Original Communications

Effect of Amino Acid Infusion on Human Postoperative Colon Protein Synthesis *in Situ*

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ABSTRACT. *Background:* Amino acids are an integral part of parenteral nutrition because of their anabolic action helping to conserve body protein after surgical stress. At the gastrointestinal tract, an adequate supply of amino acids may be particularly important because of the gut's high rate of protein turnover, cell division, and proliferation. However, no information is available about the effects of amino acids on human intestinal protein metabolism after surgery. *Methods:* Studies were performed in postabsorptive patients 8–10 days after major abdominal surgery. Mass spectrometry techniques (capillary gas chromatography/combustion isotope ratio mass spectrometry) were used to directly determine the incorporation rate of 1-[^{13}C]-leucine into colon mucosal protein. All subjects had a colostomy, which allowed easy access to the colon mucosa, and consecutive sampling from the same tissue was performed during continuous isotope infusion (0.16 μmol/kg min). Isotopic enrichments were determined at baseline and after a 4-hour infusion of amino acids or after infusion of saline (control group). *Results:* Compared with baseline, infusion of amino acids reduced fractional colon protein synthesis significantly by $-29.2 \pm 8.3\%$. This decrease was also significantly different from the corresponding (insignificant) change during saline infusion ($+19.4 \pm 26.9\%$, $p < .05$ *vs* amino acid group). *Conclusions:* After surgery, an amino acid infusion acutely reduces postoperative colon protein synthesis. This effect possibly may be attributed to interactions of specific amino acids (glutamine) with an altered intestinal immune system and enterocyte activity. (*Journal of Parenteral and Enteral Nutrition* **29:**255–261, 2005)

Amino acid supply has gained its firm place during parenteral nutrition in a wide range of patients who cannot tolerate enteral feeding. Amino acids are usually considered necessary to reduce protein loss after surgical stress. An optimal conservation of body protein will be obtained with daily administration of 1.2–1.5 g amino acids per kg body weight.[1] Potential anabolic actions of amino acids are known to occur in muscle and in splanchnic tissues.[2] At the intestinal tract, amino acid metabolism and protein turnover are particularly important because an efficient formation of new protein is essential for maintaining a high rate of cell division and proliferation and for the production of cellular structure compounds and secreted enzymes.[3,4] Unfortunately, in humans effects of specific substrates have almost exclusively been studied in the whole splanchnic bed, not allowing a differentiation between hepatic and intestinal changes.[5–7] Virtually nothing is known on the effects of amino acids on human intestinal protein metabolism *in situ*.

We sought in the present study to examine the effects of a standard amino acid infusion on colon protein synthesis using stable isotopes (1-[^{13}C]-leucine) and advanced mass spectrometry techniques. Studies were performed in patients after major abdominal surgery whose mucosal function may be altered and who are frequent candidates for parenteral nutrition.

MATERIALS AND METHODS

Subjects

Two groups of postoperative patients (control, $n = 5$; amino acid infusion, $n = 12$) with cured rectal carcinoma and colostomies were carefully screened through their medical history, physical examinations, and routine blood tests. The groups were comparable with respect to age (control, 64.8 ± 3.9 years; amino acid infusion, 63.5 ± 2.9) and body mass index (control, 24.9 ± 1.0 kg/cm^2; amino acid infusion, 27.5 ± 1.3). Patients had had limited colorectal cancer and had undergone curative, elective abdominal surgery, which had also included construction of a colostomy. All patients had a preoperative bowel preparation (orthograde flushing by oral colonoscopy fluids) and received prophylactic IV antibiotics at the time of surgery. Anesthesia consisted of epidural application of analgesic and anesthetic drugs and was continued no longer than the fifth postoperative day. The patients were studied between days 8 and 10 after surgery, when

body protein loss peaks,[8] had an uneventful postoperative course, and were free from signs of organ malfunction and local or systemic infection. No patient (before surgery) had a history of previous weight loss or clinical and laboratory signs of malnutrition or metabolic diseases. Informed consent was obtained after the experimental protocol had been explained in detail. The study was approved by the local institutional review board (protocol 134/97).

Experimental Protocol

All subjects were inpatients of the general surgical service. Before the study, patients received a mixed diet (approximately 25 kcal/kg day, of which 5 kcal/kg day were administered as protein or IV amino acids); two-thirds of the calories were administered enterally (liquids) and the other one-third parenterally. Of the calculated energy demand (25 kcal/kg day), only 60% was provided on day 1 to day 3 after surgery, 80% between day 4 and day 6, and 100% thereafter. In any case, a daily postoperative protein/amino acid intake of 1.2 g /kg was assured. Remaining nonprotein calories were given as carbohydrates between day 1 and day 6. After the sixth postoperative day, half of the nonprotein calories were given as carbohydrates, the other half as fat. Nutrition was started as parenteral nutrition. If gastrointestinal function was considered sufficient, tea per mouth was provided on postoperative days 1 and 2, clear liquids on postoperative days 3 and 4, and regular liquid food on the subsequent days. Daily oral consumption was recorded by the dieticians, and eventual caloric deficits were compensated by adjusting parenteral nutrition.

After 10 PM on the day before surgery, subjects remained postabsorptive, except for consumption of mineral water. A primed-constant infusion of 1-[13C]-leucine (Tracer Technologies, Sommerville, MA; 99.3 atom percent enrichment) was started at 7 AM the next day. The isotope infusion rate was 0.16 μmol/kg min (prime 9.6 μmol/kg). A blood sample was collected before isotope infusion to determine the background enrichment of protein-bound and free plasma leucine. Plasma background enrichments were used as an indicator of intracellular protein-bound and free leucine background enrichments.[9]

The first mucosa biopsy was performed after 180 minutes of isotope infusion, the second after 360 minutes, and the third after 600 minutes. The baseline period (period I) ranged from minutes 180–360. Between minutes 360 and 600 (after the second biopsy, period II), patients in the amino acid group ($n = 12$) received a continuous infusion of commercial amino acid solutions with comparable amino acid composition (Glamin, Baxter, Erlangen, Germany; or Parentamin/Dipeptamin, Serag-Wiessner, Naila, Germany; and Fresenius-Kabi, Bad Homburg, Germany). The total amino acid infusion rate was 67 mg/kg h. Patients in the control group remained in a fasting state (saline infusion, $n = 5$). Arterialized blood samples for measurement of amino acid concentrations were taken at the same time as mucosa biopsies. The minimum distance between biopsy sites was 2 cm. The average

biopsy size was 10 mg wet weight. All biopsies were taken from a portion of the colon that was located in the abdominal wall. No patient was included with any signs of mucosa swelling, edema, necrosis, bleeding, or malfunction. If mucosal function was unclear, we used laser Doppler flowmetry[10] to confirm the normality of mucosal flow patterns.

Procedures

Study methods and data analysis were discussed in detail previously.[9] The free and protein-bound amino acids in tissue biopsies were separated by protein precipitation. After protein hydrolysis, amino acids were separated from the accompanying impurities by cation-exchange chromatography. For capillary gas chromatography and combustion isotope ratio mass spectrometry analysis, amino acids from proteins (on average 7–8 ng) were then converted to the *N*-acetyl *n*-propyl ester. For gas chromatography and quadrupole mass spectrometry analysis, we prepared the tert-butyldimethylsilyl derivative from free intracellular amino acids. *N*-acetyl *n*-propyl-amino acid derivatives were analyzed in a capillary gas chromatography and combustion isotope ratio mass spectrometry system that consisted of a Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard) interfaced to a mass spectrometer Delta S (Finnigan MAT, Bremen, Germany). Tert-butyldimethylsilyl derivatives were analyzed by a gas chromatography and quadrupole mass spectrometry system (MSD 5971D, Hewlett-Packard). Isotopomere ratios of the sample were obtained by electron impact ionization and selected ion monitoring at mass-to-charge ratios 303 and 302. Data were expressed as tracer:tracee ratios.

Plasma amino acid levels were measured by an autoanalyzer (Beckman Instruments, Fulton, CA).

Calculations

The tissue fractional synthetic rate in the metabolic steady state was calculated by dividing the increment in protein-bound 1-[13C]-leucine tracer:tracee ratio by enrichment of the precursor pool.[9] As a substitute for the true precursor pool enrichment (tRNA-bound amino acid enrichment), we used the free homogenate 1-[13C]-leucine tracer:tracee ratio.[11] Increments of protein-bound 1-[13C]-leucine enrichment between biopsy samples were calculated from isotope ratios [13C]/[12C] using a correction factor that takes into account dilution of the label at the carboxyl position by the other carbon atoms in the derivatized leucine.[9] The fractional synthetic rate (FSR) was then calculated as follows:

$$\text{FSR} = \frac{R_L(t_{i+1}) - R_L(t_i)}{\{R_L{'}(t_i) + R_L{'}(t_{i+1})\}/2} \times \frac{1}{\Delta t} \times 60 \times 100 \qquad (\%/h)$$

$R_L(t_i)$ and $R_L(t_{i+1})$ correspond to the tracer:tracee ratio of colon protein-bound leucine in 2 subsequent samples (i and i + 1), separated by the time interval Δt (min). $R_L{'}(t_i)$ and $R_L{'}(t_{i+1})$ indicate the tracer:tracee ratios of colon free leucine in 2 subsequent samples. Average values between $R_L{'}(t_i)$ and $R_L{'}(t_{i+1})$ were used

TABLE I
Tracer:tracee ratios of 1-[^{13}C]-leucine in free intracellular leucine of colon mucosa*

	Sample 1	Sample 2	Sample 3
Control	6.67 ± 0.50	6.43 ± 0.59	7.14 ± 0.59
Amino acid	6.55 ± 0.33	6.00 ± 0.45	6.56 ± 0.35

*Values are given as % (mean ± SEM). Sample 1 was taken after 180 min, sample 2 after 360 min, and sample 3 after 600 min of isotope infusion. Patients in the amino acid group received 67 mg/kg h of amino acids between 360 and 600 min. Control patients continued to fast throughout the study.

TABLE II
Tracer:tracee ratios of 1-[^{13}C]-leucine in colon mucosa protein*

		Sample 1	Sample 2	Sample 3
Amino acid	1	0.13	0.35	0.63
	2	0.12	0.45	0.50
	3	0.22	0.45	0.76
	4	0.28	0.37	0.43
	5	0.28	0.47	0.66
	6	0.22	0.41	0.66
	7	0.32	0.61	0.85
	8	0.17	0.41	0.67
	9	0.11	0.36	0.71
	10	0.43	0.60	0.73
	11	0.30	0.53	0.82
	12	0.17	0.60	0.95
Control	1	0.16	0.50	0.78
	2	0.13	0.42	0.77
	3	0.12	0.29	0.49
	4	0.11	0.19	0.43
	5	0.19	0.35	0.66

*Values are given as percentage. Sample 1 was taken after 180 min, sample 2 after 360 min, and sample 3 after 600 min of isotope infusion. Patients in the amino acid group received 67 mg/kg h of amino acids between 360 and 600 min. Control patients continued to fast throughout the study.

as precursor enrichments for colon protein synthesis. The factors 60 (min) and 100 are needed to express the fractional synthetic rate in percentage per hour.

To analyze the speed of tracer incorporation separately in periods I and II, we calculated the absolute increase of the protein-bound tracer:tracee ratio (in percentage) per hour during the 10-hour isotope infusion by the formulas

$$\text{Period I: } \Delta R_L I \, (\%/h) = [\{R_L(t_2) - R_L(t_1)\}/\Delta t] \times 60$$

$$\text{Period II: } \Delta R_L II \, (\%/h) = [\{R_L(t_3) - R_L(t_2)\}/\Delta/t] \times 60$$

The relative change in the speed of tracer incorporation between periods I and II was calculated as

$$[(\Delta R_L II - \Delta R_L I)/\Delta R_L I] \times 100$$

Statistics

Data are expressed as mean ± SEM. Protein synthetic rates, speed of tracer incorporation and amino acid concentrations in control subjects and in subjects receiving the amino acid infusion were compared by the paired and unpaired Student's *t* test. A *p* value of .05 or less was taken as indicating a significant difference.

RESULTS

Plasma total amino acid concentration rose significantly during amino acid infusion (end of period II: 4036 ± 91 μmol/L, *p* < .05 *vs* 2679 ± 88 μmol/L at the end of period I), whereas total amino acid concentration remained unchanged in control patients (end of period II: 2838 ± 77 μmol/L, n.s. *vs* 2804 ± 92 μmol/L at the end of period I) resulting in a significant difference of amino acid concentrations between controls and subjects receiving amino acids at the end of period II (*p* < .05).

Table I presents the tracer:tracee ratios in the mucosa free homogenate leucine pool (= precursor pool for protein synthesis) in the 2 study groups. The values of protein-bound leucine enrichment at the different sampling points are presented in Table II. In control patients, tracer incorporation into mucosa protein was almost linear. The slopes (= speed of tracer incorporation = rise in tracer:tracee enrichment/hour) were identical throughout the study. Thus, tracer:tracee ratios (%) in colon mucosal protein rose by 0.069 ± 0.014 per h in period I and by 0.069 ± 0.006 per h in

period II. In contrast, the speed of tracer incorporation fell significantly when amino acids were given instead of saline in period II. In the amino acid group, tracer:tracee ratios in colon mucosal protein rose by 0.079 ± 0.008 per hour in period I and only by 0.058 ± 0.007 per hour in period II (*p* < .05). The relative change of the slopes between periods I and II in the 2 groups differed significantly between groups (control: 22.2% ± 29.0%; amino acid infusion: −25.6% ± 7.9%, *p* < .05).

After amino acid infusion, we observed a significant decrease in the fractional synthetic rate of colon mucosa (0.95% ± 0.13%/hour at the end of period II, *p* < .05 *vs* 1.34 ± 0.10 at the end of period I). In contrast, the fractional synthetic rate of colon mucosa remained unchanged throughout the study in control patients (1.00% ± 0.17%/hour at the end of period I, not significant *vs* 1.03 ± 0.05 at the end of period II). Thus, the percent change of colon protein synthesis between periods I and II differed significantly between patients receiving amino acids and control patients (Fig. 1).

DISCUSSION

In control patients, colon protein synthetic rate and the speed of tracer incorporation remained stable during the 10-hour isotope infusion. These findings are in contrast to our previous observations in ileum mucosa of comparable postoperative patients. In postsurgical ileum mucosa of the latter patients, protein synthesis declined rapidly, falling significantly from about 1.11%/hour in period I to 0.39%/hour in period II.[11]

It had been argued that this decline is artificial because of rapid mucosal tracer losses due to enzyme secretion, cell exfoliation, or protein degradation, thereby questioning the validity of the continuous tracer infusion technique for measuring protein synthesis in intestinal mucosa.[12,13] However, such a mech-
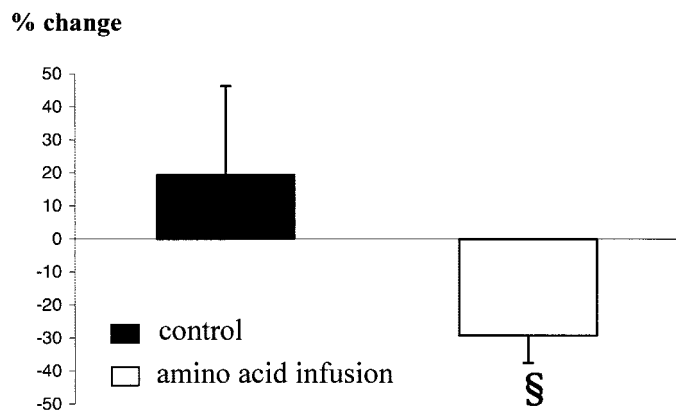
% change

F<small>IG</small>. 1. Effect of an amino acid infusion on protein fractional synthetic rate in colon mucosa. Values are given as mean ± SEM and represent percent changes between periods I and II. After a baseline period of 3 hours (period I), patients in the amino acid group received 67 mg/kg h of amino acids over 4 hours (period II), whereas control subjects (saline infusion) remained in a fasting state during the corresponding time. §$p < .05$ *vs* control.

anism would require selective loss of labeled protein or tracer. Considering the basic principles of tracer techniques, such a scenario is highly unlikely because the normal assumption is random kinetics, which leads to proportionate losses of labeled and unlabeled protein molecules no matter by which mechanism. Anything else would require a selective isotope effect that is absent in humans.[14]

In the past, a similar problem had been identified at the liver when protein synthesis was also examined *via* continuous tracer infusion.[15–17] This seeming decline of protein synthesis over time in some tissues can be explained by the existence of different protein pools with different protein turnover rates and correspondingly different speeds of isotope incorporation.[15,17] Thus in a mixed sample, enrichments of all pools will contribute to the measured total enrichment. After a certain amount of time, isotopic enrichment in each pool will reach a plateau that corresponds to the precursor pool enrichment. However, in a rapid-turnover protein pool, the maximum (plateau) enrichment may already be reached during tracer infusion. Subsequently, if the precursor pool enrichment remains constant, isotopic enrichments in this protein pool will not change anymore. Thereby, this pool will no longer contribute adequately to the increasing enrichment found in mixed samples taken at a later time. After an isotopic plateau has been reached in the rapid-turnover protein pool, only enrichments of proteins in the slow-turnover pool will continue to rise. Therefore, corresponding mixed enrichments will be lower than before because the enrichment rise in the rapid-turnover protein pool is now absent. This mechanism will result in a seeming deceleration of tracer incorporation and fall of protein synthesis during this time. Furthermore, effects of any metabolic manipulation performed after this point will remain undetectable in this rapid-turnover pool.

According to animal data, this rapidly turning over protein pool in the gut mainly consists of enzymes or epithelial glycoproteins such as lactosaminoglycans or proteoglycans, which were found to have turnover rates in the range of 20%–30%/hour, depending on the physiologic conditions.[18–22] Nevertheless, because the size of this specific pool amounts to <10% of the total protein pool,[23–25] the vast majority of proteins can still be studied in tracer infusion experiments such as ours.

Interestingly, the contribution of this rapid-turnover pool to total mucosal protein synthesis seems to vary in the intestinal tract. Our finding of a constant tracer incorporation in colon but not in ileum mucosa suggests proteins with such a high turnover rate to be less common in the large bowel. There are several experimental findings supporting this qualitative difference between ileum and colon protein synthesis. In humans, the percentage of proliferating cells is significantly lower in colon than in ileum mucosa (10.3% *vs* 17.8%).[26] Furthermore, glycoprotein production differs significantly between colon and ileum.[27] These differences are relevant when mucosal protein synthesis is determined by the flooding dose technique. Although there are various disadvantages in comparison to continuous isotope infusion, the flooding dose technique is specifically suited to examine protein synthetic rates in rapidly turning over proteins.[13] When protein synthesis is evaluated up to 10 minutes after tracer application, rat colon demonstrates a fractional synthetic rate of 62.1%/d, which is significantly lower than the corresponding rate in ileum (103.4%/d).[28]

Another key finding of our study is that we found a decrease in colon protein synthesis during infusion of standard amino acid solutions. This decline occurred despite an amino acid infusion rate that corresponded to recent clinical recommendations for patients in surgical stress and that was shown to optimally conserve total body protein.[1]

In the past, several studies elucidated the fate and metabolic effects of IV amino acids in healthy and diseased men. It is generally accepted that, compared with the fasting state, IV amino acids stimulate total body protein synthesis.[2] One portion of this stimulation takes place in skeletal muscle tissue where even a short-term amino acid infusion may double protein synthesis *in situ*.[29] However, the majority of peripherally administered amino acids are being removed in the splanchnic bed.[6,7] There is also evidence from human studies that infused amino acids predominantly stimulate splanchnic protein synthesis.[5] Presumably, a large portion of this accelerated protein synthesis can be attributed to hepatic albumin synthesis because the latter rises when amino acid supply is increased.[30,31]

The fate of the infused amino acids in different compartments of the human splanchnic region such as in the gut and their relative contribution to total splanchnic protein turnover are still unclear. Because of significant technical and methodological limitations, precise human studies are still lacking. There is, however, strong evidence from selective intestinal arteriovenous balance measurements in dogs that gut amino acid uses rise, irrespective whether proteins are administered enterally[32] or, as amino acids, parenterally.[33,34] However, this use does not necessarily reflect enhanced protein synthesis because decreased protein

breakdown or accelerated amino acid oxidation is possible.

With respect to protein synthesis, enteral protein feeding was without relevant effect in the jejunum of adult pigs,[35–37] or in the duodenum of adult man.[38] In contrast to whole proteins, enteral application of free amino acids seems to be more effective, although large amounts may be required. After a short-term application (1.25 hours) of only small amounts (0.38 g/kg), gut protein synthesis did not rise in young pigs,[36] whereas significantly more enteral amino acids (>1 g/kg) increased small-bowel protein synthesis in dogs[39] and duodenal protein synthesis in man.[40] Such an enteral dose/effect relationship may also exist for specific amino acids such as glutamine, which, when given orally at a rate of 20 mg/kg per h, did not alter human enterocyte proliferation or duodenal protein synthesis.[41,42] Only large enteral amounts (>100 mg/kg per h) were found to stimulate protein synthesis in canine and human intestinal mucosa.[40,43]

Parenteral effects of amino acids on protein synthesis are less clear and may depend on technique, site, or species. Thus, amino acid infusion increased intestinal protein synthesis in dogs[44] but not in pigs[36,45] or human duodenum.[46] Considering our rigid study design (triple mucosal biopsies in the same subjects before and during amino acid infusion), our findings strongly support the idea that a potential anabolic effect of an increased basolateral amino acid supply at the gut does not imply a rise in intestinal protein synthesis. If these actions indeed exist also in man, they are more likely to be mediated by a reduction of protein breakdown. However, it cannot be excluded that potential metabolic effects also depend on the clinical condition of the subjects.

In fact, it was surprising to observe an actual fall of gut protein synthesis during amino acid infusion. Such an observation has not been made before in any experimental setting, which, however, has never included conditions of postsurgical stress and the additional supplementation of glutamine. Considering these specific circumstances of our patients and potential nonnutrition effects of glutamine, a hypothesis might be generated to explain the observed inhibitory effects of amino acids on large-bowel protein synthesis. Besides being an important substrate for mucosal energy metabolism, glutamine also has a variety of other actions that may eventually gain relevance during postoperative stress. However, it should be noted that such a hypothesis is mostly based on experimental data from animal or *in vitro* studies.

Explanation of a putative glutamine effect requires a closer examination of the mechanisms involved in surgical injury at the intestinal tract. Surgical trauma and the associated inadequate luminal nutrition may cause profound alterations of mucosal morphology and function, including reduced abundance of digestive enzymes, diminished tight-junction integrity, or enhanced enterocyte damage and apoptosis.[47,48] The latter changes stimulate a mucosal proinflammatory response, with recruitment of inflammatory cells into the intestinal wall,[49–51] local release of proinflammatory mediators,[52,53] and subsequent compensatory stimulation of enterocyte protein synthesis and proliferation to account for the accelerated loss of enterocytes.[54,55]

Glutamine may have counteracted these inflammatory reactions and mucosal damage due to its protective antiinflammatory effects.[56,57] These effects include increased intracellular glutathione availability, thereby inhibiting NFκB activation and ultimately production of proinflammatory mediators.[58] These mechanisms have also been described in the gut,[59–62] where they lowered cytokine release from mucosal tissues and mesenteric mononuclear cells and reduced enterocyte apoptosis.[63–66] The attenuated inflammatory response and enhanced enterocyte lifespan will also attenuate the signals and the need for a compensatory acceleration of enterocyte proliferation. The glutamine-related reduction of mucosal damage and inhibitory effects on inflammatory reactions might then explain the acute fall in the accelerated colon protein synthesis during amino acid supplementation. That such immunomodulatory mechanisms of glutamine might be also effective at the human gut is suggested by a study of Van der Hulst et al,[67] who found parenteral glutamine supply to decrease the number of intraepithelial lymphocytes.

From our data, it is not possible to decide whether glutamine-containing amino acid solutions are clinically beneficial or detrimental. However, in the past, various experimental studies in different models of mucosal injury demonstrated glutamine supplementation to improve mucosal healing (eg, Wischmeyer et al,[56] Ersin et al[57]). Our data would be compatible with such a hypothesis.

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