

## Intracellular Free Amino Acid Patterns in Duodenal and Colonic Mucosa

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We report for the first time the concentrations of free amino acids in human intestinal biopsies obtained by routinely performed endoscopy. We studied 15 medical patients with no changes of the mucosa and six HIV-infected persons with duodenitis. The mean (and SD) sum of all amino acids, taurine excepted, was 61.9 (5.4) mmol/kg dry weight in duodenal biopsies of HIV-negative subjects ( $n = 11$ ) and 82.9 (0.6) mmol/kg in colonic specimens: 50% (44%) of the total (minus taurine) consisted of aspartate and glutamate and 14% (12%), of the essential amino acids. The relative amino acid pattern in duodenum and colon differed completely from that for muscle: aspartate was fourfold higher; glutamate, phenylalanine, glycine, valine, leucine, and isoleucine were about twofold higher. In contrast, glutamine amounted only to 4% (duodenum) to 14% (colon) of muscle glutamine. In duodenal biopsies of the HIV-infected persons, we found significantly ( $P < 0.01$ , except glutamine:  $P < 0.025$ ) increased concentrations of glutamate (24.1 vs 17 mmol/kg dry weight), ornithine (1.4 vs 0.4), valine (2.2 vs 1.7), and glutamine.

**Additional Keyphrases:** *tissue analysis · HIV virus infection · duodenitis · gastrointestinal disease*

Recent studies have demonstrated that the metabolism of amino acids (AA), especially of glutamine (Gln), is of

great interest with regard to the morphological and functional integrity of the intestinal wall (1, 2).<sup>3</sup>

Until now, the intracellular AA metabolism of the human gut has not been characterized precisely. In particular, it is not known whether intestinal diseases are accompanied by dysfunction of the mucosal metabolism of Gln. Thus we have investigated the pattern of free amino acids in biopsies of the intestinal mucosa obtained during routinely performed endoscopy. Here we describe our first results and give special consideration to comparisons with previously published data obtained from analysis of plasma, muscle, and liver.

### Patients and Methods

We characterized the intramucosal pattern of AAs in duodenal and colonic specimens and compared it with the pattern in plasma (3) and with the AA concentrations in muscle and liver (4, 5) of healthy volunteers.

*Patients and endoscopy:* Biopsies of duodenal and colonic mucosa were obtained with informed consent from 21 patients (seven women, 14 men; mean age 53.4, SD 15.9 y) who had to undergo gastrointestinal endoscopy because of abdominal pain (nine), suspected malignancy (three), anemia of unknown origin (two), or diarrhea (seven). Six of the patients with diarrhea were HIV-positive [stages WR 5 and 6 of the Walter Reed Classification (6)]. All others were free of cancer or infectious disease. Two of the HIV-positive patients had lost more than 5% of their original body weight during the previous three months as a consequence of anorexia.

The endoscopies were done between 0830 and 1300 hours, after a fast of at least 12 h. Specimens for AA analysis and for histological examination were taken from

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<sup>3</sup> Nonstandard abbreviations: AA, amino acid(s); EAA, essential amino acids; NEAA, nonessential amino acids; and HIV, human immunodeficiency virus.

the same region. Histological changes characteristic of duodenitis were found in all of the HIV-positive subjects. Two subjects in the HIV-seronegative group showed slight changes, but the AA data of these two patients did not differ from those for the other seronegative subjects; this contrasted with the AA data for the HIV group.

**Analysis for free intramucosal amino acids:** Specimens of intestinal mucosa were frozen in fluid nitrogen within 20 s after biopsy, then lyophilized (Lyovac GT 2 lyophilizer; Leybold-Heraeus GmbH, Köln, F.R.G.). We extracted the AA from the lyophilisate in a glass homogenizer with 250  $\mu$ L of a 30 g/L solution of sulfosalicylic acid in 0.1 mol/L lithium citrate buffer (pH adjusted with HCl to a final value of 2.2). After centrifugation, we analyzed the supernate for AA by ion-exchange chromatography (3), using an LC 5001 analyzer (Biotronik, München, F.R.G.).

**Calculations and statistics:** The AA concentrations reported for each individual are the median intramucosal concentrations found for three parallel biopsies. Group differences of the data (see Table 1) were tested for statistical significance ( $P < 0.05$ ) with the Mann-Whitney test.

## Results

Table 1 lists the absolute concentrations of the AA in duodenum and colon. The mean wet weight of the biopsies was 5.63 (SD 1.3) mg, the dry weight, 1.15 (0.26) mg. The intra-individual variations in AA concentrations of three parallel specimens ranged between 3% and 13% (except for histidine, which was 19%). Asparagine, alpha-aminobutyrate, cystine, and tryptophan were not reliably measurable. We found significant differences between duodenum and colon, both for total AA and AA pattern, both in absolute and relative terms (Table 2). Whereas the absolute amounts of total essential AAs (EAAs) are nearly identical in duodenum, colon, and skeletal muscle (4)—9.7, 8.3, and 8.8 mmol/kg dry wt, respectively—the relative pattern varies in these tissues. If arranged into four EAA groups as recommended by Waterlow and Fern (7), the

relative pattern of EAAs in duodenum tissue corresponds to that of the plasma (Table 3). In contrast, the EAA rank order in colon tissue more resembles that of skeletal muscle. The difference of the total AA amount between duodenum and colon (62 vs 83 mmol/kg dry wt) results primarily from glutamate, glutamine, glycine, and alanine, which are each 5–6 mmol/kg dry wt higher in colon than in duodenum. The relative concentration of glutamate in each, however, is identical and amounts to 28% of the total AAs: twice the value for plasma and three times the proportion in muscle. Thus glutamate shows the highest concentration of all intracellular free AA in duodenum and colon mucosa, whereas glutamine is only the fifth among the nonessential AAs (NEAAs) (Table 2). Aspartate is the AA for which the concentration differs most from that in plasma and muscle. Duodenal aspartate has nearly the same concentration as glutamate, and it also occupies the second rank in the colon. Glycine ranks third, before alanine, in contrast to plasma and skeletal muscle.

The data on HIV+ subjects show much larger standard deviations than the results for the other patients. That is why such data must be confirmed by further investigations. All HIV+ patients suffered from duodenitis. The AA concentrations of two other HIV- patients with duodenitis did not differ from that of subjects without it.

## Discussion

Our results—and especially the narrow standard deviations—demonstrate that the intracellular free AA pattern of the gastrointestinal mucosa can be characterized by analysis of mucosal biopsies. As far as we know, there is only one published study dealing with the same subject: Adibi and Mercer (8) determined 13 AAs in the jejunal mucosa from four subjects. Our rank order of the mucosal AA concentration in the duodenum (Table 2) is identical to theirs, although the absolute AA concentrations are not comparable because of differences in methodology between the two studies. According to their various physiological

**Table 1. Amino Acid Concentrations in Intestinal Mucosa Biopsies (mmol/kg dry weight)**

AA	Duodenum HIV- (n = 11)			$P^a$	Duodenum HIV+ (n = 6)		$P^b$
	Mean (SD)	Mean (SD)	Median		Mean (SD)	Median	
Taurine	23.7 (2.9)	13.3 (2.1)	14.7	***	28.6 (6.9)	32.2	
Asp	13.7 (2.1)	13.08 (1.75)	13.5		13.3 (3.5)	13.2	
Thr	1.17 (0.12)	1.78 (0.61)	1.6	*	1.98 (0.69)	1.9	*
Ser	2.20 (0.31)	2.80 (0.68)	2.6		3.6 (1.73)	3.6	**
Glu	17.03 (1.56)	24.4 (2.46)	23.3	***	24.13 (5.74)	22.3	***
Gln	2.61 (0.73)	8.18 (0.46)	8.2	***	5.65 (2.96)	4.0	**
Gly	8.06 (1.53)	12.23 (0.9)	12.1	***	8.95 (2.72)	8.8	
Ala	4.52 (0.77)	10.98 (0.75)	11.0	***	5.68 (1.39)	5.9	
Val	1.65 (0.29)	2.1 (0.67)	1.9		2.20 (0.56)	2.3	***
Ile	0.65 (0.14)	0.65 (0.1)	0.6		0.62 (0.11)	0.6	
Leu	1.77 (0.33)	1.58 (0.44)	1.5		1.37 (0.25)	1.4	*
Tyr	0.71 (0.19)	0.50 (0.1)	0.5	**	0.55 (0.1)	0.6	**
Phe	0.69 (0.10)	0.50 (0.08)	0.5		0.72 (0.11)	0.7	
Orn	0.37 (0.12)	0.65 (0.25)	0.7	***	1.38 (0.78)	1.1	***
Lys	1.67 (0.33)	2.63 (0.41)	2.5	***	2.23 (1.45)	1.6	
His	0.68 (0.16)	1.10 (0.16)	1.1	***	1.15 (0.85)	0.9	
Arg	1.25 (0.36)	1.38 (0.22)	1.4		3.48 (3.47)	1.5	
Total <sup>c</sup>	61.9 (5.4)	82.9 (0.55)	83.5	***	77.15 (13.83)	67.7	

<sup>a</sup> Colon vs duodenum. <sup>b</sup> Duodenum HIV+ vs duodenum HIV-. <sup>c</sup> Excepting taurine. \*\*\*  $P < 0.01$ ; \*\*  $P < 0.025$ ; \*  $P < 0.05$ .

**Table 2. Relative Amounts of Free Amino Acids<sup>a</sup> in Duodenum, Colon, Muscle (4), and Plasma (3)**

AA	Duodenum (n = 11)		Colon (n = 4)		Muscle (n = 16)		Plasma (n = 22)	
	Rank	% total AA	Rank	% total AA	Rank	% total AA	Rank	% total AA
<i>Essential amino acids</i>								
Leu	1	3.0	4	1.7	5	0.7	4	3.7
Val	2	2.8	2	2.0	4	1.0	1	6.8
Lys	2	2.8	1	3.0	1	3.2	2	5.7
Thr	3	1.9	3	1.9	2	1.9	3	4.1
Phe	4	1.1	7	0.6	6	0.3	7	1.7
His	4	1.1	5	1.4	3	1.3	5	2.7
Ile	5	1.0	6	0.7	6	0.3	6	2.0
Met	6	0.5	8	0.4	7	0.2	8	0.7
Total		14.2		11.7		8.9		27.4
<i>Nonessential amino acids</i>								
Glu	1	27.8	1	28.2	2	10.4	9	1.5
Asp	2	22.6	2	16.1	6	2.9	11	0.3
Gly	3	12.8	3	14.3	5	4.2	3	6.8
Ala	4	7.5	4	13.5	3	9.0	2	10.2
Gln	5	3.7	5	10.1	1	53.1	1	18.0
Ser	6	3.5	6	3.0	7	1.9	5	3.1
Pro	7	3.2	7	2.8	4	4.5	3	6.8
Arg	8	2.1	8	1.6	8	1.6	8	1.7
Tyr	9	1.3	10	0.6	12	0.4	8	1.7
Cit	10	1.0	10	0.6	13	0.4	10	1.0
Orn	11	0.5	9	0.7	10	0.9	6	2.4
Asn					9	1.0	7	2.1
Cys					11	0.5	4	3.4

<sup>a</sup> All AA (except taurine): duodenum 61.9, colon 82.9, and muscle (4) 99.8 mmol/kg dry weight; plasma 2.95 mmol/L (3).

**Table 3. Relative Amounts of Free Essential Amino Acids in Duodenum, Colon, Muscle (4), and Plasma (3)**

AA	Duodenum	Colon	Muscle	Plasma
	(n = 11)	(n = 4)	(n = 16)	(n = 22)
	% of total EAA			
BCAA <sup>a</sup>	47.8	37.6	22.5	45.5
Lys+Thr	33.1	41.8	57.3	35.8
Phe+Met	11.3	8.6	5.6	8.8
His	7.8	12.0	14.6	9.9

<sup>a</sup> BCAA, branched-chain amino acids.

functions for AA and protein metabolism, the gut, muscle, and blood plasma show different AA patterns. As an example, the highest relative portion of the EAAs can be seen in the plasma, e.g., in the transport system for vital substrates, with a two- to threefold higher relative EAA concentration than in the gut or in muscle.

Glutamine is the AA with the most pronounced concentration differences between the above-mentioned organs. Our investigation confirms results for rat small intestine (1) and human jejunum (8) that the lowest concentrations of free glutamine are measured at the location of consumption, e.g., in the duodenum. Concentrations in the duodenum amount to only 4% of free glutamine in muscle and 15% of the concentration in liver (9). Under physiological conditions, the intestinal glutamine concentrations depend predominantly on glutaminase activity of the tissue. In the rat the specific activity is similar in mucosa of duodenum, jejunum, and ileum, but much lower in stomach, cecum, and colon (10). From the higher glutamine concentrations of

colonic mucosa we deduce that this is also true for humans. In contrast, skeletal muscle has the highest concentrations of free glutamine. It is the most important source of this AA and releases the compound for removal by other organs during the postabsorptive state (11, 12). We now plan to investigate whether the availability of glutamine for intestinal consumption is impaired as a result of increased systemic glutamine catabolism (13), of disturbed muscular synthesis (14), or of glutamine-deficient artificial nutrition (15).

Next to glutamine, the concentrations of glutamate and aspartate in the intestinal tissue differ most from that of muscle or plasma. These two compounds make up about 50% of all free AA in the mucosa compared with 13% and 1.8% in skeletal muscle and plasma, respectively. Similar to the pattern for liver (5), we found almost equimolar concentrations of aspartate and glutamate in the duodenum, but not in colonic biopsies. Thus, the enzymatic activities of the upper intestine are more likely to resemble those in the liver than the distal intestine, especially the activities of the glutamate dehydrogenase/aspartate aminotransferase system (16).

Our results concerning the HIV+ patients can only be regarded as preliminary, because of the very high standard deviations compared with data for the other groups. The values for glutamate are of the most interest because of our recent investigation (3, 17) showing that HIV+ patients of stages WR 5 and 6 have significantly increased plasma glutamate. On the other hand, the enhanced intraduodenal glutamine in the HIV+ subjects leads to the assumption that the intracellular degradation of glutamine is likely to be disturbed in critically ill patients. We now are investigating whether this is indeed the case.

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