

## Response in hepatic removal of amino acids by the sheep to short-term infusions of varied amounts of an amino acid mixture into the mesenteric vein

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Under conditions of chronic supply the liver removes most amino acids (AA) in excess of net anabolic needs. Little information is available, however, on how acute alterations in AA supply (as might occur with once-daily feeding regimens) are controlled by the liver. Are these also extracted completely in a 'first-pass' manner or are there limitations to hepatic uptake? Furthermore, is the rate of removal 'saturable' (by Michaelis–Menten kinetics) over the range of supply experienced under normal feeding conditions? These questions have been addressed in a study that involved acute (4.5 h) increases in AA supply. Four sheep were prepared with trans-hepatic vascular catheters and were offered a basal diet (equivalent to 1.6×energy maintenance) throughout. On four occasions, at 7 d intervals, they were infused with various amounts of an AA mixture into the mesenteric vein over a 4.5 h period. The mixture contained fourteen AA in the proportions present in rumen microbial protein. The amounts infused were calculated to provide an additional one, two, three and four times that absorbed from the basal diet. Continuous blood collections were removed over 2 h intervals before (basal diet only) and at 0.5–2.5 and 2.5–4.5 h of AA infusion. Transfers of AA, from the digestive tract and to the liver, were calculated for both plasma and total blood. The recovery of the infused AA across the portal-drained viscera (PDV) was quantitative (100%) only for histidine and proline, the remaining AA were recovered at 56–83 %. These losses correlated ( $P < 0.001$ ) with the arterial concentrations and were probably due to removal of AA from the systemic circulation by the tissues of the digestive tract. Despite the wide range of net PDV appearances (i.e. absorbed plus infused), the percentage of most AA removed by the liver remained constant, but the percentage varied with AA (from 34 for proline to 78 for tryptophan for blood transfers). Thus, even when supply was increased 5-fold over baseline there was no indication that the transport into the liver declined, indeed the absolute removals continued to increase. In contrast, the branched-chain AA (isoleucine, leucine and valine) did not exhibit constant percentage extractions. Their percentage extractions were always the lowest (16, 10 and 25 respectively) and tended to decline at the highest infusion rates, indicative of saturation in hepatic transport and/or metabolism. The arterial concentrations of all infused AA increased ( $P < 0.001$ ) with rate of infusion, again indicative that the liver did not extract all the net AA available across the PDV. Absolute amounts removed were similar between plasma and blood, indicating that most of the hepatic transfers occurred from plasma. The fractional rates of transfer from total inflow to the liver (i.e. with re-circulated AA included) were 3- to 4-fold lower than rates based on the amounts absorbed plus infused. The highest percentage extraction for total blood inflows was for serine (27), but most were between 6 and 16, except for the branched-chain AA, which were all  $< 1$ . Use of percentage extractions based on total inflows are probably more appropriate for development of mathematical models of liver metabolism, and the current data suggest that constant values may be applied. The needs of the liver for specific mechanisms involving phenylalanine and histidine (plasma protein synthesis), glycine (detoxification of xenobiotics) and alanine (gluconeogenesis) probably also require to be included in such models.

### Liver: Portal-drained viscera: Amino acids

**Abbreviations:** AA, amino acid; PDV, portal-drained viscera.

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The net fates of absorbed amino acids (AA) include use for increased protein gain, synthesis of specific metabolites (e.g. neurotransmitters, glucose) and oxidation to waste products (mainly CO<sub>2</sub> and urea). Such catabolism usually occurs within the liver; this organ plays a vital role in maintaining AA homeostasis. Although sufficient AA must enter the peripheral circulation to support the maintenance and growth of protein stores, over-supply into the systemic plasma can lead to deleterious metabolic consequences (Hargreaves & Pardridge, 1988). Thus, the hepatic removal of AA must involve a balance between reducing the impact of peripheral hyperaminoacidaemia yet ensuring that sufficient AA are available to support protein gain.

A number of studies in both cattle and sheep (for example, see Wolff *et al.* 1972; Burrin *et al.* 1991; Koeln *et al.* 1993; Reynolds *et al.* 1994) have quantified both the absolute and the fractional hepatic removals of absorbed AA. Most of these investigations have involved chronic studies, often in metabolic steady-state, and where a balance between supply and body needs has been achieved. Under steady-state conditions those AA that are catabolised exclusively by the liver will exhibit a net splanchnic availability equal to peripheral tissue productive needs. In many practical situations, however, such as feeding twice daily (Whitt *et al.* 1996), there are diurnal fluctuations in the amount of absorbed AA, and temporal patterns of excess and deficiency occur. How, then, does the liver respond to such variations in supply? Do short-term mechanisms exist to increase (or decrease) hepatic removal (or bypass) of AA? Is removal of AA by the liver subject to constraints, e.g. 'saturation' of the transporters (or subsequent metabolic reactions), within the normal limits of nutrient supply?

Identification *in vivo* of mechanisms that might regulate hepatic removal of AA is complicated by several factors. First, hepatic extraction differs for each individual AA. Thus, low extractions are observed for the branched chain AA, while removal of other essential (e.g. phenylalanine) and non-essential AA (e.g. alanine) can be nearly complete (Lobley *et al.* 1995; Lapierre *et al.* 2000). Thus, the liver is not the exclusive site for maintenance of AA homeostasis within the body. Second, there is dispute as to whether blood or plasma is the main site of AA transfers across the splanchnic tissues (see Elwyn *et al.* 1972; Heitmann & Bergman, 1980; Houliet *et al.* 1991; Lobley *et al.* 1996; Le Floch *et al.* 1999), but most of the studies on liver transfers of AA in ruminants have reported only plasma values. If the erythrocytes make a major contribution to such exchanges, then inappropriate conclusions may have been drawn. Furthermore, most previously reported data on hepatic fractional extraction rates have been expressed relative to the amount absorbed. In practice, the total supply to the liver is dominated by AA re-circulated from peripheral tissue metabolism and, when data are expressed relative to this base, the fractional removals differ in both order and number. This factor can lead to different interpretations of the mechanisms involved (see Reynolds *et al.* 1994; Hanigan *et al.* 1998).

Some of these issues were examined in the current study, where the responsiveness of the ovine liver to acute (4.5 h) loads of AA, supplied by intra-mesenteric vein infusion,

has been quantified. The pattern of supplied AA was similar to that in rumen microbial protein and the study included a comparison of transfers from both plasma and erythrocytes.

## Materials and methods

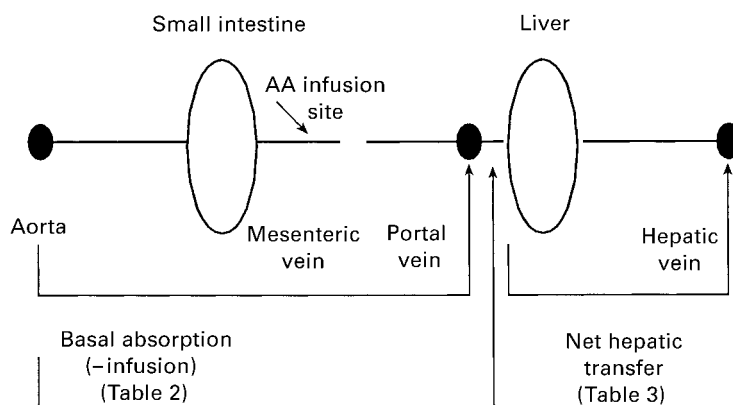
### General design

The animals and experimental design have been described in detail previously (Lobley *et al.* 1998; see Fig. 1). Briefly, four Suffolk-cross sheep were prepared with indwelling silicone rubber catheters in the aorta and the mesenteric, hepatic portal and hepatic veins. The animals were offered a basal ration of 1000 g/d fed as grass pellets (equivalent to 1.6×energy maintenance; 10.5 MJ metabolizable energy/kg DM; ×22.5 g N/kg DM; ×950 g DM/kg) supplied as twenty-four equal meals by means of automated feeders. On experimental days, animals were infused via the mesenteric vein catheter first with sterile 0.15 M-NaCl for 4 h (basal), followed by 4.5 h of an AA mixture at one of four dilutions detailed later. All animals received each of the four dilutions in a design based initially on a 4×4 Latin square. Each measurement period was separated by 1 week.

A mixture of L-AA (Ajinomoto Co. Inc., Tokyo, Japan), based on the composition of rumen microbial protein (Storm & Ørskov, 1983), was prepared as a stock solution at pH 7.4 in 0.05 M-sodium phosphate and 0.10 M-NaCl at the following concentrations (mM): alanine 104, arginine 37, glycine 91, histidine 15, isoleucine 55, leucine 75, lysine 73, methionine 22, phenylalanine 44, proline 44, serine 55, threonine 58, tryptophan 11, valine 60. Tyrosine and cysteine were omitted on the grounds of solubility and stability respectively. Aspartate, asparagine, glutamate and glutamine were excluded because their responses to AA loadings were to be tested as another component of the study (Lobley *et al.* 1998). The four infusates were prepared as follows: the stock solution alone or diluted in proportions 1:3, 1:1 or 3:1 with sterile 0.15 M-NaCl and infused at a rate of 120 g/h. The rates of infusion adopted were chosen to provide a range of AA-N, first at amounts that might normally be available to the animal, increasing to quantities in excess of the ureagenic capacity of the liver (approximately 2 mmol/min/per kg liver wet weight; see Lobley *et al.* 1998).

Blood flow was determined by downstream dilution of 0.15 M-*p*-aminohippurate infused (40 g/h) for 8.5 h into the mesenteric vein; this solution also contained 10<sup>5</sup> IU sodium heparin. Blood samples (6 g/h) were withdrawn continuously (Lobley *et al.* 1998) from the artery, hepatic portal and hepatic veins for the last 6.5 h of the study. These collections included the last 2 h of the 0.15 M-NaCl infusion ('basal' values) and then for 0.5–2.5 and 2.5–4.5 h of AA infusion.

To weighed portions of blood and plasma (prepared by centrifugation at 1000 g for 15 min at 4°C) were added known amounts of L-norleucine and samples prepared and analysed for free AA content on an LKB Alpha Plus Amino Acid Analyser (LKB Pharmacia, Uppsala, Sweden) as described previously (Lobley *et al.* 1998).



**Fig. 1.** Schematic of surgical preparation, sample sites and measurements of amino acid (AA) transfers. An infusion catheter, for both *p*-aminohippurate and the mixed AA infusion, was inserted into the mesenteric vein, while blood sampling catheters (●) were placed in the hepatic portal and hepatic veins and the aorta. Blood was withdrawn continuously at  $-2-0$  h before each AA infusion, and at  $0.5-2.5$  and  $2.5-4.5$  h after the start of AA infusion. Both basal absorption (diet alone; Table 2) and recovery of basal plus infused AA (Table 3) were calculated from the arterio-venous concentration difference and hepatic portal blood flow. Similar principles were applied to determine net transfers across the liver (Table 3) based on the differences between inflow (portal vein plus hepatic artery) and outflow (hepatic vein). PDV, portal-drained viscera.

### Calculations

Blood flow was calculated from plasma flow/(1-packed cell volume). Hepatic artery (FA) flow (blood or plasma) was determined as the difference between the flows in the hepatic vein (FH) and hepatic portal vein (FP). Net mass transfers of individual AA across the portal-drained viscera (PDV) were calculated as:

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

and across the liver as:

$$(C_h \times FH) - (C_p \times FP) - (C_a \times FA),$$

where  $C_a$ ,  $C_p$  and  $C_h$  are AA concentrations in arterial, hepatic portal vein and hepatic vein fluids (plasma or blood) respectively.

The 'recovery' of the infused AA was calculated as the difference between net PDV transfers for the AA infusion and the corresponding basal diet measurement.

### Statistics

The original design was based on a Latin square, structured as animal and week, but one infusion for each of three sheep needed to be repeated, for technical reasons, and these were performed as a fifth infusion at the end. In consequence, the data were analysed as a split-plot design that involved animal, week, fluid (blood or plasma), site (PDV absorption or liver extraction), time (basal,  $0.5-2.5$  and  $2.5-4.5$  h of infusion) and level of AA infusion as factors. All interactions were considered. In no case was there any significant effect of week (order of level of infusions), so this was excluded in order to simplify the analysis structure. The full analysis contained 192 data points; both linear and quadratic effects were also tested.

For certain comparisons linear regression analyses were also applied. Initially, sheep effects were included, but these did not yield a different, or improved, fit compared with combining all data and ignoring animal effects. These same data were also tested for quadratic responses, but none were found to be significant. All analyses were performed using Genstat for Windows (release 3.2; Lawes Agricultural Trust, Rothamsted, Herts., UK).

### Results

All the infused AA showed increased arterial concentrations ( $P < 0.001$ ; Table 1) for both plasma and blood, relative to the basal (pre-infusion) values. The increases were all linear ( $P < 0.001$ ) and only tryptophan exhibited any quadratic effects ( $P < 0.001$ ). The proportional increase, relative to basal concentration, varied considerably between AA, as shown for blood in Table 1. Glycine, the AA with the highest concentration in blood or plasma, exhibited a relatively modest increase ( $< 30\%$ ), even at the greatest rate of infusion, while isoleucine, leucine, methionine, phenylalanine and threonine were elevated at least 3-fold. Only leucine, tryptophan and valine showed time-related responses, within each infusion, with higher arterial concentrations during the  $2.5-4.5$  h of AA administration compared with the previous 2 h ( $P < 0.01$ ).

Some of the non-infused AA also responded to the infusion (Table 1). For example, glutamine concentrations increased ( $P = 0.003$ ; linear  $P = 0.002$ ), while those of glutamate decreased ( $P = 0.005$ ; quadratic  $P = 0.005$ ). There were no corresponding responses, however, in either asparagine or aspartate. Ornithine concentrations increased ( $P < 0.001$ ; linear  $P < 0.001$ ) in proportion to the AA infused, as did citrulline ( $P = 0.021$ ; linear  $P = 0.003$ ), but to a lower absolute and proportional extent. Despite

**Table 1.** Increases in arterial blood amino acid (AA) concentrations relative to basal values in response to graded levels of AA infusion into the mesenteric vein of sheep for 4.5 h (Mean values for four sheep)

Amino acid	Basal concentration ( $\mu\text{M}$ )	Level of AA infusion†				SED	Statistical significance: $P$ ‡
		1×	2×	3×	4×		
<b>Infused</b>							
Alanine	203	1.01	1.38	1.62	2.02	0.150	<0.001
Arginine*	151	1.07	1.56	1.76	2.56	0.174	<0.001
Glycine	481	1.02	1.06	1.19	1.28	0.043	<0.001
Histidine	68	1.02	1.17	1.33	1.38	0.067	<0.001
Isoleucine	96	1.36	1.83	2.64	3.29	0.197	<0.001
Leucine	163	1.32	1.79	2.37	3.05	0.176	<0.001§
Lysine	149	1.31	1.77	2.55	3.13	0.207	<0.001
Methionine	18	1.16	1.56	2.81	3.24	0.206	<0.001
Phenylalanine	72	1.36	1.71	2.34	3.14	0.170	<0.001
Proline	127	1.06	1.42	1.66	2.03	0.128	<0.001
Serine	49	0.93	1.39	1.89	1.97	0.113	<0.001
Threonine	64	1.34	2.43	4.41	4.62	0.531	<0.001
Tryptophan	26	1.07	1.22	1.54	1.36	0.051	<0.001§
Valine	256	1.29	1.69	2.17	2.66	0.125	<0.001§
<b>Non-infused</b>							
Aspartate	57	0.90	0.83	0.99	0.89	0.081	NS
Asparagine	39	0.77	0.81	0.83	0.77	0.053	NS¶
Citrulline	150	1.07	1.12	1.19	1.21	0.046	0.21
Glutamate	268	0.92	0.85	0.68	0.90	0.065	0.005
Glutamine	103	1.10	1.12	2.39	2.02	0.365	0.003
Ornithine	185	1.11	1.29	1.55	1.64	0.099	<0.001
Tyrosine	78	0.97	0.97	1.07	1.11	0.076	NS

\* Plasma values used, owing to presence of arginase in erythrocytes.

† Amounts infused (see p. 691) into the mesenteric vein to increase 'apparent absorption' of AA 1-, 2-, 3- or 4-fold above that absorbed from the basal ration; for details of experimental procedures, see p. 691.

‡ By ANOVA, with sheep treated as blocks and level×time as treatments (where time is either 0.5–2.5 h or 2.5–4.5 h of AA infusion). Total of thirty two values with 21 residual df. For all infused AA there were significant linear effects ( $P < 0.001$ ).

§ Significant effect of time:  $P < 0.01$ .

|| Also significant quadratic effect:  $P < 0.001$ .

substantial increases in arterial phenylalanine concentrations, those of tyrosine remained unaltered.

For all infused AA the amount that appeared across the PDV increased ( $P < 0.001$ ) with level of infusion (data not shown). For individual AA, the extra amounts recovered in portal blood (or plasma) were less than the amounts infused, except for histidine and proline (Table 2). For most other AA, the fractional recoveries were constant (see slopes in Table 2) but at less than 0.83, with some lower than 0.65 (arginine, isoleucine, leucine and tryptophan). Only arginine did not show constancy of recovery across the levels of infusion. The absolute amounts of infused AA apparently not recovered in the portal vein blood correlated with the corresponding arterial blood concentrations, particularly for arginine ( $P < 0.001$ ), alanine and leucine ( $P < 0.01$ ), plus valine, methionine, isoleucine and lysine ( $P < 0.05$ ). When recoveries in plasma were examined, the same trends as for blood were observed, except that <100 % of infused histidine (51.6 %) and proline (80.8 %) were recovered across the PDV and that this 'loss' was correlated with their arterial plasma concentrations ( $P < 0.05$ ; data not shown).

Comparisons of net transfers across both the PDV and the liver showed that most AA were numerically similar between blood and plasma, and not significantly different (Table 3). For the infused AA, the exceptions included glycine and methionine, where there was less apparent appearance across the PDV for blood compared with plasma ( $P = 0.004$  and  $0.002$  respectively). There were no differences in hepatic extraction. For the non-infused AA,

there was a tendency ( $P = 0.064$ ) for removal of glutamate from blood compared with a positive appearance in plasma, across the digestive tract. For all comparisons of plasma *v.* blood transfers, there were no interactions between level and time, so the pooled data are presented.

For most of the infused AA, the absolute amounts removed by the liver increased with level of infusion. In consequence, the fractions of net supply (i.e. the difference between net PDV inflow and hepatic outflow) that were removed remained constant. Linear regression equations gave the best fit, with slopes that differed significantly from zero ( $P < 0.001$ ; except for the branched-chain AA,  $P < 0.05$  or better, Table 4). There were no significant differences between the fractional extraction obtained from blood and plasma, except for alanine (plasma greater than blood,  $P < 0.05$ ) and asparagine (blood greater than plasma,  $P < 0.01$ ). The highest fractional extraction rates (for both blood and plasma) were for alanine, histidine, glycine and tryptophan, followed by methionine and phenylalanine. These extractions were all in excess of 50 % of net supply. Both lysine and threonine had substantially lower fractional extraction rates (both for blood and plasma) than did the other essential AA, except for the branched-chain AA. The proportion of net absorbed AA removed by the liver was higher during the basal diet provision only (pre-infusion) compared with the 0.5–2.5 h of infusion for phenylalanine ( $P = 0.005$ ), glycine ( $P = 0.044$ ) and alanine ( $P = 0.01$ ).

Rates of extraction for the branched-chain AA were low and, in response to increased supply, the gradients were not

**Table 2.** Apparent recovery in hepatic portal venous blood of amino acids (AA) infused into the mesenteric vein of sheep for 4.5 h\*

Amino acid	Basal absorption† ( $\mu\text{mol}/\text{min}$ )	Fractional recovery†	SE†	Percentage accounted§	Statistical significance of slope: <i>P</i> †
<b>Infused</b>					
Alanine	58.0	0.723	0.115	55.3	<0.001
Arginine	15.7	0.647	0.358	6.8	0.081
Glycine	9.6	0.665	0.161	34.1	<0.001
Histidine	7.5	1.012	0.176	50.9	<0.001
Isoleucine	21.8	0.632	0.111	50.3	<0.001
Leucine	27.8	0.630	0.105	53.3	<0.001
Lysine	29.7	0.825	0.136	53.7	<0.001
Methionine	6.4	0.729	0.108	59.1	<0.001
Phenylalanine	21.5	0.820	0.120	59.6	<0.001
Proline	27.9	1.029	0.281	28.5	<0.001
Serine	25.9	0.816	0.181	38.4	<0.001
Threonine	23.9	0.743	0.126	52.3	<0.001
Tryptophan	2.8	0.558	0.347	4.9	<0.001
Valine	25.1	0.672	0.165	33.4	<0.001
<b>Non-infused</b>					
Aspartate	7.5				
Asparagine	16.5				
Citrulline	16.9				
Glutamate	-19.9				
Glutamine	6.7				
Ornithine	2.2				
Tyrosine	16.5				

\* For details of experimental procedures, see p. 691.

† These values are the mean absorption over the 2 h before each AA infusion and represent uptake across the portal-drained viscera (PDV) from the diet alone. Data are presented from blood and represent the mean of sixteen measurements (i.e. four sheep each measured on four separate occasions).

‡ Based on linear regression analysis of net recovery of infused AA (i.e. amount appearing minus net absorption for basal diet) across the PDV (calculated as described on p. 691). Fractional recovery obtained from the slope of the regression equations (standard error also given). All data combined, no sheep effect (not significant). Total of thirty two values, 31 residual df.

§ The percentage variance that can be accounted for by the regression.

**Table 3.** Net transfers of amino acids ( $\mu\text{mol}/\text{min}$ ) across the portal-drained viscera (PDV) and liver in both plasma and blood during a 4.5 h intra-mesenteric infusion of amino acids into sheep\*

Amino acid	PDV‡		Liver‡		SED	Statistical significance: <i>P</i> §
	Plasma	Blood	Plasma	Blood		
<b>Infused</b>						
Alanine	119.9	108.8	-86.6	-80.2	10.60	NS
Arginine†	35.1		-24.4			
Glycine	90.3	60.2	-85.6	-70.7	10.88	0.004
Histidine	14.6	14.0	-11.1	-12.5	1.83	NS
Isoleucine	50.6	51.0	-0.4	-5.8	3.99	NS
Leucine	67.4	69.7	0.8	-8.5	6.00	NS
Lysine	86.4	79.1	-26.7	-29.4	7.01	NS
Methionine	24.3	17.4	-13.3	-10.4	2.15	0.002
Phenylalanine	55.1	50.4	-39.0	-35.4	4.47	NS
Proline	41.9	48.3	-3.4	-31.6	10.86	NS
Serine	75.0	68.3	-32.4	-19.8	4.81	NS
Threonine	65.4	64.8	-19.4	-21.5	5.91	NS
Tryptophan	8.6	4.7	-2.8	-4.1	3.88	NS
Valine	54.9	57.7	3.5	-5.3	7.67	NS
<b>Non-infused</b>						
Aspartate	2.8	3.6	-0.4	-2.0	3.77	NS
Asparagine	12.2	14.1	-6.7	-10.1	2.63	NS
Citrulline	15.9	14.5	0.7	0.0	3.26	NS
Glutamate	3.2	-14.7	22.5	15.6	4.57	0.064
Glutamine	-4.8	7.7	-26.9	-25.9	5.20	NS
Ornithine	1.3	7.9	20.9	-1.6	3.92	<0.001
Tyrosine	9.8	10.3	-7.5	-7.7	1.26	NS

\* For details of experimental procedure, see p. 691.

† Only plasma analysed, owing to presence of arginase in the erythrocytes.

‡ Values are the means of measurements made across all levels of amino acid infusion and during the pre-infusion periods, i.e. on basal ration alone. There were no significant differences between fluids for level of infusion, nor with the basal ration. Positive values indicate net release and negative values net uptake by the tissue beds.

§ By analysis of variance with animals/time as the block structure and site $\times$ fluid $\times$ level $\times$ time as treatment structure. There were 192 observations with 117 residual degrees of freedom. SED and *P* values presented for site $\times$ fluid interactions. *P* values compare the effect of fluid (blood v. plasma transfers).



**Table 4.** The fractional extraction rates from blood and plasma by the liver of net amino acid appearance across the portal-drained viscera for sheep infused via the mesenteric vein for 4.5 h with varying amounts of amino acids†  
(Mean values for four sheep)

Amino acid	Plasma					Blood				
	Basal transfer ( $\mu\text{mol}/\text{min}$ )	Slope	SE	Percentage variance accounted‡	Statistical significance: P§	Basal transfer ( $\mu\text{mol}/\text{min}$ )	Slope	SE	Percentage variance accounted	Statistical significance: P§
<b>Infused</b>										
Alanine	-45.1	0.772	0.058	78.9	<0.001	-48.4	0.588*	0.066	62.5	<0.001
Arginine	-15.5	0.405	0.098	25.3	<0.001	—	—	—	—	—
Glycine	-54.1	0.728*	0.087	59.3	<0.001	-36.3	0.704*	0.096	53.0	<0.001
Histidine	-6.8	0.717	0.077	64.8	<0.001	-8.4	0.684*	0.077	62.6	<0.001
Isoleucine	-4.4	0.050	0.132	LY	NS	-5.3	0.164	0.055	14.5	0.004
Leucine	-8.5	0.132	0.136	LY	NS	-9.7	0.103	0.050	6.5	0.045
Lysine	-10.8	0.382	0.079	32.1	<0.001	-15.1	0.381	0.052	52.9	<0.001
Methionine	-5.9	0.611	0.061	67.9	<0.001	-4.7	0.513	0.076	48.8	<0.001
Phenylalanine	-20.6	0.647	0.074	61.5	<0.001	-20.9	0.575	0.066	61.4	<0.001
Proline	-5.3	0.551	0.294	5.2	0.068	-22.2	0.337	0.088	22.8	<0.001
Serine	-16.6	0.470	0.062	54.7	<0.001	-12.5	0.438	0.051	61.2	<0.001
Threonine	-6.2	0.294	0.073	24.4	<0.001	-7.9	0.446	0.067	48.2	<0.001
Tryptophan	-4.5	0.715	0.142	34.0	<0.001	-2.4	0.781	0.124	45.1	<0.001
Valine	-10.9	0.257	0.127	6.2	0.049	-7.9	0.253	0.087	13.7	0.006
<b>Non-infused</b>										
Aspartate	-0.1	0.833*	0.130	46.2	<0.001	-12.0	0.550	0.204	11.8	0.010
Asparagine	-9.9	0.359	0.130	12.4	0.008	-9.8	0.839	0.063	79.0	<0.001
Citrulline	-15.2	0.543	0.106	34.9	<0.001	14.0	0.523	0.134	23.1	<0.001
Glutamate	0.1	0.237*	0.139	3.9	0.095	0.8	0.553	0.151	21.0	<0.001
Glutamine	16.0	0.163*	0.116	2.1	NS	14.1	0.503*	0.137	21.0	<0.001
Ornithine	-21.4	0.536	0.220	9.5	0.019	-16.8	0.793	0.110	52.0	<0.001
Tyrosine	14.8	0.844	0.117	52.2	<0.001	-5.3	0.807	0.074	71.3	<0.001

NS, not significant ( $P > 0.10$ ); LY, residual variance exceeds variance of Y variate.

Regression equation constant was significantly different from the origin: \* $P < 0.05$  or better.

† For details of experimental procedures, see p. 691.

‡ The percentage of variance accounted for by the regression.

§ Analysis by linear regression, forty-eight observations and 46 residual df.

significantly different from zero for plasma analyses of either leucine or isoleucine (Table 4). The blood analyses yielded lower standard errors than the plasma data. In consequence, the low fractional extraction rates of AA from blood could be determined as significantly different from zero (Table 4). Nonetheless, only a low percentage of the variance in the amounts removed by the liver for the branched-chain AA ( $\leq 15$ ) was attributable to the net supply. Indeed, a number of positive values, i.e. apparent net export from the liver, occurred for the branched-chain AA, particularly at the higher rates of infusion. During the infusions, the fractional rates of extraction of AA not included in the infusate either were maintained (e.g. glutamine) or showed a linear positive trend with small concentration changes (e.g. ornithine, citrulline).

The hepatic extraction data were also analysed against the total, rather than net, inflow to the liver (Table 5). As a result of the larger total inflow (i.e. absorbed and infused, plus AA returned via the circulation), most gradients were substantially lower (approximately 4-fold), except for serine. The order of fractional extraction rate also differed between the net and total inflow comparisons (compare Tables 4 and 5), owing to a combination of the absolute amount extracted and the changes in arterial concentration (see Table 1). For both blood and plasma, serine exhibited the highest fractional extraction rates when based on total inflows. The branched-chain AA maintained the lowest rates of extraction and were not significantly different from zero.

## Discussion

Most studies that have examined net portal absorption and hepatic removal of AA for ruminants have been of a chronic nature, where metabolic (and absorptive) steady-state conditions have been adopted (for example, see Burrin *et al.* 1991; Koeln *et al.* 1993; Reynolds *et al.* 1994; Lobley *et al.* 1995; Bruckentahl *et al.* 1997; Lapierre *et al.* 2000). Under these circumstances, the post-hepatic supply of AA will be sufficient to support both gain and oxidation by peripheral tissues, i.e. there will be no short-term changes in peripheral AA concentrations or fluxes. The present study followed the approach adopted in pregnant cows for examination of hepatic responses to acute changes in AA supply (Wray-Cahen *et al.* 1997). These acute studies allow the response of the liver to short-term diurnal AA excesses (as might occur with once or twice daily feeding; Whitt *et al.* 1996) to be assessed under conditions where non-AA energy supply remains constant.

Net inflows of AA to the liver were slightly less than expected, owing to the apparent 'loss' of infused AA as calculated from net appearance across the PDV. MacRae *et al.* (1997) reported that the majority (>80%) of AA removed to support digestive tract protein synthesis were derived from blood rather than lumen sources. In addition, plasma hyperphenylalaninaemia has been shown to inhibit phenylalanine absorption by the digestive tract (Wapnir *et al.* 1972). Thus, elevated arterial AA concentrations may

**Table 5.** The fractional extraction rates from blood and plasma of the total amino acids inflow to the liver for sheep infused via the mesenteric vein for 4.5 h with varying amounts of amino acids†  
(Means values of four sheep)

Amino acid	Plasma			P§	Blood			P§
	Slope	SE	Percentage accounted‡		Slope	SE	Percentage accounted‡	
<b>Infused</b>								
Alanine	0.294*	0.034	60.5	<0.001	0.154	0.023	47.5	<0.001
Arginine	0.107	0.290	20.0	<0.001	—	—	—	—
Glycine	0.186*	0.029	46.8	<0.001	0.131*	0.029	29.7	<0.001
Histidine	0.295*	0.050	42.0	<0.001	0.109	0.035	15.7	<0.003
Isoleucine	-0.027	0.026	0.2	NS	0.007	0.010	LY	NS
Leucine	-0.015	0.025	LY	NS	0.004	0.008	LY	NS
Lysine	0.075	0.020	22.5	<0.001	0.056	0.013	27.1	<0.001
Methionine	0.215	0.027	56.3	<0.001	0.159	0.023	50.3	<0.001
Phenylalanine	0.157	0.028	38.8	<0.001	0.103	0.015	48.1	<0.001
Proline	0.124	0.116	0.3	NS	0.046	0.031	2.5	NS
Serine	0.317*	0.042	54.9	NS	0.272*	0.028	66.1	<0.001
Threonine	0.109	0.028	22.7	<0.001	0.096*	0.014	51.4	<0.001
Tryptophan	0.141	0.074	5.2	<0.001	0.082	0.047	4.2	0.087
Valine	-0.025	0.023	0.3	NS	-0.003	0.011	LY	NS
<b>Non-infused</b>								
Aspartate	0.231	0.107	7.2	0.036	0.433*	0.106	25.1	<0.001
Asparagine	0.121	0.046	11.1	0.012	0.295*	0.059	33.6	<0.001
Citrulline	0.038	0.030	1.5	NS	0.005	0.022	LY	NS
Glutamate	-0.062*	0.079	LY	NS	0.061*	0.024	10.7	0.013
Glutamine	0.099	0.037	11.9	0.009	0.185*	0.026	50.9	<0.001
Ornithine	-0.149	0.028	36.7	<0.001	0.026	0.028	LY	NS
Tyrosine	0.139	0.037	22.3	<0.001	0.109*	0.024	30.3	<0.001

NS, not significant ( $P > 0.10$ ); LY, residual variance exceeds variance of Y variate.

Regression equation constant was significantly different from the origin: \* $P < 0.05$ , or better.

† For details of experimental procedures, see p. 691.

‡ The percentage of variance accounted for by the regression.

§ Analysis by linear regression with forty-eight observations and 46 residual df.

have led to increased use by the PDV tissues and/or lowered absorption. Either, or both, mechanisms would result in a reduction in the apparent net appearance of AA across the PDV. For a number of AA, there was no correlation between arterial concentrations and the quantities of infusate 'lost' across the PDV. Amongst these AA, threonine comprises a substantial proportion of digestive tract secretions (notably the mucins; Mukkur *et al.* 1985), while glycine and serine may be involved in  $C_1$  metabolism and nucleic acid biosynthesis in the metabolically-active, and rapidly-proliferating, mucosal cells (Perez & Reeds, 1998). In these cases, uptake and use by the digestive tract may be driven by metabolic need rather than through concentration-mediated mechanisms.

Even when the amount of AA presented to the liver is increased 5-fold that required for N equilibrium, and where the hepatic ureagenic capacity is exceeded, the removal follows a linear trend for most AA. Thus, although the absolute amount removed by the liver increased with each infusion level, this represents a constant proportion of the supply. As was also observed for cattle (Wray-Cahen *et al.* 1997), there is no obvious saturation of hepatic transport mechanisms over a range that would encompass the highest dietary AA supply.

For sheep fed grass pellets, then from comparison of the pattern of net AA supply beyond the liver with that in mixed body proteins (MacRae *et al.* 1993), free phenylalanine appears to enter the peripheral circulation in 'least excess' relative to peripheral tissue needs, followed by histidine (see Loblely & Milano, 1997). A similar situation

appears to exist under the short-term increased loading of AA adopted in the current study. The other essential AA, therefore, must accumulate in the body free pools or undergo catabolism in non-liver tissues. Extrahepatic oxidation is well documented for the branched-chain AA (discussed earlier), while porcine data indicate that lysine (Reeds *et al.* 1999) can be catabolised by the digestive tract. Nonetheless, the large increases in arterial concentrations indicate that both the oxidative and the metabolic capacities of peripheral tissues to dispose of AA excess are limited in the sheep. This factor was particularly noticeable for threonine, the arterial concentration of which increased over 4-fold at the highest rate of infusion. This increase occurs despite extrahepatic catabolism within the pancreas (pigs, Le Floch *et al.* 1999) and the kidney (rats, Ogawa *et al.* 1991; sheep, C. Germain and G.E. Loblely, unpublished results) and indicates a limited oxidative capacity within the body. Clearly, the liver is not able to play a complete and 'instantaneous' role to prevent hyperaminoacidaemia. Rather, there is a 'balance' between removal of a portion of the absorbed AA (or at least the total AA provided to the liver) and presentation to the peripheral tissues.

Most of the AA exhibited fractional absorption rates that were within the values cited in the literature for both ruminant and non-ruminants (see Wray-Cahen *et al.* 1997; Le Floch *et al.* 1999). Unfortunately, the ranges reported across studies are large and such comparisons may be rather meaningless, particularly when only single treatments are involved (rather than the incremental infusions

adopted in the current study) and where the needs for specific reactions (e.g. synthesis of plasma protein, hippurate, glucose) cannot be assessed. Such metabolic requirements may exert a variable influence on the apparent needs for specific AA. For example, although the proportion of net supply removed by the liver remained constant for most AA, a number exhibited higher fractional extraction rates when basal diet alone was supplied (similar to values observed previously for sheep, Lobley *et al.* 1995, 1996), but these extraction rates decreased during the AA infusions. This situation was observed for phenylalanine, histidine, glycine and alanine. Both phenylalanine and histidine are present in higher proportions in plasma protein (63 and 41 g/kg respectively) compared with ovine whole-body protein (38 and 22 g/kg respectively). When the basal diet alone is supplied, the demands of plasma protein synthesis for such AA results in high rates of extraction and may cause a post-splanchnic deficiency relative to the needs of peripheral tissue protein metabolism (Lobley & Milano, 1997). These demands may be quite substantial; for example, in the sheep, production of export protein can amount to 20 g/d, equivalent to 30–40 % of liver protein synthesis (Connell *et al.* 1997). There is probably a finite limit to export protein synthesis (Lobley *et al.* 1998), however, and once this limit is attained, through the exogenous infusion of AA, the anabolic demands of the liver would be lessened and a greater proportion of the AA would enter the peripheral circulation.

Analogous situations exist for glycine and alanine. A number of studies have also reported extractions of glycine by the liver in excess of the net absorption under basal diet conditions (for example, see Le Floc'h *et al.* 1999; Lapiere *et al.* 2000). Glycine plays a number of important metabolic roles, including synthesis of nucleic acids, bile salts, glutathione and serine, plus a general involvement in methyl group metabolism. Across the liver, involvement in detoxification of xenobiotics probably makes the greatest demand on glycine requirements, in particular for conversion of diet-derived benzoic acid metabolites to hippuric acid (benzoylglycine). The benzoate supply would be fixed, as the intake of the basal diet was constant, so once these needs for glycine were met the fractional extraction rates of the AA would decrease. Alanine is a minor precursor for glucose synthesis in ruminants (Heitmann & Bergman, 1978) and, once the finite needs for gluconeogenesis have been met, demands for alanine will be reduced. Thus, as with glycine, a decline in both absolute (and thus fractional) extraction rates would be expected as the AA supply is increased.

The lowest fractional net extraction rates were observed for the branched-chain AA, compatible with their sites of catabolism within the body. In both ruminants and rodents the enzymes for the catabolism of the branched-chain AA, including the rate-limiting enzyme, the branched-chain oxo-acid dehydrogenase, occur in many tissues other than liver (notably muscle, fat and those of the PDV; Goodwin *et al.* 1987). Indeed, for ruminants, <25 % of whole-body leucine oxidation occurs in the liver (Pell *et al.* 1986; Lobley *et al.* 1995). Furthermore, because >80 % of the liver branched-chain oxo-acid dehydrogenase is already in the active (dephosphorylated) form in sheep (Goodwin *et al.*

1987), the ability to increase leucine catabolism would require synthesis of new enzyme protein. Thus, as the branched-chain AA supply is increased, as in the current study, uptake and metabolism would soon reach a maximum and, therefore, the non-linear responses and low apparent fractional extraction rates observed would be expected. These low hepatic removals result in changes in branched-chain AA arterial appearance that closely reflect dietary absorption, and this may link to their putative role as regulators of peripheral tissue metabolism (see Lobley, 1998).

In practice, the liver is supplied with a mixture of newly absorbed AA and those arising from endogenous recirculation. Flows of the latter are 3–4-fold the amounts absorbed and, thus, the fractions of total AA inflow removed are much smaller than those based on net PDV transfers. Use of these values based on total inflow has been proposed as a more realistic scenario of how the liver responds to circulating AA concentrations and flows (Reynolds *et al.* 1994). Across a range of bovine studies (Reynolds *et al.* 1994; Hanigan *et al.* 1998; Lapiere *et al.* 2000; H Lapiere and R Berthiaume, personal communication), plus the present ovine investigation, the highest fractional hepatic extraction rates of total AA inflow are for alanine, arginine, methionine, phenylalanine, serine and tyrosine (all >10 % removed). The sheep data appear to show higher extraction rates for glycine, histidine, proline and threonine than the cattle studies, whereas tryptophan removal was relatively high in both sheep and the growing steers of Lapiere *et al.* (2000). Interpretation of these results is complicated by the differences in the experimental conditions employed (e.g. alterations in total food intake) and the physiological demands of the animals (for growth, pregnancy, lactation). Nonetheless, use of such values should be the preferred choice for mathematical models of liver transport and metabolism (Hanigan *et al.* 1998). What is unclear, at present, is whether the extraction rates are a response to AA concentration or to AA flows. In the current study these effects cannot be separated, because blood flow was unaltered by infusion, but both change in response to total food intake (Lapiere *et al.* 2000). The linear responses indicate that transporter capacity was not a limitation. Indeed, values of  $K_m$  and maximum velocity for both non-ruminant and ruminant hepatocytes *in vitro* (for example, see McGivan & Bradford, 1977; White, 1985; Narkewicz *et al.* 2000) indicate that neither binding nor reaction velocity will be maximal under the conditions employed in the present study, or under normal feeding regimens. Mass action effects may alter the concentration of AA within the hepatocytes, and thus influence rates of entry, particularly where  $\text{Na}^+$ -independent non-concentrative mechanisms operate. Such mass action effects have been proposed as the main drive for entry of AA into the hepatic ornithine cycle (Meijer *et al.* 1999). Use of pharmacological agents to alter blood flow, but not net absorption (thus reducing the concentration of the inflow), may help to resolve this question. Similarly, data at low intakes, where re-circulation of AA contributes an even greater proportion to total inflow, will determine whether relatively simple approaches based on concentration and/or flow can explain much of the hepatic metabolism of AA.



From early studies with dogs the hypothesis evolved that AA were transferred to the liver within the plasma, while carriage from the liver also involved the erythrocytes (Elwyn *et al.* 1972). Tests of this concept have been equivocal, with evidence both for and against (Heitmann & Bergman, 1980; Houlier *et al.* 1991; Lobley *et al.* 1996; Le Floc'h *et al.* 1999). Part of the uncertainty arises from the measurement errors that accumulate for calculation of arterio-venous differences for the different vascular fluids. Also, technical errors may arise from the action of peptidases within the erythrocytes during collection and analytical procedures (Lochs *et al.* 1990). Furthermore, changes in plasma: blood AA concentrations (Houlier *et al.* 1991) will occur even if plasma is the main source for both PDV absorption and hepatic extraction. Such changes do not necessarily mean that the AA within the erythrocytes are readily available to other tissues (Savary *et al.* 2001). Measurements, based on changes in isotopic enrichments of both plasma and blood AA indicate that erythrocytes probably play only a minor role in exchanges across the ovine splanchnic tissues (Lobley *et al.* 1996). The current data provide support for this conclusion, based on the numerical equivalence between net plasma and blood transfers.

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