

Arginine Metabolism: Enzymology, Nutrition, and Clinical Significance

Practical Recommendations for Immune-Enhancing Diets^{1,2}

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ABSTRACT Immune-enhancing diets contain nutrients that have putative benefits, including arginine, (n-3) fats, glutamine, nucleotides, and structured lipids. Although under most circumstances the systemic inflammatory response is beneficial to the host, improving the eventual outcome of injury, infection, or inflammation, excessive proinflammation (leading to cardiac, hepatic, and mitochondrial dysfunction) or excessive counterinflammation (leading to immune depression) can worsen outcome. In critically ill septic patients, the synthesis of arginine can be exceeded by its catabolism to nitric oxide (NO) and urea, rendering arginine conditionally essential. In patients with sepsis, increased production of NO increases serum nitrite and nitrate levels, whereas levels in patients with trauma and trauma with sepsis are lower than in controls. In septic patients, supplemental arginine might further increase NO levels and be potentially harmful through excessive proinflammation. However, administration of increased amounts of arginine might improve immune function in surgical and trauma patients by increasing NO production in macrophages. When the diet provides at least 1 g of the (n-3) fatty acids eicosapentaenoic and docosahexaenoic acid combined, 2-series eicosanoids (prostaglandins, prostacyclins, thromboxanes) are replaced partially by 3-series eicosanoids, and 4-series leukotrienes are replaced partially by 5-series leukotrienes that are less proinflammatory. Thus, the effects of arginine and (n-3)-fat supplementation might be expected to be complementary—arginine might improve cytokine and NO production in patients with immunodepression, whereas (n-3) fats might be beneficial when there is excessive proinflammation, particularly when supplemental arginine is supplied, by reducing cytokine-induced eicosanoid production. *J. Nutr.* 134: 2868S–2872S, 2004.

KEY WORDS: • *immune-enhancing diets* • *arginine* • *(n-3) fats* • *clinical outcome*

The increasing use of so-called immune-enhancing diets in hospitals throughout the world and the substantial number of publications in the clinical literature evaluating their effect on clinical outcomes in randomized clinical trials make it appropriate at this point to develop recommendations for their optimal utilization. Although there are a number of techniques that could be employed in such an evaluation, this review employs 3 that seem most appropriate. First is the theoretical framework as to how or why such novel substrates might be expected to act to enhance immunity, followed by a consideration of the findings in the 3 meta-analyses (1–3) already conducted that shed light on clinical indications for use, and finally an examination by post-hoc analysis of a number of potentially important and clinically relevant factors that should be considered when defining clinical guidelines for the use of these feeding therapies.

Immune-enhancing diets have as their principal components several nutrients that have putative benefits: arginine, (n-3) fats,

glutamine, nucleotides, and structured lipids. Arginine, a dibasic amino acid, is under normal conditions a nonessential or dispensable amino acid, because it can be synthesized in humans, largely through citrulline as the immediate precursor of argininosuccinate in the urea cycle. The production of carbamyl phosphate and the transfer of the carbamyl moiety to ornithine, yielding citrulline, occur in the mitochondria, whereas the hydrolysis of arginine occurs in the cytoplasm (4). Under certain conditions, such as in critically ill septic patients, the synthesis of arginine can be exceeded by its catabolism to nitric oxide (NO) and urea, rendering arginine conditionally essential (5). The principal cell functioning in the innate immune system, which is the nonspecific immune system characteristic of the main defense in primitive organisms, is the macrophage, which can recognize, phagocytose, and destroy pathogens (6,7). However, innate immunity is also a vital but somewhat less important component of the immune system in advanced organisms, in which lymphocytes function to provide specific, adaptive immunity. Under resting conditions, the use of L-arginine by the macrophage is minimal, but immune activation stimulates the active uptake of arginine by either of 2 pathways, inducible NO synthase (iNOS)⁴ or arginase I (8).

¹ Prepared for the conference "Symposium on Arginine" held April 5–6, 2004 in Bermuda. The conference was sponsored in part by an educational grant from Ajinomoto USA, Inc. Conference proceedings are published as a supplement to *The Journal of Nutrition*. Guest Editors for the supplement were Sidney M. Morris, Jr., Joseph Loscalzo, Dennis Bier, and Wiley W. Souba.

² The author receives royalties for fish oil from Novartis and Ross Products Division through his hospital.

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⁴ Abbreviations used: ARDS, adult respiratory distress syndrome; iNOS, inducible nitric oxide synthase; ICU, intensive care unit; K_m , Michaelis-Menten constant; NO, nitric oxide; Th1, T helper 1 cell; Th2, T helper 2 cell.

Expression of iNOS is stimulated by the systemic inflammatory response through the action of cytokines (IL-1, IL-2, TNF, and γ -interferon) produced by a subset of T lymphocytes, the T helper 1 (Th1) cells, which are proinflammatory and function principally to provide cellular immunity. The NO radical, which results from iNOS action on arginine, is a potent microbiocidal agent for both intracellular and extracellular pathogens (8,9). However, excessive production of NO is related to many of the untoward manifestations of septic shock, including hypotension, impaired cardiac contractility, liver damage, heightened vascular permeability, and bacterial translocation from the intestine (10).

A second pathway for arginine uptake is arginase I expression. Arginase I is the enzyme that leads to the production of ornithine, a precursor of polyamines and proline, which foster wound healing and cellular repair. Arginase I expression is upregulated in macrophages by the cytokines (IL-4, IL-10, and transforming growth factor- β) secreted by the second major type of T helper lymphocyte, Th2 cells, which primarily function in antibody response but are also antiinflammatory (8,11). Another aspect of this pathway is that arginase I reduces the availability of arginine for iNOS in M-1 macrophages as well as for the stimulation of T cells, in which capacity it serves as an important regulator of T-cell number and activation (12,13).

Thus, there are M-1 or M-2 macrophages, with M-1 macrophages expressing iNOS and thereby increasing inflammation, cellular immunity, and NO production, and M-2 macrophages expressing arginase I and thereby increasing cellular repair and antibody production but decreasing cellular immunity (6). Certain clinical conditions conform to this paradigm. Arginase I expression and activity are increased in human mononuclear cells after severe trauma, associated with IL-10 production and decreased levels of plasma arginine, its precursor citrulline, and NO metabolites (nitrite and nitrate) (11). Major surgery also presents a similar profile, characteristic of Th2 and M-2 response (14). Although not as fully explored with regard to arginase I activity in the macrophage and monocyte cell lines, sepsis is characterized by increased production of NO as reflected in serum nitrite and nitrate levels compared to controls, whereas levels associated with trauma and trauma with sepsis are lower compared to controls (15). Moreover, sepsis is also associated with lower serum arginine levels, presumably as a consequence of increased catabolism of arginine without a corresponding increase in synthesis (5). Lower circulating levels of arginine during sepsis correlate with a poorer prognosis and higher mortality rate (16). Although at normal serum arginine levels, exogenous L-arginine is not rate limiting for NO production due to the low K_m for iNOS, in experimental studies of sepsis, arginine availability becomes limiting and exogenous arginine worsens hypotension (17). Thus, a strong case could be made on a theoretical basis that supplying high-physiologic to near-pharmacologic amounts of arginine to patients with Th2, M-2 response, as found in surgical and trauma patients, should improve immune function by increasing NO production in macrophages. However, in critically ill patients with sepsis with an already exuberant Th1, M-1 response, supplemental arginine might further increase NO release and be potentially harmful through excessive proinflammation. Clinical data with arginine alone tend to support this hypothesis, because arginine supplementation to full nutrition improves immune function in surgical patients (13), whereas there is no net benefit in critically ill intensive care unit (ICU) patients (18) suggesting that some patients, presumably the less ill, may be helped and others, presumably the sickest, potentially harmed.

The (n-3) fats, found principally in cold-water fish, are the second component of immune-enhancing diets that is likely to be beneficial. Although they were long known to be helpful epidemiologically and clinically in a number of inflammatory conditions, including heart disease, sudden death, Alzheimer's disease, diabetes, IgA nephropathy, renal transplantation, regional enteritis, and ulcerative colitis (19), the important mechanisms of action were only recently identified.

One consistent action when the diet provides at least 1 g/d of eicosapentaenoic and docosahexaenoic acid combined is that 2-series eicosanoids (prostaglandins, prostacyclins, and thromboxanes) that are important second messengers in a wide variety of tissues are to some extent replaced by 3-series eicosanoids, and 4-series leukotrienes are replaced partially by 5-series leukotrienes. The 3-series eicosanoids and 5-series leukotrienes are much less proinflammatory in such actions as vasoconstriction, edema formation, and coagulation (20).

In terms of immune effects in humans, dietary fish oil supplementation decreases production of the proximate cytokines IL-1 and TNF by peripheral blood mononuclear cells and reduces the effect of endotoxin administration (21). More recently, the effect of (n-3) fats on cytokine production was determined to be immunomodulatory rather than immunodepressive, because fish oil intake decreases cytokine secretion in individuals who are genetically high producers in response to stimulus, whereas fish oil intake increases secretion in low responders (22). For a number of reasons, this makes the combination of supplemental arginine and (n-3) fats particularly likely to be complementary in action. That is, the systemic inflammatory response has both a proinflammatory component (characterized by fever, tachycardia, elevated white blood cell count with increase in immature forms, increase in phagocytosis and killing function of leukocytes, increase in reactive oxygen species, and stimulation of acute phase protein synthesis) and a counterinflammatory component, which is antiinflammatory and immune depressant, turning off inflammation and promoting wound healing.

Under most circumstances, the systemic inflammatory response that includes both components is beneficial to the host, improving the eventual outcome of injury, infection, or inflammation. However under 2 less common circumstances, excessive proinflammation, leading to cardiac, hepatic, and mitochondrial dysfunction, or excessive counterinflammation, leading to immune depression, can worsen outcome. Thus, arginine supplementation might be helpful for a Th2, M-2 response to improve cytokine and NO production, whereas (n-3) fats might be beneficial in cases of excessive proinflammation by reducing cytokine-induced eicosanoid production. There is some clinical support for this action, because in one of the early studies of the use of immune-enhancing diets in postoperative patients, the diet substantially increased TNF production while decreasing 2-series eicosanoid production, leading to a profile of improved immune function with less inflammation (23).

There is even more recent evidence for potential synergism between arginine and (n-3) fats. Arginine increases Th1 and M-1 activity, whereas one of the effects of (n-3) fats is to increase activation-induced cell death by apoptosis of activated Th1 lymphocytes specifically (24), which would tend to modulate excessive proinflammation. α -Linolenic acid, the essential fatty acid found in certain plants, such as soybean and flax, is the 18-carbon precursor for eicosapentaenoic and docosahexaenoic acid. Although dietary α -linolenic acid produces much lower levels of eicosapentaenoic and docosahexaenoic acid in tissue membranes compared to equivalent dietary amounts of the longer-chain derivatives, clinical immune-

enhancing diet studies report the same improvement in clinical outcome with either (1–3), although data on this type of fat are largely limited to trauma patients. In a recent study of critically ill patients, the ICU mortality of the subgroup with the most severe sepsis was marginally worse than that of a randomly assigned parenteral nutrition group (25). However, it should be recognized that the study was a subgroup analysis in which the mortality endpoint was changed and, most important, the fat used was high in α -linolenic acid, not eicosapentaenoic acid, and the arginine content (2%) was marginal (25).

There is little evidence for beneficial clinical effects of enteral glutamine in reducing infections or shortening hospital stay; the largest study of critically ill ICU patients with illness of moderate severity fed adequately with appropriate amounts of glutamine showed no net benefit or harm (26). However, in a small study of patients with severe burns, glutamine supplements markedly reduced bloodstream infections, particularly pseudomonas, and markedly reduced mortality, compared to randomized control subjects consuming a similar diet (27). Interestingly, the glutamine supplement was administered as frequent boluses rather than as a component of the formula diet. Bolus versus continuous feeding might be important for changing systemic blood levels of glutamine, given the extensive gastrointestinal metabolism of glutamine. Nucleotides have no proven clinical benefit in the context of feeding critically ill patients. Structured lipids do improve the tolerance of enteral feeding, and may increase the success of fish oil-based diets through their improved absorption characteristics (28), but major clinical benefits to organ function are attributable more to the fish oil component of the structured lipid than to structuring itself. Fish oil was investigated as the principal ingredient of an immune-enhancing diet in postoperative (28) and critically ill ICU patients (29). Although clinical benefits were reported, as noted above, the improvement in infection rates was more modest than those found in postoperative studies with diets containing both arginine and (n-3) fats (1–3,28). A clinical study of (n-3) fats in critically ill patients with adult respiratory distress syndrome (ARDS) showed dramatic clinical improvement in terms of pulmonary function and duration of ventilator requirement (29), but this may reflect the unique nature of ARDS to be almost exclusively an excessive proinflammatory Th1 type response, where (n-3) fats alone might be expected to be particularly effective.

There are 3 meta-analyses of the various studies of immune-enhancing diets in critically ill patients. All 3 show a marked reduction in hospital stay (by 2.5 to 3.3 d) and in infection rates (by 34 to 53%), but no substantial effect on mortality (1–3) (Table 1). The first, by Heys and colleagues (1), which was published in 1999, involved a Medline search of articles in English. This meta-analysis did not include 2 of the 3 studies

on immune-enhancing diets in critically ill ICU patients (30,31), but did include some duplicate studies. Meta-analysis of 11 studies involving 966 patients found a reduction in hospital stay of 2.5 days, a reduction in infection of 53%, and no effect on mortality, although mortality was suggestively higher in the treatment group (1). The next meta-analysis, by Beale et al. (2), also published in 1999, included all 3 ICU studies for a total of 12 studies of 1557 patients. Hospital stay was reduced by 2.9 d, ventilator duration by 2.6 d, and infections by 40%, with no effect on mortality (2). The final and most comprehensive meta-analysis included 22 studies of 2419 patients; hospital stay was reduced by 3.3 d and infections by 34%, with no effect on mortality (3).

Subgroup analysis cannot provide definitive evidence of benefit or harm of novel therapies but does provide some guidance as to their use while awaiting results of further randomized trials. Heyland et al. (3) found on subgroup analysis that postoperative patients appeared to benefit the most, and that a higher arginine intake seemed to improve results, but this last finding was largely based on an unpublished study which appears to incorporate a serious randomization error. However, the recent study cited above (25) involving severe sepsis in ICU patients with a formula using a similar arginine content is consistent with this. Thus, there is general agreement that immune-enhancing diets improve outcome in critically ill patients, with the clearest benefit in postoperative patients following surgery for upper gastrointestinal cancer that is characterized by a high risk for preoperative malnutrition and major surgical stress. Several randomized clinical trials conducted since the last meta-analysis suggest that preoperative feeding of nonmalnourished patients with upper gastrointestinal cancer (32) is also effective in terms of reducing infection and length of stay, whereas perioperative feeding is similar to preoperative with coexisting malnutrition only in terms of length of stay but is better in terms of reducing complications (33).

Immune-enhancing diets were recently reported to be modestly effective in reducing infection in colorectal surgery (34), a condition with much less risk of malnutrition and less surgical stress than in upper gastrointestinal cancer. They were also reported to reduce postoperative infection rates in head and neck cancer (35), another condition with high risk for preoperative malnutrition and major surgical stress that was included in the Heyland meta-analysis (3), and to provide clinical benefits in high-risk cardiac surgery, which is similarly characterized (36).

Any further recommendations would need to rely on subgroup analysis, which is always subject to misclassification and misattribution. However some best estimate of practice based on the literature, as it exists, with clinical judgment applied to what are likely to be the important variables is warranted. Although the factors to be selected for consideration are by their very nature arbitrary, subjective, and necessarily limited in number, it would seem that amount of diet, site of feeding (nasogastric vs. nasojejunal), underlying condition (postoperative, ICU, trauma, head injury, burn injury), and amount and type of immune nutrient likely to be the active principal should be among the most relevant.

There are 3 studies of immune-enhancing diets in critically ill ICU patients (30,31,37). Nasogastric feeding was principally used in all of these studies. Previous studies of nasogastric feeding of ICU patients found a complication rate of >60%, with adequate feeding in only a minority of patients (38). Although one might wish to employ nasojejunal feeding to increase the likelihood of successful enteral feeding, because feeding generally commences after critical illness is present in

TABLE 1

Meta-analysis of improvement in outcome in patients consuming immune-enhancing diets

Reference	Hospital stay	Ventilator	Infection rate reduction	Mortality
	d	d	%	
1	2.5	ND ¹	53	NS
2	2.9	2.6	40	NS
3	3.3	ND	34	NS

¹ ND, not determined; NS, not significant.

ICU patients, this usually requires endoscopy or fluoroscopy for feeding-tube placement, expensive techniques that are not broadly available and often delay feeding. In addition, nasojejunal feeding can lead to the uncommon but disastrous complication of small bowel infarction, particularly in the most critically ill patients with hypotension (39,40).

Bower et al. (37) reported a suggestive increase in mortality in the treatment group. However, a subgroup analysis of the group that received at least 821 mL/d of diet (31% of the study population) revealed that the length of stay decreased by 9 d in the treatment group with no effect on mortality. In the second study of ICU patients, Atkinson et al. (30) defined a priori a subgroup that received >833 mL/d of diet. In that group (25% of the study population), the length of hospital stay and ventilator time decreased by 4.5 d. In the most recent study, which used the most stringent type of statistical analysis, an intent-to-treat analysis, Galban et al. (31) reported that >84% of the subjects received >820 mL/d of diet, and rates of infection, bacteremia, and mortality decreased in the entire treatment group.

This progression of quality of evidence from the most suspect to the strongest is strikingly consistent and provides assurance in the conclusion that a minimum intake, ~50% of resting energy expenditure, is an important variable for efficacy. The mortality experience appears to track with this definition of dietary adequacy as well, with no obvious changes in mortality in the Bower et al. (37) and Atkinson et al. (30) studies and a significant reduction in the Galban et al. (31) study when this minimum amount of diet was provided. The APACHE score, which is widely used to judge severity of illness in ICU patients, suggested that those who benefited most in the Galban et al. (31) study were those with scores of 10 to 20, reflecting moderate severity of illness, with little difference in outcome in the more severely ill.

There are 5 studies of diets using a combination of arginine and (n-3) fats in patients with multiple trauma. Two studies using the same formula both report clinical benefit in terms of reduced infection rates and/or length of stay, whereas one using a similar formula reported immune benefit but no important clinical difference (41–43). The remaining 2 studies, using 2 different dietary formulas but both with a lower arginine content, reported reduced infection rates and no effect, respectively (44,45). In 2 trials with a glutamine-enriched formula but no other immune-enhancing nutrients in trauma patients, one reported a significant reduction in infection rates with no effect on length of stay (46), and the other reported no difference in either (47). Thus, one might on a theoretical basis suggest that immune-enhancing diets should help in trauma, that there is little evidence of harm, but the data provide only a strong trend in their favor at present. Perhaps as nasogastric feeding becomes increasingly likely to provide adequate nutrition in the critically ill as feeding protocols become more widely used, new studies of patients with moderate degrees of injury where benefit is most probable will define the appropriate trauma patient. This is exemplified by 2 recent studies of enteral nutrition in critically ill ICU patients in which nasogastric feeding was successfully provided to the vast majority of patients (18,26). Interestingly, arginine alone was used in one of these studies and glutamine alone in the other, in a study population with a moderately severe degree of illness, there was no benefit with either nutrient when provided singly. This suggests, as previously described in a theoretical framework, that the nutrients most likely to be effective are arginine and (n-3) fats when supplied together, in a diet containing >2 to 6% arginine by weight and at least 1 g/d of (n-3) fats, particularly eicosapentaenoic acid.

In conclusion, practical recommendations for the use of immune-enhancing diets, based on the literature and clinical interpretation, are as follows: definitely in preoperative and postoperative feeding following major surgery for patients with malnutrition, including milder degrees of malnutrition; probably for ICU patients with illness of moderate severity and APACHE scores of 10 to 20; probably for patients with multiple trauma with similar degrees of illness severity; and probably for malnourished patients with moderate systemic inflammatory response syndrome. There is insufficient evidence to make recommendations for patients with major burns or closed-head injury. There is concern regarding the potential for adverse outcomes in patients with severe sepsis, particularly when using immune-enhancing diets with only 2% arginine content, a level not much greater than the usual daily arginine intake from a normal diet. Immune-enhancing diets should be supplied for at least 3 d and optimally for 5 to 10 d, beginning as soon as possible post-trauma or -injury. Nasogastric feeding is usually possible in ICU patients, but nasojejunal feeding can be initiated in some patients with trauma and gastrointestinal surgery at the time of the initial surgery. Feeding should provide at least 820 mL/d, with an ultimate goal of 25 kcal/kg (0.1 MJ/kg). The success of feeding will generally require nursing protocols to advance feeding at regular intervals and acceptance of residuals of at least 200 mL. Nasojejun feeding should be avoided in patients who are hypotensive or who require pressors for blood pressure support. Management for good metabolic control, including glucose homeostasis (48) and renal replacement therapy where necessary to optimize protein intake, is essential (49) to realize the full clinical benefits when using these formulas.

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