Oral Administration of a Glutamine-Enriched Diet Before or After Endotoxin Challenge in Aged Rats Has Limited Effects

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ABSTRACT Numerous studies indicate beneficial effects of glutamine (Gln) in many models of catabolic adult rats. No data were available for aged rats. The effects of oral L-Gln-enriched diet were tested in endotoxemic 24-mo old rats. First, rats received for 7 d (from d0 to d7) an oral diet supplemented with either L-Gln (1g/kg·d) or casein (Cas; isonitrogenous supply) prior to lipopolysaccharide (LPS) challenge. The rats were then killed after 24 h food deprivation (from d7 to d8). Endotoxemia induced a catabolic response as shown by muscle glutamine depletion, hyperphenylalaninemia, small bowel atrophy and impaired functionality and bacterial translocation. The Gln-enriched diet did not prevent muscle Gln depletion but significantly (P < 0.05) enhanced plantaris protein content by 18% compared to the Cas-LPS rats and reduced the plasma phenylalanine-to-tyrosine ratio (1.32 ± 0.05 vs. 1.54 ± 0.10, respectively, P = 0.01). Gut translocation and histomorphology were unaffected by diet. However, Gln pretreatment reduced the fall in sucrase and glucoamylase activities in the ileum, respectively, by 55 and 63% vs. Cas supplementation (P < 0.05). In a second study, after endotoxin challenge, healthy 24-mo-old rats were then food-deprived for 2 d (from d0 to d2), received a nonpurified diet for 4 d (from d2 to d6), and then Cas or L-Gln-supplemented diet for 7 d (from d6 to d13). No beneficial effects of Gln supplementation were observed except an increase of 50 and 56% in sucrase and glucoamylase activities in the ileum of Gln-treated rats, (P < 0.01 vs. healthy rats). In conclusion, the effects of L-Gln supplementation in aged endotoxemic rats were limited. J. Nutr. 129: 1799–1806, 1999.

KEY WORDS: • aged rats • Gln-supplemented diet • endotoxemia • amino acids • muscles • small bowel

The age-related loss of muscle mass is associated with an increased risk of morbidity, mortality and disability in old age, but the mechanisms by which this occurs are not fully understood (Pohor and Kritchevsky 1998). Muscles form a massive dynamic reservoir of proteins and free amino acids especially glutamine (Gln). The metabolic importance of Gln is evident during stress, when it becomes a conditionally indispensable amino acid because endogenous de novo synthesis fails to meet increased demand (Hall et al. 1996). The loss of skeletal muscle mass and protein depletion may predispose to a Gln deficiency which could contribute to the high sensitivity of elderly people to catabolic situations. Indeed, metabolic (Je- evanandam et al. 1990) and immunological (Lesourd 1997) responses to trauma are blunted in the elderly. However, this fact may be questioned considering the unexpected finding that the muscle Gln depletion was significantly less in aged, stressed rats than in young adult rats (Farges et al. 1994, Minet-Quinard et al. 1999) in spite of a similar increase in muscle Gln synthetase activity (Meynial-Denis et al. 1996, Minet et al. 1999). A possible explanation for these unexpected results is that the relative maintenance of the muscle Gln pool in aged rats could be due to a decrease in muscle Gln flux, as previously reported by Parry-Billings et al. (1991) in soleus from healthy aged rats, perhaps secondary to impaired hormonal control (Grizard et al. 1995). This would imply that, in aged, stressed rodents, muscle is unable to supply Gln in sufficient amounts to other tissues.

Several experimental and clinical studies documented the nutritional efficiency of Gln-enriched nutrition support in the prevention and treatment of critical illness (Fürst and Stehle 1995, Neu et al. 1996). To our knowledge, no data are available for aged, stressed animals. Thus, the purpose of the present study was to test whether metabolic disorders induced by endotoxemia could be prevented or reversed by providing exogenous Gln. The experiments focused on the small bowel.
(a major site of Gln uptake and utilization) and skeletal muscle (the main tissue of Gln storage and production).

**MATERIALS AND METHODS**

**Animals**
Male Sprague-Dawley rats aged 24-mo purchased from Ifa Credo (l’Arbresle, France) were used as old rats. They were individually housed at an ambient temperature of 21–23°C with a 12-h light/dark cycle (dark from 0800 h to 2000 h) and were allowed free access to a nonpurified diet and water. The nonpurified diet (AO4; Usine d’Alimentation Rationnelle (UAR), France) contained proteins (17%), carbohydrates (59%), fats (3%), and minerals, vitamins, and water (21%). All the rats were adapted to their new environment for 15 d [from d-15 (d-15) to d 0 (d0)]. The experiments complied with the National Research Council Guide for the care and use of laboratory animals. One of us (LC) is authorized (N° 065226) by the French Ministry of Agriculture and Forestry to use this experimental model.

**Experimental designs**

**Experiment 1: prophylactic use of a Gln-enriched diet.** At the end of the acclimation period (d0), old rats (n = 14) weighed 709 ± 8 g and ate 23.8 ± 0.5 g daily. They were then randomly assigned to receive for 7 d (from d0 to d7) the nonpurified diet (chow), as described above, supplemented with either Gln (1.0 g/kg bw/day; Gln tytopolysaccharide (LPS) group; n = 7) (Fluka, Germany), or casein (Cas isonitrogenous to Gln, 1.4 g/kg bw/day; Cas-LPS group; n = 7) (UAR). Gln and Cas were mixed daily with the nonpurified diet to obtain homogeneous powders, which were given in conical ceramic troughs to avoid spillage. The intake was limited to 90% of that recorded during the second week of acclimation in order to be certain that the rats ate all the food given. Taking into account the amount of Gln contained in AO4 and in Cas and estimating that Cas contains roughly 10% Gln (Swaisgood 1982), Gln intake was 0.62 g/kg/day in the Cas-LPS group and 1.49 g/kg/day in the Gln-LPS group. Rats that ate less than the food given were excluded from the study. On d7, the two groups of rats received an i.p. injection of chromatographically purified phenol-extracted LPS (Escherichia coli, serotype O127:B8; Sigma, France) at 3 mg/kg dissolved in 9 g/L NaCl to a final concentration of 1 mg/mL. This dose was previously shown to induce a catabolic state in aged rats (Farges et al. 1996). Subsequently, the rats were food-deprived for 24 h (d7 to d8) because we previously observed that old endotoxemic rats do not eat during this period (Farges et al. 1994, 1996) and because this food deprivation period contributes greatly to the catabolic state induced by LPS (Lasnier et al. 1996). This was also verified in a preliminary set of experiments where LPS- and fasted-treated rats were compared to saline-injected rats (data not shown), all the rats having been previously fed chow ad libitum. The rats were then killed (n = 5 in Cas-LPS group and n = 6 in Gln-LPS group). This results were recorded daily. The cause of mortality in LPS-treated rats was not explored. The rats concerned were lethargic before death and did not exhibit any sign of pain. The experimental designs are summarized in Figure 1.

**Experiment 2: Effects of a Gln-enriched diet administered after LPS challenge.** On day 0 (d0), the rats (n = 27) weighed 718 ± 15 g and ate 26.9 ± 0.9 g daily. They were given an i.p. injection of LPS (E. coli serotype O127:B8) at 1.5 mg/kg. The rats were then fasted for 48 h (from d0 to d2) since they do not eat anyway during this period (Farges et al. 1996), and fasting increases catabolic response to stress. Because of the marked anorexia, they were subsequently refed with the chow-supplemented Gln or Cas (LPS-Gln group; n = 6) in each group). This experiment was conducted twice. Since the two replicates yielded similar results (Student’s t-test, P > 0.05), these were pooled.

For experiments 1 and 2, a common control group of healthy 24-mo-old rats with free access to the chow was used (n = 5). For these two experiments, food consumption and total body weight were recorded daily. The cause of mortality in LPS-treated rats was not explored. The rats concerned were lethargic before death and did not exhibit any sign of pain. The experimental designs are summarized in Figure 1.

**Blood treatment and removal of tissues**

At the end of the experimental periods, all rats were in the postabsorptive state or starved and were killed by decapitation after general anesthesia with diethyl ether (Prolabo, Fontenay-sous-Bois, France). Blood samples, collected in heparinized tubes, were rapidly centrifuged and the plasma deproteinized with sulfosalicylic acid (50 g/L). The supernatants were stored at −80°C until analysis. In the first experiment only, the mesenteric lymph nodes (MLN) were removed under sterile conditions.

The small intestine extending from the ligament of Treitz to the ileoceleal junction was promptly resected. Its total length was determined by vertical suspension with an attached 3 g weight, and the intestine was cut at its mid-point, resulting in two portions, jejunum and ileum. Pieces 1 cm long were removed in proximal parts of the jejunum and the ileum for histomorphometric examination. The following 20 cm of the jejunum and the ileum were divided into two segments used for hydrolysis activity determination and free amino acid (AA) content analysis, respectively. Each segment was cut off, flushed with ice-cold saline buffer, everted, and the mucosa scraped, weighed and frozen in liquid nitrogen.

Finally the soleus, a muscle composed of type I fibers, the extensor digitorum longus (EDL) and the plantaris, muscles composed of a majority of type II fibers, were promptly removed, weighed and frozen in liquid nitrogen. All samples were stored at −80°C until analysis.
Analytical methods

Plasma AA. Plasma samples were analyzed for amino acid (AA) concentrations by ion-exchange chromatography with ninhydrin detection on an AA analyzer (model 630C; Beckman Instruments, Palo Alto, CA). Our laboratory participates in the European Control Quality Scheme (ERNDIM, Brussels, Belgium), and our results indicate the accuracy and reproducibility of the measurements with a between-batch reproducibility ranging from 2 to 8% for various AA.

Tissue AA and protein contents. Frozen tissues were pulverized and homogenized in ice-cold 10% trichloroacetic acid containing 0.5 mmol/L EDTA (1 mL/100 mg of tissue). The acid-soluble fraction containing free AA was separated by centrifugation from precipitated proteins. AA concentrations were determined in the supernatant as described above. Fat was removed from the protein precipitate with alcohol/ether (50/50). The homogenate was dissolved in 1 mol/L of NaOH (4 mL/100 mg of tissue) at 40°C for 12 h and protein content was determined by the method of Lowry et al. (1949).

MLN culture. Mesenteric lymph nodes were cultured on a Muler Hinton agar and on deoxycholate medium for the isolation of Gram-negative enteric bacilli (Diagnostic Pasteur, France). The plates were examined after 24 h incubation at 37°C.

Histology. After fixation in Bouin’s solution, the samples were dehydrated and embedded in paraffin. Tissue cross sections (5 mm) were cut, dehydrated, and embedded in paraffin. Tissue cross sections (5 mm) were mounted on clean slides and stained with hematoxylin and eosin. Villus height and crypt depth were measured with a semi-automatic image analyzer (Bio-conv, Lyon, France). Only villi cut from their base to the top with a single epithelial layer at their tip and crypts with a visible lumen along their entire depth were kept for analysis.

Hydrolase activity determinations. Enzyme assays were performed on frozen mucosa homogenized in 4 vol (4x the mucosal weight) of 0.01 mol/L NaH2PO4, K2HPO4 buffer, pH 6.1. An aliquot was used to determine protein content using albumin as standard (Gornall et al. 1949). Sucrase and glucoamylase activities were determined by a modification of Dahlquist’s technique (Géard et al. 1979). Leucine aminopeptidase was measured as described previously (Ahnen et al. 1982).

Statistical analysis. All results are expressed as mean ± SEM. Statistical significance was determined by one way ANOVA followed by the Newman-Keuls test or by Student’s t-test using PC-SM software (Programme Conversational de Statistiques pour les Sciences et le Marketing, Version 5.0, Deltasoft, Grenoble-Meylan, France). Differences were considered significant when P < 0.05.

RESULTS

Experiment 1: prophylactic administration of a Gln-enriched diet

Body weight, food intake and tissue weight. At the time the rats received Gln-enriched or isonitrogenous Cas-enriched diets, food intake did not differ between the two groups. They consumed 97 and 93% of the food given, respectively. Thus any differences between these two groups did not originate from different intakes. However, during this period, these groups had lower food intakes than the control group fed ad libitum (21.0 ± 1 and 20.9 ± 0.5 g for Gln-LPS and Cas-LPS groups, respectively, vs. 27.9 ± 1.5 g in healthy rats, P < 0.01). Soon after endotoxemia developed, the rats became lethargic and displayed symptoms of illness (piloerection, chromodactyly). One and two rats died in the Gln-LPS and in the Cas-LPS group, respectively, (P > 0.05). Post-LPS body weight losses were 7% and 5%, respectively. One healthy rat died during the experiment (for an unidentified reason). Whatever the diet, the rats showed an increase in body weight (Table 1).

Plasma AA concentrations. Plasma serine and glycine concentrations were lower in both Gln-LPS and Cas-LPS groups (P < 0.01 vs. Healthy) (Table 1) and in LPA rats (P < 0.05 vs. NaCl, data not shown). Whatever the nutritional treatment, the LPS challenge induced hyperphenylalaninemia. The phenylalanine-to-tyrosine ratio, reflecting the degree of catabolism during infection (Wannemarcher et al. 1976), was significantly greater in all LPS-treated groups compared with their respective control group (P ≤ 0.01), but less in the Gln-LPS group than in the Cas-LPS group (P ≤ 0.01) (Table 1). LPS-treatment induced hypoglutaminemia (data not shown), while no significant effect was observed in either Gln-LPS or Cas-LPS groups compared with their controls. Glutamate concentrations were markedly lower in LPS-treated rats except in those receiving Gln.

Tissue protein contents. Muscle and small bowel mucosa total protein contents were not significantly different among groups (data not shown). However, the plantaris total protein concentration was higher in the Gln-LPS rats than in the Cas-LPS group (209 ± 11 vs. 171 ± 6 mg/g, P ≤ 0.05).

Tissue free AA concentrations. The combined effect of LPS challenge and 24 h food deprivation induced a muscle Gln depletion in both the EDL and the plantaris (data not shown). Similar results were observed for the prophylactic study; Gln- or Cas-enriched diets did not prevent the muscle Gln depletion (EDL: −47%, plantaris: −38%) (Table 2). Whatever the diet, Gln concentration in the soleus was not altered by endotoxemia (P > 0.05). Glutamate concentrations were not significantly reduced in LPS-treated rats except in the EDL of LPS rats compared with NaCl-treated rats (data not shown) and of Cas-LPS rats compared with healthy controls. The concentrations of some other nonessential AA (histidine, arginine, glycine, serine) were significantly lower (P ≤ 0.01) in the muscles of the LPS-treated groups, whereas levels of essential AA (phenylalanine, branched-chain AA, methionine) tended to be or were greater in LPS, Gln-LPS or Cas-LPS groups compared with their respective controls.

### TABLE 1

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Healthy (n = 5)</th>
<th>Cas-LPS (n = 5)</th>
<th>Gln-LPS (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>µmol/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>333 ± 34</td>
<td>139 ± 13**</td>
<td>187 ± 8*</td>
</tr>
<tr>
<td>Asn</td>
<td>115 ± 11</td>
<td>74 ± 11*</td>
<td>94 ± 7</td>
</tr>
<tr>
<td>Glu</td>
<td>173 ± 4</td>
<td>103 ± 24*</td>
<td>184 ± 20†</td>
</tr>
<tr>
<td>Gln</td>
<td>848 ± 70</td>
<td>750 ± 68</td>
<td>768 ± 12</td>
</tr>
<tr>
<td>Gly</td>
<td>541 ± 93</td>
<td>221 ± 14**</td>
<td>260 ± 11**</td>
</tr>
<tr>
<td>Arg</td>
<td>137 ± 7</td>
<td>89 ± 10**</td>
<td>105 ± 8</td>
</tr>
<tr>
<td>Phe/Tyr</td>
<td>0.86 ± 0.03</td>
<td>1.54 ± 0.10*</td>
<td>1.32 ± 0.02**</td>
</tr>
</tbody>
</table>

1 Data expressed as mean ± SEM. 2 Other amino acids did not differ among groups. Healthy: rat fed ad libitum with chow. Gln-LPS: glutamine-enriched diet administered before endotoxin treatment. Cas-LPS: casein-enriched diet (isonitrogenous control) given before endotoxin treatment. * P ≤ 0.05 and ** P ≤ 0.01 vs. Healthy. † P ≤ 0.05 and ‡ P ≤ 0.01 vs. CAS-LPS (ANOVA and Newman-Keuls test).
Gln levels varied according to the small bowel segment considered: while no difference was observed in the jejunum, ileal Gln concentration was significantly enhanced in the Gln-LPS and Cas-LPS rats (Table 3). Jejunal free serine, glycine and alanine concentrations were reduced in the Gln-LPS and Cas-LPS groups (P ≤ 0.01 vs. healthy rats). The citrulline level was significantly lower in the Cas-LPS group compared with both control and Gln-LPS rats. In the ileum, glycine concentrations were reduced in the Cas-LPS group compared with both control and Gln-LPS rats. The citrulline level was significantly lower in the Cas-LPS group compared with both control and Gln-LPS rats.

Small bowel morphometry. In comparison with healthy rats, LPS treatment and starvation resulted in lower total height of the jejunum and total villus height of the ileum from old rats fed the Cas-enriched chow diet. Gln supply did not prevent small bowel atrophy (Fig. 2). Crypt depth in both intestinal segments was unaffected by the nutritional treatment and the LPS challenge. The total villus height showed a significant marked reduction in both the jejunum and the ileum of Gln-LPS and Cas-LPS groups (P ≤ 0.01 and P ≤ 0.05 vs. healthy group).

Small bowel hydrolase activities. In the jejunum, hydrolase activities were lower in rats given the LPS challenge (P ≤ 0.01 vs. healthy rats) (Fig. 3). Gln supplementation did not prevent or limit the fall in enzyme activities. In the ileum, hydrolase activities were also affected by the stress, but sucrase and glucoamylase activities were greater in the rats receiving Gln (P ≤ 0.05 vs. Cas-LPS group) (Fig. 3).

### Table 2

**Muscle concentrations of selected free amino acids in LPS-treated rats receiving a prophylactic Gln-enriched diet**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>extensor digitorum longus</th>
<th>Plantaris</th>
<th>Soleus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy (n = 5)</td>
<td>Cas-LPS (n = 5)</td>
<td>Gln-LPS (n = 5)</td>
</tr>
<tr>
<td></td>
<td>µmol/g</td>
<td>µmol/g</td>
<td>µmol/g</td>
</tr>
<tr>
<td>Ser</td>
<td>1.03 ± 0.09</td>
<td>0.40 ± 0.06**</td>
<td>0.50 ± 0.04**</td>
</tr>
<tr>
<td>Glu</td>
<td>1.09 ± 0.19</td>
<td>0.51 ± 0.05*</td>
<td>0.75 ± 0.11</td>
</tr>
<tr>
<td>Gln</td>
<td>4.77 ± 0.30</td>
<td>2.48 ± 0.30**</td>
<td>2.53 ± 0.21**</td>
</tr>
<tr>
<td>Gly</td>
<td>2.94 ± 0.43</td>
<td>1.28 ± 0.05**</td>
<td>1.51 ± 0.09**</td>
</tr>
<tr>
<td>Cit</td>
<td>0.29 ± 0.05</td>
<td>0.16 ± 0.03</td>
<td>0.11 ± 0.02**</td>
</tr>
<tr>
<td>His</td>
<td>0.40 ± 0.01</td>
<td>0.24 ± 0.05*</td>
<td>0.24 ± 0.03*</td>
</tr>
<tr>
<td>Arg</td>
<td>0.35 ± 0.05</td>
<td>0.06 ± 0.02**</td>
<td>0.15 ± 0.06**</td>
</tr>
</tbody>
</table>

1 No significant difference for the other amino acids. Data expressed as mean ± SEM. Healthy: rat fed ad libitum with chow. Gln-LPS: glutamine-enriched diet administered before endotoxin challenge. Cas-LPS: casein-enriched diet (isonitrogenous to Gln supplementation) given before endotoxin treatment. * P ≤ 0.05 vs. Healthy and ** P ≤ 0.05 vs. Cas-LPS (ANOVA and Newman-Keuls test).

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**Table 3**

**Jejunum and ileum concentrations of selected free amino acids in LPS-treated rats receiving a prophylactic Gln-enriched diet (prophylactic study)**

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Jejunum (n = 5)</th>
<th>Cas-LPS (n = 5)</th>
<th>Gln-LPS (n = 5)</th>
<th>Ileum (n = 5)</th>
<th>Cas-LPS (n = 5)</th>
<th>Gln-LPS (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/g</td>
<td>µmol/g</td>
<td>µmol/g</td>
<td>µmol/g</td>
<td>µmol/g</td>
<td>µmol/g</td>
</tr>
<tr>
<td>Ser</td>
<td>0.87 ± 0.08</td>
<td>0.39 ± 0.04*</td>
<td>0.49 ± 0.14*</td>
<td>0.59 ± 0.04</td>
<td>0.47 ± 0.04</td>
<td>0.34 ± 0.06*</td>
</tr>
<tr>
<td>Asn</td>
<td>0.85 ± 0.15</td>
<td>0.30 ± 0.08*</td>
<td>0.46 ± 0.15</td>
<td>0.36 ± 0.03</td>
<td>0.12 ± 0.05*</td>
<td>0.21 ± 0.08</td>
</tr>
<tr>
<td>Gln</td>
<td>0.54 ± 0.08</td>
<td>0.46 ± 0.06</td>
<td>0.50 ± 0.06</td>
<td>0.41 ± 0.02</td>
<td>0.61 ± 0.03*</td>
<td>0.64 ± 0.08*</td>
</tr>
<tr>
<td>Gly</td>
<td>1.92 ± 0.27</td>
<td>0.97 ± 0.07*</td>
<td>1.11 ± 0.19*</td>
<td>1.74 ± 0.28</td>
<td>1.07 ± 0.07*</td>
<td>1.09 ± 0.09*</td>
</tr>
<tr>
<td>Ala</td>
<td>2.28 ± 0.17</td>
<td>1.48 ± 0.10*</td>
<td>1.78 ± 0.19*</td>
<td>1.93 ± 0.16</td>
<td>1.63 ± 0.09</td>
<td>1.84 ± 0.15</td>
</tr>
<tr>
<td>Cit</td>
<td>0.26 ± 0.03</td>
<td>0.16 ± 0.02*</td>
<td>0.26 ± 0.03**</td>
<td>0.21 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>0.23 ± 0.03</td>
</tr>
</tbody>
</table>

1 No significant variation for the other amino acids. Data expressed as mean ± SEM. Healthy: rat fed ad libitum with chow. Gln-LPS: glutamine-enriched diet administered before endotoxin challenge. Cas-LPS: casein-enriched diet (isotrophic to Gln supplementation) given before endotoxin treatment. * P ≤ 0.05 vs. Healthy and ** P ≤ 0.05 vs. Cas-LPS (ANOVA and Newman-Keuls test).
Experiment 2: Effects of a Gln-enriched diet administered after LPS challenge

Body weight, food intake and tissue weight. Soon after endotoxemia developed, the rats became lethargic and displayed symptoms of severe illness. The LPS challenge induced a catabolic state with a reduced food intake. During the wash-out period (from d2 to d6), on d4 with free access to the rat chow the endotoxemic rats ate only 37% of d0 intake ($P < 0.05$ vs. healthy group), but on d6 when the nutritional supplementation started they had normalized their food intake compared to the healthy group. During the supplementation period (from d6 to d13), the daily food intakes for the three groups did not differ (22.8 $\pm$ 1.0 g; 22.4 $\pm$ 0.8 g; 25.0 $\pm$ 0.9 g in LPS-Gln, LPS-Cas and healthy control rats, respectively). Endotoxemia associated with the reduced food intake led to a body weight loss of 10% on d2 compared with their d0 body weight. When the feeding was resumed (from d2 to d13), body weight changes were similar for both endotoxemic groups, but body weight remained significantly lower compared with controls ($P < 0.01$, data not shown). Before the beginning of supplementation, the mortality rate was about 52%; afterward one Gln-treated rat died.

Skeletal muscle weights were not different across the three groups (data not shown).

Plasma AA concentrations. Irrespective of the supplementation employed, plasma concentrations ($\mu$mol/L) of four essential AA and of tyrosine were significantly lower in the LPS-Cas and LPS-Gln rats compared with healthy ad libitum rats: methionine: 40 $\pm$ 2, 36 $\pm$ 2 vs. 49 $\pm$ 3, $P < 0.05$; isoleucine: 66 $\pm$ 6, 65 $\pm$ 2 vs. 87 $\pm$ 7, $P < 0.05$; leucine: 95 $\pm$ 8, 93 $\pm$ 3 vs. 129 $\pm$ 9, $P < 0.05$; phenylalanine: 54 $\pm$ 3, 54 $\pm$ 3 vs. 73 $\pm$ 6, $P < 0.01$ and tyrosine 61 $\pm$ 4, 57 $\pm$ 5 vs. 86 $\pm$ 10, $P < 0.05$. No differences among the three groups were noted for other AA. The Phe/Tyr ratio and the total plasma AA concentration did not differ in the three groups (data not shown).

Tissue protein contents. EDL plantaris and soleus protein concentrations did not differ but in contrast, the soleus protein content was significantly lower in LPS-Gln and LPS-Cas rats than in the healthy group (42 $\pm$ 3 mg/muscle; 38 $\pm$ 5 mg/muscle vs. 50 $\pm$ 3 mg/muscle, $P < 0.01$).

Tissue free AA concentrations. For the free intramuscular AA, Gln supplementation had no effect on the EDL-free AA pattern compared with Cas-supplemented and ad libitum-fed rats (data not shown); plantaris concentrations of asparagine, ornithine, and histidine were significantly lower in both the LPS-Gln and Cas-LPS groups compared with healthy rats (Table 4). In the soleus, besides the lower levels of asparagines and histidine in the two LPS-treated groups, threonine, lysine, and arginine concentrations were specifically reduced in the LPS-Gln group. Most striking was the depletion of Gln levels in the LPS-Gln-treated rats compared with the two other groups ($P < 0.01$) (Table 4).

No significant variations due to the nutritional treatment

FIGURE 2 Mucosal morphometry in the prophylactic study. (A) jejunum and (B) ileum. TH: total height, VH: villus height, CD: crypt depth. Data are expressed as mean $\pm$ SEM, $n = 5–6$; * $P < 0.05$ and ** $P < 0.01$ vs. Healthy (ANOVA and Newman-Keuls test). Healthy: fed ad libitum with the rat chow. Gln-LPS: glutamine-enriched diet provided before endotoxin administration. Cas-LPS: casein-enriched diet (isotitrogenous to Gln) provided before endotoxin administration. LPS: lipopolysaccharide.

FIGURE 3 Mucosal total hydrolase activities in rats supplemented with glutamine and then challenged with lipopolysaccharide (LPS). (A) jejunum and (B) ileum Sucr.: sucrase, Glucoam.: glucoamylase, Apep.: aminopeptidase. Data are expressed as mean $\pm$ SEM, $n = 5–6$. ** $P < 0.01$ vs. Healthy and * $P = 0.05$ vs. Cas-LPS (ANOVA and Newman-Keuls test). Groups are as defined in Figure 2.
were observed in either jejunal or ileal AA concentrations (data not shown).

**Small bowel histology and hydrolase activities.** For the morphometry, no variation occurred in either the jejunal or ileal segments (data not shown). Total hydrolase activities were not modified by the supplemented diet in the jejunum. In contrast, in the ileum, sucrase and glucoamylase activities were significantly higher in the rats fed the Gln-enriched diet than in the healthy ad libitum group \((P = 0.01\) vs. healthy) (Fig. 4).

**DISCUSSION**

**Endotoxemic model in aged rats.** We examined the effects of a Gln-enriched diet provided before or after inducing endotoxia in old rats. Endotoxia induced with *E. coli* LPS can be used as a reliable model of infection since it reproduces many of the metabolic disturbances that occur in clinical sepsis (Glausser et al. 1991). LPS is the primary pathogenic factor associated with Gram-negative bacteria. The rationale for the use of such a model is that the incidence of bacterial sepsis, which increases with age, and mortality due to sepsis remain high, particularly for the elderly (Gardner et al. 1980).

The underlying mechanism of the vulnerability to infections was ascribed to the breakdown of anatomic barriers, to underlying host diseases and to the declining capacity of the immune system (Gardner et al. 1980, Lesourd 1997). It became clear that LPS does not injure host tissues directly but rather, through the action of a variety of inflammatory mediators such as cytokines. The great sensitivity of aged animals to sepsis, mortality up to 100% in some studies, was related to an excessive tumor necrosis factor-α, interferon-γ and nitric oxide production (Chorinchath et al. 1996, Tateda et al. 1996, Kuchnaroff et al. 1997), resulting from perturbed endogenous glucocorticoid control of cytokine production (Chorinchath et al. 1996, Tateda et al. 1996). The prevalence of malnutrition is high in septic elderly patients (Naber et al. 1997). The nutritional status, the occurrence of complications, and the underlying diseases constitute a triangle in which the original cause is unclear. Because anorexia induced by endotoxin shock is long-lasting in aged rats as we previously reported (Farges et al. 1996) and the nutritional depletion is usually caused by the
joint action of an underlying disease and a dietary deficiency, our experimental model combined both the LPS challenge and 24–48 h fasting. These endotoxicemic rats were compared to a control group of healthy, freely fed, aged rats.

**Prophylactic administration of a Gln-supplemented diet.**
Gln, a nonessential AA, is attracting widespread attention because of its relevance to numerous metabolic processes and its potential role in the prevention and treatment of critical illness (Furst and Stehle 1995, Neu et al. 1996). However, to date all the available data are derived from studies in young adult animals. Gln supplementation before LPS challenge did not exert a protective effect on the muscle Gln depletion either in the EDL or the plantaris but increased protein concentration in the plantaris. The relationship between Gln content and protein synthesis is controversial. A striking correlation between the size of muscle-free Gln pool and protein turnover rates was found in some studies (Ardawi and Magoun, 1991, McLennan et al. 1987, Millward et al. 1988). However, no direct relationship between these variables was found in other catabolic states such as burn injury (Le Boucher et al. 1997) or sepsis induced by cecum ligation and puncture (Fang et al. 1995).

In our model, aged endotoxicemic rats were catabolic as shown by muscle branched-chain AA and phenylalanine accumulation as the result of muscle protein breakdown (Je-evanandan 1995). In agreement with the plasma AA pattern previously described in old stressed rats (Milakofsky et al. 1996) and in geriatric trauma patients (Je-evanandan et al. 1990), the decrease in nonessential AA such as serine, Gln, glycine and arginine suggests a central utilization greater than their peripheral release. Gln supplementation contributed to a smaller decrease in the plasma phenylalanine to tyrosine ratio, suggesting a limitation of protein wasting (Wannemacher et al. 1976).

In the small bowel, Gln appears to be essential for the integrity of the mucosal barrier as well as the maintenance of its metabolic and immune function (Souba et al. 1990). Consequently, when muscle de novo synthesis is not sufficient, the needs of enterocytes and immune cells are no longer met, leading to immune and gut dysfunction. There is considerable experimental evidence linking the failure of the gut barrier to multiple organ failure syndrome (Soeters 1996). However, the ability of Gln to prevent bacterial translocation is controversial (Bark et al. 1995, Gianotti et al. 1995, Klimberg et al. 1990). Although our model is different from others in the literature because it focuses on the aged rat thus rendering comparisons difficult, notably we found no significant difference in survival rate, mucosal atrophy or bacterial translocation between the two groups supplemented and then LPS treated.

The response of the gut to sepsis is characterized by a marked reduction in gut Gln utilization associated with impaired Gln transport and a decrease in glutaminase activity (Salloum et al. 1991). Since glutaminase is preferentially located in the proximal intestine, this may induce a high Gln availability to the ileum and therefore explain the elevated ileal-free Gln concentration that we observed. At the functional level, whereas Gln supplementation did not exert a protective effect against the stress in the jejunum, it preserved the functional capacities of the ileum. Our results confirm the reduced adaptive capacities of the jejunum of senescent rats (Aceville et al. 1991). The increase in disaccharidase activities in the ileum of rats previously fed the Gln-supplemented diet might be related to the presence of luminal stimuli and especially the presence of AA that enhance brush border hydrolase activities and are normally hydrolyzed and absorbed upstream by the proximal intestine. These results are also consistent with the fact that 90% of the brush border enzymes are synthesized from luminal AA (Babst et al. 1993, Poullain et al. 1991) and with the anabolic properties of Gln (Higashiguchi et al. 1995). By comparison, the low enzyme activity observed for LPS-Cas rats might result from the enhanced degradation by pancreatic proteases previously stimulated by the casein supply (Goda et al. 1988). The lack of variation in aminopeptidase activity might be related to its slow turnover compared with disaccharidase turnover and also to a defect in dietary protein hydrolysis by pancreatic enzymes leading to a lower intraluminal formation of oligopeptides, which stimulate brush border aminopeptidase activity (Raul et al. 1987). In addition, it was reported (Greenberg and Holt 1986) that pancreatic function is impaired during aging.

**Effects of a Gln-supplemented diet administered after LPS challenge.** As discussed above, aged stressed rats characteristically display a long-lasting marked anorexia followed by a period of very heterogeneous intake (Farges et al. 1996). For this reason, in the experiment described here, a long period was allowed after LPS challenge before starting the supplementation. Therefore, at the time of killing, the effects observed were probably more representative of chronic malnutrition than of LPS treatment, per se, as shown by the low plasma Phe/Tyr ratio. In addition, considering the decreased plasma levels of most essential AA, the general nitrogenous homeostasis remained impaired. Clearly, the rats were not only catabolic but malnourished (Cynober et al. 1992). We performed another experiment starting the supplementation earlier (Bérand et al., unpublished data) in which the rats were starved for 24 h after endotoxemia and thereafter fed by continuous intragastric enteral nutrition supplemented either with Gln or with Cas. Unfortunately, all the rats died during the first 48 h. The LPS injection after the gastroscope tube implantation may have been too stressful for old rats. The polymeric diet may have been poorly tolerated and use of a semi-elementary diet should be considered. Finally the flow rate of the enteral mixture administered may have been too high. Since this type of nutrition is well-tolerated in young adult rodents (Raul et al. 1995), this mortality underlines the extreme sensitivity of old rats and the difficulty in managing models of old stressed rats. The single significant effect of Gln was as in experiment 1, an improvement of the hydrolytic activities in the distal part of the small bowel.

Many studies involving young adult stressed rats indicate the beneficial effects of Gln supplementation (Ardawi 1991, Furst and Stehle 1995, Neu et al. 1996). Using aged stressed rats, we were unable to reproduce these results. However, none of the models described in the literature is exactly the same as those used in this study, and the effect of Gln supplementation may depend on the type and extent of stress in experimental models. It would therefore be interesting to compare the response of aged rats to that of young adult rats with the same experimental design. Hence, we must be cautious in assuming that the age of animals is solely responsible for the weak effects of Gln-supplemented diet.

In conclusion, administration of a Gln-enriched diet before endotoxemia challenge did not prevent muscle Gln depletion and small bowel atrophy in aged rats; it only preserved the intestinal function in the ileum. When administered after LPS challenge, Gln had only a marginal effect. Contrary to numerous experimental or clinical studies suggesting anabolic effects of Gln-supplemented diets, the effects of Gln administration may be limited in old stressed rats.
We gratefully acknowledge F. Gossé and J. Nacey for their skilled technical assistance and M. Robery for typing the manuscript.

LITERATURE CITED


