

## Immune-modulatory actions of arginine in the critically ill

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Current trials of immune-enhancing diets suggest several beneficial clinical effects. These products are associated with a reduction in infectious risk, ventilator days, ICU and hospital stay. However, methodological weaknesses limit the inferences we can make from these studies. Furthermore, improvements in outcomes were largely seen in surgical patients and in patients who tolerated critical amounts of formula. We propose that the beneficial findings cannot easily be extrapolated to other patient populations since there is suggestion from clinical trials that the sickest patients, especially those with severest appearances of sepsis, shock and organ failure may not benefit or may even be harmed. In these conditions we hypothesize that systemic inflammation might be undesirably intensified by immune-enhancing nutrients like arginine in critically ill patients. In this paper, we review the purported effects of arginine on the immune system and organ function to understand the scientific rationale for its inclusion into enteral feeding products. We conclude that patients with the most severe appearances of the systemic inflammatory response syndrome should not receive immune-enhancing substrates which may aggravate systemic inflammation and worsen clinical outcomes.

**Arginine: Nitric oxide: Inflammation: Critical illness: Enteral nutrition**

### Introduction

Nutrition support may have a modulating effect on the underlying illness by its salutary effects on the immune system and organ function. In this context, immunonutrition is appealing as a novel approach to favourably modulate the immune (dys)function associated with critical illness. The concept of 'immunonutrition' has been developed to supply specifically defined substrates that promote certain biochemical pathways as they become depleted due to their extensive consumption. Enteral formulations have developed to such an extent that they contain selected substrates such as glutamine, nucleotides, arginine and *n*-3 fatty acids as well as selenium, vitamins E, C, A and beta-carotene in supra normal concentrations. Clinical demonstration of the enhancing action of these immune-modulating diets on individual parameters of cellular defense has already been successful, with patients mostly examined during post-operative or post-traumatic stress (Cerra, 1991; Daly *et al.* 1992; Kemen *et al.* 1995; Moore *et al.* 1994). However, a modifying effect on selected parameters of the immune response does not, in itself, justify the use of an expensive

enteral nutritional formula that may not be free from side-effects. Thus, the reinforcement of cellular defense functions has to be reflected by improvements in clinical outcome such as a reduction in infection rate, length of hospital or ICU stay or savings in treatment costs.

Up until the early 1990s there were no investigations in critically ill patients available supporting these results from the clinical perspective although data from animal experiments already suggested that supplementing enteral formula with RNA, *n*-3 fatty acids, arginine and glutamine can improve outcome (Heyland *et al.* 1994). This state of affairs had changed in the last decade. In 1998, Zaloga (Zaloga, 1998) referred to thirteen prospective, randomized clinical studies in which an immune-modulating enteral diet was compared with a standard one. Twelve of these thirteen studies indicated an improved outcome in the group that received the experimental therapy. The benefits of these immune-modulating diets are seen as a reduced incidence of infectious complications, a reduced duration of ventilation, ICU and hospital stay, and reduced hospitalization costs. Subsequently, two meta-analyses, one guided by Beale *et al.* (Beale *et al.* 1999) and one by Heys *et al.* (Heys *et al.* 1999),

**Abbreviations:** NO, nitric oxide; iNOS, inducible NO synthase; cNOS, constitutive isoforms; SIRS, systemic inflammatory response syndrome.

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confirmed these findings with regard to the reduction of infection rate and length of stay. A third, more comprehensive and recent meta-analysis (Heyland *et al.* 2001) evaluated twenty-two studies of immunonutrition. The investigators found that immunonutrition may decrease infectious complication rates; however, the treatment effect varies depending on the intervention, the patient population and the methodological quality of the study.

### Do unresolved questions exist in immunonutrition?

In an editorial published in 1998 (Zaloga, 1998), the author makes an unequivocal recommendation for immune-modulating enteral nutritional solutions: 'Using an evidence-based approach, the use of immune-enhancing formulas in critically ill patients represents a level I recommendation.' However, there are several shortcomings in the design of some of these trials that weaken, if not invalidate, such an inference. Of the existing trials of immunonutrition, only 12/22 (55%) were double-blinded and in only 5/22 (23%) was randomization concealed. Frequently the control groups did not receive isocaloric and/or isonitrogenous treatments and the study groups were not stratified with regard to the severity of the illness. Finally, only 10/22 (45%) performed an intention-to-treat analysis. The majority of significant findings were observed in analyses based on events that occur after randomization, such as on the amount of enteral feeds received. Analyses classified by variables measured *after baseline* (such as compliance or tolerance) are more likely to mislead rather than inform (Oxman & Guyatt, 1992; Yusuf *et al.* 1991). There may be an interaction between compliance or tolerance to enteral feedings and the study intervention resulting in a bias that can not be compensated for. It is recommended that no study subjects should be withdrawn from the analysis because of compliance reasons (Friedman *et al.* 1991). With the exception of the Galban study (Galban *et al.* 2001), no randomized trial of immunonutrition has demonstrated a statistically significant difference in clinically important outcomes, compared to controls, in an intention-to-treat analysis.

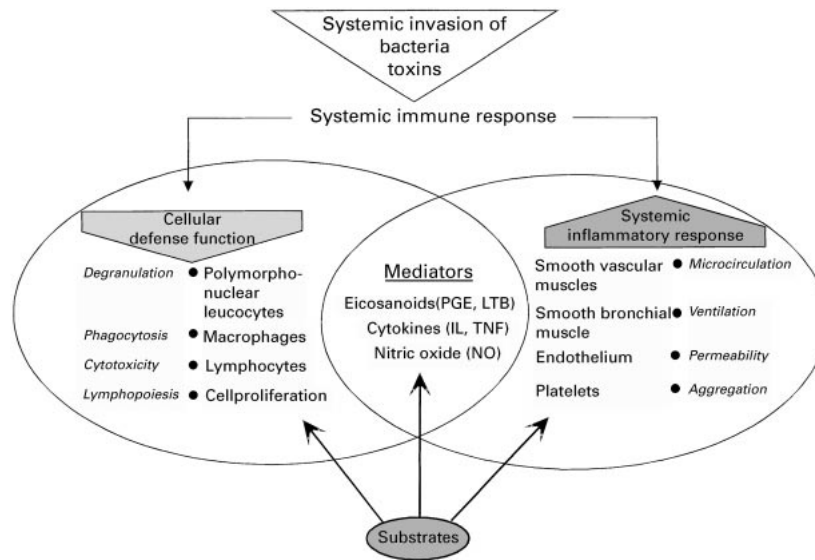
In addition to limitations to the validity of these studies, one must consider the limitations in the generalizability of results. Since the majority of studies have been carried out on operative or post-traumatic patient groups, Nelson (Nelson, 1998) points to the faulty comparability between different patient populations. She points out that we must be careful not to overextrapolate data from one patient population to another because patients with shock, sepsis and organ failure may be systematically different. This notion is supported by the results of the most recent meta-analysis of immunonutrition (Heyland *et al.* 2001) that found that the treatment effect in surgical patients was significantly different than the treatment effect in critically ill patients. Indeed, the best estimate of treatment effect in critically ill patients was consistent with no effect or perhaps a trend towards harm (RR, 1.18; 95% CI, 0.88–1.58) (Heyland *et al.* 2001).

Besides the results of the meta-analyses, there are findings from individual studies that support the notion that immune-enhancing diets may do more harm than good in

critically ill patients. In a large multi-centered, double-blind, randomized trial, Bower and colleagues compared Impact to Osmolite HN in critically ill patients (Bower *et al.* 2001). Of the 326 patients that were randomized, forty-seven (14%) were dropped from the primary analysis. When including only those patients that received feeds, more patients who received the experimental formula died (24/153, 15.7%) than in the control group (12/143, 8.4%) although the investigators do not report the *P* value in the actual publication (chi-square, *P*=0.055). In the subgroup analysis, the mortality rate in the septic group who received the experimental feed was three times that of septic patients who received control feeds (11/44[25%] v. 4/45[8.9%], *P*=0.051). In an unpublished study of immunonutrition (Ross Products Division of Abbott Laboratories, 1996), 170 critically ill patients were randomized to receive either an experimental diet consisting of supplemental arginine, omega-3 fatty acids and vitamins A, E and beta carotene or a isonitrogenous control feed. There were significantly more deaths in the group that received the experimental formula (20/87, 23.0%) compared to the control group (8/83, 9.6%, *P*=0.03). Despite similar baseline demographics, including APACHE II scores, there were more patients with pneumonia at baseline in the group that received the experimental formula compared to controls. It was in this subgroup (patients with pneumonia at baseline) where the excess deaths occurred in the experimental group (10/26, 38.5%) compared to the control group (0/9, 0%). Furthermore, Saffle *et al.* (Saffle *et al.* 1997) indicated higher mortality in a subgroup of burns patients with inhalation trauma receiving the immune enhancing formula. Finally, Mendez *et al.* (Mendez *et al.* 1996; Mendez *et al.* 1997) demonstrated an increased length of stay in ICU and in the hospital, and reported increased ventilation periods associated with the use of immune-enhancing diets. In particular, the authors point to an increased incidence of pulmonary organ failure during immunonutrition. Although the majority of these adverse results do not reach the significance level, taken as a whole they indicate a trend that at this time does not support the use of currently available immune-modulating formulas in the *most seriously ill, infected patients*. Further research needs to define the underlying mechanism by which immunonutrition may be harmful. However, it may relate to the effect of some immune-enhancing agents on the immune system.

### Actions of immune modulating substrates on areas of immune defense

In highly simplified fashion, the immune defense system can be divided into two areas: the cellular defense function and the local or systemic inflammatory response (Fig. 1). Once pathogens enter the circulation they initially may boost cellular defense mechanisms (induced by release of tumor necrosis factor-alpha, interleukin [IL]-1 $\beta$ , IL-6, IL-8 and other cytokines). However, in the long term this is followed by the suppression of immune functions that cover the specific and non-specific cellular immune response. During this stage of illness, immunosuppressive mediators may cause anergy to skin test antigens, impaired antibody production and diminished phagocytosis rendering patients



**Fig. 1.** Effects of invading pathogens on the systemic immune response and its modulation by substrates with immune-modulating action. Modified from Suchner *et al.* 2000.

at increased risk for additional infectious morbidity and mortality (Bone, 1996; Zedler *et al.* 1999). Defined nutritional substrates can enforce the cellular and humoral defense system via modified mediator formation or interference with intracellular signal transduction.

In contrast the inflammatory response almost predictably becomes amplified as indicated by the exaggeration of pertinent changes outlined in Fig. 1. Essential components of the inflammatory immune response are represented by the activation of cascade systems, such as the coagulatory or the complement system. Moreover, mediators are involved including cytokines, eicosanoids, platelet activating factor, nitric oxide (NO) as well as vasoactive amines and kinins. Cascade systems, as well as mediators, participate in the initiation and perpetuation of the inflammatory immune response which leads to changes to the endothelium, the smooth vascular and bronchial muscles, and cellular metabolism. This in turn may affect the microcirculation, pulmonary gas exchange, vascular permeability as well as substrate utilization, and subsequently may impair organ function.

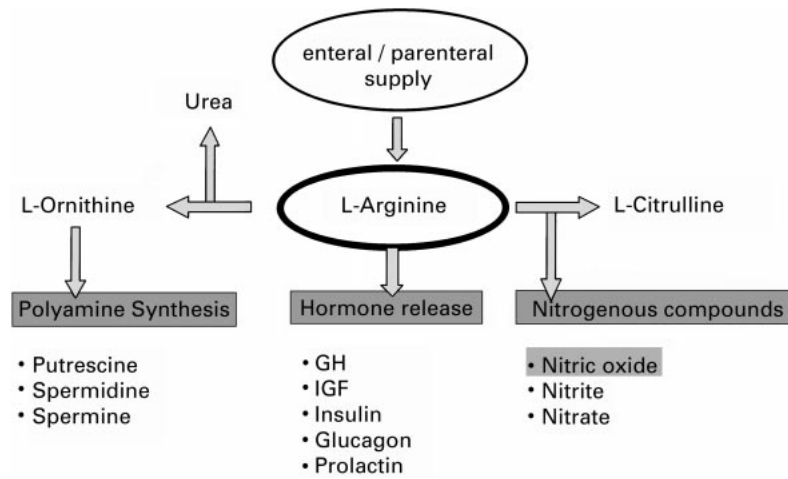
The combined stress induced changes on cellular defense function and the inflammatory response, especially if exaggerated, can lead to protein-calorie malnutrition, increased infectious morbidity, prolonged ventilatory dependence, greater length of hospital stay, and increased mortality (Herrmann *et al.* 1992; Reinhardt *et al.* 1980; Windsor & Hill, 1988). Within the framework of these events, experimental and clinical data lend credence to the idea of understanding defined substrates as 'pharmacologically effective agents', by which the cellular defense function can be restored and the inflammatory response can be modulated (Suchner *et al.* 1995). Since defined nutritional substrates are also the precursors of highly active pro- and antiinflammatory mediators, their supply may precipitate either an attenuation or an augmentation of the severity of the inflammatory immune response

depending on the quantitative and qualitative choice of their administration.

In the light of the results of clinical studies, we propose the hypothesis that substrates employed with the objective of stimulating the cellular defense function, should not induce a concomitant augmentation of systemic inflammation if applied to patients with severe appearances of systemic inflammatory response syndrome (SIRS) or sepsis. Therapeutic agents that suppress the inflammatory response in sepsis have been recently shown to be associated with improved survival (Bernard *et al.* 2001). Since the most common added nutrient to immune-enhancing formulas is arginine, below we elude to the immunomodulatory actions of arginine. We will focus on the rationale as well as the available experimental and clinical evidence favoring the notion that arginine exerts both salutary and harmful actions if applied to systemically infected patients.

### Benefits and risks of arginine as an immune enhancing substrate

Arginine is considered a non essential amino acid although its availability is reduced during trauma and sepsis (Barbul *et al.* 1983; Kirk & Barbul, 1990; Nirgiotis *et al.* 1991). Arginine promotes profound secretagogue actions (Barbul *et al.* 1981; Rettura *et al.* 1979) since it induces the release of somatotropin and prolactin from the hypophysis and promotes the pancreatic release of insulin (Fig. 2). In addition, the production of insulin-like-growth-factor (IGF) and the release of anti-insulinemic hormones like glucagon, somatostatin, pancreatic polypeptides and catecholamines are enhanced by arginine (Barbul, 1986). As it is metabolized to citrulline, L-arginine also gives rise to the formation of nitric oxide (NO), nitrites, and nitrates. Moreover, arginine is the precursor of growth factors like putrescine, spermine and spermidine. Via the formation of glutamate, arginine can yield increased amounts of proline



**Fig. 2.** Effects of arginine on polyamine synthesis, hormone release, and the production of nitrogenous compounds.

and hydroxyproline, which are required for the synthesis of connective tissue. Clinical studies evaluating the effects of supplemental enteral arginine supply have already demonstrated net nitrogen retention, increased protein synthesis and improved wound healing (Barbul, 1986; Barbul, 1990).

NO is produced by a family of enzymes called nitric oxide synthases (NOS), which exist in constitutive and inducible isoforms (Salzman, 1995). Shared and unshared characteristics are outlined in Fig. 3. The production of NO is under the control of three distinct NO-synthase (NOS) genes. The type 1 and 3 constitutive isoforms (cNOS) are always present, calcium-dependent and produce low levels of NO intermittently (Schmidt *et al.* 1995). In contrast, the type 2 inducible isoform (iNOS) is activated by cytokines and endotoxins. It is not calcium-dependent (Szabo, 1995) but substrate and cofactor availability are the rate limiting factors. Once induced, iNOS produces large amounts of NO for prolonged periods of time and the excessive production of NO by iNOS is thought to escalate derangements in the

structural and functional integrity of the intestinal mucosa (Salzman, 1995; Szabo, 1995), the liver (Isobe *et al.* 1999; Isobe *et al.* 2000; Wang *et al.* 1998) and the kidney (Ling *et al.* 1999) and do have adverse effects on gastrointestinal motility (Konturek *et al.* 1995).

*Effect of arginine on the immune response*

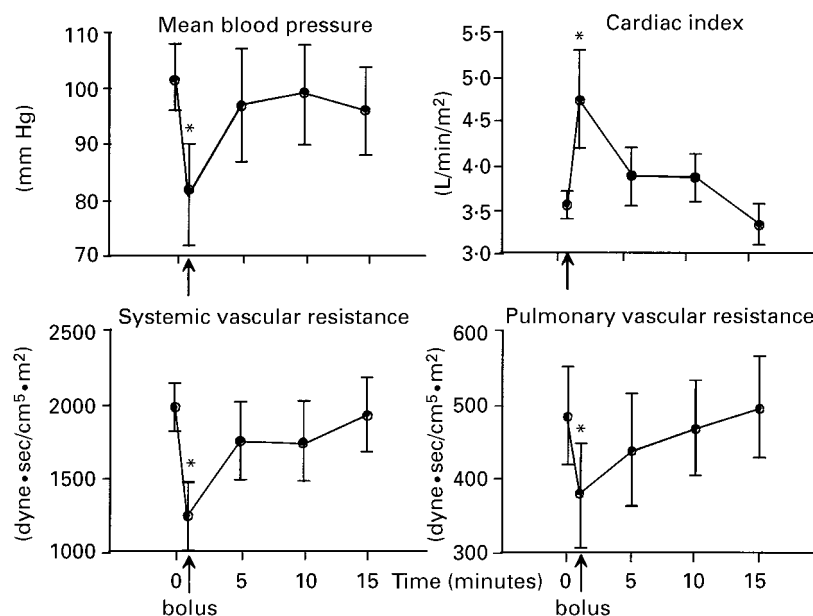
Arginine was demonstrated to be of significance in the chronically as well as in the critically ill because of its potential role in immunomodulation (Evoy *et al.* 1998; Kirk & Barbul, 1990). In particular, arginine derived NO plays an essential role in the regulation of inflammation and immunity according to recent reports (Albina, 1996).

Current evidence indicates that arginine enhances the depressed immune response of individuals suffering from injury, surgical trauma, malnutrition or sepsis. The effect of arginine is on parameters of the *cellular defense function*, presumably by means of cNOS mediated NO formation. An

<u>Shared characteristics</u>		
Substrate:	L-arginine	
Cofactors:	NADPH, FAD, FMN, BH4	
Heme group:	Protoporphyrin IX	
Enzyme structure:	Homodimer	
<u>Unshared characteristics</u>		
	cNOS	iNOS
Expression	Always present	Must be induced
Chromosomal location	ecNOS:7; ncNOS:12	iNOS:17
Rate limiting	Calcium / calmodulin binding	Substrate / cofactor availability
Product quantity	Small / intermittent	Large / sustained

**Fig. 3.** Characteristics of nitric oxide synthase isoforms. Modified from Moncada & Higgs, 2001.





**Fig. 4.** Hemodynamic changes induced by L-arginine (200 mg/kg i.v.) during sepsis in humans;  $P \leq 0.05$  v. minute 0. Redrawn from Lorente *et al.* 1993a.

exogenous enteral supply of arginine is accompanied by an increased lymphocyte (Barbul *et al.* 1981) and monocyte (Barbul *et al.* 1981; Cerra *et al.* 1990; Daly *et al.* 1988) proliferation, enhanced T-helper cell formation (Barbul, 1990; Cerra *et al.* 1990; Daly *et al.* 1988), an activation of macrophage cytotoxicity, the reinforcement of the activity of the natural killer cells, an increased phagocytosis as well as an increased cytokine production (Kirk & Barbul, 1990; Reynolds *et al.* 1988; Reynolds *et al.* 1990). These salutary effects of arginine on cellular defense function led to its inclusion into current concepts of immune enhancing formulas designed to reduce the incidence of infectious morbidity and mortality in critically ill and immune compromised patients.

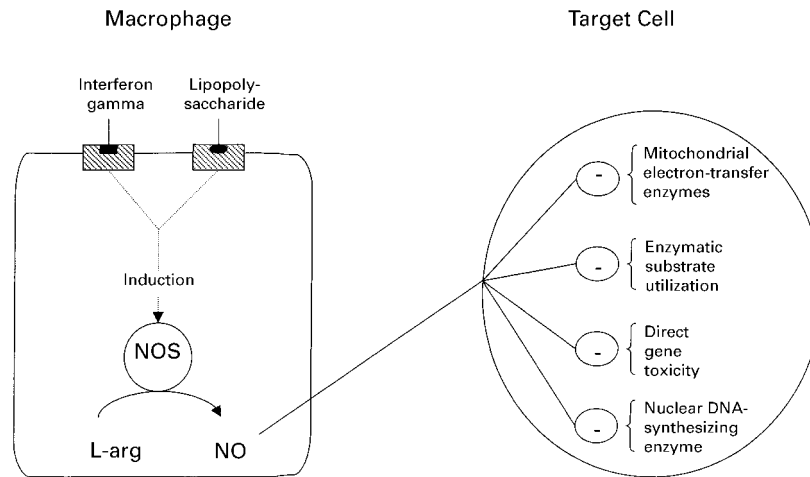
Contemporaneously, arginine enhances the *systemic inflammatory response* by means of an unbalanced NO release. Since iNOS is prominent in inflammatory conditions, it was already implicated as the predominant producer of NO from arginine during immune response. NO contributes to negative ino- and chronotropism at the heart (Lowenstein *et al.* 1994), impaired coagulation (de Graaf *et al.* 1992; Radomski *et al.* 1990), and vascular dilatation (Lorente *et al.* 1993a; Lorente *et al.* 1993b). Whereas vasodilatation restricted to local, microvascular regions may positively impact on regional immune defense and wound healing, the release of large quantities of NO may cause systemic vasodilatation with therapeutically refractory hypotension. As shown in Fig. 4, the administration of L-arginine to patients with severe SIRS and sepsis caused transient hypotension, and increased cardiac index while it decreased both systemic and pulmonary vascular resistance (Lorente *et al.* 1993a).

In addition, NO has a cytotoxic effect since it is a non-specific effector that inhibits growth or kills cells in an untargeted fashion (Lepoivre *et al.* 1991; Lowenstein *et al.* 1994; Wink *et al.* 1991). The major mechanisms of cytostasis and cytotoxicity induced by nitric oxide are

illustrated in Fig. 5. The key enzymes which are inhibited by NO are summarized in Fig. 6. In particular the enzyme cytochrome-c-oxidase, the terminal enzyme of the mitochondrial respiratory chain (complex IV), has been demonstrated to be exquisitely sensitive to nitric oxide (NO) at even low physiological concentrations. It is known that exogenous administration of low concentrations of NO inhibits cytochrome-c-oxidase in a variety of cells and isolated mitochondria. Up until now, however, we can only assume what the biological consequences of such an interaction might be since the mechanisms underlying the sensing of acute variations in oxygen concentration have not been fully elucidated. Cumulative evidence indicates that a haem protein is involved in this regulatory process (Bunn & Poyton, 1996), and cytochrome-c-oxidase has been proposed as a candidate (Duchen & Biscoe, 1992; Wilson *et al.* 1994).

#### *Effects of arginine derived NO production on cell respiration— implications for the critically ill*

Patients who are subjected to a critically lowered oxygen delivery are prone to turn into a state of oxygen supply dependency. In this state, the rate of oxygen consumption varies with its concentration at the tissue level suggesting the existence of an intracellular mechanism that controls cell respiration. Recently, it was demonstrated (Clementi *et al.* 1999) that NO generated by vascular endothelial cells modulates cell respiration to acute changes in oxygen concentration. The induced inhibition is competitive with oxygen and is fully reversible even after several hours (Clementi *et al.* 1998). This action, indeed, occurs at the cytochrome-c-oxidase level. The process depends on influx of calcium indicating that cNOS is involved. Apparently, NO plays a physiological role in adjusting the capacity of this enzyme to use oxygen allowing cells to adapt to acute changes of oxygen supply.



**Fig. 5.** Mechanisms of cytosclerosis and cytotoxicity induced by nitric oxide. Modified from Moncada & Higgs, 2001.

If, indeed, NO is in control of the mitochondrial enzymes, then this may be a general biological mechanism of regulation of cell respiration. This line of reasoning would explain, at least in part, why basal oxygen consumption is increased in a whole animal when treated with a NOS inhibitor (Shen *et al.* 1994; Shen *et al.* 1995). Although a basal generation of NO may be suitable to exert control during resting conditions, it is likely that the activation of the inducible NO synthase may result in an increased NO production and subsequently in a further increased inhibition of mitochondrial enzymes. This, however, implies that the classical paradigm about the independence of oxygen consumption along a wide range of oxygen concentrations should be corrected in terms of NO induced interferences. It remains to be investigated under which conditions cells further down-regulate their oxygen requirement. Evidence in favor of a role of endogenous nitric oxide (NO) as a limiting factor of cell respiration has been derived from experiments in tissues activated with cytokines and bacterial products in which NO is generated

continuously in large quantities by the inducible NO synthase (iNOS). In these conditions, NO-induced inhibition of cell respiration is persistent and attributable to nonselective inhibition of various mitochondrial enzymes, including complexes I–IV in the respiratory chain. Such inhibition contributes to the pathological actions of NO (Bolaños *et al.* 1997). Inhibition of oxygen consumption by 50% has been reported to occur at ratios of NO–oxygen concentrations ranging from 1:500 to 1:150 (Boveris *et al.* 1999; Brown & Cooper, 1994).

These only recently appreciated mechanisms of the NO induced inhibition of cellular respiration provide new insights into the principles of energy metabolism in patients with severe sepsis. In these patients a pathologic oxygen uptake/supply dependency was suggested to result in tissue hypoxia and a supra-normal oxygen delivery was proposed (Shoemaker & Appel, 1994). This hypothesis, however, is controversial since no skeletal muscle hypoxia was detected in patients with severe SIRS or sepsis. In contrast, the skeletal muscle  $pO_2$  of critically ill patients was demon-

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- Enzyme inhibition of energy metabolism
    - glycolysis / gluconeogenesis → Glyceraldehyde-3-phosphate dehydrogenase
    - TCA cycle → cis-Aconitase
    - Electron transfer → Ubiquinone-reductase
    - Cytochrome-c-oxidase
  - Enzyme inhibition of DNA synthesis
    - nucleotides → Ribonucleotide-reductase
  - Enzyme inhibition of detoxification
    - Biotransformation → Cytochrome P450
- 

**Fig. 6.** Possible targets of NO-mediated inhibition of key enzymes. Modified from Moncada & Higgs, 2001.

strated to be significantly higher on days with septic state than on days with intermediate or nonseptic states (Boekstegers *et al.* 1993). More recent data provide additional evidence that suggest that skeletal muscle  $pO_2$  increases as sepsis becomes more severe (Boekstegers *et al.* 1994a), indicating decreased oxygen utilization, whereas a reduction of skeletal muscle  $pO_2$  might be an early indicator of improvement of sepsis (Boekstegers *et al.* 1994b). A decreased oxygen utilization within the cell, which may result from a 'downregulation' of oxygen-dependent metabolic pathways, might account for decreased oxygen extraction by peripheral tissue in severe sepsis. Recent findings support the view that this bioenergetic part of septic organ failure is caused by disturbances of the mitochondrial function. As outlined in Fig. 6 the key enzymes inhibited by NO include those of the tricarboxylic acid cycle (TCA) cycle, glycolysis and the electron transfer chain, favouring the notion that NO may play a key role in regulating energy metabolism and oxygen utilization. A well established model of septic baboons was used to investigate key enzymes of energy metabolism and to answer the question whether or not impairment of mitochondrial oxidation may occur under these conditions (Gellerich *et al.* 1999). Dependent on sepsis severity, a reduction of the activity of NADH:cytochrome-c-reductase (Complex I+III) and succinate:cytochrome-c-reductase (Complex II+III) was found in the tissue of surviving animals. An even more severe reduction of the enzyme activities was found in animals with lethal septic shock where additionally a diminished activity of phosphofructokinase was present. Thus, there is an increasing body of evidence that high cellular levels of NO may contribute to the impaired substrate utilization that needs to be dealt with in the critical care environment. Moreover, just as NO can be successfully employed by the organism against invading cells, it apparently may also damage the body's own healthy ones and possibly promote organ dysfunction.

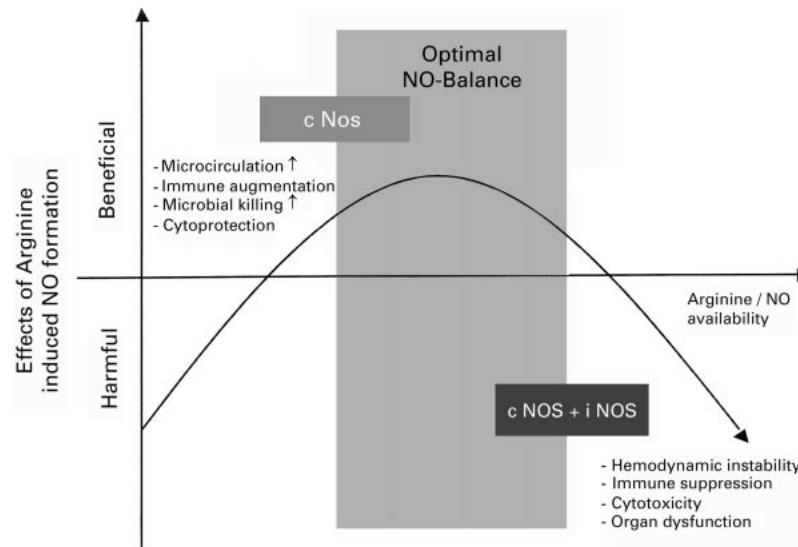
#### *Significance of NO in gut failure formation*

The decisive action by which endotoxin is thought to cause bacterial translocation is by an ischemia-reperfusion injury of the gut (Deitch *et al.* 1989; Navaratnam *et al.* 1990; Xu *et al.* 1993). The mechanisms leading to gut barrier failure and how loss of gut barrier function promotes distant organ injury in the critically ill remain to be fully determined. Current evidence indicates that cNOS-derived endogenous NO production reduces the sequela of acute gastrointestinal inflammation (Alican & Kubes, 1996). It was shown that NO, formed by calcium-dependent constitutive NO-synthase, plays a crucial role in maintaining vascular integrity as well as mucosal barrier function (Kubes, 1992; Kubes & Granger, 1992; Whittle *et al.* 1990). Moreover, NO generators significantly reduce mucosal injury shortly after ischemia-reperfusion (Kurose *et al.* 1994) while N-nitro-L-arginine methyl ester (L-NAME), a potent inhibitor of NO-synthase, greatly exacerbates intestinal injury and increases mucosal barrier dysfunction associated with ischemia-reperfusion (Kubes, 1993). These results suggest that basal nitric oxide production is important in minimizing mucosal barrier dysfunction in these models.

However, in more advanced SIRS and sepsis, induction of iNOS and the production of large amounts of NO may cause direct intestinal mucosal injury and intestinal barrier dysfunction (Schmidt *et al.* 1995), again highlighting the potential dual role NO may play in preventing and also inducing intestinal injury. Based on recent experimental data (Salzman, 1995), it seems that NO may be involved in endotoxin-induced gut injury, as it may contribute to a direct injurious effect on the gut mucosa, potentially mediated via reactive nitrogen intermediates such as peroxynitrite (Beckman *et al.* 1990). NO rapidly reacts with the superoxide anion to form the peroxynitrite anion ( $ONOO^-$ ), a highly reactive oxidizing agent capable of causing tissue damage. Since NO also contributes to the incidence of deamination-related genetic mutation (Wink *et al.* 1991) the combined actions precipitate in the generation of an NO induced cytotoxicity. Therefore, as shown in a variety of experimental trials, the excessive iNOS driven release of the pluripotent signalling and effector molecule NO appears to be a factor contributing to intestinal epithelial damage, hyperpermeability, and bacterial translocation. For example, the upregulation of iNOS-messenger RNA (mRNA) expression has been documented in the small intestine of rats challenged with endotoxin (Chen *et al.* 1996; Cook *et al.* 1994; Unno *et al.* 1997a) and in cultured enterocytic monolayers incubated with interferon- $\gamma$  (IFN- $\gamma$ ) (Salzman *et al.* 1996) or IFN- $\gamma$ +interleukin-1 $\beta$  (IL-1 $\beta$ ) (Kolios *et al.* 2001; Salzman *et al.* 1996). Furthermore, evidence was obtained that excessive production of NO contributes to increased gut mucosal permeability in rats challenged with lipopolysaccharide (Chen *et al.* 1996; Unno *et al.* 1997a) or cytokines (Chavez *et al.* 1999). Moreover, it was shown that cytokine-induced hyperpermeability is largely dependent on increased NO-production and also requires the availability of  $O_2$ . This suggests that  $ONOO^-/ONOOH$  may be an important intermediate in this phenomenon (Chavez *et al.* 1999). In addition, NO-donors from exogenous sources directly increase the permeability of enterocyte monolayers *in vitro* (Salzman *et al.* 1995; Unno *et al.* 1997b). On the other hand, specific iNOS inhibitors are capable of blocking the endotoxin-induced bacterial translocation (Mishima *et al.* 1999) as well as the increase in permeability induced in cell monolayers if incubated with IFN- $\gamma$  (Unno *et al.* 1995). Also iNOS knockout mice (iNOS $^{-/-}$ ) are resistant to endotoxin-induced gut injury and bacterial translocation (Mishima *et al.* 1997). In summary, endotoxin-induced gut mucosal injury seems to be mediated through increased NO-production by activated iNOS. In the light of this conclusion an extra arginine load provided by artificial nutrition appears to be of questionable benefit and might even be harmful.

#### **Defining the optimal NO availability related to the clinical setting**

From a clinical perspective, the NO-mediated immunomodulating effect of arginine has two different outcomes, largely a function of the existing patho-physiological circumstances (Fig. 7). Based on improvements in immune function and microcirculation, supplemental administration



**Fig. 7.** Schematic representation of the consequences of an unbalanced nitric oxide availability derived from arginine.

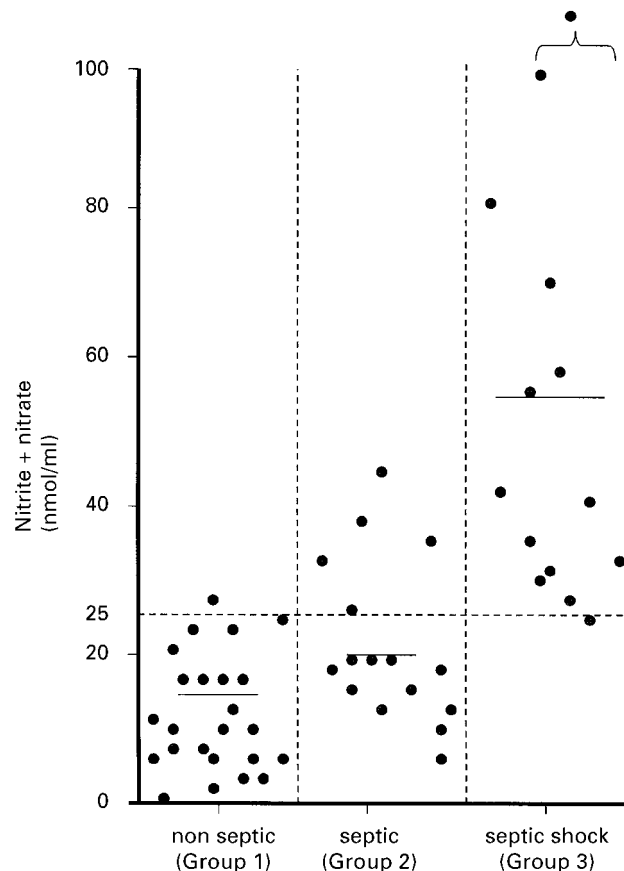
of arginine can possibly lead to a reduction in infectious morbidity if a systemic induction of the inducible NO-synthase is absent. However, exogenous supply of arginine may result in excessive production of NO, if cytokine-induced activation of the inducible enzyme system is present. In such an event we cannot — from our present perspective — rule out an exacerbation of the clinical appearance of SIRS or sepsis by the supplemental use of arginine, including hemodynamic instability and immunologically, or metabolically provoked cellular damage. At present, it is not possible to assess optimal NO availability for the individual patient as no suitable tools for evaluation exist.

#### NO-formation and -inhibition in the experimental and clinical setting

A variety of clinical studies have shown that adults, children and even neonates with the sepsis syndrome have an increased serum concentration of nitrite and nitrate (Doughty *et al.* 1998; Shi *et al.* 1993; Wera *et al.* 1997). As shown in Fig. 8 increased plasma nitrite and nitrate concentrations were found particularly in patients with septic shock (Gomez-Jimenez *et al.* 1995). These concentrations correlated directly with endotoxin concentration and cardiac output, and inversely with systolic blood pressure. More recently a study of eighty polytrauma patients documented that the plasma concentrations of NO increased as the magnitude of the septic response increased (Rixen *et al.* 1997). Because prolonged exposure of cells to large amounts of NO may cause cellular damage (Salzman, 1995), inhibit cellular respiration (Stadler *et al.* 1994), cause maldistribution of regional blood flow (Thiermermann, 1994), increase gut permeability (Salzman, 1995), and result in the increased production of the oxidant peroxynitrite (Beckman *et al.* 1990), there are many ways that an increase in NO-production could adversely affect outcome.

Based on these findings the potential relationship

between NO-production and infection or septic states has been investigated in the clinical (Petros *et al.* 1994; Schilling *et al.* 1993) and experimental (Szabo, 1995) setting using a number of drugs that inhibit NOS-activity.



**Fig. 8.** Plasma nitrate plus nitrite levels in different groups of critically ill patients;  $P < 0.001$  v. group 1 and 2. Redrawn from Gomez-Jimenez *et al.* 1995.



Although protective effects of the inhibition of NO-production were revealed (Ruetten *et al.* 1996), it was also shown that inhibition of NOS-activity may not be protective (Evans *et al.* 1994), or even detrimental (Park *et al.* 1996). The reasons for these conflicting experimental observations appear to be based on the use of different NOS-inhibitors, some of which have differential effects on cNOS and iNOS. The selective action of the drug, however, is of importance because the inhibition of cNOS is potentially deleterious, but inhibition of iNOS may be beneficial in models of circulatory shock, sepsis, or endotoxemia (Salzman, 1995; Schmidt *et al.* 1995). If it is confirmed by future trials that selective inhibition of iNOS activity might be of benefit in the severe septic patient, the concept of providing arginine, as the precursor of NO formation, in pharmacological dosages has to be reconsidered in that particular clinical setting. However, because NO synthase, NO mediated immunofactors, and intracellular arginase are restricted to distinct compartments according to current knowledge, this kind of reasoning gave rise to the assumption that supplemental arginine may not affect extracellular NO concentration (Moncada *et al.* 1991). Nevertheless, we have to bear in mind that this important regulatory measure of the cellular NO generation might not be operative in critically ill patients.

### Concluding remarks

Currently available enteral nutrition solutions with an immune-modulating effect are first-generation products. Their multi-nutrient composition mainly rests on evidence derived from experimental models rather than from clinical evaluation of the proposed actions of the single nutrients. Future research needs to investigate the immune-modulating effects of the individual substrates in humans as well as their effective mechanisms, in order to develop second-generation formulas based on the improved understanding of 'substrate pharmacology' (Zaloga, 1998). Indeed the modulating effect of selected nutritional substrates on immune response parameters should be regarded as insufficient evidence for clinical adoption if used as an exclusive proof of benefit. Given the potential for harm, it must be demonstrated that these substances have positive effects on clinically important end-points (infection frequency, ventilation period, length of ICU and hospital stay, and mortality).

Considering the available data evaluating enteral immuno-enhancing formulas, it certainly can be argued that significant improvements in most of the outcome variables can be demonstrated in surgical patients. However, patients with most severe appearances of SIRS, sepsis and organ failure show a rather disadvantageous outcome. Based on the evidence provided, we put forward the hypothesis that supplemental arginine, at least partly, may be responsible for these undesirable findings as it may aggravate an already ongoing systemic inflammation. Although the rationale for the concept of 'immunonutrition' is based upon the targeted supply of key nutrients as they become depleted due to their extensive consumption, we have to bear in mind that the availability of some substrates might be deliberately kept at low levels since high

concentrations can be harmful in certain circumstances. Thus, not any lowered availability of a substrate has to be replenished because adequate substrate levels have to be defined in accordance with the underlying pathophysiology. Referring to 'normal values' can be misleading if patients with severe SIRS and sepsis have to be treated. Moreover, in comparison to other immune-modulating substrates such as glutamine, we believe that arginine has been insufficiently investigated as yet with regard to its significance in inflammatory events. While no adverse effects (experimentally and clinically) have been reported for glutamine so far, both a benefit as well as a reduction in survival rates has been reported for arginine in experimentally-induced sepsis (Gonce *et al.* 1990; Heyland *et al.* 1994). Therefore, immune-modulating interventions which presently may include high extra loads of arginine should only be undertaken with care and under controlled study conditions if administered to patients with complex immune-pathological conditions like *severe SIRS, sepsis, or organ failure*.

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