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Glutamine-Containing Dipeptides in Parenteral Nutrition

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ABSTRACT. Of the total pool of muscle free intracellular amino acids, glutamine represents about 60%. During catabolic stress, a marked reduction (50%) of this pool occurs; the depletion is not reversible by therapeutic efforts or conventional nutritional means. If maintenance of the intracellular glutamine pool promotes conservation of muscle protein, there is a theoretical case for use of glutamine supplements in the parenteral nutrition of patients with injury and infection. Glutamine is too unstable and poorly soluble for addition to existing preparations in its native form, but this drawback can be overcome by the use of synthetic stable and highly soluble glutamine-containing dipeptides. *In vivo* studies in humans and animals provide firm evidence that a synthetic glutamine-containing dipeptide, L-alanyl-L-glutamine (Ala-Gln), is readily hydrolyzed following its intravenous administration. The results also indicate a safe and efficient use of Ala-Gln as a source of free glutamine in parenteral nutrition. In clinical studies, nitrogen balance was more positive in catabolic patients receiving a peptide-supplemented solution than in control patients given isonitrogenous, isoenergetic total parenteral nutrition. Muscle glutamine concentrations were markedly decreased in the control groups. The intracellular concentrations were not influenced following severe injury, but were maintained in postoperative trauma. It is inferred that the increased intestinal requirement and cellular demand for metabolic fuel during catabolic stress is matched by an enhanced demand on muscle glutamine, resulting in intracellular glutamine depletion. Thus, the delivery of adequate amounts of glutamine is essential to maintain the integrity of intestinal mucosa and rapidly proliferating cells, to preserve the muscle glutamine pool, and to improve overall nitrogen economy during conditions of stress. (*Journal of Parenteral and Enteral Nutrition* **14**:118S–124S, 1990)

The first reliable data on intracellular concentrations of free amino acids in human muscle tissue were published in 1974.[1] In normal human muscle, free glutamine constitutes 61% of the total intracellular amino acid pool. After elective operations[2] or major injury[3] and burns,[4] intramuscular glutamine concentration declined considerably irrespective of nutritional efforts. These results were subsequently confirmed in other surgical and infected patients.[5–9]

It was concluded that reduction of the muscle free glutamine pool is a typical feature of injury and that the extent and duration of the depletion are proportional to the severity of the illness. It was also hypothesized that lack of this major intracellular nonessential nitrogen source might be a limiting factor for optimal protein synthesis.[10,11] Glutamine was thus considered to be a mandatory component in intravenous amino acid solutions, even though its poor stability and its instability during storage (heat sterilization) might make its direct addition to presently available preparations difficult.

FREE GLUTAMINE IN PARENTERAL SOLUTIONS? A CRITICAL ISSUE!

The first expressed concern about the physiologic role of glutamine in humans appeared in 1914.[12] Before that time, organic chemists had already determined that this amino acid is instable in aqueous solutions.[13,14] The effect of temperature,[15] pH,[15] and various anions[16] on the nonenzymatic deamidation of free glutamine was critically evaluated and the relevance of these factors was scrutinized in the context of biologic fluids.[17,18] The decomposition of free glutamine is quantitative and yields the cyclic product pyroglutamic acid (pGlu) and ammonia (Fig. 1).[19]

It is difficult to measure glutamine deamidation by directly determining changes in free glutamine concentration, because methodologic variations exceed the actual changes in concentration. The rate of decomposition of glutamine can be assessed, however, by measuring pGlu formation. Accordingly, the influence of temperature and duration of storage on pGlu formation was evaluated for two concentrations of free glutamine (Fig. 2). As shown, about 1 mM pGlu was formed during 24 hours at 28°C, about 10 times the initial concentration of this compound. The results of these experiments conform well with earlier observations[15–18] and demonstrate that formation of pGlu is augmented with increasing pH and temperature.[18,20]

Based on present knowledge about the possible neurotoxic effects of pGlu in humans,[21,22] it seems inconceivable that the amounts detected would be harmful, but the maximal levels of this solute that can be formed have not yet been determined.

The low solubility of free glutamine (35 g/liter at 20°C) is an additional problem. In clinical practice, supplementation with glutamine as presently proposed (20–57 g) would considerably increase the volume of fluid to be infused. Furthermore, when glutamine is added to highly

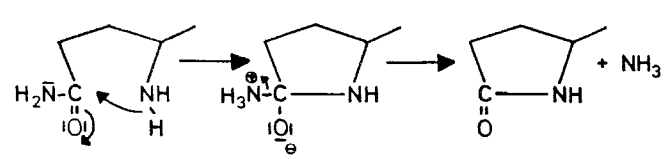

FIG. 1. Suggested mechanism for the formation of pGlu from glutamine.

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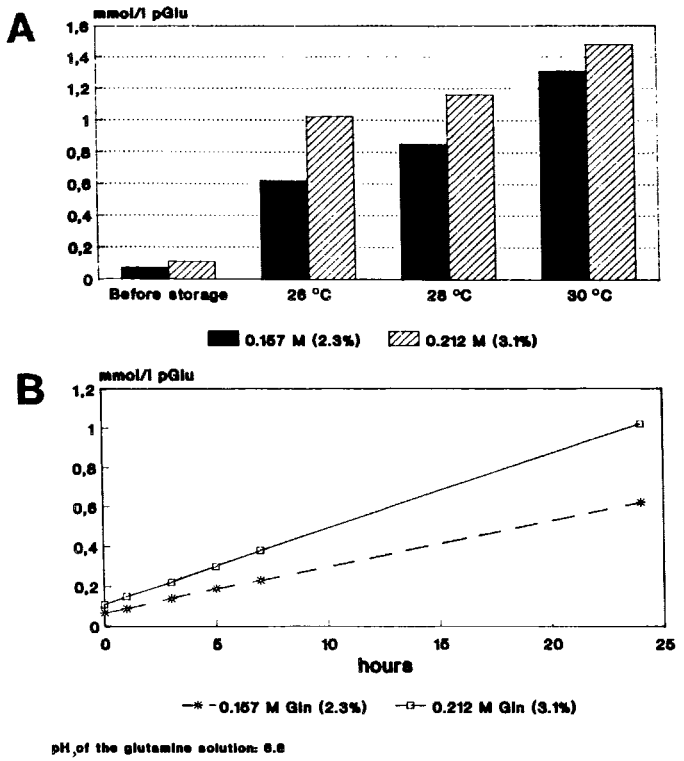

FIG. 2. Stability of glutamine during storage. Influence of temperature (*A*) and duration of storage (*B*) on pGlu liberation.

concentrated amino acid preparations, it must be assumed that its solubility will decrease and its rate of decomposition will increase (higher ionic strength).

All these drawbacks can be overcome by use of synthetic glutamine-containing stable and highly soluble dipeptides. As our knowledge of extraintestinal peptide assimilation has grown, synthetic short-chain peptides have become new candidates for inclusion as substrates in parenteral nutrition solutions.[23,24] There is convincing evidence for an extraintestinal assimilation of di- and tripeptides. After parenteral injection, they are rapidly cleared from plasma without being accumulated in tissues or lost appreciably in urine. This rapid clearance does not appear to be affected by bilateral nephrectomy or enterectomy.[23,24]

SYNTHESIS AND CHARACTERIZATION OF GLUTAMINE-CONTAINING DIPEPTIDES

Our basic research plan has attempted to combine the synthesis and characterization of nutritionally relevant di- and tripeptides with investigations aimed at examining *in vivo* uptake and subsequent utilization of these compounds. These investigations have been combined with trials to evaluate the nutritional benefit of the peptides in suitable animal models and, finally, to appraise their value in the treatment of catabolic patients.

The glutamine-containing peptide L-alanyl-L-glutamine (Ala-Gln) was synthesized by chemical methods[25] and subsequently purified.[25–27] The purity of the final product approached 100%, and the molecular structure was fully confirmed by field-desorption mass spectrometry and protein magnetic resonance spectrometry.[25,28] The water solubility of Ala-Gln is 16-fold higher (568 g/liter H_2O; 20°C) than that of the free amino acid glutamine (36 g/liter H_2O; 20°C).

Trials are now in progress to develop biotechnologic synthesis procedures that employ proteases of plant origin as biocatalysts.[29,30] Clear advantages of this novel approach include high stereospecificity of the reaction, short reaction time and, thus, simple subsequent purification. However, the major benefit of enzyme-catalyzed peptide synthesis is the economical, low-price production of the desired short-chain peptides. These factors might be prerequisite for a rational industrial production of these peptides and, subsequently, of amino acid/peptide solutions for parenteral nutrition.

Indeed, great attention was paid to controlling the stability of the synthetic peptide during storage and heat-sterilization. No liberation of ammonia or formation of pGlu or of L-alanyl-L-glutamic acid was detected.[14,25] Thus, the synthetic peptide Ala-Gln satisfies each of the criteria required for inclusion in future parenteral solutions.

IN VIVO UTILIZATION OF ALA-GLN

Animal experiments (rats and dogs) with glutamine-containing dipeptides (Ala-Gln and Gly-Gln) provide convincing evidence that the peptides are easily available and that their constituent amino acids are instantly incorporated into various tissue proteins.[31,32] During continuous total parenteral nutrition (TPN) in rats, parenterally administered Ala-Gln provides free glutamine for maintenance of its intra- and/or extracellular pools.[33] Interestingly, the net efflux of muscle glutamine can be considerably reduced with intravenous Ala-Gln in catabolic dogs, indicating that parenteral provision of this peptide decreases muscle loss of glutamine during stress.[34]

There are considerable species differences in tissue free amino acid concentrations. Although it is frequently possible to make qualitative conjectures about human metabolism based on the results of animal studies, a quantitative understanding requires direct measurements of amino acid pattern in humans.[10] In recent kinetic studies by Albers et al,[35] *in vivo* utilization of intravenously administered Ala-Gln was measured after a bolus injection (130 mg/kg body weight [BW]) in healthy volunteers. The rate of disappearance of Ala-Gln from plasma and the simultaneous liberation of the constituent free amino acids are shown in Figure 3. Kinetic data are given in Table I. Disappearance of the peptide was accompanied by a prompt and equimolar increase in the concentrations of free alanine and glutamine. The estimated elimination half-lives were 8.4 ± 2.6 min for alanine and 12.0 ± 5.5 min for glutamine.

In a subsequent study in similar subjects, an admixture of amino acids and Ala-Gln was infused continuously at a rate of 125 mg of amino acid-peptide/kg/hr (23.8 mg of Ala-Gln).[36] Infusion of the peptide-supplemented solution resulted in an instant substantial increase in the concentrations of the constituent amino acids (Fig. 4). During the steady state, the increment in free glutamine

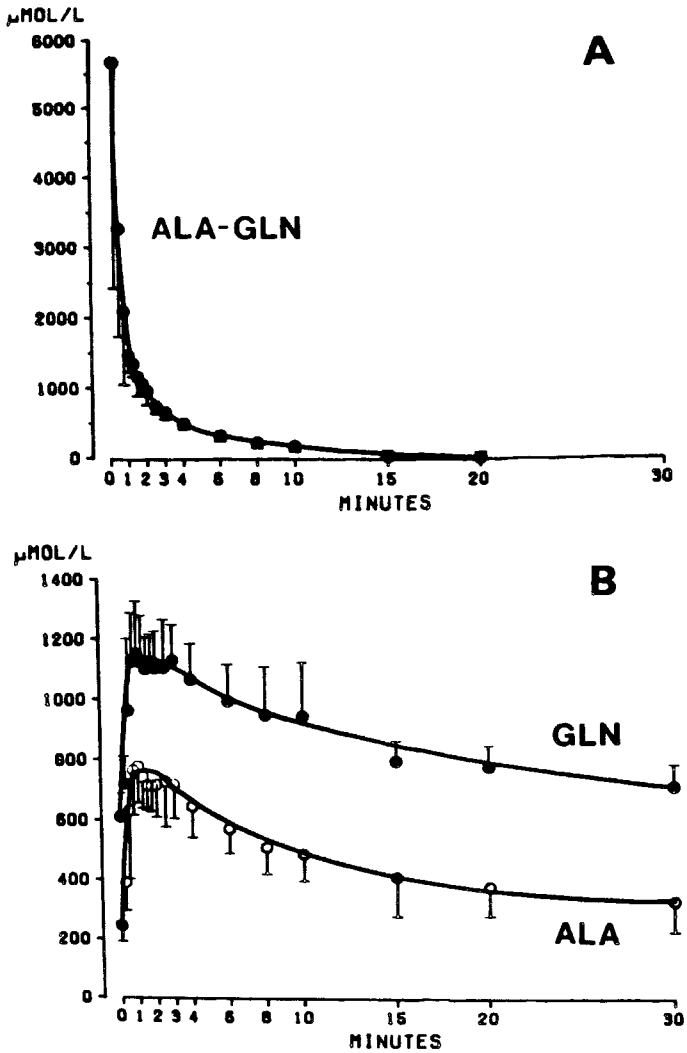

FIG. 3. Time course of disappearance of Ala-Gln from plasma (*A*) and liberation and subsequent elimination of free glutamine and free alanine (*B*) after bolus injection of Ala-Gln in 10 healthy subjects (mean ± SD). Adapted from Reference 35 with permission.

TABLE I
Calculated kinetic data for Ala-Gln[35]

r^2	0.975 ± 0.024
k_{el} (min^{-1})	0.185 ± 0.024
$t_{1/2}$ (min)	3.80 ± 0.50
V (liters)	10.52 ± 2.43
V′ (liters/kg)	0.140 ± 0.028
Cl (liters/min)	1.92 ± 0.36

Results are mean ± SD (N = 10).

over the basal value was 33 ± 2.2%. After completion of the infusion, the free glutamine concentration fell to baseline values. During the entire infusion period, the plasma concentrations of Ala-Gln were at trace levels and the dipeptide could not be recovered in the urine. These results suggest a virtually quantitative hydrolysis and subsequent utilization of infused Ala-Gln. Infusion of the solution was not accompanied by any side effects and the volunteers reported no complaints.

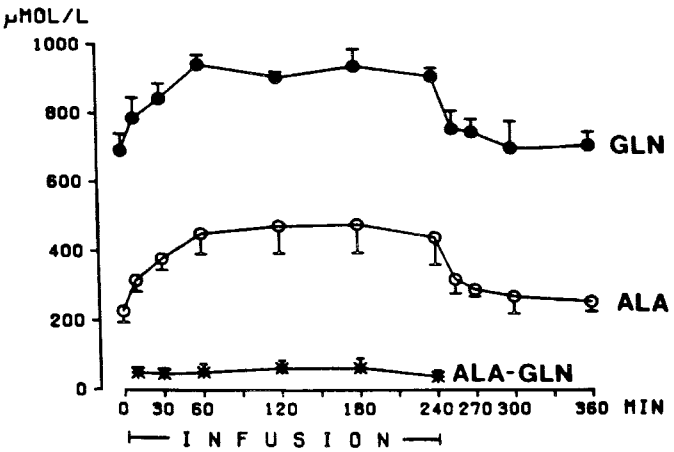

FIG. 4. Plasma concentrations of alanine, glutamine, and Ala-Gln before, during, and after continuous intravenous infusion of an amino acid solution supplemented with Ala-Gln (mean ± SD). Adapted from Reference 36 with permission.

IN VIVO PEPTIDE HANDLING

Site of Hydrolysis

The occurrence in Albers' studies of equimolar increments of alanine and glutamine, as well as the prompt liberation of these amino acids, suggests extracellular hydrolysis of the infused dipeptides.[35,36] Intracellular cleavage of dipeptides has been repeatedly demonstrated after enteral supply of the peptides, occurring preferentially at the site of the renal or intestinal brush border.[37] Intracellular hydrolysis after intravenous provision of the peptides would require an equimolar efflux/reabsorption of the liberated amino acids from tissues or organs, a difficult proposition to accept given the heterogeneity of the various transport systems and the intermediary metabolism of the constituent amino acids. Considerable hydrolase activity in plasma,[24,38] as well as a recent report describing hepatic assimilation of dipeptides by enzymes located on the liver sinusoidal plasma membranes,[39] supports the concept of an extracellular hydrolysis. Indeed, Hundal and Rennie[40] identified a dipeptide hydrolase from plasma membrane in skeletal muscle.

Current studies employing a newly developed *in vitro* assay for plasma hydrolase activity demonstrate that glutamine-, tyrosine-, and cystine-containing peptides are substrates for human plasma free peptidases, the rate of hydrolysis clearly depending on the amino acid composition of the peptides.[41] Calculation of the total plasma hydrolase capacity against Ala-Gln and Gly-Tyr revealed 149 μmol/min and 104 μmol/min, respectively. These high capacities may explain in part the extreme short plasma half-lives of these peptides observed in previous studies *in vivo*.

Prolonged or Fast Elimination of Dipeptides: A Benefit or Disadvantage

Glycyl dipeptides are claimed to be superior to other dipeptides because they have longer half-lives than do dipeptides with alternative N-terminal amino acid residues.[24,38] This belief in their superiority rests on the

assumption that, due to their longer half-lives, a greater proportion of infused glycyl dipeptides can reach the tissues in intact form, but no peptide has ever been detected in any tissue. Furthermore, as emphasized above, a growing body of evidence supports the notion of extracellular hydrolysis of parenterally supplied dipeptides.

Rapid elimination of the peptides offers the obvious advantage that substantial amounts can be provided without their accumulation in body fluids, perhaps thus avoiding the possible risk of undesirable pharmacologic and/or physiologic side effects.[42] This prediction is verified by the fact, that despite its presence as a substantial peptide load, the plasma concentration of Ala-Gln remained at trace levels throughout the infusion, accompanied by apparent steady-state concentrations of the constituent free amino acids.[36] This stable amino acid/peptide ratio allows an estimation of the plasma clearance rates of the dipeptides.[43] Such calculations reveal an estimated metabolic clearance rate for Ala-Gln of 35.9 ± 9.5 ml/min/kg. The estimated total body plasma clearance was 2.9 ± 0.9 liters/min, which is in fair agreement with that obtained from the bolus study.[35] Importantly, these values are very similar to those obtained with the dipeptide glycyl-L-tyrosine (33.7 ml/min/kg and 2.7 ± 0.9 liters/min).[36] Together, these data obtained from experiments with different protocols and underlying assumptions strongly indicate, by their consonance, that these dipeptides are handled similarly after intravenous infusion.

In general, we believe that speculations concerning the correlation between dipeptide structure and affinity for hydrolysis[24,38] are unhelpful. An appropriate evaluation of the influence of structure on hydrolysis behavior would require consideration of the effects of variation of about 400 possible structures. Indeed, given the intention to improve clinical nutrition by using intravenously administered dipeptides, marginal differences in half-lives are of little importance. We submit that the demonstration of specific or selective organ utilization (*vide infra*) of a given dipeptide or amino acid/dipeptide combination is of much greater importance.

USE OF GLUTAMINE-CONTAINING DIPEPTIDES (ALA-GLN) IN CATABOLIC PATIENTS

As emphasized, profound intracellular depletion is characteristic of injury and other hypercatabolic conditions. Two recent observations suggest that glutamine is involved in regulation of muscle protein balance—the striking direct correlation between muscle glutamine concentration and the rate of protein synthesis[44] and the positive effect of glutamine on protein anabolic processes *in vitro*.[45] If maintenance of the intracellular glutamine pool promotes conservation of muscle protein, there is an obvious indication for use of glutamine supplements in the parenteral nutrition of patients with injury and infection.

Major Uncomplicated Operation

The effect of Ala-Gln-supplemented TPN on postoperative nitrogen balance and intracellular muscle free glutamine concentrations was investigated in 12 patients undergoing elective resection of carcinoma of the colon or rectum.[46] They were randomly allocated to a peptide test or a control group. The patients received isonitrogenous (0.23 g N/kg BW/day) and isoenergetic (166 kJ/kg BW/day) TPN over 5 days; the nonprotein energy (140 kJ/kg BW/day) came equally from glucose and a fat emulsion (20% Intralipid, Kabi-Vitrum, Sweden). Patients in the peptide group received TPN supplemented with the dipeptides Ala-Gln (280 mg/kg BW/day; 54 mg N/kg BW/day) and glycly-L-tyrosine (50 mg/kg BW/day; 5.9 mg N/kg BW/day) and the control group received corresponding amounts of free alanine and glycine nitrogen.

With the use of the Ala-Gln-containing solution, intramuscular glutamine concentration remained close to preoperative values (18.9 ± 1.2 vs 17.5 ± 1.0 mmol/liter intracellular water); with the control solution, it decreased significantly (19.8 ± 0.9 vs 12.0 ± 0.6 mmol/liter intracellular water) (Fig. 5). The day-to-day N balance was significantly better in patients receiving the peptide on each day of the study. The cumulative N balances were −7.1 ± 2.2 vs −18.1 ± 1.7 g N at completion (Fig. 6).

Severe Accidental Injury

In preliminary studies, eight patients with severe accidental injury were investigated in cooperation with Drs. G. Guarnieri and G. Toigo, Trieste, Italy. On the day of admission, the patients were treated as necessary with fluid, electrolytes, and whole blood transfusions. After resuscitation, the necessary surgical procedures were performed. Thus, nutritional therapy was not started until 2 or 3 days after the trauma. Four patients received TPN providing 310 mg of amino acid nitrogen and 165 kJ/kg BW/day and four others were given isonitrogenous and

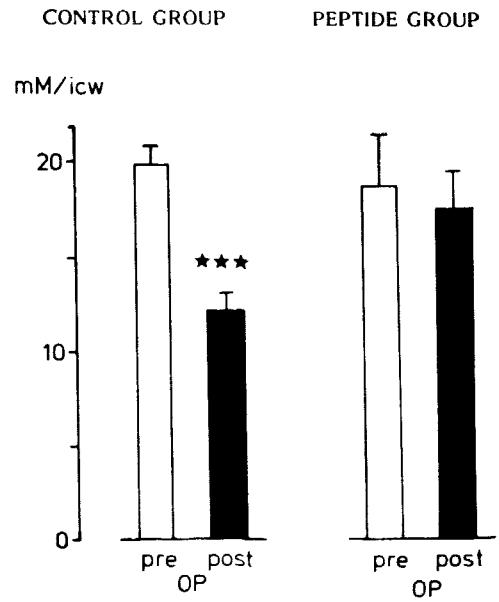

FIG. 5. Intracellular muscle glutamine concentrations (millimoles/liter intracellular water [icw]) (mean ± SEM) in control patients and in patients receiving Ala-Gln-supplemented TPN. ***$p < 0.001$.

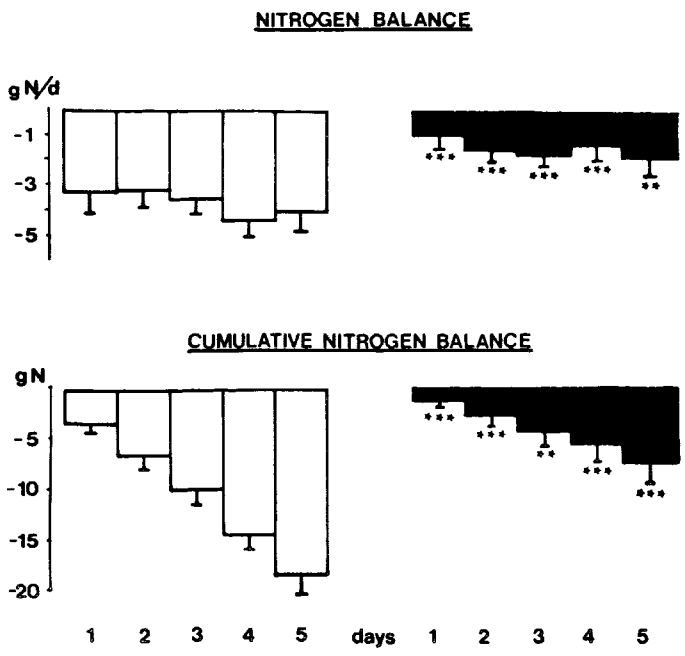

FIG. 6. Day-to-day and cumulative nitrogen balances in 12 patients undergoing major uncomplicated operations. They received Ala-Gln-supplemented TPN (n = 6) (*filled bars*) or a conventional control solution (n = 6) (*open bars*) (mean ± SEM). $^{***}p < 0.001$; $^{**}p < 0.01$.

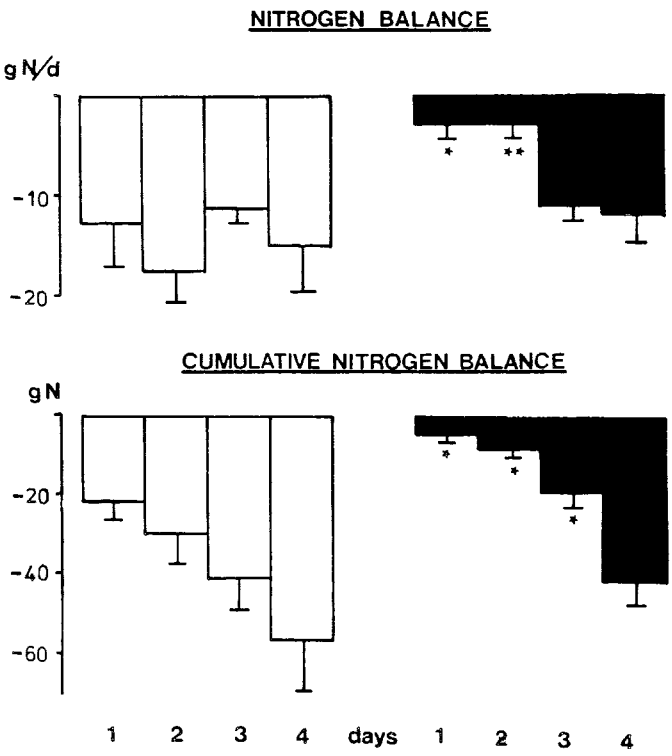

FIG. 7. Day-to-day and cumulative nitrogen balance in patients (severe accidental injury) receiving Ala-Gln-supplemented TPN (*filled bars*) or a conventional control solution (*open bars*) (mean ± SEM). $^{*}p < 0.05$; $^{**}p < 0.01$.

isoenergetic nutrition, but with 52 mg/kg BW/day of amino acid nitrogen provided as Ala-Gln-nitrogen. Muscle biopsies were performed and blood samples were obtained 2–3 days after injury (ie, at the commencement of the nutritional therapy) and on the fifth day of treatment. Interestingly, despite the daily provision of about 20 g of Ala-Gln, corresponding to 13 g of glutamine, no appreciable influence on the intracellular muscle glutamine concentration could be observed. Nevertheless, as shown in Figure 7, cumulative N balance was significantly better in the peptide group. This positive result is entirely due to improvement of the nitrogen balance on days 1 and 2 (Fig. 7).

INTRAMUSCULAR GLUTAMINE DEPLETION DURING CATABOLIC STRESS: A REAPPRAISAL

Recent animal studies have demonstrated that glutamine consumption by the intestinal tract is markedly increased during catabolism.[47–49] Extrapolating from animal data, in a 70-kg patient, about 10–14 g of glutamine are taken up by the gastrointestinal tract per day.[47] Including the uptake of about 4 g of glutamine per day by the dog kidney[50] and considering the daily requirement of glutamine by the rapidly proliferating cells,[51,52] the total glutamine influx may be about 14–20 g/day. During stress, the reported value of muscle glutamine efflux varies within the range of 9–13 g/day, depending upon the severity of the catabolic stimulus.[47–49,53] Thus, the estimated glutamine consumption appears to exceed release by about 5–6 g/day. This figure matches almost exactly the extent of muscle glutamine depletion in our surgical patients treated with conventional TPN over the 3-day period. Accordingly, in these patients, provision of about 12–13 g of glutamine per day as the dipeptide Ala-Gln almost abolished trauma-induced muscle glutamine depletion and greatly amended the nitrogen balance.[46] These results may indicate that the increased intestinal requirement and the cellular demand for metabolic fuel in postoperative patients can be met by a daily provision of 12–13 g of glutamine per day.

In contrast, after severe accidental injury the same amount of Ala-Gln (20 g/day) did not influence the muscle free intracellular glutamine pool, although the nitrogen economy was improved in the immediate post-injury phase. Tentatively, thus, the increased intestinal requirement and the cellular demand for metabolic fuel are partly compensated, but not met, in these patients.

Interestingly, Wilmore and co-workers obtained very similar results by supplementing with 20 g of glutamine per day the nutrition of patients undergoing bone marrow transplantation and total body irradiation. Improvement of nitrogen balance with this amount was restricted to the first few days of the study and was followed by successive deterioration of the retention. Administration of 40 g of glutamine per day, however, resulted in a prolongation of the beneficial effect throughout the study (Wilmore, personal communication). Thus, support of 40–60 g of Ala-Gln per day might be a more realistic recommendation for injured and critically ill patients.

According to the original hypothesis, the fate of intramuscular glutamine was to supply hepatic processes with carbon and nitrogen, and any deficit in glutamine could limit these processes during catabolic stress.[2,5,6] However, in light of our present knowledge, a revision of this

hypothesis may be indicated. Glutamine may serve primarily as an obligatory nutrient necessary for normal maintenance of the intestinal mucosa and, presumably, of proliferating cells.[47–53] The reportedly increased intestinal requirement for metabolic fuel during catabolic stress might be matched by an enhanced demand for muscle glutamine and lead to intracellular glutamine depletion. This line of reasoning leads to the conclusion that the delivery of adequate amounts of glutamine is essential to maintain the integrity of the mucosa and of rapidly proliferating cells, to preserve the muscle glutamine pool, and to improve overall nitrogen economy during conditions of stress.

FUTURE ROLE FOR PEPTIDES IN PARENTERAL NUTRITION

Peptides might be considered as brand new candidates for parenteral nutrition. Their potential use is based on the assumption that tailored amino acid solutions will increase the benefits of intravenous nutrition during episodes of catabolism and in patients with specific diseases. Undoubtedly, this new approach has introduced a new dimension. The explosion of new information concerning the assimilation of peptides is probably a necessary prelude to the eventual routine use of intravenous peptides in clinical practice.[54]

This review sought to elaborate the potential nutritional need for glutamine peptides in parenteral nutrition. Indeed, we have studied only one peptide, L-alanyl-L-glutamine, and the value of other glutamine-containing dipeptides remains to be elucidated. It is highly probable that the potential utilization of a peptide by target tissues will vary according to its structure and its biologic effects.[34,41,54] Specific diseases may lead to certain amino acid deficiencies or to antagonisms or imbalances in various organ tissues; these conditions might selectively cause a nutritional need for one or more specific peptides that are appropriate for use only in that specific condition to support the attenuated tissue.

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