Intestinal Protein Supply Alters Amino Acid, but Not Glucose, Metabolism by the Sheep Gastrointestinal Tract[1,2]

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ABSTRACT This study was intended to establish the extent which amino acids (AAs) and glucose are net metabolized by the gastrointestinal tract (GIT) of ruminant sheep when intestinal protein supply is varied. Wether sheep ($n = 4$, 33 ± 2.0 kg) were fitted with catheters for measurement of net absorption by the mesenteric (MDV) and portal-drained (PDV) viscera and a catheter inserted into the duodenum for casein infusions. Sheep received a fixed amount of a basal diet that provided adequate metabolizable energy (10.9 MJ/d) but inadequate metabolizable protein (75 g/d) to support 300-g gain per day. Four levels of casein infusion [0 (water), 35, 70, and 105 g/d], each infused for 5.5 d, were assigned to sheep according to a 4 × 4 Latin square design. [methyl-$^{2}H_3$]leucine was infused (8 h) into the duodenum while [1-^{13}C]leucine plus [6-$^{2}H_2$]glucose were infused (8 h) into a jugular vein. With the exception of glutamate and glutamine, net absorption of AAs increased linearly ($P < 0.05$, $R^2 = 0.46$–1.79 for MDV; $P < 0.05$, $R^2 = 0.6$–1.58 for PDV) with casein infusion rate. Net absorption by the PDV accounted for <100% of the additional supplies of leucine, valine, and isoleucine (0.6–0.66, $P < 0.05$) from casein infusion, whereas net absorption by the MDV accounted for 100% of the additional essential AA supply. Glucose absorption (negative) and utilization of arterial glucose supply by the GIT remained unchanged. There was a positive linear ($P < 0.05$) relation between transfer of plasma urea to the GIT and arterial urea concentration (MDV, $P < 0.05$, $r = 0.90$; PDV, $P < 0.05$, $r = 0.93$). The ruminant GIT appears to metabolize increasing amounts of the branched-chain AAs and certain nonessential AAs when the intestinal supply of protein is increased. J. Nutr. 136: 1261–1269, 2006.

KEY WORDS: • *sheep* • *gastrointestinal tract* • *amino acid* • *urea* • *glucose*

In ruminants, metabolism by the gastrointestinal tract (GIT)[4] represents the single largest metabolic fate of amino acids (AAs), glucose, and other energy substrates in the body. Depending upon the AA, the GIT metabolizes 5–100% of the AA supply disappearing from the small intestines, and 50–100% of intestinal disappearance of glucose (1–3). The nutritional importance of GIT metabolism is well known, yet there are still important aspects of GIT metabolism that remain largely unresolved. With regard to AA metabolism, it could be hypothesized that, at a minimum, net metabolism of essential AAs by the GIT occurs at a fixed rate and an amount relative to their compositions in the endogenous proteins lost from the GIT. Thus, once this "service cost" is satisfied, absorption of AAs across the GIT should approximate to 100% of their additional intestinal supplies. Current information suggests that this may not be the case, however, particularly with regard to certain nonessential AAs (4–9).

Net absorption of AAs by the small intestines of ruminants has been determined from the product of net arteriovenous difference and blood flow measurements across the mesenteric-drained viscera (MDV). Most often, however, net metabolism by the whole GIT is determined from measurements across the portal-drained viscera (PDV), which represents the sum of MDV plus rumen and hind-gut metabolism. This measure accounts for intestinal metabolism as well as that occurring by the rumen and hind-gut tissues, the latter of which derive AAs almost exclusively from the arterial blood supply (1). Although this arterial use of AAs by the PDV is considered a consequence of GIT metabolism, this arterial use of AAs represents potential competition with peripheral tissues (e.g., muscle, mammary gland). Based on PDV net flux measurements (dairy cows: (3,4,10); sheep: (1)) net recovery of infused casein, or

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[4] Abbreviations used: AA, amino acid; APE, atom percent excess; BW, body weight; GC-MS, gas chromatography-mass spectrometry; GIT, gastrointestinal tract; MDV, mesenteric-drained viscera; PAH, p-amino-hippuric acid; PDV, portal-drained viscera.

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free AAs, or absorbed essential AAs, is generally found to be lowest for the branched-chain AAs (BCAA: leucine, valine and isoleucine; 10–62%), followed variously by methionine (33–69%), histidine (39–92%), phenylalanine (36–96%), lysine (30–66%), tryptophan (31–88%) and threonine (41–63%). By contrast, intestinal supplies of the nonessential AAs glutamine and glutamate have been found to be almost completely metabolized by the GIT, even when the supplies of these AAs are increased (4–9). These studies examined PDV nutrient use in response to only one level of protein, AAs or feed intake, and so it has not been possible to establish response relations of intestinal supply and net absorption of individual AAs. In consequence, it is not known whether GIT metabolism occurs at a fixed or variable rate for individual AAs, and nor is it known whether this GIT metabolism ultimately leads to differential supplies of AAs to the liver, and beyond to peripheral tissues, for anabolic use.

The aim of this study is to answer 2 central questions regarding AA utilization by the ruminant GIT: *1*) are certain AAs preferentially utilized, and *2*) are certain AAs metabolized at fixed or variable rates relative to changes in their intestinal supply? A secondary aim relates to observations that the GIT of ruminants net metabolizes the intestinal supply of glucose (5,11). For this reason, the net balance of glucose across the GIT is often negative. In this respect, a goal was to determine whether glucose utilization is reduced or unaffected by changes in the intestinal supply and GIT utilization of AAs.

MATERIALS AND METHODS

Sheep and surgery. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland. Four mixed-breed (Polypay × Dorsett, 33 ± 2.0 kg body weight (BW), 5 to 6-mo-old) wether lambs were prepared under general anesthesia with vascular catheters (Silastic, 0.64 mm i.d., 1.19 mm o.d.) placed in the distal and proximal mesenteric vein, the hepatic portal vein, and a femoral artery. The tip of the proximal mesenteric vein blood sampling catheter was positioned ~5 cm from the ileo-cecal vein junction at the most proximal point where the venous drainage from proximal small intestine enters the mesenteric arcade (see 12). The tip of the distal mesenteric vein catheter was positioned ~30 cm upstream of the proximal catheter for infusion of the blood flow marker para-aminohippuric acid (PAH). A catheter (polyvinyl chloride, 2.4 mm i.d. × 4.0 mm o.d.) was also inserted into the proximal duodenum, the tip ~10 cm from the pylorus, for infusion of casein. From 2 wk postsurgery, feed (**Table 1**) was delivered in equal portions every 2 h via an automatic feeder. Sheep were fed to 2 times energy maintenance intake (10.9 MJ/d, ~80 g dry matter/kg $BW^{0.75}$) a pelleted ration that was adequate in energy (calculated 10.2 MJ metabolizable energy/kg dry matter) but which was low in protein (10.4% crude protein, dry matter) and provided only ~60% of the metabolizable protein intake (~75 g/d) required to support 300 g of gain per day (13).

Casein infusion. Sheep were placed in individual metabolic crates equipped with automatic feeders and fresh water was provided daily. Sheep were randomly assigned to 4 levels of casein infusion (0, 35, 70, and 105 g/d in 1.0 L water) according to a balanced 4 × 4 Latin square design. Rates of casein infusion were designed to increase metabolizable protein supply from 60 (control, 75g/d) to 110% (180 g/d, 105 g casein/d level) of that required to support 300 g/d gain (13). Each period lasted 10 d, with casein infused during the last 5.5 d. On day 1 and 3 of each 10-d experimental period, sheep were allowed to exercise in floor pens for at least 2 h/d. In addition, a 1-wk rest period (for exercise) in floor pens separated periods 2 and 3.

Tracer infusion and blood sampling. On day 4 of casein infusions, a temporary catheter (polyvinyl chloride, 0.8 mm i.d., 1.20 mm o.d.) was inserted (10 cm) into a jugular vein. On the last day, a sterile solution containing PAH (0.1 mol/L) and heparin (235 kIU/L) was infused (8 h, 20 mL/h) into the distal mesenteric vein catheter, and into the jugular vein a sterile solution containing [1-^{13}C]leucine (1.5 g/L) and [6-$^{2}H_2$]glucose (5 g/L) was infused (8 h, 20 mL/h). At the same time, [methyl-$^{2}H_3$]leucine (30 mg/h) was infused into the duodenum along with water or casein infusions. Over the final 4 h, blood samples were continuously withdrawn (5 mL/h) over 1-h periods from the artery, proximal mesenteric vein, and portal vein by peristaltic pump and collected into sealed syringes submerged in an ice bath. Each syringe was mixed by gentle hand rolling, and the plasma separated by centrifugation (1000 × *g* for 15 min at 4°C).

TABLE 1

Ingredients and nutrient composition of the experimental diet[1]

	Diet
	g/kg
Ingredient	
Alfalfa meal 17%	301
Beet pulp, dried	200
Wheat straw	467
Ammonium chloride	5
Vitamin mineral premix[2]	16
Sodium phosphate[3]	11
Nutrient composition	
Dry matter	911
Crude protein	95
Acid detergent fiber	358
Neutral detergent fiber	521
Starch	39
Crude fat	24
Total digestible nutrients	610
Net energy for maintenance, *MJ/kg*	5.44
Net energy for gain, *MJ/kg*	3.23

[1] As fed.
[2] Shepherd's Pride (Renaissance Nutrition), provided per kg premix: calcium, 220 g; salt, 160 g; sulfur, 31 g; phosphorus, 30 g; magnesium, 27 g; potassium, 24 g; iron, 1820 mg; zinc, 2700 mg; manganese, 240 mg; iodine, 40 mg; cobalt, 35 mg; selenium, 24 mg; vitamin A, 682,799 IU; vitamin D, 137,574 IU; and vitamin E, 1,774 IU.
[3] XP-4 (Astaris LLC), sodium acid pyrophosphate and monosodium phosphate anhydrous per kg of premix: phosphorus, 260 g; and sodium, 193 g.

Plasma amino acid and urea concentration. These were determined by isotope dilution with gas chromatography-mass spectrometry (GC-MS) as previously described (14). To a known weight (0.5 g) of fresh plasma was added an equal known weight of a solution containing 0.2 mg hydrolyzed [U-^{13}C]algae protein powder (99 atoms %; Martek Biosciences), 100 nmol [indole-$^{2}H_5$]tryptophan, 200 nmol [5-^{15}N]glutamine, 25 nmol [methyl-$^{2}H_3$]methionine, and 3 μmol [$^{15}N_2$]urea, and the samples stored frozen (−20°C). Thawed samples were deproteinized by addition (1 mL) of sulfosalicylic acid (15% w:v), the supernatant desalted by cation (AG-50, H^+ form) exchange, and AAs and urea eluted with 2 mol/L NH_4OH followed by water. For urea analysis, 20 μL of this eluate was dried under a stream of N_2, and urea converted to the *t*-butyldimethylsilyl derivative prior to GC-MS (HP 5973N Mass Selective Detector, Agilent). The remaining eluate was lyophilized to dryness, and AAs converted to their *t*-butyldimethylsilyl derivative. Under electron impact mode, the following ions (*m/z*) were monitored: urea 231, 233; alanine 260, 263; glycine 246, 248; valine 288, 293; isoleucine 302, 308; proline 286, 291; methionine 292, 295; serine 390, 393; threonine 404, 408; phenylalanine 234, 242; aspartate 302, 304; glutamate 432, 437; lysine 300, 306; histidine 440, 446; glutamine 168, 169; tyrosine 302, 304; and tryptophan 244, 249. For leucine isotope enrichment and concentration, ions at 302, 303 ([1-^{13}C]leucine), 305 ([$^{2}H_3$]leucine), and 308 ([$^{13}C_6$]leucine, internal standard) were monitored. Calibration curves were generated from gravimetric mixtures of labeled and unlabeled AA. For leucine, correction was also made for the spillover of [1-^{13}C]leucine (*m/z* 303)

into [$^2H_3$]leucine (m/z 305), and spillover of [$^2H_3$]leucine into [$^{13}C_6$]leucine (m/z 308). All enrichments were expressed as atoms percent excess (APE) relative to background natural abundance.

Glucose concentration and enrichment. To a known weight (0.150 g) of fresh plasma was added an equal known weight of a solution containing 0.6 mmol [$^{13}C_6$]glucose. The aldonitrile pentaacetate derivative of glucose was formed (15), and ions at 242, 244 ([$^2H_2$]glucose) and 247 ([$^{13}C_6$]glucose) monitored by GC-MS under electrical impact mode. This derivative of glucose loses carbon-1 under electron impact to yield m/z 247, which corresponds to the internal standard [$^{13}C_6$]glucose. Calibration curves were generated, and corrections made for spillover of m/z 244 ([$^2H_2$]glucose) to m/z 247 ([$^{13}C_6$]glucose). Enrichments are expressed as APE above background.

PAH concentration. Plasma concentrations of PAH were determined in duplicate using 0.25 g plasma and employing gravimetric procedures (16).

Calculation of net fluxes of amino acids. Net absorption or removal of an AA by the mesenteric (MDV; mostly small intestines) and portal (PDV; whole GIT) drained viscera was calculated as the product of plasma flow (F; [kg/(kg BW · h)]) and plasma venoarterial concentration difference (μmol/kg plasma) as appropriate. The incremental recovery by the MDV and PDV of individual AAs infused as casein into the duodenum was determined from the slope of the regression of net flux rate against duodenal casein–AA infusion rate [μmol/(kg BW · h]. The intestinal digestibility of casein was assumed to be 100% (17).

Calculation of leucine fluxes. Rate leucine of appearance (Leu R_a) was calculated from arterial plasma leucine enrichment (E_A) when the leucine tracer was infused into the jugular vein ([^{13}C]leucine; systemic Leu R_a) or into the duodenum ([$^2H_3$]leucine; whole body Leu R_a):

$$\text{Leu } R_a = [(E_I/E_A) - 1] \times IR,$$

where E_I is the APE of the leucine tracer infused ([$^2H_3$] or [1-^{13}C]), and IR is isotope infusion rate per kg BW per h. The difference between whole body Leu R_a and systemic Leu R_a is leucine removed by the gut and liver during its first pass. Fractional first-pass arterial utilization of leucine by the MDV or PDV was calculated from jugular infusion of [^{13}C]leucine:

$$\text{Fractional arterial utilization} = ([A] \times E_A - [V] \times E_V)/([A] \times E_A),$$

where [A] is arterial and [V] is MDV or PDV concentration of leucine as appropriate. First-pass arterial utilization by the MDV or PDV was calculated as:

$$\text{First-pass arterial utilization} = [A] \times F \times \text{fractional arterial utilization},$$

where F is plasma flow rate for the MDV or PDV as appropriate. Fractional first-pass intestinal utilization of leucine was computed from [$^2H_3$]leucine tracer balance across the PDV, corrected for [$^2H_3$]leucine, recycled, and sequestered on second-pass by the PDV from the arterial circulation (i.e., [^{13}C]leucine arterial removal):

$$\text{Fractional first-pass intestinal utilization} = (([V] \times E_V) - ([A] \times E_A) + ([A] \times E_A \times \text{fractional arterial utilization} \times F))/IR,$$

where IR is the rate of [$^2H_3$]leucine infusion into the duodenum.

Calculation of glucose utilization. Net removal of plasma glucose by the MDV and PDV was calculated as above for AAs. Glucose rate of appearance (gluconeogenesis, glucose recycling, and absorption) was calculated as described for Leu R_a. First-pass arterial utilization of glucose by the MDV and PDV was calculated as:

$$\text{First-pass arterial glucose utilization} = (([A] \times E_A - [V] \times E_V)/([A] \times E_A)) \times [A] \times F,$$

where E is the enrichment (APE) of [$^2H_2$]glucose in the artery (A) and vein (V; MDV or PDV).

Statistical analysis. For all data, ANOVA assumptions were checked prior to analysis. Data were analyzed by 3-way ANOVA for a 4×4 Latin square design using the MIXED procedure of SAS (version 8.0, SAS Institute), in which the infusion level was the fixed effect and sheep and experimental period are random effects. The following linear mixed model was used:

$$Y_{ijk} = \mu + T_i + R_j + C_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observed value for the k^{th} sheep, the j^{th} period and the i^{th} treatment, μ is the grand mean, T_i is the treatment effect for the i^{th} treatment, R_j is the period effect for the j^{th} period, C_k is the sheep effect for the k^{th} sheep, and ε_{ijk} is the random error associated with Y_{ijk}. When a significant treatment effect was detected, means were compared using Tukey-Kramer multiple comparison test. Backward stepwise regression was performed in which a third-order model was tried first, and, if not significant, the analysis was repeated with a lower-order model until significance was reached. The 95% CI were calculated for the slopes of the regression equation. Significance of differences between first-pass utilization and net absorption was assessed by Student's t test. Data are presented as least square means ± SEM and differences were considered significant at $P \leq 0.05$.

RESULTS

Sheep. Sheep consumed their feed allowances with no refusals throughout the experiment and over the period of experimentation (55 d) sheep gained ~75 g/d. The MDV measurements for one sheep were omitted due to catheter tip misalignment noted postmortem, and for another sheep the PDV data were omitted for the last 2 treatment periods (35 and 105 g casein/d) due to PDV catheter blockage.

Plasma concentrations. Casein infusion significantly increased ($P < 0.05$) arterial concentrations (**Table 2**) of the BCAA, methionine, phenylalanine, and proline, whereas glycine concentration decreased ($P < 0.05$). Plasma urea also increased ($P < 0.001$) with level of casein infusion, with values doubling between the control and the 105 g/d casein infusion level.

MDV net fluxes. Casein infusion ($P < 0.05$) increased the net appearance in the MDV of all the essential AA (**Table 3**), except for tryptophan. Among the nonessential AAs, the net

TABLE 2

Plasma arterial concentrations of amino acids and urea in sheep infused with increments of casein into the duodenum[1]

	Casein infusion, *g/d*					
	0	35	70	105	SEM	*P*-value
			μmol/kg			
Essential AAs						
Valine	204^c	296^b	370^b	450^a	18.6	0.0001
Leucine	153^c	209^b	244^{ab}	281^a	13.0	0.0007
Isoleucine	89^b	118^a	134^a	139^a	6.4	0.0002
Methionine	24^b	33^{ab}	38^{ab}	40^a	3.6	0.0431
Threonine	95	134	140	109	21.2	NS
Phenylalanine	48^b	57^b	61^{ab}	72^a	3.5	0.0044
Lysine	155	202	194	175	18.3	NS
Histidine	56	75	71	69	6.7	NS
Tryptophan	40	55	59	90	19.0	NS
Nonessential AAs						
Alanine	169	170	144	131	13.4	NS
Glycine	533^a	427^b	361^{bc}	313^c	37.2	0.0003
Proline	84^c	136^b	142^b	192^a	8.6	0.0001
Serine	55	62	61	54	11.2	NS
Aspartate	16	20	17	17	2.4	NS
Glutamate	120	133	109	118	6.4	NS
Glutamine	275	284	259	277	20.2	NS
Urea, *mmol/kg*	6.35^c	8.52^b	10.36^b	13.52^a	0.604	0.0001

[1] Values are least-square treatment means ± SEM, $n = 16$. Concentrations are μmol/kg plasma; casein infused in increments of 0, 35, 70, and 105 g/d. Means in a row without a common superscript letter differ, $P < 0.05$. NS, not significant, $P > 0.05$.

TABLE 3

Plasma flow and net absorption of amino acids across the MDV of sheep infused with increments of casein into the duodenum[1]

	Casein infusion, *g/d*					
	0	35	70	105	SEM	*P*-value
Plasma flow, *kg/(kg BW · h)*	1.082	1.116	1.373	1.567	0.2330	NS
Net absorption, *μmol/(kg BW · h)*						
Essential AAs						
Valine	43^{b}	45^{b}	95^{a}	87^{a}	11.4	0.0044
Leucine	51^{b}	61^{b}	129^{a}	116^{a}	10.5	0.0008
Isoleucine	35^{b}	39^{b}	74^{a}	70^{a}	7.0	0.0031
Methionine	13^{c}	15^{bc}	30^{ab}	32^{a}	3.3	0.0078
Threonine	35^{b}	42^{ab}	70^{a}	67^{ab}	9.0	0.0249
Phenylalanine	30^{b}	33^{b}	64^{a}	61^{a}	6.4	0.0051
Lysine	48^{b}	52^{b}	101^{a}	97^{a}	10.1	0.0043
Histidine	14^{b}	14^{b}	23^{ab}	34^{a}	5.8	0.0158
Tryptophan	7	2	25	33	6.9	NS
Nonessential AAs						
Alanine	69^{b}	77^{ab}	129^{a}	125^{ab}	12.8	0.0220
Glycine	61	66	88	83	11.1	NS
Proline	31^{b}	46^{b}	102^{a}	105^{a}	7.7	0.0005
Serine	55	55	95	94	13.9	NS
Aspartate	3	9	11	14	3.4	NS
Glutamate	20	24	40	38	9.7	NS
Glutamine	−3	−4	45	25	24.1	NS
Urea	−175	−204	−373	−361	68.7	NS

[1] Values are least-square treatment means ± SEM, $n = 16$. Positive values denote net release (absorption into blood) and negative values denote net removal from blood. Means in a row without a common superscript letter differ, $P < 0.05$. NS, not significant, $P > 0.05$.

appearances of only alanine ($P < 0.05$) and proline ($P < 0.001$) increased with casein infusion level. The incremental efficiency of absorption (Δ net absorption ÷ Δ casein–AA infusion) was computed based on the slope of regressing net AA absorption against the casein–AA infusion rate for that AA (see **Fig. 1**). The 95% CI were generated to test for unity, i.e., a slope of 1 = 100% recovery of the infused AA. Net MDV absorption (**Table 4**) of all essential and nonessential AAs, except for glutamate and glutamine, was linearly ($P < 0.05$) related to their casein–AA infusion rates. The incremental efficiency of absorption across the MDV fell within a narrow range (0.87–1.05) for all essential AAs, excluding tryptophan. For the nonessential AAs, values ranged from 0.46 for aspartate to 1.79 for alanine. Because of the wide 95% CI associated with each AA measurement, however, none of the slopes were significantly different from unity for the MDV (small intestines). Thus, recovery of AA across the MDV was not different from 100%.

PDV net fluxes. Net PDV absorption (**Table 5**) of leucine, isoleucine, methionine, phenylalanine, alanine, proline, and aspartate increased ($P < 0.05$) in a linear relation with their casein–AA infusion rates. Based on the regression slopes, the incremental efficiency of absorption (**Table 6**) across the PDV ranged from 0.60 for valine and isoleucine to 0.93 for phenylalanine, with a high of 1.58 for alanine. For the BCAA, the upper confidence limits were <1 ($P < 0.05$), indicating that <100% of the additional supplies of these AAs were recovered in blood by the PDV.

For the MDV and PDV, net removal of urea from plasma was not affected by casein infusion although, numerically, the values were higher. The correlation of urea removal with plasma urea concentration was significant for the MDV ($P = 0.0379$, $r = 0.90$) and PDV ($P = 0.0124$, $r = 0.93$).

Leucine metabolism. Rate of casein infusion increased ($P < 0.01$) systemic and whole body leucine R_a (**Table 7**). As a proportion of whole body leucine R_a, the MDV accounted for 0.16 (± 0.059), whereas casein infusion increased the proportion (0.33–0.60 ± 0.060, $P < 0.033$) of whole body leucine R_a partition to the PDV. Although net absorption of leucine

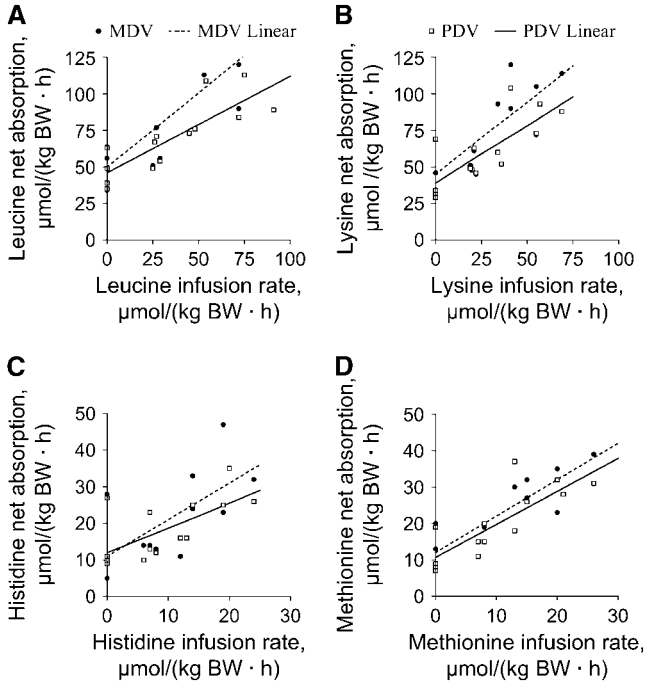

FIGURE 1 Plots of MDV ($n = 12$) and PDV ($n = 14$) net absorption rates of leucine (*A*), lysine (*B*), histidine (*C*) and methionine (*D*) against their rates of infusion as casein into the duodenum of sheep (see Tables 5 and 6 for linear regression analysis).

TABLE 4

Linear mixed-effect model predictions of the intercept, slope, and CI describing the relation between net MDV absorption of amino acids and the rate of casein–amino acid infusion into the duodenum of sheep[1]

	Intercept, *a*	SE_a	Slope, *b*	SE_b	*P*-value[2]	R^2	95% CI for *b*
Essential AAs							
Valine	39	10.7	0.87	0.223	0.0045	0.69	(0.55, 1.21)
Leucine	50	11.4	1.01	0.233	0.0014	0.65	(0.73, 1.28)
Isoleucine	33	6.3	0.88	0.191	0.0017	0.71	(0.43, 1.30)
Methionine	12	2.7	1.00	0.198	0.0005	0.72	(−0.02, 1.94)
Threonine	36	8.1	0.89	0.239	0.0073	0.73	(0.36, 1.44)
Phenylalanine	29	6.1	1.05	0.267	0.0041	0.65	(0.41, 1.66)
Lysine	45	9.5	0.99	0.236	0.0029	0.68	(0.62, 1.35)
Histidine	11	5.6	1.00	0.231	0.0024	0.81	(−0.10, 2.01)
Tryptophan	1	5.7	4.74	1.352	0.0056	0.55	(1.39, 7.84)
Nonessential AAs							
Alanine	66	10.7	1.79	0.449	0.0025	0.56	(0.91, 2.63)
Glycine	62	10.2	0.96	0.391	0.0388	0.26	(−0.26, 2.23)
Proline	29	8.1	0.77	0.121	0.0001	0.78	(0.46, 1.07)
Serine	51	12.1	1.06	0.397	0.0280	0.42	(0.30, 1.80)
Aspartate	4	3.2	0.46	0.098	0.0051	0.29	(−0.94, 1.86)
Glutamate	20	7.5	0.23	0.131	NS	0.21	(−0.14, 0.58)
Glutamine	−2	19.8	0.47	0.418	NS	0.18	(0.02, 0.86)

[1] Values were derived from the model: $\hat{y} = a + bx$, where x is the casein–amino acid infusion rate in μmol/(kg BW · h), *a* is the intercept, and *b* is the slope of the regression ($n = 12$). SE_a, standard error of intercept *a* estimate; SE_b, standard error of slope *b* estimate; NS, not significant, $P > 0.05$.

[2] Probability that the slope estimate $\neq 0$.

by the PDV increased with casein infusion, there was also increased ($P < 0.05$) first-pass arterial utilization of leucine by the PDV. For the MDV, net absorption of leucine increased with casein infusion, but there was no change in the rate of first-pass arterial utilization. Fractional first-pass intestinal utilization was computed from PDV, rather than MDV, flux measurements to avoid introduction of errors due to site of luminal tracer infusion and location of the downstream MDV sampling catheter. Fractional first-pass intestinal utilization of leucine was higher (Student's *t* test, $P < 0.05$) than fractional first-pass arterial utilization, but neither was altered by casein infusion.

Glucose metabolism. Plasma glucose concentration increased ($P < 0.05$) with the casein infusion rate (**Table 8**), whereas plasma glucose R_a remained unchanged. The relation of glucose R_a and casein infusion rate was linear ($P < 0.05, R^2 = 0.76$), with daily glucose R_a increasing by 0.188 g glucose/g casein infused. Intestinal casein infusion did not alter the amount and proportion of whole body glucose R_a partitioned to the PDV. The PDV accounted for ~0.38 of whole body glucose R_a, whereas the MDV accounted for a smaller but increasing (0.11–0.24) proportion in response to casein infusion. Because of the higher rates of first-pass arterial utilization of plasma glucose, the net balances of glucose across the PDV and MDV were negative (i.e., arterial utilization > net absorption) and these were not significantly affected by casein infusion. With first-pass arterial utilization of glucose by the PDV (Student's *t* test, $P < 0.001$) and MDV (Student's *t* test, $P < 0.0011$), both exceeded net glucose removal, indicating that glucose was absorbed from the small intestines only to be removed from the blood on second pass.

DISCUSSION

Despite the numerous studies in the literature, there is a lack of information in ruminants and other farm species regarding the patterns of AA utilized by the GITs, and whether this pattern of use fluctuates or remains unchanged with intestinal protein supply. In part, the availability of such information has been limited by analytical and technical constraints of existing methodologies. Therefore, to address the questions we posed, the methods we used for measuring blood flow and substrate concentrations needed to be sufficiently accurate and sensitive for detection of biologically and statistically significant changes in the flux of AAs, urea, and glucose by the GIT. Herein, gravimetric (i.e., weighing) procedures and isotope dilution with GC-MS were used to quantify plasma AAs, urea, and glucose concentrations. Based on the isotope-dilution technique, the coefficient of determination was 0.56–3.93% for individual AAs, 0.12% for urea, and 0.33% for glucose. By contrast, conventional AA analyzers may reach a precision of only 1–4% (18), and this variance is further propagated when measurements of both arterial and venous concentrations are combined for detecting often small (2–15 μmol/L) arteriovenous differences across tissue beds (19), such as in the present study.

It was also important that our measurements of GIT metabolism were made over a wide range of intestinal protein supplies, spanning from marginal to above the estimated metabolizable protein requirements for maintenance and growth of sheep with this body weight. This was achieved by infusing casein into the duodenum at 4 levels (0, 35, 70, and 105 g/d). The highest level of protein supply was predicted to raise total protein supply (diet + casein infusion) to 110% of the metabolizable protein supply required to support gain of 300 g/d for sheep of this size (13). Furthermore, to acquire a more representative estimate of the efficiency of AA absorption, net fluxes for each AA were plotted against their respective casein–AA infusion rates (Fig. 1 and Tables 5 and 6). Regression slopes were analogous to the net efficiency of absorption above the basal level of protein intake in this study. CI (95%) were then computed from the slope relationships, and tests against unity (i.e., 100%) were performed for each AA.

TABLE 5

Plasma flow and net absorption of amino acids across the PDV of sheep infused with increments of casein into the duodenum[1]

	Casein infusion, *g/d*					
	0	35	70	105	SEM	*P*-value
Plasma flow, *kg/(kg BW · h)*	2.795	2.515	2.488	3.055	0.3065	NS
Net absorption, *μmol/(kg BW · h)*						
Essential AAs						
Valine	32	39	58	69	9.7	NS
Leucine	46^{c}	60^{bc}	86^{ab}	95^{a}	7.8	0.0043
Isoleucine	27^{c}	35^{bc}	49^{ab}	56^{a}	4.4	0.0035
Methionine	11^{c}	15^{bc}	27^{ab}	30^{a}	3.1	0.0043
Threonine	25	32	43	56	7.5	NS
Phenylalanine	31^{b}	38^{b}	51^{ab}	62^{a}	5.3	0.0113
Lysine	41	52	72	85	9.7	NS
Histidine	14	14	19	29	3.5	NS
Tryptophan	7	1	9	11	4.2	NS
Nonessential AAs						
Alanine	61^{c}	80^{bc}	101^{ab}	119^{a}	7.8	0.0020
Glycine	39	56	47	75	9.7	NS
Proline	24^{c}	42^{bc}	77^{ab}	93^{a}	9.1	0.0010
Serine	61	60	87	81	22.9	NS
Aspartate	−2^{b}	4^{ab}	9^{ab}	22^{a}	7.1	0.0380
Glutamate	6	21	34	23	12.5	NS
Glutamine	−6	10	73	13	44.1	NS
Urea	−587	−625	−671	−1072	124.7	NS

[1] Values are least-square treatment means ± SEM, $n = 14$. Positive values denote net release (absorption into blood) and negative values denote net removal from blood. Means in a row without a common superscript letter differ, $P < 0.05$. NS, not significant, $P > 0.05$.

We highlight 3 observations from the slopes of the regression analysis. First, the net absorption data for each essential AA best fit a linear relation ($P < 0.005$; Fig. 1 and Tables 5 and 6) in response to casein infusion. We had hypothesized that, because the level of metabolizable protein intake (~75 g/d) of the basal ration was low relative to growth requirements, net AA requirements of the GIT may not have been met and, in consequence, net absorption would not increase until perhaps

TABLE 6

Linear mixed-effect model predictions of the intercept, slope and CI describing the relation between net PDV absorption of amino acids and the rate of casein–amino acid infusion into the duodenum of sheep[1]

	Intercept, *a*	SE_a	Slope, *b*	SE_b	*P*-value[2]	R^2	95% CI for *b*
Essential AAs							
Valine	30	7.4	0.60	0.159	0.0044	0.66	(0.37, 0.82)
Leucine	46	5.7	0.66	0.126	0.0002	0.69	(0.48, 0.86)
Isoleucine	27	3.6	0.60	0.109	0.0004	0.76	(0.31, 0.91)
Methionine	11	2.5	0.91	0.197	0.0010	0.66	(0.29, 1.64)
Threonine	24	6.1	0.75	0.186	0.0029	0.71	(0.36, 1.10)
Phenylalanine	29	4.0	0.93	0.175	0.0004	0.77	(0.55, 1.40)
Lysine	39	6.7	0.78	0.188	0.0021	0.62	(0.55, 1.05)
Histidine	12	2.7	0.67	0.225	0.0109	0.43	(0.02, 1.46)
Tryptophan	4	3.8	0.77	0.701	NS	0.56	(−1.26, 3.16)
Nonessential AAs							
Alanine	60	5.2	1.58	0.223	<0.0001	0.82	(0.26, 2.94)
Glycine	39	7.7	1.20	0.484	0.0347	0.45	(−0.72, 3.17)
Proline	23	6.8	0.67	0.108	<0.0001	0.76	(0.20, 1.15)
Serine	58	18.4	0.61	0.532	NS	0.55	(−0.51, 1.82)
Aspartate	−3	7.2	0.95	0.161	0.0011	0.95	(−1.10, 3.27)
Glutamate	10	9.2	0.24	0.172	NS	0.14	(−0.31, 0.80)
Glutamine	−1	35.8	0.58	0.648	NS	0.43	(−0.17, 1.14)

[1] Values were derived from the model: $\hat{y} = a + bx$, where x is the casein–amino acid infusion rate in μmol/(kg BW · h), *a* is the intercept, and *b* is the slope of the regression ($n = 12$). SE_a, standard error of intercept *a* estimate; SE_b, standard error of slope *b* estimate; NS, not significant, $P > 0.05$.

[2] Probability that the slope estimate $\neq 0$.

TABLE 7

Whole body and gastrointestinal tract fluxes of leucine in sheep infused with increments of casein into the duodenum[1]

	Casein infusion, *g/d*					
	0	35	70	105	SEM	*P*-value
Leucine R_a						
Systemic R_a (A)	163[c]	187[bc]	216[ab]	244[a]	14.9	0.0075
Whole body R_a (B)	236[b]	251[b]	286[ab]	318[a]	19.6	0.0111
First-pass splanchnic utilization (B – A)	73	64	64	79	11.5	NS
Fractional first-pass splanchnic utilization ((B – A)/B)	0.297	0.263	0.246	0.239	0.0287	NS
PDV metabolism						
Net absorption	46[c]	60[bc]	86[ab]	95[a]	7.8	0.0043
First-pass arterial utilization	53[b]	62[b]	70[b]	146[a]	7.7	0.0001
Proportion of whole body R_a	0.330[b]	0.340[b]	0.330[ab]	0.598[a]	0.0600	0.0330
Fractional first-pass arterial utilization	0.13	0.12	0.11	0.16	0.023	NS
Fractional first-pass intestinal utilization	0.32	0.32	0.20	0.27	0.060	NS
MDV metabolism						
Net absorption	51[b]	61[b]	129[a]	116[a]	10.5	0.0008
First-pass arterial utilization	24	30	36	36	11.1	NS
Proportion of whole body R_a	0.15	0.17	0.17	0.15	0.059	NS
Fractional first-pass arterial utilization	0.16	0.13	0.10	0.10	0.028	NS

[1] Values are in units of μmol/(kg BW · h) unless otherwise indicated. Values are least-square treatment means ± SEM ($n = 16$), MDV ($n = 12$), and PDV ($n = 14$) fluxes. Positive values denote net release (absorption into blood) and negative values denote net removal from blood. Means in a row without a common superscript letter differ, $P < 0.05$. R_a, rate of appearance; NS, not significant, $P > 0.05$.

the 70 g casein infusion level. Even with the limited observations in this study (see Fig. 1), there appears to be no indication of an inflection in net absorption, but this will need to be further confirmed at lower protein intakes and with a larger number of observations.

Second, the 95% CI of the slopes indicated that essential AA absorption by the MDV (small intestines) was effectively 100% (i.e., slope = 1) whereas absorption of the BCAA by the PDV was significantly <100% (i.e., slope <1). Also approaching significance were the slopes for threonine (slope 0.75, 95% CI 0.36–1.10) and lysine (slope 0.78, 95% CI 0.55–1.05) for the PDV. We interpret these data as a demonstration that the small intestines (MDV) were in net balance with respect to essential AA utilization, irrespective of the rate of intestinal protein supplies above the maintenance requirement. The net efficiency of absorption of the BCAA by the PDV was <100% (slope ranges: 0.60–0.66, 95% CI 0.31–0.91) across the protein infusion levels with, in consequence, greater net metabolism (e.g., lost as protein into the GIT or catabolized) of the BCAA as intestinal protein supply increased. In fact, for leucine, as representative of the BCAA, the majority of the increased use by the GIT occurred from the arterial blood supply (Table 7), mainly by the non-MDV tissues (i.e., rumen and hind gut). MacRae et al. (1,2) made similar observations in sheep (~35 kg

TABLE 8

Whole body and gastrointestinal tract metabolism of glucose in sheep infused with increments of casein into the duodenum[1]

	Casein infusion, *g/d*					
	0	35	70	105	SEM	*P*-value
Arterial concentration, *μmol/kg*	4.249[b]	4.283[b]	4.540[a]	4.567[a]	0.1086	0.0103
Plasma R_a	690	730	783	814	49.5	NS
PDV metabolism						
Net absorption	−110	−111	−128	−216	49.8	NS
First-pass arterial utilization	255	247	302	322	76.0	NS
Proportion of plasma R_a	0.37	0.35	0.41	0.38	0.101	NS
Fractional arterial utilization	0.02	0.02	0.03	0.02	0.007	NS
MDV metabolism						
Net absorption	−25	−66	−37	−70	23.303	NS
First-pass arterial utilization	83	143	187	119	31.495	NS
Proportion of plasma R_a	0.11[b]	0.21[ab]	0.24[a]	0.14[ab]	0.041	0.022
Fractional arterial utilization	0.02	0.03	0.03	0.02	0.005	NS

[1] Values are in units of μmol/(kg BW · h) unless otherwise indicated. Values are least-square means ± SEM ($n = 16$), MDV ($n = 12$), and PDV ($n = 14$) fluxes. Positive values denote net release (absorption into blood) and negative values denote net removal from blood. Means in a row without a common superscript letter differ, $P < 0.05$. R_a, rate of appearance; NS, not significant, $P > 0.05$.

BW) that were fed alfalfa pellets (15.6% crude protein) at 2 levels of intake (maintenance vs. 1.5 × maintenance). In that report, recoveries of the essential AAs in the MDV was ~100% (mean 106%, range 74–131%), whereas recoveries by the PDV were significantly less (mean 69%, range 45–96%) than the disappearance of the AAs from the small intestines (duodenum to ileum).

Third, for both glutamate and glutamine, net absorption by the MDV and PDV were far less than their intestinal supplies and net absorption was not increased by casein infusion. We find it of interest that the BCAA, glutamate, and glutamine were utilized by the whole GIT in greater amounts as the supply of protein to the small intestines increased. The primary fates of AAs utilized by the GIT are catabolism and incorporation into constitutive and secretory proteins (6,17). Except for the threonine-rich mucoproteins (20), the AA composition of GIT protein is not likely to fluctuate. In this respect, we consider it unlikely that the higher removals of the BCAAs, glutamate, and glutamine by the GIT relates to their increased incorporation into GIT proteins (and endogenous losses). If the additional removal of these AAs had been directed toward GIT protein synthesis, we should have also observed a corresponding increase in the net removal of other essential AAs, which we did not observe. The more likely explanation is that these AAs were catabolized either by luminal microbes or by gut tissues either from the luminal, arterial, or both aspects of the GIT.

The BCAA have been shown to be oxidized by the GIT of ruminants (19) and considerable evidence indicates extensive catabolism of glutamate and glutamine by the GIT of ruminants and monogastric species (4–9). For both leucine and glutamate, oxidation by the GIT fluctuates in direct relation to intestinal protein supply (dairy cattle: (21); piglets: (8)). To date, it remains unresolved whether AA catabolism occurs directly by the GIT tissues or by microbes in the intestinal lumen. The present data indicate nearly complete metabolism of glutamate and glutamine by the MDV tissue (small intestines), but it was not possible to determine the extent of luminal vs. arterial use. However, based on leucine kinetic data, most of the increased use of leucine by the GIT occurred from the arterial supply in response to casein infusion. Therefore, we suggest that the GIT tissues, but not luminal microbes, were responsible for metabolism of the BCAA.

Numerous studies in rats (see 9) and piglets ((6) and (22)), under conditions of adequate food intake, have shown that AAs are the major substrates (50–70%) contributing to oxidative energy generation by the intestinal mucosa. By contrast, glucose contributes relatively less (30–40%) (23). Similar information is not complete for ruminant animals and, to date, investigations in vivo have been limited to glutamate and glutamine (24), leucine (25,26) and lysine, methionine, and phenylalanine (19). Given that the GIT of the sheep appears to metabolize greater amounts of the BCAA, glutamate, and glutamine as protein supply is increased, it was logical to consider whether metabolism of other nutrients would be reciprocally reduced, namely, glucose. The majority of glucose supplied to the ruminant GIT is from the arterial blood, and so it is commonly observed that the net balance of glucose across the MDV and PDV is negative. There is, of course, some glucose available to the small intestinal lumen from dietary starch escaping the rumen and from bacterial polysaccharides (27). It is apparent from the comparison of unidirectional and net removals of glucose (Table 8) that glucose was presented to the small intestinal lumen and absorbed into the MDV and PDV blood drainages (i.e., unidirectional > net removal).

Net removal and first-pass arterial utilization of glucose by the GIT remained constant across the levels of casein infusion, suggesting that glucose use by the PDV was not spared by the additional protein supply. This is perhaps not surprising for 2 reasons. First, Van der Schoor et al. (8) observed that glucose oxidation by the PDV of piglets remained the same at 2 levels of protein intake (9.6 vs. 21.6 g/(kg · d)). And, second, catabolism of BCAA carbon skeletons occurs mainly via acetyl-CoA, and for these AAs to spare glucose from complete catabolism necessitates that glucose is also metabolized via this route. In a study conducted with isolated sheep rumen epithelial and small intestinal mucosal cells (28), we observed that glucose contributed to only 1–5% of acetyl-CoA flux, despite the fact that glucose made a significant contribution to pyruvate flux (8–38%). Therefore, it would not be expected that increased metabolism of the BCAA by the GIT would lead to sparing glucose from complete catabolism. In contrast, we have shown that sheep rumen and intestinal cells partially catabolize glucose to pyruvate, and thence to alanine (28), and that net production of alanine by intestinal cells is increased in the presence of glucose (29). In this connection, the high and increased net release of alanine by the PDV with casein infusion may reflect greater partition of glucose toward alanine synthesis and release.

In summary, AA and glucose metabolism by the MDV and PDV was examined in growing sheep in response to incremental increases in protein supply to the small intestines that spanned the range of metabolizable protein supplies from marginal to well above requirements for maintenance and growth. The efficiency of absorption above maintenance of all the essential AAs, except the BCAA, was effectively 100%, and this remained fixed at levels of protein supply above maintenance. By contrast, the efficiency of absorption the BCAA, glutamate, and glutamine were <100%, and for glutamate and glutamine high rates of net metabolized by the GIT were maintained even at upper levels of intestinal protein supply. Despite the excess metabolism of these AAs, glucose utilization by the GIT was not altered, and therefore not spared by AA supply. The basis for the greater metabolism of the BCAA, glutamate, and glutamine with increased intestinal protein supply, remains uncertain. In practical terms, the nearly complete catabolism of these nonessential AAs, and to some extent aspartate, by the GIT necessitates that they be synthesized de novo from carbon skeletons and nitrogen that likely derive from absorbed essential AAs.

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