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Can a glutamate-enriched diet counteract glutamine depletion in endotoxemic rats?

Chantal Chambon-Savanovitch,* Marie-Chantal Farges,* Francis Raul,† François Blachier,‡ Paule Davot,* Luc Cynober,* and Marie-Paule Vasson*

**Biochemistry, Molecular Biology and Nutrition Laboratory, EA 1742, Pharmacy School and Research Center for Human Nutrition, Clermont-Ferrand, France; †CFJ INSERM 95-09, IRCAD, Strasbourg, France; and ‡INRA, Department of Nutrition, Food and Security, Jouy-en-Josas, France*

The study evaluated whether a glutamate-enriched diet would restore glutamine tissue pools and maintain tissue trophicity in endotoxemic rats. For this purpose, young male Sprague-Dawley rats received an intraperitoneal injection of lipopolysaccharide (LPS) from Escherichia coli *at 3 mg/kg body weight. After 24 hours of food deprivation, the rats were enterally refed for 48 hours using Osmolite® enriched with glutamate at 4 g/kg/d (LPS-Glu group,* n = *7) or glycine isonitrogenous to glutamate (LPS-Gly group,* n = *7). A control group (healthy group,* n = *7) had free access to a standard rodent diet. Tissue weights and protein contents were significantly lower in both LPS-treated groups than in the healthy group. No plasma or tissue accumulation of glutamate was observed except in the liver. Glutamine concentrations were increased in the jejunum, liver, and plasma in the LPS-Glu group versus the other two groups (*$P < 0.05$*). Conversely, they were depleted in muscles of the endotoxemic groups versus the healthy group (*$P < 0.05$*). Villus height was significantly greater in the LPS-Glu group than in the LPS-Gly group in the jejunum (*$P < 0.05$*), but not in the ileum. In conclusion, a glutamate-enriched diet administered enterally to endotoxemic rats can counteract glutamine depletion in the splanchnic area but not in muscles. In addition, glutamate displayed a trophic effect restricted to the jejunum.* (J. Nutr. Biochem. 10:331–337, 1999)

Keywords: glutamate; glutamine; enteral diet; endotoxemic rats; intestine; muscles

Introduction

Glutamine, the most abundant free intracellular amino acid (AA) after taurine, plays major physiologic roles. In particular, it is an important fuel for enterocytes[1] and immune cells.[2] Glutamine also provides ammonia for acid-base balance in the kidney[3] and is the precursor for purine and pyrimidine synthesis.[1] During sepsis, an increased glutamine requirement for kidney, liver, and immune system cells is observed. Glutamine is then made available by an elevated rate of synthesis and release from skeletal muscles and lungs[4] and by a decreased rate of its intestinal utilization associated with the decrease in mucosal glutaminase activity.[5] However, during serious or long illness, glutamine producing tissues are unable to meet increased needs and thus, this AA becomes conditionally essential.[6] In these conditions, as previously described by us and other authors,[7–9] the administration of exogenous glutamine to humans or animals restores AA concentrations to normal, improves nitrogen balance, and sustains tissue structures and functions, particularly in the intestine. However, the enrichment of liquid nutritional products with free glutamine is difficult because of its relatively poor solubility and stability.[10] This drawback has stimulated research into glutamine analogs such as dipeptides (e.g., Glycyl-Gln or Alanyl-Gln) and glutamine precursors (e.g., ornithine α-ketoglutarate).[7] Enteral or parenteral supplementation with these latter substances are, in a variety of catabolic states, as beneficial as glutamine itself.[7,11] In contrast, little research

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Address correspondence to Docteur Marie-Paule Vasson, Laboratoire de Biochimie, Biologie Moléculaire et Nutrition, Faculté de Pharmacie, 28, place Henri-Dunant - B.P. 38, 63001 Clermont-Ferrand Cedex 1, France.
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has focused on the more direct precursor of glutamine via glutamine synthetase (i.e., glutamate). This AA plays a pivotal role in the intermediary metabolism: It is linked to the citric acid cycle and gluconeogenesis through glutamate dehydrogenase and transaminases. Furthermore, glutamate is the precursor of a key regulator of ureagenesis, N-acetyl Glu,[12] of glutathione and of several AAs such as proline and ornithine, which are involved in collagen and polyamine synthesis, respectively.[13] Glutamate is also a neurotransmitter and the precursor of another transmitter, γ-aminobutyric acid. This property underlies the intensive debate about the potential neurotoxicity of glutamate. It has been implicated in the Chinese restaurant syndrome described in adult humans and caused by intake of a large dose (3 g or more) of monosodium L-glutamate.[14]

Concerning in vivo glutamate exchanges and metabolism, Darmaun et al.[15,16] described different weakly mixing pools, as shown after intravenous administration of a tracer: There exists an interorgan transport of glutamate but this pool is poorly metabolized into glutamine.[15] This was confirmed in a cultured fibroblast model: There is a compartmentalization of glutamate pools so that exogenous glutamate does not mix with the endogenous pool. This latter is solely responsible for glutamine synthesis.[16] In addition, after an oral administration of glutamate in healthy subjects, this AA was mainly catabolized in the splanchnic area, giving rise to only limited appearance of glutamine in the systemic circulation.[17]

Hence, in physiologic conditions, exogenous glutamate is considered a poor substitute for glutamine, with a possible deleterious potential. Consequently, in catabolic states, little research has focused on its usefulness as a precursor of glutamine. However, results obtained in physiologic conditions cannot be extrapolated to stress situations in which interorgan glutamate/glutamine flows and related enzyme activities change. Accordingly, the aim of this study was to determine whether an enteral glutamate-enriched diet could restore glutamine tissue pools in endotoxemic rats and to evaluate if such a diet would modulate the intestinal trophicity.

Material and methods

Animals

Twenty-one 3-week-old male Sprague-Dawley rats (Iffa Credo, L'Arbresle, France) were used. For 1 week (d7 to d0) the rats were maintained in individual cages and given free access to a standard diet (UAR AO_3, Villemoisson-Sur-Orge, France) and water. During the experimentation, the rats were submitted to an inverted light cycle of 12 hours on/12 hours off. Body weight and food intake were recorded daily. This study was conducted in compliance with the Guide for the Care and Use of Laboratory Animals (NRC 1985) and our laboratory is authorized by the French Ministry of Agriculture and Forestry to use the model described here.

Experimental design

At the end of the acclimatization period (d0), the rats were randomly assigned to three groups. The first group ($n = 14$) received an intraperitoneal (IP) injection of lipopolysaccharide (LPS) from *Escherichia coli* (serotype 0127: B8; Sigma, Saint-Quentin-Fallavier, France) at 3 mg/kg body weight. After 24 hours of food deprivation (d0 to d1), the rats were enterally refed for 48 hours (d1 to d3) using Osmolite® (Abbott-Ross, Rungis, France) enriched with glutamate at 4 g/kg/d (LPS-Glu group, $n = 7$) or with glycine at 2 g/kg/d (LPS-Gly group, $n = 7$) making the two groups isonitrogenous. The remaining seven rats were used as a control group (healthy group) and were given free access to the standard rodent diet throughout the experiment. The rationale for this experimental design was recently described elsewhere.[18] The selected dose of glutamate (4 g/kg) is in the range of those used for studies on animal models involving glutamine or glutamine precursors such as ornithine α-ketoglutarate.[7,11,12] Total glutamate content in the regimen was 4.80 g/kg/d, taking into consideration glutamate contained in the Osmolite diet. In a preliminary experiment using the same experimental model, we administered three different doses of glutamate: 1, 2, and 4 g/kg. The highest dose was not deleterious but induced more marked biological effects. Therefore, we considered the dose of 4 g/kg as the best to explore the effects of glutamate supplementation in our model.

Urine was collected and body weight was measured daily. At d3, after overnight fasting, animals were euthanized by decapitation. Blood samples were collected in heparinized tubes and rapidly centrifuged, and the plasma was deproteinized with sulfosalicylic acid (50 g/L). Supernatants were stored at −80°C until AA analysis. The abdominal cavity was opened and the liver was quickly removed. The small intestine, from the ligament of Treitz to the ileocecal junction, was promptly resected, cut in the middle, and flushed with ice-cold 9 g/L saline. Pieces 1 cm long were removed in the proximal part of the jejunum and the ileum for histomorphometric examination. A 10-cm segment of the jejunum and of the ileum was everted to scrape the mucosa for free AA content analysis. The tibialis and extensor digitorum longus (EDL) muscles were then rapidly removed from hindlimbs. Tissues were weighed and quickly frozen in liquid nitrogen before storage at −80°C until used.

Analytical methods

Urinary variables. Nitrogen was quantified by chemoluminescence using an Antek 7000 apparatus (Antek, Houston, TX USA). Nitrogen balance (N mg/24 h) was calculated as the difference between daily nitrogen intake and daily urinary nitrogen excretion. 3-Methylhistidine (3-MH) was analyzed by ion-exchange chromatography as described below for AA analysis. Creatinine (creat) was estimated by Jaffe's reaction. These results were expressed as 3-MH:creat ratio.

Plasma amino acids. Deproteinized plasma samples were analyzed for AA concentrations with an AA autoanalyzer

(6300 Beckman, Palo Alto, CA USA) by ion-exchange chromatography with ninhydrin detection as previously described.[19] Our results in the European Quality Control Scheme (ERNDIM) indicate the accuracy of the plasma AA determination.

Tissue amino acid and total protein contents. Free AA concentrations of tissues (liver, muscles, intestinal mucosa) after acid extraction were measured as previously described.[19] The tissue protein contents after delipidation and basic dissolution were assayed by Gornall's method.[19,20]

Intestinal morphometric study. After fixation in Bouin's solution, the intestine pieces were dehydrated and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin. Villus length and crypt depth were measured with a semi-automatic image analyzer (MOP/AM 01 System, Kontron, Munich, Germany). Villus height is the distance from the crypt-villus junction to the villus tip and crypt depth is the distance from the crypt-villus junction to the bottom of the crypt. The morphologic studies were blinded (i.e., the operator was not aware about the group location of the samples).

Statistical analysis

Results are expressed as means ± SEM. Statistical analysis was performed by analysis of variance followed by a Newman-Keüls test (PCSM, version 5, Deltasoft 1992, Meylan, France). In addition, a Student's *t*-test was made to specifically assess significant differences between LPS-Glu group and its nitrogenous control, the LPS-Gly group. A *P*-value of less than 0.05 was considered to indicate a significant difference among groups.

Results

Soon after the LPS challenge, all the rats displayed pathologic signs (i.e., piloerection, diarrhea, and chromodacryorrhea), which disappeared within approximately 24 hours.

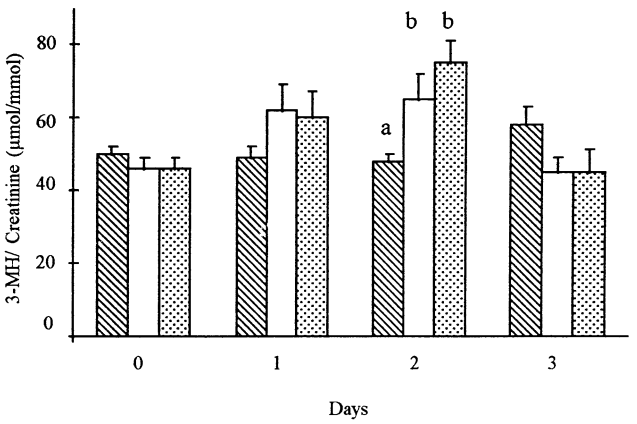

Figure 1 Urinary 3-methyl-histidine (3-MH):creatinine ratio in endotoxemic and healthy rats. Means ± SEM; analysis of variance and Newman-Keüls: For each day, bars with different superscripts mean a significant difference at $P < 0.05$. Student *t*-test between LPS-Gly and LPS-Glu groups was not significant. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate. ▨ Healthy ☐ LPS-Gly ▩ LPS-Glu

During the days following LPS injection (d0 to d3), the two endotoxemic groups significantly lost approximately 10% of their body weight ($P < 0.05$ vs. healthy; *Table 1*). The weights of tissues and organs also were significantly decreased in both the LPS-treated groups except in the ileum ($P < 0.01$ vs. healthy; *Table 1*). No significant difference was observed between the LPS-Glu group and the LPS-Gly group.

At d1 after endotoxemia, nitrogen balance became negative in the two endotoxemic groups (LPS-Gly: −162 ± 15 mg/24 h, LPS-Glu: −160 ± 13 mg/24 h), and significantly lower than in the healthy group (426 ± 27 mg/24 h; $P <$ 0.01). From d2 to d3, nitrogen balance became positive but remained significantly lower in the endotoxemic groups than in the healthy rats with no difference between the LPS-Gly group (5 ± 11 mg/24 h) and the LPS-Glu group (45 ± 10 mg/24 h) at d3.

The 3-MH:creat ratio was significantly greater at d2 for the two endotoxemic groups versus the healthy group (*Figure 1*; $P < 0.05$) and normalized at d3 with no difference according to supplementation.

As shown in *Table 2*, total protein contents in intestine,

Table 1 Total body, organ, and tissue weights

Weights (g)	Healthy	LPS-Gly	LPS-Glu
Body weight			
d0	121 ± 1[a]	121 ± 1[a]	119 ± 2[a]
d1	136 ± 1[a]	115 ± 2[b]	112 ± 3[b]
d2	142 ± 1[a]	115 ± 2[b]	113 ± 2[b]
d3	150 ± 2[a]	112 ± 2[b]	111 ± 2[b]
Jejunum (10 cm)	0.49 ± 0.02[a]	0.33 ± 0.01[b]	0.34 ± 0.02[b]
Ileum (10 cm)	0.49 ± 0.03[a]	0.39 ± 0.03[a]	0.40 ± 0.03[a]
Liver	5.89 ± 0.11[a]	4.23 ± 0.14[b]	4.38 ± 0.23[b]
EDL	0.13 ± 0.01[a]	0.11 ± 0.00[b]	0.11 ± 0.00[b]
Tibialis	0.53 ± 0.01[a]	0.41 ± 0.01[b]	0.43 ± 0.01[b]

Means ± SEM. $n = 7$. Analysis of variance + Newman-Keüls: Data within a line with different superscripts mean a significative difference at $P < 0.01$.
Student's *t*-test between LPS-Gly and LPS-Glu groups was not significant.
EDL–extensor digitorum longus. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate

Table 2 Tissue protein contents

Protein (mg)	Healthy	LPS-Gly	LPS-Glu
Jejunum (10 cm)	69 ± 3[a]	42 ± 1[b]	44 ± 2[b]
Ileum (10 cm)	67 ± 5[a]	51 ± 4[b]	52 ± 4[b]
Liver	2,186 ± 74[a]	1,470 ± 56[b]	1,488 ± 88[b]
EDL	44 ± 2[a]	34 ± 2[b]	32 ± 2[b]
Tibialis	198 ± 6[a]	154 ± 6[b]	160 ± 3[b]

Means ± SEM, $n = 7$. Analysis of variance + Newman-Keüls: Data within a line with different superscripts mean a significative difference at $P < 0.05$.
Student's *t*-test between LPS-Gly and LPS-Glu groups was not significant.
EDL–extensor digitorum longus. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate

Table 3 Plasma and tissue free glutamate concentrations

Glutamate	Healthy	LPS-Gly	LPS-Glu
Plasma (μmol/L)	136 ± 13[a]	112 ± 5[a]	165 ± 32[a]
Jejunum (μmol/g)	3.59 ± 0.18[a,b]	3.20 ± 0.12[a]	3.84 ± 0.21[b*]
Ileum (μmol/g)	4.39 ± 0.24[a]	3.07 ± 0.19[b]	3.66 ± 0.28[b]
Liver (μmol/g)	3.37 ± 0.20[a]	3.92 ± 0.51[a]	5.47 ± 0.38[b*]
EDL (μmol/g)	1.76 ± 0.17[a,c]	1.00 ± 0.04[b]	1.43 ±0.09[c*]
Tibialis (μmol/g)	1.36 ± 0.10[a]	0.74 ± 0.08[b]	1.06 ± 0.05[c*]

Means ± SEM. $n = 7$. Analysis of variance + Newman-Keüls: Data within a line with different superscripts mean a significative difference at $p < 0.05$.
Student's *t*-test between LPS-Gly and LPS-Glu groups: $^*P < 0.05$.
EDL–extensor digitorum longus. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate

liver, and muscles (EDL and tibialis) were significantly smaller in the two endotoxemic groups than in the healthy one, with no difference between endotoxemic groups supplemented with Glu or Gly.

In the plasma, no significant variation of glutamate was observed for the two endotoxemic groups versus the healthy group (*Table 3*). Jejunal glutamate concentration was greater in the LPS-Glu group than in the LPS-Gly group, whereas ileal glutamate content was lower in both endotoxemic groups than in the healthy group ($P < 0.05$; *Table 3*). In the liver, glutamate concentration was significantly higher in the LPS-Glu group than in the other two groups. In skeletal muscles, glutamate concentration was increased both in the EDL and the tibialis of LPS-Glu group versus the LPS-Gly group, although glutamate content in the tibialis was not replenished in the LPS-Glu group compared with the healthy group.

Interestingly, glutamine concentration was more elevated in the plasma, jejunum, and liver of the LPS-Glu group compared with the two other groups ($P < 0.05$; *Figure 2*). In contrast, glutamine concentration was diminished significantly in both the EDL and the tibialis in the endotoxemic groups compared with the healthy group ($P < 0.05$). However, glutamate supplementation afforded a partial recovery of glutamine pool for the tibialis.

The patterns of other AAs metabolically related to glutamate such as alanine, proline, ornithine, citrulline, and arginine also were studied.

Alanine concentrations in plasma, jejunum and liver were significantly higher in the LPS-Glu group than in the other two groups ($P < 0.05$; *Table 4*). In the ileum, alanine content was higher in the LPS-Glu group than in the LPS-Gly group ($P < 0.05$). In skeletal muscles, alanine concentrations in both the EDL and the tibialis were more elevated in the LPS-Glu group than in the other two groups ($P < 0.05$).

The concentration of proline was augmented in the LPS-Glu group versus the other two groups in the plasma and the EDL ($P < 0.05$; *Table 5*). The concentration of this AA was unmodified in the intestine and liver. In addition, in the two muscles studied, a significant increase of proline was observed for the LPS-Glu group versus the LPS-Gly group ($P < 0.05$).

No significant variation of ornithine concentration was

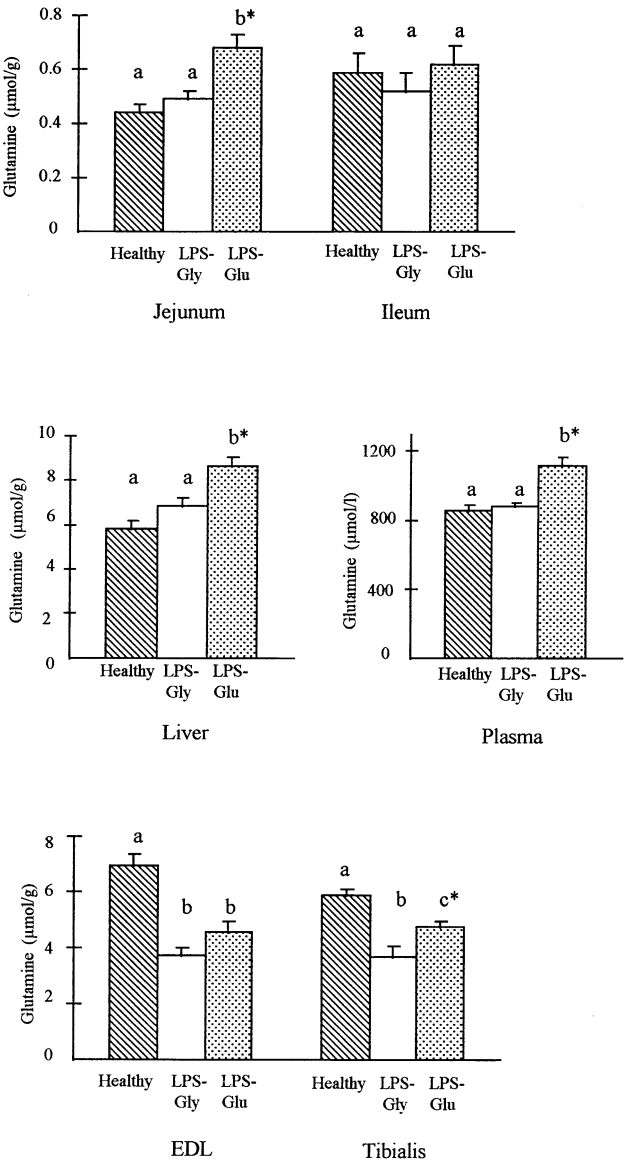

Figure 2 Free glutamine concentrations in plasma and tissues from endotoxemic and healthy rats. Means ± SEM; analysis of variance and Newman-Keüls: For each tissue, bars with different superscripts mean a significant difference at $P < 0.05$. Student *t*-test between LPS-Gly and LPS-Glu groups: $^*P < 0.05$. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate. ▨ Healthy □ LPS-Gly ▨ LPS-Glu

observed (data not shown) except in plasma, where it was greater ($P < 0.05$) in the LPS-Glu group (92 ± 4 μmol/L) than in the LPS-Gly group (76 ± 4 μmol/L) and the healthy group (66 ± 3 μmol/L).

Citrulline levels were unchanged in the plasma and the jejunum (data not shown) but were decreased ($P < 0.05$) in the EDL for the two endotoxemic groups versus the healthy one (healthy group, 0.39 ± 0.02 μmol/g; LPS-Gly group, 0.26 ± 0.01μmol/g; LPS-Glu group, 0.34 ± 0.02 μmol/g) with a significant increase in the LPS-Glu group versus the LPS-Gly group ($P < 0.05$).

Glycine concentration was dramatically augmented ($P < 0.05$) in the LPS-Gly group compared with the other two

Table 4 Plasma and tissue free alanine concentrations

Alanine	Healthy	LPS-Gly	LPS-Glu
Plasma (μmol/L)	374 ± 36[a]	447 ± 20[a]	658 ± 37[b*]
Jejunum (μmol/g)	2.59 ± 0.12[a]	2.85 ± 0.11[a]	3.20 ± 0.11[b*]
Ileum (μmol/g)	2.61 ± 0.15[a,b]	2.39 ± 0.08[a]	2.93 ± 0.15[b*]
Liver (μmol/g)	2.48 ± 0.09[a]	1.77 ± 0.09[b]	2.03 ± 0.07[c*]
EDL (μmol/g)	2.70 ± 0.23[a]	2.46 ± 0.08[a]	4.24 ± 0.19[b*]
Tibialis (μmol/g)	3.10 ± 0.13[a]	2.79 ± 0.20[a]	4.37 ± 0.15[b*]

Means ± SEM. $n = 7$. Analysis of variance + Newman-Keüls: Data within a line with different superscripts mean a significative difference at $p < 0.05$.
Student's *t*-test between LPS-Gly and LPS-Glu groups: *$P < 0.05$.
EDL–extensor digitorum longus. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate

groups for both plasma (healthy group, 434 ± 27 μmol/L; LPS-Gly group, 1,290 ± 100 μmol/L; LPS-Glu group, 523 ± 31 μmol/L; $P < 0.05$) and tissues (data not shown).

Serine patterns were similar to glycine in plasma (healthy group, 285 ± 16 μmol/L; LPS-Gly group, 604 ± 48 μmol/L; LPS-Glu group, 421 ± 12 μmol/L; $P < 0.05$) and tissues (data not shown).

For arginine, no variation was observed for the three groups studied except in the muscles, where concentrations of this AA were lower ($P < 0.05$) in the two endotoxemic groups than in the healthy group (i.e., for EDL, healthy group, 0.34 ± 0.05 μmol/g; LPS-Gly group, 0.13 ± 0.02 μmol/g; LPS-Glu group, 0.18 ± 0.02 μmol/g).

Concerning morphometry, total height, villus height, and crypt depth of jejunum were significantly smaller in the two endotoxemic groups than in the healthy group (*Figure 3*). However, the villus height was significantly greater in the LPS-Glu group than in the LPS-Gly group ($P < 0.05$) and no difference in the depth of the crypts was detected between these groups. In contrast, no significant change was observed for ileal morphometry (*Figure 3*).

Discussion

This study explored whether an enterally administered glutamate-enriched diet can restore glutamine tissue pools in endotoxemic rats and whether any intestine trophic effect is induced by a such diet.

Table 5 Plasma and tissue free proline concentrations

Proline	Healthy	LPS-Gly	LPS-Glu
Plasma (μmol/L)	228 ± 30[a]	224 ± 22[a]	309 ± 16[b*]
Jejunum (μmol/g)	0.57 ± 0.04[a]	0.57 ± 0.04[a]	0.66 ± 0.06[a]
Ileum (μmol/g)	0.52 ± 0.05[a]	0.40 ± 0.02[a]	0.52 ± 0.03[a*]
Liver (μmol/g)	0.16 ± 0.01[a]	0.14 ± 0.01[a]	0.17 ± 0.01[a]
EDL (μmol/g)	0.35 ± 0.04[a]	0.27 ± 0.02[a]	0.47 ± 0.04[b*]
Tibialis (μmol/g)	0.38 ± 0.04[a,b]	0.29 ± 0.03[a]	0.46 ± 0.04[b*]

Means ± SEM. $n = 7$. Analysis of variance + Newman-Keüls: Data within a line with different superscripts mean a significative difference at $p < 0.05$.
Student's *t*-test between LPS-Gly and LPS-Glu groups: *$P < 0.05$.
EDL–extensor digitorum longus. LPS, lipopolysaccharide; Gly, glycine; Glu, glutamate

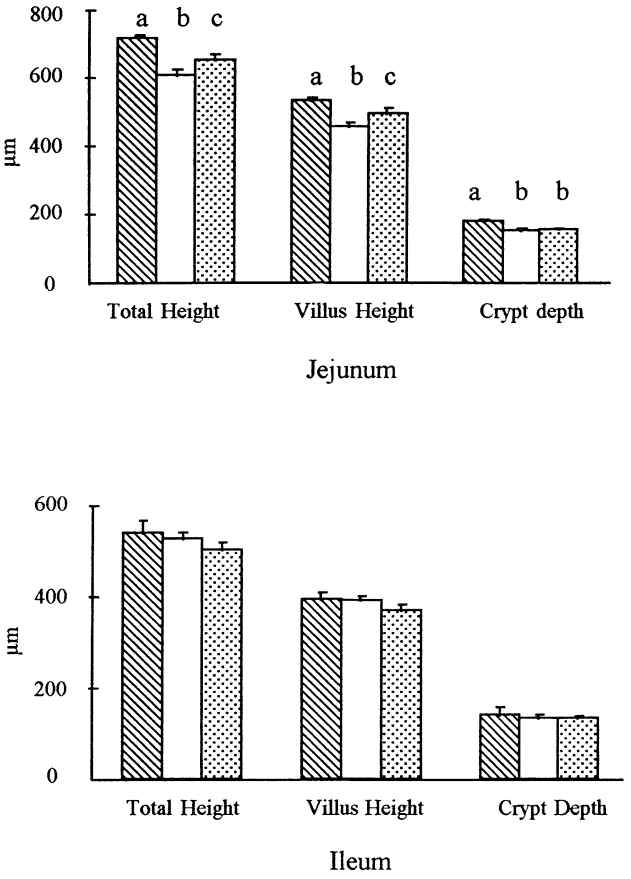

Figure 3 Jejunal and ileal morphometry of endotoxemic and healthy rats. Means ± SEM; analysis of variance and Newman-Keüls: For each parameter, bars with different superscripts mean a significant difference at $P < 0.05$. Student *t*-test between LPS-Gly and LPS-Glu groups was not significant. ▧ Healthy □ LPS-Gly ▨ LPS-Glu

In our experimental model, the effects of dietary supplementation with glutamate were compared with those observed with an isonitrogenous supplementation in the form of glycine as control. There is no consensus on the choice of compounds for a "nitrogen placebo," because the concept of inert nitrogen is still controversial.[6] We selected glycine because of its wide use as an isonitrogenous control.[18,21–23] The LPS-Gly group was characterized by modifications of glycine and serine concentrations in all tissues. The accumulation of glycine could reflect a low rate of metabolism of this AA, which can result in stimulation of the serine-threonine-dehydratase activity converting glycine into serine. This may explain the marked increase in serine concentrations observed in the LPS-Gly group as already described.[24]

The administration of a single dose of LPS induces a catabolic state comparable to bacterial infection[18] and constitutes a reproducible model of stress.[25] Stress induced by LPS administration and food deprivation led to altered nitrogen balance, body weight loss, and decreased organ and tissue weights accompanied by a diminution of their protein content.

In glutamate-supplemented rats, elevation of glutamate concentrations (relative to LPS-Gly and/or healthy rats) was

more marked in the intestine and liver than in the plasma and muscles, confirming that the splanchnic area is the primary site of storage and metabolism of glutamate and that little of this AA reaches the systemic circulation.[15,17,26]

Rats receiving a glutamate-enriched diet presented higher glutamine concentrations in the jejunum compared with the other two groups, thus exerting an endogenous glutamine-sparing effect that may correspond, in part at least, to the glutamate-induced glutaminase inhibition.[27] Alternatively, glutamine could arise from glutamate because glutamine synthetase seems to be expressed in this tissue during sepsis,[28] whereas in parallel glutaminase activity is decreased.[1] It is reasonable to predict from the literature[13] that glutamine concentration in the portal vein is increased, and this should contribute to high glutamine levels in the liver as observed here. Additionally, glutamate may be taken up by perivenous hepatocytes, which contain high glutamine-synthetase activity.[29] Glutamine metabolism in the liver during sepsis is controversial. For some authors, activation of glutaminase occurs[30] and the liver remains a glutamine consumer organ. For others, in relation with acidosis,[3] the liver switches to glutamine production, both uptake by periportal cells and glutaminase activity being inhibited. High glutamine levels in the liver and increase in plasma glutamine concentrations observed in this study are consistent with the second hypothesis. This is further supported by data from Hasebe et al.[31] who suggested, in a model of burned rats receiving an enterally administered glutamate-enriched diet, that glutamate first was oxidized in enterocytes and that it may be converted into glutamine in the liver through an increased glutamine-synthetase activity.

In skeletal muscles, the glutamine pool was depleted in endotoxemic rats, which is in agreement with Augsten et al.,[32] who showed a similar decrease in muscle glutamine concentration in endotoxemic rats. This classical response to injury is associated with an accelerated muscle glutamine release, although at the same time glutamine synthesis is increased. In our model of moderate trauma, enhanced muscle glutamine efflux together with switching of liver to glutamine production must meet other organ requirements because plasma glutamine augmentation was not different from healthy animals.

The plasma and tissue increase in alanine concentration in the LPS-Glu group is probably related to the high alanine-aminotransferase activity in the gut and liver.[33] α-Ketoglutarate most likely enters the tricarboxylic acid cycle in the enterocyte[34] and alanine is subsequently used as a substrate for gluconeogenesis.[35]

As shown in our study, glutamate also can act quantitatively as a carbon precursor for other metabolites such as proline and ornithine, which in turn is converted into citrulline. The gut probably plays a central role in these metabolic conversions[13] but the complexity of the pathways involved and the tissue specificity of the enzyme equipment preclude further speculation.

Glutamate-enriched diet administration produced clear effects on trophicity in the jejunum, whereas the effects were weak in the ileum and muscles. It is noteworthy that almost the same pattern of action was observed for glutamine concentrations. This supports the common assumption of a link between glutamine pools and tissue trophicity[7,11] and would suggest a jejunal preferential absorption. The weak (or absent) effect of glutamate on the glutamine pool in the muscles is also consistent with the lack of effect on nitrogen balance, because it is well known that most excreted nitrogen comes from muscles in trauma situations. This also suggests that glutamine derived from glutamate in the jejunum may be responsible for better maintenance of villus height in glutamate-treated endotoxemic rats, as was demonstrated after glutamine administration itself in various models of trauma inducing intestinal atrophy.[36]

In conclusion, as described in physiologic conditions, exogenous glutamate is also mainly metabolized in the splanchnic area in pathologic situations. However, glutamine generation seems greater in this latter situation, possibly owing to modifications of enzyme activities (i.e., expression of glutamine synthetase in the intestine or inhibition of glutaminase in the liver).[3,31] This might explain the jejunal effect of the glutamate-enriched diet. However, the lack of effect of this diet on muscle and nitrogen balance obtained in our experimental model does not argue for the specific use of glutamate as a nutrition supplement in trauma.

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