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Newsletter

Amino Acids Link News

Newsletter of Ajinomoto Co., Inc. Amino Acid Department

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Contents

Amino Acids in the 21st Century (2) ~Glutamine: another key immunonutrient~

Entitled "Amino Acids in the 21st Century," the previous article in the series reviewed the immune-enhancing activity and applications of arginine. Another amino acid that plays an important role in enhancing the immune system is glutamine. In this issue, we introduce glutamine and its functions.

From the perspective of nutritional science, glutamine, like arginine, is not an essential amino acid. Nevertheless, glutamine is the most abundant amino acid in the body. Glutamine plays major roles in accelerating muscle metabolism, in preserving muscle, in promoting wound healing and in cell proliferation. It also serves as the nutritional source for gastrointestinal cells and as the major energy source for the immune system. In times when the demand for glutamine rises rapidly—following onset and spread of a disease or after a burn, wound, or surgical procedure—the amount of glutamine synthesized by the body itself is inadequate. For this reason, glutamine is called a semi-essential amino acid.

Glutamine

●Chemical Composition :

$$H_2N-CO-CH_2-CH_2-\underset{\displaystyle NH_2}{\underset{|}{CH}}-COOH$$

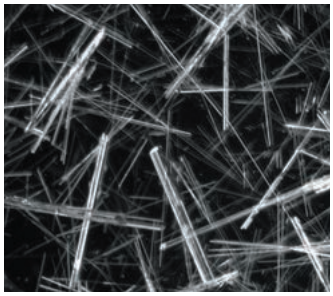
●Crystal Structure: Rhombic system

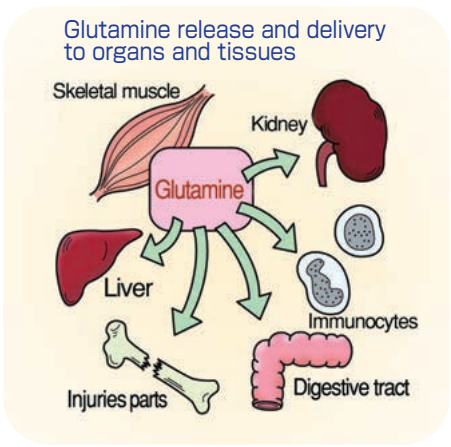
●pH of the aqueous solution: (pH 4.5 to 6.0)

●Taste: Umami

●Properties: Slightly soluble in water and insoluble in ethanol and diethyl ether. Glutamine is used in powder form or as a dipeptide, since it quickly decomposes in an aqueous solution.

●Crystal photograph: (X100)





●Major activities and applications of glutamine

Activity	Typical applications
(1) Activation of immune system	Immunonutritional diets and sports supplements
(2) Improvement of liver functions (suppression of alcohol-induced liver damage)	Diets designed to improve liver functions and suppress alcohol-induced liver damage
(3) Growth and preservation of and energy source for gastrointestinal cells	Drugs for treating gastric and duodenal ulcers
(4) Synthesis of muscle proteins	Sports supplements

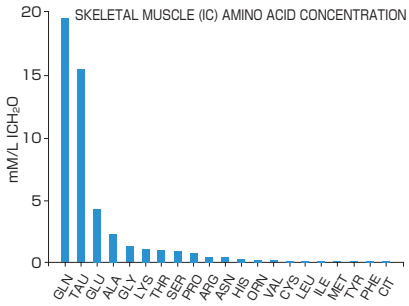
Glutamine's Physiological Functions

Kazunori Mawatari,
Amino Acid Department, Ajinomoto Co., Inc.

Glutamine as the energy source for the digestive tract and for protection of the mucosa

Glutamine is the most abundant amino acid among the intracellular free amino acids in skeletal muscles (Fig. 1) and various organs. However, except for its role as a nitrogen carrier, its major roles within the body remained unclear until recently. Significant progress in research into glutamine function during the 1980s showed that glutamine serves as the energy source for the digestive tract; particularly the enteric tract. This knowledge fueled dramatic progress in research into glutamine's physiological functions during the following decade. Frequently used by practitioners as the main ingredient for anti-inflammatory ulcer drugs due to its suppression of gastric ulcers, glutamine was widely known in Japan for its gastric mucosa-protecting effects well before such research was undertaken. Okabe *et al.* reported the dramatic effectiveness of glutamine administration in suppressing development of rat gastric ulcers induced by aspirin megadoses (Fig. 2), a finding that paved the way for use of glutamine in clinical applications.

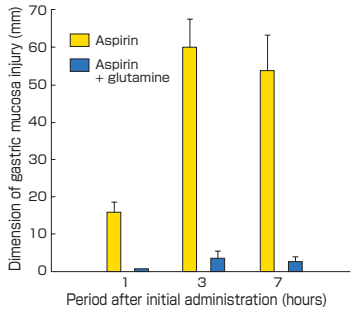
Fig. 1 Free amino acid concentration in skeletal muscle



(Lacey JM, *et al.* Is glutamine a conditionally essential amino acid? Nutri Rev 48(8):297, 1990)

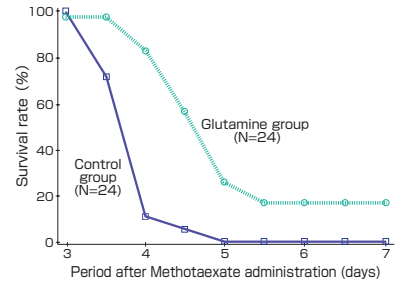
As described above, D.W. Wilmore *et al.* later showed that during infectious periods—for example, immediately after surgery—glutamine stored in skeletal muscle is released and delivered to the digestive tract, where it plays critically important roles as an energy source for the digestive tract and in the repair of damaged tissue, preventing the invasion of bacteria into the body (Fig. 3). Their study also revealed that the digestive tract was able to preserve the shape of microvilli, tissues responsible for absorption of nutrients, using only glutamine as its nutritional source. These results suggest glutamine's potential for improving the prognosis of patients who suffer from atrophy of digestive tract mucosa and the resulting postoperative invasion of bacteria into the body, atrophy of digestive tract mucosa caused by IVH over extended periods, and septicemia, as well as those who have received bone marrow transplants.

Fig. 2 Effects of glutamine administration on aspirin-induced gastric mucosa injury in rats



(S. Okabe, *et al.* Inhibitory effect of L-glutamine on gastric irritation and back diffusion of gastric acid in response to aspirin in the rat. Digestive Disease 20(7):626 1975)

Fig. 3 Effects of glutamine ingestion on survival rate of rats administered a chemotherapeutic drug



(Fox AD, *et al.* Effect of a Glutamine-Supplemented Enteral Diet on Methotrexate-Induced Enterocolities. JPEN 12(4):325, 1988)

Activity as nutrient involved in the immune system.

In 2000, D.W. Wilmore held a symposium on the metabolism of glutamine and its applications in clinical settings, in which he reviewed the advantages of glutamine learned to date. Paul A. M. van Leeuwen *et al.* had demonstrated the usefulness of glutamine derived from peptide infusions (Ala-Gln). He also explained the benefits of using glutamine as an enteral nutrient, based on a review of numerous clinical results showing that glutamine administration not only reduces the atrophy of digestive tract mucosa, but in many instances, increases resistance to infectious disease.

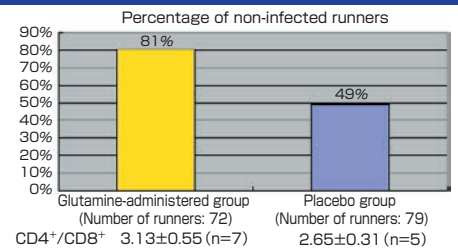
Yamamoto *et al.* showed that the administration of glutamine to experimental animals increases the number of S-phase cells in the cell cycle of peripheral blood lymphocytes. That is, glutamine administration increases the proliferation potency of peripheral blood lymphocytes. P. Newsholme *et al.* showed that, compared with sugar, glutamine is an energy source more easily consumed by immunocytes, such as macrophages, lymphocytes, and neutrophils. It also promotes cytokine production in macrophages. These results all point to the vital role of glutamine in the

immune system, and in particular, the usefulness of glutamine ingestion for those with weakened immune response, such as with patients after surgery. A.P. Houdijk *et al.* reported that administration of an enteral nutrient formulation containing glutamine in larger supplements to severely ill patients with wounds reduces the incidence of pneumonia and septicemia. J. Neu *et al.* reported that glutamine suppresses the incidence of septicemia in premature infants. Both studies demonstrated that glutamine administration reduces both the term of hospitalization and medical costs.

Roles of glutamine during exercise

According to a study by L.M. Castell *et al.* at Oxford University, in a double-blind test, more than 150 marathon runners were provided with 5g of either glutamine, or dextrin as a control, immediately after a marathon and two hours thereafter. The incidence of infectious diseases such as colds during the following one-week period was monitored by a physician according to predetermined standards. Results revealed that although about half of the runners in the control group contracted some type of infectious disease, the rate of incidence for runners in the glutamine-ingestion group was significantly lower, at less than 20% (Fig. 4). The study also showed that the ratio of $CD4^+$(helper cells)/$CD8^+$(suppressor cells) in peripheral blood lymphocytes of these runners improved dramatically, indicating that glutamine improves resistance to infectious disease in those weakened by extended physical exertion.

Fig. 4 Effects of glutamine administration on incidence of infectious diseases for marathon runners during the 7-day period following completion of a marathon

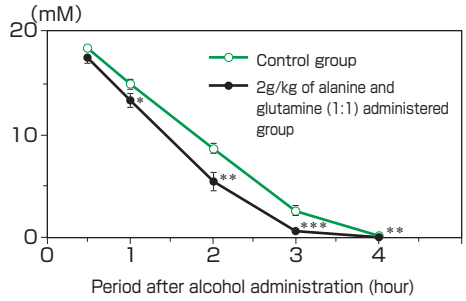


(L.M.Castell, *et al.* The effect of oral glutamine supplementation on athletes after prolonged exhaustive exercise. Nutrition 13:738,1997)

Expanding potential of glutamine

In our Ajinomoto Laboratory, we recently discovered that rats with alcoholic hepatopathy selectively ingest glutamine and alanine solutions when presented with numerous amino acid choices. Further study showed administration of a mixture of glutamine and alanine accelerated liver regeneration in rats, and accelerated the dissipation of plasma alcohol concentrations in rats to whom alcohol had been administered (Fig. 5). Glutamine is stored in abundant quantities in skeletal muscle under normal conditions, serving as the major nutritional source for immunocytes when the immune system is weakened. Glutamine may also be used in a similar manner by hepatic cells during hepatopathy. Further research into glutamine is expected to disclose numerous other applications of this versatile amino acid.

Fig. 5 Effects of combined alanine and glutamine administration on blood alcohol concentrations in rats administered alcohol



(Mawatari K, *et al.* Amelioration of oral intake of both alanine and glutamine on ethanol metabolism and motor activity in rats after acute ethanol loading. The 10th congress of the international society for biological research on alcoholism. 2000. Alcohol Clin Exp Res. 24(5):215A, 2000)

Profile of Kazunori Mawatari

Received Master's degree from Food Science and Technology Dept., Graduate School of Engineering, Kyushu Univ. in 1984.
Assumed a position at Life Science Laboratories, Ajinomoto Co., Inc, in the same year. Pharmaceutical Development Dept., in 1992.
Health & Nutritional Business Development Dept., in1994.
AminoScience Laboratories in 1999.
Amino Acid Dept. from 2002.

Glutamine's Immune-enhancing Activity

As with arginine, glutamine's various actions have been studied over a long period of time. Its immune-enhancing activities are highly regarded with respect to clinical application. The Following below is a question and answer session about glutamine that took place during a talk with Dr. Kazuhiko Fukatsu, associate professor at the National Defense Medical College, who has been involved in clinical medicine as a surgeon and in research into immunonutrition for some time.

The most useful and applicable immunonutrient

Q.1 Dr. Fukatsu. When did you become interested in immunonutrition? What triggered your interest?

A.1 Well, some patients showed poor prognoses due to unexpected postoperative complications, even when surgeries were completed successfully. In particular, once

severe pulmonary disorders and pneumonia occur, very high mortality rates result. Are there any new methods for treating and preventing these conditions? That question guided me to research into surgical in sult and biological responses. Dr. Hideaki Saito, former professor at the University of Tokyo, who directed our research group, had been interested in surgical nutrition for quite some time. He was involved in research into arginine, glutamine, and enteral nutrition. So, from those days, I was also involved in research into surgical nutrition, including immunonutrition and host defense mechanisms.

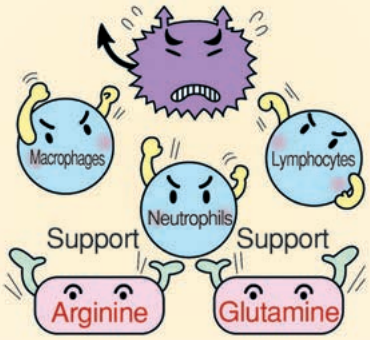
Although their purpose is to treat patients, surgical operations result in a sort of traumatic injury to the body and tend to incite subsequent abnormal biological response, particularly after major operations. Excessive inflammatory response may destroy tissue and result in organ failure. In contrast, excessive anti-inflammatory response, or insufficient increase in normal inflammatory response may reduce resistance to infection, leading to severe complicated infections and ultimately to organ failure. Patients who have poor immunocompetence tend to contract complicated infections even when antibiotics are administered prophylactically. Once they begin to suffer severe infections, resolution is extremely difficult, even with the current advanced medical techniques and drugs. Under these circumstances, immunonutrition has attracted widespread attention since it modulates excessive inflammatory response and increases resistance to infectious disease. Although many unsuccessful results involving recent anti-mediator therapies (Note 1) have been reported, nutritional therapy based on immunonutrients offers the greatest potential benefits in modulating biological response and the rate of effectiveness in clinical settings, for which, much evidence has already been accumulated in actual clinical settings.

Note 1) Anti-cytokine and anti-endotoxin therapies

Q.2 What ingredients are effective immunonutrients?

A.2 Immunonutrition formulations contain nutrients such as arginine, glutamine, ω-3 fatty acids, and nucleic acids. Although arginine and glutamine are nonessential amino acids, they are referred to as conditionally essential amino acids, since demand for them increases dramatically following major surgical procedures. Thanks to numerous studies, the supplementary effects are well understood. In addition to these nutrients, enteral nutritional preparations (including those ingested orally) may also be regarded as important immunonutrients.
Administration of enteral nutrients in the early stages to patients with trauma reduces infection complications. The mechanism may be explained by the increase in mucosal immunity of the body (including enteric immunity) and the function of the enteric tract as a barrier, as well as by the preservation of the biologically protective potential of the abdominal cavity as triggered by enteral nutrition.

Immunopotentialization by arginine and glutamine



Potentialization of immunocytes
Secretion of pituitary hormones such as growth hormone (GH), etc.
Substrate for NO, etc.

+

Potentialization of immunocytes
Function of enteric tract as a barrier — enhancement of mucosal immunity
Improvement of protein metabolism, etc.

Arginine and glutamine have different functions

Q.3 Arginine and glutamine are both amino acids. How do they differ in their action as immunonutrients?

A.3 Both arginine and glutamine potentialize immunocytes such as lymphocytes, macrophages, and neutrophils, preserving the immunocompetence of the body following major surgical procedures and promoting wound healing. Arginine increases secretion of the growth hormone, insulin, which improves metabolism, and also modulates biological response during major surgical procedures by acting as a substrate for nitrogen monoxide, which resuls in various physiological benefits, including improved microcirculation and bactericidal action. Arginine does not preserve the function of the enteric tract as a barrier, but since glutamine is the substrate providing energy for the intestinal tract, it is a powerful preservative of its function as a physical and immunological barrier. In addition, glutamine is a building block for glutathione, an antioxidant, which protects the body from oxidative stress.

Energy source for immunocytes that exhibit a range of actions

Q.4 Could you explain the action of glutamine in the body more specifically?

A.4 Glutamine is the most abundant amino acid in the body, representing 60% of the free amino acids in muscle. Since immunocytes use glutamine as a major energy source, the demand for glutamine increases following major surgical procedures. Without external supplementation, glutamine is rapidly released from the amino acid pool in the muscle into the blood stream, resulting in destruction of skeletal muscle.

Without enteral administration of nutrients during major surgical procedures, the supply of glutamine from the gut lumen into the intestinal tract is cut off and blood glutamine concentrations are reduced, thus impairing an important energy substrate for the intestinal tract. This weakens the

physical and immunological barrier of the gut, resulting in more frequent bacterial translocation (Note 2) and reduced mucosal immunity.

Glutamine administration during invasive surgical procedures likely results in the following: 1) potentialization of immunocytes (such as proliferation of lymphocytes; promotion of the differentiation of B-lymphocytes to antibody-producing cells; enhancement in phagocytotic bactericidal potential, cytokine producing potential, active oxygen-producing potential, and antigen presentation potential of macrophages; potentialization of phagocytotic bactericidal potential, and active oxygen-producing potential of neutrophils; and so on); 2) suppression of the destruction of skeletal muscles; and 3) preservation of the enteric tract barrier (Table 1).

Note 2) Invasion of the bacteria and toxins present in the enteric canal into the body

Table 1 Functions of glutamine

1. Preserves the enteric tract's function as a barrier
2. Prevents bacterial translocation
3. Enhances enteric and mucosal immunities
4. Serves as an energy-providing substrate for lymphocytes, macrophages, neutrophils
5. Promotes wound healing
6. Improves protein metabolism
7. Serves as a building-block for antioxidant, glutathione
8. Serves as a raw material for nucleic acids (nitrogen source for purine and pyrimidine)

Is combined use of arginine and glutamine effective?

Q.5 What is the advantage of ingesting both arginine and glutamine?

A.5 Arginine exhibits an immunopotentialization action that depends on its concentration, but excessive administration suppresses immunocompetence. Glutamine is unstable in solution and not sufficiently soluble in water, making it difficult to prepare glutamine solutions at high concentrations. Simultaneous use of these amino acids may provide nutrient formulations that more effectively increase immunocompetence than those containing only a single amino acid. Glutamine as well as arginine, the substrate for NO, have been reported to be necessary for NO production. The combined use of these amino acids may improve immunopotentialization effects and microcirculation effected by NO. Arginine alone cannot adequately preserve the enteric tract's function as a barrier. Glutamine may be added to augment arginine.

Q.6 What are the typical effects of immunonutrient administration?

A.6 Some immue-enhancing diets (IEDs), which are reinforced by combined use of several immunonutrients, are widely used in clinical settings, mainly in the U.S. and Europe. Results have shown that such diets are effective among those who have undergone elective surgery: 1) reducing the incidence rate of postoperative complications and infections; 2) reducing the term of hospitalization; and 3) reducing the severity of infectious disease.
However, in studies of critically ill patients who demand intensive care, results vary depending on reports, due to the diversity of the pathological conditions of the patients.

Growing interest in immunonutrition research

Q.7 What future do you see for use of immunonutrients?

A.7 In the summit meeting of ASPEN in 2000, administering IEDs periodically to undernourished patients during surgical procedures was recommended, but not mentioned in connection with well-nourished patients. However, since Braga *et al.* demonstrated the effectiveness of preoperative administration even among well-nourished patients, this application will likely expand. Preoperative administration of IEDs would be effective even for well-nourished patients in cases of major surgical procedures, or operations among patients who may have infectious diseases.

On the other hand, it's not clear just now whether administering IEDs to patients with septicemia or multiple organ failure is effective. Opinions regarding administration to patients with such diseases are currently divided, I think due to the wide range of patient conditions among those classified into the crude categories of septicemia and organ failure. In our studies using gut ischemia reperfusion models, intravenous administration of glutamine during enteric ischemia caused excessive activation of leucocytes in peripheral blood, worsening organ injury and increasing mortality rates. From this point, we need to discuss what pathological conditions will respond to immunonutrients, and when, as well as the criteria. In addition, we need to ascertain the most suitable IED formulations (containing specific immunonutrients) for the specific pathological conditions of individual patients.

Profile of Dr. Kazuhiko Fukatsu

Graduated from Faculty of Medicine, Univ. of Tokyo in 1988
Dept. of Surgery, Ibaraki Prefectural Central Hospital in 1989
1st Dept. of Surgery, Univ. of Tokyo in 1992
Dept. of Surgery, Univ. of Tennessee in 1998
Assistant, Dept. of Colon and Rectal Surgery (former 1st Dept. of Surgery), Univ. of Tokyo in 2000
National Defense Medical College Research Institute in 2002
Currently, associate professor, Division of Basic Traumatology