Role of Membrane Transport in Interorgan Amino Acid Flow Between Muscle and Small Intestine

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In the fasting state, amino acids are released from the periphery to be used in splanchnic tissues. To understand the mechanism of such interorgan substrate exchange at the tissue level, we have determined the relationships between inward and outward amino acid transport and intracellular amino acid kinetics in the small intestine and skeletal muscle of postabsorptive anesthetized dogs. In the gut, amino acids appearing intracellularly (from inward transport, protein degradation, and absorption from the lumen) were used for protein synthesis more efficiently ($P < .05$) than in muscle (phenylalanine, 55% ± 5% *v* 13% ± 3%; lysine, 70% ± 7% *v* 28% ± 3%). In contrast, in muscle, amino acids appearing intracellularly (from inward transport and protein degradation) were preferentially ($P < .05$) released into the bloodstream, as opposed to being incorporated into protein (phenylalanine, 87% ± 4%; lysine, 72% ± 3%). Inward transport accounted for a greater ($P < .05$) proportion of total intracellular amino acid appearance in the gut than in muscle (leucine, 63% ± 3% *v* 37 ± 3%; valine, 75% ± 5% *v* 53% ± 3%; phenylalanine, 66% ± 1% *v* 50% ± 4%; lysine, 52% ± 2% *v* 31% ± 2%). We conclude that differences in transmembrane amino acid transport kinetics in both the inward and outward directions contribute to the net flow of amino acids from the muscle to the gut in the fasting state.

IN THE SMALL INTESTINE, the rate of protein synthesis is rapid[1] to maintain cell proliferation and digestive enzyme production. In the jejunal mucosa of both fed and starved rats, the fractional protein synthesis rate was greater than 100% per day,[2] whereas in skeletal muscle it was approximately 5% per day.[3] An adequate availability of free amino acids in small intestine is needed in the intracellular space to maintain such a rapid rate of protein synthesis. Precursors for protein synthesis can derive directly from intracellular proteolysis or from the extracellular space via transmembrane transport systems.[4,5] Small intestine cells have membrane amino acid transport systems at both the blood-facing and luminal sides.[6-10] However, the small intestine relies mostly on amino acid supply from the bloodstream in the fasting state. Thus, the active release of essential amino acids from skeletal muscle[11-14] represents a fundamental homeostatic mechanism during fasting that enables the maintenance of gut protein synthesis.

The present study was undertaken to evaluate in the gut and skeletal muscle the role of transmembrane amino acid transport in the regulation of net amino acid flow from the periphery to splanchnic tissues in the postabsorptive state. We have determined in the gut and muscle the rates of inward and outward amino acid transport and protein synthesis in relation to rates of total intracellular amino acid appearance. The study was conducted using a three-pool model of regional amino acid and protein metabolism that we recently developed in leg muscle, but which is applicable to any body region.[15] This model involves systemic continuous infusion of labeled amino acids and steady-state measurement of amino acid enrichments and concentrations in arterial and venous plasma and in the free tissue water, obtained by biopsy.

MATERIALS AND METHODS

Isotopes

L-[1-^{13}C]leucine (99% enriched), L-[1,2-$^{13}C_2$]leucine (99% enriched), and L-[$^{2}H_8$]valine (97.5% enriched) were purchased from Merck (Montreal, Canada): L-[α-^{15}N]lysine (99% enriched), L-[1,2-$^{13}C_2$,6,6-$^{2}H_2$]lysine (98% enriched), and L-[1-^{13}C]phenylalanine (99% enriched) were purchased from Tracer Technologies (Somerville, MA). L[1-^{13}C]valine (99% enriched), L-[ring-$^{2}H_5$]phenylalanine (98% enriched), and L-[2,3,3,3-$^{2}H_4$]alanine (98% enriched) were purchased from Cambridge Isotope Laboratories (Woburn, MA).

Experimental Design

The experimental protocol was approved by the Institutional Animal Care and Use Committee of The University of Texas Medical Branch. Four healthy adult mongrel dogs (25 ± 2 kg) of either sex maintained on a regular dog chow diet were studied in the postabsorptive state. The dogs were anesthetized with 30 mg · kg^{-1} sodium pentobarbital. Additional doses of pentobarbital (1 mg · kg^{-1} · h^{-1}) were administered throughout the experiment to maintain a constant level of anesthesia. A Swan-Ganz catheter was placed through a venotomy on the right external jugular vein for measurement of cardiac output. Polyethylene catheters were inserted into the left jugular vein for isotope infusions and into the left carotid artery for blood sampling. A small branch of the superior mesenteric vein was cannulated through a median incision for blood sampling. A femoral vein was also cannulated for the purpose of sampling the leg arteriovenous difference in order to develop a model across the leg muscle tissue. Results from these data have already been presented.[15] Venous catheters were kept patent with an infusion of 0.9% saline.

Before starting the tracer infusion, two blood samples (at −10 and 0 minutes) were drawn from the carotid artery to measure plasma background isotope enrichments. After background samples were obtained, the following priming doses were administered as a bolus injection over approximately 1 minute: 15.0 ± 1.5 μmol/kg L-[1-^{13}C]leucine, 15.0 ± 1.5 L[1-^{13}C]valine, 4.4 ± 0.4 L-[ring-$^{2}H_5$]phenylalanine, and 22.0 ± 2.0 L-[α-^{15}N]lysine. Immediately after the priming doses, the tracers were infused continuously for 6

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hours. Isotope infusion rates were 0.25 ± 0.03 μmol · kg^{-1} · min^{-1} L-[1-^{13}C]leucine, 0.25 ± 0.03 L[1-^{13}C]valine, 0.11 ± 0.01 L-[ring-$^{2}H_5$]phenylalanine, and 0.24 ± 0.02 L-[α-^{15}N]lysine. After 3, 4, 5, and 6 hours of tracer infusion, blood samples were drawn from the carotid artery and mesenteric and femoral veins and placed into tubes containing lithium heparin. During isotope infusions, biopsies (1 to 2 g) of leg muscle (after 4 hours) and of full-thickness gut wall (after 6 hours) were taken using a frozen iron clamp. The tissues were immediately frozen in liquid nitrogen and stored at −80°C until analysis. Plasma was quickly separated and frozen for later analysis.

Analysis of Samples

Concentrations of unlabeled leucine, valine, phenylalanine, lysine, and alanine, as well as the enrichment of infused tracers, were simultaneously determined by gas chromatography–mass spectrometry both in plasma samples and in total free water of tissue specimens using the internal standard approach.[15,16] (For a detailed description of the analytical method, see Biolo et al.[15]) Isotopic enrichment of infused tracers and internal standards was determined in plasma and tissue free water using a Hewlett-Packard (Palo Alto, CA) 5985 gas chromatograph–mass spectrometer as acetyl-*n*-propyl esters.[15,16] Data for enrichments are expressed as the tracer to tracee ratio (TTR).[16,17] TTR was calculated as the difference between isotopic abundance of sample and background, with correction for the contribution of isotopomers of small weight to the apparent enrichment of isotopomers with a greater mass.[17] This approach considers the tracee to be only the naturally occurring amino acid, rather than the sum of all other tracers (ie, the infused tracer and the internal standard). TTR is analogous to the term "specific activity" when radioactive tracers are used. The relation between TTR and atom percent excess is discussed elsewhere.[15-17]

Calculations

We have recently described a model that allows the quantification of intracellular amino acid kinetics, including transmembrane amino acid transport and incorporation into and release from protein.[15] This model can be applied to tissues with clearly identified venous drainage, as is the case with leg muscle and small intestine. All kinetic parameters are defined in terms of the basic three-pool model shown in Fig 1. The arterial pool (A) is the blood upstream from the tissue, and the venous pool (V) is the blood downstream from the tissue. Compartment T is the free amino acid pool in muscle or gut tissues. A, V, and T are connected by arrows that indicate the unidirectional rate of movement (micromoles per minute) of amino acid from one compartment to another. Further definitions necessary for the derivation of this model are as follows: C_A, C_V, and C_T, concentrations (μmol/L) in compartments A, V, and T; E_A, E_V, and E_T, TTR in compartments A, V, and T; and BF, blood flow across the tissue. Since phenylalanine and lysine are not oxidized either in muscle or small intestine,[18] their irreversible loss across tissues ($F_{0,T}$) equals their incorporation into protein. De novo appearance across skeletal muscle ($F_{T,0}$) of all the essential amino acids studied, leucine, valine, phenylalanine, and lysine, equals their release from protein breakdown. De novo amino acid appearance across the gut ($F_{T,0}$) equals release from protein breakdown plus absorption from the lumen. In fact, intraluminal digestion of proteins from bacteria, exfoliated cells, and secreted enzymes occurs also in the postabsorptive state.[19] Total intracellular appearance (Ra_T) of essential amino acids is defined as $F_{T,A}$ plus $F_{T,0}$. The legend for Fig 1 contains the remaining essential definitions for the model.

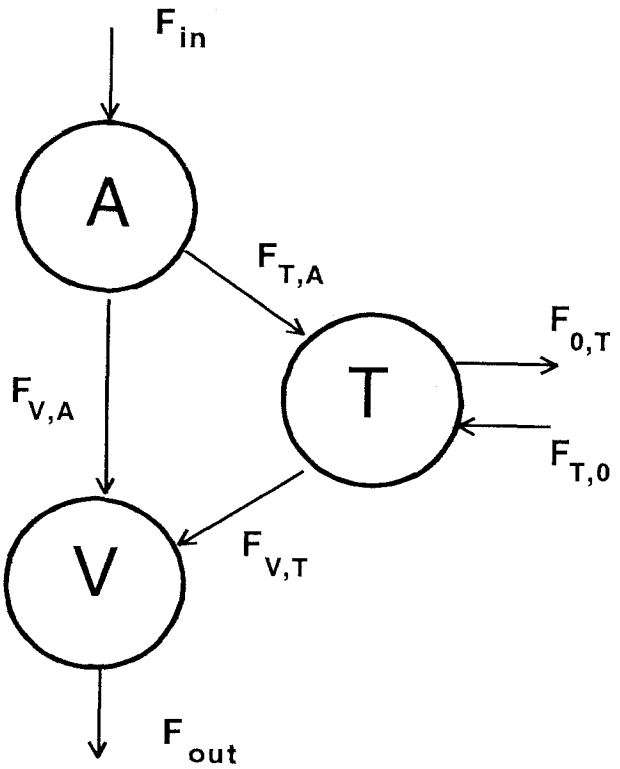

Fig 1. Three-compartment model of tissue amino acid kinetics. F_{in}, rate of arterial amino acid delivery to muscle or small intestine from systemic circulation; F_{out}, rate of amino acid exit via femoral or superior mesenteric vein; $F_{T,0}$, rate of amino acid entry into intracellular space T from vascular compartment A, ie, inward transport; $F_{V,T}$, rate of exit from T to V, ie, outward transport; $F_{V,A}$, rate of shunting from artery to vein; $F_{0,T}$, rate of amino acid incorporation into protein, in the case of phenylalanine and lysine that are not oxidized in gut or muscle[18]; $F_{T,0}$, de novo appearance of essential amino acids from protein degradation in the case of muscle tissue, or the sum of protein degradation and absorption from the lumen in the case of small intestine.

Each kinetic parameter is defined as follows (see Biolo et al[15] for the derivation of the equations):

$$F_{in} = C_A \cdot BF, \quad (1)$$

$$F_{T,A} = \{[(E_T - E_v)/(E_A - E_T)] \cdot C_V + C_A\} \cdot BF, \quad (2)$$

$$F_{V,A} = \{-[(E_T - E_V)/(E_A - E_T)] \cdot C_V\} \cdot BF, \quad (3)$$

$$F_{V,T} = \{[(E_T - E_V)/(E_A - E_T)] \cdot C_V + C_V\} \cdot BF, \quad (4)$$

$$F_{out} = C_V \cdot BF, \quad (5)$$

$$F_{0,T} = [(C_A \cdot E_A - C_V \cdot E_V)/E_T] \cdot BF, \quad (6)$$

$$F_{T,0} = \{[C_A(E_A - E_T) - C_V(E_V - E_T)]/E_T\} \cdot BF, \text{ and} \quad (7)$$

$$Ra_T = F_{T,A} + F_{T,0}. \quad (8)$$

Each kinetic parameter is a function of blood flow across that tissue. In this experiment, we could not measure blood flow across the small intestine, and absolute kinetic rates therefore could not be calculated. Nonetheless, physiologically relevant factors can be derived by expressing certain kinetic parameters in relation to other parameters, thus canceling out BF in the calculation. In this light, the rate of amino acid incorporation into protein ($F_{0,T}$, Eq 6)

was related to the total intracellular amino acid rate of appearance (Ra_T, Eq 8). $F_{0,T}/Ra_T$ expresses the rate of protein synthesis in relation to total intracellular free amino acid availability. The rate of outward amino acid transport ($F_{V,T}$, Eq 4) was related to the total intracellular amino acid rate of appearance (Ra_T, Eq 8). $F_{V,T}/Ra_T$ expresses the ability of outward transport to release amino acids into the bloodstream in relation to their intracellular availability. Intracellular free amino acids may derive from both inward transport ($F_{T,A}$, Eq 2) and de novo intracellular appearance ($F_{T,0}$, Eq 7). We calculated from equations 2, 7, and 8 the relative contributions to the total intracellular rate of appearance of inward transport from plasma and de novo appearance, $F_{T,A}/Ra_T$ and $F_{T,0}/Ra_T$, respectively. These figures express the relative importance of amino acid supply from plasma and intracellular appearance from protein breakdown and luminal absorption (if any) for gut and muscle protein synthesis. All these expressions are independent of the rate of regional blood flow. Results are expressed as the mean ± SEM. Statistical comparisons of mean values were performed using the paired *t* test.

RESULTS

Between 3 and 6 hours of tracer infusion, the dogs were in a steady-state condition, meaning that cardiac output (data not shown) and plasma concentrations (Table 1) and enrichments (Table 2) of labeled and unlabeled amino acids in the carotid artery, as well as in the mesenteric and femoral veins, were essentially constant. Figure 2 shows the arteriovenous difference of plasma amino acid concentrations across hindlimb and gut tissues. Arterial concentrations of leucine, valine, phenylalanine, and lysine (Table 1) were lower than in the femoral vein and higher than in the superior mesenteric vein, indicating net leg release and gut uptake of essential amino acids (Fig 2). Alanine was released from both leg and gut tissues (Fig 2, Table 1). Tissue amino acid concentrations in the gut were lower than in muscle (Table 1; $P < .05$ for leucine, valine, lysine, and alanine). Table 2 lists amino acid isotopic enrichments in artery, veins, and tissues. Tissue enrichments were consistently lower than in veins and artery because of intracellular tracer dilution from unlabeled sources (eg, protein breakdown).

Table 1. Concentration (μmol/L) of Free Amino Acids in Plasma (C_A, F_V, and SM_V), Muscle, and Gut

	Time (h)			
	3	4	5	6
Leucine				
C_A	148 ± 13	144 ± 9	153 ± 13	152 ± 15
F_V	175 ± 16	176 ± 12	176 ± 19	173 ± 13
SM_V	130 ± 8	134 ± 12	139 ± 16	139 ± 16
M_T		310 ± 31		
G_T				263 ± 36*
Valine				
C_A	219 ± 7	220 ± 11	235 ± 15	234 ± 15
F_V	243 ± 11	244 ± 9	252 ± 11	242 ± 10
SM_V	195 ± 8	201 ± 11	218 ± 18	202 ± 14
M_T		374 ± 40		
G_T				288 ± 23*
Phenylalanine				
C_A	75 ± 7	75 ± 4	82 ± 8	85 ± 5
F_V	94 ± 10	109 ± 10	106 ± 9	97 ± 7
SM_V	72 ± 3	77 ± 4	78 ± 2	76 ± 4
M_T		147 ± 15		
G_T				110 ± 9
Lysine				
C_A	191 ± 21	186 ± 20	197 ± 17	209 ± 18
F_V	231 ± 27	254 ± 25	258 ± 15	257 ± 11
SM_V	186 ± 20	191 ± 15	199 ± 16	195 ± 18
M_T		708 ± 98		
G_T				328 ± 20*
Alanine				
C_A	226 ± 38	244 ± 57	263 ± 71	221 ± 48
F_V	327 ± 58	354 ± 61	354 ± 58	328 ± 32
SM_V	276 ± 37	262 ± 36	265 ± 40	284 ± 57
M_T		2,551 ± 430		
G_T				1,088 ± 203*

Abbreviations: C_A, carotid artery; F_V, femoral vein; SM_V, superior mesenteric vein; M_T, tissue water in muscle; G_T, tissue water in gut.
*$P < .05$, gut *v* muscle.

Leg and small intestine amino acid and protein kinetics were calculated after 4 and 6 hours of tracer infusion, respectively, using corresponding plasma and tissue measurements (Tables 1 and 2). Table 2 lists the fractions of intracellular phenylalanine and lysine rates of appearance that were either used for muscle and gut protein synthesis or released into the bloodstream via outward transport systems. In muscle, only a small portion of total intracellular amino acid appearance was used for protein synthesis, whereas in small intestine, protein synthesis accounted for a large fraction of total intracellular amino acid availability. In contrast to the gut, muscle intracellular amino acids were preferentially released into the bloodstream. Table 4 lists the contribution of inward transport and de novo appearance to the total intracellular amino acid rate of appearance in muscle and small intestine. Values were variable among different amino acids, but in each case the contribution of inward transport from plasma to the total intracellular amino acid rate of appearance was greater in intestine than in muscle.

DISCUSSION

We have studied, in the small intestine and in skeletal muscle, the relationships between rates of protein synthesis and the ability of membrane transport systems to release intracellular amino acid into the bloodstream or to take up circulating amino acids. We found that in muscle, intracellular amino acids appearing from protein breakdown were preferentially released into the bloodstream instead of being reincorporated into protein. In the small intestine, intracellular amino acids were largely derived from transport from plasma and were used for protein synthesis more efficiently than in muscle. Thus, differences in transmembrane amino acid transport kinetics in small intestine and muscle contribute to the net amino acid transfer from muscle to small intestine in the postabsorptive state.

Arteriovenous catheterization studies across the small intestine are complicated by the fact that even in the postabsorptive state, intraluminal digestion of proteins from bacteria, exfoliated cells, and secreted enzymes results in the absorption of free amino acids[19] that cannot be detected by the arteriovenous difference technique. This absorption of unlabeled amino acids contributes to the

Table 2. Isotopic Enrichment of Free Amino Acids in Plasma (C_A, F_V, and SM_V), Muscle, and Gut

	Time (h)			
	3	4	5	6
Leucine				
C_A	0.0931 ± 0.0136	0.0914 ± 0.0103	0.0950 ± 0.0077	0.0952 ± 0.0104
F_V	0.0619 ± 0.0079	0.0570 ± 0.0073	0.0756 ± 0.0110	0.0746 ± 0.0103
SM_V	0.0825 ± 0.0094	0.0810 ± 0.0097	0.0811 ± 0.0097	0.0843 ± 0.0101
M_T		0.0388 ± 0.0051		
G_T				0.0600 ± 0.0061
Valine				
C_A	0.1063 ± 0.0146	0.1065 ± 0.0133	0.1112 ± 0.0120	0.1091 ± 0.0135
F_V	0.0825 ± 0.0101	0.0791 ± 0.0113	0.0934 ± 0.0107	0.0904 ± 0.0098
SM_V	0.0960 ± 0.0134	0.0976 ± 0.0135	0.1004 ± 0.0131	0.1038 ± 0.0122
M_T		0.0562 ± 0.0084		
G_T				0.0795 ± 0.0070
Phenylalanine				
C_A	0.0987 ± 0.0077	0.0966 ± 0.0087	0.1017 ± 0.0061	0.0998 ± 0.0082
F_V	0.0688 ± 0.0098	0.0628 ± 0.0079	0.0706 ± 0.0121	0.0735 ± 0.0115
SM_V	0.0857 ± 0.0099	0.0863 ± 0.0099	0.0890 ± 0.0099	0.0915 ± 0.0089
M_T		0.0486 ± 0.0067		
G_T				0.0660 ± 0.0058
Lysine				
C_A	0.1058 ± 0.0122	0.1067 ± 0.0110	0.1098 ± 0.0078	0.1071 ± 0.0101
F_V	0.0807 ± 0.0115	0.0742 ± 0.0112	0.8737 ± 0.0144	0.0810 ± 0.0135
SM_V	0.0961 ± 0.0097	0.0979 ± 0.0103	0.0993 ± 0.0104	0.1003 ± 0.0114
M_T		0.0335 ± 0.0058		
G_T				0.0557 ± 0.0072

NOTE. Data are expressed as TTR. Abbreviations are as in Table 1.

intracellular tracer dilution and cannot be distinguished from amino acid release from intracellular breakdown of endogenous protein. Thus, in the cells of the small intestine, de novo appearance of essential amino acids can be both from endogenous proteolysis and from absorption of free amino acids from the enteric lumen, not only in the postprandial state but also during fasting. Absorption of unlabeled amino acids from the intestinal lumen will result in an overestimation of protein breakdown, when calculated using the arteriovenous balance technique. In addition, epithelial cell exfoliation causes a loss of protein that is not detected by the arteriovenous difference. Therefore, arteriovenous catheterization of the small intestine does not produce a reliable estimation of the net protein balance, because the rate of protein degradation cannot be calculated. On the other hand, estimation of protein synthesis is not affected by these problems, because precursor enrichment for synthesis is measured directly.

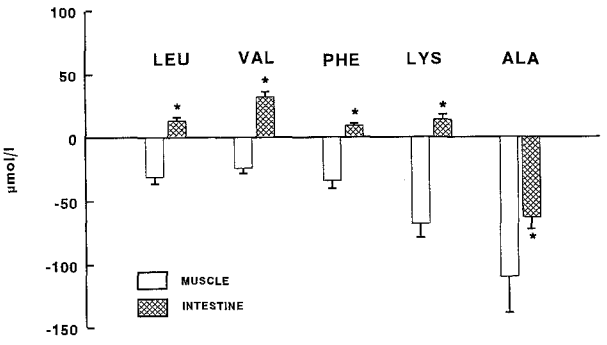

Fig 2. Arteriovenous difference of amino acid concentrations across small intestine and leg muscle. *$P < .05$, small intestine *v* muscle.

The precise rate of blood flow in the small intestine was unknown, so it was not possible to calculate absolute values of amino acid and protein kinetics in small intestine. Nonetheless, cardiac output was stable throughout our study, and others have shown that barbiturate anesthesia does not produce major disturbances in splanchnic circulation.[20,21] Therefore, values for blood flow could be estimated, thereby enabling approximate values for kinetics to be calculated. Liard measured blood flow in several dog tissues using radioactive microspheres.[22] He found that the

Table 3. Fraction of Ra_T Used for Protein Synthesis ($F_{O,T}/Ra_T$) or Transported Into the Bloodstream ($F_{V,T}/Ra_T$) in Muscle and Small Intestine

	Phenylalanine	Lysine
Muscle		
$F_{O,T}/Ra_T$	0.13 ± 0.03	0.28 ± 0.03
$F_{V,T}/Ra_T$	0.87 ± 0.04†	0.72 ± 0.03†
Intestine		
$F_{O,T}/Ra_T$	0.55 ± 0.05*	0.70 ± 0.07*
$F_{V,T}/Ra_T$	0.45 ± 0.06*	0.30 ± 0.08*

*$P < .05$, intestine *v* muscle.

†$P < .05$, $F_{V,T}/Ra_T$ *v* $F_{O,T}/Ra_T$.

Abbreviation: Ra_T, total intracellular appearance of essential amino acids, ie, the sum of inward transport from plasma ($F_{T,A}$) and de novo appearance ($F_{T,O}$) (from protein degradation in the case of muscle tissue, or from protein degradation plus absorption from the lumen in the case of small intestine).

*$P < .05$, intestine *v* muscle.

†$P < .05$, $F_{V,T}/Ra_T$ *v* $F_{O,T}/Ra_T$.

Table 4. Fractional Contribution of $F_{T,A}$ and $F_{T,O}$ to Ra_T in Muscle and Small Intestine

	Leucine	Valine	Phenylalanine	Lysine
Muscle				
$F_{T,A}/Ra_T$	0.37 ± 0.03	0.53 ± 0.03	0.50 ± 0.04	0.31 ± 0.02
$F_{T,O}/Ra_T$	0.63 ± 0.03	0.47 ± 0.03	0.50 ± 0.04	0.69 ± 0.02
Intestine				
$F_{T,A}/Ra_T$	0.63 ± 0.01*	0.75 ± 0.05*	0.66 ± 0.01*	0.52 ± 0.02*
$F_{T,O}/Ra_T$	0.37 ± 0.01*	0.25 ± 0.05*	0.34 ± 0.01*	0.48 ± 0.02*

*$P < .05$, intestine *v* muscle.

Abbreviations: $F_{T,O}$, de novo appearance of essential amino acids from protein degradation in the case of muscle tissue, or the sum of protein degradation and absorption from the lumen in the case of small intestine; Ra_T, total intracellular appearance of essential amino acids, ie, the sum of de novo appearance ($F_{T,O}$) and inward transport from plasma ($F_{T,A}$).

average blood flow in small intestine was 67.3 mL · min^{-1} · 100 g^{-1}, whereas muscle blood flow was only 6.1. Assuming these values in our study, as well as assuming that phenylalanine content in small intestine proteins is the same as that in muscle (250 nmol/mg dried protein),[15] we calculated the fractional rate of synthesis in muscle and small intestine. The average fractional rate of synthesis was 52.3% per day in small intestine and 2.2% per day in muscle. These values are in agreement with previous studies that used different techniques.[1-3,23-25] Because blood flow is 10 times greater in gut than in muscle,[22] absolute values of amino acid transport are approximately five to eight times higher per gram of tissue in gut than in muscle.

Gut tissue obtained by a full-thickness biopsy is not homogeneous and contains both mucosal epithelial cells and smooth muscle of submucosa. This heterogeneity could present a problem in the interpretation of the data, since the different components of the gut could have different enrichments and transport rates, at least in theory. However, we eliminated this problem by simultaneously taking both full-thickness biopsies of the gut wall and epithelial cells by scraping gut mucosa; we found that amino acid enrichment in the acid-soluble free pool of full-thickness gut homogenate did not significantly differ from that in the mucosa. This situation could be expected to be different after eating, because absorption of unlabeled amino acids might decrease the mucosal cell enrichment, while not affecting the submucosal cells to the same extent.

As described earlier, enrichment and concentration of free amino acids in gut and muscle were measured in the total free water of the whole tissue. These values were assumed to be representative of the intracellular pool. However, a fraction of the total free water space is the extracellular space. Exact values of amino acid enrichment and concentration in the extracellular space are unknown, but are between the intracellular and plasma values in vein. Thus, our measurements presumably overestimate intracellular enrichment and underestimate the concentration. These errors lead to an underestimation of protein synthesis (see Eq 6 and Eq 7) and to an overestimation of transmembrane transport rate (see Eq 2 and Eq 4). However, the upper limit for the interstitial fluid value is the corresponding venous value. Assuming that venous values are equal to interstitial values, intracellular enrichment and concentration can be corrected as described previously.[15,26] When these "corrected" values are used in the model calculation, there is an increase in the ratio between amino acid utilization for protein synthesis and total intracellular appearance ($F_{0,T}/Ra_T$) in both muscle (phenylalanine +5%, lysine +8%) and gut (phenylalanine +12%, lysine +9%). These changes in the calculated values represent the maximum possible error in the results shown earlier. Nonetheless, even using these corrected values for tissue measurements, differences between gut and muscle kinetics still remain significant, thus confirming the conclusions of the present report.

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