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## Pharmaconutrition Review: Physiological Mechanisms

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### Abstract

The search to improve outcomes in critically ill patients through nutrition support has steadily progressed over the past 4 decades. One current approach to this problem is the addition of specific nutrients as primary therapy to improve host defenses and improve the outcome of critically ill patients. The field is referred to as “pharmaconutrition,” with the hope of focusing investigations on each nutrient to understand its pharmacological effects on immune and clinical outcomes. The purpose of this review is to describe some of the known physiological mechanisms of pharmaconutrients such as glutamine, arginine,  $\omega$ -3 fatty acids, and selenium. (*JPEN J Parenter Enteral Nutr.* 2013;37:51S-65S)

### Keywords

immunonutrition; pharmaconutrition; immune modulation; nutrition therapy

Critical illness poses significant challenges to patients who must respond effectively to systemic inflammation, potentially infectious organisms, altered immunity, and metabolic changes resulting in hypermetabolism. The inability to maintain adequate nutrient delivery during these hypercatabolic conditions renders the patient susceptible to significant nutrient deficiencies, which may increase the risk for infection, organ failure, and mortality.<sup>1–4</sup> Since the ability of patients to meet their needs by eating is usually not possible, the use of nutrition support through parenteral and enteral feeding is frequently employed to provide the required amounts of caloric and nutrient needs. The field of nutrition support has progressed in knowledge and complexity over the past 45 years from simply solving the problem of safely providing enough micronutrients and macronutrients to meet metabolic demands to the current search for individual nutrient regimens to optimize immune function and cell recovery.

Initially, the search for specialized formulas focused on enteral products enriched with various combinations of metabolic substrates (eg, glutamine, arginine, antioxidants, nucleotides, and/or  $\omega$ -3 fatty acids). The enteral products were referred to as *immunonutrition* or *immune-enhancing diets* (IEDs) and were formulations or “cocktails” of nutrients in various concentrations. Since these products contained different combinations of assorted nutrients, the exact contribution of each individual nutrient could not be determined in humans. In 2008, Jones and Heyland<sup>5</sup> suggested we shift our concept of immunonutrition toward a standardized assessment of specific nutrients administered at pharmacological levels, emphasizing an emerging term called *pharmaconutrition*. This framework suggested nutrients be studied as therapeutic agents administered in physiologic and supraphysiologic (ie, pharmacologic)

doses, thus shifting the focus of specialized nutrition support to a study of active therapeutics. The difficulty in defining an optimal combination of agents and the optimal dose of each agent remains an impossible task. Nevertheless, it appears that specific pharmaconutrients do improve clinical outcomes in surgical and intensive care unit (ICU) patients.

There are conflicting results in IED data with regard to infection, morbidity, length of stay (LOS), mortality, and other outcome parameters. Potential explanations for discrepancies include variable effects of individual nutrients dependent on dose, the very real possibility of nutrient interactions, and that different hypermetabolic states may dictate different metabolic needs. Similarly, an individual’s genome may influence

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metabolic requirements and immune response. Each factor limits our ability to evaluate individual nutrient effects. For example, arginine supports lymphocyte function, but is also a nitric oxide precursor. Therefore, in addition to other confounding variables, arginine may improve outcomes in one type of patient while potentially harming another. Although the controversy regarding arginine is heatedly debated and the conclusions are controversial, clinicians must be mindful that substances potent enough to help can also be potent enough to harm and consider each nutrient with full regard to dosage, route, timing, and duration in distinct patient subpopulations. The purpose of this review is to cover potential beneficial physiological mechanisms for several individual pharmacutrients and briefly describe some of the current clinical data in critically ill and surgical patients. Current nutrient guidelines from the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.) and the Canadian Critical Care Nutrition Group (CCCNG) are included; however, this article is not intended to provide specific recommendations for any patient populations regarding nutrient dosing, timing, or duration.

## Macronutrients

### *Glutamine*

Glutamine is the most abundant free amino acid in circulation (500–900  $\mu\text{mol/L}$ ) under normal circumstances.<sup>6</sup> Under conditions of critical illness or with gastrointestinal (GI) disorders, glutamine levels may not be adequate to meet demands and become conditionally essential.<sup>7-9</sup> During critical illness, muscle cells metabolize branched-chain amino acids (BCAAs) obtained from intracellular protein catabolism.<sup>10-12</sup> As the BCAAs are deaminated, nitrogen is transaminated onto either pyruvate, creating alanine, or onto  $\alpha$ -ketoglutarate (which is produced in the Krebs cycle), which assimilates 2 ammonia groups to produce glutamine. Although alanine and glutamine constitute only 7% of normal muscle protein, they account for 60%–70% of the amino acids released into the circulation by skeletal muscle cells in stress, sepsis, or injury. Glutamine is central to cellular energy, cell proliferation, renal acid base regulation, and nitrogen and carbon metabolism.<sup>13,14</sup> Glutamine also supports regulation of glucose metabolism through improved insulin sensitivity in trauma patients and provides carbon backbones required for glucose production through gluconeogenesis.<sup>15</sup>

Within the intestine, glutamine is particularly important as the preferred fuel source by enterocytes and for the vast mass of immune cells within the gut-associated lymphoid tissue (GALT), where up to 50% of available glutamine is metabolized following enteral feeding.<sup>16-19</sup> Once activated, immune cells such as lymphocytes and macrophages use increased quantities of glutamine for energy, in addition to glucose.<sup>20</sup> Glutamine is also necessary for purine and pyrimidine synthesis of DNA and messenger RNA during immune cell

proliferation.<sup>21</sup> Limited glutamine availability prevents expression of membrane receptors and cytokines, suppressing the efficiency of immune response.<sup>22</sup> Since glutamine is used as a fuel source by enterocytes, colonocytes, and other cells in the gut mucosa, glutamine depletion impairs gut architecture and increases gut barrier permeability.<sup>16,20,23</sup> Maintenance of enterocyte absorption with enteral glutamine may explain the clinical observation of increased formula volume tolerance in patients with glutamine supplementation.<sup>24</sup> Glutamine is also important in nervous system function since it crosses the blood-brain barrier to serve as a chief precursor to the neurotransmitter  $\gamma$ -aminobutyric acid (GABA), and glutamine supplementation enhances GABA concentrations.<sup>25</sup>

In addition to metabolic contributions, glutamine is a precursor to the antioxidant glutathione, promotes heat shock protein (HSP) responses, and modulates gene regulation linked to apoptosis and signal transduction.<sup>26-29</sup> Glutathione expression is high in the intestinal mucosa, and the reduced activity of this antioxidant leads to mucosal degradation, diarrhea, malabsorption, and failure to thrive. Glutamine administration enhances glutathione levels.<sup>30-32</sup> The expression of HSP in particular is protective during cellular stress through prevention of cell damage and death in the splanchnic bed and other organs. The synthesis of key HSPs, including HSP-70, requires adequate glutamine levels, and HSP-72 has been demonstrated to correlate in a dose-dependent fashion with glutamine availability in human mononuclear cells.<sup>33-36</sup> Laboratory experiments have demonstrated that glutamine administration decreases levels of circulating interleukin (IL)-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and reduces nuclear factor (NF)- $\kappa$ B activity following sepsis, effects that are dependent on HSP-70.<sup>37,38</sup> Administration of glutamine in clinical and basic research settings has revealed improved tissue HSP expression.<sup>28,39</sup> Animal models of ischemia-reperfusion and sepsis have demonstrated that glutamine administration maintains metabolic function and survival of cardiac muscle and lung tissues.<sup>35,40</sup>

Numerous clinical studies have examined the use of enteral and parenteral glutamine in critically ill patients. The most recent meta-analysis, performed by the CCCNG, examined 21 combined enteral and parenteral studies involving 1564 patients and determined a significant reduction in mortality from 20% to 14.7% (risk ratio [RR], 0.75; 95% confidence interval, 0.61–0.93).<sup>41</sup> Improvements were also observed with LOS (RR, 0.79; 95% CI, 0.68–0.93) and infectious complications (RR, –2.56; 95% CI, –4.39 to –0.74). Unfortunately, similar effects were not observed when only 8 enteral studies involving 691 patients were examined alone, with the exception of LOS.<sup>41</sup> It should be noted that enterally fed patients generally represent a more heterogeneous patient population compared with parenterally fed patients, and this should be considered when critically interpreting clinical data. Two single-center clinical trials performed a decade ago in ICU patients examining parenteral glutamine observed a significant reduction in mortality, but this result was not confirmed in a

more recent randomized prospective study of ICU patients receiving intravenous (IV) glutamine dipeptide.<sup>42-44</sup> Unfortunately, most clinical data used the more stable glutamine dipeptide that is not currently available in the United States. However, the use enteral glutamine in severely burned patients decreased the rate of infection and improved gut function and survival,<sup>45,46</sup> and therefore current guidelines recommend enteral glutamine in burn and trauma patients,<sup>47,48</sup> but there is insufficient evidence to advise enteral glutamine in other critical illness. The route and dose of glutamine administration should be considered carefully since enteral glutamine is rapidly metabolized by the splanchnic bed at low doses and may not reach the portal vein. The effectiveness of glutamine use with parenteral nutrition (PN) across all critically ill patient subgroups is still undetermined, but its use is recommended in all parenterally fed patients.<sup>47,48</sup>

### Arginine

Arginine is a dibasic conditionally essential amino acid included in many pharmaconutrition formulations. In the urea cycle, arginine is derived from arginosuccinate and is further metabolized to produce urea and the amino acid ornithine. De novo biosynthesis of arginine uses citrulline as a precursor, and citrulline is provided by glutamine metabolism in the intestine. Arginine is used in the biosynthesis of polyamines<sup>49</sup> and proteins such as creatine and agmatine,<sup>50</sup> and it serves as the precursor for nitric oxide (NO; nitrogen monoxide), a potent vasodilator that is released in circulation with a half-life of several seconds.<sup>51-53</sup> In addition to precursor and biosynthetic roles, arginine stimulates certain physiological processes and is therefore also a regulatory molecule. For instance, elevated arginine levels increase collagen synthesis and growth hormone production in laboratory experiments, suggesting arginine may stimulate wound repair.<sup>49,54</sup> Animal and cell culture models also demonstrate that high arginine concentrations improve glucose clearance and insulin sensitivity by influencing pancreatic  $\beta$ -cell insulin release as well as glucose uptake by peripheral tissues in response to insulin.<sup>55,56</sup> Arginine also regulates lymphocyte function, especially T cells.<sup>50</sup> In vitro cell culture conditions deficient in arginine result in arrested T-lymphocyte cell development,<sup>57</sup> whereas elevated levels of arginine stimulate lymphocyte proliferation through increased expression of surface T-cell receptors (TCRs) and cytokines.<sup>58,59</sup> Arginine is required for lymphocyte memory following antigenic exposure and  $\zeta$ -chain expression.<sup>60</sup> The  $\zeta$ -chain, along with  $\alpha/\beta$  or  $\gamma/\delta$  heterodimers and CD3, makes up the TCR complex required for coupled antigen recognition, and low TCR complex expression results in impaired adaptive immune responses.<sup>60</sup>

Arginine is the substrate for 2 myeloid cell enzymes that are upregulated during immune activation: inducible NO synthase (iNOS) and arginase.<sup>50</sup> iNOS produces NO, an important cell signaling molecule that regulates vasodilation of blood vessels and vascular permeability. The importance of this role in

vasodilatation is underscored by the finding that low levels of NO production can lead to ischemic organ damage and pulmonary hypertension.<sup>61,62</sup> As an active antimicrobial compound within intra- and extracellular compartments, NO is also directly bactericidal and used by leukocytes and macrophages to destroy microbial pathogens.<sup>63</sup> During amplified immune response, iNOS expression and NO levels are stimulated in Th1-supporting macrophages (M-1) by proinflammatory cytokines, including IL-1, IL-2, TNF- $\alpha$ , and interferon (IFN)- $\gamma$ .<sup>64,65</sup> Critical illness can lead to high levels of these proinflammatory cytokines that upregulate iNOS expression of NO concentrations. Elevated NO levels are observed concomitantly in septic shock where hypotension, cardiac insufficiency, and increased tissue and endothelial permeability may precede organ failure.<sup>66</sup> Cytotoxic effects attributed to NO include damage to cell structures, inactivation of metabolic pathways, lipid peroxidation, and alterations in gene expression.<sup>67,68</sup> In contrast to iNOS, the other myeloid enzyme, arginase, converts arginine to ornithine and shunts available arginine away from NO production.<sup>50</sup> Arginase is expressed in Th2-supporting macrophages (M-2) following stimulation by Th2 anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, and transforming growth factor- $\beta$  (TGF- $\beta$ ).<sup>64,69</sup> Ornithine produced by arginase can be used to synthesize polyamines and proline, which are needed for wound healing.<sup>70</sup> However, a key role for arginase during Th2 responses may be limiting NO production.<sup>71</sup>

Recent findings suggest the type of response generated, Th1 or Th2, is influenced by the nature of injury.<sup>72</sup> For instance, trauma and major surgery patients appear to generate Th2 responses, whereas septic patients appear to generate Th1 responses.<sup>72</sup> Consistent with the role of arginine in NO production, some laboratory studies have demonstrated negative effects with arginine supplementation in animal models of inflammation, infection, and injury.<sup>73,74</sup> However, clinical data suggest that septic patients,<sup>75</sup> but not trauma or elective surgery patients,<sup>76</sup> may have elevated circulating NO levels, supporting the case for specific Th1 vs Th2 responses in specific injury. At least 5 meta-analysis studies have been performed on the available accumulative clinical data for arginine.<sup>77-81</sup> Each demonstrates reductions in overall infectious complications, 4 demonstrate reduced LOS, and 2 demonstrate decrease in ventilator days. In general, the data suggest that most ICU patient and elective surgery populations may benefit from arginine with fewer infections. However, based on the data, 1 highly controversial current recommendation is to withhold arginine supplementation in septic patients because of the potential harm induced by NO production and the increased formulation costs. Furthermore, hemodynamically unstable ICU patients should not receive additional arginine or enteral formulas in general.<sup>82</sup>

### Leucine

Leucine is 1 of the 3 BCAAs, along with isoleucine and valine. BCAAs are metabolized directly by skeletal muscle and, unlike

other amino acids, do not reenter the circulation following muscle proteolysis. Leucine is the only dietary amino acid that promotes cell growth in the body.<sup>83</sup> Laboratory experiments demonstrate that leucine stimulates protein synthesis in skeletal, cardiac, and intestinal muscle, as well as other tissue and cell types, through stimulation of the mammalian target of rapamycin (mTOR) pathway.<sup>83,84</sup> Animal experiments demonstrate that enteral leucine stimulates protein synthesis in a rat model of sepsis or burn.<sup>85</sup> Since critical illness is characterized by muscle catabolism and a 30% loss of lean muscle is associated with a significant increase in mortality,<sup>86-88</sup> the addition of leucine to nutrition support formulas has the potential to slow muscle proteolysis in the critically ill.

BCAAs and their ketoacid derivatives have been used in clinical studies in the critically ill over the past 30 years. In burn patients, BCAA supplementation sustained mild inhibition of protein catabolism in some studies, as assessed by reduced urinary 3-methylhistidine/creatinine ratios, but these studies were confounded by small sample size and the use of all 3 BCAAs.<sup>89,90</sup> Likewise, some larger studies in trauma, ICU, and septic patients have shown improvements in nitrogen balance or the half-life of visceral proteins but few improvements in mortality.<sup>91-94</sup> Furthermore, the vast majority of these studies used valine and isoleucine, in addition to leucine, and we now know that these other BCAAs do not stimulate protein synthesis and instead compete with leucine for transport into muscle cells and use shared metabolic pathways.<sup>95</sup> Considering these findings, more studies are needed that focus on leucine alone. De Bandt and Cynober<sup>96</sup> suggested that future work should consider glutamine status when investigating leucine since muscle proteolysis induced by low glutamine levels may override any positive effects leucine has on protein synthesis and nitrogen balance. Larger controlled studies are needed to determine the effects of leucine, with balanced nutrition support, to determine its effect in respectively stratified critically ill patient subgroups.

### *$\omega$ -3 Fatty Acids*

The  $\omega$ -3 fatty acids are isolated from cold-water fish species, flaxseed, and canola oil and are used for their proposed anti-inflammatory properties.<sup>97</sup> The  $\omega$ -3 fatty acids are polyunsaturated fatty acids with a carbon-carbon double bond at the third carbon. Mammals cannot synthesize the carbon-carbon double bonds at either the third or sixth carbon, making these compounds essential in the diet.<sup>97</sup> The 3 most important  $\omega$ -3 fatty acids are  $\alpha$ -linolenic acid (ALA; 18 carbons, 3 double bonds), eicosapentaenoic acid (EPA; 20 carbons, 5 double bonds), and docosahexaenoic acid (DHA; 22 carbons, 6 double bonds). These  $\omega$ -3 fatty acids are vital to normal cognitive function but are also inserted in cell membranes where they compete with  $\omega$ -6 fatty acids in the form of arachidonic acid (AA; 20 carbons, 4 double bonds).<sup>5,97-99</sup> Under healthy conditions, the ratio of dietary  $\omega$ -3/ $\omega$ -6 fatty acids does not appear to have dramatic

effects on health. However, during cellular stress or damage, these fatty acids are released from the membranes by phospholipases and are converted by cyclooxygenase and lipoxygenase enzymes into powerful secondary messenger hormones, eicosanoids.<sup>100,101</sup> Eicosanoids mediate tissue responsiveness to injury and pathogens but can become harmful when overexpressed or produced chronically. Following sufficient tissue repair or pathogen elimination, anti-inflammatory compounds are produced that resolve the inflammatory processes and promote homeostasis. These anti-inflammatory compounds include the resolvins and protectins, which are derived from the  $\omega$ -3 fatty acids EPA and DHA.<sup>102</sup> Cyclooxygenase function is the target of numerous nonsteroidal anti-inflammatory drugs, such as aspirin, highlighting the significance of this inflammatory pathway.

Eicosanoids derived from  $\omega$ -6 fatty acids promote 2- and 4-series eicosanoids, such as prostaglandin E2, prostaglandin A2, and leukotriene B4, which are highly proinflammatory. For example, these compounds mediate platelet coagulation, pain, redness, and swelling.<sup>100,101</sup> Circulating prostaglandin E2 functions directly on the hypothalamus to induce fever, loss of appetite, and fatigue.<sup>103</sup> However,  $\omega$ -3 fatty acids promote the less inflammatory eicosanoids of the 3- and 5-series, such as prostaglandin E3 and leukotriene B5. High doses of  $\omega$ -3 or limited intake of  $\omega$ -6 fatty acids appear to shift the balance in favor of less systemic inflammation in at least 3 competitive ways.<sup>100,101</sup> First, the  $\omega$ -3 fatty acids displace AA, so there is less  $\omega$ -6 fatty acid proportionally within the cell membrane. Second,  $\omega$ -3 fatty acids compete for cyclooxygenase and lipoxygenase enzymes, so less AA is catalyzed into proinflammatory eicosanoids. And third, the effect of 3- and 5-series eicosanoids directly counteracts the effects of the 2- and 4-series eicosanoids. Furthermore,  $\omega$ -3 fatty acids decrease the release of proinflammatory cytokines IL-1 and TNF in peripheral blood mononuclear cells and reduce iNOS expression in macrophages.<sup>29,104</sup> Considering the  $\omega$ -3 fatty acid potential to reduce NO overproduction by limiting iNOS, adding  $\omega$ -3 fatty acids to enteral formulas may benefit septic patients who are expressing Th1/M-1 inflammatory responses.

A clinical study revealed a rapid (<1 hour) and sustained (2–3 days) enrichment of leukocyte phospholipid  $\omega$ -3 fatty acid content following IV bolus infusion in healthy adults, demonstrating the effectiveness of  $\omega$ -3 administration.<sup>105</sup> Because of the competitive effects between  $\omega$ -6 and  $\omega$ -3 fatty acids, the recommended ratio is 2:1–4:1 ( $\omega$ -6: $\omega$ -3),<sup>106</sup> and this should be considered when interpreting clinical data. One unpublished and at least 3 published clinical trials examining  $\omega$ -3 fatty acid formulations in critically ill patients have demonstrated beneficial outcomes.<sup>107-109</sup> Unfortunately, the formulations in these studies also include other compounds, such as antioxidant combinations, so there has not been a thorough analysis of  $\omega$ -3 formulation effects alone. Even so, a meta-analysis of these studies including 554 patients revealed significant benefits in mortality, where  $\omega$ -3 treatment reduced

mortality from 35% to 23.5% (RR, 0.67; 95% CI, 0.51–0.87) compared with the standard diets.<sup>41</sup> Further analysis of 340 critically ill patients from the same meta-analysis demonstrated significant reductions in LOS (RR, 4.49; 95% CI, –6.49 to –2.47) and ventilator days (RR, –5.83; 95% CI, –7.96 to –1.70) compared with a standard diet. A recent clinical study with 106 septic patients that combined enteral  $\omega$ -3 fatty acids with antioxidants demonstrated significant reductions in septic severity and cardiovascular and respiratory failure compared with controls,<sup>110</sup> but there were no differences in overall mortality between groups. The use of  $\omega$ -3 fatty acid in patients with acute respiratory distress syndrome (ARDS) does appear to decrease mortality rates.<sup>111</sup> In addition, improved pulmonary function and reduced lung edema have occurred with  $\omega$ -3 supplementation in both clinical and laboratory studies.<sup>112,113</sup> These findings led to the recommendation by A.S.P.E.N. and CCCNG to include enteral  $\omega$ -3 fatty acids (with antioxidants) in patients with acute lung injury (ALI) and ARDS.<sup>41,47,48</sup> However, formulation, dosage, and duration remain controversial.

## Micronutrients

### *Vitamin C (Ascorbic Acid)*

Vitamin C is an important redox compound that reduces radical forms of  $\alpha$ -tocopherol (vitamin E), glutathione, hydroxyl, and superoxide, as well as radicals in the cell cytoplasm and mitochondria. Animal models demonstrate that vitamin C infusion protects against ischemia-reperfusion injury of the liver and skeletal muscle.<sup>114–116</sup> Other models have also demonstrated positive effects of vitamin C administration in ARDS and systemic inflammatory response syndrome (SIRS) that were attributed to free radical scavenging activity.<sup>117,118</sup> In addition to limiting radicals, vitamin C is important for the synthesis of collagen, carnitine, and norepinephrine, and low levels of vitamin C increase bruising and delay wound healing.<sup>119</sup> In vitro, vitamin C reduces activation of NF- $\kappa$ B through TNF- $\alpha$  modulation<sup>120</sup> and spontaneously destroys histamine, a molecule associated with respiratory tract infection, allergic pathologies, and bronchial asthma.<sup>121</sup> Animal models demonstrate that vitamin C protects against vascular leakage, epithelial barrier disruption, and increased alveolar fluid levels during sepsis.<sup>118,122</sup> Burn patients are subjected to increased oxidative stress and the need for increased collagen synthesis. A clinical study using high-dose vitamin C in severely burned patients demonstrated reduced wound edema, resuscitation fluid volume requirements, and respiratory failure.<sup>119,123,124</sup> However, no large well-controlled studies have examined the effect of vitamin C alone, without other antioxidants, on immune function, vascular repair, or wound healing in other critically ill patients. Parenteral dosing of 200 mg/d is recommended during critical illness.<sup>48,125</sup> The current enteral dose recommendation for vitamin C is 125–250 mg/d, with supplemental recommendations

of an additional 500–3000 mg/d.<sup>47,125</sup> Trauma and burn patients routinely receive elevated doses of 1000 mg/d,<sup>125</sup> and some clinical data suggest 1000–2000 mg/d may be required to maintain normal levels of plasma vitamin C (55–100  $\mu$ mol/L) in postoperative ICU patients. Parenteral dosages of 3000–6000 mg/d for 2 days or 3000 mg/d for several weeks may be beneficial without producing adverse effects or impaired renal function.<sup>123,124</sup>

### *Selenium*

Selenium is an essential trace mineral required in microgram quantities that is incorporated into the amino acid selenocysteine. It is an important cofactor in at least 25 selenoproteins, including important immune, endocrine, and antioxidant enzymes.<sup>126–128</sup> Selenium is most readily bioavailable in the form of inorganic salts, such as selenate and selenite,<sup>129,130</sup> and recent advances have been made in identifying novel biomarkers of selenium status.<sup>131</sup> The precise role of selenium in humoral immunity remains unclear, but selenium-deficient rodents have demonstrated reduced lymphocyte function and decreased immunoglobulin (Ig) M, IgG, and IgA production. Low selenium status in humans is associated with decreased IgG and IgM titers in circulation.<sup>132</sup> Likewise, macrophages and neutrophils rely on selenoprotein-dependent generation of reactive oxygen species (ROS), and reduced selenium availability decreases neutrophil function.<sup>133,134</sup>

The most recognized role of selenium is the antioxidant function of glutathione. There are 8 glutathione peroxidase (GP) isoforms, including cytosolic GPx1, gastrointestinal GPx2, and plasma GPx3.<sup>127</sup> The plasma GPx3 correlates with the circulating form of selenium, selenoprotein P (SePP), and these proteins are markers of selenium status.<sup>135,136</sup> Deficiency of GPx3 and SePP is associated with increased mortality in septic clinical patients.<sup>136</sup> Clinical data also demonstrate that low GPx3 levels inversely correlate with C-reactive protein (CRP), procalcitonin (PCT), and the Sequential Organ Failure Assessment score.<sup>137,138</sup> CRP is released by the liver as an acute-phase protein and is a marker of inflammation that binds dying cells and activates complement. PCT is a precursor for calcitonin produced by the thyroid and neuroendocrine cells in the intestine and lung that responds to infection and systemic inflammation. Elevated levels of CRP and PCT suggest increased systemic inflammation when GPx3 levels are low. Selenium is also a cofactor in thioredoxin reductases (TRxR), enzymes found ubiquitously in mammals that are necessary for defense against oxidative damage and controlling the redox molecules hydrogen peroxide and NO.<sup>139</sup> Furthermore, selenium is required for regulation of thyroid hormones through iodothyronine deiodinase proteins. Poor selenium status following trauma correlates with non-hypothyroid-related levels of thyroid T3, effects that are reversed with selenium supplementation.<sup>140</sup>

Clinical studies have used megadose selenium, typically around 1000 µg/d, with the aims of reducing infection and organ dysfunction. Such doses are high considering that the World Health Organization recommends a recommended daily intake at 70 µg/d, with a risk of toxicity at 900 µg/d. However, clinical data suggest selenium levels can decrease 40%–70% during septic shock, and serum levels <0.7 µmol/L are associated with significantly greater mortality and organ failure. Metabolic activity and urinary losses of nutrients are increased in critical illness and can be further accelerated by the use of statins and corticosteroids that suppress selenium status.<sup>141,142</sup> Furthermore, acute renal failure treatment with continuous venovenous hemodiafiltration treatment can significantly deplete plasma selenium concentrations.<sup>143</sup> Considering some patients have preexisting dietary deficiency in addition to catabolic stress, the typical regimen is a bolus infusion followed by continuous infusion for 7–10 days but not longer than 2 weeks.<sup>144</sup> Using this model, a clinical trial involving 189 septic and SIRS patients demonstrated significant reductions in mortality in several patient subgroups, and treatment significantly maintained whole-blood selenium markers.<sup>145</sup> Yet, there remains controversy over the safety of high-dose selenium in PN, and current CCCNG guidelines<sup>41</sup> suggest there are insufficient data to make specific recommendations for the use of selenium in PN in critically ill patients, particularly considering its requirement and contraindications are undefined in specific patient populations, such as smokers and alcoholics, in whom liver function may be impaired. However, A.S.P.E.N.<sup>47</sup> supports the use of selenium and other antioxidants in PN at lower doses for all critically ill patients requiring nutrition support. The current dosing range suggestion for the critically ill is 70–100 µg/L in enteral nutrition (EN) and 100–400 µg/d in PN.<sup>125,146</sup>

## Zinc

Zinc is a trace mineral with cofactor roles in catalytic, structural, and regulatory proteins throughout the body that are especially important in immune function and implicated in wound healing.<sup>147,148</sup> Zinc deficiency can occur with high-calorie PN and EN without appropriate zinc supplementation and may present with skin rash, abnormal sense of taste or smell, delayed wound healing, and the loss of hair.<sup>149–152</sup> In children, zinc deficiency can lead to failure to thrive. Zinc is a vital cofactor to DNA and RNA polymerase, and zinc deficiency can cause result in arrested cell growth cycles.<sup>147,153</sup> Clinically, deficiency can manifest as reduced natural killer cell numbers and function, as well as greater risk of infection and mortality.<sup>149</sup> Human studies have suggested that temporary and mild zinc deficiency decreases the CD4/CD8 lymphocyte ratio and function.<sup>154</sup> In ICU patients, zinc plasma levels are inversely correlated with the serum cytokines IL-6 and IL-8, which are indicative of inflammatory stress.<sup>155</sup> Zinc deficiency in vitro increases the level of IL-1β and TNF-α in promyeloid cells through increased redox stress.<sup>156</sup> Within the intestine,

zinc has important antimicrobial roles as cofactors in matrix metalloproteinase (MMP) enzymes, including MMP-7, which activates antimicrobial defensins in intestinal Paneth cells.<sup>157</sup> Zinc also plays a structural role in intestinal tight-junction proteins, which holds enterocytes and other epithelial cells of the mucosa together, and deficiency increases mucosal barrier permeability and bacterial translocation.<sup>158,159</sup>

Zinc status is assessed by measuring serum concentration, which is normally lower than levels in intracellular compartments; however, drug regimens can affect serum levels, leading to artificial status results. Other biomarkers for zinc status have been reviewed,<sup>160</sup> but it remains unclear which are appropriate in the critically ill. Leukocyte zinc levels may be a reliable indicator of zinc status in the critically ill, but this analysis is challenging to perform.<sup>161</sup> The normal range for serum zinc is 80–160 µg/dL, and levels <60 µg/dL require zinc replacement. Optimal dosing for zinc has not been determined,<sup>162</sup> but recommended zinc intakes are 2.5–5 mg/d in PN, 11–19 mg/L in EN, and an additional 12–30 mg/d with diarrhea or fistulas.<sup>48,125</sup> Up to 36 mg/d has been administered to burn patients without deleterious adverse effects, and careful monitoring of zinc status in fistula patients may be required to ensure adequate status.<sup>163</sup>

## Magnesium

Magnesium is a divalent metal ion that is an essential cofactor for >300 enzymes, including those involved in protein synthesis, nucleic acid synthesis, and mitochondrial membrane stabilization.<sup>164</sup> Magnesium deficiency is associated with dizziness, muscle cramping and weakness, and fatigue. Renal reabsorption is usually highly efficient, but patients with poor renal function may be at increased risk of deficiency without magnesium supplementation.<sup>165</sup> Low magnesium levels are associated with significantly greater mortality in ICU patients, and very low serum magnesium is associated with an increased incidence of cardiac dysrhythmias.<sup>166</sup> Accordingly, adequate magnesium is essential in preventing idiopathic mitral valve prolapse and hypertension.<sup>167</sup> In addition, similar to zinc, magnesium has essential roles in maintaining intestinal tight-junction protein integrity, and magnesium deficiency elevates liver and intestinal proinflammatory cytokine TNF-α and IL-6 levels.<sup>168</sup> Magnesium is also the cofactor for the enzyme arginase in myeloid cells and liver tissue.<sup>164</sup> There are currently no specific recommendations for clinical use, but magnesium status should be checked to ensure proper administration in patients with acute (type 1 and 2) intestinal failure as well as acute and chronically ill patients supported with specialized IV nutrition.

## Prebiotics, Probiotics, and Synbiotics

Probiotics are viable bacteria or yeast microorganisms of human origin that are proposed to benefit the host when added as dietary supplements.<sup>169</sup> Prebiotics are fermentable soluble

dietary fibers, such as inulin and fructooligosaccharides, which are metabolized by the microbiome and favor growth of “beneficial” bacteria in the gut.<sup>170</sup> *Synbiotics* is the term applied to nutrition products that contain both prebiotics and probiotics, with the aim of inducing additive beneficial effects in the host when consumed.<sup>170</sup>

Although the specific mechanisms by which prebiotics and probiotics exert their effects remain loosely defined, it is likely they function largely by improving gut health and may provide stability in some conditions of critical illness during the use of antibiotics and decreased enteral intake.<sup>171,172</sup> For example, at the most basic level, specific probiotic organisms and prebiotic substrates induce favorable shifts in intestinal microbiome composition, which outcompete pathogens for growth and mucosal attachment.<sup>173,174</sup> Similarly, certain prebiotics and probiotics may improve the GI barrier function directly by promoting goblet cell mucus production and secretion and release of selective antimicrobial products that protect the mucosa from adherent pathogenic microorganisms.<sup>175-180</sup> Since pathogens can influence absorption and ion balance at the mucosal interface, alterations in microbiome composition can result in diarrhea or bloating.<sup>181</sup> Probiotics also improve digestive function through increased nutrient availability and absorption, the production of short-chain fatty acids that fuel colonocytes, and the synthesis of vitamins K and B<sub>12</sub>, thiamin, and riboflavin.<sup>182-184</sup> Vitamin deficiency has been accelerated for B and K vitamins in germ-free or antibiotic-treated rodents, suggesting the microbiome makes significant contributions toward the hosts status of these compounds.<sup>185-187</sup>

Recent interest has also focused on the immunomodulatory properties of pre- and probiotics, potentially through the GALT, which contains up to 70% of immune cells in the body.<sup>188-192</sup> The use of prolonged PN reduces the number of Peyer’s patch and lamina propria lymphocytes, mucosal Th2 cytokines, the mucosal secretory immunoglobulin-A (sIgA) transport protein, and polymeric immunoglobulin receptor (pIgR).<sup>193-196</sup> These effects result in reduced luminal sIgA and loss of the major limb of specific immunity in GI luminal fluid. sIgA is anti-inflammatory through bacterial opsonization, which prevents bacterial mucosal attachment. The presence of sIgA inhibits expression of virulence factors by gut bacteria.<sup>197</sup> Certain prebiotic and probiotic mixtures augment tissue anti-inflammatory cytokines and improve macrophage activation and antigen presentation, normalizing parameters of GALT function and stimulating the release of sIgA on the gut lumen.<sup>198-200</sup>

The study of pre- and probiotics and their effects on human health is complicated by the extremely complex interactions that take place at the host-microbial interface. The human intestine contains up to 100 trillion bacterial cells, with the potential to influence expression of >600,000 genes in addition to their effects on GALT function.<sup>201</sup> Furthermore, microbial diversity and composition among individuals are highly variable and depend on environment, diet, genetics, the source of

colonization at birth, and the patients’ global health. Despite these variables, 3 bacterial phyla dominate the microbiome composition: Bacteroides, Firmicutes, and Actinobacteria.<sup>202,203</sup> Common probiotics include species from the Firmicutes and Actinobacteria phyla.<sup>201</sup>

Clinical trials investigating the effects of pre- and probiotics have studied synbiotic mixtures. Collectively, studies to date have resulted in inconsistent outcomes in general ICU settings, likely due to the variable effects that individual bacterial species exert on the host.<sup>47</sup> In addition, the use of synbiotic mixtures is advised against in pancreatitis, where their use has been associated with worse outcomes. However, there have been promising results with synbiotic formulations in some patient populations, including transplant,<sup>204,205</sup> major abdominal surgery,<sup>206</sup> and trauma patients,<sup>207,208</sup> in whom treatment reduced overall infection and intestinal permeability. Also, certain prebiotic soluble fibers alone may benefit stable critically ill patients with diarrhea by improving bifidobacteria levels,<sup>209</sup> since low levels of this organism are associated with diarrhea. Due to these discrepancies in general ICU patients, no recommendations are currently given by A.S.P.E.N. or CCCNG for the use of probiotics, prebiotics, or synbiotics in critically ill patients.<sup>41,47,48</sup>

## Nucleotides

Nucleotides are small molecular weight compounds found in all cells that are used to synthesize RNA and DNA. During rapid cell growth and proliferation, nucleotides can become limiting if de novo synthesis and scavenging pathways do not compensate.<sup>210,211</sup> Under normal conditions, pancreatic enzymes digest dietary RNA from plant and animal food sources and recover approximately 2%–5% of ingested RNA.<sup>212</sup> Supplementing nucleotides can reduce the necessity of de novo synthesis. Low nucleotide availability can have deleterious effects on immune function since effective adaptive mediated immune responses require rapid cell proliferation. Mice fed nucleotide-free diets have significantly reduced Th2 immune responses, including antibody production to T-lymphocyte-dependent antigen processing.<sup>213</sup> In contrast, nucleotide availability shifts lymphocyte populations toward Th2 cytokine profiles, as opposed to Th1, and enhance macrophage-lymphocyte interactions, which support adaptive immune response.<sup>214,215</sup> Laboratory studies using enteral nucleotides have also demonstrated beneficial outcomes on GI parameters, including increased villus height, mucosal protein, and brush-border enzymes.<sup>216</sup> Functionally, nucleotide supplementation limits bacterial translocation and maintains the mucosal barrier in rodent studies.<sup>217-219</sup> Clinical studies have not examined the effect of nucleotides alone in critical patients; rather, nucleotides have been added with other nutrition support compounds, including glutamine, arginine, and  $\omega$ -3 fatty acids. Those studies found no effect on mortality but did demonstrate benefits on LOS and infection that were most



evident in the malnourished. There are currently no recommendations for the use of nucleotides in critically ill patients.

## Appropriate vs Inappropriate Monitors of Nutrition Status

A thorough history and physical examination, including recent body weight and dietary changes, remains the most important monitor of nutrition therapy. Nutrition status is a balance between preexisting nutrition status and the degree of hypermetabolism and time until consuming an adequate diet. In patients undergoing elective general surgery with no inflammation, a low baseline serum albumin level correlates with increased complications when studied in large patient populations.<sup>220</sup> However, as the magnitude of surgery increases, smaller drops in serum albumin are needed for increased complications. Since patient outcome results from the interactions between the extent of preexisting malnutrition, the magnitude of injury, and the degree and duration of hypermetabolism, several techniques to quantify the degree of stress have been studied, including measurement of the metabolic rate, assessing the nitrogen losses vs nitrogen intake, the level of serum cytokines, and sequential measurements of serum protein levels. Each technique has a drawback. For example, the metabolic rate is a measurement of oxygen consumption and CO<sub>2</sub> production. Unfortunately, in the most critically ill or injured patients, these measurements are not valid if chest tubes are in place, a high fraction of inspired oxygen or high levels of positive end-expiratory pressure are used during mechanical ventilation, or there are air leaks around a tracheostomy or an endotracheal tube. Nitrogen balances are flawed in that nitrogen administered is overestimated while nitrogen loss is underestimated. Quantifying nitrogen losses by assessing levels in stool or open abdominal wounds is almost always inaccurate. Levels of serum cytokines ignore the local production of these individual cytokines and more likely measure spillover from local sites into systemic circulation. For example, in 1992, Ohzato et al<sup>221</sup> measured IL-6 levels following aortic surgery and noted a peak in IL-6 levels at 24–48 hours, which returned to normal by 72 hours. However, Baigrie et al<sup>222</sup> harvested simultaneous splanchnic and systemic samples simultaneously during similar surgeries and showed that portal levels of IL-6 were significantly higher than the systemic levels. The relevance of this is that hepatic protein synthesis is directed by IL-6 to switch from constitutive to acute-phase protein production, providing elevations in serum fibronectin, CRP, and  $\alpha$ -1 acid glycoprotein while driving serum albumin and prealbumin levels lower. IL-6 levels within the splanchnic circulation correlate with CRP levels, the degree of injury, and the metabolic rate.

We studied sequential serum constitutive and acute-phase protein levels on days 1, 4, 7, and 10 in a study of trauma patients randomized to enteral and parenteral feeding.<sup>223</sup> Serum albumin and  $\alpha$ -1 acid glycoprotein dropped immediately and

remained low, unresponsive to recovery. CRP and prealbumin recovered as the patient improved, suggesting their use as a monitor of recovery. A lower infection rate occurred in enterally fed compared with parenterally fed patients. The protein responses were evaluated relative to the presence or absence of infection by group. Serum albumin levels in infected patients in both groups were significantly lower than their uninfected cohorts and remained depressed even in uninfected patients for the entire 10-day period of study. Prealbumin levels dropped by day 4 compared with day 1 in both groups but recovered in uninfected patients in either group. CRP levels gradually dropped over the 10 days in uninfected patients regardless of the route of feeding but remained elevated in all infected patients. These results confirmed that following injury, serum albumin and  $\alpha$ -1 acid glycoprotein are not useful since outcome—good or bad—does not affect them. Sequential prealbumin and CRP levels appear to discriminate between infected and uninfected patients. These responses, however, do not reflect nutrition status but rather the presence or absence of complications.

The appropriate monitor for nutrition status in critically ill patients is unknown. Preoperative albumin may be a useful marker in large populations to predict their likelihood of infection, but the relevance to an individual is less precise. A thorough history and physical examination, by an informed clinician, is probably the best predictor of nutrition status.

## Conclusions

The concept of pharmaconutrition advocates investigating the effects of pharmacologic doses of individual nutrients on immune function and clinical outcomes, rather than “cocktail” formulations. The various dosages, durations, and efficacy of nutrient supplementation make this an extremely difficult and expensive undertaking, especially given heterogeneous patient populations and the need for a plethora of treatment arms. However, significant clinical evidence suggests that specific nutrients can improve clinical outcomes when provided to the select patient subpopulations. Updated systematic reviews of the current clinical data and recommendations are available at <http://www.nutritioncare.org/library.aspx> and <http://criticalcarenutrition.com> through A.S.P.E.N. and CCCNG, respectively.

## Discussion

**Robert Martindale:** So, for our recommendations we could say that albumin in the postop setting is useless. In the preop setting albumin still may be of some predictive value but only as a surrogate marker of outcome and not a true marker of nutritional status.

**Kenneth Kudsk:** Some people do come in severely malnourished and their albumin levels are normal but if you give them a liter or two of fluid and dextrose, it plummets.

## On Glutamine

**Paul Wischmeyer:** The ReDox trial taught us who is helped, who isn't helped, and who is harmed by dipeptide glutamine. You did not separate out the surgical patients with glutamine and PN, but the clinical data are very positive for glutamine in PN for postoperative surgical patients. If you provide PN for a prolonged period of time, you probably impair the immune system and the gut in particular. Providing glutamine probably helps. We added the data from the Ziegler GLND trial to our meta-analysis and there still was a positive effect in hospital mortality with glutamine in PN. We have 11 new trials since the last derivation of the Canadian guidelines and Ziegler's GLND trial was one of them. Ziegler's trial was only 150 patients, so it is not definitive by any means, and it excludes the major body of patients who we think benefit. If you go back to old trials in surgery patients with cancer who received glutamine, more than 50% of those trials in the critical care setting showed benefit. If you take out the cancer patients, those people were hurt. ReDox patients, none of whom had cancer, were harmed potentially. That makes physiologic sense because the cancer patient has less glutamine and is a greater malnutrition risk. So I think we need to be thinking about our surgery patients differently: cancer vs no cancer.

**Robert Martindale:** And so just for the group knows, the ReDox trial evaluated antioxidants and glutamine. The REDOX trial had 1200 patients, a big trial not yet published, but the data are available. The Ziegler NIH-funded trial is finished and not published yet.

**Steve McClave:** Several recent glutamine studies were negative: the Wernerman, Signet, ReDox, and GLND NIH trials. My question is should we not focus so much on the parenteral, but rather focus instead on enteral glutamine and resuscitating the gut? Should the focus shift to using enteral glutamine clinically to prepare the gut for enteral feeding?

**Paul Wischmeyer:** We must understand the parenteral glutamine trials using PN. We should not count ReDox. ReDox was an unusual study using both an enteral and parenteral combination. It was given in a manner completely exclusionary of enteral nutrition. In fact, there is statistical data from post hoc analysis that if you don't get fed enteral calories adequately that glutamine is bad for you. Giving glutamine in exclusion of other nutrients is bad for you. Similarly, fish oil in exclusion of other nutrients didn't work in the Omega study. I don't think we are losing ground in the study of parenteral glutamine.

**Daren Heyland:** I am struggling with the applicability of this conversation to the elective surgical patient because all of those studies you mentioned, including ReDox, are the sick critically ill patients. There are a lot of patients undergoing elective surgery getting parental nutrition, who don't apply at all to our multi-organ failure people.

**Robert Martindale:** Marco Braga's study involved cancer patients.

**Paul Wischmeyer:** That was a preop study.

**Robert Martindale:** Ziegler's GLND study was elective cardiovascular surgery.

**Paul Wischmeyer:** Ziegler's subjects were sick ICU patients. Ziegler's GLND trial was all surgical ICU. There is a large body of data for IV glutamine that is beneficial in the non-critically ill surgical patient.

**Leah Gramlich:** My question relates to scope. Identifying preop, intraop and postop strategies is needed to achieve our goal. We need to ensure that every major surgical patient has the opportunity to receive appropriate nutrition intervention. We need to think about levels of complexity. The number of patients in an ICU is a fraction of the number of patients receiving surgical care. We want to have the biggest impact on the greatest number of surgical patients. And we should strive for simplicity. I personally think we need to know how to package this immunonutrition discussion. It is confusing enough in the critically ill patient population where well-designed, prospective randomized intervention trials have been conducted to ask explicit questions. We are making lots of extrapolations that I think is going to be a huge barrier to uptake at the level of the system and the provider. The vast majority of surgeons and anesthesiologists out there are totally old school. If you talk to them about arginine and glutamine you are going to lose the audience. Don't fast them preop and start simple feeding postop. I think we need to scale our message to the audience. Target this discussion to achieve the most benefit for the maximal number of surgical patients.

**Marco Braga:** I would like to ask about the unpublished trial on glutamine. Because I will speak about glutamine during my presentation, I would mention the A.S.P.E.N. Guidelines from 2009 and the results of the multi-center study in Italy that we published with surgery in over 400 patients. We showed no benefit at all. So, I am interested about which kind of population was involved in these studies; were they undernourished, elective cancer patients? What was the timing and dose of glutamine that they gave?

**Paul Wischmeyer:** There are 2 trials that I think are being referred to here, GLND and ReDOX. Ziegler's GLND trial had 150 patients, so it was a relatively small trial. They looked at surgical ICU patients who did not have renal failure or liver failure and were not in shock when they were enrolled, but they had to be in the ICU.

**Marco Braga:** And why were they in the ICU?

**Paul Wischmeyer:** They could have been in the ICU because they had shock or because they had postoperative complications. The glutamine wasn't started until the shock resolved.

But they couldn't have active renal failure until about midway through the trial when we added CVVH patients. If you were on CVVH actively you could get this intervention. But you couldn't have renal failure without CVVH, and you couldn't have chronic renal failure coming in. So these were patients without organ failure for the most part who were getting glutamine. They could not have cancer, or that could not be their primary diagnosis or their admitting surgical reason. They got about 20 grams of glutamine a day IV for the period that they had to be on PN. So they could have entered the study 4 days into the ICU, 10 days into the ICU or 40 days into the ICU stay, whenever they got started on PN. They excluded all of the cancer patients. The ReDox study included very sick patients. The evidence that we have still supports that the elective surgical patient who receives postoperative PN is benefited from glutamine. I think the cancer patient undergoing elective surgery, in particular, is more likely to benefit with the data that we have. The other question is whether they should receive a probiotic.

### On Probiotics

**Paul Wischmeyer:** One trial was the probiotic trial in pancreatitis that resulted in more deaths with the probiotics. We can explain the pancreatitis trial. That trial failed miserably because of very huge methodologic flaws.

**Robert Martindale:** Why don't you summarize it quickly?

**Paul Wischmeyer:** That trial was unique. The Propatria trial included patients with severe pancreatitis who were fed with a postpyloric tube in the jejunum with a probiotic mixture that has never been tested in a human. The probiotic consisted of 9 different organisms, many of which had not been given a human therapeutically. It was also given with fiber.

**Robert Martindale:** Soluble and insoluble?

**Paul Wischmeyer:** Yes. There were 3 things about that trial that are worth mentioning. First, if you talked to the surgeons who operated on the patients who died, they found a bezoar of fiber near the feeding tube surrounded by dead gut. Their motility was slowed. Instead of giving the probiotics like all of the other trials did by giving it by mouth and feeding into the stomach, they gave it postpyloric. So with stasis and poor motility, the bacteria probably sat there and proliferated. We don't know what these bacteria did in humans, but they probably used that fiber as a culture medium and killed the gut. Second, the trial had an interim analysis to evaluate mortality. The *BMJ* review of that trial indicated that there was a signal of potential harm in one group and benefit in the other. They should have stopped that trial, but didn't. The *BMJ* article thus brought up ethical concerns. Third, if you look at the *HRQ*'s review of all 622 probiotic trials done in humans, that pancreatitis trial is the only one to show risk to a human. So there are some unique issues about the Propatria trial.

**David Flum:** Could you clarify the probiotic study positives?

**Paul Wischmeyer:** The trials weren't always positive for benefit, but they didn't show risk. There actually was a signal in the critically ill patients of benefit of probiotics reducing adverse events. In this massive review, the *HRQ* looked across all of the trials in humans at any age. The signal shown in this analysis indicated that there was no risk at all except for that one Propatria trial.

**Robert Martindale:** I think the problem with that trial was an infusion method and delivery issue. I think the data are superb for use of probiotics in a surgical patient, both preoperatively for prevention and immediately postop in the population of patients who get antibiotics. I think the probiotic area is one that we can go after. The problem with the probiotics is that the literature is full of quackery. Multiple different organisms are used, of which we know very little. We know that there are probably many organisms that are superb probiotics, such as *Lactobacillus reuteri*, *plantarum*, *casei*, *rhamnosus*, and *GG*. Those are the big 5 for which we have the data in the form of prospective randomized clinical trials published as of May 2012. Out of 11,800 patients with antibiotic-associated diarrhea, about half represented a surgical population. Prophylactic probiotics significantly decreased antibiotic-associated diarrhea. Results were published just last month in *Critical Care Medicine*. So we have very large trials in large surgical populations, but we have got to be careful with our definition.

**Paul Wischmeyer:** If we are going to make recommendations, I don't know if we can make any around specific pharmacconutrients for surgical patients. If we are going to make recommendations, then I think we need to be very thoughtful about what the ReDox study and Ziegler's GLND trial have taught us. We can make recommendations, but we need to be precise.

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