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 1-13C 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 13CO2, corrected for sequestration of [13C]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 O2 consumption (Burrin et al. 1989) and as much as 65 % to whole-body protein synthesis (WBPS; MacRae et al. 1997a). 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 desquamated 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. 1996b; 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 leucine?
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. 1997b; 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 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.

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**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–splanchnic 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 hind gut, 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 1-13C-labelled form of one of the AA. Rates of infusion were: L-[1-13C]leucine (99·1 atom %; Isotec Inc., Woburn, MA, USA) 0·70 mmol/h; L-[1-13C]methionine (98·7 atom %; Isotec Inc., Miamisburg, OH, USA) 0·23 mmol/h; L-[1-13C]phenylalanine (99 atom %; Mass Trace Inc.) 0·33 mmol/h + L-[2H4]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-[1-13C]lysine (99 atom %; Isotec Inc.) or L-[1-13C, 2H2]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 [1,13C]bicarbonate (0·30 mmol/h; 98 atom %; Isotesc Inc.).
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 CO2 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 17O2 that would yield 13C17O18O with a mass of 45; this would interfere with the quantification of 13CO2. Atom % excess values were calculated by difference from the natural abundance samples.

Blood haemoglobin, P02, Pco2, 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. NH3 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-13C]algae powder (99 atom %; Martek Biosciences Corp., Colombia, MD, USA), 200 nmol [5,15N]glutamine (99 atom %, Mass Trace Inc.), 100 nmol [indole-2,15N]tryptophan (99 atom %, Cambridge Isotope Laboratories) and 3.48 μmol [15N2]urea (99 atom %; Mass Trace Inc.). These samples were mixed and then frozen (−80°C) until analysis as the t-butylmethylislyl 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 sulfoalicylic 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-methylpentanate, MOP) and 2-aminobutyrate (2-oxobutyrate) extracted and converted to the quinoxanol t-butyldimethylsilyl derivative prior to GC–MS (Calder & Smith, 1988). The AA and other metabolites were then eluted with 2 ml 2 M NH4OH followed by 1 ml deionised water. From the combined eluate, 80 μl was removed for urea analysis after drying under N2 gas, while the remainder was freeze-dried. The urea, AA and amino-derivatives were converted to the t-butyldimethylsilyl 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 [1-13C]lysine and 300, 303 for [1-13C, 2H2]lysine; phenylalanine 336, 337; tyrosine 466, 467 (for [1-13C]tyrosine synthesised from [1-13C]phenylalanine), 470 for m+4 ions from [2H4]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:

\[ (M_m - M_p) \times PF_m \text{ or } p, \]

where M is metabolite (AA, urea, NH3 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 Ie represents enrichment (molar fraction) of AA or bicarbonate infused and I is the rate of infusion (mmol/min), while Ea is the mean enrichment of the metabolite (mol fraction) in arterial plasma. Outflow from plasma...
AA pool (tracee + tracer; mmol/h) was calculated as:

\[
(I_e/I_a) \times 1.
\]

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:

\[
(E_a \times \text{CO}_2 \times \text{WBFCO}_2)/(E_d \times 1),
\]

where \(E_a\), CO\(_2\), is the enrichment (atom % excess) of CO\(_2\) in arterial plasma, WBFCO\(_2\) is whole-body flux (production; mmol/h) of CO\(_2\) and \(E_d\) is the enrichment of the infusion.

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}\text{C}]\text{tyrosine}:[1 - ^{13}\text{C}]\text{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:

\[
\text{WBPS} \times 24 \times 0.1 \times \text{MM}_{aa}/(\% \text{ AA in protein}),
\]

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 \(^{13}\text{CO}_2\) appearance/min) was calculated as:

\[
\text{PF}_m \times p \times ((B_m \times p \times E_{\text{CO}_2}, m \times p) - (B_a \times E_{\text{CO}_2}, a)),
\]

where B is the relevant plasma bicarbonate concentration (mm). During the bicarbonate infusions, the appearance of total \(^{13}\text{CO}_2\) 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 digestate tract tissues. The sequestration (sq) was determined for each sheep from:

\[
((B_a \times E_{\text{CO}_2}, a) - (B_m \times p \times E_{\text{CO}_2}, m \times p))/(B_a \times E_{\text{CO}_2}, a).
\]

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:

\[
\text{AOAA} + (\text{sq} \times \text{arterial inflow} ^{13}\text{C} \text{bicarbonate})
\]

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:

\[
(TO_{\text{tracer}+\text{tracee}})/(WBO_{\text{tracer}+\text{tracee}}) = (TO_{\text{tracee}})/(WBO_{\text{tracee}}),
\]

so that:

\[
\text{TO} = WBO_{\text{tracee}} \times WBO_{\text{tracer}+\text{tracee}}/WBO_{\text{tracer}+\text{tracee}}.
\]

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

\[
\text{TPS} = \text{WBPS}_{\text{tracee}} \times \text{TPS}_{\text{tracer}+\text{tracee}}/\text{WBPS}_{\text{tracer}+\text{tracee}},
\]

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 NH3 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 NH3 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 NH3 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 NH3 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 NH3 (Sarraseca et al. 1998).

In terms of total N absorbed (i.e. NH3 + AA-N), the fraction from NH3 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 + NH3 – 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† |
|---|---|---|---|---|---|
| Non-essential AA | MDV | PDV | SED | Statistical significance of effect (ANOVA): P† | PDV:MDV‡ |
| Alanine | 4·062 | 3·198 | 0·219 | 0·002 | 0·823<sup>bc</sup> |
| Aspartate | 0·612 | 0·594 | 0·043 | NS | 1·014<sup>a</sup> |
| Glutamate | 1·572 | 1·302 | 0·069 | 0·003 | 0·839<sup>bc</sup> |
| Glutamine | 0·684 | 1·710 | 0·107 | <0·001 | 0·738<sup>bc</sup> |
| Glycine | 3·642 | 2·682 | 0·157 | <0·001 | 0·655<sup>a</sup> |
| Proline | 1·638 | 1·074 | 0·067 | <0·001 | 0·772<sup>bc</sup> |
| Serine | 2·442 | 1·872 | 0·089 | <0·001 | 0·767<sup>bc</sup> |
| Tyrosine | 1·560 | 1·158 | 0·082 | <0·001 | 0·0670 |
| Essential AA | | | | | |
| Histidine | 0·774 | 0·546 | 0·029 | <0·001 | 0·711<sup>b</sup> |
| Isoleucine | 2·130 | 1·290 | 0·088 | <0·001 | 0·610<sup>cd</sup> |
| Leucine | 3·450 | 1·902 | 0·180 | <0·001 | 0·559<sup>de</sup> |
| Lysine | 2·280 | 1·590 | 0·110 | <0·001 | 0·714<sup>e</sup> |
| Methionine | 0·732 | 0·480 | 0·046 | <0·001 | 0·679<sup>cd</sup> |
| Phenylalanine | 1·872 | 1·482 | 0·089 | 0·001 | 0·811<sup>a</sup> |
| Threonine | 2·154 | 1·344 | 0·097 | <0·001 | 0·629<sup>bcd</sup> |
| Tryptophan | 0·510 | 0·258 | 0·024 | <0·001 | 0·478<sup>d</sup> |
| Valine | 2·634 | 1·572 | 0·109 | <0·001 | 0·589<sup>d</sup> |
| 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<sup>§</sup> | 40·50 | 37·52 | 3·072 | NS | |

<sup>a,b,c,d,e</sup> Mean values within a column and subsection (i.e. non-essential and essential AA compared separately) with unlike superscript letters were significantly different (P<0·001).

<sup>‡</sup> For details of procedures, see p. 620.

<sup>†</sup> 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).

<sup>‡</sup> 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.

<sup>§</sup> Calculated as (AA-N + ammonia) – urea-N.
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 ($P<0.01$), except for aspartate, where appearances were not different, and glutamine, where net disappearance occurred across both the MDV and PDV, with greater removal ($P<0.001$) 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 ($P<0.001$) between the essential AA. A greater proportion of net uptake into the mesenteric vein appeared in the portal vein for phenylalanine (0.81, $P<0.01$ v. all other AA), while the lowest value was observed for tryptophan (0.48, $P<0.05$ for leucine, $P<0.01$ for valine, $P<0.001$ for the other essential AA). While histidine and lysine had similar PDV: MDV appearance ratios, these were higher ($P<0.01$) than observed for threonine, isoleucine, valine and leucine. Similarly, methionine and threonine ratios were greater ($P<0.01$) 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 ($P<0.05$ for glutamate, $P<0.01$ for the non-essential AA) than all the others. Proline had the lowest ratio (0.66) and this was lower ($P<0.05$) than that observed for alanine (0.79) and glutamate (0.83).

Isotope kinetics

Whole-body irreversible loss rate and oxidation. Whole-body CO₂ 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. 1996b; Lapierre et al. 2002), due to the larger amounts of isotope infused in order to be able to detect the differences in CO₂ isotopic enrichments across the intestinal tract more precisely. Estimates of phenylalanine oxidation based on hydroxylation to tyrosine were lower than those calculated from $^{13}$CO₂ production (0.23 v. 0.49 mmol/h, $P=0.013$). 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, $\text{SED} 21$), but with greater estimates ($P<0.05$) based on methionine compared with lysine.

Gastrointestinal metabolism

CO₂ transfers across the gastrointestinal tract. Natural abundance enrichments (atom %) of blood CO₂ differed between sample sites (1.09058, 1.09028, 1.09052 (SED 0.00003, 118 residual df, $P<0.001$) 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 $^{13}$CO₂ across the PDV, uncorrected for isotope sequestration, was positively different from zero ($P<0.001$) 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 $^{13}$CO₂ 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 ($P<0.001$), but only numerically so for methionine.

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**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†

<table>
<thead>
<tr>
<th>WB fluxes (mmol/h)</th>
<th>Leu</th>
<th>Lys</th>
<th>Met</th>
<th>Phe</th>
<th>(Tyr)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
<td>SEM</td>
<td>Mean</td>
</tr>
<tr>
<td>ILR†</td>
<td>7.76</td>
<td>0.10</td>
<td>4.92</td>
<td>0.27</td>
<td>1.99</td>
</tr>
<tr>
<td>CO₂ entry rate</td>
<td>978</td>
<td>7.1</td>
<td>4.21</td>
<td>0.27</td>
<td>1.99</td>
</tr>
<tr>
<td>WBO</td>
<td>1.93</td>
<td>0.14</td>
<td>0.61</td>
<td>0.10</td>
<td>0.50</td>
</tr>
<tr>
<td>WBO/ILR</td>
<td>0.25</td>
<td>0.02</td>
<td>0.12</td>
<td>0.02</td>
<td>0.25</td>
</tr>
<tr>
<td>ILRps‡</td>
<td>5.83</td>
<td>0.07</td>
<td>4.31</td>
<td>0.19</td>
<td>1.49</td>
</tr>
<tr>
<td>WBPS (g/d)†</td>
<td>270</td>
<td>244</td>
<td>310</td>
<td>284</td>
<td></td>
</tr>
</tbody>
</table>

* † For details of procedures, see p. 619.
‡ Phenylalanine infusion also included [4-¹⁳]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 ILRps 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).
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 80% for leucine and 33% for methionine. These represented 25% and 10% of WBO. These values can be converted into trace 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**

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†

<table>
<thead>
<tr>
<th>$[1-^{13}$C] infusate</th>
<th>$^{13}$CO$_2$ production (μmol/h)</th>
<th>Leucine</th>
<th>Lysine</th>
<th>Methionine</th>
<th>Phenylalanine</th>
<th>SED</th>
<th>Statistical significance of effect (ANOVA): $P_\text{‡}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncorrected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDV</td>
<td>9.7$^a$</td>
<td>−5.4$^b$</td>
<td>0.0$^{ab}$</td>
<td>−5.1$^b$</td>
<td>5.46</td>
<td>0.098</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDV</td>
<td>32.2$^{**}$</td>
<td>−4.7$^b$</td>
<td>−4.9$^b$</td>
<td>−8.4$^b$</td>
<td>3.69</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Corrected</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDV</td>
<td>19.6$^a$</td>
<td>−3.5$^b$</td>
<td>4.0$^c$</td>
<td>−1.5$^b$</td>
<td>6.48</td>
<td>0.040</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PDV</td>
<td>52.0$^{**}$</td>
<td>−0.0$^b$</td>
<td>7.6$^c$</td>
<td>−1.2$^b$</td>
<td>4.55</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Dose oxidised (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDV</td>
<td>2.9$^a$</td>
<td>−1.4$^c$</td>
<td>1.8$^b$</td>
<td>−0.5$^{bc}$</td>
<td>1.05</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td>PDV</td>
<td>7.6$^{**}$</td>
<td>−0.3$^b$</td>
<td>3.3$^c$</td>
<td>−0.4$^c$</td>
<td>0.89</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WB</td>
<td>30.9$^a$</td>
<td>17.1$^{1b}$</td>
<td>33.1$^a$</td>
<td>21.7$^c$</td>
<td>1.67</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>WB oxidation (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDV</td>
<td>9.5$^a$</td>
<td>−7.6$^c$</td>
<td>5.0$^{ab}$</td>
<td>−2.2$^{bc}$</td>
<td>3.92</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>PDV</td>
<td>24.8$^{**}$</td>
<td>−0.9$^c$</td>
<td>9.9$^b$</td>
<td>−1.9$^b$</td>
<td>3.58</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

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 blocking 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 80% for leucine and 33% for methionine. These represented 25% and 10% of WBO. These values can be converted into trace 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.
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 4. Isotopic enrichments (molar % excess) of primary and secondary metabolites in arterial (A), portal vein (PV) and mesenteric vein (MV) plasma following infusion of 1-13C-labelled amino acids into the hepatic vein* (Mean values for four sheep)

<table>
<thead>
<tr>
<th>Amino acid infused</th>
<th>A</th>
<th>MV</th>
<th>PV</th>
<th>SED</th>
<th>Statistical significance of effect (one-way ANOVA): P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1-13C]leucine</td>
<td>8.04±</td>
<td>5.65±</td>
<td>6.46±</td>
<td>0.297</td>
<td>0.001</td>
</tr>
<tr>
<td>[1-13C]MOP</td>
<td>6.79±</td>
<td>6.60±</td>
<td>6.86±</td>
<td>0.042</td>
<td>0.004</td>
</tr>
<tr>
<td>MOP:leucine</td>
<td>0.85±</td>
<td>1.20±</td>
<td>1.06±</td>
<td>0.054</td>
<td>0.003</td>
</tr>
<tr>
<td>Lysine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1-13C]lysine‡</td>
<td>5.41±</td>
<td>4.20±</td>
<td>4.57±</td>
<td>0.242</td>
<td>0.010</td>
</tr>
<tr>
<td>[1-13C]AAA‡</td>
<td>2.74±</td>
<td>2.87±</td>
<td>2.85±</td>
<td>0.113</td>
<td>NS</td>
</tr>
<tr>
<td>AAA:lysine</td>
<td>0.53±</td>
<td>0.74±</td>
<td>0.64±</td>
<td>0.052</td>
<td>0.026</td>
</tr>
<tr>
<td>Methionine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1-13C]Methionine</td>
<td>10.58±</td>
<td>6.45±</td>
<td>7.83±</td>
<td>0.185</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>[1-13C]HC</td>
<td>7.59±</td>
<td>7.60±</td>
<td>7.68±</td>
<td>0.055</td>
<td>NS</td>
</tr>
<tr>
<td>HC:methionine</td>
<td>0.74±</td>
<td>1.21±</td>
<td>1.01±</td>
<td>0.046</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>[1-13C]JAB</td>
<td>2.82±</td>
<td>2.61±</td>
<td>2.71±</td>
<td>0.217</td>
<td>NS</td>
</tr>
<tr>
<td>[1-13C]OB</td>
<td>1.53±</td>
<td>1.30±</td>
<td>1.71±</td>
<td>0.136</td>
<td>NS</td>
</tr>
<tr>
<td>OB:AAB</td>
<td>0.62±</td>
<td>0.52±</td>
<td>0.68±</td>
<td>0.116</td>
<td>NS</td>
</tr>
<tr>
<td>[1-13C]Homoserine</td>
<td>0.88±</td>
<td>0.89±</td>
<td>0.72±</td>
<td>0.121</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine + [4H4]Tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[1-13C]Phenylalanine</td>
<td>8.15±</td>
<td>5.14±</td>
<td>6.28±</td>
<td>0.218</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>[1-13C]Tyrosine‡</td>
<td>1.41±</td>
<td>0.90±</td>
<td>1.11±</td>
<td>0.043</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AAA:phenylalanine</td>
<td>0.17±</td>
<td>0.18±</td>
<td>0.18±</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>AAA:phenylalanine</td>
<td>2.82±</td>
<td>2.61±</td>
<td>2.71±</td>
<td>0.217</td>
<td>NS</td>
</tr>
</tbody>
</table>

MOP, 4-methyl-2-oxopentanoate; AAA, 2-aminoadipate; HC, homocysteine; AAB, 2-aminoisobutyrate; OB, 2-oxobutyrate.

*Mean values within a row with unlike superscript letters were significantly different (P<0.05).
†Values 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 [1-13C, 2H2].

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)

<table>
<thead>
<tr>
<th>Protein synthesis (mmol/h)</th>
<th>Leucine</th>
<th>Lysine</th>
<th>Methionine</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDV†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial precursor</td>
<td>1.23± 0.22</td>
<td>0.88± 0.23</td>
<td>0.36± 0.14</td>
<td>0.43± 0.15</td>
</tr>
<tr>
<td>Venous precursor</td>
<td>1.83± 0.24</td>
<td>1.21± 0.32</td>
<td>0.64± 0.26</td>
<td>0.69± 0.21</td>
</tr>
<tr>
<td>PDV</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial precursor</td>
<td>2.77± 0.11</td>
<td>1.60± 0.27</td>
<td>0.63± 0.08</td>
<td>0.91± 0.11</td>
</tr>
<tr>
<td>Venous precursor</td>
<td>3.45± 0.17</td>
<td>1.89± 0.32</td>
<td>0.85± 0.11</td>
<td>1.19± 0.11</td>
</tr>
<tr>
<td>MDV:PDV†</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arterial precursor</td>
<td>0.48± 0.08</td>
<td>0.47± 0.09</td>
<td>0.50± 0.09</td>
<td>0.42± 0.07</td>
</tr>
<tr>
<td>Venous precursor</td>
<td>0.53± 0.08</td>
<td>0.52± 0.12</td>
<td>0.60± 0.14</td>
<td>0.53± 0.08</td>
</tr>
<tr>
<td>Fraction of WBPS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDV†</td>
<td>0.28± 0.03</td>
<td>0.19± 0.05</td>
<td>0.27± 0.07</td>
<td>0.16± 0.04</td>
</tr>
<tr>
<td>PDV</td>
<td>0.59± 0.04</td>
<td>0.39± 0.05</td>
<td>0.52± 0.08</td>
<td>0.37± 0.05</td>
</tr>
</tbody>
</table>

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. 1996b; 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, 1996b; 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 GIT). 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 other only 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. 1996a). 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. 1997a; 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. 1997a). 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.
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 (Wolf & 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). Threonine has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine. Reports have shown that the GIT has specific, and substantial, demands for certain non-essential AA, including glutamate and glutamine.

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. 1997b; 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. mucus, 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. 1997b; 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. 1997b) and cattle (0·38–0·76; Berthiaume et al. 2001). In long-term infusions with [1,13C]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. 1997b; 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 mucus, 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 shows 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
Essential amino acid metabolism

plasma. A popular candidate for this role is plasma MOP, the o xo-acid of leucine, and this is commonly used as a surrogate intracellular precursor during [1-14C]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; Lapiere 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-amino-adipate, 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-amino-adipate 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. 1997a). 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. 1997a), 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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References


