Effects of enteral glutamine on gut mucosal protein synthesis in healthy humans receiving glucocorticoids

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Glutamine is the major fuel for enterocytes (28, 32), and it promotes growth, metabolism, structure, and function of intestinal mucosa, especially in situations of gut injury (27, 32). Numerous studies have shown the benefits of glutamine enteral or parenteral nutritional supplementation on the gut barrier (14, 21, 25, 26, 30). In vitro, glutamine stimulates intestinal cell proliferation (24, 26). In animal studies, glutamine supply decreases the gut mucosal alterations observed after prolonged starvation (14, 21) or during experimental enterocolitis (25) by increasing the weight of the mucosa, the height of villi, and DNA and protein content. In humans, glutamine-enriched parenteral nutrition has been reported to maintain villus height and to limit the increase of gut permeability (30). The mechanism by which glutamine exerts its beneficial effects on gut mucosa is not totally known, but it could be partly due to a stimulation of protein synthesis, as shown in animal studies in vitro (11, 12) and in vivo (29, 33). Glutamine is well absorbed in human intestine (8), but its effect on gut mucosal protein synthesis has not been documented.

The aim of this study was to investigate, in the fed and fasted states, the effect of enteral glutamine supplementation on gut mucosal protein synthesis during a hypercatabolic state induced by treatment with glucocorticoids in healthy young volunteers. The study of healthy subjects made hypercatabolic by glucocorticoid administration represents a good model of what is observed in a pathologic situation during trauma or surgical stress, because cortisol stimulates both whole body protein catabolism and intestinal protein catabolism (3, 7) and glutamine intestinal utilization (1, 9).

MATERIALS AND METHODS

Subjects and experimental design. Thirty-two healthy volunteers, male and female, participated in the study. They were 21.2 (range 20–25) yr old and had a mean body mass index of 22.1 (range 18.1–25.1) kg/m². All subjects were in good general health without any hepatic, renal, or cardiac dysfunction or any medical or surgical digestive history. None was taking medication on a regular basis. The study was approved by the local ethical committee, and the subjects gave written informed consent. The subjects were divided into four groups. Over 5 days, all subjects consumed a controlled diet providing 35 kcal·kg⁻¹·day⁻¹ and 1.85 g protein·kg⁻¹·day⁻¹. The meals were prepared by the hospital dietary unit. On days 6 and 7, two groups continued to be fed...

THE INTESTINE NOT ONLY PLAYS a primary role in the digestion and absorption of nutrients but is also essential as a barrier to enteric flora protecting the host from intraluminal bacteria and their toxins. This intestinal barrier can be altered during intestinal pathologies, drug therapies, hypercatabolic situations, long-term fasting, or prolonged total parenteral nutrition (17, 31). This disruption of the intestinal barrier could result in bacterial translocation and favor an acute hypercatabolic state leading to multiple organ failure (31).
orally with regular food (F groups), whereas the other two groups were fed enterally with a liquid diet (Sondalis Iso, Nestlé Clinical Nutrition, Sèvres, France), under medical supervision in the clinical study unit (TF groups). The two diets provided 35 kcal·kg⁻¹·day⁻¹ and 1.31 g protein·kg⁻¹·day⁻¹. In addition, all four groups received orally either 0.5 g·kg⁻¹·day⁻¹ of glutamine (F-Gln and TF-Gln groups) or a 50:50 (wt/wt) mixture of alanine and glycine (0.55 g·kg⁻¹·day⁻¹) as nitrogen equivalent (F-Neq and TF-Neq groups). During days 6 and 7, all subjects received 0.42 mg/kg of prednisolone orally two times per day, at 8:00 AM and 8:00 PM.

The study was started at 8:00 AM on day 8 to measure intestinal mucosal protein synthesis. A nasogastric feeding tube was placed in the subjects of the two F groups. The F groups were studied in the fasted state, after a 12-h overnight fast, whereas enteral nutrition was maintained at the same rate (93 ml/h) in TF groups. The F-Gln and TF-Gln groups received glutamine (0.02 g·kg⁻¹·h⁻¹) via the gastric route either in normal saline (120 ml/h) for the F-Gln group or in the liquid diet for the TF-Gln group, whereas the other two groups received the nitrogen equivalent (0.022 g·kg⁻¹·h⁻¹) i.e. normal saline (120 ml/h) for the F-Neq group or in the liquid diet for the TF-Neq group, over 4 h. A primed (equivalent to 1 h of infusion), continuous intravenous infusion of tracers was performed, providing 6 mol·kg⁻¹·h⁻¹ for [¹³C]leucine (99% mle percent enrichment (MPE), Mass Trace, Woburn, MA) and 5 mol·kg⁻¹·h⁻¹ for L-[ring-²H₅]phenylalanine (90% MPE, Mass Trace) over 5 h. The tracers had been tested for sterility and pyrogenicity. Tracers were diluted in saline in the hospital pharmacy on the evening before the study and kept at 4°C until use. The subjects of the four groups received an intravenous infusion of hydrocortisone at a rate of 140 µmol·kg⁻¹·h⁻¹ over 4 h.

Arterialized blood samples were taken before the infusion (baseline sample) and at regular intervals during the last 2 h of infusion, from a vein in the hand (placed in a heating box) not used for the intravenous infusions. One hour after the end of enteral infusion but with intravenous tracer infusion being maintained, an upper endoscopy was performed (Olympus XQ10). Mucosal biopsies were taken from the distal duodenum, immediately frozen in liquid nitrogen, and stored at −80°C until being analyzed.

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 isolated from the supernatant. The supernatant was prepared as plasma to measure free amino acid enrichment (6). 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 protein enrichment.

Enrichment of [¹³C]leucine, [¹³C]ketoisocaproate ([¹³C]KIC), and [³H₅]phenylalanine was determined in the plasma and mucosal intracellular free amino acid pools and in the mucosal proteins by gas chromatography-mass spectrometry (MSD 5972, Hewlett Packard, Palo Alto, CA), using tert-butylidimethylsilyl derivatives (6). Appropriate standard curves were run simultaneously with determination of enrichment.

Calculations. The fractional synthesis rate (FSR) of duodenal mucosal protein was calculated as (19) FSR (%/day) = \( \left( \frac{E_p}{E_i} \right) \times 1\text{h} \times 24 \times 100 \), where \( E_i \) is the isotopic enrichment achieved in tissue protein at time t (% corrected for baseline values obtained in normal duodenal biopsies from ambulatory patients undergoing endoscopy for medical reasons), \( E_p \) is the enrichment of the precursor pool at plateau (% precursor pool used was the intracellular free amino acid pool), and t is the duration of the tracer infusion (h).

Statistical analysis. Results are expressed as means ± SE. To evaluate the effects of glutamine, feeding or fasting state, and tracer, statistical differences were assessed using a nonparametric analysis of variance and a nonparametric paired or unpaired test as appropriate (Mann-Whitney and Wilcoxon).

RESULTS

On the morning of tracer infusion, cortisolemia was 1,332 ± 135, 1,387 ± 188, 1,448 ± 183, and 1,155 ± 116 nmol/l (normal range = 250–850 nmol/l) in F-Gln, F-Neq, TF-Gln, and TF-Neq groups, respectively. The plateau of plasma enrichment was obtained after 3 h of infusion for the two tracers. Enrichment in the free amino acid pools and in mucosal proteins is reported in Table 1. Intracellular free phenylalanine enrichment was 24, 34, 30 and 34% of plasma phenylalanine enrichment, and intracellular free leucine enrichment was 29, 30, 25 and 29% of plasma leucine enrichment for F-Gln, F-Neq, TF-Gln, and TF-Neq groups, respectively (no significant difference between groups). Plasma KIC enrichment was 81, 77, 74, and 74% of plasma leucine enrichment for F-Gln, F-Neq, TF-Gln, and TF-Neq groups, respectively.

Values of FSR calculated with the free intracellular amino acids as precursor pool are reported in Table 2. Regardless of the tracer used to calculate FSR, there was no statistically significant difference between the four treatment groups. However, there was a trend for a higher FSR with Gln in the postabsorptive state (+49%) with leucine as tracer, although it failed to reach significance (P = 0.058).

When the values obtained with each tracer in a given group were compared, phenylalanine and leucine gave similar FSR for the groups studied in the postabsorptive state (103.7 ± 15.5 vs. 130.4 ± 22.4 and 75.9 ± 11.0 vs. 87.4 ± 9.3%/day, phenylalanine vs. leucine for F-Gln and F-Neq groups, respectively). In contrast, FSR obtained with phenylalanine were significantly lower than those obtained with leucine.

Table 1. Enrichment in different precursor pools and in mucosal protein for phenylalanine and leucine tracers

<table>
<thead>
<tr>
<th>Precursor Pool</th>
<th>F-Gln</th>
<th>F-Neq</th>
<th>TF-Gln</th>
<th>TF-Neq</th>
</tr>
</thead>
<tbody>
<tr>
<td>[¹³C]phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma aa</td>
<td>9.98 ± 0.66</td>
<td>9.57 ± 0.71</td>
<td>8.82 ± 0.40</td>
<td>8.47 ± 0.25</td>
</tr>
<tr>
<td>Intracellular aa</td>
<td>2.40 ± 0.33</td>
<td>3.27 ± 0.52</td>
<td>2.61 ± 0.28</td>
<td>2.82 ± 0.41</td>
</tr>
<tr>
<td>Protein</td>
<td>0.47 ± 0.04</td>
<td>0.45 ± 0.02</td>
<td>0.41 ± 0.04</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>[³H₅]phenylalanine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma aa</td>
<td>5.04 ± 0.37</td>
<td>4.99 ± 0.29</td>
<td>4.04 ± 0.11</td>
<td>4.31 ± 0.12</td>
</tr>
<tr>
<td>Intracellular aa</td>
<td>4.10 ± 0.34</td>
<td>3.83 ± 0.25</td>
<td>2.99 ± 0.17</td>
<td>3.18 ± 0.12</td>
</tr>
<tr>
<td>Protein</td>
<td>1.49 ± 0.24</td>
<td>1.40 ± 0.20</td>
<td>1.02 ± 0.13</td>
<td>1.23 ± 0.19</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mol% enrichment. aa, Amino acids; KIC, ketoisocaproate; F-Gln, orally fed subjects given glutamine studied in the fasted state; F-Neq, orally fed subjects given alanine and glycine as nitrogen equivalent studied in the fasted state; TF-Gln, enterally fed subjects given glutamine studied in the fasted state; TF-Neq, enterally fed subjects given alanine and glycine as nitrogen equivalent studied in the fasted state.
were two- to threefold higher than in the previous study. The FSR calculated with leucine in the present study was considered to be close to the true precursor pool (20), as indicated by the intracellular free amino acid as precursor pool, which was observed in the previous study (4). With the difference between the FSR of the fed and fasted groups, fasting duration is a major issue because no comparison was made using the same analytic methods and with a similar protocol except for the fasted state, which was a 36-h fast in the previous study and a 12-h fast in our study. However, we do not think that this difference could be explained by a dilution of the tracer in the intracellular pool by the unlabeled amino acids released by proteolysis. This is in agreement with Park et al. (22), who found a decrease of gut mucosal weight and protein content in rats receiving glucocorticoids, indicating that proteolysis was increased even more than protein synthesis. Accordingly, dexamethasone increased gut mucosal protein fractional breakdown but had no significant effect on fractional protein synthesis in neonatal pigs (5).

In addition to this stimulating effect on mucosal protein synthesis, corticoids probably also stimulate intestinal mucosal proteolysis, because the leucine intracellular-to-plasma enrichment ratios are lower in hypercortisolemic subjects compared with nonhypercortisolemic subjects (4). When phenylalanine is used as tracer, the effect of corticoids is less marked. This may be related to a difference in metabolism of phenylalanine and leucine in splanchic tissues (16).

The effects of glutamine supply on gut FSR have not been documented previously in humans.

We decided to study healthy subjects treated with glucocorticoids because some studies in healthy humans have shown that hypercortisolemia induced a 15–25% increase of whole body proteolysis during fasting (3, 7) or fed (3) state and thus reproduced what could be observed in hypercatabolic situations such as surgery or sepsis. To our knowledge, there are no data about the effects of hypercatabolic situations on intestinal protein synthesis rate in humans, but in animals sepsis increases intestinal protein synthesis both in vitro (12) and in vivo (13, 33). Moreover, the stimulating effects of glutamine on intestinal protein synthesis seem to be more important during hypercatabolic situations than in physiological situations (10, 12, 33). Thus, in our study, the subjects were given glucocorticoids and became markedly hypercortisolemic (see RESULTS). By comparing the results of this study with those obtained in a recent study performed in healthy, nonhypercortisolemic humans (4), we can attempt to determine the effects of glucocorticoids on fractional protein synthesis rate of intestinal mucosa. Of course, this is an indirect comparison, but the two studies were performed using the same analytic methods and with a very similar protocol except for the fasted state, which was a 36-h fast in the previous study and a 12-h fast in this study. However, we do not think that this difference in fasting duration is a major issue because no difference between the FSR of the fed and fasted groups was observed in the previous study (4). With the intracellular free amino acid as precursor pool, which is considered to be close to the true precursor pool (20), the FSR calculated with leucine in the present study were two- to threefold higher than in the previous study (4). This was true for both the fasted state (130 and 87%/day vs. 40%/day in previous study) and the fed state (126 and 100%/day vs. 48%/day in previous study). Our observation of an increased FSR in gut mucosa of hypercortisolemic subjects is consistent with that obtained during sepsis in animal studies (12, 13, 33). When phenylalanine is used as tracer, the effect of corticoids is less marked. This may be related to a difference in metabolism of phenylalanine and leucine in splanchic tissues (16).

The effects of glutamine supply on gut protein synthesis have only been studied previously in animal models in vitro and in vivo. In nonhypercatabolic animals, glutamine does not influence gut protein FSR when given intravenously (10, 15) but increases FSR when given by the enteral route (29). Studies in vitro have also shown that glutamine enhanced enterocyte protein synthesis (11) and that this effect was maximal during sepsis (12). Accordingly, in septic rats, an intravenous glutamine supply enhanced FSR of gut mucosal protein (33). To our knowledge, no previous study had been performed in humans to evaluate the effect of glutamine supply on gut mucosal protein synthesis. In our study, in the postabsorptive state, the FSR of gut mucosal protein were 87 and 76%/day in the control group and 130 and 104%/day in the glutamine group with leucine and phenylalanine as tracers, respectively. The difference approached significance with leucine (P = 0.058). We may have failed to attain statistical significance because we studied a limited number of subjects and there was a rather wide interindividual variation of the gut mucosa FSR within and between our four groups, which were unpaired. The stimulating effect of glutamine on gut FSR might have been more easily detectable in subjects with higher doses of cortisone or more potent catabolic synthetic glucocorticoids such as dexamethasone (5). Alternatively, larger doses of glutamine should be evaluated in humans. Finally, a key issue remains the potential effect of glutamine on mucosal protein breakdown, because the final catabolic or anabolic effect depends on the balance between FSR and fractional breakdown rate (FBR). Measurement of FBR is hardly possible in humans, and to our knowledge there are no animal data examining this effect.

### Table 2. Fractional synthesis rate of gut mucosal proteins calculated with free intracellular amino acids as precursor pool for phenylalanine and leucine tracers

<table>
<thead>
<tr>
<th></th>
<th>[H₄]phenylalanine</th>
<th>[¹³C]leucine</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-Gln</td>
<td>103.7 ± 15.5</td>
<td>130.4 ± 22.4</td>
</tr>
<tr>
<td>F-Neq</td>
<td>75.9 ± 11.0</td>
<td>87.4 ± 9.3</td>
</tr>
<tr>
<td>TF-Gln</td>
<td>83.5 ± 16.9*</td>
<td>126.0 ± 22.7</td>
</tr>
<tr>
<td>TF-Neq</td>
<td>69.6 ± 5.6*</td>
<td>100 ± 11.2</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in%/day. *P < 0.05, phenylalanine vs. leucine.
The potential ability for glutamine but not other amino acids to stimulate gut mucosal protein synthesis could be explained by its specific metabolism in the small intestine. Indeed, uptake of glutamine by the mucosal cells of the small intestine is far greater than that of the other amino acids, and several enterocyte membrane transport systems have a high affinity for glutamine (28, 32). From studies on glutamine enterocyte metabolism, we know that the activity of the glutaminase that catalyzes the hydrolysis of glutamine to glutamate and ammonia is very high in the enterocytes and a great proportion of glutamine is metabolized in the tricarboxylic acid cycle via the formation of glutamate and α-ketoglutarate, providing a major energy source for the enterocytes (28, 32). The majority of glutamine nitrogen is used to produce ammonia, alanine, and citrulline and is not directly incorporated in synthesized proteins (28, 32). In an in vitro study (11), stimulation of enterocyte protein synthesis was observed with glutamine but not with any of the other amino acids tested; moreover, this effect was similar to that obtained with acetocacetate and 3-hydroxybutyrate, known enterocyte fuels, and disappeared in the presence of a glutaminase inhibitor. Thus glutamine likely stimulates gut protein synthesis by provision of energy rather than by acting as a substrate for protein synthesis. In addition, glutamine is a precursor for nucleotide synthesis and facilitates nucleic acid synthesis (28) together with stimulation of cell proliferation (24, 26). Thus both the number of protein-synthesizing cells and their energy provision may have been supported by glutamine administration.

We studied subjects in both fasted and fed states. As observed in our previous study (4), there was no effect of nutritional state (fasting or feeding) on gut mucosa FSR. This suggests again that amino acids other than glutamine have minimal effects on gut protein synthesis (11). As previously reported, we also observed that the FSR calculated with phenylalanine are lower than those calculated with leucine, the difference being statistically significant in the fed state. This probably reflects a different behavior of the two amino acids in the intestinal mucosa (16).

In conclusion, during physiological hypercortisolism in humans, gut mucosal protein FSR is high. In this situation, a moderate enteral glutamine supply failed to demonstrate a significant effect on gut mucosal protein synthesis in the postabsorptive state and during feeding.

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