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REducing Deaths due to OXidative Stress (The REDOXS© Study): rationale and study design for a randomized trial of glutamine and antioxidant supplementation in critically-ill patients

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Critically-ill patients experience an extent of hyperinflammation, cellular immune dysfunction, oxidative stress and mitochondrial dysfunction. Supplementation with key nutrients, such as glutamine and antioxidants, is most likely to have a favourable effect on these physiological derangements, leading to an improvement in clinical outcomes. The results of two meta-analyses suggest that glutamine and antioxidants may be associated with improved survival. The purpose of the present paper is to report the background rationale and study protocol for the evaluation of the effect of high-dose glutamine and antioxidant supplementation on mortality in a large-scale randomized trial in 1200 mechanically-ventilated, critically-ill patients. Patients admitted to an intensive care unit (ICU) with clinical evidence of severe organ dysfunction will be randomized to one of four treatments in a 2×2 factorial design: (1) glutamine; (2) antioxidant therapy; (3) glutamine and antioxidant therapy; (4) placebo. The primary outcome for this study is 28 d mortality. The secondary outcomes are duration of stay in ICU, adjudicated diagnosis of infection, multiple organ dysfunction, duration of mechanical ventilation, length of stay in hospital and health-related quality of life at 3 and 6 months. A novel design feature is the combined use of parenteral and enteral study nutrients dissociated from the nutrition support. The therapeutic strategies tested in the randomized trial may lead to less morbidity and improved survival in critically-ill patients. The trial will be conducted in approximately twenty tertiary-care ICU in Canada and the first results are expected in 2009.

Enteral nutrition: Critical illness: Glutamine: Antioxidants

The relationship between nutrient deficiency and altered immune status has been recognized for years. In critically-ill patients nutrient deficiencies can predispose patients to impaired immune function and higher risk of developing infectious complications, organ dysfunction and death. Consequently, over the last few decades numerous experimental studies have explored the immune-modulating properties of nutrients such as glutamine, arginine, *n*-3 fatty acids and others. Several nutrition formulas supplemented with one or more of these nutrients have been developed and are currently available. 'Immunonutrition', 'immune-enhancing diets' and other terms have been used to describe these products. Unfortunately, these products have been developed without a sound scientific understanding of what effect these nutrients have on clinically-important outcomes in the critical care setting (Heyland, 2002).

The purpose of the research programme has been to identify key nutrients likely to have a positive effect on clinical outcomes in critically-ill patients and determine their clinical efficacy in the context of a large-scale randomized trial. Given that the majority of randomized trials in this area are small and underpowered to detect a difference in mortality or infectious complications, meta-analyses have been used as a tool to determine a more precise estimate of treatment effect. The authors' research

Abbreviations: ICU, intensive care unit; ROS, reactive oxygen species; RR, risk ratio; SF-36, a multipurpose survey of general health status consisting of eight domains and thirty-six items.

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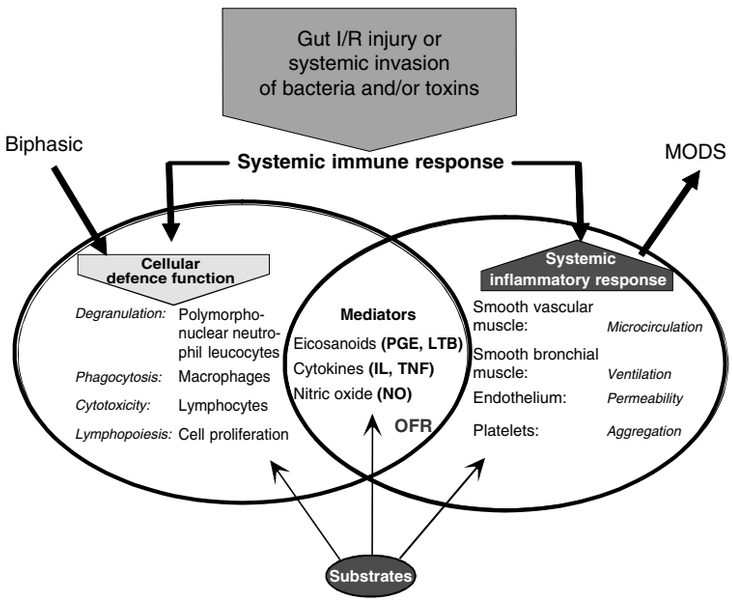

Fig. 1. The relationship between the systemic and cellular immune responses. I/R, ischaemic–reperfusion; PGE, prostaglandin E, LTB, leukotriene B; OFR, oxygen free radicals; MODS, multiple organ dysfunction syndrome.

to date suggests that arginine supplementation in critical illness is associated with more harm than good (Heyland & Samis, 2002) and is not recommended, whereas the data evaluating *n*-3 fatty acids are limited to one industry-sponsored clinical trial in patients with acute lung injury that suggests a potential benefit, leading to inconclusive clinical recommendations (Gadek *et al*. 1999). Of all nutritional interventions tested in critically-ill patients, the largest reduction in mortality that has been observed is associated with glutamine and antioxidant supplementation (for details of the meta-analyses conducted by the authors' group on these interventions and the most recent version of the meta-analyses, see Critical Care Nutrition, 2006). Considerable basic scientific mechanistic research in this area supports the hypothesis that these nutrients may improve the outcomes of critically-ill patients. The purpose of the present paper is to describe the rationale and protocol for a large-scale multicentre randomized clinical trial that will evaluate the effect of both supplemental glutamine and antioxidant strategies in critically-ill patients (REducing Deaths due to OXidative Stress; The REDOXS© Study; ID # NCT00133978).

The scientific basis of immunonutrition

The immune system

The purpose of this section is to give a brief review of the scientific rationale for immune-modulating nutrients. In a very simplistic model the host response to invading micro-organisms can be divided into two arms: (1) cellular defence that includes both innate (non-specific) immunity and adaptive (specific) immunity; (2) the systemic inflammatory response (Fig. 1). The cellular defence function includes all the functions of polymorphonuclear granulocytes, macrophages and lymphocytes as well as their proliferation behaviour (Fig. 1). By contrast, the systemic inflammatory response, which is triggered by immune-competent cells, works mainly at the tissue level (Fig. 1). The systemic inflammatory reaction is characterized by effects of mediators, free radicals and activated immune cells on metabolism, endothelium, platelets and smooth muscle of the vascular and bronchial systems.

Both arms of the immune response are stimulated at an early stage and almost occur in parallel (Fig. 2). Of note, the initiation of the immune response has to be considered as a sequential event, since the development of the systemic inflammatory response follows the activation of the cellular defence function. The severity of the inflammatory deterioration varies in accordance with the magnitude of the infectious, traumatic or ischaemic insult.

Viewed over time, the triggered response of the cellular defence function is a biphasic phenomenon with an initial hyperactive phase, which may overshoot the requisite response, followed by depression of cellular defence function. The special features of stress-induced cytokine release are assumed to make a major contribution to this biphasic response of cellular defence systems. When immune cells encounter microbial by-products after invasion or reactive oxygen species (ROS) from ischaemia–reperfusion injury, these cells become activated. Numerous proinflammatory cytokines, such as TNFα, IL-1β, IL-6 and IL-8, and other mediators including thromboxanes, leukotrienes, platelet-activating factor, prostaglandins, NO and complement are released and serve to augment the cellular immune response. At the same time, these proinflammatory mediators are known to cause effects on tissues other than the immune cells. NO is known to be cytotoxic and oxygen

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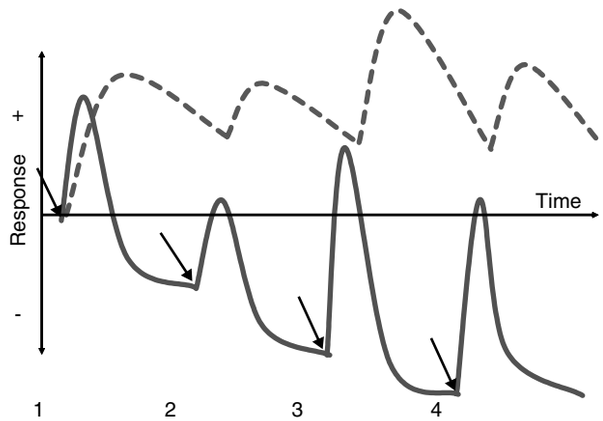

Fig. 2. The relationship between the cellular immune response (——) and the systemic inflammatory response (– – –) to multiple insults (↓). +, Positive; –, negative.

free radicals, including peroxynitrite, may cause harm in tissues and organs whereas leukotrienes may increase vascular permeability (Kolaczkowska *et al*. 2002). Moreover, activated leucocytes migrate from the bloodstream into tissues where they are capable of prompting severe tissue damage.

Following the initial proinflammatory phase, a parallel compensatory anti-inflammatory response ensues, primarily as a result of the release of IL-10, IL-4, prostaglandin E2, soluble TNF receptors and IL-1 receptor antagonists (Bone, 1996). Although the temporal relationship between a proinflammatory and anti-inflammatory cytokine response has not been fully delineated, anti-inflammatory mediators are known to suppress the cellular immune response. The anti-inflammatory response may overwhelm the proinflammatory state, leading to a functional state of depressed immune response. Manifestations of this state include anergy to skin-test antigens, impaired antibody production and diminished phagocytosis, which render patients at increased risk for additional infectious morbidity and mortality (Astiz & Rackow, 1998).

Thus, suppressed immune function of the cellular defence system may prompt a new episode of infection and subsequently may trigger a new peak of the systemic inflammatory response (Fig. 2). Indeed, a similar chain of events could be elicited by a renewed episode of ischaemia and reperfusion. Over time, patients may move repeatedly between cellular defence-activating or -suppressing states. Usually, the overshooting release of proinflammatory cytokines is only short lived, whereas the suppression of the cellular defence function, mediated by anti-inflammatory cytokines and other mediators, persists much longer. The more insults that are encountered, the more pronounced the suppression will be (Fig. 2). In contrast, no biphasic characteristic for the systemic inflammatory response has been reported yet. Although it can wax and wane depending on the severity of the insult, the mode of the insult (shock, injury or infection) and whether additional insults occur, the inflammatory response does not usually reach baseline until the critical illness is resolved (Fig. 2). This characteristic may be related to continuously-enhanced levels of proinflammatory triggers, such as NO, peroxynitrite and other free radicals, as well as eicosanoids and other lipid mediators. Thus, the potential exists for a patient to present with manifestations of systemic inflammation, and also to suffer from depression or hyporesponsiveness of the cellular immune function at the same time.

Special role of the gastrointestinal tract, regional ischaemia–reperfusion injury and reactive oxygen species

Impairment of the gastrointestinal tract plays a central role in the pathogenesis of infection and sepsis, and even the failure of other distant organs. As summarized by Deitch (2001), the gastrointestinal tract is one of the first organs exposed to shock and the last to be resuscitated if circulatory failure arises. Previously, the focus of gastrointestinal tract dysfunction has centred on the concept of bacterial translocation, which has not been well documented. Recent studies suggest that ischaemia–reperfusion of the gastrointestinal tract may play an important role in the initiation and perpetuation of organ dysfunction. Numerous observations in haemorrhagic shock, trauma and burns suggest that regional ischaemia–reperfusion injury to the gastrointestinal tract has to be considered a predominant region of ROS formation, mediator generation and leucocyte priming (Deitch, 2001). Moreover, recent animal studies suggest that these gastrointestinal tract-derived factors may reach the systemic circulation via the lymphatic duct rather than the portal–hepatic system and thereby cause distant organ injury (Deitch, 2001). These gastrointestinal tract-derived factors not only contribute to distant organ failure but also to infection, since they contribute to the suppression of the cellular defence function.

Given the whole range of factors impacting on the stress-induced immune response, ROS are assumed to play a key role in the underlying pathophysiology. When O_2 availability is limited in tissue of vital organs by hypoperfusion, the cells shift from aerobic to anaerobic metabolism, thereby lowering the cellular energy charge. As a result, increased ATP hydrolysis, a subsequent increase in AMP levels and finally an accumulation of the purine metabolites are found in ischaemic tissues. At the same time xanthine dehydrogenase is converted to xanthine oxidase, either by reversible oxidation or irreversible proteolytic degradation. During reperfusion, as O_2 is reintroduced, rapid oxidation of purines producing urate and superoxide radicals can develop. This superoxide can then secondarily generate the highly-toxic hydroxyl radical, again facilitated via an Fe-catalysed reaction. Reperfusion of ischaemic tissues can further generate ROS, mainly by the activity of the cellular xanthine oxidase. Furthermore, during activation of the immune response neutrophils, macrophages and other immune-competent cells may activate a plasma membrane-associated NADPH oxidase system capable of oxidizing NADPH to NAD^+, leading to further generation of superoxide radicals. Spontaneous dismutation of the superoxide radical yields H_2O_2 and O_2 at physiological pH.

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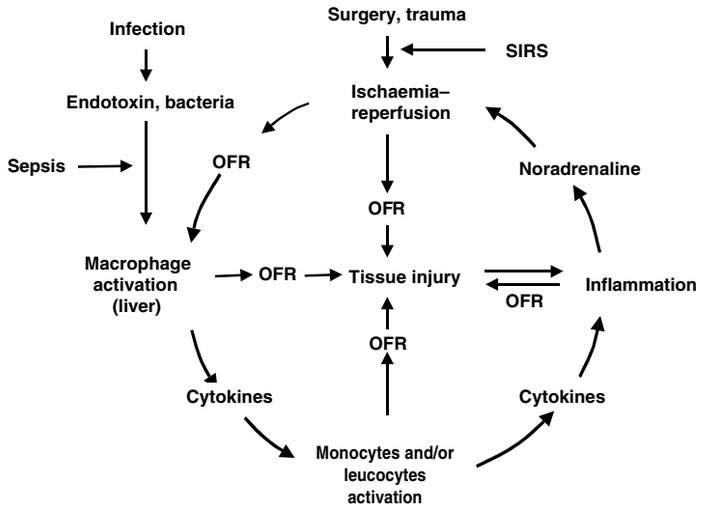

Fig. 3. The vicious cycle of oxygen free radical (OFR) production in critically-ill patients. SIRS, systemic inflammatory response syndrome.

ROS not only lead to direct damage of cellular components but also trigger the release of cytokines that further activate the inflammatory cascades (Grimble, 1994). Free radicals activate resident macrophages or Kupffer cells, which release inflammatory cytokines (TNF, IL-1, IL-6; Vendemiale *et al*. 1999). These proinflammatory mediators, in turn, elicit activation and influx of inflammatory cells (monocytes and leucocytes) into tissues and organs, and may directly cause mitochondrial dysfunction leading to further ischaemia and tissue injury. Furthermore, the activated Kupffer cells also produce large amounts of oxygen free radicals, whereby a vicious cycle of inflammation, cellular activation and ROS generation is created (Fig. 3).

A recent study in critically-ill patients demonstrates a relationship between increased inflammatory mediators, antioxidant depletion, reduced respiratory chain complex I activity in the mitochondria and low cellular ATP levels (Brealey *et al*. 2002). These observations correlate with severity of disease and clinical outcome, and support the notion that mitochondrial dysfunction resulting from bioenergetic failure may be an important factor in the underlying pathophysiology in critically-ill patients with a systemic inflammatory response and multi-organ dysfunction (Brealey *et al*. 2002).

What are the implications for clinical use of immunonutrition?

'Metabolic resuscitation' of the gastrointestinal tract by providing adequate nutrition, in general, and defined immuno-modulating substrates specifically, to maintain gastrointestinal tract barrier integrity and function and to reduce regional oxidative stress, will have to be considered key therapeutic strategies. However, within a given patient over time or across different patient populations the severity of 'gastrointestinal tract failure', the amount of bacterial translocation, the extent of cellular immune dysfunction, the balance of inflammation–anti-inflammation and the regional and systemic generation of ROS will vary. Despite the cause of the initial perturbation, the mechanism of tissue injury is most probably related to the extent of cytokine activation and ROS formation. Thus, the treatment effect of various substrates or nutrients will vary depending on the underlying pathophysiology of the host and whether the substrate influences cellular immune function and/or the synthesis of inflammatory mediators and/or the generation of ROS. A minimum level of key nutrients (glutamine, arginine and *n*-3 fatty acids) is required for immunocompetence. However, particularly in the case of arginine via excessive NO production, this nutrient may have adverse effects on patients with hyper-inflammation (Heyland & Samis, 2002). Excessive amounts of *n*-3 fatty acids may have immunodepressant effects via eicosanoid synthesis (Thies *et al*. 2001).

Given this heterogenous and variable treatment response, the clinical trials of immunonutrition in patients undergoing surgery (or in patients with AIDS, obesity, etc.) cannot be examined and the results generalized to other populations, such as critically-ill patients. Generally, patients undergoing elective surgery experience minimal activation of cytokines and some extent of suppression of the cellular defence function following surgical stress, putting them at higher risk for acquired infectious morbidity and mortality. It follows that nutrients that stimulate the cellular defence system may reduce infectious complications in the patient undergoing elective surgery. In contrast, the associated changes to the systemic inflammatory response accompanying critical illness are far more intense, complex, variable and less-well defined, but best characterized by an over-amplified inflammatory response, probably as a result of an excess of NO and ROS and excessive availability of lipid mediators. Thus, nutrients that further stimulate the systemic inflammatory response may be deleterious in critically-ill patients. In fact, novel therapies that have been shown to be effective in the early

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phase of critical illness decrease the inflammatory response associated with critical illness, not stimulate it (Bernard *et al*. 2001). What is emerging in the critical care literature is the notion of hyperinflammation and cellular immune dysfunction coexisting in the same patient or patient population at the same time. Hence, for critically-ill patients nutrients that augment cellular defence (specific and non-specific immune function), ameliorate ROS and support mitochondrial function without a collateral increase in the inflammatory response are most likely to be beneficial. Review of the literature indicates that glutamine and antioxidant strategies are most likely to be beneficial to critically-ill patients with hyperinflammation, cellular immune dysfunction, oxidative stress and mitochondrial dysfunction.

Glutamine: scientific rationale and review of the literature

The amino acid glutamine plays a central role in N transport within the body, is a fuel for rapidly-dividing cells (particularly lymphocytes and enterocytes), is a precursor to glutathione and has many other essential metabolic functions. Under normal physiological conditions glutamine is synthesized in sufficient amounts by the skeletal muscle and therefore is considered non-essential. It has been hypothesized that glutamine may become a conditionally essential amino acid in patients with catabolic disease, as studies have shown that glutamine levels drop following major surgery (Parry-Billings *et al*. 1992; Blomqvist *et al*. 1995) and during critical illness (Parry-Billings *et al*. 1990; Planas *et al*. 1993). Lower levels of glutamine have been associated with immune dysfunction (Oehler *et al*. 2002) and increased mortality (Roth *et al*. 1982).

As a preferred substrate for enterocytes, glutamine has been shown to support the normal immunological structure and function of the gastrointestinal tract. In animal studies glutamine deprivation is associated with loss of intestinal epithelial integrity (Potsic *et al*. 2002), while glutamine supplementation decreases gastrointestinal tract mucosal atrophy during total parenteral nutrition (Ardawi, 1992; Platell *et al*. 1993; Khan *et al*. 1999), preserves both intestinal and extra-intestinal IgA levels (Kudsk *et al*. 2000), prevents lymphocyte and glutathione depletion in the Peyer's patches (Manhart *et al*. 2001) and does not increase NO production induced by proinflammatory cytokines (Marion *et al*. 2003). However, in relation to bacterial translocation in animal models studies of parenteral or enteral glutamine-supplemented formulas show mixed results. Some studies have shown decreased translocation (Zapata-Sirvent *et al*. 1994; Gianotti *et al*. 1995) while others have demonstrated no such effect (Barber *et al*. 1990; Bark *et al*. 1994). Other studies have demonstrated that glutamine administration in animals can protect against septic shock following endotoxaemia. This protection may be mediated via enhanced tissue heat-shock protein expression (Wischmeyer *et al*. 2001*a*; Coffier *et al*. 2002) and/or attenuated proinflammatory cytokine release (Wischmeyer *et al*. 2001*b*). Tissue levels of ATP and ADP are commonly depleted during shock and may lead to cell death or apoptosis. In shock and myocardial injury reperfusion models glutamine supplementation preserves glutathione levels, ATP:ADP and NAD:NADH content, and reduces lactate accumulation (Dhar *et al*. 2003; Wischmeyer *et al*. 2003; Singleton *et al*. 2005). Regardless of the mechanism, several animal studies have demonstrated improved survival associated with glutamine supplementation in models of sepsis (Ardawi, 1991; Inoue *et al*. 1993; Suzuki *et al*. 1993; Naka *et al*. 1996).

A meta-analysis of trials of glutamine supplementation in critically-ill patients has been undertaken, as several studies have been conducted since the original meta-analysis was published (Novak *et al*. 2002). When the results of all these trials are combined a significant reduction in mortality (risk ratio (RR) 0·75 (95% CI 0·59, 0·96); $P = 0{\cdot}02$), infectious complications (RR 0·79 (95% CI 0·63, 0·98); $P = 0{\cdot}04$) and length of stay (d) in the intensive care unit (ICU; weighted mean difference $-4{\cdot}50$ (95% CI $-8{\cdot}28$, $-0{\cdot}72$); $P = 0{\cdot}02$) in critically-ill patients is observed (Critical Care Nutrition, 2006). In summary, the recent review of clinical studies suggests that glutamine supplementation is safe and may be associated with a reduction in mortality in critically-ill patients. However, there are several reasons why the overall results should be viewed as hypothesis generating rather than hypothesis confirming. First, relative to other meta-analyses the review contains few trials with even fewer observed clinical end points. Second, while an attempt has been made to obtain results generated from an intention-to-treat analysis, this approach is not possible in the majority of the cases. For the aforementioned reasons, the results of the meta-analysis are believed to be unstable, and they require confirmation or refutation. However, given that the upper 95% CI around the effect on mortality is $<1{\cdot}00$, it can be concluded with reasonable confidence that glutamine supplementation is safe (excludes harm). Thus, the proposal is to move forward and rigorously test this hypothesis that glutamine supplementation improves the survival of critically-ill patients.

Antioxidant supplementation: scientific rationale and review of the literature

Increasingly, oxidative stress is being recognized as central to the underlying pathophysiology of critical illness, particularly the development of organ failure. ROS and reactive nitrogen–oxygen species have clearly identified roles in modulating cell signalling, proliferation, apoptosis and protection. However, ROS and reactive nitrogen–oxygen species are also capable of attacking proteins, polysaccharides, nucleic acids and PUFA, resulting in cellular damage and mitochondrial dysfunction (Lovat & Preiser, 2003). In man there is a complex endogenous defence system designed to protect tissues from ROS–reactive nitrogen–oxygen species-induced cell injury. Special enzymes such as superoxide dismutase, catalase and glutathione peroxidase (including their cofactors Se, Zn, Mn and Fe), SH group donors (i.e. glutathione) and their

precursors (i.e. glutamine) and vitamins (i.e. vitamins E and C, and β-carotene) form a network of functionally overlapping defence mechanisms. In critically-ill patients there are reduced stores of antioxidants, reduced plasma or intracellular concentrations of free electron scavengers or cofactors and decreased activities of enzymic systems involved in the detoxification of ROS (Metnitz *et al*. 1999; Therond *et al*. 2000). The more severe the insult, the larger the depletion of antioxidants appears to be (Alonso de Vega *et al*. 2002; Motoyama *et al*. 2003). These described observations are not mere epiphenomena, as low endogenous stores of antioxidants are associated with an increase in free radical generation, an augmentation of the systemic inflammatory response, subsequent cell injury, increased morbidity and even higher mortality in the critically-ill patient (Goode *et al*. 1995; Quasim *et al*. 2003).

A systematic review of trials has recently been conducted to examine whether supplementing critically-ill patients with antioxidant nutrients will improve their survival (Heyland *et al*. 2005). The studies that were included were those that were randomized, reported on clinically-important end points in critically-ill patients and compared various trace elements and vitamins to placebo. Eleven articles that met the inclusion criteria were identified, most of which studied the effects of Se either alone or in combination with other trace elements and vitamins, while others looked at the effects of Zn and vitamins A, C and E. When the results of all the trials were aggregated, overall antioxidants were found to be associated with a significant reduction in mortality (RR 0·65 (95% CI 0·53, 0·80); $P<0·0001$) but no effect on infectious complications (RR 0·90 (95% CI 0·65, 1·24); $P = 0·51$). In further hypotheses-generating subgroup analyses (Heyland *et al*. 2005) it was observed that Se supplementation may be associated with a significant reduction in mortality (RR 0·59 (95% CI 0·32, 1·08); $P = 0·09$) while non-Se antioxidants have no effect on mortality (RR 0·73 (95% CI 0·41, 1·29); $P = 0·3$). Using the median dose of Se provided in each study as the cut-off, studies that provided a higher dose (500–1000 μg/d) of Se were found to be associated with a greater treatment effect (RR 0·52 (95% CI 0·24, 1·14); $P = 0·10$) than studies that used lower doses (159–380 μg/d; RR 1·47 (95% CI 0·20, 10·78); $P = 0·7$).

Se may be the cornerstone of the antioxidant defence system in acute conditions. The majority of patients with sepsis or shock have low plasma Se levels that correlate inversely with the severity of systemic inflammatory response syndrome and subsequent outcome (Forceville *et al*. 1998). Patients with a low plasma Se level are three times more likely to die compared with those with a higher plasma level (Forceville *et al*. 1998). Supplementing with Se may improve clinical outcomes as Se is an essential cofactor in glutathione enzymic function and has favourable effects on cellular immune function (Rayman, 2000). However, the results cannot be extrapolated to say that other trace elements and vitamins are not of value. There were too few studies to confirm this point and it requires further investigation. Furthermore, Se, glutathione, vitamin E and vitamin C may function synergistically to regenerate both water-soluble and fat-soluble antioxidants (Kelly, 1994). Thus, provision of a combination of supplemental antioxidant micronutrients (i.e. a multimodal approach) at an early stage in the course of acute disease may be superior to single micronutrients and may improve clinical outcome. Moreover, administration of single antioxidants may introduce disturbances in the entire system of overlapping antioxidant defences, as antioxidants may turn into pro-oxidants if auxiliary systems for radical scavenging are missing (Kelly, 1994). For example, ascorbate recycles the tocopheryl radical to tocopherol. Thus, ascorbate serves as a biochemical link between the Se–glutathione peroxidase system and vitamin E (Burk & Hill, 1999). To generate sufficient intracellular glutamate to ensure adequate amounts of glutathione, sufficient amounts of glutamine need to be provided as well (Manhart *et al*. 2001; Flaring *et al*. 2003). Considering all these implications, it is postulated that combinations of antioxidants would provide a larger treatment effect than single micronutrient strategies.

In summary, it appears that antioxidants, particularly parenteral Se at high doses, may be associated with a significant reduction in mortality in critically-ill patients. As with the glutamine meta-analysis, the results are incompatible with harm, yet do not prove benefit; the results of this meta-analysis are more hypothesis generating than hypothesis confirming. The biological rationale and clinical trial data that have been systematically reviewed clearly justify moving forward with this large randomized controlled trial.

Lessons learned from previous studies of immunonutrition

Patient population

One of the main reasons for the failure of some previous individual studies of specialized nutrients to demonstrate a treatment effect is that the studies targetted the wrong patient population. The ability to demonstrate a treatment effect probably correlates with severity of illness and nutrient deficiency. Oudemans-van Straaten *et al*. (2001) have demonstrated that the presence of shock and older age correlates strongly with low plasma glutamine levels and a higher mortality. Other studies suggest a relationship between low glutamine levels, malnutrition and poor patient outcomes (Van Der Hulst *et al*. 1994; Sacks, 1999). The majority of patients with sepsis or shock have low plasma Se levels that correlate inversely with the severity of systemic inflammatory response syndrome and the subsequent outcome (Forceville *et al*. 1998). Patients with a low plasma Se level are three times more likely to develop ventilator-associated pneumonia, multi-organ failure or to die compared with those with a higher plasma level (Forceville *et al*. 1998). Furthermore, a small randomized trial of Se supplementation in patients with severe systemic inflammatory response syndrome or sepsis suggests that the most critically-ill patients may derive the greatest benefit (Angstwurm *et al*. 1999). While the overall mortality is not statistically significant between groups (seven of twenty-one with Se *v*. eleven of twenty-one without Se), in a *post hoc* analysis of patients with an APACHE II score

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(Knaus *et al*. 1985) >20 Se supplementation was found to be associated with a significant reduction in mortality (four of eleven *v*. eight of nine, $P = 0{\cdot}05$; Angstwurm *et al*. 1999). One potential reason why the largest study of enteral glutamine has failed to show a treatment effect could be that the patients were not sick enough (average APACHE II score of 14 and average clinician's probability of survival 90%; Hall *et al*. 2003). Thus, to optimally design a clinical trial to demonstrate the treatment effect of glutamine and Se, it is necessary to enrol patients who are seriously ill (as evidenced by the presence of shock and other organ failure) and malnourished.

Route of delivery

Previous randomized trials that have failed to demonstrate a treatment effect may relate to route of delivery of the key nutrients. A number of the large randomized trials have provided the key nutrients enterally and have demonstrated no treatment effect, while the meta-analyses suggest that the larger treatment effect is associated with parenteral delivery of glutamine and Se (Novak *et al*. 2002; Heyland *et al*. 2005). However, given the major role of the gastrointestinal tract as a source of cytokine and leucocyte activation and ROS formation, the provision of the key nutrients directly to the lumen of the gastrointestinal tract makes biological sense. Furthermore, studies of glutamine supplementation in patients with burns support the concept that enteral glutamine has a positive treatment effect; enteral glutamine increases plasma glutamine levels, improves permeability, decreases endotoxin levels, reduces infections secondary to Gram-negative infections, reduces hospital stay and reduces mortality (Garrel *et al*. 2003; Zhou *et al*. 2003). The purpose of the proposed study is not to determine whether there is a different treatment effect between enteral or parenteral provision of these key nutrients. Rather, given the cost and complexity of the study, the intervention has been designed to use both parenteral and enteral provision of nutrients to maximize the opportunity of demonstrating a treatment effect, if one truly exists. If such an effect is observed, additional studies could be performed to determine whether the benefit is derived from the parenteral or enteral components, or partially from both.

Dose of nutrients

An additional reason why some previous randomized trials have failed to demonstrate a treatment effect may relate to inadequate dosing. When provided enterally and combined with the enteral nutrition product, given that these sick patients may have trouble tolerating their enteral feeds, reducing the intake of enteral feeds limits the intake of these key nutrients. For the purposes of the proposed trial the provision of these key nutrients has been dissociated from the provision of enteral (or parenteral) nutrition. This approach is unique and is believed to represent a major conceptual advance in the design of studies in this area.

The results of the glutamine and antioxidant meta-analysis suggest that a higher dose of glutamine and Se given parenterally is associated with a greater treatment effect (Novak *et al*. 2002; Heyland *et al*. 2005). There are several difficulties in providing a high amount of free glutamine to critically-ill patients because of problems with limited solubility and stability, especially in patients with volume-restricted conditions. Nevertheless, recent advances in glutamine delivery have overcome some of these challenges, making the provision of bioavailable glutamine practical, even at higher doses.

The upper limit or maximal tolerable dose of Se is unknown. The Se doses used in the trials with beneficial mortality effects have been moderate (300–1000 μg/d) and delivered for limited periods of time. These quantities correspond to five to twenty times the recommended parenteral nutrition intakes. Recommended or standard doses of these micronutrients are based on requirements and metabolism in healthy subjects and have little meaning in critically-ill patients. At high doses vitamin C, vitamin E and Se have been shown to have some pro-oxidant properties (Spallholz, 1997; Abuja, 1998); thus, more is not necessarily better. If correction of an altered circulating antioxidant status is the target, it is probably achieved with moderately-high doses in the critically-ill population, but more research is needed to determine the optimal dose, particularly when given in combination with glutamine.

Thus, in order to optimally design a clinical trial to demonstrate whether a treatment effect of glutamine exists, it is necessary to provide the highest possible dose. However, before embarking on a multicentre trial of high-dose glutamine and antioxidant supplementation, a dose-escalating study has been performed to determine the maximal tolerable dose of glutamine and Se, particularly when used in combination.

Summary of rationale

The presence of underlying critical illness results in perturbances of gastrointestinal structure and function and immunological competence, a reduction in antioxidant capacity and impairments to mitochondrial function. Careful systematic review of the literature suggests that supplementing critically-ill patients with high-dose glutamine and antioxidants provided both enterally and parenterally may have a large positive effect on survival. Given the inherent weaknesses in making clinical inferences from these meta-analyses, a large randomized trial is warranted to determine whether or not supplementing these key nutrients truly offers a survival advantage to this critically-ill patient population.

Research questions

In critically-ill patients with severe organ dysfunction, what is the effect of glutamine supplementation compared with placebo on 28 d mortality?

In critically-ill patients with severe organ dysfunction, what is the effect of antioxidant supplementation compared with placebo on 28 d mortality?

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Design architecture

Study design: a randomized controlled trial

The proposed study is a multicentre prospective double-blind randomized trial of 1200 critically-ill patients with clinical evidence of organ failure from their acute illness. Patients will be randomized to one of the four treatments within the 2×2 factorial design: glutamine; antioxidant therapy; glutamine and antioxidant therapy; placebo. Factorial designs may be considered when it is possible to give the treatments together without modification (i.e. they do not interfere with each other or potentiate each other's treatment effect or toxicity). There are no randomized trials comparing the effect of glutamine and antioxidants alone or together; although there may be some theoretical rationale for why an interaction may exist, there is no clinical evidence to support this assertion. A factorial design will enhance the efficiency of the study, allowing two questions to be answered in one large trial and the benefit of combination therapy (glutamine and antioxidants compared with placebo) to be explored. Patients will be stratified according to centre and presence of cardiovascular dysfunction (given that shock is associated with the lowest plasma glutamine levels and higher mortality rate than other risk factors; Oudemans-van Straaten *et al.* 2001).

Patient population

Mechanically-ventilated adult patients (≥18 years old) admitted to ICU will be considered eligible if they have two or more of the following organ failures related to their acute illness:

an arterial O_2 partial pressure : inspired O_2 concentration of ≤300;
clinical evidence of hypoperfusion defined as the need for vasopressor agents (noradrenaline, adrenaline, vasopressin, ≥5 μg dopamine/kg body weight per min or ≥50 μg phenylephrine/min) for ≥2 h;
in patients without known renal disease, renal dysfunction defined as a serum creatinine ≥171 μmol/l or a urine output of <500 ml in the previous 24 h (or 80 ml in the previous 4 h if a 24 h period of observation is not available). In patients with acute or chronic renal failure (pre-dialysis), an absolute increase of ≥80 μmol/l from baseline or pre-admission creatinine or a urine output of <500 ml in the previous 24 h (or 80 ml in the previous 4 h) will be required;
a platelet count of $\leq 50 \times 10^9$/l.

Patients who meet one or more of the following criteria will be excluded:

>24 h from admission to ICU;
patients who are moribund (not expected to be in ICU for >48 h because of imminent death);
a lack of commitment to full aggressive care (anticipated withholding or withdrawing treatments in the first week);
absolute contraindication to enteral nutrients (e.g. gastrointestinal perforation, obstruction or no gastrointestinal tract access for any reason);
patients with severe acquired brain injury: (a) significant head trauma (defined as an injury, in the opinion of the investigator, that represents a severe, disabling or fatal brain injury); (b) grade 4 or 5 subarachnoid haemorrhage; (c) stroke resulting in coma and intubation; (d) post-cardiac arrest with suspected significant anoxic brain injury;
seizure disorder requiring anticonvulsant medication;
cirrhosis, Child's class C (Child & Turcotte, 1964) liver disease;
metastatic cancer or stage IV lymphoma with life expectancy <6 months;
routine elective cardiac surgery (patients with complicated peri-operative course requiring pressors, intra-aortic balloon pump, ventricular assist devices can be included);
patients with primary admission diagnosis of burns (>30% body surface area);
weight <50 kg or >200 kg;
pregnant patients or lactating with the intent to breast-feed;
previous randomization in the proposed study;
enrolment in a related ICU interventional study.

These criteria are designed to include those sick patients who are likely to benefit from the therapeutic intervention to be tested in the proposed study. For the nutrients to have a beneficial effect they have to be delivered as soon after the injury as possible. Patients who only receive a few days of the study nutrients are not likely to derive any treatment benefit. The criteria defining organ failure are according to standard definitions (Moreno *et al.* 1999) and are similar to those used in a recent study of patients with severe sepsis (Bernard *et al.* 2001). In the proposed study the provision of enteral nutrition to these critically-ill potentially-hypoperfused patients will not be attempted early in their course but key enteral nutrients will be provided to ameliorate their metabolic stress. There is supportive evidence from observational studies that enteral solutions can have a favourable effect on haemodynamics, and that they are absorbed and utilized in patients with circulatory compromise (Revelly *et al.* 2001).

Patients not likely to benefit from the intervention (patients not likely to survive beyond 48 h, patients already in shock for >24 h, patients with significant end-stage disease and patients with significant brain injury) and patients with no gastrointestinal tract access will be excluded. The amount of glutamine provided may be excessive in small malnourished patients or patients with cirrhosis, so they will also be excluded (Oppong *et al.* 1997). Patients with renal dysfunction will be enrolled, as they are likely to benefit from the study nutrients, and guidelines will be provided to manage the disproportionate elevation in serum urea in patients receiving study solutions pre-dialytic.

Description of experimental manoeuvre

Random allocation

A centralized randomization system at the Kingston General Hospital Clinical Evaluation Research Unit will be

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used to allocate patients to study treatments, stratifying by site and presence of shock. Allocation will be random and concealed, and will be blinded to everyone except the pharmacist at each site, who will be responsible for preparing study samples and delivering them to the ICU in a blinded fashion. Variable (four to twelve) undisclosed block randomization will be used at each centre to avoid imbalance in the number of subjects assigned in each group and in each strata. Study participants will be unaware of block size.

Intervention

Glutamine supplementation. Patients randomized to receive glutamine supplementation will receive parenterally 0·35 g glutamine/kg body weight per d (provided as 0·50 g dipeptide alanyl-glutamine (Dipeptiven®; Fresenius Kabi, Bad Homburg, Germany)/kg body weight per d) or respective placebo solution. Dipeptiven® is a solution (20 %, w/v) of the glutamine-containing dipeptide, N(2)–L-alanyl-L-glutamine and has been registered in Europe and several non-European countries since 1995. In order to reach the higher optimal dose of glutamine supplementation, as determined in a recently-completed dosing study (DK Heyland, R Dhaliwal, A Day, J Drover, H Cote and P Wischmeyer, unpublished results), an additional 30 g glutamine (as alanyl-glutamine and glycine-glutamine dipeptides)/d delivered enterally or the respective placebo solution will be provided.

Antioxidant supplementation. In addition, at the time of enrolment into the study patients will be randomized to receive an antioxidant cocktail or placebo. Patients will receive 500 μg Se parenterally and the following vitamins and minerals administered enterally (mg): Se, 300 μg; Zn, 20; β-carotene, 10; vitamin E, 500; vitamin C, 1500; or a placebo. As previously outlined, the provision of a combination of endogenous antioxidant micronutrients that includes high-dose Se at an early stage in the course of acute disease may be safer and more efficacious than monotherapy alone. Furthermore, recent studies have confirmed the safety and tolerability of enteral antioxidants at the doses being provided (in combination with glutamine; Senkal *et al*. 2004; Schroeer *et al*. 2005).

Patients in each of the study groups will receive both an enteral and parenteral component to the intervention. All study solutions will be provided continuously over a 20–24 h period. To minimize the complexity and workload around the interventions a parenteral and an enteral component will be prepared for administration to patients in each group. For example, patients randomized to receive both glutamine and antioxidants will receive an intravenous bag of glutamine and Se premixed in saline (9 g NaCl/l) at the local pharmacy and a ready-made blinded enteral solution that contains both glutamine and antioxidants.

It is proposed to use a saline placebo for both enteral and parenteral nutrients. To maintain blinding the active and placebo ingredients will be identical in volume, colour, smell and consistency. The use of a non-isonitrogenous placebo is justified because there are no studies documenting that a difference in a few grams of protein or N will impact on the survival of critically-ill patients. If the nutrients tested in the proposed study have a beneficial effect on mortality, it is not plausible that it can be attributed to differences in protein administration. In addition, amino acids are not inert substances, and using other amino acids to balance the protein dose associated with glutamine administration may interfere with mechanisms of action and clinical outcomes. Finally, there is sufficient background rationale to argue that the mechanisms of action are not related to protein metabolism.

Given that the sooner study medications are started, the more likely it will be that they will have a treatment effect, all else being equal, the proposal is to identify, consent and initiate study procedures within 24 h of admission to ICU. Clearly, resuscitative strategies will take priority, but the parenteral nutrients will begin as soon as possible once intravenous access is available. The parenteral nutrients will continue until death, discharge from ICU or for a maximum of 28 d. The enteral nutrients will begin once the patient is resuscitated (adequate volume status and on stable or decreasing doses of vasopressors) and there is a nasogastric tube or feeding tube in place. Unless there is an absolute contraindication to enteral nutrients (intestinal perforation, bowel obstruction, etc.), the enteral study solution will continue throughout the duration of stay in the ICU and will be stopped when the nasogastric tube or feeding tube is permanently removed or when the patient dies, is transferred to another hospital or discharged from ICU, for a maximum of 28 d. Both the parenteral and enteral nutrients will be continued for a minimum of 5 d even if the patient is transferred outside the ICU but within the hospital. Independent of study nutrients, all patients will be fed according to the Canadian clinical practice guidelines for nutrition support (Heyland *et al*. 2003*a*).

Cointerventions

In order to minimize the influence of other treatments provided to critically-ill patients, it is important that co-interventions that are likely to influence study outcomes are standardized. The proposal is to actively disseminate and ensure compliance with nutrition support practices and the approach to weaning patients from mechanical ventilation in participating ICU. In relation to nutrition support, recently-developed evidence-based clinical practice guidelines (Heyland *et al*. 2003*a*) will be followed and materials to maximize compliance with these guidelines will be distributed. Standardizing the approach to weaning patients from mechanical ventilation has been shown to markedly reduce length of stay in ICU compared with a non-standardized approach (Ely *et al*. 1999). Given that duration of mechanical ventilation and length of stay in ICU are secondary end points, all participating centres will adopt this weaning protocol for the purposes of the proposed study.

Description of outcomes measures

Given that the meta-analysis of glutamine and antioxidant supplementation has demonstrated a significant reduction and a trend towards reduced mortality, respectively,

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associated with these nutrients, the primary outcome for the proposed study is 28 d mortality. The secondary outcomes include duration of stay in ICU, development of infectious complications, multiple organ dysfunction, duration of mechanical ventilation, length of stay in hospital, antibiotic use and costs of care.

It is planned to follow study patients prospectively while in the ICU, documenting the compliance with study nutrients, reasons for interruptions and development of infectious complications. Following death, discharge from the ICU or after 28 d the charts will be reviewed by two investigators (the site research nurse and the site investigator) to evaluate and categorize all infectious complications. With the exception of pneumonia, Center for Disease Control (Anonymous, 1989) definitions of infectious complications acquired in the ICU will be used. For classifying pneumonia definitions that are currently being used in the multicentre trial of ventilator-associated pneumonia (Canadian Critical Care Trials Group, unpublished results) will be used. These forms will be reviewed by the methods centre personnel to ensure logic and consistency across sites.

In addition, there is some evidence that the effect of aggressive nutrition support and glutamine supplementation will extend beyond the acute phase of the illness (Griffiths *et al*. 1997; Taylor *et al*. 1999). In an effort to better understand the impact of study treatments on longer-term survival and quality of life, surviving study patients will be followed for 6 months. At 3 and 6 months post randomization patients discharged from hospital will be contacted to assess their survival status and whether they have resumed normal activities, and to administer a multipurpose survey of general health status consisting of eight domains and thirty-six items (SF-36; Ware, 1996) over the telephone. All but one of the thirty-six items is aggregated into eight subscales that can also be clustered to form two higher-order scales, the physical health and mental health component scores. Each subscale is scored from 0 to 100 (100 is optimal). The SF-36 is suitable for self-administration or for administration by a trained interviewer in person or by telephone. The SF-36 has been used in a variety of patient populations and the norms for age, gender and fourteen chronic diseases have been published (Ware, 1996). Compared with other generic health status instruments the SF-36 has been shown to have better feasibility, internal consistency, content validity and discriminative ability and is more responsive to clinical improvement (Essink-Bot *et al*. 1997). Recently, Heyland *et al*. (2001) have demonstrated that the SF-36 has good reliability and validity when used to measure health-related quality of life in survivors of critical illness. This approach has been used in existing studies examining the longer-term follow up of acute respiratory distress syndrome and sepsis. Heyland *et al*. (2003*b*) justify only following patients for 6 months because most of the improvements in quality of life will have occurred by then.

Justification of sample size

In the recently-completed dose-escalation study a 28 d mortality rate of 30% was observed in the control group (DK Heyland, R Dhaliwal, A Day, J Drover, H Cote and P Wischmeyer, unpublished results). This outcome is similar to another recently-published therapeutic trial of 1690 patients with severe sepsis in which the control group mortality was reported to be 30·8% (Bernard *et al*. 2001) and is consistent with the published literature on critically-ill patients with multiple organ failure (Moreno *et al*. 1999). To recap, the recently-updated meta-analysis examining the effect of glutamine on survival (Critical Care Nutrition, 2006) has found a RR of acute death of 0·75 in patients receiving glutamine compared with control patients. It has also found that the RR of acute death with antioxidant supplementation is 0·65 compared with controls. Thus, a relative risk reduction of 25% by either glutamine or antioxidant supplementation alone is considered to be plausible and clinically important. Assuming a two-sided 5% significance level and a 30% baseline mortality rate, if both treatments have a relative risk reduction of 25% (assuming no interaction), 80% power to detect an effect for each intervention would be achieved if 1200 patients are enrolled (see Table 1). The power of detecting one intervention decreases slightly as the effect size of the other intervention increases, but when no interaction is present, acceptable power is maintained over a plausible range of effect sizes.

Table 1. Sample size justification: power for n_{total} 1200 assuming a baseline mortality rate of 30% and no interaction between treatments*

Relative risk reduction of other factor (%)	Relative risk reduction of factor tested (%)			
	20	25	30	35
0	65‡	84†	95†	99†
20	60‡	80†	93†	98†
25	61‡	80†	93†	98†
30	59	79‡	92†	98†
50	53	73‡	87†	96†

*Estimates of the power for testing the effect of one intervention in the possible presences of an effect of the other intervention. The effect sizes are described in terms of relative risk reductions in 28 d mortality from a baseline (control group) risk of 30%.
†High power (>80%) is achieved.
‡Acceptable power (60–80%) is achieved.

With a sample size of 1200, statistical power to detect an interaction will be lacking. However, the effect an interaction might have on the power to detect main treatment effects has been considered. If the interaction is positive, the power would increase. In such a scenario an overestimate of the magnitude of the treatment effect of either nutrient in isolation would be achieved, but this aspect is less relevant given that the combined treatment would be recommended anyway. If the interaction is negative, the power of the study would be reduced to suboptimal levels. In the worst-case scenario the mortality reduction in the combination group is assumed to be no greater than the best treatment effect of one nutrient alone (i.e. no additional benefit with the addition of the second agent). In the presence of such a large negative interaction, with the base-case assumptions, there would be insufficient

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Table 2. Sample size justification: power for n_{total} 1200 assuming a baseline mortality rate of 30% and a large negative interaction*

Relative risk reduction of other factor (%)	Relative risk reduction of factor tested (%)			
	20	25	30	35
0	65‡	84†	95†	99†
20	22	44	68‡	87†
25	23	33	58	80†
30	23	33	45	70‡
50	25	35	49	62‡

*Estimates similar to Table 1 except that a large negative interaction, which is considered the worst plausible, is assumed. In this worst-case scenario, the combination arm has a 28 d mortality rate equal to the best of the two single intervention arms. The first row of this table is identical to Table 1 since this type of interaction only exists if both interventions have some effect.
†High power (>80%) is achieved.
‡Acceptable power (60–80%) is achieved.

Table 3. Sample size justification: power for n_{total} 1200 assuming a baseline mortality rate of 30% and a moderate negative interaction*

Relative risk reduction of other factor (%)	Relative risk reduction of factor tested (%)			
	20	25	30	35
0	65‡	84†	95†	99†
20	39	64‡	83†	95†
25	40	58	79‡	92†
30	39	56	73‡	89†
50	37	55	70‡	84†

*Estimates similar to Tables 1 and 2. This table makes an assumption intermediate to that of Tables 1 and 2. Here it is assumed that the combination arm has a mortality rate that is the midpoint between what would be expected without any interaction (Table 1) and what is seen under the worst plausible case (Table 2).
†High power (>80%) is achieved.
‡Acceptable power (60–80%) is achieved.

power unless the relative risk reduction is ≥35% (see Table 2). It is more plausible that the negative interaction is modest in size. With an interaction half the size of the worst plausible scenario there would be >70% power to detect a relative risk reduction of 30% if the other treatment resulted in a relative risk reduction of ≤50% (see Table 3). It is not feasible to power this trial to allow for a very large negative interaction. However, there is some theoretical rationale that, if present, an interaction is likely to be positive, because both glutamine (as a glutathione precursor) and Se (as an important cofactor of glutathione peroxidase) may combine synergistically to enhance antioxidant capacity in critically-ill patients. It is realized that trying to secure the resources to conduct a trial to protect against an extremely unlikely negative interaction is not feasible. In summary, given the range of plausible scenarios, it is believed that 1200 patients is an adequate and yet feasible sample size.

Proposed statistical analysis

The primary end point of the proposed study is 28 d mortality. The primary analysis will employ logistic regression with terms for presence of cardiovascular dysfunction (the stratification factor), both treatments and their interaction. If the treatment interaction is potentially clinically relevant and significant at $\alpha \leq 0{\cdot}10$, then the effect of each supplement will be examined in both subgroups of the other supplement at $\alpha/2$. If no significant treatment interaction is identified, then the interaction term will be dropped from the model and the two main treatment effects will be tested marginally over both levels of the other treatment. To account for the interim analyses, the final analysis will be performed at a significance level of 4·4%. Although only the primary analysis testing strategy will be used to formally determine the efficacy of the interventions, 28 d morality will be reported for each treatment combination (marginally and conditionally) even if no interaction is identified (McAlister *et al*. 2003). The secondary end points of ventilator-free days and ICU-free days within the first 28 d will be compared by arm as has been previously proposed (Bernard *et al*. 1994) using the Wilcoxon-Mann-Whitney test. Duration of stay in the ICU and duration of mechanical ventilation will be compared between arms using a survival analysis approach. Patients who die in the ICU will be censored after 6 months. Adjudicated diagnosis of infection and presence of multiple organ dysfunction will be analysed using the logistic regression approach described for the primary analysis. Health-related quality of life will be described at 3 and 6 months post randomization, performed two ways: (1) including only surviving discharged patients; (2) setting all values of dead or hospitalized patients to 0. The robustness of the health-related quality of life analysis to missing data will be assessed by describing the missing data pattern in detail and performing a sensitivity analysis that will incorporate multiple imputation and a range of assumptions. As before, study outcomes will be compared in the subgroups defined by the presence of aetiology of shock.

All patients will be analysed as randomized in accordance with the intent-to-treat principle. However, a secondary efficacy analysis including only patients who received a minimum of 5 d of study nutrients is also planned. All tests will be two-sided and all analysis will be performed using SAS version 9.1 or later versions (SAS Institute Inc. Cary, NC, USA).

Interim analyses will be performed after finalized data are available for 600 and 900 subjects. The results of the interim analyses will be reviewed by a Data Monitoring Committee who will not disclose any of the efficacy results unless an early stopping decision is made. The method of Lan & DeMets (1983) with O'Brien-Fleming boundaries will be used to apply a formal stopping rule to the primary end point. This method maintains the overall type I error rate at 5%, and yet only minimally reduces the overall power (see Table 4). These boundaries will require significance levels of 0·3% and 1·8% at the first and second interim analyses respectively. The final primary analysis will be performed at a significance level of 4·4%. The glutamine and antioxidant factors will be tested separately and distinct early stopping decisions will be made for each intervention. If only one of the interventions meets the stopping criteria, then the trial will continue, but patients will only be randomized to the remaining intervention.

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Table 4. Sample size justification: operating characteristics of interim analyses assuming a 25% relative risk reduction of each intervention*

Analysis	Sample size	α (%)			Power	
		Nominal	Incremental	Cumulative	Incremental (%)†	Cumulative (%)‡
Interim 1	600	0·3	0·3	0·3	16·3	16·3
Interim 2	900	1·8	1·6	1·9	36·8	53·1
Final	1200	4·4	3·1	**5·0**	26·2	**79·3**

*The effect of performing two interim analyses on the operating characteristics of this study. The nominal α is the significance level used as the significance criteria at each analysis. The cumulative α is the chance of making a type I error by the end of each analysis. It may be seen that the overall chance of making a type I error is maintained at 5% throughout the study. The inclusion of two interim analyses reduces the overall power of the study from 80·0% to 79·3%.
†The chance of obtaining a significant result given that a significant result was not obtained at an earlier analysis.
‡Provides the probability that a significant result is obtained by the end of each analysis.

Summary

Having systematically reviewed the literature on numerous nutrients and nutritional interventions, the use of glutamine supplementation and antioxidants are most likely to lower mortality among critically-ill patients. In collaboration with the Canadian Critical Care Trials Group, preparation is underway to move forward with a large-scale multicentre trial of glutamine and antioxidants in the critical care setting (The REDOXS© study, the first results of which are expected to be available in 2009). This study represents an important paradigm shift for nutrition and critical care practitioners, as it emphasizes the potential importance of nutrients, rather than nutrition, in improving the outcomes of critically-ill patients. Furthermore, if the trial is positive, the results will be used to inform the clinical practice of nutrition support around the world.

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