Enteral glutamine stimulates protein synthesis and decreases ubiquitin mRNA level in human gut mucosa

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Submitted 6 September 2002; accepted in final form 1 April 2003

Materials and methods

Subjects and experimental design

Twenty healthy volunteers, male and female, participated in the study. They were 22 (median, range 20–25) yr old and had a mean body mass index of 21.7 kg/m² (19.5–23.9). All subjects were in good general health with no hepatic, renal, and DNA and protein content. In humans, glutamine-enriched parenteral nutrition maintained villus height and limited the increase of gut permeability (42). Treatment of patients with glutamine, growth hormone, and diet modifications after gut resection was reported to be beneficial on water and electrolyte absorption in an early uncontrolled study (5), but this has not been confirmed by more recent controlled studies (36).

Additionally, enteral infusion of a high glutamine load in volunteers altered whole body leucine fluxes in a manner indicating a reduced protein oxidation and an increased protein synthesis (16). The beneficial effects of glutamine on gut mucosa could be partly due to a stimulation of protein synthesis as shown in animal studies, in vitro (17) and in vivo (41). In previous studies, we have shown that glutamine was well absorbed in human intestine (13) and stimulated ~40% duodenal protein synthesis in hypercatabolic subjects, although this effect only approached statistical significance (4). Furthermore, an improvement of protein balance in the gut could result from an inhibition of proteolysis. In main organs such as the liver and the muscle, the degradation of proteins results from the activity of the three major systems, the lysosomal (cathepsins), the Ca⁺²-activated (calpains), and the ATP-ubiquitin-dependent proteolytic pathways. These pathways have been reported in rat intestinal mucosa (35), but data in human mucosa are still limited. The aim of this study was to investigate the effects of enteral glutamine on gut mucosal protein synthesis and on the mRNA levels for the main proteolytic systems in healthy volunteers. The effects of glutamine on whole body protein turnover were also assessed.

Materials and methods

Subjects and experimental design

Twenty healthy volunteers, male and female, participated in the study. They were 22 (median, range 20–25) yr old and had a mean body mass index of 21.7 kg/m² (19.5–23.9). All subjects were in good general health with no hepatic, renal,
or cardiac dysfunction or any medical or surgical digestive past history. The study was approved by the local ethics committee, and the subjects gave their written informed consent. The subjects were divided into two groups of 10 subjects (group 1 and group 2), and each subject was studied on two occasions in random order. During 3 days, all subjects consumed a controlled diet providing 30 kcal and 1.2 g protein-kg body wt−1 day−1. The meals were prepared by the hospital dietary unit. The study was started at 8:00 AM on day 4 after a 12-h overnight fast. A nasogastric feeding tube was placed in the stomach. Subjects from group 1 received during 6 h either glutamine (0.8 mmol·kg body wt−1·h−1 in saline) or the same infusion rate of normal saline (4.5 ml·kg body wt−1·h−1), in a random order with a 15-day interval among studies. Subjects from group 2 received either glutamine (0.8 mmol·kg body wt−1·h−1) or an isotonic and isoosmolar mixture of amino acids (Gly/Ala/Ser/Pro/Asp/Asn/His, 2/2/4/4/7/10/10). We conceived this amino acid solution instead of a balanced solution with all 20 amino acids because we wanted this control solution to be both isonitrogenous and isoosmolar to glutamine to limit any interference due to fluid movements and variations of intracellular cell volume. Thus enterally infused solutions were isoosmolar (saline: 308 mosM; glutamine: 311 mosM; nonessential amino acids: 310 mosM), and rate of fluid infusion was similar (4.5 ml·kg body wt−1·h−1). After baseline blood and air samples were obtained, a bolus infusion of tracers was performed providing 6 μmol·kg body wt·[1-^2H_5]phenylalanine [90% mol percent enrichment (MPE); Mass Trace], and 6 μmol·kg body wt·[1-13C]leucine (99% MPE; Mass Trace) and 6 μmol·kg body wt·[2-^2H_4]tyrosine (99% MPE; Mass Trace). After the bolus, 6 μmol·kg body wt−1·h−1 of [1-^2H_2]phenylalanine and 6 μmol·kg body wt−1·h−1 of [2-13C]leucine were intravenously infused during 6 h. In addition, subjects from group 1 received 6 μmol·kg body wt−1·h−1 of [1-^13C]leucine (99% MPE; Mass Trace) via the nasogastric tube during 6 h. Tracers were performed for sterility and pyrogenicity and were diluted in saline in the hospital pharmacy on the evening before study and kept at 4°C until use.

Arterialized blood samples were taken from a vein in the contralateral hand of the intravenous infusions, the hand being placed in a heating box, before the infusion (baseline sample) and at regular intervals during the last 2 h of infusion. Before and during isotope infusion, carbon dioxide production was repeatedly measured over 20-min periods by means of indirect calorimetry (Deltatrac II; Datex, Helsinki, Finland); the variation coefficient of V\textsubscript{CO2} measurement was <10%. Breath samples were collected in bags for analysis of \textsuperscript{18}O\textsubscript{2} enrichment by infrared spectrometry (IRIS; Wagner).

The upper endoscopy (Olympus XQ10) was performed, for practical and safety reasons, 30 min after the end of the intravenous tracer and enteral amino acid infusions. At endoscopy, intragastric residual volumes ranged from 20 to 150 ml, which were aspirated before biopsy sampling in the duodenum. Eight mucosal biopsies were taken from the distal duodenum: two biopsies were fixed in formalin for histological analysis and two series of three biopsies were immediately frozen in liquid nitrogen, either as such for isozone analysis or sunk in guanidine isothiocyanate for mRNA analysis, and stored at −80°C.

Sample Analysis

Mucosal tissue samples were processed as previously described (4). Briefly, mucosal tissue samples were quickly rinsed in ice-cold 0.9% NaCl and immediately ground. Proteins were precipitated with 10% TCA, and free amino acids were then separated from the supernatant. The supernatant was prepared as plasma to measure free amino acid enrichment. The protein pellet was dissolved in 1 M NaOH and then hydrolyzed in 6 M HCl at 110°C for 18 h to allow analysis of the enrichment of amino acid released from protein hydrolysis.

Calculations

The fractional synthesis rate (FSR) of duodenal mucosal protein was calculated as follows: FSR (%/day) = (Et − E0)/E0 × 100, where Et is the enrichment in tissue protein at time t (%); E0 is the natural abundance of the labeled amino acid in intestinal mucosal protein (%); Ep is the enrichment of the precursor pool at plateau (%); and t is the duration of the tracer infusion (h). The baseline isotope enrichment was determined in normal duodenal biopsies from ambulatory patients undergoing endoscopy for medical reasons. The precursor pool used was the intracellular free amino acid pool. Whole body leucine, KIC, and phenylalanine fluxes were calculated according to standard isotopic dilution equations as previously described (8), by using steady-state plasma enrichment level during the last 2 h of continuous tracer infusion. Although [\textsuperscript{13}C]leucine enrichment was measured, tyrosine kinetics were estimated from phenylalanine kinetics as previously described (40).

Plasma amino acid concentrations. Plasma amino acid concentrations were measured by using an amino acid analyzer (Biotronik LC3000; Eppendorf).

Plasma hormone concentrations. Plasma concentrations of insulin and IGF-I were analyzed by radioimmunoassay.

RT-PCR. Mucosal RNA were extracted from biopsies by a modified extraction method as previously described (9). The quality and quantity of total RNA were determined by spectrophotometry by using the absorbance at A\textsubscript{260}/A\textsubscript{280} nm. The integrity was also controlled by visualization of 18S and 28S ribosomal bands. RNA expression of ubiquitin, cathepsin D, and m-calpain was studied by RT-PCR. The RT reaction was performed as previously described (9). RT products were amplified by PCR by using sense and antisense primers specific for proteolytic systems and GPDH as an internal standard: ubiquitin, 5'-GTTGAGCCCAAGTGACAC-3' and 5'-CTCTGAGACCCGAGC-5'; cathepsin D, 5'-CATTGTTGACACAGGACCTT-3' and 5'-GATAAGCCGGCGGATGAAGA-5'; m-calpain, 5'-TTGACAACTGCGTCTGAGGAG-3' and 5'-TGACAACTGCGTCTGAGGAG-5'; GPDH, 5'-GTACATCCGACACTTGGTG-3' and 5'-GAGCGAAGTGGATGCGGACT-5'. The PCR reaction mixture (25 μl) consisted of sense and antisense primers (50 pmol each), 1 unit of Thermoprime Plus ADN Polymerase (ABgene), 200 μM of each of the four dNTPs, 1 × PCR buffer (Promega) supplemented with 2.5 mM MgCl\textsubscript{2} (Promega), 0.5 μCI of \textsuperscript{32}PdATP (Amersham Pharmacia Biotech), and 5 μl of RT cDNAs. The initial DNA amplification was performed by 21 (ubiquitin, cathepsin D, and GPDH) or 28 cycles (m-calpain) consisting of denaturation for 30 s at 95°C, primer annealing for 40 s at 60°C, and primer extension for 40 s at 72°C by using a thermal cycler (model PTC 200; MJ Research).
PCR products were electrophoresed on a 6% polyacrylamide gel. After 2 days of exposure the autoradiogram bands were analyzed by an image analyzer (Biocon; Lecophor). The levels of amplified product were normalized to constant amounts of GAPDH mRNA. The number of PCR cycles in each system was chosen within linear phase to use this assay as a relative measure of gene expression. For each RNA, RT-PCR was performed three times.

Statistical Analysis

Results are expressed as median (range). To evaluate the effects of glutamine, amino acids, or saline, statistical analyses were assessed by using a nonparametric paired (Wilcoxon) or unpaired test (Mann Whitney U-test).

RESULTS

Group 1: Glutamine vs. Saline Infusion

Plasma, intracellular, and protein enrichments are displayed in Table 1. In group 1, FSR was increased after glutamine infusion compared with saline infusion whatever tracer was used (Fig. 1): intravenous tracers ([2H5]phenylalanine and P = 0.06 for [13C]leucine) or enteral tracer (P < 0.05 for [2H3]leucine). The plateau of plasma enrichments was obtained after 3 h of infusion for the different tracers (data not shown). After glutamine infusion, both leucine endogenous rate of appearance (Ra) and non-oxidative disposal (Table 2) decreased by 10% (P < 0.05) compared with saline infusion. Phenylalanine and leucine fluxes were not different between glutamine and amino acid infusion, except for a slight yet significant (P < 0.05) increase of leucine oxidation after glutamine infusion (Table 2).

The mRNA level of ubiquitin was significantly decreased (P < 0.05, Table 3) after glutamine infusion compared with saline infusion, whereas cathepsin D and m-calpain mRNA levels remained unchanged.

Enteral glutamine infusion increased plasma glutamine concentration compared with baseline (P < 0.05, Table 4). Plasma essential amino acid concentrations decreased after both saline and glutamine infusions compared with baseline (P < 0.05); plasma essential amino acid concentrations were significantly lower after glutamine compared with saline infusion (P < 0.05).

Enteral glutamine infusion increased plasma insulin concentration at 6 h compared with saline (P < 0.05) but had no influence on IGF-1 concentration (Table 5).

Group 2: Glutamine vs. Amino Acids Infusion

In group 2, whatever the tracer used, FSR was increased to the same extent by glutamine and amino acid infusion compared with saline infusion (P < 0.05, unpaired test); there was no significant difference for FSR after glutamine or amino acid infusion (Table 1). Phenylalanine and leucine fluxes were not different between glutamine and amino acid infusion, except for a slight yet significant (P < 0.05) increase of leucine oxidation after glutamine infusion (Table 2).

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Table 1. Isotopic enrichment in different pools in 13CO2 and VCO2 production

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<tr>
<th></th>
<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td></td>
<td>Saline</td>
<td>Glutamine</td>
</tr>
<tr>
<td>[2H3]phenylalanine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma aa</td>
<td>11.8(8.7–13.6)</td>
<td>12.9(8.0–14.6)</td>
</tr>
<tr>
<td>Intracellular aa</td>
<td>3.7(3.0–5.0)</td>
<td>3.4(1.8–5.0)</td>
</tr>
<tr>
<td>Protein</td>
<td>0.7(0.6–0.9)</td>
<td>0.9(0.7–1.2)</td>
</tr>
<tr>
<td>[2H3]tyrosine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma aa</td>
<td>1.6(1.4–2.2)</td>
<td>1.7(0.9–2.2)</td>
</tr>
<tr>
<td>[13C]leucine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma aa</td>
<td>5.5(4.9–7.0)</td>
<td>6.2(4.2–7.3)</td>
</tr>
<tr>
<td>Intracellular aa</td>
<td>1.6(1.3–2.5)</td>
<td>1.4(1.2–1.8)</td>
</tr>
<tr>
<td>Protein</td>
<td>0.3(0.2–0.3)</td>
<td>0.4(0.2–0.6)</td>
</tr>
<tr>
<td>13CO2</td>
<td>0.010(0.007–0.013)</td>
<td>0.011(0.006–0.015)</td>
</tr>
<tr>
<td>VCO2</td>
<td>159(133–205)</td>
<td>163(126–211)</td>
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</table>

Values are medians (range) from 7–9 subjects. Plasma, intracellular amino acids (aa), protein, and expired 13CO2 enrichments are expressed in mol %excess. Volume of expired CO2 (VCO2) is expressed as milliliters per minute. [2H3]leucine enteral infusion was performed only in subjects from group 1.
Table 2. Whole body phenylalanine and leucine fluxes

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<th>Group 1</th>
<th>Group 2</th>
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<tbody>
<tr>
<td></td>
<td>[3H]phenylalanine</td>
<td></td>
</tr>
<tr>
<td>Ra</td>
<td>39(32–54)</td>
<td>36(27–43)</td>
</tr>
<tr>
<td>HOxRd</td>
<td>0.9(0.6–1.7)</td>
<td>0.8(0.5–1.0)</td>
</tr>
<tr>
<td>NHoxPD</td>
<td>43(37–58)</td>
<td>41(32–49)</td>
</tr>
<tr>
<td>[13C]leucine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ra</td>
<td>89(80–104)</td>
<td>79(63–90)*</td>
</tr>
<tr>
<td>OxRd</td>
<td>13(11–19)</td>
<td>13(10–19)</td>
</tr>
<tr>
<td>NoxLD</td>
<td>86(76–100)</td>
<td>78(67–84)*</td>
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</table>
| Values are medians (range) from 7–9 subjects. Phenylalanine and leucine endogenous rate of appearance (Ra), rates of oxidation (OxRd), and hydroxylation (HOxRd), rate of nonoxidative leucine disposal (NOxLD), and rate of nonhydroxylative phenylalanine disposal (NHoxPD) are expressed as μmol·kg body wt \(^{-1}\)·h \(^{-1}\). *P < 0.05 (paired test), glutamine vs. saline infusion (group 1) and glutamine vs. amino acid infusion (group 2). †P < 0.05 (unpaired test), amino acid (group 2) vs. saline (group 1) infusion.

The ubiquitin mRNA level was significantly decreased after glutamine compared with amino acid infusion (P < 0.05, Table 3). The intraindividual comparison of mRNA expression (Fig. 2) showed that the mRNA level decreased in 9 of 10 subjects for ubiquitin and in 6 of 10 subjects for cathepsin D. However, cathepsin D and m-calpain mRNA expression was not statistically different after glutamine or amino acid infusion.

Plasma nonessential amino acids increased after both amino acids and glutamine infusions compared with baseline (P < 0.05, Table 4). Plasma glutamine concentration was also increased after both amino acid and glutamine infusions compared with baseline (P < 0.05). Essential amino acid concentration was decreased only after glutamine infusion compared with baseline (P < 0.05).

Plasma insulin concentration at 6 h was increased to the same extent by glutamine and amino acids compared with saline (P < 0.05, Table 5). IGF-I concentration was not significantly influenced.

DISCUSSION

The effects of glutamine supply on gut protein synthesis have been studied previously mainly in animal models. In nonstressed animals, glutamine did not influence gut protein FSR when given intravenously to rats (15) or dogs (25) but increased FSR when given by the enteral route to piglets (41). Glutamine also enhanced protein synthesis in vitro in isolated enterocytes from rats (17).

Table 3. mRNA levels for ubiquitin, cathepsin D, and m-calpain in human duodenal mucosa

<table>
<thead>
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<th>Group 1</th>
<th>Group 2</th>
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<tr>
<td></td>
<td>Saline</td>
<td>Glutamine</td>
</tr>
<tr>
<td></td>
<td>Amino acids</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>100(80–110)</td>
<td>100(80–110)</td>
</tr>
<tr>
<td>Cathepsin D</td>
<td>100(80–154)</td>
<td>100(80–154)</td>
</tr>
<tr>
<td>m-Calpain</td>
<td>100(18–151)</td>
<td>100(18–151)</td>
</tr>
<tr>
<td></td>
<td>110(31–152)</td>
<td>100(18–151)</td>
</tr>
<tr>
<td></td>
<td>100(21–132)</td>
<td>100(21–132)</td>
</tr>
<tr>
<td></td>
<td>85(15–144)</td>
<td>85(15–144)</td>
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</tbody>
</table>

Values after glutamine infusion are medians (range) expressed as percent of saline infusion (group 1) and percent of amino acids infusion (group 2). *P < 0.05 (paired test), glutamine vs. saline infusion (group 1) and glutamine vs. amino acid infusion (group 2).

Available studies (3, 29) on human gut mucosal protein synthesis are still limited and have measured the FSR of gut mucosal protein mainly in the postabsorptive state in healthy humans. In these studies, duodenal protein FSR approached 30–50% per day, a value much higher than that of other tissues such as liver or muscle (32). Surprisingly, feeding had no effect on gut FSR (3). In celiac patients, gut FSR was still higher than in healthy subjects, probably due to the high proliferation rate of the mucosa (28). In contrast, jejunal FSR was reduced in short-bowel patients treated with a somatostatin analog (31). In a previous study from our group (4), a moderate enteral glutamine supply increased gut FSR ~40% in hypercatabolic humans, an effect however, that only approached statistical significance (P = 0.069) in this small group of subjects. In controlled clinical studies with glutamine in patients with short-bowel syndrome, only modest effects on gut absorptive functions were reported; however, gut protein synthesis was not assessed in these studies (5, 36). In the present study, by using both intravenous and enteral tracers (group 1), a high load of enteral glutamine increased markedly (almost two-fold) mucosal protein FSR compared with saline. Gut mucosal FSR measured with the enteral leucine tracer was still higher than with both intravenous tracers, which is in accordance with previous reports (44) showing that gut mucosa preferentially incorporates luminal amino acids for its protein synthesis. In group 2, FSR was increased to a similar extent by infusion of an isonitrogenous mixture of amino acids, indicating that a high load of glutamine or nonessential amino acids is able to stimulate gut FSR. In the present study, intravenous infusion of tracers was stopped 30 min before sampling of biopsies. The calculation of FSR relies on the enrichment in the amino acid intracellular pool and in the mucosal protein at the time of sampling of the biopsy. Although it cannot easily be measured repeatedly in the duodenum, it can be assumed that intracellular enrichment increases over time in parallel to plasma enrichment; achievement of a steady state of duodenal intracellular free amino acid enrichment after 2 to 3 h as for plasma enrichment (3, 4) looks likely. In our present study, although the enrichment may
begin to decline both in the intracellular and mucosal protein pool over 30 min after the end of tracer infusion, this seems unlikely to induce a major bias in the calculation of FSR and especially hamper its comparison among groups studied with the same protocol. Indeed, both leucine and phenylalanine intravenous tracers, although having different behavior in the splanchic bed, lead to similar estimations of FSR and showed its increase in response to glutamine. In addition, we have compared this study with previous ones by means of tissue/plasma enrichment ratios. Indeed, we have compared this study with previous ones using tracers, although having different behavior in the literature, this seems unlikely to induce a major bias in the present study averaged 0.31 and 0.26 in the saline and glutamine groups, respectively, which is quite similar to 0.34 and 0.24 in the control and glutamine groups, respectively, from our previous study (4). Similarly, the ratio of intracellular to plasma enrichment in the present study averaged 0.31 and 0.26 in the saline and glutamine groups, respectively, which is quite similar to 0.34 and 0.24 in the control and glutamine groups, respectively, from our previous study (4). Thus it seems likely that the FSR results in our present study are good estimates and should not differ markedly from those measured during ongoing tracer infusion. In addition, in the present study, subjects from group 1 also received an enteral infusion of tracer, [3H]leucine, together with intravenous [3H]phenylalanine and [13C]leucine. This enteral tracer infusion was stopped at the same time as the enteral glutamine or saline infusion, i.e., 30 min before biopsies were taken. Yet, at the beginning of the endoscopy, intragastric residual volumes ranged from 20 to 150 ml. So [3H]leucine was still present in the gastrointestinal lumen at the time of biopsy sampling. With the use of [3H]leucine enrichment in intracellular pool and mucosal protein pool, calculated FSR confirms the results obtained with intravenous tracers.

Table 5. Plasma insulin and IGF-I concentrations

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Glutamine</th>
<th>Amino acids</th>
<th>Glutamine</th>
</tr>
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<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin, &amp;p;M</td>
<td>20 (10–31)</td>
<td>39 (22–51)</td>
<td>30 (17–73)</td>
<td>36 (14–74)</td>
</tr>
</tbody>
</table>

Values are medians (range). Plasma insulin and IGF-I concentrations were assessed after 6-h enteral infusion. *P < 0.05 (paired test), glutamine vs. saline infusion (group 1); †P < 0.05 (unpaired test), amino acids (group 2) vs. saline (group 1).
essential amino acids, whereas the fed group in the study by Bouteloup-Demange et al. (3) received a mixed protein meal. These authors observed no significant effect of feeding on mucosal FSR. It is difficult, however, to make a direct comparison between the present study and that from Bouteloup-Demange et al. (3), because in the latter, subjects received a complete energy and protein diet. Among other differences with our amino acid mixture, this diet included branched-chain amino acids, which may have favored protein synthesis in the muscle, or in the liver, rather than in the gut (26). We also observed that our control nonessential amino acid mixture did stimulate gut FSR as potently as glutamine. This could be due either to the anabolic effects of one or several of the amino acids contained in the mixture or to the stimulation of glutamine de novo synthesis from these amino acids in the gut. Indeed, glutamine synthetase is very active in human stomach and also present in human duodenal mucosa (21). It may thus be hypothesized that the nonessential amino acid mixture did stimulate gut FSR as potently as glutamine. This could be due either to the anabolic effects of one or several of the amino acids contained in the mixture or to the stimulation of glutamine de novo synthesis from these amino acids in the gut.

In this latter study, glutamine was compared with a high glycine load. In the present study, glutamine infusion, but not amino acid mixture infusion, induced a decrease of plasma essential amino acids. This was likely due to the increase in insulin secretion and its suppressive effect on whole body proteolysis as suggested from the leucine Ra data (Table 2).

Protein metabolism in the gut results from the balance between synthesis and degradation. However, the regulation of proteolysis in human gut has been poorly documented until now, and we are not aware of studies reporting the mRNA levels for components of the proteolytic pathways in human gut. The influence of nutrients on gut proteolysis has been studied only in animals (1, 30). Several pathways for the degradation of protein have been described in major organs such as the liver and the muscle (39), including the lysosomal, Ca\(^{2+}\)-activated, and ATP-ubiquitin-dependent proteolytic pathways. This last pathway is believed to be the most important one (39). In the gastrointestinal tract, little information is available, although all three processes have been identified in the small intestine of the rat (19, 35). The expression of the components of proteolytic pathways was increased during fasting in rats (35) but not during short-term underfeeding in ewes (30). Increased gut proteolysis may contribute to mucosal atrophy in the absence of adequate nutritional supply, in addition to a reduction of mucosal protein synthesis (43).

Cathepsin D is the major aspartic proteinase of the lysosomal compartment, contributing to the degradation of long-lived cytoplasmic proteins. It has been reported that cathepsin D may contribute to intestinal damage and to the activation of systemic inflammatory response after hemorrhagic shock in rat (14). During total parenteral nutrition in rats, intestinal cathepsin
activity increases and could be implicated in gut barrier alteration (24). In the present study, the mRNA level for cathepsin D in duodenum was not modified by glutamine infusion.

The Ca\(^{2+}\)-activated calpain system is ubiquitous in cells but has rarely been documented in intestine. The mRNA level for m-calpain in intestine increases during fasting in rats (35). In addition, m-calpain has been implicated in the differentiation of enterocytes (19).

The use of a calpain inhibitor in a model of experimental colitis in rat reduced the degree of colitis (11). In the present study, the mRNA level for m-calpain in intestine increases during fasting in rat (12). Therefore, it is possible that the use of an inhibitor of the ubiquitin pathway may be critical to limit NF-\(\kappa\)B activation (23).

Indeed, the inhibiting effect of glutamine on intestinal protein synthesis (6). On the other hand, insulin reduces muscular ATP-ubiquitin-dependent proteolytic pathway activity (10); however, in the present study, the inhibiting effect of glutamine on intestinal protein synthesis was observed after both glutamine and amino acid infusion. Indeed, recent data indicate that insulin can support intestinal protein synthesis (6). On the other hand, insulin reduces muscular ATP-ubiquitin-dependent proteolytic pathway activity (10); however, in the present study, the inhibiting effect of glutamine on intestinal protein synthesis (6).

In summary, enteral infusion of glutamine in humans stimulates protein synthesis in a nonspecific way but may limit mucosal proteolysis through a specific inhibition of the ubiquitin pathway. Glutamine may thus be beneficial to gut protein balance in humans. Whether the antiproteolytic effect of glutamine may also contribute to the regulation of the inflammatory response in intestinal mucosa deserves further investigation.

The skillful assistance of Brigitte Maurer and Dany Laforest (Laboratory of Medical Biochemistry, CHU Rouen) for tape analysis of isotope preparations (Prof. Philippe Arnaud). The authors also thank Richard Medeiros for his advice in editing the manuscript.

DISCLOSURES

This study was supported, in part, by Grant 96-035HP from Conseil Regional Haute-Normandie and from Institut de Recherche sur les Maladies de l’Appareil Digestif.

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