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Oxidation of essential amino acids by the ovine gastrointestinal tract

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It is not known if the ruminant animal gastrointestinal tract (GIT) can oxidise essential amino acids (AA) other than leucine. Therefore, the oxidation of four essential AA (leucine, lysine, methionine and phenylalanine), supplied systemically as labelled 1-^{13}C forms, was monitored across the mesenteric-drained viscera (MDV; small intestine) and portal-drained viscera (PDV; total GIT), as part of a 4 × 4 Latin square design, in four wether sheep (35–45 kg) fed at 1·4 × maintenance. Oxidation was assessed primarily by appearance of $^{13}CO_2$, corrected for sequestration of [^{13}C]bicarbonate. The GIT contributed 25 % ($P<0·001$) and 10 % ($P<0·05$) towards whole-body AA oxidation for leucine and methionine respectively. This reduced net appearance across the PDV by 23 and 11 % respectively. The contribution of MDV metabolism to total PDV oxidation was 40 % for leucine and 60 % for methionine. There was no catabolism of systemic lysine or phenylalanine across the GIT. Production and exchange of secondary metabolites (e.g. 4-methyl-2-oxo-pentanoate, homocysteine, 2-aminoadipate) across the GIT was also limited. Less AA appeared across the PDV than MDV ($P<0·001$), indicative of use by tissues such as the forestomach, large intestine, spleen and pancreas. The PDV: MDV net appearance ratios varied ($P<0·001$) between AA, e.g. phenylalanine (0·81), lysine (0·71), methionine (0·67), leucine (0·56), histidine (0·71), threonine (0·63) and tryptophan (0·48). These differences probably reflect incomplete re-absorption of endogenous secretions and, together with the varied oxidative losses measured, will alter the pattern of AA net supply to the rest of the animal.

Gastrointestinal tract: Oxidation: Leucine: Methionine: Lysine: Phenylalanine: Sheep

It is well recognised that the gastrointestinal tract (GIT) makes a major contribution to whole-body energy and protein metabolism. For example, in sheep the GIT contributes approximately 25 % total O_2 consumption (Burrin *et al.* 1989) and as much as 65 % to whole-body protein synthesis (WBPS; MacRae *et al.* 1997*a*). While this high metabolic activity is undoubtedly necessary to ensure effective organ function to meet the demands of digestion, absorption and provision of an innate immune defence, this may incur a cost. Incomplete resorption from the lumen of endogenous secretions and desquamed cells can result in reduced amino acid (AA) availability to other tissues, both in total amount and the relative composition (van Goudoever *et al.* 2000; Lapierre & Lobley, 2001). The GIT also can catabolise (oxidise) AA, as has been clearly demonstrated, in both ruminant and non-ruminant animals, for glutamate and glutamine (Wolff & Bergman, 1972; Reeds *et al.* 2000; Reeds & Burrin, 2001). Such catabolism is probably linked to the energy needs of the GIT (Reeds *et al.* 2000).

The extent to which essential AA may be catabolised by the GIT is less clear, with results available for only a few AA. In ruminant animals, for example, reported studies have only used leucine, which is catabolised by the GIT with the extent determined by a range of factors that include diet quantity (Pell *et al.* 1986; Yu *et al.* 2000), diet quality (Lobley *et al.* 1996*b*; Lapierre *et al.* 2002), the presence of intestinal tract parasites (Yu *et al.* 2000) and use of antibiotics directed against the GIT microflora (MacRae *et al.* 1999). In consequence, 25–50 % of leucine absorption may be oxidised across the GIT. The question, then, is do the other essential AA behave similarly to

Abbreviations: AA, amino acid; GIT, gastrointestinal tract; MDV, mesenteric-drained viscera; MOP, 2-oxo-4-methylpentanoate; PDV, portal-drained viscera; WBO, whole-body oxidation; WBPS, whole-body protein synthesis.

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leucine? There is evidence that lysine is also oxidised by the pig GIT, provided that dietary protein supply is high (van Goudoever *et al.* 2000). In contrast, threonine is not catabolised in young pigs (Burrin *et al.* 2001), indicative of preferential rather than global responses.

A further issue relates to the site of any GIT oxidation. Does this occur predominantly in the tissues of the small intestine? If so, then the absolute amount of essential AA appearance in the mesenteric vein should be less than absorption of diet-derived AA (i.e. disappearance from the lumen of the small intestine). This is apparently not the case with sheep and cattle (MacRae *et al.* 1997*b*; Berthiaume *et al.* 2001), but reduced amounts do appear in the portal vein. While one explanation might involve metabolism of AA by the rumen tissues, there are similar quantitative and relative losses across the pig GIT (van Goudoever *et al.* 2000). Such results would imply that the GIT has a major impact on the amount of AA available to peripheral tissues and treatments that reduce the metabolic activity of the digestive tract would enhance growth or lower protein requirements for maintenance of lean mass. It is necessary, therefore, to determine if AA are oxidised by the GIT or may be lost by other routes, e.g. non-reabsorption of endogenous secretions.

The current study addresses this issue in sheep and investigates the capability of both the small intestine and total GIT to oxidise four essential AA extracted from the systemic circulation. Leucine was selected as representative of the branched-chain AA and because of known catabolism by the GIT. Methionine and lysine were also examined as either are often considered first limiting in typical ruminant animal diets (National Research Council, 2001) and any losses during absorption will restrict further any potential anabolism. In addition, conversion of methionine to homocysteine, and then probably to cysteine, occurs in both the ovine (Lobley *et al.* 1996*a*) and digestive tract of human subjects (Stegink & den Besten, 1972). Phenylalanine was chosen to represent the aromatic AA and, for which catabolism in non-hepatic tissues is know to occur in other species (Bush *et al.* 2003). In addition to these direct measurements of oxidation, from differences in net appearances in the mesenteric and portal veins of other individual AA, the impact of oxidative or secretory losses could be assessed.

Materials and methods

Animals and diet

Four Suffolk cross wether lambs (35–45 kg, 8–10 months old) were prepared with indwelling silicone rubber catheters in the aorta, portal vein, hepatic vein and two in the mesenteric vein as described previously (Lobley *et al.* 1995). For the two mesenteric vein catheters, the more cranial was inserted via a side-branch approximately 0·20 m from the gastro–splenic junction and the tip advanced to be 0·05 m distal to that junction. The more distal catheter was inserted via a side-branch at least 0·25 m from the insertion of the other catheter and the tip advanced 0·10 m into the mesenteric vein in the direction of blood flow. This preparation allowed blood to be collected across the mesenteric-drained viscera (MDV) and the portal-drained viscera (PDV; Fig. 1). The MDV drained blood from the small intestine between a point approximately 0·50 m caudal to the pylorus through to the ileum. The PDV also drained those tissues plus the forestomachs, the first 0·50 m of the duodenum, the hindgut, spleen and pancreas. Animals were allowed 2 weeks to recover from surgery and then were adjusted to metabolism cages and offered 1·2 kg grass pellets (as fed)/d (964 g DM/kg; 23 g N and 10·5 MJ estimated metabolisable energy/kg DM), supplied as twenty-four equal portions at hourly intervals by means of automated feeders. In addition, 50 g mixed concentrate–forage diet (g/kg: hay 500, barley 300, molasses 100, fishmeal 90, vitamins and minerals 10; 11·7 g N/kg as fed) were given each morning. Therefore, total N intake was 798 mmol/h.

Each sheep was studied on five separate occasions. The first four periods comprised part of a 4 × 4 Latin square design that involved quantification of the metabolism of the four AA. Each measurement period involved a 10 h intra-hepatic vein infusion of a 1-^{13}C-labelled form of one of the AA. Rates of infusion were: L-[1-^{13}C]leucine (99·1 atom %; Mass Trace Inc., Woburn, MA, USA) 0·70 mmol/h; L-[1-^{13}C]methionine (98·7 atom %; Isotec Inc., Miamisburg, OH, USA) 0·23 mmol/h; L-[1-^{13}C]phenylalanine (99 atom %; Mass Trace Inc.) 0·33 mmol/h + L-[$^{2}H_{4}$]tyrosine (99 atom %; Isotec Inc.) 0·07 mmol/h. For lysine, limited availability meant that different forms were infused (0·23 mmol/h) in separate sheep; either L-[1-^{13}C]lysine (99 atom %; Isotec Inc.) or L-[1-^{13}C, $^{2}H_{2}$]lysine (98 atom %; Cambridge Isotope Laboratories, Andover, MA, USA). Following the last infusion of the 4 × 4 Latin square design, all animals received a fifth 10 h infusion of sodium [1-^{13}C]bicarbonate (0·30 mmol/h; 98 atom %; Isotec Inc.).

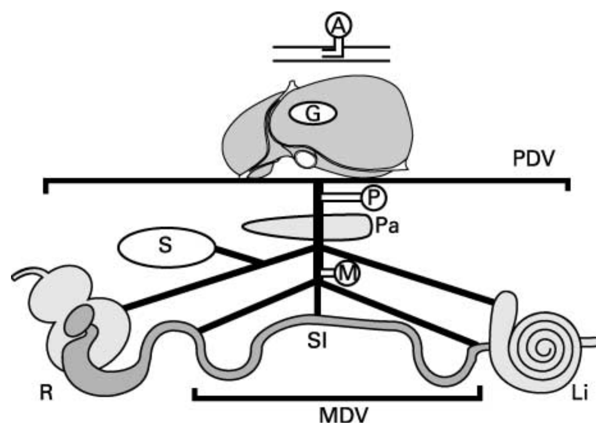

Fig. 1. Vascular drainage of the gastrointestinal tract and positions of catheters for blood sampling. All blood to the gastrointestinal tract is derived from arterial sources, represented by aortal blood (catheter A). The mesenteric-drained viscera (MDV) supplies blood to the mesenteric vein (catheter M) from the small intestine (SI) between approximately 0·50 m caudal to the pylorus and the ileum. The portal-drained viscera (PDV) includes blood in the portal vein (catheter P) derived from the MDV plus forestomachs (from rumen (R) to first 0·50 m of duodenum), large intestine (LI), spleen (S) and pancreas (Pa). Secretions derived from saliva and the gall bladder (G, as bile inflows) enter the rumen and small intestine respectively, but are derived from vascular sources beyond the PDV.

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All infusates were prepared in 200 g sterile 0·15 M-NaCl and infused at 20 g/h into the hepatic vein, except for the phenylalanine–tyrosine mixture which was dissolved in 400 g sterile 0·15 M-NaCl and infused at 40 g/h. A minimum of 4 d was left between each infusion. A solution of sterile 0·1 M-sodium *p*-aminohippurate in 0·05 M-sodium phosphate pH 7·4 containing 250 IU heparin/g (Leo Laboratories Ltd, Princes Risborough, Bucks., UK) was infused (40 g/h) into the distal mesenteric vein catheter between 3·5–10·0 h of labelled AA or bicarbonate infusion. During all infusions the animals continued to be fed on an hourly basis, i.e. all measurements involve absorptive periods.

Continuous integrated blood samples (11 ml) were withdrawn hourly from each of the cranial mesenteric vein, portal vein and aortal catheters between 6–9 h of AA infusion and collected into syringes over ice (Lobley *et al.* 1995). On the day preceding each infusion of labelled material, a solution of sterile 0·15 M-NaCl containing 250 IU heparin/g was infused into the mesenteric vein for 2 h, with 3 × 40 min integrated samples withdrawn (7 ml) from the other three catheters to provide natural abundance (‘background’) samples for bicarbonate and AA enrichments. All samples from natural abundance and labelled AA infusions were mixed in the syringes and triplicate samples (1 ml) injected into evacuated red-topped Vacutainers (Beckton Dickinson, Plymouth, Devon, UK) containing 0·9 ml lactic acid. These were immediately mixed to liberate CO_2 and, on the same day, enrichments measured, based on ion intensities at masses 44, 45 and 46 by dual-inlet gas isotope MS (SIRA 12; VG Isogas, Middlewich, Ches., UK). Calculated atom % values included the Craig correction to allow for the presence of atmospheric $^{17}O_2$ that would yield $^{12}C^{17}O^{18}O$ with a mass of 45; this would interfere with the quantification of $^{13}CO_2$. Atom % excess values were calculated by difference from the natural abundance samples.

Blood haemoglobin, P_{O_2}, P_{CO_2}, pH and packed cell volume were determined in duplicate using an ABL625 Blood Gas Analyser (Radiometer, Copenhagen, Denmark). The remaining blood was then centrifuged at 1000 ***g*** for 15 min at 4°C to obtain plasma. NH_3 concentrations were determined, in duplicate, in 0·4 ml fresh plasma by the glutamate dehydrogenase reaction using a Kone Dynamic Selective Analyser (Kone, Espoo, Finland). The *p*-aminohippurate concentrations in 0·7 g fresh plasma were determined gravimetrically as described previously (Lobley *et al.* 1995). To a further 0·7 g plasma was added a known weight (0·3 g) of a solution containing 0·6 mg hydrolysed [U-^{13}C]algae powder (99 atom %; Martek Biosciences Corp., Colombia, MD, USA), 200 nmol [5-^{15}N]glutamine (99 atom %, Mass Trace Inc.), 100 nmol [indole-^{15}N]tryptophan (99 atom %, Cambridge Isotope Laboratories) and 3·48 μmol [^{15}N^{15}N]urea (99 atom %; Mass Trace Inc.). These samples were mixed and then frozen (−80°C) until analysis as the *t*-butyldimethylsilyl derivatives to determine AA and urea concentrations as molal concentrations by isotope dilution with GC–MS, as described previously (Calder *et al.* 1999).

Another portion of plasma (1 g) was frozen (−80°C) for later analysis of the enrichments of the AA and appropriate metabolites. The plasma samples for subsequent analysis of methionine had 0·1 ml 100 mM-dithiothreitol added before freezing to allow subsequent release and analysis of homocysteine bound to plasma protein and, on thawing, this plasma was left at room temperature for 30 min before deproteinisation. All thawed plasma was deproteinised with 0·15 ml sulfosalicylic acid (480 g/l) and, after centrifugation, the supernatant fraction was applied to 0·7 ml AG-50 resin (100–200 mesh × 8) in the H^+ form followed by 1 ml deionised water wash. The eluate plus washings were collected and the oxo-acids of leucine (2-oxo-4-methylpentanoate, MOP) and 2-aminobutyrate (2-oxobutyrate) extracted and converted to the quinoxanol *t*-butylmethylsilyl derivative prior to GC–MS (Calder & Smith, 1988). The AA and other metabolites were then eluted with 2 ml 2 M-NH_4OH followed by 1 ml deionised water. From the combined eluate, 80 μl was removed for urea analysis after drying under N_2 gas, while the remainder was freeze-dried. The urea, AA and amino-derivatives were converted to the *t*-butylmethylsilyl derivatives and analysed by GC–MS using a Hewlett Packard HP5989A Engine (Hewlett Packard, Avondale, PA, USA) as described previously (Calder & Smith, 1988). The respective m/z monitored were: leucine 302, 303; methionine 321, 322; lysine 300, 301 for [1-^{13}C]lysine and 300, 303 for [1-^{13}C, ^{2}H$_2$]lysine; phenylalanine 336, 337; tyrosine 466, 467 (for [1-^{13}C]tyrosine synthesised from [1-^{13}C]phenylalanine), 470 (for m+4 ions from [^{2}H$_4$]tyrosine); homocysteine 420, 421; 2-aminobutyrate 274, 275; homoserine 404, 405; 2-aminoadipate 446, 447 or 449 depending on the labelled lysine infused. For the oxo-acids, the m/z ions monitored were: MOP 259, 260; 2-oxobutyrate 231, 232. All calculations of molar % excess were as described by Campbell (1974), based on the ratio differences against the natural abundance values.

Calculations

Net transfers (μmol/h) across the MDV or PDV were calculated as:

$$(M_{m\ or\ p} - M_a) \times PF_{m\ or\ p},$$

where M is metabolite (AA, urea, NH_3 or bicarbonate) concentration (μM), PF is plasma flow (kg/h from gravimetric *p*–aminohippuric acid measurements) and m is mesenteric, p is portal and a is arterial sample respectively. For these, and subsequent trans–organ calculations, values were calculated for individual plasma flows and concentration differences for each of the three hourly periods on each measurement day and then the mean value used for subsequent statistical analyses.

Whole-body tracee AA irreversible loss rate (mmol/h) was calculated from:

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

where I_e represents enrichment (molar fraction) of AA or bicarbonate infusate and I is the rate of infusion (mmol/min), while E_a is the mean enrichment of the metabolite (mol fraction) in arterial plasma. Outflow from plasma

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AA pool (tracee + tracer; mmol/h) was calculated as:

$$(I_e/E_a) \times I.$$

It was assumed that the equivalent of the infused dose was in excess of body needs and was oxidised, with tracee and tracer metabolised similarly. Therefore, whole–body tracee oxidation (WBO) of AA (mmol/h) was calculated as:

$$(\text{whole–body outflow} \times \text{fraction dose oxidised}) - \text{dose infused}.$$

The fraction of the dose oxidised was calculated as:

$$(E_{a,\ CO_2} \times WBF_{CO_2})/(E_d \times I),$$

where $E_{a,\ CO_2}$ is the enrichment (atom % excess) of CO_2 in arterial plasma, WBF_{CO_2} is whole–body flux (production; mmol/h) of CO_2 and E_d is the enrichment of the infusate.

For phenylalanine, an alternative approach could be adopted based on the production of tyrosine (as the 1-^{13}C form) from hydroxylation of phenylalanine, the first stage in the degradative pathway. Total tyrosine (mmol/h) from phenylalanine is given by:

$$\text{tyrosine outflow (tracee + tracer)} \times \text{fraction of tyrosine from phenylalanine},$$

where the fraction converted is obtained from the relative enrichments of [1 − ^{13}C]tyrosine:[1 − ^{13}C]phenylalanine ratio. The tracee tyrosine from phenylalanine is then obtained by subtracting the dose of phenylalanine infused (again it is assumed that this is all oxidised).

Use of AA for WBPS (mmol/h) was calculated as irreversible loss rate − WBO. This was converted into g protein synthesised/d by:

$$WBPS \times 24 \times 0{\cdot}1 \times MM_{aa}/(\%\ \text{AA in protein}),$$

where MM_{aa} is the molecular mass of the AA, and (% AA in protein) is the average content (g/100g) of the AA in body protein (MacRae *et al.* 1993).

Apparent oxidation of AA (AOAA) across the gut (mmol $^{13}CO_2$ appearance/min) was calculated as:

$$PF_{m\ or\ p} \times ((B_{m\ or\ p} \times E_{CO_2,\ m\ or\ p}) - (B_a \times E_{CO_2,\ a})),$$

where B is the relevant plasma bicarbonate concentration (mM). During the bicarbonate infusions, the appearance of total $^{13}CO_2$ label in the venous drainage of the MDV and PDV was less than that in the arterial inflow. This was due to sequestration (isotopic exchange) of label by the digestive tract tissues. The sequestration (sq) was determined for each sheep from:

$$((B_a \times E_{CO_2,\ a}) - (B_{m\ or\ p} \times E_{CO_2,\ m\ or\ p}))/(B_a \times E_{CO_2,\ a}).$$

On average, this represented 3·3 % of arterial inflow of labelled plasma bicarbonate for both PDV and MDV. This is greater than reported sequestration across the PDV of dogs (2 %; Gresham *et al.* 2000). While this may reflect species differences, the canine study is unusual in that whole–body bicarbonate recovery exceeded 97 %, whereas in the ovine (Ram *et al.* 1999) and human subjects (see Leijssen & Elia, 1996) lower recoveries (i.e. higher sequestration) of <85 % are reported.

Oxidation (tracer + tracee) by the MDV or PDV tissues was then calculated as:

$$AOAA + (Sq \times \text{arterial inflow}\ ^{13}C\ \text{bicarbonate})$$

To obtain from this the oxidation of tracee by the tissue (TO, mmol/h) it is assumed that the partition of oxidation between body organs is the same for tracer and tracee such that:

$$(TO_{tracer+tracee})/(WBO_{tracer+tracee}) = (TO_{tracee})/(WBO_{tracee}),$$

so that:

$$TO = WBO_{tracee} \times TO_{tracer+tracee}/WBO_{tracer+tracee}.$$

Similar reasoning was applied for trace AA use for protein synthesis (mmol/h) across the MDV and PDV:

$$TPS = WBPS_{tracee} \times TPS_{tracer+tracee}/WBPS_{tracer+tracee},$$

where TPS is the total protein synthesis. For these calculations, various pools (and corresponding enrichments) were selected as being most representative of the precursor for protein synthesis. This was usually taken as the artery for comparison with whole-body flux calculations or the appropriate vein (see p. 619). These values could be converted into values expressed as g protein/d by a similar equation as that used for whole-body flux calculations.

Statistics

All comparisons were by ANOVA using GenStat for Windows (version 6, release 6.1.0.200; Lawes Educational Trust, Rothamsted, Herts., UK). For main effects, animals and periods were treated as blocks, but if period was found not to be significant (the usual situation) then the data were re–analysed with period omitted. For net movements of individual AA, NH_3 and urea, animal and day of sampling were treated as blocks and site of sample (MDV and PDV) as treatment. The PDV:MDV appearance ratio was examined for groups of AA (essential or non–essential), again blocked for animal and day of sampling. These data were restricted to three sheep only, due to problems with the placement of the mesenteric catheter tip. For isotope–related measurements, data were again analysed with sheep and period as blocks and the latter subsequently removed if found not to be significant. The enrichments of natural abundance bicarbonate in blood between the various sites were also compared. This involved the mean of triplicate analysis for three samples (number), from each blood vessel at five periods (before each infusion). These were analysed with animal, period and number as blocks and with vessel as treatment.

Results

Mass transfers across the gastrointestinal tract

Plasma flows and non-amino-acid-N transfers. In one sheep the tip of the cranial mesenteric catheter tip was

found adhered to the vessel wall at analysis post mortem. For this sheep, MDV flows and analyses were not included in the data.

There was no effect of either sheep or AA infusion on PDV plasma flow (1·64 (SD 0·19) kg/min). In contrast, for MDV flow (0·83 (SD 0·18) kg/min), there were effects between sheep ($P=0·023$), although not between AA infusion. The animal effect was also apparent ($P=0·014$) for the MDV:PDV plasma flow ratios (0·50 (SD 0·12)). The animal differences in MDV flow may relate to position of the catheter tip during sampling and whether caecal vein inflow was included or not. With such surgical preparations, catheter tip placement is always slightly uncertain due to the flexibility of the GIT and the physical movements associated with the free postural changes permitted during the measurements.

There was net production ($P<0·001$) of NH_3 across the GIT, with approximately 42 % absorbed from the small intestine into the mesenteric vein (Table 1). In contrast, there was consistent uptake ($P<0·001$) of urea-N by the GIT with approximately 45 % of total removal across the GIT occurring across the MDV (Table 1). These results indicate that it would be erroneous to think at all NH_3 appearing in the portal vein is derived from rumen fermentation. Clearly other mechanisms, such as deamination of AA in the small intestine (Windmueller & Spaeth, 1980; Gate *et al.* 1999) can be quantitatively important. The proportions of NH_3 absorbed from the rumen and the lower digestive tract might vary with both the nature of feed offered and the feeding frequency. The observation that non-salivary urea entry to the GIT is also not exclusive to the rumen agrees with other reports (see Lapierre & Lobley, 2001). While urea-N removal accounted, numerically, for approximately 70 % of the NH_3 appearance across both the MDV and PDV, direct determination of the fate of the urea-N would require use of isotope kinetics. It is known, however, that under similar experimental conditions at least 40 % of urea-N entry to the GIT is returned to the liver in the form of NH_3 (Sarraseca *et al.* 1998).

In terms of total N absorbed (i.e. NH_3 + AA-N), the fraction from NH_3 increased ($P<0·001$) from 0·31 across the MDV to 0·63 for the whole GIT. When allowance was made for urea-N inputs, however, the relative amounts were 0·10 and 0·35 respectively; this probably reflects the net fates of absorbed dietary-N better. An ‘apparent’ digestibility value of 0·47 can be determined from the PDV N-balance value ($AA-N + NH_3 - N - urea-N$; Table 1) expressed against N intake (79·8 mmol/h). These do not include N ‘digested’ in other forms e.g. nucleic acid-N, amino sugars, nitrate and, most notably, arginine and citrulline. These latter will probably contribute 8·3 mmol N/h to PDV net absorption (from Lobley *et al.* 2001) and would increase the digestibility value to 0·57. This compares with digestibility values of 0·63 determined

Table 1. Net absorption (mmol/h) of amino acids (AA), urea and ammonia across the mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) of sheep offered 1200 g grass pellets/d*

	MDV	PDV	SED	Statistical significance of effect (ANOVA): *P*†	PDV:MDV‡
Non-essential AA					
Alanine	4·062	3·198	0·219	0·002	0·823[bc]
Aspartate	0·612	0·594	0·043	NS	1·014[a]
Glutamate	1·572	1·302	0·069	0·003	0·839[ab]
Glutamine	−0·684	−1·710	0·107	<0·001	–
Glycine	3·642	2·682	0·157	<0·001	0·738[bc]
Proline	1·638	1·074	0·067	<0·001	0·655[c]
Serine	2·442	1·872	0·089	<0·001	0·772[bc]
Tyrosine	1·560	1·158	0·082	<0·001	0·767[bc]
SED					0·0670
Essential AA					
Histidine	0·774	0·546	0·029	<0·001	0·711[b]
Isoleucine	2·130	1·290	0·088	<0·001	0·610[cd]
Leucine	3·450	1·902	0·180	<0·001	0·559[de]
Lysine	2·280	1·590	0·110	<0·001	0·714[b]
Methionine	0·732	0·480	0·046	<0·001	0·670[bc]
Phenylalanine	1·872	1·482	0·089	0·001	0·811[a]
Threonine	2·154	1·344	0·097	<0·001	0·629[bcd]
Tryptophan	0·510	0·258	0·024	<0·001	0·478[e]
Valine	2·634	1·572	0·109	<0·001	0·589[cd]
SED					0·0326
Others					
Ammonia	15·54	37·38	2·538	<0·001	
Urea-N	−11·46	−25·62	2·016	<0·001	
AA-N	35·16	21·90	1·518	<0·001	
N balance§	40·50	37·52	3·072	NS	

[a,b,c,d,e] Mean values within a column and subsection (i.e. non-essential and essential AA compared seperately) with unlike superscript letters were significantly different ($P<0·01$).
* For details of procedures, see p. 620.
† Mean values based on four measurement days for each of four sheep, ANOVA with animal and day as blocks and site as treatment, with 11 residual df (four missing values, all MDV results for one sheep).
‡ Values of ratios based on three sheep only, by ANOVA with animals and day of infusion treated as blocks, sample site as treatment, 66 and 88 residual df for non-essential and essential AA respectively.
§ Calculated as (AA-N + ammonia) − urea-N.

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by N balance for the same diet in other studies (results not shown).

Net amino acid absorption. For the AA examined, net appearance across the MDV exceeded PDV appearance ($P<0{\cdot}01$), except for aspartate, where appearances were not different, and glutamine, where net disappearance occurred across both the MDV and PDV, with greater removal ($P<0{\cdot}001$) across the latter (Table 1).

The PDV : MDV appearance ratio yields the proportion of net AA absorbed across the small intestine that is then either catabolised or used to support metabolism in the other sections of the GIT i.e. the forestomachs, hindgut, pancreas and spleen. This ratio differed ($P<0{\cdot}001$) between the essential AA. A greater proportion of net uptake into the mesenteric vein appeared in the portal vein for phenylalanine (0·81, $P<0{\cdot}01$ *v.* all other AA), while the lowest value was observed for tryptophan (0·48, $P<0{\cdot}05$ for leucine, $P<0{\cdot}01$ for valine, $P<0{\cdot}001$ for the other essential AA). While histidine and lysine had similar PDV: MDV appearance ratios, these were higher ($P<0{\cdot}01$) than observed for threonine, isoleucine, valine and leucine. Similarly, methionine and threonine ratios were greater ($P<0{\cdot}01$) than for leucine, although the branched-chain AA were not different from each other. There were also differences in the PDV: MDV appearance ratios for the non-essential AA with aspartate (at unity) greater ($P<0{\cdot}05$ for glutamate, $P<0{\cdot}01$ for the other non-essential AA) than all the others. Proline had the lowest ratio (0·66) and this was lower ($P<0{\cdot}05$) than that observed for alanine (0·79) and glutamate (0·83).

Isotope kinetics

Whole-body irreversible loss rate and oxidation. Whole-body CO_2 entry rate was consistent between sheep (CV 1·5 %), while whole-body irreversible loss rates for the AA were more variable (CV from 3 % for leucine to 13 % for methionine) (Table 2). Both the absolute (0·49–1·93 mmol/h) and fractional rates (0·12–0·25) of tracee oxidation differed markedly between AA. The fractional rates of combined tracee + tracer oxidation of leucine were greater (38 %) than normally reported (e.g. Lobley *et al.* 1996*b*; Lapierre *et al.* 2002), due to the larger amounts of isotope infused in order to be able to detect the differences in CO_2 isotopic enrichments across the intestinal tract more precisely. Estimates of phenylalanine oxidation based on hydroxylation to tyrosine were lower than those calculated from $^{13}CO_2$ production (0·23 *v.* 0·49 mmol/h, $P=0{\cdot}013$). The whole-body irreversible loss rate used for protein synthesis differed between AA (range 1·49–5·83 mmol/h), but when corrected for the proportion of each AA in body mixed protein, then rates (as g protein synthesis/d) were more similar (range 244–310 g/d, SED 21), but with greater estimates ($P<0{\cdot}05$) based on methionine compared with lysine.

Gastrointestinal metabolism

CO_2 transfers across the gastrointestinal tract. Natural abundance enrichments (atom %) of blood CO_2 differed between sample sites (1·09058, 1·09028, 1·09052 (SED 0·00003, 118 residual df, $P<0{\cdot}001$) for arterial, mesenteric and portal vein samples respectively) (Table 3). Arterial and portal vein enrichments were similar, but both were greater than for mesenteric vein samples.

During 1-^{13}C-labelled AA infusions, apparent appearance of $^{13}CO_2$ across the PDV, uncorrected for isotope sequestration, was positively different from zero ($P<0{\cdot}01$) only for leucine (Table 3). For lysine and phenylalanine, mean values were negative, similarly for MDV measurements. Most of this could be attributed to sequestration of ^{13}C from arterial bicarbonate inflow. This was consistent across the four sheep: 0·033 (SD 0·002) of the PDV inflow and 0·033 (SD 0·005) of the MDV inflow. Correction for sequestration approximately doubled $^{13}CO_2$ appearance from leucine and all values for methionine were positive (Table 3). For lysine, corrected oxidation averaged zero, while for phenylalanine the mean values were slightly negative (but not significantly different from zero). Oxidation across the PDV was larger than across the MDV for leucine ($P<0{\cdot}001$), but only numerically so for methionine.

Table 2. Whole-body amino acid (AA) kinetics and oxidation during infusion of [1-^{13}C]leucine, [1-^{13}C]lysine, [1-^{13}C]methionine and [1-^{13}C]phenylalanine*†

(Mean values with their standard errors for four sheep)

			Leu		Lys		Met		Phe		(Tyr)†	
WB fluxes (mmol/h)	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
ILR‡			7·76	0·10	4·92	0·27	1·99	0·13	3·31	0·19	2·92	0·24
CO_2 entry rate	978	7·1										
WBO			1·93	0·14	0·61	0·10	0·50	0·04	0·49	0·04		
WBO/ILR			0·25	0·02	0·12	0·02	0·25	0·02	0·17	0·01		
ILR_{ps}§			5·83	0·07	4·31	0·19	1·49	0·11	2·82	0·13		
WBPS (g/d)¶			270		244		310		284			

WB, whole-body; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Tyr, tyrosine; ILR, irreversible loss rate; WBO, whole-body oxidation; WBPS, whole-body protein synthesis.

* For details of procedures, see p. 619.

† Phenylalanine infusion also included [$^2H_4$]tyrosine.

‡ Tracee ILR were based on mean values of arterial enrichments for each of four sheep.

§ ILR − WBO (tracee only).

¶ WBPS as g protein synthesis/d from ILR_{ps} expressed as mmol/h x *f*, where is a factor of 44·0, 56·5, 198·7 or 101·8 for Leu, Lys, Met and Phe respectively (from MacRae *et al.* 1993).

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Table 3. Production of $^{13}CO_2$ (μmol/h) across the mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) during infusion of [1-^{13}C]leucine, [1-^{13}C]lysine, [1-^{13}C]methionine and [1-^{13}C]phenylalanine†

(Mean values for four sheep (three only for MDV) with mean values of triplicate analysis of three samples taken hourly)

$^{13}CO_2$ production (μmol/h)	[1-^{13}C] infusate					
	Leucine	Lysine	Methionine	Phenylalanine	SED	Statistical significance of effect (ANOVA): *P*‡
Uncorrected						
MDV	9·7^{a}	−5·4^{b}	0·0^{ab}	−5·1^{b}	5·46	0·098
PDV	32·2^{a*}	−4·7^{b}	−0·4^{b}	−8·4^{b}	3·69	<0·001
Corrected						
MDV	19·6^{a}	−3·3^{b}	4·0^{b}	−1·5^{b}	6·48	0·040
PDV	52·0^{a*}	−0·0^{b}	7·6^{b}	−1·2^{b}	4·55	<0·001
Dose oxidised (%)						
MDV	2·9^{a}	−1·4^{c}	1·8^{ab}	−0·5^{bc}	1·05	0·022
PDV	7·6^{a*}	−0·3^{b}	3·3^{c}	−0·4^{c}	0·89	<0·001
WB	30·9^{a}	17·1^{b}	33·1^{a}	21·7^{c}	1·67	<0·001
WB oxidation (%)						
MDV	9·5^{a}	−7·6^{c}	5·0^{ab}	−2·2^{bc}	3·92	0·019
PDV	24·8^{a*}	−0·9^{c}	9·9^{b}	−1·9^{c}	3·58	<0·001

WB, whole-body.
[a,b,c] Mean values with a row with unlike superscript letters were significantly different ($P<0·05$).
Mean values were significantly different from those of MDV (comparisons between MDV and PDV were for three sheep only by ANOVA with animals as blocks and amino acid × site as treatment, 14 residual df): *$P<0·05$.
† For details of procedures, see p. 620.
‡ Animal as block and amino acid as treatment (no period effect), 9 residual df for PDV and 6 residual df for MDV (one animal missing).

In part, the absolute appearance of $^{13}CO_2$ is a function of the dose infused: a better reflection of the response at both the whole-body and GIT level is obtained when the data are expressed relative to the amount of labelled AA supplied. The proportion of the dose oxidised by the PDV was 8·0 % for leucine and 3·3 % for methionine. These represented 25 % and 10 % of WBO. These values can be converted into tracee oxidation by the GIT and, under the dietary conditions employed in the present study, this amounted to 0·52 and 0·05 mmol/h for leucine and methionine respectively. Thus, net absorption was reduced by 25 and 11 % respectively. In contrast, for neither lysine nor phenylalanine was oxidation across the PDV different from zero, even though WBO accounted for 16 and 22 % of the dose respectively.

Metabolite enrichments

Enrichments of the infused 1-^{13}C-labelled AA and [$^{2}H_4$]-tyrosine were all greater ($P<0·01$) in the arterial plasma than either of the mesenteric or portal venous samples. This is due to dilution, either from unlabelled AA absorbed from the feed or released from protein degradation within the GIT tissues. Enrichments in the mesenteric vein were lower ($P<0·01$) than in the portal vein for all AA except lysine. In contrast, during the leucine infusion, MOP enrichments were similar between arterial and portal veins, but slightly lower (4 %, $P=0·004$) for the mesenteric vein. As a consequence, the MOP: leucine ratio increased from 0·85 in arterial plasma to 1·20 in the mesenteric vein ($P=0·003$) (Table 4). During lysine infusions, the 2-aminoadipate: lysine enrichment ratio in arterial plasma (0·53) was lower ($P<0·001$) than for the corresponding MOP : leucine ratio. Similarly to MOP, however, the enrichment of 2-aminoadipate did not alter across the GIT and, in consequence, the ratio against lysine increased to 0·74 ($P=0·03$) in the mesenteric vein. Glutamate enrichments remained low in all plasma samples (<0·4 molar % excess, results not shown).

As with MOP and 2-aminoadipate, the product of methionine metabolism, homocysteine, also had a lower enrichment in arterial plasma than the parent molecule (ratio 0·74). Again, there was no change in homocysteine enrichment across the GIT, but the homocysteine : methionine ratio increased to unity and greater ($P<0·001$) for the portal and mesenteric veins respectively. A later metabolite of the degradation pathway, 2-aminobutyrate, had enrichments <40 % those of homocysteine, while the corresponding oxo-acid, 2-oxo-butyrate, was lower still. The enrichment of neither 2-aminobutyrate nor 2-oxo-butyrate was changed during passage across the digestive tract. During methionine infusion, label incorporation into homoserine was observed, but this also was unchanged by transit across the GIT and did not exceed 0·14 of the corresponding [1-^{13}C]methionine enrichment.

During infusion of [1-^{13}C]phenylalanine, [1-^{13}C]tyrosine was formed. Although the enrichment of the latter differed ($P<0·001$) between the three sample sites, the ratio against the corresponding [1-^{13}C]phenylalanine was constant (0·17–0·18). This yielded values of 0·51 (SD 0·08) mmol/h for whole-body phenylalanine hydroxylation.

Gastrointestinal tract amino acid gross fluxes. As expected, estimates of protein synthesis across both the MDV and PDV were higher ($P<0·001$) when corresponding venous, rather than arterial, free AA enrichments were chosen as precursor. The contribution of the MDV to total GIT (i.e. PDV) protein synthesis was 42–50 % based on arterial precursor and 52–60 % based on venous plasma across all AA (Table 5). When all data were compared with arterial free AA as precursor, then the contribution of the MDV to WBPS ranged from 16–28 % for the MDV and between 37–59 % for the PDV, between the

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Table 4. Isotopic enrichments (molar % excess) of primary and secondary metabolites in arterial (A), portal vein (PV) and mesenteric vein (MV) plasma following infusion of 1-^{13}C-labelled amino acids into the hepatic vein*

(Mean values for four sheep)

Amino acid infused	A	MV	PV	SED	Statistical significance of effect (one-way ANOVA): *P*†
Leucine					
[1-^{13}C]leucine	8·04^a	5·65^b	6·46^c	0·297	0·001
[1-^{13}C]MOP	6·79^a	6·60^b	6·86^a	0·042	0·004
MOP:leucine	0·85^a	1·20^b	1·06^c	0·054	0·003
Lysine					
[1-^{13}C]lysine‡	5·41^a	4·20^b	4·57^b	0·242	0·010
[1-^{13}C]AAA‡	2·74	2·87	2·85	0·113	NS
AAA:lysine	0·53^a	0·74^b	0·64^{ab}	0·052	0·026
Methionine					
[1-^{13}C]Methionine	10·58^a	6·45^b	7·83^c	0·185	$<$0·001
[1-^{13}C]HC	7·59	7·60	7·68	0·055	NS
HC:methionine	0·74^a	1·21^b	1·01^c	0·046	$<$0·001
[1-^{13}C]AAB	2·82	2·61	2·71	0·217	NS
[1-^{13}C]OB	1·53	1·30	1·71	0·136	NS
OB:AAB	0·62	0·52	0·68	0·116	NS
[1-^{13}C]Homoserine	0·88	0·89	0·72	0·121	NS
Phenylalanine + [^{2}H$_4$]tyrosine					
[1-^{13}C]Phenylalanine	8·18^a	5·14^b	6·28^c	0·218	$<$0·001
[1-^{13}C]Tyrosine	1·41^a	0·90^b	1·11^c	0·043	$<$0·001
[1-^{13}C]Tyrosine:[1-^{13}C]Phenylalanine	0·17	0·18	0·18	0·006	NS
[^{2}H$_4$]Tyrosine	2·21^a	1·63^b	1·90^c	0·040	$<$0·001

MOP, 4-methyl-2-oxopentanoate; AAA, 2-aminoadipate; HC, homocysteine; AAB, 2-aminobutyrate; OB, 2-oxobutyrate.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures, see p. 620.
† Based on mean values of three plasma samples taken from each sheep during infusion of respective labelled amino acids, animals as blocks (no period effect) and site of sampling as treatment, 5 residual df (one missing value).
‡ Alternatively, where appropriate lysine or AAA labelled with [1-^{13}C, ^{2}H$_2$].

various AA. Absolute rates of synthesis, based on venous enrichments as precursor, also varied between the AA and ranged from 62–104 g/d for the MDV and 97–143 for the PDV. Corresponding values based on arterial enrichments were lower by 15–44 %.

Discussion

There were two main questions asked in the present study. First, does the ovine GIT oxidise essential AA other than leucine? Second, do differences in oxidation (or any other mechanism) between the MDV and non-MDV tissues lead to differences in the pattern of AA presented to the liver and beyond?

Which amino acid does the gastrointestinal tract oxidise?

There is little doubt that the mammalian GIT can catabolise leucine (e.g. Pell *et al.* 1986; Lobley *et al.* 1995; Yu *et al.* 2000; van der Schoor *et al.* 2001) and, in ruminant animals,

Table 5. Protein synthesis across the mesenteric-drained viscera (MDV) and portal-drained viscera (PDV) based on use of either plasma arterial or venous enrichments of leucine, lysine and methionine for sheep offered 1000 g grass pellets*

(Mean values with their standard errors for four sheep)

	Leucine		Lysine		Methionine		Phenylalanine	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Protein synthesis (mmol/h)								
MDV†								
Arterial precursor	1·23	0·22	0·88	0·23	0·36	0·14	0·43	0·15
Venous precursor	1·83	0·24	1·21	0·32	0·64	0·26	0·69	0·21
PDV								
Arterial precursor	2·77	0·11	1·60	0·27	0·63	0·08	0·91	0·11
Venous precursor	3·45	0·17	1·89	0·32	0·85	0·11	1·19	0·11
MDV:PDV†								
Arterial precursor	0·48	0·08	0·47	0·09	0·50	0·09	0·42	0·07
Venous precursor	0·53	0·08	0·52	0·12	0·60	0·14	0·53	0·08
Fraction of WBPS								
MDV†	0·28	0·03	0·19	0·05	0·27	0·07	0·16	0·04
PDV	0·59	0·04	0·39	0·05	0·52	0·08	0·37	0·05

WBPS, whole-body protein synthesis, based on arterial enrichments.
* For details of procedures, see p. 620.
† Values based on three sheep only.

this oxidation can exceed that across the liver (Lobley *et al.* 1996*b*; Lapierre *et al.* 2002). Leucine oxidation appears to vary with absolute intake (Pell *et al.* 1986; Yu *et al.* 2000) as well as both protein (van der Schoor *et al.* 2001) and leucine supply (Lapierre *et al.* 2002). Furthermore, challenges that either increase (Yu *et al.* 2000) or reduce (MacRae *et al.* 1999) the metabolic demand on the GIT also alter the catabolism of leucine. This sensitivity means that the proportion of whole-body leucine oxidation attributable to GIT metabolism can vary between 0 and 50 % (Lobley *et al.* 1995, 1996*b*; van der Schoor *et al.* 2001; Lapierre *et al.* 2002). The current results show a mid-range (26 % of total oxidation), but this may be influenced by the dose of leucine infused (8 % of whole-body leucine flux; 20 % of apparent absorption across the MDV). The net result was that the oxidation by the GIT lowered net absorption of leucine by 25 %.

The contribution of the MDV (small intestine) to PDV (whole GIT) leucine oxidation was approximately 40 %, similar to the only other report in lambs (Yu *et al.* 2000). In that study, although both the MDV and non-MDV tissues (forestomach, large intestine, spleen and pancreas) were responsive to intake, the largest changes in leucine oxidation, induced by the presence of parasites in the upper small intestine, were across the MDV. By analogy, the sensitivity of leucine oxidation to protein intake in pigs (van der Schoor *et al.* 2001) probably occurs across the small intestine.

Although leucine has proved the most popular AA as a tracer for metabolic studies (due to lower cost and a simple two-step metabolic pathway to liberate the carboxyl-C as CO_2), it may not be a suitable marker for other AA. This is because the enzymes responsible for transamination and decarboxylation of the branched-chain AA are widely distributed across tissues, such as liver, muscle, fat, the mammary gland as well as the GIT (Goodwin *et al.* 1987; Papet *et al.* 1988; Bequette *et al.* 1996). In contrast, the catabolism of other essential AA is either restricted to the liver or a limited number of other tissues (Le Floc'h *et al.* 1997; Mabjeesh *et al.* 2000). Oxidative losses of these AA across the GIT, therefore, cannot be assumed just because leucine is catabolised. The reason why leucine oxidation is widespread between tissues is unclear, but may involve a role as a signal of nutrient supply (Lobley, 1998). Indeed, leucine has recently been shown to interact directly with the signal cascades that regulate protein metabolism (Kimball & Jefferson, 2002). In addition, the branched-chain AA act as N donors in transamination reactions (de Lange *et al.* 1992) and the resultant oxo-acids are sensitive to oxidation unless rapidly re-aminated (Cheng *et al.* 1985).

Of the other AA examined, labelled CO_2 release across the GIT was observed only for methionine, but this only represented 10 % of WBO, with a similar loss in terms of net absorption. As with leucine, methionine oxidation occurred across both the MDV and non-MDV. Again the question can be asked: 'what role would methionine catabolism play in the GIT?'. Methionine acts as an intermediate in methyl group transfers with the product, homocysteine, either being re-methylated to methionine (thus conserving the carboxyl-C) or converted to cysteine. The latter involves the trans-sulfuration pathway, with formation from the methionine-C of 2-aminobutyrate, which is then oxidised. In sheep, tissues with an active methionine–homocysteine cycle include not only the liver and kidney, but also the duodenal mucosa (Lobley *et al.* 1996*a*). The presence of this cycle is important for tissues based on proliferative cells with a high demand for synthesis of new membrane lipids and regulation of gene expression (Wajed *et al.* 2001). This would certainly include the GIT, where extensive cellular divisions occur (Attaix & Meslin, 1991). Indirect evidence suggests that cysteine may be synthesised within the human GIT (Stegink & den Besten, 1972) and, if so, methionine-C would be catabolised.

Intestinal catabolism of enteral-supplied [^{13}C]lysine accounted for 31 % of WBO in pigs fed high-protein diets (van Goudoever *et al.* 2000), but this declined to zero when a low-protein diet was offered. Interestingly, in both pigs (van Goudoever *et al.* 2000) and sheep (current study) no lysine oxidation occurred when the tracer was supplied parenterally. Thus, any GIT lysine metabolism may be restricted to cells that interface with the lumen or may arise from microbial action. In this regard, recent results indicate that isolated porcine enterocytes can oxidise lysine *in vitro* (Ball, 2002). Such site-specific catabolism within the GIT may be unique to lysine, however, because leucine can be oxidised from enteral and systemic sources in both sheep and pigs (MacRae *et al.* 1997*a*; van der Schoor *et al.* 2001), while in contrast, threonine is not degraded by the porcine GIT, regardless of whether supply is enteral or parenteral (Burrin *et al.* 2001).

Although the two methods to estimate whole-body phenylalanine oxidation gave different absolute values, as has also been observed in studies with well-fed human subjects (Sanchez *et al.* 1996), neither approach, i.e. $^{13}CO_2$ release nor change in the enrichment ratios for [1-^{13}C]tyrosine : [1-^{13}C]phenylalanine across the MDV or PDV, gave any indication of catabolism by the GIT. This contrasts with a recent study in pigs (Bush *et al.* 2003) where 34–43 % of phenylalanine was catabolised when the tracer was supplied enterally, although the authors were unable to distinguish between oxidation on 'first pass' and losses of the AA (or synthesised tyrosine) re-circulated to the GIT via the blood.

The results from sheep (current study) and pigs (van Goudoever *et al.* 2000; Burrin *et al.* 2001; van der Schoor *et al.* 2001; Bush *et al.* 2003) illustrate two major points. First, although the GIT catabolises some essential AA, this may not be a universal phenomenon and, even when it occurs, the magnitude may vary between AA. Therefore, care must be exercised before making general conclusions from studies based on the fate of a single AA. Second, catabolism may differ if the AA are supplied from the lumen rather than the systemic circulation. This is despite the fact that 80 % of the AA flux through GIT tissues is from plasma origin (MacRae *et al.* 1997*a*). In this context, the issues of how much AA are presented for absorption as free AA, as opposed to di- and tri-peptide forms, and the impact of metabolism by GIT bacteria need to be considered.

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Pattern of absorbed amino acids

Differential oxidation should lead to the pattern of absorbed AA being different from that disappearing from the small intestine. Indeed, early studies with sheep did report such a phenomenon (Tagari & Bergman, 1978) and findings in sheep (Wolff & Bergman, 1972), rodents (Windmueller & Spaeth, 1980) and pigs (Reeds *et al.* 2000; Reeds & Burrin, 2001) have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports for net losses of essential AA present a more confused picture, however. For example, in pigs the proportion of dietary methionine supply that appears in the portal vein varies with age (48–95 %), while threonine recovery remains low (38–52 %; Rerat *et al.* 1992; Stoll *et al.* 1998). Similarly, in sheep and cattle the recovery across the PDV of individual essential AA that disappeared from the small intestine varied between 43 and 95 % (from MacRae *et al.* 1997*b*; Berthiaume *et al.* 2001).

These comparisons of ruminant animals are confounded, however, by possible over-estimation of small intestine disappearance due to flow of endogenous protein at the duodenum (Siddons *et al.* 1985; Ouellet *et al.* 2002). Such inflows may arise from either post-splanchnic (saliva) sources or from the non-MDV portion of the PDV (rumen, abomasal secretions). The latter will distort the ratio of PDV appearance: small intestine disappearance (MacRae *et al.* 1997*b*; Berthiaume *et al.* 2001). Further complications arise from endogenous proteins secreted directly into the small intestine. These may arise from the pancreas, bile or the cells that line the lumen. Re-absorption of any of these sources occurs from the small intestine (into the MDV drainage), but those proteins of pancreatic origin will have derived their AA from the non-MDV vasculature of the PDV. Thus, a ‘double accounting’ occurs, with removal from the PDV (and lowered portal vein concentrations) and increased absorption across the MDV (and higher mesenteric vein concentrations). Bile secretions (from post-PDV sources) will augment both MDV and PDV appearance, but will alter the PDV:MDV ratio because the absolute increase adds to different net flows. Similarly, secretions from the epithelial cells of the small intestine (e.g. mucins, sucrase) will also perturb the MDV:PDV appearance ratios if not completely re-absorbed. These differences between site of synthesis and re-absorption of endogenous secretions have two consequences. First, the ratio of MDV : small intestine disappearance for essential AA can exceed unity, as observed in both sheep and cattle (0·74–1·32, MacRae *et al.* 1997*b*; Berthiaume *et al.* 2001), depending on the magnitude of secretions from inputs such as pancreas and bile. Indeed, theoretically this ratio should be larger than observed practically (Ouellet *et al.* 2002; G Zuur, F Yu, RL Coop, LA Bruce, GE Lobley and JC MacRae, unpublished results), indicating that other mechanisms leading to AA loss are also occurring. Second, the ratio of essential AA appearance between PDV: MDV should always be less than unity, regardless of whether AA oxidation occurs. This is indeed the case in both sheep (0·55–0·77; from Seal & Parker, 1996; MacRae *et al.* 1997*b*) and cattle (0·38–0·76; Berthiaume *et al.* 2001). In long-term infusions with [1-^{13}C]leucine, the impact of pre-jejunum secretions was predicted to decrease the PDV: MDV ratio for leucine by a minimum of 18–24 % (G Zuur, F Yu, RL Coop, LA Bruce, GE Lobley and JC MacRae, unpublished results). This would account for approximately half of the decrease observed in both sheep and cattle (0·61–0·69, Seal & Parker, 1996; MacRae *et al.* 1997*b*; Berthiaume *et al.* 2001). Much of the remainder would be accounted by non-MDV oxidation of leucine (Yu *et al.* 2000; current study).

The impact of endogenous secretions on both absolute and relative supply will vary between AA. For example, threonine has often been reported to have either low (Rerat *et al.* 1992; Stoll *et al.* 1998) or variable (see Lapierre & Lobley, 2001) recoveries during absorption. Usually, this is attributed to poor re-absorption of mucins, produced from goblet cells and rich in threonine, valine and proline (Mukkur *et al.* 1985; Lien *et al.* 1997). In the absence of threonine oxidation (sheep assumed to be the same as pigs; Burrin *et al.* 2001), then such secretions probably account for most of the 37 % difference between the MDV and the PDV appearances. Although much attention has focused on threonine losses, especially in non-ruminant animals (Burrin *et al.* 2001), the current data indicate that tryptophan showed the lowest PDV:MDV ratio. Few data are available for this AA because of analytical difficulties, but the results suggest that there are substantial requirements for tryptophan by the non-MDV sections of the PDV. Whether this relates to endogenous secretions or metabolic needs of tissues such as the rumen remains to be elucidated. It is also possible that the GIT as a whole may operate to regulate (or at least influence) total supply to the liver and beyond. Thus, if AA were subjected to low rates of catabolism across the small intestine, this may be ‘compensated’ by greater metabolic use in other parts of the GIT. In the absence of measurements of AA flow at the duodenum and small intestine disappearance it is not possible to take this argument further.

Overall, there is little doubt that, in combination, AA oxidation and the pattern of endogenous secretion and re-absorption across the GIT can alter both the absolute and relative amounts supplied to the liver and peripheral tissues. Whether these are sufficient to constrain productive performance remains unclear but the demands for threonine may leave little or none available to support growth (van Goudoever *et al.* 2000), while enhanced catabolism of AA under challenge situations may also slow protein gain (Yu *et al.* 2000).

Gastrointestinal protein synthesis and metabolite formation

The use of arterio–venous approaches to measure protein synthesis is complicated by the heterogeneity of the GIT tissues and selection of a plasma metabolite that best represents the enrichment of the intracellular precursor. Ideally, what is required is a metabolite produced within the cells from the precursor AA and then exported to the

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plasma. A popular candidate for this role is plasma MOP, the oxo-acid of leucine, and this is commonly used as a surrogate intracellular precursor during [1-^{13}C]leucine infusions to allow quantification of WBPS (Matthews *et al.* 1982). For this, it is assumed that outflows from the various tissues to the plasma MOP are in the same proportion as their relative rates of protein synthesis. From direct measurements, plasma MOP has been claimed to reflect enrichments of free leucine in a diverse range of tissues, including muscle (Watt *et al.* 1992; but see Chinkes *et al.* 1996), pancreas (Bennet *et al.* 1993) and liver (Barazzoni *et al.* 1999). Nonetheless, very little exchange of MOP between plasma and the splanchnic tissues occurs in either human subjects (Biolo & Tessari, 1997) or ruminant animals (Lobley *et al.* 1995; Lapierre *et al.* 1999???). This was confirmed in the current study where MOP enrichments were lowered by only 3 % in the mesenteric vein while leucine enrichments decreased by 30 %.

The situation is similar for homocysteine and 2-aminoadipate, intracellular products of methionine and lysine metabolism respectively. Plasma enrichments were unchanged across MDV and PDV despite a 22–39 % decrease in the respective AA enrichments. This may reflect either limited metabolite production within the GIT tissues and/or low rates of exchange with the plasma. So, although the arterial plasma enrichments of MOP, homocysteine and 2-aminoadipate were 0·85, 0·74 and 0·53 of leucine, methionine and lysine respectively, these probably reflect metabolism in tissues other than the GIT. As such, it is probably not appropriate to use these as precursors. Unfortunately, the other common option, based on AA enrichments in either the artery or vein, yielded a wide range of estimates for protein synthesis (e.g. MDV 47–76 g/d, PDV 88–133 g/d, based on arterial enrichments), although they are similar to values obtained by the large dose procedure for the total ovine GIT (70–101 g/d; Lobley *et al.* 1994).

Although the small intestine tissues probably comprised only 30 % of total GIT mass (Burrin *et al.* 1990), they have higher fractional synthesis rates (Lobley *et al.* 1994) and hence contributed 0·42–0·50 of protein synthesis in the complete digestive tract. These ratios are compatible with other values based on either direct isotope incorporation studies (0·41–0·50; Lobley *et al.* 1994), but lower than other arterio–venous measurements based on leucine kinetics (Yu *et al.* 2000). The contribution of the total GIT to WBPS was within the range of 0·45–0·65 reported based on a multi-tracer technique (MacRae *et al.* 1997*a*). This contribution was similar regardless of whether the arterial enrichment of the AA or the corresponding metabolite was adopted as precursor. Thus, the contribution of the GIT to protein dynamics is even greater than for energy metabolism (19–28 %; Burrin *et al.* 1989). Whether there is a penalty associated with this high protein turnover remains a matter of debate (MacRae *et al.* 1997*a*), but results in both the pig and now the sheep suggest that not all essential AA have an obligate oxidation across the GIT. Therefore, any losses that do occur are more probably associated with specific consequences of incomplete re-absorption of endogenous secretions and cellular desquamation, rather than being a general feature of high rates of protein turnover within intestinal tissues.

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