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Addition of Glutamine Does Not Improve Protein Synthesis and Jejunal Mucosa Morphology in Non-Hypercatabolic Stress

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Summary

To investigate the effect of glutamine-enriched total parenteral nutrition (TPN) on the protein synthesis and morphology of jejunal mucosa in non-hypercatabolic stress, sixty-two male Sprague-Dawley rats were subjected to surgical stress by femoral fracture. The rats were divided into 3 groups and received TPN for 8 days. One group received a standard amino acid solution without glutamine, the second group a standard solution enriched with glycine and glutamic acid, and the third group a standard solution enriched with glycyl-glutamine. All regimens were isocaloric and isonitrogenous-nitrogen (2.2 g/kg.day), glucose (150 Kcal/kg.day), and lipids (150 Kcal/kg.day). There were no statistically significant differences in jejunal mucosal thickness, DNA content, protein content, fractional synthesis rate or absolute protein synthesis among the groups after eight days of parenteral nutrition. In conclusion, the addition of glutamine to TPN did not influence either protein metabolism or morphology of the jejunal mucosa in non-hypercatabolic surgical stress.

Key Words

Rat – Protein metabolism – Jejunum – Nutrition – Glutamine dipeptide

Introduction

Glutamine is the most abundant amino acid in the body and it plays a fundamental role in different physiological processes. It is a precursor of hepatic ureagenesis and renal ammoniagenesis. It acts as cellular fuel in tissues with a fast turnover (intestine, skin, immune system) and is a potential regulator of protein synthesis (Darmaund 1992). Glutamine levels decline markedly in many different catabolic disease states and, recently, it has been suggested that glutamine may be a conditionally essential dietary nutrient rather than a non-essential amino acid (Smith and Wilmore 1990). The available total parenteral nutrition formulas (TPN) do not contain glutamine because it is not stable in free form and may decompose into pyroglutamic acid and ammonia. This problem can be obviated using dipeptides of glutamine (Fürst *et al.* 1990, Fürst and Stehle 1993).

The gut is the principal organ of glutamine uptake and metabolism in the body with smaller amounts being consumed by the liver, kidneys, adipocytes and white blood cells (Souba and Austgen 1990). Gut fuel use has several unique features. Arterial and luminary fuels provide nutrition for the enterocyte. This factor and the heterogeneity of cell types within the gut make it difficult to define its fuel use. Studies *in vitro* suggest that enterocytes, not immune cells, are responsible for must gut glutamine metabolism (Newsholme and Carrie 1994). Studies performed in rats suggested that parenteral infusion of glutamine partially reverses the small bowel atrophy associated with the infusion of solutions of conventional parenteral nutrients (Platell *et al.* 1993). Similar results were obtained in humans, in which glutamine prevents the deterioration of gut permeability and preserves mucosa structure (van der

Hulst *et al.* 1993). In models of bacterial translocation, exogenous glutamine helped to maintain the structural and functional integrity of the gut, by impeding bacterial translocation through the intestinal barrier (Burke *et al.* 1989). However, Spaeth *et al.* (1993) found that TPN supplementation with glutamine did not seem to improve gut barrier function or mucosal immunity in unstressed rats.

Recently, considerable scientific interest in glutamine was focused on the possibility of impaired intestinal glutamine metabolism during critical illness and the potential consequences for intestinal function (Souba *et al.* 1990). The importance of glutamine in the metabolism of enterocytes and inflammatory cells, and its functional role during stress and sepsis, has been established (Smith and Wilmore 1990, Chen *et al.* 1994). Concentrations of circulating glutamine are decreased after operative stress, despite an augmented release of glutamine by skeletal muscle and lung, with profound intramuscular glutamine depletion (Souba *et al.* 1990). These observations are consistent with an accelerated postoperative uptake of glutamine by other tissues. Studies in dogs show that following operative stress, glutamine consumption by the intestinal tract is increased by 75 % (Souba and Wilmore 1983).

The increased intestinal requirement and cellular demand for metabolic fuel during catabolic stress are matched by an enhanced demand of muscle glutamine, resulting in intracellular glutamine depletion. Thus, the delivery of adequate amounts of glutamine may be important for maintaining the integrity of the intestinal mucosa and rapidly proliferating cells, to preserve the muscle glutamine pool, and to improve nitrogen economy in this condition (Fürst *et al.* 1990).

Studies of the effect of glutamine-enriched TPN formulas have been performed under conditions of hypercatabolic stress (sepsis) (Ardawi 1992a, Yoshida *et al.* 1992), surgical trauma (Fürst *et al.* 1990), cancer and organ transplantation (Ziegler *et al.* 1992). However, little information using models of non-hypercatabolic stress are available.

The aim of this study was to investigate the effect of total parenteral nutrition supplemented with glutamine on jejunal mucosa in a model of non-hypercatabolic stress. Mucosal status was investigated by studying the morphology of the jejunal mucosa and by measuring the rate of protein synthesis.

Material and Methods

The experiments included 62 male Sprague-Dawley rats, with an initial body weight of 170.8 ± 14.3 g. The rats were housed individually in metabolic cages in a controlled environment (constant humidity, temperature 21 ± 2 °C, light-dark cycle 12 h). Standard chow and water were supplied during the five-day adaptation period.

On the fifth day after arrival, the animals were subjected to surgical stress by femoral fracture under intraperitoneal anaesthesia (5 mg/100 g body weight of sodium pentobarbitone), followed by Kirschner pin insertion into the medullary canal of both fragments (Schwartz *et al.* 1987). After surgery, a catheter was inserted under sterile conditions into the cava vein *via* the jugular vein, using the operative technique described by Weeks and Davis (1964). The catheter was tunnelled subcutaneously to the back of the rat and connected to a syringe pump (Perfusor Secura, Braun).

The catheter was made as described by Roos *et al.* (1981): a 2.5 cm silicone tube (inner diameter 0.12 mm, Dow Corning MI, USA) connected to the end of a coiled polyethylene PE-10 tube (0.28 mm inner diameter, Intramedic, Clay Adams, Becton Dickinson) extended with 20 cm of PE-20 tube (0.38 mm inner diameter). The silicone portion of the catheter was inserted into the superior cava vein *via* the jugular vein. The nutritional substrates were mixed under a laminar air flow, and the solutions kept in individual sterilized syringes (50 ml) which were changed every day. The TPN solutions were administered for 8 days, beginning at 1500 h on day 1 and ending at 1100 h on day 9.

The rats were divided into three groups. Group A (n = 21) received a standard TPN mixture consisting of glucose (150 Kcal/kg.day), lipids (150 Kcal/kg.day, Intralipid, Pharmacia), and amino acids (2.2 g N/kg.day, Vamin 18 EF, Pharmacia, Stockholm, Sweden). Groups B (n = 20) and C (n = 21) received isonitrogenous and isocaloric standard TPN solutions. Twenty percent of nitrogen were replaced with glycine (gly) and glutamic acid (glu) in ratio 93.5 : 6.5 in group B, and with glycyl-glutamine (gly-glu, Pharmacia) in group C. We supplemented gly in group B because it is well tolerated by rats, which received 1 g gly/g N and the remaining N was supplemented as glutamic acid. Groups B and C had the same amino acid composition except glutamine, glutamic acid and glycine.

For the determination of nitrogen balance in the rats, the animals were placed in metabolic cages that allowed separate collection of urine and faeces. Urine was collected during 24-hour periods. The nitrogen content in the urine was measured by a micro-Kjeldahl method (Peters and Van Slike 1932) (measured by Pharmacia, Germany). The nitrogen input, excretion and balance were determined daily during the experimental period for each group of rats.

After eight days of parenteral nutrition, the jejunal protein synthesis was measured in all the groups with L-1-^{14}C-leucine using a flooding dose technique (McNurlan *et al.* 1979). The isotope solution was prepared with labelled (1850 GBq/mol) and unlabeled leucine and had a final concentration of 100 μmol and 555 kBq/ml. The dose administered was 1 ml/100 g body weight. In each group, 5 animals were killed 2 min after the isotope injection and the remaining rats after 10 min.

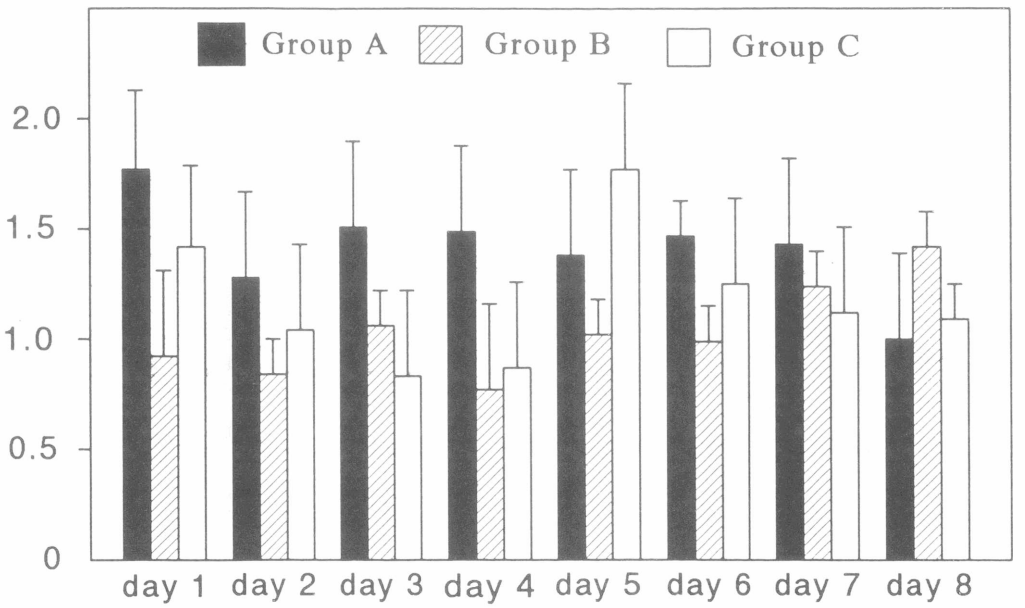

Fig. 1
Nitrogen balance (g N/kg.day, mean ± S.D.) of the different groups. At each day the nitrogen balance was positive in all the groups. There were no statistically significant differences. Group A, received the standard amino acid solution; group B, standard solution + glu + gly; group C, standard solution + gly-gln.

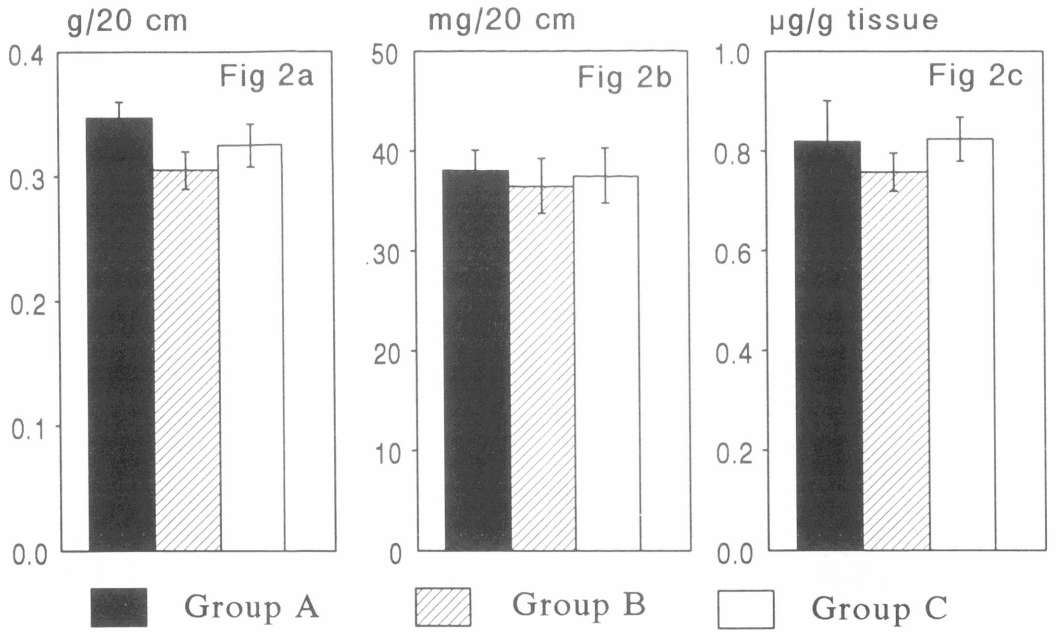

Fig. 2
Fig. 2a; weight of 20 cm of jejunum. Fig. 2b; protein content of 20 cm of jejunum. Fig. 2c; DNA content of 20 cm of jejunum. Data are means ± S.E.M. There were no statistically significant differences among the groups. Group A, received the standard amino acid solution; group B, standard solution + glu + gly; group C, standard solution + gly-gln.

Jejunal mucosa was obtained by removal of the proximal small intestine. The first 30 cm distal to the pylorus were discarded and the following 20 cm were taken after being stretched with a 3.5 g weight. The mucosa was separated from the serosa by slicing the intestine longitudinally and scraping the luminal side with a microscope slide. The sample was then transferred to a polypropylene tube and stored at −20 °C for analysis of protein synthesis and DNA content (Munro 1969). The adjacent 10 cm were put into

formol to study the microscopic morphology of the jejunal mucosa (by the Toxicology Department, Pharmacia, Sweden). For histological evaluation of isolated segments, tissues were fixed in 10 % v/v formalin embedded in paraffin and stained with hematoxylin-eosin. Villus height and crypt depth were determined on tissue cross-sections by using an ocular micrometer. Measurements were performed in triplicate. All differences were statistically evaluated by ANOVA.

Results

Nitrogen balance. The nitrogen balance was positive in all the groups on all the days studied (Fig. 1). There were no statistically significant differences among the groups.

Jejunal weight, protein and DNA content. There were no statistically significant differences in the jejunal weight, protein content and DNA content among the groups (Fig. 2).

Protein synthesis. The fractional synthesis rate (Ks %) and the absolute protein synthesis rate (mg protein synthesized per day) are shown in Fig. 3. No statistically significant differences were found among the groups.

Histopathology. No differences of biological significance were observed in the villus height and crypt depth among the studied groups (Table 1).

Table 1

Histopathology of jejunal mucosa in individual experimental groups

	Villus height (μm)	Crypt depth (μm)
Group A	425 (390–570)	148 (117–195)
Group B	365 (335–434)	150 (121–171)
Group C	407 (344–604)	167 (118–203)

Values are means (range). There were no statistically significant differences among the groups. Group A, received standard amino acid solution; group B, standard solution + glu + gly; group C, standard solution + gly-gln

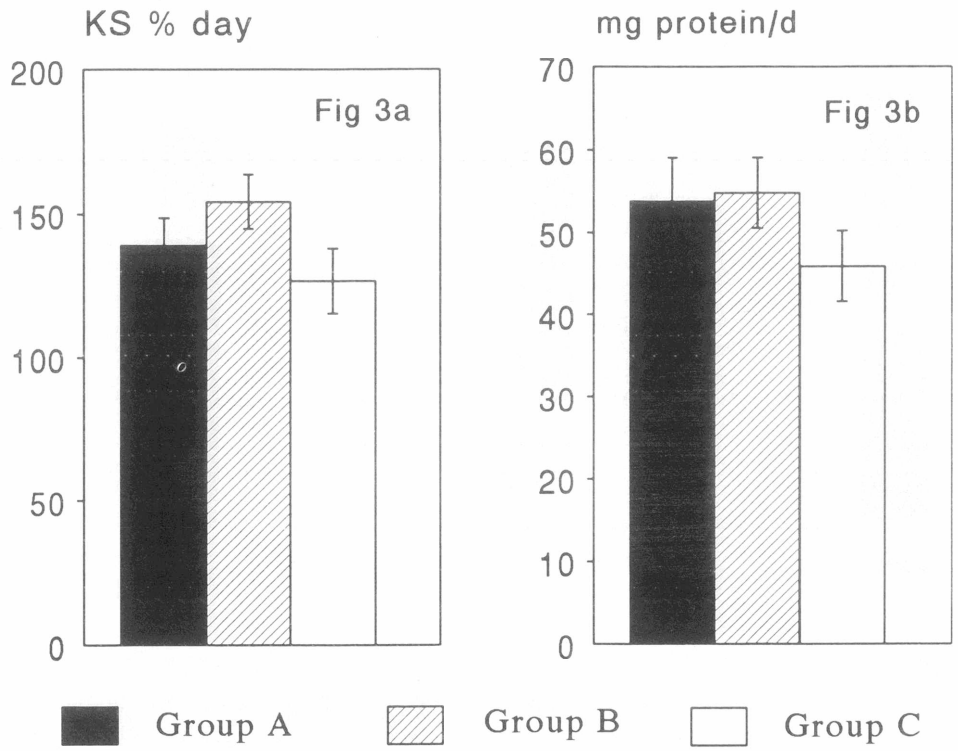

Fig. 3
Jejunal protein synthesis. Fig. 3a; jejunal fractional synthesis rate (Ks % day). Fig 3b; jejunal protein synthesis (mg of protein synthesized per day). Data are means ± S.E.M. There were no statistically significant differences among the groups. Group A, received the standard amino acid solution; group B, standard solution + glu + gly; group C, standard solution + gly-gln.

Discussion

It has been found that the addition of glutamine to TPN formulas improves the nitrogen balance in patients who had undergone major surgery (Hammarqvist *et al.* 1990, Stehle *et al.* 1989), severe accidental injury (Fürst *et al.* 1990) and allogeneic bone marrow transplants (Ziegler *et al.* 1992). Improved nitrogen balance has also been observed in septic rats fed glutamine-enriched TPN (Ardawi 1991, 1992a, 1992b). In our stress model, a positive nitrogen balance was seen in all the groups throughout the study period, with no statistically significant differences among the groups. The absence of a negative nitrogen balance shows that this stress model was not hypercatabolic. Presumably, glutamine does not affect nitrogen balance in this situation. Discrepant results were found by Jacobs *et al.* (1988) with a less severe stress model in which a jugular catheter was inserted as the only experimental injury in rats. In that study, glutamine-supplemented TPN was found to be more beneficial to the cumulative nitrogen balance than conventional TPN. This disagreement cannot be attributed to differences in the nitrogen or caloric intake between the two studies (2.3 g/kg.day and 257 Kcal/kg.day vs 2.2 g/kg.day and 300 Kcal/kg.day).

In hypercatabolic stress, experimental studies have demonstrated trophic effects of glutamine on the intestinal mucosa during TPN treatment. The addition of glutamine produced a pronounced increase of crypt depth and villous height in dogs with extensive small bowel resection (Gouttebel *et al.* 1992). In septic rats, TPN glutamine addition (Ardawi 1992b) markedly increased jejunal weight, the DNA and protein content, villus height, crypt depth, and the net intestinal use of glutamine and production of ammonia. In contrast, Vanderhoof (1992) found that 14 days after small bowel resection, the weight and protein and DNA content, in the duodenum of glutamine-supplemented animals (orally) were significantly lower than in non-supplemented rats, suggesting that high (5 %) concentrations of glutamine in the diet can have negative effects on intestinal adaptation. In the present work, the administration of glutamine-supplemented TPN did not have trophic effects on the intestinal mucosa in non-hypercatabolic stress. This was demonstrated by the fact that there was no difference in mucosal thickness, villus height, crypt depth, DNA content, protein content and protein synthesis. Similar results regarding the DNA content, protein content and mucosal thickness were found by Jacobs *et al.* (1988).

As far as protein synthesis is concerned, Ardawi (1992a) found increased rates of leucine incorporation into liver proteins *in vitro*, in response to glutamine-supplemented parenteral nutrition in septic rats. However, Yoshida *et al.* (1992) showed that the addition of glutamine to TPN did not increase protein synthesis in the jejunal mucosa of septic rats. This finding agrees with our results that glutamine supplementation does not affect protein synthesis in the jejunal mucosa.

It was previously reported by our group that the intraenterocytic glutamine levels were not decreased in the same non-hypercatabolic situation (López-Hellín *et al.* 1989). This fact may explain the lack of trophic effects on the intestinal mucosa with glutamine addition in the present study. These results suggest that, during non-hypercatabolic stress, glutamine is not an essential amino acid and is therefore not required in the TPN formulas used in this condition. In summary, the data obtained in this study indicate that glutamine dipeptide supplied parenterally does not improve protein metabolism or the morphology of jejunal mucosa in non-hypercatabolic surgical stress.

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