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Basic nutritional investigation

Arginine-enriched diet limits plasma and muscle glutamine depletion in head-injured rats

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Abstract

Objective: Injury is associated with a depletion in glutamine (GLN) pools, which may contribute to impairment of immune and nutritional statuses. Total parenteral nutrition enriched with arginine (ARG) is able to generate GLN in surgical patients. We hypothesized that this same concept may be applicable to enteral administration and could be extended to muscle GLN reserves. This study investigated the ability of an enteral formula enriched with ARG to restore the GLN pools in an experimental model of head injury.

Methods: Twenty-five male Sprague-Dawley rats were randomized into 4 groups: *ad libitum* access to food, head injury plus free access to nutrition, head injury plus standard enteral nutrition (Sondalis), and an immune-enhancing diet (IED). The two enteral diets were adjusted to be isocaloric (290 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1}) and isonitrogenous (3.29 g $\cdot$ kg^{-1} $\cdot$ d^{-1}) and were delivered for 4 d (24 h/24 h). After sacrifice, plasma and muscle amino acids were determined.

Results: Head injury was associated with a large depletion of muscle and plasma GLN pools that were restored by IED administration but not by the standard diet. Moreover, the IED but not the standard diet improved or normalized ornithine and glutamate pools, suggesting that the modification of GLN pools is related to ARG administration.

Conclusion: In our model of head injury, our IED, a diet without free GLN, is efficient in restoring the plasma and muscle pools of GLN, probably due to its high ARG content.

Keywords: Immune enhancing diet; Trauma; Enteral nutrition; Ornithine; Glutamate

Abbreviations: AA, amino acid; AL, ad libitum; HI, head injury; GLN, glutamine; ARG, arginine; IED, immune enhancing diet; HIS, head-injury receiving Sondalis; HIC, head-injury receiving the IED; EDL, extensor digitorum longus.

Introduction

Head injury (HI) is one of the most severe injuries encountered in intensive care units. Clinical [1,2] and experimental [3] studies have well described that HI is characterized by protein wasting and a depletion of free amino acid pools, in particular glutamine (GLN). This is of particular importance because it has been suggested that hypoglutaminemia may contribute to the impairment of the im-

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mune system observed in catabolic states [4]. However, although there is a line of evidence to suggest that GLN requirements are high in stressed patients, there is still controversy about the form that should be used to provide GLN (i.e., GLN itself or a precursor such as GLN-containing dipeptides or ornithine α-ketoglutarate [5,6]). This is related to the fact that GLN administered enterally is largely metabolized by the gut in the first passage and only minimal amounts enter the general blood circulation. The magnitude of splanchnic extraction of GLN in healthy subjects is reported to be around 60% to 80% [7] and may be even higher in trauma patients in whom liver gluconeogenesis is activated.

We previously demonstrated that total parenteral nutrition enriched with arginine (ARG) was able to improve plasma GLN pools in surgical patients [8]. However, this property of ARG as a GLN precursor has not been documented when given by an enteral route, and the effect of ARG administration on muscle GLN pools is not known. In this context, the present study investigated the ability of Crucial (an enteral formula enriched with ARG) to restore plasma and muscle GLN pools in a validated experimental model of HI by temporal fluid percussion [3]. We chose this model because it induces reproducible nutritional alterations that mimic most metabolic and immunological disturbances observed in clinical practice [3,9].

Materials and methods

Animal care and experimentation complied with French and European Community regulations (Official Journal of the European Community L 358, 18/12/1986), and two of the authors are authorized by the French government to use animal models of stress and to perform surgical operations in rats (CC, no. 75.456; CM, no. 75.522).

Animals and study design

Fifty-two male Sprague-Dawley rats (300–325 g; Charles River, L'Arbresle, France) were housed individually in a controlled temperature environment (21 ± 1°C), with a 12-h light-dark cycle (lights on 0800–2000 h, light off 20:00–8:00 h). They were maintained on a standard chow diet (17% protein, 3% fat, 59% carbohydrate, 21% water, fibers, vitamins, and minerals; A04, Usine d'Alimentation Rationnelle, Epinay-sur-Orge, France) and were given *ad libitum* access to water throughout a 6-d acclimatization period. After this acclimatization period, the rats were housed in individual metabolic cages and, after an overnight fast (D-8), they were randomized to an *ad libitum*-fed group of healthy rats (AL, $n = 7$) or a group of rats subjected to gastrostomy ($n = 45$). Rats in the AL group underwent no treatment and were fed *ad libitum* throughout the study. This group was the absolute control group.

Surgical procedure for enteral nutrition (D-7).

Seven days before HI, the rats undergoing gastrostomy were anesthetized with isoflurane (1.5 L of O_2/4% isoflurane; Minerve, Esternay, France). Gastrostomy was performed as described previously [10]. Rats received a single intramuscular injection of an anti-inflammatory drug (ketoprofen, 10 mg/kg) 20 min before the end of surgery. The rats were then given *ad libitum* access to food and water and housed in metabolic cages for a 6-d recovery period.

Head trauma (D0).

Seven days after gastrostomy (D0), HI was performed by fluid percussion as previously described [3]. In brief, the scalp was incised and a 3-mm craniotomy was performed lateral to the right temporoparietal cortex (stereotaxic coordinates: 3.5 mm anterior and 6 mm above the interaural line) with a dental drill, taking care to leave the *dura mater* intact. A 3-mm polyethylene tube was placed over the *dura mater*, fixed securely into the craniotomy site using dental cement (Paladur, Hanau, Germany), and connected to a solenoid valve (Danfoss, Nordborg, Denmark). The opposite end of the valve was connected to a high-performance liquid chromatographic pump (Gilson, Roissy, France). The system was filled with sterile water and providing a calibrated outflow pressure of 1.6–1.8 bar. The solenoid valve was controlled with a timer (Omron, Kyoto, Japan) and the opening for 20 ms triggered the percussion directly onto the *dura mater*. The cortical pressure applied was measured extracranially with a pressure transducer (Emka Technologies, Paris, France) connected to an oscilloscope (DSO 400, Gould, les Ulis, France). Immediately after fluid percussion, the tube was removed, the scalp was sutured, and the animals were kept at 26–28°C to recover from the anaesthesia. The animals received 10 mL of saline intraperitoneally to prevent the acute renal failure that occurs after trauma [3]. The rats were maintained at 26–28°C for 4 h [11] before being transferred to metabolic cages.

Nutrition program

Rats were placed in metabolic cages and randomized into three groups: a control group that received 0.9% NaCl by enteral route at a constant rate of 0.3 mL/100 g of body weight per hour and had free access to the standard chow diet (HI group). This control group allowed us to assess HI-related nutritional impairments (trauma control group). Sodium chloride was perfused to prevent the renal failure previously observed in this model [3]. Food intake was recorded daily. A second group received enteral administration of the standard diet Sondalis HP (HIS group; Nestlé Clinical Nutrition, Noisiel, France), and the third group received enteral administration of the immune-enhancing diet (IED) Crucial (HIC group; Nestlé Clinical Nutrition; Table 1). The

Table 1
Diet composition (per 100 mL)

	Sondalis HP + caseinate	Crucial
Proteins (g)	6.8	9.4
Caseinate added (g)	4.69	
Free taurine (mg)		15
L-carnitine (mg)		15
Free L-arginine (g)		1.45
Arginine bound to caseinate (g)	0.45	0.37
Total arginine, free and bound (g)	0.45	1.82
Total nitrogen (g)	1.7	1.7
Carbohydrates (g)	16.9	13.5
Lipids (g)	5.2	6.6
MCT (g)	2.5	3.4
ω-6 (g)	1.1	0.77
ω-3 (g)	0.15	0.38
ω-6/ω-3 ratio	7.33	2.03
Sodium (mg)	90	120
Potassium (mg)	169	190
Chloride (mg)	146.6	170
Calcium (mg)	74.4	100
Phosphorous (mg)	81.2	100
Magnesium (mg)	31.6	36
Iron (mg)	1.47	1.8
Zinc (mg)	1.47	3.6
Copper (μg)	0.15	0.2
Manganese (mg)	0.32	0.32
Fluoride (mg)	0.15	0.18
Iodine (μg)	14.66	15
Molybdenum (μg)	11.28	15
Chromium (μg)	10.6	9.8
Selenium (μg)	6.77	9.8
Vitamin A (μg RE)	68	210
β-Carotene (μg RE)		54
Vitamin D (μg)	0.38	1.5
Vitamin E (mg TE)	1.47	6.5
Vitamin K (μg)	4.5	8.3
Vitamin C (mg)	7.89	60
Thiamin (mg)	0.15	0.18
Riboflavin (mg)	0.18	0.2
Niacin (mg)	1.8	1.8
Vitamin B5 (mg)	0.74	
Vitamin B6 (mg)	0.21	0.25
Folic Acid (μg)	27	36
Vitamin B12 (μg)	0.45	0.45
Biotin (μg)	14.66	4.5
Choline (mg)	31.5	30

MCT, medium chain triglycerides.

Sondalis HP diet was isovolumic and was rendered isonitrogenous and isocaloric with respect to the IED by addition of a casein hydrolysate (Sigma, Saint-Quentin Fallavier, France). Enteral nutrition was introduced on day D0, 4 h after injury, at a flow rate of 0.5 mL/h. The flow rate was then gradually increased until reaching a maximum on day D1, and enteral nutrition was infused 24 h/24 h at a constant rate of 0.8 mL/100 g of body weight per hour for 4 d, providing 290 kcal $\cdot$ kg^{-1} $\cdot$ d^{-1} and 3.29 g of nitrogen $\cdot$ kg^{-1} $\cdot$ d^{-1}. On day 4 (D4), nutrition was stopped 2 h before the rats were sacrificed. All rats were then anesthetized with isoflurane and sacrificed by beheading.

Procedures and analytical methods

Blood collection and muscle removal

Blood was collected on sodium heparinate. Three muscles of the hind limbs (soleus, extensor digitorum longus, and gastrocnemius) were rapidly removed, weighed, frozen in liquid nitrogen, and then stored at −80°C until amino acid (AA) analysis.

Plasma AA concentration

Blood samples were rapidly centrifuged, and plasma was deproteinized with a 30% (w/v) sulfosalicylic acid solution. The supernatants were stored at −80 ° until analysis. AAs were separated and quantified by ion exchange chromatography using an amino acid autoanalyzer (Aminotac model, Jeol, Tokyo, Japan).

Muscle AA concentration

Muscles were ground and deproteinized with 10% trichloroacetic acid containing 0.5 mM ethylene-diaminetetra-acetic acid. The supernatants were stored at −80°C until analysis. AAs were separated and quantified by ion exchange chromatography using the Aminotac autoanalyzer.

Statistical analysis

Statistical analysis was performed by using analysis of variance and Duncan's test. $P < 0.05$ was considered statistically significant. Data are expressed as mean ± SEM. PCSM software was used (Deltasoft, Grenoble, France).

Results

Of the 45 rats that underwent gastrostomy and HI, 23 died after HI. Failure to supply adequate continuous enteral nutrition occurred in 4 of the 22 remaining rats. Hence, 18 rats (six in the HI group, six in the HIS group, and six in the HIC group) were studied. All seven rats in the AL group were analyzed.

Head injury induced a significant decrease in plasma GLN concentrations that was partly corrected by the standard enteral nutrition and by the IED (AL versus HI versus HIS versus HIC, $P < 0.05$; Fig. 1). ARG concentrations in plasma remained unchanged in the HI and HIS groups and significantly increased in the HIC group compared with all other groups (HIC versus AL, HI, and HIS, $P < 0.05$; Fig. 1).

Head injury induced a significant decrease in GLN content of the *gastrocnemius* and *extensor digitorum longus* (HI versus AL, $P < 0.05$; Fig. 2). The standard enteral nutrition

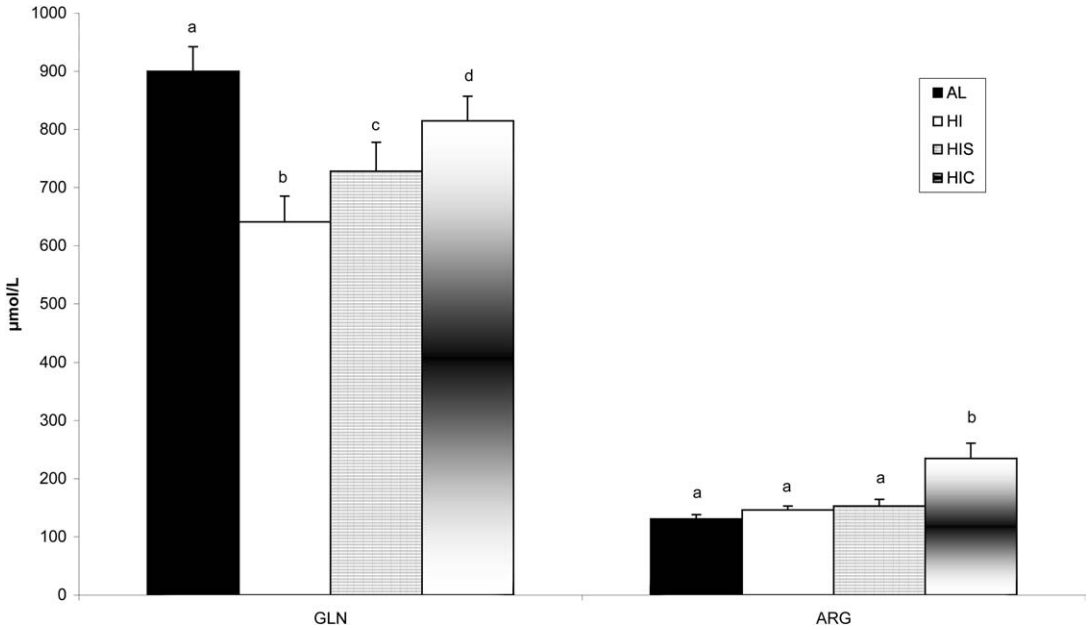

Fig. 1. GLN and ARG concentrations in plasma from rats in the AL, HI, HIS, and HIC groups. The results are expressed in micromoles per liter as means ± SEM. Values with different superscript letters in each histogram are different at $P < 0.05$ (analysis of variance and Duncan's test). AL, *ad libitum*; ARG, arginine; GLN, glutamine; HI, head injury; HIC, head injury plus Crucial diet; HIS, head injury plus Sondalis HP diet.

had no effect on GLN content, whereas the IED partly limited the decrease (Fig. 2). GLN content in the soleus was similar in all groups.

Muscle ARG content was similar in all but the HIC group, where ARG content was significantly increased in all three muscles studied (HIC versus AL, $P < 0.05$; Fig. 3).

Muscle and plasma ornithine contents were normalized or significantly increased in the HIC rats compared with control and other HI groups (Table 2). Concerning glutamate, plasma and *gastrocnemius* concentrations were normalized by the IED administration. There were no between-group differences in *extensor digitorum longus* and *soleus* muscles (Table 2).

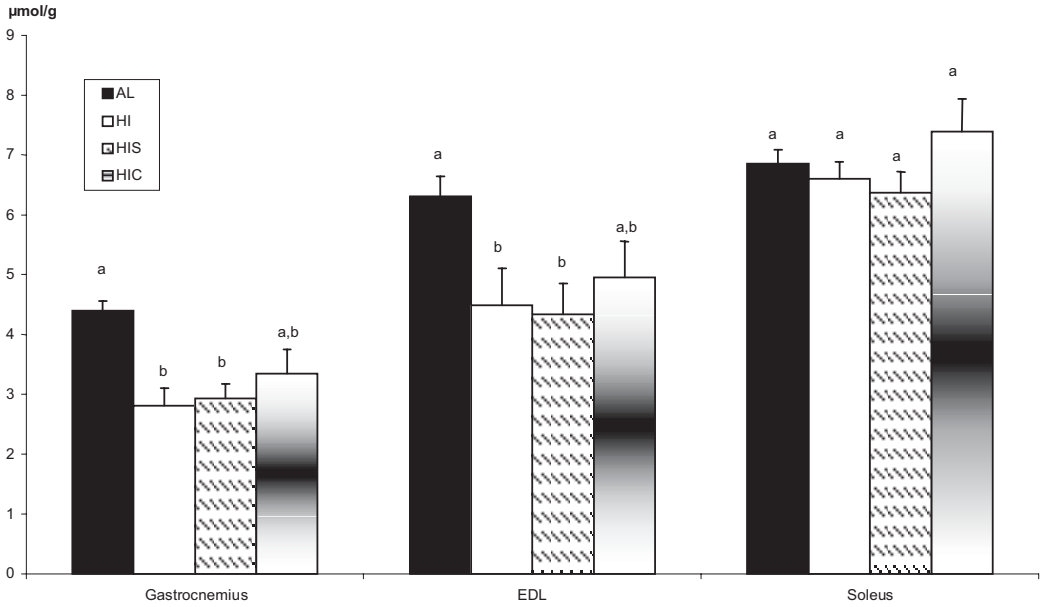

Fig. 2. Arginine concentrations in muscles (gastrocnemius, EDL, and soleus) from rats in the AL, HI, HIS, or HIC groups. The results are expressed in micromoles per gram as means ± SEM. Values with different superscript letters in each histogram are different at $P < 0.05$ (analysis of variance and Duncan's test). AL, *ad libitum*; EDL, *extensor digitorum longus*; HI, head injury; HIC, head injury plus Crucial diet; HIS, head injury plus Sondalis HP diet.

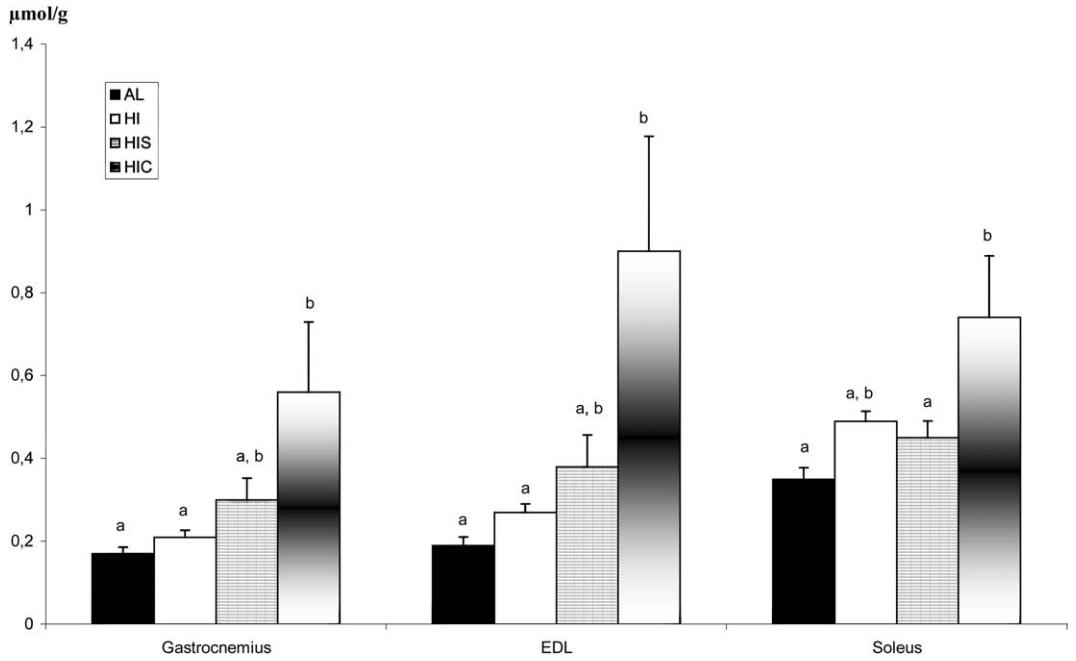

Fig. 3. Glutamine concentrations in muscles (*gastrocnemius*, EDL, and soleus) from the rats in the AL, HI, HIS, and HIC groups. The results are expressed in micromoles per gram as means ± SEM. Values with different superscript letters in each histogram are different at $P < 0.05$ (analysis of variance and Duncan's test). AL, *ad libitum*; EDL, *extensor digitorum longus*; HI, head injury; HIC, head injury plus Crucial diet; HIS, head injury plus Sondalis HP diet.

Table 2
Glutamate and ornithine concentrations in plasma and muscles (*gastrocnemius, EDL, and soleus*) in rats in the AL, HI, HIS, and HIC groups*

	Ornithine	Glutamate
Plasma		
AL	62 ± 7[a]	128 ± 21[a,b]
HI	60 ± 7[a]	84 ± 6[a]
HIS	71 ± 10[a]	128 ± 19[a,b]
HIC	110 ± 25[b]	190 ± 36[b]
Gastrocnemius		
AL	0.051 ± 0.004[a]	1.22 ± 0.08[a]
HI	0.056 ± 0.005[a,b]	0.96 ± 0.10[b]
HIS	0.043 ± 0.008[a]	1.12 ± 0.08[a,b]
HIC	0.119 ± 0.040[b]	1.29 ± 0.05[a]
EDL		
AL	0.059 ± 0.010[a,b]	1.00 ± 0.06
HI	0.054 ± 0.010[a]	0.88 ± 0.06
HIS	0.048 ± 0.010[a]	0.91 ± 0.08
HIC	0.163 ± 0.050[b]	1.09 ± 0.12
Soleus		
AL	0.066 ± 0.010[a]	3.23 ± 0.16
HI	0.071 ± 0.010[a,b]	3.39 ± 0.14
HIS	0.070 ± 0.006[a]	3.26 ± 0.09
HIC	0.137 ± 0.030[b]	3.50 ± 0.21

AL, *ad libitum*; EDL, *extensor digitorum longus*; HI, head injury; HIC, head injury plus Crucial diet; HIS, head injury plus Sondalis HP diet

* The results are expressed in micromoles per liter (plasma) and micromoles per gram (muscles) and presented as means ± SEM. Values with different superscript letters are significantly different at $P < 0.05$ (analysis of variance and Duncan's test).

Discussion

Head injury is associated with hypermetabolism and hypercatabolism, resulting in a negative nitrogen balance [1,12]. This leads to protein wasting, which compromises clinical outcome, thereby increasing morbidity and mortality. Moreover, it is also known that HI is accompanied with GLN deprivation [13] because the reserves and endogenous synthesis are not sufficient to cover the requirements of patients during critical illness. Because the efficacy of enteral GLN supplementation in terms of GLN pool restoration has not been clearly established due to major splanchnic metabolism, it is necessary to develop alternative strategies to adequately cover GLN requirements. We hypothesized that an ARG-enriched diet would be able to restore plasma and muscle GLN pools in a model of HI. As expected, HI was associated with a significant decrease in GLN pools. IED administration increased ARG concentrations in plasma and muscles. We also observed a clear improvement in GLN pools, which was particularly marked in the muscles. This confirms our hypothesis that ARG may be suitable to replenish GLN pools when given by an enteral route. The tissues involved in this metabolism of ARG into GLN remain to be established; however, because only the liver possesses all the enzymes required (i.e., arginase, ornithine aminotransferase, and GLN synthetase; Fig. 4), this organ may well be a good candidate through cooperation between periportal and perivenous hepatocytes [14]. Either way, it is highly likely that the increase in GLN pools stems

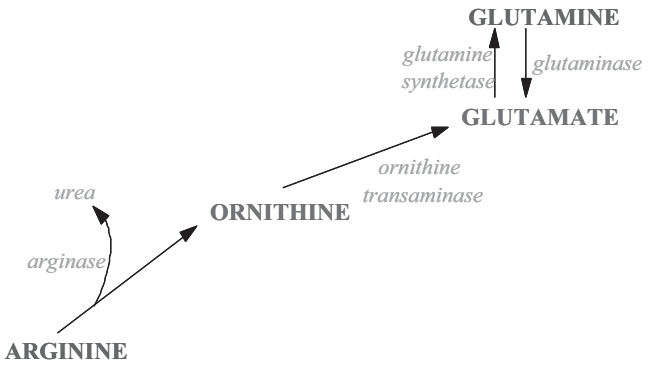

Fig. 4. Metabolic pathway of arginine and glutamine.

from ARG administration, because concentrations of the two intermediate metabolites (i.e., ornithine and glutamate; Fig. 1) increased in plasma and muscle.

The preservation of normal GLN pools can be considered an important goal in nutritional care for maintaining protein stores and immune functions [5]. This concept is supported by our recent work, where we showed that this IED is able to preserve lymphocyte function in HI rats [9]. Although it is recognized that IED is indicated in head trauma [13], further studies are required to confirm the efficacy of this treatment in preventing infectious complications.

The results of this study raise the question as to how a standard enteral nutrition also modifies plasma GLN concentrations, albeit not to the same extent as the IED. The standard enteral nutrition supplies ARG and GLN in protein-bound form and ensures higher intake of these amino acids than in traumatized rats, which is ascribed to oral feeding because the traumatized rats have severe anorexia. Higher plasma GLN concentrations in the HI groups should result from higher GLN intake rather than from ARG intake, because ornithine and glutamate concentrations were not different between HI and HIS rats.

A confounding factor is that the IED is not enriched in ARG alone but also in ω-3 fatty acids and antioxidant micronutrients. However, there are no data to support the idea that either of these can interfere with GLN pools. A possible option was to study a standard diet supplemented only with ARG. However, such a diet does not exist on the market, and thus the results obtained using this approach would have had only limited clinical relevancy.

In conclusion, in our model of HI, the IED studied, a diet that does not contain free GLN, was efficient in restoring the plasma and muscle GLN pools, probably through its high ARG content. In addition, in a trauma setting, this study is the first to extend this concept to enteral nutrition and to include muscle, which forms the GLN reserve for the rest of the body.

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