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Effect of intake on whole body plasma amino acid kinetics in sheep

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Abstract — While both the quantity and quality of food ingested are potent regulators of whole body protein metabolism in ruminants, little data are available on responses across a wide range of intakes. The current study examined the responses in whole body protein flux (PrF) to such intake changes and compared these with the responses across the hind-quarters (in a companion study). Six growing sheep (6–8 months, 30–35 kg) received each of four intakes of dried grass pellets (0.5, 1.0, 1.5 and 2.5 times maintenance energy; M) for a minimum of 7 days. At each intake, a mixture of U-^{13}C amino-acids (AA) was infused intravenously for 10 h. Arterial plasma and blood were obtained over the last 4 h of infusion and the concentrations and the enrichments of thirteen ^{13}C labelled AA were determined. The absolute values for plasma Irreversible Loss Rate (ILR) but also converted PrF varied between the AA. PrF values were lower for histidine, methionine, aspartate, glycine and proline (range 68 to 174 g·d^{-1} at 1.5 M) than for isoleucine, leucine, valine and glutamate (range 275 to 400 g·d^{-1} at 1.5 M). These discrepancies may be explained by (1) the differential AA removal by the splanchnic tissues, (2) the de novo synthesis of the non-essential AA, (3) the transfer of AA from the erythrocytes or plasma to the tissues. The first two assumptions require further investigation whereas recent work has shown a minor role for AA transfers between erythrocytes and tissues. For most AA, ILR and PrF responded linearly to intake but curvilinear responses were observed for phenylalanine, lysine, leucine, isoleucine and tyrosine. These differences were not due to hind-quarter metabolism and may involve the digestive tract and liver.

intake / whole body / kinetics / ovine / amino acid

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1. INTRODUCTION

In ruminants, and other mammals, whole body and tissue amino acid (AA) fluxes are sensitive to many physiological and nutritional stimuli. While intake has been shown to be a potent regulator of such fluxes (e.g. Pell et al. [1]; Young et al. [2]; Harris et al. [3]) there is still controversy as to how different tissues might respond and the effect this has on whole body kinetics. This may be because of the technique and tracer AA used (e.g. Tessari et al. [4, 5]) or because tissues may respond differently to changes in the nutrient supply, or both.

Whole body protein metabolism in larger animals, including humans, is often estimated based on continuous infusion of a single labelled AA (usually an essential AA), during steady-state conditions [6, 7]. The advantages include limited surgical invasion, use of longitudinal studies and lower tracer costs compared with the large dose procedure [8, 9]. This technique is, however, subject to some limitations [10], especially when a single AA is monitored (such as leucine or phenylalanine [11–13]), which may, or may not, be distributed similarly between the different metabolic routes (protein synthesis, oxidation or synthesis of metabolites: [5, 9, 14]). To date, there have been relatively few comparisons between kinetic data obtained with a number of different AA (e.g. Lobley et al. [10]; MacRae et al. [15]) and even less that have investigated the responses across a wide range of intakes.

From various data, based on the kinetics of a limited number of amino acids (especially phenylalanine and leucine), it has been proposed [7, 16] that ruminant peripheral tissue protein metabolism responds in a curvilinear manner as intake is increased between fasting and supra-maintenance levels. This was then postulated to relate to muscle tissue responsiveness to the anabolic polypeptide hormones, insulin and insulin-like-growth factor-1, with differential responses in protein synthesis and degradation when intake is altered. Unfortunately, the individual experiments available from the literature for such an analysis only provide a limited number of intakes [16]. Furthermore, whether the same pattern of response occurs for other tissues (and thus whole body protein metabolism) has not been examined. Metabolic responses and utilisation of the AA by other tissues (notably the liver and the digestive tract) may differ with intake [10, 17, 18]. As a consequence, the influence of the tissues that may respond curvilinearly to intake (e.g. muscle) can be masked when whole body flux is measured, if more metabolically active tissues, such as the splanchnic tissues, do not respond similarly.

The current study quantified the effect of four intakes, between 0.5 to 2.5 times maintenance, on the whole body AA kinetics in the growing lamb. The kinetics were based on the infusion of a multiple U-^{13}C-labelled AA mixture that permits the simultaneous evaluation and comparison of the responses of 15 AA, both essential and non-essential, to intake. The contribution of hindquarter metabolism, based on data reported previously [19], to whole body responses as altered intake was also assessed. Finally, the absolute and relative changes associated with selection of either plasma or whole blood AA fluxes was also investigated.

2. MATERIALS AND METHODS

2.1. Animals and treatments

Six Suffolk cross wether lambs (age 6–8 months, 30–35 kg live weight) were surgically prepared with a polyvinyl or silicone rubber catheter into the aorta as previously described [19]. This catheter was used for arterial blood sampling. A temporary polyvinyl catheter was inserted into the jugular vein for infusion of labelled algal hydrolysate. If, for surgical reasons, either

of the aortal catheters lost patency they were replaced by either a temporary catheter into an auricular artery (14 infusions) or the right ventricle (6 infusions). Animals were offered grass pellets (10 MJ estimated metabolisable energy (ME)·kg^{-1} dry matter (DM); 26 g nitrogen·kg^{-1} DM) supplied daily as twenty-four equal portions each hour using automated feeders. The sheep were offered each of four levels of feed intake (corresponding to approximately 0.5, 1.0, 1.5 and 2.5 × energy maintenance (M), based on daily M requirements of 400 kJ/kg body weight$^{0.75}$) in a Latin Square order, with two repeated animal sequences. The Latin Square design involved pairing of the lower two intakes (0.5 and 1.0 E) and the higher two (1.5 and 2.5 E), with the order randomised within and between pairs. This option was selected to minimise any compensatory growth effects, or dietary upsets, consequent to moving between dietary extremes during adjacent measurement periods. Each level of intake was offered for 2 weeks prior to measurements, except for the 0.5 M ration that was fed for 1 week, but was preceded for 7 d with the M intake. Measurements of amino acid kinetics were made on the last day of each period and involved infusion of the U-^{13}C-labelled hydrolysate.

2.2. Infusions and blood sampling

The preparation of the [^{13}C]AA from Celtone-C (Martek Biosciences Corporation, Columbia, MD, USA) was as previously described for algal biomass [10, 20]. Briefly, the dried hydrolysate was diluted in 0.05 M sodium phosphate buffer 0.10 M sodium chloride (100 mL per 1.0 g original Celtone-C powder), filtered under aseptic conditions and 100 mL freeze-dried portions. Immediately prior to use (the day of the infusion) one of these portions was re-suspended in 400 mL sterile water and re-filtered (0.2 µ filter) under aseptic conditions. The AA composition of the hydrolysate was determined by ion-exchange chromatography (Alpha Plus Amino Acid Analyser; Pharmacia-LKB Biochrom Ltd, Cambridge), with L-norleucine as the external standard. The Celtone-C powder contained 300 µmol L-lysine per g at an enrichment of 99 mole % excess (mpe). The concentration of each infusate was determined individually.

The hydrolysate was continuously infused (40 mL infusate·h^{-1}) into the jugular vein over a 10 h period regardless of the level of intake. Hourly integrated blood samples were withdrawn continuously by a peristaltic pump from the aortal catheter (or that placed in an auricular artery or the heart) between hours 5 and 9 of infusion [20].

2.3. Laboratory analyses

Approximately, 7 mL of blood was centrifuged at 1000 *g* for 10 min. A further 1 g of plasma was stored in a micro-centrifuge tube at –20 °C for later preparation and enrichment analyses as the n-butyl heptafluorylbutyryl (HFB) derivatives by chemical ionisation gas chromatography-mass spectrometry (GCMS) as described previously [10], with the D and L proportions of lysine resolved by a chiral column [10]. To another 1 g of plasma was added 0.3 parts, by weight, of a diluted solution of the hydrolysate infusate for measurement of AA concentration by the isotope dilution technique using the t-butyldimethylsilyl derivatives and electron impact GCMS [21]. The blood samples were processed similarly to plasma samples, but involved the addition of an equal volume of ice-cold pure water to induce haemolysis of the samples. Samples of blood and plasma were obtained on the day prior to infusion to provide background (natural abundance) samples for analyses. Due to the preparation of the algal infusate by acid hydrolysis, data are not available for glutamine, asparagine and tryptophan.

2.4. Calculations

Whole body irreversible-loss rate (ILR; mmol·h^{-1}) for the AA was calculated from:

$$ILR = (99/mpe_{b(or\ p)} - 1) \times I,$$

Where b and p represent the enrichment (mpe) of free AA, in either blood or plasma respectively, and I is the rate of [L-^{13}C]AA infusion (mmol·h^{-1}) in the hydrolysate.

The ILR values were converted to equivalent daily protein flux (PrF; g·d^{-1}) by:

$$PrF = ILR \times 24 \times MW_{AA}/(\text{protein content}_{AA})$$

Where MW_{AA} is the molecular weight of AA (g·mol^{-1}) and $\text{protein content}_{AA}$ is the weight of the AA per 100 g ovine mixed protein deposited during growth (g·100 g^{-1} protein) (from MacRae et al. [22]).

AA kinetics across the hind-quarters were calculated as described previously [19], but with the enrichment of the AAs in the artery (rather than the vena cava) selected as the precursor pools. This allowed more direct comparison with the whole body flux data.

2.5. Statistics

Comparisons were made by ANOVA using Genstat 4.1 for Windows (Lawes Educational trust, Rothamstead, Herts). For the main effects, the animals were treated as blocks with period × intake as the treatment. In all cases, there was no effect of period and this was excluded. The data were also analysed for both linear and quadratic effects.

3. RESULTS

All sheep recovered well from the surgery and during the study consumed all the ration offered. The mean body weight changes (±SEM g·d^{-1}) during each 2-week period of feeding were the following: 0.5 M, –321 ± 60.5, 1.0 M, –24 ± 39.8, 1.5 M, +48 ± 57.3, 2.5 M +381 ± 79.6.

Haematocrit values were unaffected by intake (mean plasma proportion 69% ±0.9, data not shown). Kinetic data are presented as ILR (mmol·h^{-1}), but the equivalent whole body protein fluxes (PrF, g·d^{-1}) can be obtained from the conversion factors detailed in the table legends. The statistical effects obtained were identical for both ILR and the corresponding PrF. ILR calculated from plasma enrichments increased with intake ($P < 0.001$) for essential (Tab. I), non-essential (Tab. II) and branched chain (BCAA, Tab. III) AA. This increase was linear for all AA, but quadratic (curvilinear) responses were also observed for phenylalanine ($P = 0.008$, Tab. I) and leucine ($P = 0.049$, Tab. III), with trends for lysine ($P = 0.059$, Tab. I), isoleucine ($P = 0.072$, Tab. III) and tyrosine ($P = 0.091$, Tab. II). For these AA, whole body fluxes at 2.5 × M intake were much greater than the values obtained at 1.5, 1.0 and 0.5 M intakes. A similar pattern was observed for AA fluxes in blood, with intake-related curvilinear increases for phenylalanine ($P = 0.013$), lysine ($P = 0.041$) and leucine ($P = 0.012$), with a trend for proline ($P = 0.079$) (data not shown).

In absolute terms, plasma PrF values for essential AA at 0.5 M ranged between 101 g·d^{-1} for methionine to 190 g·d^{-1} for valine and, at 2.5 M, between 218 g·d^{-1} for methionine to 453 g·d^{-1} for isoleucine (Tabs. I and III). At any intake, the lowest PrF were observed for methionine, histidine, threonine and lysine while the highest values were for phenylalanine plus the BCAA. PrF based on blood enrichments were greater than the corresponding plasma values. These PrF values ranged between 147 g·d^{-1} for methionine to 245 g·d^{-1} for leucine at 0.5 EM and from 286 g·d^{-1} for methionine and 546 g·d^{-1} for phenylalanine at 2.5 EM (full data not shown).

For non-essential AA (Tab. II), the plasma PrF values were more variable than for essential AA, ranging from 45 g·d^{-1} for proline to 301 g·d^{-1} for glutamate at 0.5 M

Table I. Effect of feed intake (× energy maintenance, M) on whole body flux (ILR, mmol·h^{-1} and protein flux (PrF, g·d^{-1}) in six sheep using essential amino acids: phenylalanine, histidine, threonine, lysine and methionine as the tracer, in plasma. Contribution of the hindlimb protein synthesis to the overall protein flux in plasma (%)[a].

Amino acid	Feed intake				P values			SED
	0.5 M	1.0 M	1.5 M	2.5 M	Intake	Linear	Quad.	
Phenylalanine								
ILR plasma (mmol·h^{-1})	1.36	1.62	1.98	3.31	<0.001	<0.001	0.008	0.14
PrF plasma (g·d^{-1})	158	188	230	386	<0.001	<0.001	0.008	16.2
Hindlimb contribution plasma (%)[a]	23.0	19.7	17.7	14.8				
Histidine								
ILR plasma (mmol·h^{-1})	0.79	0.90	1.03	1.44	<0.001	<0.001	NS	0.09
PrF plasma (g·d^{-1})	134	151	174	242	<0.001	<0.001	NS	14.8
Hindlimb contribution plasma (%)[a]	26.1	23.6	22.1	21.7				
Threonine								
ILR plasma (mmol·h^{-1})	1.99	2.72	3.32	4.81	<0.001	<0.001	NS	0.28
PrF plasma (g·d^{-1})	139	189	231	335	<0.001	<0.001	NS	19.2
Hindlimb contribution plasma (%)[a]	21.4	18.9	19.2	20.7				
Lysine								
ILR plasma (mmol·h^{-1})	2.40	2.93	3.37	5.21	<0.001	<0.001	0.059	0.23
PrF plasma (g·d^{-1})	153	187	215	332	<0.001	<0.001	0.059	14.9
Hindlimb contribution plasma (%)[a]	16.5	15.9	17.7	16.8				
Methionine								
ILR plasma (mmol·h^{-1})	0.43	0.68	0.65	0.92	<0.001	<0.001	NS	0.06
PrF plasma (g·g^{-1})	101	162	155	218	<0.001	<0.001	NS	13.5
Hindlimb contribution plasma (%)[a]	19.1	10.5	17.5	16.3				

[a]Hindlimb protein synthesis was calculated using the arterio-venous technique as previously described by Hoskin et al. [19] .

Table II. Effect of feed intake (× energy maintenance, M) on whole body flux (ILR, mmol·h^{-1}) and protein flux (PrF, g·d^{-1}) in six sheep using non essential amino acids: alanine, glycine, serine, proline, aspartate, glutamate and tyrosine as the tracer, in plasma. Contribution of the hindlimb protein synthesis to the overall protein flux in plasma (%).

Amino acid	Feed intake				P values			SED
	0.5 M	1.0 M	1.5 M	2.5 M	Intake	Linear	Quad.	
Alanine								
ILR plasma (mmol·h^{-1})	4.60	5.49	6.76	9.16	<0.001	<0.001	NS	0.73
PrF plasma (g·d^{-1})	161	192	237	321	<0.001	<0.001	NS	25.7
Hindlimb contribution plasma (%)[a]	15.9	15.3	15.7	12.7				
Glycine								
ILR plasma (mmol·h^{-1})	5.38	6.40	6.95	9.42	<0.001	<0.001	NS	0.28
PrF plasma (g·d^{-1})	129	154	167	226	<0.001	<0.001	NS	6.8
Hindlimb contribution plasma (%)[a]	24.8	16.3	16.7	15.2				
Serine								
ILR plasma (mmol·h^{-1})	2.24	3.14	3.74	5.45	<0.001	<0.001	NS	0.36
PrF plasma (g·d^{-1})	138	193	230	335	<0.001	<0.001	NS	22.1
Hindlimb contribution plasma (%)[a]	19.3	17.4	17.8	17.0				
Proline								
ILR plasma (mmol·h^{-1})	0.97	1.18	1.48	2.33	<0.001	<0.001	NS	0.16
PrF plasma (g·d^{-1})	45	54	68	107	<0.001	<0.001	NS	7.4
Hindlimb contribution plasma (%)[a]	31.2	27.9	27.6	22.7				
Aspartate								
ILR plasma (mmol·h^{-1})	1.69	2.02	2.81	4.33	<0.001	<0.001	NS	0.37
PrF plasma (g·d^{-1})	76	91	127	196	<0.001	<0.001	NS	16.8

Table II. (*continued*).

Amino acid	Feed intake				P values			SED
	0.5 M	1.0 M	1.5 M	2.5 M	Intake	Linear	Quad.	
Hindlimb contribution plasma (%)[a]	nd	nd	nd	nd				
Glutamate								
ILR plasma ($mmol \cdot h^{-1}$)	9.23	10.75	12.26	16.00	<0.001	<0.001	NS	0.79
PrF plasma ($g \cdot d^{-1}$)	301	351	401	523	<0.001	<0.001	NS	25.8
Hindlimb contribution plasma (%)[a]	nd	nd	nd	nd				
Tyrosine								
ILR plasma ($mmol \cdot h^{-1}$)	0.99	1.32	1.57	2.65	<0.001	<0.001	0.094	0.14
PrF plasma ($g \cdot d^{-1}$)	158	213	253	426	<0.001	<0.001	0.094	23.2
Hindlimb contribution plasma (%)[a]	22.5	18.1	17.7	15.7				

[a] Hindlimb protein synthesis was calculated using the arterio-venous technique as previously described by Hoskin et al. [19].

Table III. Effect of feed intake (× energy maintenance, M) on whole body flux (ILR, mmol·h^{-1}) and protein flux (PrF, g·d^{-1}) in six sheep using branched chain amino acids: valine, isoleucine, leucine as the tracer, in plasma. Contribution of the hindlimb protein synthesis to the overall protein flux in plasma (%)[a].

Amino acid	Feed intake				P values			SED
	0.5 M	1.0 M	1.5 M	2.5 M	Intake	Linear	Quad.	
Valine								
ILR plasma (mmol·h^{-1})	2.84	3.51	4.42	6.54	<0.001	<0.001	NS	0.43
PrF plasma (g·d^{-1})	190	235	295	437	<0.001	<0.001	NS	28.9
Hindlimb contribution plasma (%)[a]	21.2	17.7	20.2	23.9				
Leucine								
ILR plasma (mmol·h^{-1})	3.23	3.84	4.70	7.52	<0.001	<0.001	0.049	0.38
PrF plasma (g·d^{-1})	164	195	238	381	<0.001	<0.001	0.049	19.5
Hindlimb contribution plasma (%)[a]	22.4	23.0	23.2	24.2				
Isoleucine								
ILR plasma (mmol·h^{-1})	1.51	1.92	2.36	3.89	<0.001	<0.001	0.074	0.20
PrF plasma (g·d^{-1})	176	224	275	453	<0.001	<0.001	0.074	23.8
Hindlimb contribution plasma (%)[a]	20.1	18.1	21.9	20.9				

[a] Hindlimb protein synthesis was calculated using the arterio-venous technique as previously described by Hoskin et al. [19].

and from 107 $g \cdot d^{-1}$ and 523 $g \cdot d^{-1}$ for the same AA at 2.5 M. Again blood PrF were greater than the corresponding values based on plasma. These PrF values ranged between 87 $g \cdot d^{-1}$ for proline to 738 $g \cdot d^{-1}$ for glutamate at 0.5 M and from 186 $g \cdot d^{-1}$ for proline to 1319 $g \cdot d^{-1}$ for glutamate at 2.5 M (full data not shown).

Based on the use of the arterial AA enrichments (either plasma or blood) as the common precursor, the hind-quarter contribution to the whole body protein flux was estimated. Within each AA and precursor pool, this contribution was unaltered by the intake. For data based on plasma enrichments and concentrations, at 0.5 M, the hind-quarter contribution to total protein flux ranged from 16% for lysine and alanine to 31% for proline; at 2.5 M, the values ranged from 12–16% for phenylalanine, alanine and lysine to 24% for valine and leucine.

4. DISCUSSION

The difference in the estimation of whole body protein flux, or protein synthesis in the muscle, using different amino acids as tracers has already been extensively studied using the flooding dose [6] or the continuous infusion [3–6, 10, 23] techniques. These studies concluded that the calculation of the protein fluxes could vary depending on the AA used as a tracer.

The current experiment covered a greater number of AA and a wider range of intakes than those reported previously and thus allowed a more extensive evaluation of any differences between the tracers. This also included comparisons between essential and non-essential AA, where only limited information is available (e.g. Biolo et al. [17]; Chung et al. [24]). The data at 1.5 M were similar to those from a comparable intake in an earlier study [10], but were lower for some non essential AA (plasma ILR = 10.34 $mmol \cdot h^{-1}$ vs. 6.95 $mmol \cdot h^{-1}$ for glycine, 17.12 $mmol \cdot h^{-1}$ vs. 12.26 $mmol \cdot h^{-1}$ for glutamate, 5.43 $mmol \cdot h^{-1}$ vs. 2.81 $mmol \cdot h^{-1}$ for aspartate). The differences for the dicarboxylic AA may relate to preparation procedures for the HFB-derivatives, where hydrolysis of plasma glutamine and asparagine can occur leading to lower enrichments and higher ILR.

When ILR were converted to PrF, there were disparities between the various AA. In agreement with previous observations at a single intake [10], the plasma PrF could be separated into 3 groups with histidine, methionine, aspartate, glycine and proline having the lowest values (ranging from 68.2 to 174.1 $g \cdot d^{-1}$ at 1.5 M); isoleucine, valine and glutamate having the highest values (ranging from 274.9 to 400.5 $g \cdot d^{-1}$ at 1.5 M) and leucine, lysine, phenylalanine, threonine, alanine, serine and tyrosine having intermediate values (ranging from 214.5 to 252.9 $g \cdot d^{-1}$ at 1.5 M). This hierarchy was maintained across all the intakes. The disparities between the PrF obtained for the different AA can be explained in different ways:

(a) Estimates of whole body flux are based on enrichments of systemic (arterial) AA and are influenced by the differential removal of absorbed AA by the splanchnic tissues. Both the gastrointestinal tract (GIT) and liver can modify the amount of AA available to peripheral tissues and circulation. The GIT does remove large quantities of glutamine and glutamate [18, 25–27]. Furthermore, between 30 and 80% of AA disappearing from the lumen do not appear in the portal vein [15, 27–29] and are used as energy substrates to support protein turnover within the tissues of the digestive tract. Although, the essential AA do not appear to be used preferentially by the GIT [15, 27] this is not the case for the liver where net removals may range from 10–100% [30, 31]. Hepatic metabolism therefore will alter both the amount and pattern of AA that enters the systemic circulation and is available to [32]. For example, the ovine liver removes small

amounts of absorbed BCAA [30, 31] but a large proportion of histidine and phenylalanine are removed to support the synthesis of export proteins [33]. For the peripheral tissues (especially muscle), the BCAA have a major role in the regulation of protein synthesis [34, 35] and hepatic bypass may act as an important signal of increased nutrient (protein) supply.

Further differences in tissue extractions relate to the site of amino acid catabolism, with the liver being the main site for several essential AA (e.g. methionine, histidine, phenylalanine) while others (e.g. the BCAA, lysine and threonine) can be metabolised by other tissues (e.g. Papet et al. [36]; LeFloc'h et al. [37]). The gut tissues are also capable of AA oxidation (leucine: [38]) and contain the enzymes responsible for the transamination and dehydrogenation of the BCAA [36]. The data concerning the catabolism of other essential AA in the digestive tract remain scarce (see Lobley [39]). The non essential AA are also catabolised across the PDV (alanine, glutamate and glutamine: [40]). Consequently, the relative contribution of the gut, the liver and the peripheral tissues to each AA oxidation is difficult to evaluate.

Together, these metabolic fates of AA across the splanchnic tissues dilute the inflow of AA into the peripheral circulation and alter the apparent systemic flux.

(b) While flows through the plasma (or blood) pools arise from the absorption plus the release from protein breakdown, in the case of the essential AA, there is also a potential input from the de novo synthesis for the non-essential AA. This will increase the apparent PrF. The extent of synthesis and inter-conversion varies with species as well as the nutritional and physiological state (e.g. Wolff et al. [18]; Chung et al. [24]). For sheep, although serine is converted to glycine [41], the converse does not occur ([42] and unpublished results). This will lead to higher PrF for serine compared with glycine. Under these circumstances glycine would function as a 'quasi' essential AA and yields PrF values similar to many of the essential AA, as observed in the current study.

(c) The transfer of AA from the erythrocytes or plasma to the individual tissues can vary and alter the apparent flux. Whether direct exchange occurs between erythrocytes and tissue is still a matter of a debate both in the liver [10, 37, 43, 44] and the hind-quarter [20]. However, recent work from Lobley et al. [31] provides support for the hypothesis that the erythrocytes probably play a minor role in the AA exchanges across the splanchnic tissues in sheep. Similarly, companion data from the current study indicated that the majority of lysine transfers (> 90%) across the hind-quarters is from plasma [20]. The minor role of erythrocytes in lysine metabolism appears to extend to the other AA because net uptakes were similar between plasma and blood (data not shown), while the hind-quarter contributions to the daily protein flux were lower when blood rather than plasma transfers were calculated. Any involvement of the erythrocytes will increase the apparent flux transfers due to the low enrichments of red blood cell AA [10, 20], as was reflected in the higher ILR when the results were expressed with whole blood rather than plasma.

Across the different intakes, the ILR (and PrF) increased regularly and in a linear manner for the majority of the AA. For some AA (phenylalanine, lysine, leucine, isoleucine and tyrosine), this increase followed a curvilinear pattern, but this effect was dominated by the greater proportional increase in the fluxes at 2.5 M compared with the differences obtained between 0.5, 1 or 1.5 M. Whole body protein metabolism is the result of the accumulation of the different metabolic events occurring in the tissues (> 85% from the GIT, liver, muscle, skin and bone [8]). The relative proportion of each tissue to overall protein metabolism could vary with the level of intake, due to

differences in AA disposal to the tissues and/or the sensitivity of the tissues to the anabolic or catabolic hormones. Muscle has been postulated to exhibit a curvilinear response to intake [16] and this would then account, in part, for the pattern of whole body protein flux. More detailed companion analysis [19] of muscle responses in the current animals did not show any curvilinear effect over the intake range for either protein synthesis or breakdown. Furthermore, for muscle-dominated effects on curvilinearity then the proportional contribution of hind-quarter metabolism to whole body AA flux might be expected to increase. In fact, the proportion remained fairly constant (or tended to decrease) even when the conditions changed from net catabolism to net anabolism [19]. Of course, such responses may be masked due to the changes in the ratio of enrichments for 'true' precursor: blood (or plasma) as intake varies, but this would have entailed the measurement of the aminoacyl tRNA which was out-of the scope of the current study of low enrichment multiple tracers.

Non-linear responses in whole body ILR may be due to the impact of other tissues, particularly since the contribution of the hind-quarters (≈ 20%) is less important than that of the splanchnic tissues, for example (30–60% [8, 45–47]). These are also sensitive to intake [12, 48, 49] and these values can increase by approximately 40% when intake is raised from 1 to 2 M [49]. Interestingly, these increases in absolute protein synthesis are associated with greater tissue mass, since the fractional rates of protein synthesis for the splanchnic tissues tend to be insensitive to intake [42, 50]. What regulates such splanchnic utilisation is not known, nor do we know how much is used to support anabolic compared with catabolic processes. This requires further investigation.

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REFERENCES

[1] Pell JM, Calderone EM, Bergman EN. Leucine and α-cetoisocaproate metabolism and interconversions in fed and fasted sheep. Metabolism 1986, 35: 1005–1016.

[2] Young VR, Gucalp C, Rand WM, Matthews DE, Bier DM. Leucine kinetics during three weeks at submaintenance-to-maintenance intakes of leucine in man: adaptation and accomodation. Hum Nutr Clin Nutr 1987, 41: 1–18.

[3] Harris PM, Skene PA, Buchan V, Milne E, Calder AG, Anderson SE, Connell A, Lobley GE. Effect of food intake on hind-limb and whole-body protein metabolism in young growing sheep: chronic studies based on arterio-venous techniques. Br J Nutr 1992, 68: 389–407.

[4] Tessari P, Barazzoni R, Zanetti M, Vettore M, Normand S, Bruttomesso D, Beaufrere B. Protein degradation and synthesis measured with multiple amino acid tracers in vivo. Am J Phys 1996, 271: E733–E741.

[5] Tessari P, Barazzoni R, Zanetti M. Differences in estimates of forearm protein synthesis between leucine and phenylalanine tracers following unbalanced amino acid infusion. Metabolism 1999, 48: 1564–1569.

[6] Obled C, Barre F, Millward DJ, Arnal M. Whole body protein synthesis: studies with different amino acids in the rat. Am J Phys 1989, 257: E639–E646.

[7] Lobley GE. Protein metabolism and turnover. In: Forbes JM, France J (Eds), Quantitative aspects of ruminant digestion and metabolism. CAB International, Wallingford, 1993. p 313–339.

[8] Attaix D, Aurousseau E, Bayle G, Rosolowska-Huszcz D, Arnal M. Contribution of liver, skin and skeletal muscle to whole body protein

synthesis in the young lamb. Br J Nutr 1988, 60: 77–84.

[9] Lobley GE, Harris PM, Skene PA, Brown D, Milne E, Calder AG, Anderson SE, Garlick PJ, Nevison I, Connell A. Responses in tissue protein synthesis to sub– and supra-maintenance intake in young growing sheep: comparison of large-dose and continuous-infusion techniques. Br J Nutr 1992, 68: 373–388.

[10] Lobley GE, Connel A, Revell DK, Bequette BJ, Brown DS, Calder AG. Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through use of a multiple U-^{13}C-labelled amino acid mixture. Br J Nutr 1996, 75: 217–235.

[11] Reeds PJ, Fuller MF, Cadenhead A, Lobley GE, McDonald JD. Effects of changes in the intakes of protein and non-protein energy on whole body protein turnover in pigs. Br J Nutr 1981, 45: 539–546.

[12] Lapierre H, Bernier JF, Dubreuil P, Reynolds CK, Farmer C, Ouellet DR, Lobley GE. The effect of intake on protein metabolism across splanchnic tissues in growing beef steers. Br J Nutr 1999, 81: 457–466.

[13] Liu SM, Lobley GE, MacLeod NA, Kyle DJ, Chen XB, Orskov ER. Effect of long-term protein excess or deficiency on whole-body protein turnover in sheep nourished by intragastric infusion of nutrients. Br J Nutr 1995, 73: 829–839.

[14] Obled C, Arnal M. Age-related changes in whole-body amino acid kinetics and protein turnover in rats. J Nutr 1991, 121: 1990–1998.

[15] MacRae JC, Bruce LA, Brown DS, Farningham DAH, Franklin M. Absorption of amino acids from the intestine and their net flux across the mesenteric– and portal-drained viscera of lambs. J Anim Sci 1997, 75: 3307–3314.

[16] Lobley GE. Nutritional and hormonal control of muscle and peripheral tissue metabolism in farm species. Livest Prod Sci 1998, 56: 91–114.

[17] Biolo G, Tessari P, Inchiostro S, Bruttomesso D, Fongher C, Sabadin L, Fratton MG, Valerio A, Tiengo A. Leucine and phenylalanine kinetics during mixed meal ingestion: a multiple tracer approach. Am J Phys 1992, 262: E455–E463.

[18] Wolff JE, Bergmann EN, Williams HH. Net metabolism of plasma amino acids by liver and portal-drained viscera of fed sheep. Am J Physiol 1972, 223: 438–446.

[19] Hoskin SO, Savary IC, Zuur G, Lobley GE. Effect of feed intake on ovine hind-limb protein metabolism based on 13 amino acids and arterio-venous techniques. Br J Nutr 2001, 86: 577–585.

[20] Savary IC, Hoskin SO, Dennisson N, Lobley GE. Lysine metabolism across the hindquarter of sheep; effect of intake on transfers from plasma and the red blood cells. Br J Nutr 2001, 85: 565–573.

[21] Calder AG, Garden KE, Anderson SE, Lobley GE. Quantification of blood and plasma amino acids using isotope dilution electron impact gas chromatography/mass spectrometry with U-^{13}C amino acids as internal standards. Rapid Commun Mass Spectrom 1999, 13: 2080–2083.

[22] MacRae JC, Walker A, Brown D, Lobley GE. Accretion of total protein and individual amino acids by organs and tissues of growing lambs and the ability of nitrogen balance techniques to quantitate protein retention. Anim Prod 1993, 57: 237–245.

[23] Barrett EJ, Revkin JH, Young LH, Zaret BL, Jacob R, Gelfand RA. An isotopic method for measurement of muscle protein synthesis and degradation in vivo. Biochem J 1987, 245: 223–228.

[24] Chung M, Teng C, Timmerman M, Battaglia FC. Production and utilization of amino acids by ovine placenta in vivo. Am J Phys 1998, 274: E13–E22.

[25] Souba WW. Glutamine a key substrate for the splanchnic bed. Ann Rev Nutr 1991, 11: 285–308.

[26] Stoll B, Henry PJ, Reeds PJ, Yu H, Jahoor F, Burrin DG. Catabolism dominates the first-pass intestinal metabolism of dietary essential amino acids in milk protein-fed piglets. J Nutr 1998, 128: 606–614.

[27] Berthiaume R, Dubreuil P, Stevenson M, McBride BW, Lapierre H. Intestinal disappearance and mesenteric and portal appearance of amino acids in dairy cows fed ruminally protected methionine. J Dairy Sci 2001, 84: 194–203.

[28] Tagari H, Bergman EN. Intestinal disappearance and portal blood appearance of amino acids in sheep. J Nutr 1978, 108: 790–803.

[29] Seal CJ, Parker DS. Effect of intraruminal propionic acid infusion on metabolism of mesenteric– and portal-drained viscera in growing steers fed a forage diet. II. Ammonia, urea, amino acids, and peptides. J Anim Sci 1996, 74: 245–256.

[30] Wray-Cahen D, Roberts S, Metcalf JA, Backwell FRC, Bequette BJ, Brown DS, Sutton JD, Lobley GE. Hepatic response to increased exogenous supply of plasma amino acids by infusion into the mesenteric vein of Holstein-Friesian cows in late gestation. Br J Nutr 1997, 78: 913–930.

[31] Lobley GE, Bremner DM, Brown DS. Response in hepatic removal of amino acids by the sheep to short-term infusions of varied amounts of an amino acid mixture into the mesenteric vein. Br J Nutr 2001, 85: 689–698.

[32] Lobley GE, Sinclair KD, Grant CM, Miller L, Mantle D, Calder AG, Warkup CC, Maltin CA. The effect of breed and level of nutrition on whole-body and muscle protein metabolism in pure-bred Aberdeen Angus and Charolais beef steers. Br J Nutr 2000, 84275–84284.

[33] Nieto R, Lobley GE. Integration of protein metabolism within the body and between organs. Proceedings of the VIIIth International Symposium on Protein Metabolism and Nutrition, 1999.

[34] Garlick PJ, Grant I. Amino acid infusion increases the sensitivity of muscle protein synthesis in vivo to insulin: effect of branched chain amino acids. Biochem J 1988, 254: 579–584.

[35] Wester TJ, Lobley GE, Birnie LM, Lomax MA. Insulin stimulates phenylalanine uptake across the hind limb in fed lambs. J Nutr 2000, 130: 608–611.

[36] Papet I, Grizard J, Bonin D, Arnal M. Regulation of branched chain amino acid metabolism in ruminants. Diabetes Metab 1992, 18: 122–128.

[37] Le Floc'h N, Mézière N, Sève B. Whole blood and plasma amino acid transfers across the portal drained viscera and liver of the pig. Reprod Nutr Dev 1999, 39: 433–442.

[38] Lapierre H, Blouin JP, Lobley GE, Reynolds CK, Dubreuil P, Bernier JF. Effect of protein degradability on protein splanchnic metabolism in dairy cows. Proc Nutr Soc 1997, 56: 162A.

[39] Lobley GE. Protein turnover – what does it mean for animal production? In: Amino acids: meat, milk and more!: CSAS – Symposium – SCSA: Quebec. 2002. p 1–15.

[40] Gate JJ, Parker DS, Lobley GE. The metabolic fate of the amino-N group of glutamine in the tissues of the gastrointestinal tract in 24 h-fasted sheep. Br J Nutr 1999, 81: 297–306.

[41] Liu SM, Mata G, Figliomeni S, Powell BC, Nesci A, Masters DG. Transsulfuration, protein synthesis rate and follicle mRNA in the skin of young Merino lambs in response to infusions of methionine and serine. Br J Nutr 2000, 83: 401–409.

[42] Connell A, Calder AG, Anderson SE, Lobley GE. Hepatic protein synthesis in sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. Br J Nutr 1997, 77: 255–271.

[43] Elwyn DH, Launder WJ, Parikh HC, Wise EM. Roles of plasma and erythrocytes in interorgan transport of amino acids in dogs. Am J Phys 1972, 222: 1333–1342.

[44] Houlier ML, Patureau-Mirand P, Durand D, Bauchart D, Lefaivre J, Bayle A. Transport des acides aminés dans l'aire splanchnique par le plasma sanguin et le sang chez le veau préruminant. Reprod Nutr Dev 1991, 31: 399–410.

[45] Lobley GE, Milne V, Lovie JM, Reeds PJ, Pennie K. Whole body and tissue protein synthesis in cattle. Br J Nutr 1980, 43: 491–502.

[46] Cherel Y, Attaix D, Rosolowska-Huszcz D, Belkhov R, Robin JP, Arnal M, Le Maho Y. Whole body and tissue protein synthesis during brief and prolonged fasting in the rat. Clin Sci 1991, 81: 611–620.

[47] Lapierre H, Bernier JF, Dubreuil P, Reynolds CK, Farmer C, Ouellet DR, Lobley GE. The effect of feed intake on splanchnic metabolism in growing beef steers. J Anim Sci 2000, 78: 1084–1099.

[48] McNurlan MA, Garlick P. Influence of nutrient intake on protein turnover. Diabetes Metab Rev 1989, 5: 165–189.

[49] Lobley GE, Connell A, Milne E, Newman AM, Ewing TA. Protein synthesis in splanchnic tissues of sheep offered two levels of intake. Br J Nutr 1994, 71: 3–12.

[50] Merry BJ, Holehan AM, Lewis SEM, Goldspink DF. The effects of ageing and chronic dietary restriction on in vivo hepatic protein synthesis in the rat. Mech Ageing Dev 1987, 39: 189–199.