Effect of enteral glutamine on leucine, phenylalanine and glutamine metabolism in hypercortisolemic subjects

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1Groupe de Biochimie et Physiopathologie Digestive et Nutritionnelle and Institut Fédératif de Recherches Multidisciplinaires sur les Peptides No. 23, Faculté de Médecine-Pharmacie, 76183 Rouen Cedex; and 2Laboratoire de Nutrition Humaine, Université d’Auvergne, Centre de Recherche en Nutrition Humaine, 63000 Clermont-Ferrand, France

CLAEYSSENS, Sophie, Corinne Bouteiloup-Demange, Pierre Gachon, Bernadette Hecketserweiler, Eric Lerebours, Alain Lavoinne, and Pierre Déchelotte. Effect of enteral glutamine on leucine, phenylalanine and glutamine metabolism in hypercortisolemic subjects. Am J Physiol Endocrinol Metab 278: E817–E824, 2000.—The effect of enteral Gln on protein and Gln metabolism was investigated during experimental hypercortisolemia. Four groups of subjects that had received corticosteroids and either enteral Gln (0.5 g·kg⁻¹·day⁻¹ for 2 days) or isonitrogenous Ala-Gly were studied in a fasted or in a fed state. In either state, enteral Gln, compared with Ala-Gly, induced no statistically significant change in the endogenous rate of Leu appearance, an index of proteolysis, Leu oxidation, and nonoxidative Leu disposal, an index of protein synthesis, as studied by kinetics of [¹⁵N]Leu. Similar data were obtained from kinetics of [²H₅]Phe, resulting in an unchanged protein balance in both cases. In contrast, enteral Gln significantly decreased the endogenous rate of Gln appearance and Gln de novo synthesis in the fed state (P < 0.05) as estimated by the kinetics of [¹³C]Gln. This decrease in Gln de novo synthesis induced by Gln could contribute to spare amino acids in hypercatabolic patients.

protein metabolism; glutamine; stable isotopes; cortisol

GLN IS THE MOST abundant free amino acid in the body and is involved in various metabolic pathways. Its role in the regulation of protein metabolism has been suggested by numerous studies in animals and humans. In particular, intracellular free Gln concentration has been found to be correlated with the rate of muscle protein synthesis in experimental animal studies (24, 25), and this effect has been shown to be specific for this amino acid (19, 24). However, this correlation remains controversial since it was not found to be present in some animal (30, 31) and human studies (18). On the other hand, in healthy humans, enteral Gln has been reported to increase protein synthesis, as assessed by the nonoxidative Leu flux (15), whereas it has no effect on whole body protein breakdown, as assessed by the endogenous rate of Leu appearance (11, 15). Likewise, enteral Gln has been shown to decrease Gln de novo synthesis (14), which might contribute to its anabolic effect on protein synthesis.

In recent years, evidence has increased supporting the concept that Gln becomes a conditionally indispensable amino acid in critical illness and prevents protein wasting when exogenously supplied (13, 22, 32). Thus, in humans in a catabolic state, administration of either Gln, α-ketoglutarate, or a Gln-containing dipeptide has been shown to improve the clinical outcome and nitrogen balance (12, 26, 33). However, the mechanisms whereby Gln improves nitrogen balance and possibly affects whole body protein metabolism in patients in a catabolic state remain unclear (12, 17, 23). In these patients, measurements of whole body protein metabolism have shown that protein breakdown matches or overcomes protein synthesis (1, 7). Furthermore, the concomitant increase in plasma cortisol concentration has been shown to play a significant role in these changes (1). Indeed, when cortisol was administered to healthy subjects at a level that was similar to that found in critical illness, whole body protein breakdown was increased (2, 6, 10, 16), whereas protein synthesis slightly increased (6) or remained unchanged (2, 16), resulting in negative protein balance. This was associated also with an increased Gln flux through an increase of de novo synthesis (6, 10).

In the present study, we investigated the effects of an enteral Gln administration on protein and Gln metabolism in healthy subjects given cortisol to mimic the critical illness-induced protein hypercatabolism. The effect of Gln administration on protein metabolism was assessed from the kinetics of two indispensable amino acids, Leu and Phe. The effect of Gln administration was evaluated in the fasted and fed states compared with that induced by an isonitrogenous Ala-Gly administration.

MATERIALS AND METHODS

Subjects

Thirty-two healthy volunteers (24 males, 8 females) were included and randomly allocated to four groups of eight subjects (groups A, B, C, and D). They were 22.1 (20–25) yr
old and had a body mass index of 19.9 (18.1–25.1) kg/m². None of the subjects had a previous history of surgery, intestinal disease, or any hepatic, renal, digestive, cardiac, or metabolic dysfunction. All subjects were considered healthy after physical examination and screening with standard blood tests. No subjects were on regular medication. After clinical screening, subjects gave written informed consent to participate in the study. The protocol was reviewed and approved by the regional Ethical Committee of Haute-Normandie (Comité consultatif pour la Protection de la Personne dans la Recherche Biomédicale).

Diet and Experimental Procedure

The four groups of subjects underwent a 5-day prestudy equilibration diet followed by 2 days of prednisolone treatment, along with a calibrated diet plus a supplement consisting of either Gln or an isonitrogenous Ala-Gly mixture (Table 1). During the 5-day prestudy equilibration diet, all subjects received a standardized diet prepared by the Rouen University Hospital dietary kitchen on an outpatient basis. This diet provided 1.9 g protein · kg⁻¹ · day⁻¹ and 35 kcal · kg⁻¹ · day⁻¹ in three meals. Nonprotein energy was supplied as 35% lipids and 65% carbohydrates. The calorie and protein sources were the same for all volunteers over the 5 days of pretreatment diet. On the last evening, subjects had their dinner at 8:00 PM and remained fasted afterward.

On the morning of day 1, all subjects were admitted at 8:00 AM to the metabolic research ward. Their resting energetic expenditure (REE) was measured by indirect calorimetry (Deltatrac II; Datex Instrumentation, Helsinki, Finland) after an overnight fast; all subjects had REE within ±5% of predicted REE according to Harris and Benedict equations, and there was no difference for REE between groups (data not shown). After measurement of REE, the subjects from group B underwent a basal isotopic study (see below for details) to serve as controls for the evaluation of the effects of corticosteroids. In this group, the corticosteroid treatment was initiated only after the end of the isotopic study. All of the subjects received oral prednisolone (0.84 mg · kg⁻¹ · day⁻¹, given as 0.42 mg/kg 2 times/day, morning and evening) on day 1 and 1.4 g oral prednisolone to allow adequate steady-state conditions. During the 5-day pretreatment period before days 1–3 of study, subjects from groups A and B received a standardized oral diet further on (35 kcal · kg⁻¹ · day⁻¹, 1.4 g protein · kg⁻¹ · day⁻¹), whereas subjects from groups C and D were fed enterally via a nasogastric tube with a commercially available liquid diet (Sondalis Iso; Nestlé Clinical Nutrition, Sèvres, France) at a continuous rate providing 35 kcal · kg⁻¹ · day⁻¹ and 1.4 g protein · kg⁻¹ · day⁻¹. On the evening of day 2, subjects from groups A and B had their dinner and remained fasted afterward. In contrast, in subjects from groups C and D, enteral nutrition was maintained at the same rate used previously.

On days 1 and 2, subjects from groups B and D were given an oral Gln (0.5 g · kg⁻¹ · day⁻¹) supply. Subjects from groups A and C received an oral isonitrogenous supply of Ala-Gly (50:50 wt%, 0.55 g · kg⁻¹ · day⁻¹). Gln and Ala-Gly supplements were split in equal doses given with meals in groups A and B and were continuously infused with enteral solution in groups C and D. Thus the total protein intake provided by the oral or enteral diet and the supply during days 1 and 2 were maintained at the same level (1.9 g · kg⁻¹ · day⁻¹) as during the pretreatment period. Because our aim was to study groups in the fed state in adequate steady-state conditions, we used a commercially available enteral solution in groups C and D. Supplementation of Gln or Ala-Gly were then added to this solution.

At 8:00 AM on the morning of day 3, baseline blood and breath samples were collected, and the rate of CO₂ production (V̇CO₂) was measured by indirect calorimetry. Afterward, subjects from groups B and D received Gln (0.02 g · kg⁻¹ · h⁻¹) either in normal saline (group B) or in the liquid diet (group D) through a nasogastric tube. Subjects from groups A and C received an isonitrogenous infusion of Ala-Gly (0.022 g · kg⁻¹ · h⁻¹) either in normal saline (group A) or in the liquid diet (group C) via a nasogastric tube. These infusion rates were calculated to maintain over the 6 h of study on day 3 the same state of Gln or Ala-Gly as on days 1 and 2. For subjects from groups C and D, infusion of the liquid diet was also maintained on the morning of day 3 at the same rate as on days 1 and 2, thus providing 35 kcal · kg⁻¹ · day⁻¹ and 1.4 g protein · kg⁻¹ · day⁻¹ in the diet.

Isotopic Study

On the morning of day 1, control subjects (group B) underwent an isotopic study in the fasted state. In all treated groups, the isotopic study was carried out on the morning of day 3. Subjects from groups A and B were in the fasted state, whereas those from groups C and D were in the fed state. Tracers (Tracer Technologies, Sommerville, MA or Euriso-top, Saint Aubin, France) had been previously checked for purity, sterility, and the absence of pyrogen. Tracer solutions of the study were prepared by the hospital pharmacy in sterile conditions within 24 h of use and were stored at 4°C. In the morning, two short intravenous catheters were inserted, one in the antecubital fossa for isotopic and corticosteroid infusion and the other in a vein of the contralateral dorsal hand kept in a heating box for arterialized venous blood sampling. After two baseline blood and air samples for V̇CO₂ were obtained, a primed (corresponding to 1 h of infusion) constant infusion of L-[ring-¹³C]Phe [5 µmol · kg⁻¹ · h⁻¹, 90 moles percent excess (MPE)], L-[¹³C]Leu (6 µmol · kg⁻¹ · h⁻¹, 99 MPE), L-α-amino-¹⁵N]Gln (7 µmol · kg⁻¹ · h⁻¹, 99 MPE), and L-[¹⁵N]Tyr (5 µmol · kg⁻¹ · h⁻¹) was given intravenously over a 4-h period in all subjects. The isotope continuous infusion started at the same time as enteral administration of Gln or Ala-Gly supply in a saline or liquid diet. Arterialized blood samples (5 ml in

Table 1. Diet and experimental procedure

<table>
<thead>
<tr>
<th>Day 2</th>
<th>Day 3</th>
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<tr>
<td>(0.84</td>
<td>(140 µg · kg⁻¹ · h⁻¹)</td>
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<tr>
<td>mg · kg⁻¹ · day⁻¹</td>
<td>iv hydrocortisone)</td>
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<table>
<thead>
<tr>
<th>Cortisol</th>
<th>Fasted</th>
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<tr>
<td>+ Ala-Gly</td>
<td>Oral diet</td>
</tr>
<tr>
<td>+ Gln</td>
<td>Oral diet</td>
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<tr>
<td>Fed</td>
<td>Oral diet</td>
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<table>
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<tr>
<th>Oral prednisolone)</th>
<th>Enteral diet</th>
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<tbody>
<tr>
<td>No diet</td>
<td>enteral Ala-Gly</td>
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<tr>
<td>No diet</td>
<td>enteral Gln</td>
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<tr>
<td>Enteral diet</td>
<td>enteral Ala-Gly</td>
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<tr>
<td>Enteral diet</td>
<td>enteral Gln</td>
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</table>

During the 5-day pretreatment period before days 1–3 of study, subjects received a standardized oral diet. On the morning of day 1 control subjects underwent an isotopic study in the fasted state.
heminized tubes) were drawn at 150, 180, 200, 220, and 240 min after the initiation of the infusion and were centrifuged immediately at 4°C. The plasma was stored at -80°C until analyzed. Before and during isotope infusion, CO2 production was repeatedly measured by means of indirect calorimetry, and breath samples were collected in a Douglas bag and then transferred to Vacutainer tubes. For the four groups, additional blood samples were drawn at the end of tracer infusion for measurement of blood hormones and substrate levels.

Analytical Methods

The enrichments of plasma amino acids and metabolites were determined by electron impact ionization GC-MS (MSD 5972; Hewlett Packard, Palo Alto, CA) using the n-butyl ester N-trifluoroacetyl derivat for [1-13C]Leu, the trimethysilyl quinoxaline derivat for α-[1-13C]ketosoproprate (KIC), the methyl ester trifluoroacetyl derivat for [3H]Phe, [3H]Tyr, and [4H]Tyr as previously described (20), and the N-acetyl, n-propyl ester derivat for [4N]Gln as previously described (10). Appropriate standard curves were run simultaneously to the sample analysis. The breath sample [13CO2] enrichment was determined as previously described (2) by isotope ratio-mass spectrometry (VG Isotech; Fisons Instrument, Middletown, UK). Plasma concentrations of glucose and urea were analyzed by an enzymatic method, insulin and glucagon were analyzed by RIA, and cortisol was analyzed by chemiluminescent immunoassay. The plasma amino acid concentrations, except that of tryptophan, were determined on an amino acid autoanalyzer (Beckman, Anaheim, CA).

Calculations

Whole body protein turnover. Whole body protein metabolism was assessed from the kinetics of two indispensable amino acids (IAA), Leu and Phe, to give two independent estimates.

The whole body total appearance rate was determined at steady state using the equation

$$R_a = i(E_p / E_i - 1)$$

(1)

where $R_a$ is the rate of amino acid appearance into plasma (µmol·kg$^{-1}·$h$^{-1}$), $E_i$ and $E_p$ are the amino acid tracer enrichments (MPE) in the infuse and in the plasma at steady state, and $i$ is the tracer infusion rate (µmol·kg$^{-1}·$h$^{-1}$). In the fasted state, IAA were totally derived from whole body proteolysis. Thus the whole body total appearance rate represented the endogenous appearance rate of the IAA and gave an estimate of whole body protein breakdown. In the fed state, IAA derived from whole body proteolysis and from exogenous intake arising from the hydrolysis of dietary proteins. Because both splanchnic (gut and liver) and peripheral organs contribute to whole body protein and Gln turnover, the endogenous amino acid appearance rate was calculated by subtracting the total rate of dietary amino acid infusion from the total amino acid appearance rate, assuming a quantitative absorption of the enterally infused amino acids. The appearance rate of Leu ($R_{aLeu}$) was calculated using plasma Leu enrichment or using the reciprocal pool model with plasma KIC enrichment.

An estimate of protein synthesis was determined by subtracting the rate of whole body amino acid catabolism [i.e., Leu oxidation ($Ox_{Leu}$) or Phe hydroxylation ($HOx_{Phe}$)] from the total amino acid appearance rate. Although Leu is exclusively catabolized through oxidation to expired CO2, mainly in the muscle, Phe is almost entirely catabolized by hydroxylation in Tyr in the liver.

Leu model. $Ox_{Leu}$ (µmol·kg$^{-1}·$h$^{-1}$) was calculated using the formula

$$Ox_{Leu} = F^{13C}CO_2 \times (1/E_p - 1/E_i) \times 100$$

(2)

where $F^{13C}CO_2$ is the rate of CO2 produced by the oxidation of the [1-13C]Leu (µmol·kg$^{-1}·$h$^{-1}$) calculated as previously described (5). Finally, the rate of nonoxidative Leu disposal (NOLD), and estimation of protein synthesis, was calculated by subtracting the rate of whole body $Ox_{Leu}$ from the total $R_{aLeu}$.

Phe-Tyr model. $HOx_{Phe}$ to Tyr (µmol·kg$^{-1}·$h$^{-1}$) was derived as described (27) from the equation

$$HOx_{Phe} = R_{aTyr} \times (E_p / E_i) \times R_{aPhe} (i_{Phe} + R_{aPhe})$$

(3)

where $R_{aTyr}$ and $R_{aPhe}$ are the endogenous Tyr and Phe appearance rates (µmol·kg$^{-1}·$h$^{-1}$) estimated independently by the primed constant infusions of [3H]Tyr and [3H]Phe, respectively; $E_p$ and $E_i$ are the enrichments of [3H]Tyr and [3H]Phe, respectively, in plasma; and $i_{Phe}$ is the rate of infusion of [3H]Phe (µmol·kg$^{-1}·$h$^{-1}$). The rate of nonhydroxylative Phe disposal (NHOPD), an estimate of whole body protein synthesis, was calculated by subtracting $HOx_{Phe}$ from $R_{aPhe}$.

Gln kinetics. The whole body total appearance rate of Gln was determined at steady state using Eq. 1. In the fasted state, the whole body total appearance rate of Gln, a dispensable amino acid (DAA), has two endogenous and one exogenous component. The endogenous components arise from proteolysis-induced release and de novo synthesis, and the exogenous component arises from free Gln supplement. The endogenous rate of Gln appearance ($R_{aGln}$) was calculated by subtracting the rate of free supplemental Gln from the total Gln appearance rate. The de novo synthesis rate of Gln ($D_{Gln}$) can be determined by subtracting the estimate of its proteolysis-induced release from its endogenous appearance rate, as previously described (10). The rate of Gln release from proteolysis was estimated from the average of the endogenous appearance rate of the IAA Leu and Phe, and $D_{Gln}$ was calculated finally using the equation

$$D_{Gln} = R_{aGln} - k_{Gln}(R_{aLeu}/k_{Leu} + R_{aPhe}/k_{Phe}) / 2$$

(4)

where the concentrations of Gln ($k_{Gln}$), Leu ($k_{Leu}$), and Phe ($k_{Phe}$) were assumed to be 95, 61, and 26 mmol amino acid/100 g protein, respectively (10). Recently, lower concentrations of Gln in muscle proteins have been reported in a preliminary form (21). However, because this factor could influence the absolute $D_{Gln}$ but not the comparison between groups, we decided to base our evaluation on standard figures. In the fed state compared with the fasted state, the whole body total appearance rate of Gln has one additional exogenous component (exogenous Gln arising from dietary protein). Thus the endogenous Gln appearance rate was calculated by subtracting both the rate of dietary Gln infusion and the rate of free supplemental Gln from the total Gln appearance rate.

Statistics

Results were expressed as means ± SE. Plasma amino acid enrichment was considered to be at steady state when a coefficient of variation of <10% was obtained over the final period of tracer infusion. Groups were compared using a Kruskal-Wallis test, and statistical difference among the groups was assessed using a post hoc Bonferroni test.
RESULTS

Plasma Hormone and Substrate Concentrations

In the fasted state, the plasma cortisol concentrations were significantly higher in the two cortisol-treated groups given Ala-Gly or Gln supplementation than in the control group (P < 0.05, Table 2). The plasma glucagon, urea, and glucose concentrations similarly, but not significantly, increased in the Ala-Gly and Gln groups (+35%, +31, and +20%, respectively) vs. the control group. The plasma insulin concentration was not significantly modified in the Ala-Gly and Gln groups vs. the control group.

During meal infusion, the plasma cortisol, glucagon, and urea concentrations in the Ala-Gly and Gln groups were not different from values obtained in the fasted state. In contrast, the plasma insulin and glucose concentrations were higher in the fed than in the fasted groups, but the difference was statistically significant in the Ala-Gly group only (P < 0.05).

Gln vs. Ala-Gly administration did not significantly change the plasma hormone and substrate concentrations in the fasted or in the fed groups, although a trend toward an increase in the plasma insulin concentration was seen in the fasted Gln group (+93%) vs. the fasted Ala-Gly group.

Plasma Amino Acid Concentrations

The mean values of the plasma amino acid concentrations are shown in Table 3. The plasma concentrations of the sum of DAA and IAA are shown in Fig. 1. In the fasted state, the plasma DAA concentration was significantly higher in the Ala-Gly group (+34%, P < 0.05) but not in the Gln group (+24%, not significant (NS)) vs. the control group. In contrast, the plasma IAA concentration was significantly higher in the Gln group (+27%, P < 0.05) but not in the Ala-Gly group (+16%, NS) vs. the control group.

During meal infusion, the plasma DAA and IAA concentrations in the Ala-Gly and Gln groups were not different from values obtained in the fasted state.

Gln vs. Ala-Gly administration did not significantly affect the plasma DAA and IAA concentrations in the fasted or in the fed groups, although a trend toward an increase in the plasma IAA concentration could be seen in the groups given Gln. Gln vs. Ala-Gly administration did not affect the plasma Gln concentration in the fasted or in the fed groups (Table 3). In contrast, Ala-Gly vs. Gln administration significantly increased the plasma Gln concentration, but not the Ala concentration, in the fasted and in the fed groups.

Leu and Phe Kinetics

In the fasted state, the Leu and Phe kinetics similarly changed in the Ala-Gly and Gln groups vs. the control group. Indeed, a slight, but nonsignificant, increase in RaLeu and RaPhe, which provide indexes of proteolysis, was observed in the Ala-Gly and Gln groups vs. the control group (Table 4). Likewise, a similar change was also observed in OxLeu and HOxPhe and in NOLD and NHOPD, which provide indexes of protein synthesis. Similar results were obtained whether Leu kinetics were estimated from the plasma enrichment in KIC (Table 4) or Leu (data not shown). The protein balance estimated from Leu kinetics (NOLD − RaLeu) slightly, but not significantly, decreased in the Ala-Gly (−22.1 ± 7.4 µmol·kg⁻¹·min⁻¹) and Gln groups (−19.0 ± 4.0 µmol·kg⁻¹·min⁻¹) vs. the control group (−14.2 ± 2.5 µmol·kg⁻¹·min⁻¹). The protein balance as estimated from Phe kinetics yielded similar results.

During meal infusion compared with the fasted state, Leu and Phe kinetics similarly changed in the Ala-Gly and Gln groups. Indeed, the RaLeu and the RaPhe decreased (RaPhe P < 0.05), whereas a trend toward an increase in the NOLD and NHOPD (about +12 and +19%, respectively) was seen in the Ala-Gly- and

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Table 2. Plasma hormone and substrate concentrations in control or cortisol-treated groups receiving Ala-Gly or Gln

<table>
<thead>
<tr>
<th></th>
<th>Cortisol, nM</th>
<th>Insulin, µM</th>
<th>Glucagon, ng/l</th>
<th>Urea, mM</th>
<th>Glucose, mM</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fasted</td>
<td>251 ± 58</td>
<td>35 ± 7</td>
<td>56 ± 11</td>
<td>3.8 ± 1.2</td>
<td>4.3 ± 1.0</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>+Ala-Gly</td>
<td>1,387 ± 377*</td>
<td>28 ± 17</td>
<td>76 ± 17</td>
<td>5.0 ± 0.8</td>
</tr>
<tr>
<td>+Gln</td>
<td>1,333 ± 357*</td>
<td>54 ± 19</td>
<td>74 ± 19</td>
<td>5.3 ± 0.9</td>
<td>5.3 ± 0.3</td>
</tr>
<tr>
<td>Fed</td>
<td></td>
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</tr>
<tr>
<td>+Ala-Gly</td>
<td>1,155 ± 285</td>
<td>184 ± 94†</td>
<td>94 ± 30</td>
<td>4.9 ± 0.8</td>
<td>6.5 ± 0.6†</td>
</tr>
<tr>
<td>+Gln</td>
<td>1,448 ± 520</td>
<td>190 ± 116</td>
<td>85 ± 39</td>
<td>4.9 ± 0.9</td>
<td>6.8 ± 1.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects. *P < 0.05 compared with control group, †P < 0.05 compared with fasted groups.
GLUTAMINE AND PROTEIN METABOLISM IN HYPERCORTISOLEMIA

Table 3. Plasma amino acid concentrations in control or cortisol-treated groups receiving Ala-Gly or Gln

|                | Pro | Tau | Asp | Ser | Gln | Gly | Ala | Cit | Cys | Tyr | Orn | His | Arg | Thr | Val | Met | Ile | Leu | Phe | Lys |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| **Control**    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Fasted         | 185 | 76  | 15  | 123 | 57  | 525 | 210 | 257 | 23  | 34  | 67  | 79  | 74  | 40  | 115 | 192 | 22  | 49  | 127 | 74  | 148 |
|                | ±76 | ±44 | ±7  | ±28 | ±19 | ±105| ±64 | ±60 | ±7  | ±9  | ±9  | ±25 | ±19 | ±9  | ±27 | ±44 | ±4  | ±10 | ±28 | ±16 | ±36 |
| **Cortisol**   |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Fasted         | 184 | 62  | 12  | 206 | 52  | 721 | 470 | 361 | 24  | 31  | 63  | 66  | 79  | 68  | 144 | 212 | 31  | 56  | 151 | 79  | 172 |
| +Ala-Gly       | ±86 | ±16 | ±4  | ±22 | ±15 | ±143 | ±67 | ±105 | ±4  | ±9  | ±15 | ±18 | ±10 | ±18 | ±20 | ±24 | ±8  | ±9  | ±24 | ±13 | ±10 |
| +Gln           | 218 | 57  | 12  | 136 | 59  | 778 | 203 | 340 | 35  | 30  | 61  | 82  | 91  | 100 | 180 | 231 | 35  | 60  | 164 | 93  | 173 |
|                | ±32 | ±11 | ±4  | ±19 | ±17 | ±151 | ±34 | ±73 | ±8  | ±10 | ±21 | ±10 | ±31 | ±26 | ±41 | ±58 | ±18 | ±36 | ±17 | ±19 |     |
| Fed            | 202 | 50  | 11  | 174 | 55  | 683 | 425 | 428 | 32  | 20  | 66  | 69  | 75  | 82  | 155 | 219 | 36  | 63  | 153 | 94  | 155 |
| +Ala-Gly       | ±67 | ±19 | ±3  | ±38 | ±14 | ±51  | ±122 | ±127 | ±10 | ±15 | ±8  | ±7  | ±8  | ±23 | ±50 | ±39 | ±6  | ±11 | ±25 | ±16 | ±18 |
| +Gln           | 278 | 55  | 16  | 153 | 48  | 749 | 237 | 541 | 36  | 16  | 73  | 103 | 93  | 82  | 265 | 233 | 40  | 65  | 161 | 89  | 173 |
|                | ±57 | ±10 | ±4  | ±22 | ±12 | ±89  | ±374 | ±931 | ±12 | ±11 | ±10 | ±26 | ±11 | ±31 | ±75 | ±42 | ±4  | ±8  | ±18 | ±14 | ±23 |

Values are means ± SE; n = 8 subjects. Plasma amino acid concentrations are expressed as µM. *P < 0.05 compared with control group. †P < 0.05 compared with fasted groups. ‡P < 0.05 compared with Ala-Gly administration.

Gln-fed groups. In contrast, the OxLeu increased in the Gln (P < 0.05) and Ala-Gly groups, and the HOxPhe slightly decreased in the Ala-Gly and Gln groups. The protein balance, as estimated from Leu kinetics, was significantly higher (P < 0.05) in the Ala-Gly and Gln-fed groups (+5.4 ± 5.2 and +4.1 ± 8.7 µmol·kg⁻¹·min⁻¹, respectively) than in the fasted groups (−22.1 ± 7.4 and −19.0 ± 4.0 µmol·kg⁻¹·min⁻¹, respectively). The protein balance as estimated from Phe kinetics gave similar results.

Gln vs. Ala-Gly administration did not significantly affect the Leu and Phe kinetics in the fasted or fed groups.

Gln Kinetics

In the fasted state, RaGln and DGln were not different in the Ala-Gly, Gln, and control groups (Fig. 2). In all of these groups, the RaGln fraction that could be attributed to de novo synthesis or proteolysis averaged 60 or 40%, respectively.

During meal infusion, RaGln or DGln in the Ala-Gly or Gln groups was not different from values obtained in the fasted groups. The RaGln fraction that could be attributed to de novo synthesis or proteolysis averaged 70 or 30%, respectively, in the Ala-Gly group and 60 or 40%, respectively, in the Gln group.

Gln vs. Ala-Gly administration significantly decreased RaGln and DGln in the fed groups (P < 0.05), whereas a similar but not significant trend was seen in the fasted groups. Because Gln vs. Ala-Gly administration did not modify the extent of proteolysis, these Gln-induced decreases in RaGln were accounted for only by a decrease in DGln.

DISCUSSION

We investigated the influence of enteral Gln administration on whole body protein metabolism in healthy cortisol-treated humans. Cortisol was administered to reproduce the conditions of an accelerated protein breakdown as observed during critical illness. The effect of Gln was evaluated in the fasted and fed states and was compared with that obtained with an isonitrogenous Ala-Gly administration.

Previous studies in cortisol-treated subjects (2, 6, 10, 16) in the fasted state have demonstrated changes in the plasma concentrations of hormones and substrates and in whole body protein metabolism. In our study, some of these changes were observed in the Ala-Gly group in the fasted state, which was the control group for cortisol treatment. Indeed, the administration of a cortisol dose that was similar to that usually administered in other studies resulted in the expected...

Table 4. Leu and Phe kinetics in control or cortisol-treated groups receiving Ala-Gly or Gln

<table>
<thead>
<tr>
<th></th>
<th>Leu</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ra</td>
<td>Ox</td>
</tr>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>124.1 ± 12.6</td>
<td>19.6 ± 2.4</td>
</tr>
<tr>
<td>Cortisol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasted</td>
<td>140.6 ± 22.6</td>
<td>27.7 ± 7.2</td>
</tr>
<tr>
<td>+Ala-Gly</td>
<td>141.2 ± 14.9</td>
<td>25.2 ± 3.4</td>
</tr>
<tr>
<td>+Gln</td>
<td>125.7 ± 11.3</td>
<td>43.5 ± 5.1</td>
</tr>
<tr>
<td>Fed</td>
<td>134.0 ± 33.8</td>
<td>48.0 ± 4.8†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 subjects. Endogenous rate of appearance (Ra), rates of oxidation (Ox) and hydroxylation (HOx), rate of nonoxidative Leu disposal (NOLD), and rate of nonhydroxylicative Phe disposal (NHOPD) are expressed as µmol·kg⁻¹·h⁻¹. 1P < 0.05 compared with fasted groups.
increases in the plasma cortisol, glucagon, glucose, IAA, and DAA concentrations. Changes in the Gln and whole body protein metabolism were also observed, albeit to a lesser extent. Indeed, in the Ala-Gly group, the increases in RaGln and DGln (19 and 13%, respectively) represented one-half of the values previously reported (2, 6). Likewise, proteolysis (as estimated by RaLeu and RaPhe) increased by 12% after 60 h of cortisol treatment, whereas previous studies have reported a 15–25% increase in proteolysis after cortisone administration for 64 h (10) or prednisone administration for 7 days (2). Moreover, the observed increased rate of OxLeu (41%) in the Ala-Gly group was also moderate (50–60% in Refs. 2 and 16). In fact, the weak cortisol effect on protein metabolism observed in our study was similar to that reported in which an equivalent dose of hydrocortisone was administered for only 17 h (6). This slight cortisol effect could result from particular conditions of our protocol that might have limited the cortisol-induced hypercatabolism. First, the duration of cortisol treatment in our study might have been too short. Second, the level of protein intake before and during cortisol treatment was higher than in most previous experiments (1.9 g protein·kg\(^{-1}\)·day\(^{-1}\) compared with 0.8, 1.2, and 1.6 g protein·kg\(^{-1}\)·day\(^{-1}\), in Refs. 10, 2 and 6, and 16, respectively).

During meal infusion, changes in whole body protein metabolism when compared with the fasted state were in agreement with those previously reported in healthy subjects (5, 9, 28), postsurgery catabolic patients (7), or cortisol-treated subjects (2, 16). Indeed, proteolysis was lower during meal infusion than in the fasted state, whereas protein synthesis was moderately higher, which resulted in an improved protein balance. However, whereas OxLeu was higher as previously described, HOXPhe was lower during meal infusion than in the fasted state. This discrepancy might be explained by the specific metabolism of these two amino acids. Indeed, it has been demonstrated that, during meal infusion, the splanchnic uptake of dietary Phe is greater than that of Leu (+60% vs. +25%; see Refs. 3 and 4). Therefore, it can be assumed that, in the fed groups, dietary Phe is mainly utilized for protein synthesis in the splanchnic bed and that a limited hydroxylation in the liver might preserve endogenous Phe. To our knowledge, Gln kinetics during the transition from the fasted to the fed state has only been described in healthy subjects (9). Namely, RaGln has been shown to increase with parenteral or enteral feeding. We have now shown that enteral feeding has no significant influence on RaGln and DGln in cortisol-treated volunteers. This result might be explained by the fact that the RaGln was already stimulated by cortisol treatment.

We hypothesized that the effects of Gln administration may be conditioned by systemic signals depending on the nutritional status of the body, and, therefore, they were investigated in the fasted and in the fed state. For instance, only the changes in DGln might have been influenced by the nutritional state: the Gln-induced decrease in DGln was more pronounced in the fed state but more pronounced in the fasted state. This was probably independent of the insulinenia. Indeed, even if the plasma insulin concentration was twofold higher during meal infusion than in the fasted state, when proteolysis was maximally stimulated, than in the fasted state, when protein synthesis was maximally stimulated, and when proteolysis released amino acids and protein synthesis remained unchanged.
Even though an anabolic effect of Gln could be exerted at the site of a particular organ, our study failed to clearly demonstrate a direct anabolic effect of Gln on whole body protein metabolism in cortisol-treated subjects either in the fasted or in the fed state. This might be explained by some of our study conditions. In this way, the outcome of our statistical test has been limited first because of the small size of the population in each group and second by the fact that subjects and controls were unpaired. The choice of distinct subjects and controls was justified by the sampling of intestinal biopsies at the end of the isotopic study to measure the protein synthesis in the intestinal mucosa. On the other hand, the choice of the Ala-Gly as a control for the Gln administration has probably not influenced the results. This choice can be justified by the fact that specific effects of Ala or Gly on the protein and amino acid turnover had not been reported before this study was initiated. Since then, to our knowledge, only one study has documented the effects of Gly used as a control Gln administration (15). In this latter study, it was shown that a high dose of enteral Gln increased its plasma concentration by 10-fold and moderately decreased both proteolysis and protein synthesis in healthy subjects in a fasted state. In contrast, an isonitrogenous administration of Gln did not change proteolysis and increased protein synthesis. Accordingly, it could be supposed that, if Gln had exerted specific effects on the conditions of our study, differences in the effects of Ala-Gly and Gln administration on the protein metabolism should have been amplified rather than reduced. However, because the corticoid-induced increased proteolysis was the same after Ala-Gly and Gln administration, and given that the plasma Gly concentration increased only twofold, it is likely that Gly administration did not exert specific effects on protein metabolism in the conditions of our study. Furthermore, the duration of the enteral administration of Gln could be considered critical. Indeed, in contrast to the study previously mentioned in which a similar daily amount of enteral Gln administered only during 5 h doubled the plasma Gln concentration and increased whole body protein synthesis in healthy fasted humans (15), in our study Gln was administered during 2 days, resulting in unchanged plasma Gln concentration and protein synthesis. Likewise, in critically ill patients in the fed state, Gln administration at a dose and for a period that was similar in our study did not affect either the whole body protein synthesis, proteolysis, or the plasma Gln concentration (23). Therefore, it cannot be excluded that a Gln-induced anabolic effect on whole body protein synthesis in hypercatabolic subjects might require an acute enteral administration of a high load of Gln, inducing an increase in the plasma Gln concentration. Alternatively, a depletion in the body Gln pool might then be a necessary condition for Gln to exert an anabolic effect. Although the Gln pool was not evaluated in our study, the cortisol treatment had only a moderate hypercatabolic effect on protein and Gln metabolism, as discussed above, and unlikely affected the Gln pool.

In conclusion, under the conditions of our study, enteral administration of Gln in humans with physiological hypercortisolism induced a decline in $\Delta_{\text{Gln}}$, in the fed state that may have contributed to spare amino acids. These results, together with a favorable clinical report (17), warrant a further evaluation of Gln-supplemented enteral nutrition in patients at risk of protein wasting.

We acknowledge Marie Chassagne and Bernard Dieu for tracer preparation and Brigitte Maurer and Michèle Patenêtre for technical help. We are also grateful to Richard Medeiros for editorial assistance. We thank the dieticians for the preparation of the menus and the nurses for their help during the isotopic studies.

This study was supported by a grant of the Ministère chargé de la Santé, Direction des Hopitaux (PHRC 1994 no. 94087 FM), and by the Fondation pour l’Association Charles Nicolle.

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Received 16 March 1999; accepted in final form 22 November 1999.

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