980); as a preferred fuel for cells of the intestinal mucosa (Souba, 1991); and an obligate nutrient for the immune system (Calder, 1994). Additional functions include involvement in maintenance of acid–base balance (Meijer *et al.* 1990); prevention of peripheral hyperammonaemia (Häussinger *et al.* 1992); and a putative role in regulation of cellular macronutrient metabolism (vom Dahl & Häussinger, 1996). Alteration of glutamine status, therefore, may have marked effects on whole-body metabolism.

In non-ruminant animals, increased hepatic extraction of glutamine occurs when amino acid supply is in excess of the requirement for tissues (Meijer *et al.* 1990; Häussinger *et al.* 1992). This is to provide sufficient NH_3 to maintain ornithine-cycle activity and allow catabolism of the amino acid surplus. In ruminant animals, however, rumen fermentation normally results in excess NH_3 production and limited amino acid available for absorption. Under these circumstances, hepatic glutamine net production may occur, this time as a mechanism to detoxify NH_3 (Wray-Cahen *et al.* 1997). This putative species difference may also extend to the use of glutamine by the digestive tract where, in fasted animals, the limited result available suggest that more glutamine is catabolised to NH_3 in rats than sheep (cf. Windmueller & Spaeth, 1980; Gate *et al.* 1999). In the case of the rat, such elevation in NH_3 production at non-hepatic sites, may assist the hepatic removal of amino acid excesses. In contrast, when NH_3 availability is already plentiful, as is the case for the ruminant animal due to microbial fermentation in the foregut, avoidance of

Abbreviations: AA, amino acid mixture infusion; AMM, ammonium bicarbonate infusion; CONT, water infusion; ILR, irreversible loss rate; PDV, portal-drained viscera.

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NH_3 excess is desirable because of the known metabolic toxicity (see Lobley *et al.* 1995).

The hypotheses described earlier, and implied species differences, are based on limited information, however. This is particularly so when the metabolic involvement of the amido(5)-N is considered. This moiety is key to a number of the reactions described and yet, even in non-ruminant animals, only a few studies *in vivo* have been conducted (e.g. Darmaun *et al.* 1986; Cooper *et al.* 1988). Until recently, the studies of Heitmann & Bergman (1978) were the only source of data available for kinetics of glutamine fluxes across the splanchnic tissues; the information, however was restricted to the C-skeleton metabolism through use of [^{14}C]glutamine. Some information on the metabolic fate of the 5-N of glutamine across the digestive tract of sheep is now available (Gate *et al.* 1999), but this is only for fasted lambs.

Removal of excesses of either NH_3 and/or amino acids is universally recognised as important to all animals, but what is less clear are the possible implications for glutamine requirement and status. The current study was designed, therefore, to test whether the glutamine-based mechanisms for controlling entry of excess NH_3 or amino acids into the ornithine cycle described for non-ruminant animals also operate in sheep. The protocol simulated nutritional extremes by providing, through chronic (4 d) abomasal infusion, either ammonium bicarbonate (AMM) or a mixture of amino acids (AA) in addition to a basal diet (CONT).

Materials and methods

Animals and diets

Six Suffolk cross wether sheep (mean live weight 39 SD 3·1 kg,) were surgically prepared with chronic indwelling catheters in the aorta and portal, hepatic and mesenteric veins, as described previously (Lobley *et al.* 1995). An additional Degania silicone rubber catheter (1·5 mm i.d., 3·2 mm outer diameter; Merck Ltd, Poole, Dorset, UK) was implanted directly in the abomasum to allow post-rumen infusions. Animals were allowed at least 3 weeks recovery after surgery before allocation to the experiment, at which time a temporary polyvinly chloride catheter (0·8 mm i.d., 1·5 mm outer diameter; Critchley Electrical Products, Auburn, New South Wales, Australia) was inserted into an exterior jugular vein.

Animals were adapted to metabolic cages and offered 47 g grass pellets/kg$^{0·75}$ per d (1·1 × energy maintenance requirements) (grass pellets contained:10·5 MJ metabolisable energy/kg DM; 22·0 g N/kg DM; 960 g DM/kg), supplied from automated feeders as hourly portions.

Experimental design

For each animal the experimental protocol involved three consecutive abomasal infusions of ammonium bicarbonate (AMM; 365 μmol/min, 23·4 μmol/kg$^{0·75}$ per min), an L-amino acid mixture (AA; based on rumen microbial protein composition, as described previously (Lobley *et al.* 1998), at a rate of 730 μmol amino acid-N/min (46·8 μmol AA-N/kg$^{0·75}$ per min)) and water (CONT). The AMM and AA infusates were prepared in water. Three animals were infused in the order AMM, CONT, AA while the other three received the reverse sequence (AA, CONT, AMM). Each infusion was for 4 d at a rate of 60 g/h, allowing at least 10 d between the AMM and AA treatments. The CONT infusion was allocated in the middle of the other two treatments to allow maximum time spacing between AA and AMM infusion, and was started at least 3 d after finishing the previous treatment.

The amounts infused were designed to obtain a relationship for liver uptake of NH_3-N: amino acid-N of approximately 2:1 for the AMM infusion, and 1:2 for the AA respectively, assuming this relationship was approximately 1:1 in the case of water infusion (CONT), according to previous observations in animals under similar conditions (Milano, 1997) and assuming that half the amount of amino acid-N infused reached the liver. The infusions were also calculated to maintain urea production below the maximum hepatic capacity (approximately 2 μmol urea N/g liver per min; Symonds *et al.* 1981; Orzechowski *et al.* 1987).

Stable isotope infusion

Pyrogen-free L-glutamine solution (36 mM in sterile 0·15 M -NaCl; BUFA bv, Spodefell Ltd, London, UK) was infused into the jugular catheter at a rate of 6 μmol/min during the first 3 of treatment. This was to avoid changes in glutamine pool sizes or fluxes when the [^{15}N]glutamine was infused. On the fourth day the L-glutamine infusion was substituted by 100 ml 24 mM [5-^{15}N]glutamine (99 atom %; Mass Trace Inc., Woburn, MA, USA) that contained 60 kU sodium heparin (CP Pharmaceuticals, Wrexham, Wales, UK), and which was infused at 6 μmol/min (15 g/h) for 6 h. Over the same period a sterile solution of 0·1 M-sodium *p*-aminohippurate in 0·05 M-sodium phosphate buffer (final pH 7·4) was infused into the mesenteric vein at 40 g/h to measure blood flow through splanchnic tissues.

Sample collection

Over 1 h intervals, between 2 and 6 h of [5-^{15}N]glutamine infusion, blood was continuously withdrawn from the aorta and hepatic and portal veins (each 12 ml/h) as described previously (Lobley *et al.* 1995). Blood samples for determination of metabolite natural abundance were taken 24 h before the start of each infusion of [5-^{15}N]glutamine.

Chemical analyses

Analyses of blood pH and pO_2 were performed on an ABL3 Blood Gas Analyzer (Radiometer, Copenhagen, Denmark) and packed cell volume was determined by haematocrit. Blood samples were maintained on ice and centrifuged at 1000 ***g*** for 15 min at 4°C to prepare plasma. Blood and plasma DM were obtained by freeze-drying known weights of material (blood was first haemolysed by a 1:1 addition of water). Plasma flow (g/min) was determined

gravimetrically by downstream dilution of *p*-aminohippurate (Lobley *et al.* 1995).

Plasma glutamine and urea concentrations were determined by isotope dilution (Milano *et al.* 2000) following addition of known amounts of 0·75 mmolal [5-^{15}N]glutamine and 3 mmolal [$^{15}N_2$]urea (99 atom %; Isotec Ltd, Miamisburg, OH, USA), with the mixture stored at −20°C until analysis. Enrichments of glutamine (in isolated plasma and from isotope dilution) and urea (isotope dilution only) were determined as the tributyldimethylsilyl derivatives (Calder & Smith, 1988) and analysed by GC–MS in the electron impact mode. For [5-^{15}N]glutamine enrichment, fragment ions of m/z 168 and 169 were monitored; these contain ^{15}N associated only with the 5-N (amide) group (Williams & Wolfe, 1994). For urea concentration, fragment ions of m/z 231 and 233 were monitored. Incorporation of ^{15}N into urea *in vivo* were too low to be detected by GC–MS approaches, so these were determined by GC–combustion MS, as described previously (Obitsu *et al.* 2000).

NH_3 concentration was measured in fresh plasma using a Kone Dynamic selective Analyzer (Kone Instruments, Espoo, Finland) by the glutamate dehydrogenase reaction. $^{15}NH_3$ enrichment was determined by enzymatic conversion (glutamate dehydrogenase) of plasma NH_3 to norvaline, following the procedure described by Nieto *et al.* (1996).

Calculations and statistics

For blood transactions, blood flow (BF) was calculated from plasma flow (PF) and packed cell volume (PCV) as:

$$BF = PF/(1 - PCV).$$

Blood O_2 content was determined from haemoglobin content and saturation (see Lobley *et al.* 1995). Mass transfers of metabolites across the portal–drained viscera (PDV) and liver were calculated as:

$$(C_p - C_a) \times PF,$$

and

$$[C_h \times HF] - [C_p \times PF] - [C_a \times AF],$$

respectively, where PF is hepatic portal vein plasma (or blood) flow, HF is hepatic vein plasma (or blood) flow and AF is hepatic artery plasma (or blood) flow (calculated as the difference between hepatic vein and portal vein flows, i.e. HF − PF); and C_a, C_p and C_h represent the concentrations of metabolites in the artery, hepatic portal and hepatic vein plasma respectively. Similarly, isotope transfers (utilisation) across PDV and liver were determined as:

$$(C_p \times E_p - C_a \times E_a) \times PF,$$

and

$$[C_h \times E_h \times HF] - [C_p \times E_p \times PF] - [C_a \times E_a \times AF],$$

respectively, where E is the enrichment (mol % excess) of the metabolite in the appropriate plasma sample. These isotope transfers were converted to glutamine movements by division with the assumed precursor. In this case, the glutamine enrichment in the venous outflow was chosen (P for PDV; and H for liver). PDV and hepatic productions of glutamine were calculated as the sum of utilisation plus net glutamine transfer and represents *de novo* glutamine production plus glutamine from protein degradation.

The 5-N glutamine whole-body irreversible loss rate (ILR or flux) was calculated as:

$$((E_i/E_a) - 1) \times I,$$

where E_i and I represent the enrichment (mol % excess) and rate of infusion (μmol/min) of the [5-^{15}N]glutamine infusate respectively. When the proportion of whole–body ILR attributable to the organs was estimated, all the transfers were calculated based on the common precursor, arterial glutamine enrichment. Blood NH_3 concentration was assumed equal to that in plasma (Milano, 1997). Urea transfers were calculated based on blood water flow and plasma water urea concentration (Lobley *et al.* 1998). Data were subjected to two–way ANOVA (Genstat for Windows, release 3·2; Lawes Agricultural Trust, Rothamsted, Herts, UK), with animals treated as blocks, for the effect of treatment (AMM, CONT and AA) and period. The experimental design adopted allowed eight residual d.f. for all analyses. In most instances, period was found to be not significant and all data were re-analysed by one-way ANOVA, with ten residual d.f. for a full data set (except for indicated missing values). Significance between treatments was assessed by Fisher's least significant difference procedure. For comparisons of different variables within one treatment, one-way ANOVA was also used.

Results

Animals showed normal behaviour during all infusions. Initial preliminary studies revealed occasional increases in body temperature (1·0–1·5°C) when unlabelled L-glutamine from different commercial sources was infused. This problem disappeared with introduction of the pyrogen-free grade L-glutamine.

For all animals, each value represents the mean of the four hourly integrated measurements, except for one sheep where, during the AA infusion, only three sampling points were used due to problems with one sample. For another sheep, during infusion CONT, the data are based on two integrated 1 h samples because of difficulties in withdrawal of blood from the portal catheter. Blood, and plasma, flows (g/min) were not significantly different between the treatments (Table 1). The mean value obtained for hepatic artery flow was 6 % (range 2–9 %) of total hepatic flow, comparable with previous observations (Lobley *et al.* 1995, 1996, 1998).

In all the blood samples, pH was unchanged by treatment (results not shown). Arterial pH remained >7·4 for all infusions, thus mild acidosis associated with other ammonium salts (e.g. the chloride; Lobley *et al.* 1995) was avoided. O_2 consumptions by PDV and liver were of similar magnitude and not significantly affected by treatment (Table 1).

Table 1. Effect of 4 d infusion of ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) into the abomasum, on plasma and blood flow and oxygen exchanges across portal-drained viscera (PDV) and liver of six sheep fed a basal ration of grass pellets*

Abomasal infusion...	AMM		CONT		AA		SED†	Statistical significance of effect: *P*
	Plasma	Blood	Plasma	Blood	Plasma	Blood		
Flow (g/min)								
Arterial	131	189	30	92	102	113	55	NS
Portal	1331	1957	1471	2086	1407	2033	99	NS
Hepatic	1461	2086	1500	2177	1515	2145	67	NS
O_2 exchange (mmol/min)								
PDV	−2·6		−2·9		−2·6		0·2	NS
Liver	−2·4		−2·5		−2·7		0·2	NS

* For details of infusion and procedures, see p. 358.
† Based on one-way ANOVA with 10 residual d.f.

Ammonia and urea concentrations and transfers

NH_3 concentrations in arterial or hepatic venous plasma were not significantly affected by treatment (Table 2). During infusion of AMM, hepatic portal venous NH_3 concentrations were higher than during CONT or AA infusion (increased by +82 and +37 % respectively, $P<0·01$). Hepatic portal NH_3 concentrations were also greater during AA compared with CONT infusion ($P<0·05$). Both NH_3 appearance across the PDV ($P<0·01$) and liver removal ($P<0·05$) were significantly increased by AMM treatment (Table 3). Compared with CONT, the net increase in PDV NH_3 appearance during AMM infusion of 389 μmol NH_3/min was close to the amount infused (365 μmol NH_3/min). Net liver NH_3 removal tended to exceed net NH_3 PDV appearance in CONT and AA infusions. This indicates a contribution of peripheral tissues to NH_3 production, as was reflected in the greater differential in NH_3 concentrations between arterial compared with hepatic venous plasma (for CONT $P<0·05$; for AA $P=0·10$).

Blood water urea concentrations were significantly greater (1·9-fold, $P<0·001$) for all samples during AMM and AA infusions compared with CONT (Table 2). The increases in either NH_3 or amino acid load to the liver were clearly reflected, not only in urea concentrations, but also in urea release into the hepatic vein (Table 3). In both cases, liver urea-N appearance was nearly double that observed with CONT infusion ($P=0·026$). There was consistent net urea uptake by the PDV, but this was not significantly different between treatments. The PDV uptake represented 47, 53 and 36 % of liver urea production for AMM, CONT and AA treatments respectively. In the case of CONT and AA infusions, removal of urea-N by the digestive tract accounted numerically for more than 95 % of net NH_3-N PDV appearance, and more than 80 % for treatment AMM.

The proportions of urea-N released by the liver which could be apparently accounted by NH_3 removal were 66, 65 and 51 % for AMM, CONT and AA treatments, with no significant differences. Compared with CONT treatment, the extra liver NH_3 uptake:urea-N output ratio were 0·66 and 0·42 for AMM and AA infusions respectively.

Glutamine concentration and mass transfers

The AA infusion induced a considerable decrease (16 to 26 %, $P<0·02$ or better) in glutamine concentrations at all sample sites (Table 4). Net glutamine mass transfers across the PDV and liver were not significantly different between treatments, although both were numerically greater during the AA infusion (NB this mixture did not contain glutamine).

[5-^{15}N]Glutamine transfers

Mean values for 5-N glutamine whole-body flux did not

Table 2. Effect of 4 d infusion of ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) into the abomasum, on plasma ammonia and blood water urea concentration of six sheep fed a basal ration of grass pellets*

Abomasal infusion...	AMM	CONT	AA	SED†	Statistical significance of effect: *P*
NH_3 concentration (μM)					
Arterial	87	87	130	35	NS
Portal	489[a]	269[b]	356[c]	25	<0·001
Hepatic	74	42	51	15	NS
Urea concentration (mM)					
Arterial	8·61[a]	4·41[b]	8·44[a]	1·04	0·004
Portal	8·42[a]	4·31[b]	8·32[a]	1·03	0·004
Hepatic	8·81[a]	4·48[b]	8·62[a]	1·06	0·003

[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of infusion and procedures, see p. 358.
† Based on one-way AVOVA, 10 residual d.f.

Table 3. Effect of 4 d infusion of ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) into the abomasum, on ammonia and urea transfers across portal-drained viscera (PDV) and liver of six sheep fed a basal ration of grass pellets*

Abomasal infusion...	AMM	CONT	AA	SED†	Statistical significance of effect: *P*
NH_3 (μmol/min)					
PDV	772[a]	383[b]	460[b]	89·8	0·004
Liver	−793[a]	−483[b]	−638[ab]	97·4	0·030
Urea-N (μmol/min)					
PDV	−637	−364	−481	125·0	NS
Liver	1361[a]	685[b]	1329[a]	226·8	0·026
NH_3-N:urea-N					
Liver	0·66	0·65	0·51	0·07	NS

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of infusions and procedures, see p. 358.
† Based on one-way ANOVA, 10 residual d.f. except for NH_3-N:urea-N (d.f. 9, one missing value).

differ significantly between infusions (Table 5). The [5-^{15}N]glutamine enrichment ratios within the three sample sites differed between treatments (Table 5). Compared with CONT, the AA infusion resulted in a lower portal:arterial ratio ($P<0·02$) while the hepatic:arterial enrichment ratio for AMM was also reduced ($P<0·03$).

Glutamine production across the PDV (the sum of absorption from the GIT lumen plus that released from cell turnover) was unaffected by treatment. Glutamine utilization by the liver exceeded that by the PDV ($P<0·02$), with net splanchnic utilization similar (104 *v.* 101 μmol/min) for CONT and AA infusions and these tended to be lower than for the AMM treatment (140 μmol/min; $P=0·22$, SED 40·4). Hepatic production of glutamine was less than utilisation for all treatments ($P<0·001$). Hepatic glutamine production during AMM infusion was significantly greater ($P<0·02$) than for CONT and AA. As a proportion of whole-body glutamine flux, PDV utilisation was 19–44 %, while the total splanchnic glutamine utilization was 45–70 % of total flux.

Enrichments of $^{15}NH_3$ in hepatic artery, hepatic portal vein and hepatic vein were 14, 9 and 10 % those of the respective glutamine values. These values are similar to those derived from comparisons of net glutamine utilisation with NH_3 absorption (5–12 %) across the digestive tract. Nonetheless, this appearance of labelled NH_3 for all three treatments (Table 6) was the equivalent of 70–115 % ^{15}N extracted as glutamine. Across the liver, $^{15}NH_3$ was removed, with the amounts similar across all treatments, despite the differences in net NH_3 movements (cf. Table 3). PDV $^{15}NH_3$ appearance accounted for 112 and 61 % hepatic $^{15}NH_3$ uptake during AMM and CONT infusions but was only 13 % during AA treatment.

Mean [^{15}N]urea enrichments obtained in plasma from the artery and portal and hepatic veins are shown in Fig. 1. Because of the relatively short duration of infusion (6 h), compared with the half-life of body urea (about 4–6 h; Sarraseca *et al.* 1998) 'plateau' values for plasma [^{15}N]urea were not achieved. Across all treatments, [^{15}N]urea enrichments in the hepatic venous plasma were higher than for arterial and hepatic portal venous samples ($P<0·001$, Fig. 1). The [^{15}N]urea enrichment was higher during the CONT infusion in all the vessels sampled, when compared with the other two treatments, but significant only against AA values ($P<0·05$; Fig. 1).

For AMM and CONT treatments there was net removal of [^{15}N]urea by the PDV (Table 6). In contrast, PDV [^{15}N]urea utilisation was lower ($P<0·02$) and close to zero for the AA infusion. The amount of ^{15}N transferred to the PDV as urea was approximately 0·5 that absorbed as NH_3. For all three treatments, there was hepatic production of [^{15}N]urea, and this was greater ($P<0·05$) for AMM infusion compared with CONT and AA. The amount of ^{15}N released from the liver as urea exceeded the amount extracted as glutamine by 30–100 %, but when $^{15}NH_3$ removal was included as a ureagenic-N precursor, then a more than adequate ^{15}N balance was achieved (Table 6). The amount of ^{15}N removed as gluta-

Table 4. Effect of 4 d infusion of ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) into the abomasum, on glutamine concentration and glutamine mass transfers across portal-drained viscera (PDV) and liver of six sheep fed a basal ration of grass pellets*

Abomasal infusion...	AMM	CONT	AA	SED†	Statistical significance of effect: *P*
Glutamine concentration (μM)					
Arterial	246·5[a]	238·7[a]	183·3[b]	18·75	0·014
Portal	245·0[a]	237·9[a]	199·6[b]	10·41	0·003
Hepatic	223·7[a]	223·0[a]	165·0[b]	16·46	0·007
Glutamine transfers (μmol/min)					
PDV	−1·4	0·8	24·9	14·80	NS
Liver	−33·2	−21·4	−48·3	14·97	NS

[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of infusions and procedures, see p. 358.
† Based on one-way ANOVA, 10 residual d.f.

Table 5. Kinetics of 5-^{15}N glutamine in six sheep infused for 4 d into the abomasum with ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) and fed a basal ration of grass pellets*

Abomasal infusion...	AMM	CONT	AA	SED†	Statistical significance of effect: *P*
Glutamine-5-N whole-body flux (μmol/min)	173·0	166·2	177·1	13·37	NS
5-[^{15}N]glutamine enrichment ratios (%)					
Portal:Arterial	86^{ab}	89^{a}	85^{b}	1·1	0·019
Hepatic:Arterial	76^{a}	80^{b}	78^{ab}	1·2	0·024
Hepatic:Portal	89^{a}	91^{ab}	92^{b}	1·3	0·100
Splanchnic glutamine movements (μmol/min)					
PDV					
Utilisation	−55·2	−45·2	−22·6	16·50	NS
Production	53·2	46·0	47·4	4·63	NS
Liver					
Utilisation	−84·4	−58·2	−78·5	15·09	NS
Production	51·3^{a}	36·7^{b}	30·2^{b}	5·8	0·013

PDV, portal drained viscera.
^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of infusions and procedures, see p. 358.
† Based on one-way ANOVA, 10 residual d.f.

Table 6. Effect of 4 d infusion of ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) into the abomasum on ^{15}N transfers across portal-drained viscera (PDV) and liver of six sheep fed a basal ration of grass pellets*

Abomasal infusion...	AMM	CONT	AA	SED†	Statistical significance of effect: *P*
^{15}N–NH_3 transfers (μmol/min)					
PDV‡	1·70	1·19	0·25	0·97	NS
Liver‡	−1·52	−1·95	−1·86	0·34	NS
^{15}N -urea transfers (μmol/min)					
PDV§	−0·99^{a}	−0·55^{ab}	0·08^{b}	0·29	0·014
Liver§	3·91^{a}	2·74^{b}	2·74^{b}	0·46	0·044
Hepatic ^{15}N transfers					
Urea-^{15}N: glutamine-^{15}N§	1·64	2·02	1·32	0·53	NS
Urea-^{15}N: glutamine-^{15}N+NH_3-^{15}N‡	0·87^{a}	0·82^{ab}	0·71^{b}	0·05	0·031

^{a,b}Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of infusions and procedures, see p. 358.
† Based on one-way ANOVA.
‡ 6 d.f.
§ 10 d.f.

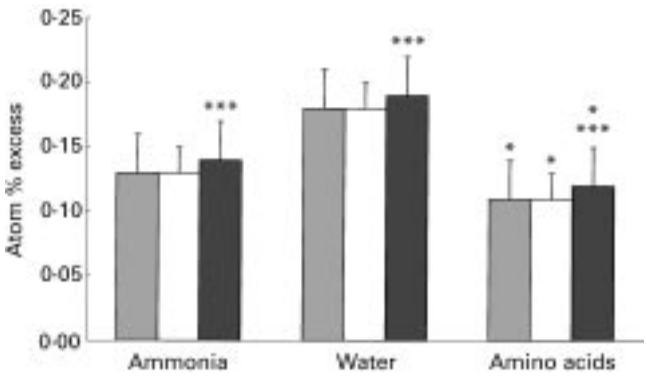

Fig. 1. Changes in ^{15}N-urea enrichment in arterial (■), portal (□) and hepatic vein (■) plasma in six sheep infused for 4 d into the abomasum with ammonium bicarbonate (AMM), water (CONT) or amino acids (AA) and [5-^{15}N]glutamine into the jugular vein. For details of infusions and procedures, see p. 358. Mean values were significantly different from the corresponding values for the CONT infusion: *$P<0·05$. Mean hepatic values were significantly different from those of arterial and portal values for all treatments: ***$P<0·001$.

mine plus NH_3, relative to that released as urea, was greater ($P<0·05$) for AA compared with CONT and AMM infusions. These latter calculations are based on only four animals, because it was not possible to determine $^{15}NH_3$ enrichments in two sheep.

Discussion

Metabolic principles

The complex interactions between liver removal of NH_3 and amino acids with urea production and glutamine metabolism are achieved through the unique architecture of the liver. The majority of hepatocytes are periportal and contain the enzymes of the ornithine cycle along with mitochondrial glutaminase. Glutaminolysis helps maintain high concentrations of intra-mitochondrial NH_3, necessary for the continuous synthesis of carbamoyl phosphate (Meijer *et al.* 1985). Synthesis of urea involves input of one N atom via carbamoyl phosphate and another N atom through aspartate. If aspartate-N is in excess then extra NH_3 can be produced by glutaminolysis. The less

abundant perivenous cells lack glutaminase and the ornithine-cycle enzymes, but instead contain cytosolic glutamine synthetase. When NH_3 supply exceeds the capacity of the ornithine cycle (periportal) then this is used as a substrate for glutamine synthesis (perivenous). Therefore, excess NH_3 supply may lead to an increase in glutamine synthesis (Lobley *et al.* 1995), while amino acid excess may increase hepatic glutamine use (Häussinger, 1990; Wray-Cahen *et al.* 1997). Both mechanisms may alter glutamine status for other metabolic functions within the liver or other tissues.

Whole-body glutamine metabolism

In sheep, most (>90 %) of the glutamine in whole blood is present in the plasma (N Roy and G Lobley, unpublished results), whereas a large fraction is present in erythrocytes of human subjects (Darmaun *et al.* 1986). In the current study, therefore, plasma transfers will represent the majority of inter-organ exchanges. Despite the relatively large excess of NH_3 and amino acids whole-body 5-N glutamine fluxes were unaltered, even though there was a marked decrease in arterial glutamine during AA treatment. Such a decrease may reflect either lowered production (release) or increased utilisation by tissues (e.g. the liver). Whole-body glutamine fluxes were similar (256–272 μmol/kg per h) to those observed in human subjects (268–283 μmol/kg per h; Darmaun *et al.* 1986), but considerably lower than those found in rats (900–1300 μmol/kg per h; Squires & Brosnan, 1983; for review see Curthoys & Watford, 1995). Compared with other published result in sheep, present values (256–272 μmol/kg per h; 638–681 μmol/$kg^{0·75}$ per h) exceed those for [U-^{14}C]glutamine ILR in fed adult sheep (211–224 μmol/kg per h; 570–604 μmol/$kg^{0·75}$ per h; Heitmann & Bergman, 1978) both on a live weight or metabolic body weight ($kg^{0·75}$) basis. This suggests that flow of recycled glutamine (i.e. converted to glutamate and then resynthesised) through the plasma pool may occur in the sheep, conserving the C skeleton but exchanging the amido-N.

The current values for fed sheep are greater than observed for the 24 h fasted ovine (175 μmol/kg per h; 432 μmol/$kg^{0·75}$ per h; Gate *et al.* 1999), indicating that nutritional state does alter the inter-organ fluxes of the amido-N of glutamine. Because there is little net absorption of glutamine, the ILR differences can reflect changes in metabolism of tissues beyond the digestive tract (AMM and CONT treatments). However, PDV tissues could be implicated as well in the ILR change, as can be deduced from the numerical effect obtained in both net and total portal fluxes obtained with treatment AA. This is contrary to the findings of Heitmann & Bergman (1978) for the C skeleton, but supports results for human subjects, where inter-organ glutamine metabolism responds markedly to starvation as well as several pathophysiological situations, e.g. metabolic acidosis, critical illness and catabolic diseases (Souba, 1991). Certainly, the peripheral tissues contribute to both glutamine release and synthesis (fat, Frayn *et al.* 1991; muscle, lungs and kidneys, Curthoys & Watford, 1995), as could be indicated by the higher numerical concentrations in arterial compared with hepatic venous plasma.

Glutamine splanchnic metabolism

The gastrointestinal tract is an avid net consumer of glutamine. In the rat, these tissues have little capacity to synthesise glutamine, but contain high glutaminase activity, particularly in the mucosa of the small intestine (James *et al.* 1998). The pancreas and gut lymphatic tissue also utilise substantial amounts of glutamine (Souba, 1991). During AMM and CONT treatments, all dietary and some systemic glutamine was removed by the digestive tract. In fed and fasted sheep, the arterio-venous net utilisations were the same (Heitmann & Bergman, 1978), i.e. apparent removal from arterial sources was independent of intake. Nonetheless, total utilisation by the digestive tract must have been greater in the fed state, due to glutamine available from the diet. These earlier results implied there was an obligate need by the tissues of the digestive tract for arterial glutamine (e.g. to support serosal metabolism). In contrast, fasted lambs (Gate *et al.* 1999) showed greater rates of extraction (−45 μmol/min) from the arterial inflow than the CONT sheep (1 μmol/min) in the current study. As the diet will provide approximately 30 μmol digestible glutamine, these results suggest an absolute need by the gut that can be met by appropriate combinations of dietary and arterial sources. The reduced glutamine uptake from plasma by the basolateral membranes of the digestive tract tissue during AA infusion (as reflected by the numerically higher glutamine appearance), may be associated with the lower arterial concentrations, as rate of delivery is a known regulator of transport (Rennie *et al.* 1994). Alternatively, some of the metabolic functions that glutamine fulfils may be substituted by other amino acids present in the AA infusate. For example, use of the glutamine C skeleton for synthesis of other nonessential amino acids (e.g. alanine, proline; Windmueller & Spaeth, 1980) may be 'spared' due to their exogenous provision.

Gross utilisations across the PDV followed the same pattern as net uptake and values from CONT and AMM animals were similar to other results (2·75–3·96 μmol/$kg^{0·75}$ per min; Heitmann & Bergman, 1978; Gate *et al.* 1999). The gastrointestinal tract contributed 0·19–0·44 to whole-body glutamine ILR. The values for CONT and AMM treatments are slightly higher than that obtained from C-skeleton fluxes (0·43 and 0·44 respectively, *v.* 0·33–0·39; Heitmann & Bergman, 1978) and clearly below for AA infusion (0·19). The values were also below that observed for the amido-N ILR in fasted lambs (0·47; Gate *et al.* 1999), although absolute PDV utilisation was similar between the two studies (−43 *v.* −45 μmol/min from CONT treatment). The comparison of the present study against control animals would suggest that glutamine metabolism is more sensitive to intake in non-PDV rather than PDV tissues.

The current results emphasise the ability of the liver to function both as a consumer and producer of glutamine. The numerical increases in hepatic glutamine utilisation (by 35–45 % above basal conditions) during both AA

(NS) and AMM administration, at first, might appear contradictory. For AA infusion, provision of additional NH_3 in the liver mitochondria (via glutaminase action) is necessary to ensure an equal supply of carbamoyl phosphate when aspartate inflows to the ornithine cycle are augmented during deamination of amino acids. This hepatic use of glutamine could be reflected by a decrease in plasma concentrations. Paradoxically, hepatic glutaminase activity can also be augmented when the NH_3 supply is increased (as with AMM infusion). Indeed, for the perfused rat liver, addition of NH_3 stimulated the production of urea from glutamine by 2-fold (Taylor & Rennie, 1988). This process again involves ensuring that flows of N atoms into the ornithine cycle are matched. In ruminant animals, much of the excess NH_3-N is converted into glutamate and, subsequently, aspartate (Luo *et al.* 1995; Milano *et al.* 2000).

Liver glutamine production represented 0·17–0·30 whole-body ILR, again lower than values reported previously for [^{14}C]glutamine kinetics (0·38; Heitmann & Bergman, 1978). Hepatic production of glutamine was significantly elevated during the AMM infusion, this probably reflects the role of the perivenous cells as the final, high affinity scavenger, to ensure that post-hepatic blood has low NH_3 concentration. Nonetheless, this remained the minor route for NH_3 removal and only accounted for 6 % of hepatic extraction (NB this contribution would be doubled if the glutamine were synthesised from 2-oxoglutarate rather than from glutamate).

Despite the dynamic responses to AMM and AA supplementation, there were numerical changes in net transfers of glutamine across the liver, but not significantly different from control. This appears to contradict the suggestion for non-ruminant animals that ureagenic removal of excess amino acids will result in utilisation of glutamine (Häussinger, 1990). Rather, there is an elevation of the intra-hepatic glutamine cycle, but where increased utilisation by the periportal cells is counterbalanced by additional synthesis within the perivenous hepatocytes. Other studies present a confused picture with respect to net utilisation of glutamine to support amino acid catabolism. For example, in pregnant cows, acute (6 h) infusion of L-amino acids into the mesenteric vein increased liver output of urea and net uptake glutamine (Wray-Cahen *et al.* 1997). In contrast, a similar study in sheep elevated ureagenesis but not glutamine extraction (Lobley *et al.* 1998). In other ovine experiments, liver glutamine extraction was unaffected by changes in either the amount (Heitmann & Bergman, 1978) or quality (Lobley *et al.* 1996) of diet offered. Hepatic production of glutamine was observed with NH_4Cl infusions but this involved mildly acidotic conditions (Lobley *et al.* 1995). One explanation for these differences between studies may involve the relative inputs of amino acids and NH_3 to the liver. For example, in the current study, for the basal diet (CONT) NH_3 was dominant contributor (65 %) to liver urea-N synthesis. Even when amino acid supply was doubled (AA treatment), NH_3-N removal by the liver was still 51 % of urea-N output. Therefore, the experimental conditions may not have been extreme enough to create a net demand on glutamine.

Urea and ammonia kinetics

Based on the capacity of the ornithine cycle in ruminant animals (2 μmol urea N/g liver per min; Symonds *et al.* 1981; Orzechowski *et al.* 1987), and a liver mass of 1·6–1·9 % of live weight (Lobley *et al.* 1994), near maximal synthesis of urea was probably achieved under AMM and AA infusions. That the amido-N of glutamine contributes to this urea synthesis is clearly demonstrated from the liver ^{15}N balance. Separately, neither hepatic extraction of $^{15}NH_3$ nor [^{15}N]glutamine were sufficient to account for the ^{15}N released as urea. For example, liver removal of $^{15}NH_3$ would only account for a maximum of 42–46 % of [^{15}N]urea synthesis, while glutamine would provide up to 50–76 %. Of course, NH_3 is still the predominant ureagenic precursor, but because of the much lower enrichment than glutamine in the current study makes a smaller contribution to ^{15}N transfers. In rat hepatocytes *in vitro*, the amido-N of glutamine provides up to 65 % urea-N, when present either as the sole substrate or in a medium that simulated the extracellular fluid (Nissim *et al.* 1992). Such extremes are unlikely to operate *in vivo*, but show the potential importance of glutaminolysis in maintaining ornithine-cycle activity during conditions of metabolic stress.

Urea entry to the digestive tract ranged from 36–53 % of hepatic production, in the lower range of values reported for ruminant animals (Lapierre & Lobley, 2001). This contribution of N to the digestive tract was similar (83–104 %) to the amount absorbed as NH_3. In practice, however, usually only 30–50 % of absorbed NH_3 is derived from such urea sources (Sarraseca *et al.* 1998), thus other metabolites are important precursors of NH_3. In the fasted rodent, 40 % of metabolised glutamine-N is absorbed as NH_3 (Windmueller & Spaeth, 1980) but, in both fasted (Gate *et al.* 1999) and fed (current study) sheep, only 5–10 % of the NH_3 is derived from the amido-N of glutamine. This represents less than 25 % of the glutamine amido-N extracted from the arterial plasma and supports the concept that glutamine metabolism is directed towards anabolic fates, such as synthesis of nucleic acids and proteins, in the digestive tract tissues of ruminant animals (Gate *et al.* 1999).

The incremental increases in hepatic urea-N production for AMM and AA (675 and 644 μmol/min compared with CONT) were greater than the additional liver removals of NH_3 (310 and 155 μmol/min respectively). Thus, other N-sources, probably amino acids, were required to support ureagenesis during both infusions. While this is understandable for the AA treatment, such extra demands during AMM infusion again raises the issue of whether NH_3 detoxification involves additional amino acid catabolism (cf. Parker *et al.* 1995; Lobley & Milano, 1997).

In conclusion, splanchnic glutamine utilisation was shown to account for the majority of whole-body plasma glutamine flux, under conditions which simulated nutritional extremes in ruminant animals. The PDV tissues had a lower demand for glutamine when amino acids were infused, indicative of sparing by other metabolites. Hepatic glutamine utilisation increased during both NH_3

and amino acid infusion, while production increased by 40 %, compared with basal conditions, when ammonium bicarbonate was infused. This provided an effective strategy to prevent peripheral hyperammonaemia. Peripheral glutamine production contributed to the balance of whole-body glutamine metabolism and varied with the form of nutrient supply.

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