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# Protein Kinetics and Metabolic Effects Related to Disease States in the Intensive Care Unit

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## Abstract

Evaluating protein kinetics in the critically ill population remains a very difficult task. Heterogeneity in the intensive care unit (ICU) population and wide spectrum of disease processes creates complexity in assessing protein kinetics. Traditionally, protein has been delivered in the context of total energy. Focus on energy delivery has recently come into question, as the importance of supplemental protein in patient outcomes has been shown in several recent trials. The ICU patient is prone to catabolism, immobilization, and impaired immunity, which is a perfect storm for massive loss of lean body tissue with a unidirectional flow of amino acids from muscle to immune tissue for immunoglobulin production, as well as liver for gluconeogenesis and acute phase protein synthesis. The understanding of protein metabolism in the ICU has been recently expanded with the discovery of how the mammalian target of rapamycin complex 1 is regulated. The concept of "anabolic resistance" and identifying the quantity of protein required to overcome this resistance is gaining support among critical care nutrition circles. It appears that a minimum of at least 1.2 g/kg/d with levels up to 2.0 g/kg/d of protein or amino acids appears safe for delivery in the ICU setting and may yield a better clinical outcome. (*Nutr Clin Pract*. 2017;32(suppl 1):21S-29S)

## Keywords

proteins; amino acids; critical illness; intensive care unit; metabolism; anabolic resistance; rapamycin complex 1

Traditionally, nutrition support for the hospitalized population has focused on delivery of adequate energy. The importance of protein has only recently again surfaced as a major factor in providing "adequate" nutrition therapy for the critically ill patient. When parenteral nutrition (PN) became routinely clinically available in the 1970s, the ability to deliver adequate energy was no longer an issue. In those early days of PN, it was incorrectly presumed that caloric delivery was the answer to malnutrition, and the importance of protein delivery was lost in the rush to supply "hyperalimentation." Described by his work with traumatic long bone fractures, Sir David Cuthbertson clearly exposed the extensive loss of lean body tissue that was associated with bedrest, finding that the loss of lean body tissue was greater than that which would result from only local inflammatory response of the fracture. Cuthbertson appropriately concluded that bedrest and relative immobility associated with facture therapy resulted in a systemic effect that induced excessive nitrogen loss following bone fracture.[1] Despite this seminal work almost 100 years ago, it was not until the 1990s, when Frank Cerra presented the concept of "septic autocannibalism," that attention returned to the importance of nitrogen delivery in the critically ill patient.[2]

## Background

Essentially, all body protein is functional, with no storage form of protein available (with the exception of a small amount in the gut that is available between meals). Skeletal muscle is the largest pool of protein available and thus serves as the primary source of amino acids for synthesis of acute phase proteins, production of

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immunoglobulins, and support for gluconeogenesis during times of stress and catabolism. In the intensive care unit (ICU) setting, total-body total protein synthetic rates change little, while a dramatic increase in protein degradation is seen. Within the muscle compartment, the accelerated protein degradation is uniformly distributed between cellular proteins having contractile function and mitochondrial proteins.[3] Protein metabolism is a large consumer of daily energy expenditure, and it has been estimated that protein metabolism in catabolic states is responsible for approximately 15%–20% of the total resting energy expenditure.[4]

Most recent studies and major nutrition and critical care societies recommend between 1.2 and 2.0 g/kg/d of protein for ICU patients.[5] Despite these widely accepted guidelines, a large observational trial reported only about 0.6 g/kg/d of protein is actually delivered to the ICU patients.[6] The investigators also reported better outcomes in those patients who received the higher protein levels within the study.

The ICU sets the patient up for the "perfect storm" of massive muscle protein loss by immobilizing an already catabolic patient, essentially unloading the muscle, leading to a decrease in muscle protein synthesis, with an increase in muscle degradation and apoptosis. This deleterious combination of events subsequently leads to a decrease in force of contraction and muscle mass. Classic studies by Plank and Hill[7] and Plank et al[8] demonstrated that up to 16% total-body protein is lost at 21 days, with 67% of that loss coming from skeletal muscle. ICU-acquired weakness (ICUAW) has been well studied and has both a neurogenic and muscular component.[9] The studies reviewing ICUAW and the large quantity of muscle loss are confirmed by others showing the rapidity of muscle loss in the ICU.[10] Many factors contribute to ICUAW, including immobilization, hyperglycemia, corticosteroids, neuromuscular blockade, and systemic inflammatory conditions.[11] In addition to the factors associated with ICUAW, the heterogeneous nature of the ICU population must be taken into consideration, including age, sex, body habitus, pre-ICU diet, nutrition state, and genetic variability. A recent prospective trial of 63 critically ill adults (the majority with sepsis), all expected to stay in the ICU for 7 days and require mechanical ventilation for at least 48 hours, used serial ultrasound of the rectus femoris as well as muscle biopsies to follow muscle protein loss. At 7 days, ultrasound demonstrated a 10% decrease in muscle cross-sectional area, histological analysis reported a 17.5% decrease in muscle fibers, and the ratio of muscle cellular protein to DNA ratio decreased 29%.[12] Interestingly, significant inflammatory changes were noted in the muscle biopsies at the completion of the study. These patients were being fed during the period covered by the study, although the protein level was felt to be suboptimal at approximately 0.6 g/kg/d.[13] It appeared that some muscle groups degraded at an accelerated rate compared with others. Jung et al,[14] using a volumetric computed tomography (CT) method, recently reported that core muscle groups, including the diaphragm, were seen to lose volume at an accelerated rate compared with peripheral muscles.

## Protein Kinetics in the ICU—A Confusing Picture

Factors that influence the protein and amino acid (AA) kinetics in human physiology are numerous and can be best evaluated by attempting to break them down into body compartments. The complexity of the kinetics goes up almost exponentially in the critical care setting, when various organs may show increased synthetic rates, while others show increased catabolic rates. Total-body protein kinetics will require consideration of processes, such as luminal mucosal uptake, hepatic uptake from portal circulation and systemic circulation, endothelial cell uptake, and end-organ uptake. Each of these compartments has endogenous regulators.[15] Variability of patients and the heterogeneity of the disease states add to the complexity of determining protein kinetics for ICU patients. Factors such as age, sex, body habitus, preexisting nutrition state, muscle loading, route of nutrition therapy, and even the patient's microbiome all play a part in determining total-body protein kinetics.[16–20] Extensive discussion of all of these factors is beyond the scope of this review. Only kinetics in specific disease states will be reviewed here.

In the clinical setting of the ICU, it is common to observe a decrease in protein or AA intake, which can be either intentional or unintentional. The unintentional low protein delivery is usually a consequence of the clinical condition, the aggressiveness of the ICU team regarding nutrition delivery, frequent nutrition provision interruptions, inadequate protein content within the formulas, and the ability to tolerate enteral nutrition (EN) or PN. The inflammatory state of the patient and the insulin resistance commonly associated with critical illness have been previously discussed. Muscle unloading and lack of resistance exercise are other major factors affecting protein kinetics in the ICU. Kinetics of protein and AA absorption depend on multiple transport systems. In the gut lumen, the epithelial transporters are all noted to be limited by the extent of mucosal compromise. The luminal protein transporter PepT1 is primarily responsible for the majority of luminal protein uptake and is noted to be the first transporter system to be influenced by mucosal compromise.[21,22] Interestingly, it is also the first transporter to return when function is restored. Little has been described in the literature about the effects of mucosal compromise and AA or peptide transport in ICU patients. In general, studies of cellular protein kinetics have shown that the intracellular transporters in both the cytosol and mitochondria are shown to be altered in the ICU, depending on severity of illness.[23]

Essentially every organ studied in evaluating protein kinetics in the ICU population, including the liver, kidney, brain, and muscles, has demonstrated alterations in AA transport systems associated with critical illness.[15,23] These changes are felt to be irrespective of substrate levels observed in most cases. In the plasma compartment, there is a balance between inflow

consisting of AA absorption, endogenous AA breakdown, and de novo synthesis and the outflow consisting of protein synthesis and AA oxidation. Plasma AAs are usually below normal in the hyperdynamic patient, with the exception of severe sepsis or septic shock, and in conditions where liver hypoperfusion is present. In these conditions, the plasma AA levels are widely variable. This decrease in plasma AAs does not result from a decrease in muscle AA release as previously discussed but is believed to be from the stimulated uptake and clearance from the serum into the core organs, primarily the liver. The overall picture of AA kinetics in the unfed critically ill catabolic patient is one of rapid protein turnover of muscle compartments to supply the splanchnic organs, primarily the liver for supporting gluconeogenesis, and the bone marrow, spleen, and lymph nodes to support immune function and immunoglobulin synthesis.[15,23,24] With this unidirectional flow of nitrogen substrate from muscle, it is not surprising that a large amount of data now supports the concept that poor outcomes result in a population of ICU patients with sarcopenia or low muscle mass (as demonstrated by axial imaging).[12,25] A reduced lean body tissue to adipose ratio on axial imaging at the level of the third lumbar vertebra has been shown to adversely affect outcomes for colorectal, esophageal, and pancreatic cancer, as well as lymphoma and hepatoma.[26–28]

Protein kinetics studies have traditionally been done with meticulous nitrogen balance studies.[29] These studies have offered a better understanding of total-body protein balance, but they have a number of drawbacks and give us essentially no clear information about mechanisms of variations in synthesis and degradation (ie, protein turnover). Without understanding mechanisms, the nitrogen balance studies limit any detailed understanding of protein kinetics.[30] A relatively recent addition to the study of protein kinetics in the ICU has been the use of indicator AA oxidation methods, using the stable isotopes of carbon-13 ($^{13}C$).[15]

## Regulation of Protein Synthesis

As early as 1975, reports in the literature suggested that not all AAs regulate protein metabolism in the same manner.[31] In subsequent years, leucine and branched-chain amino acids (BCAAs) were shown to be a primary anabolic stimulus for protein synthesis. In an effort to better understand the mechanisms of anabolic signals of the BCAA or leucine, it became clear that the mammalian target of rapamycin (mTOR) was a key regulator. Recently, mTOR has become the focus of studies evaluating cellular protein kinetics. The mTOR complex is currently considered the central regulator that integrates nutrient signals, anabolic growth factors such as insulin, cellular energy status, and the oxidative stress level of the cell. Although any of these regulators can alter rates of protein synthesis, adequate AA substrate is required for optimal activation of the complex, and the regulation is especially sensitive to arginine, leucine, and glutamine.[32] When the mammalian target of rapamycin complex 1 (mTORC1) is activated, it co-locates with the lysosome membrane, which is thought to be the ideal location to sense the energy state of the cell. Its co-localization also allows for a steady supply of AA, even in nonfed states when there is a lack of exogenous AA supply.[32] Another important participant in this regulation is the lysosomal AA transporter SLC38A9.[32] This transporter is often referred to as a "transceptor," as it serves as both receptor and transporter.[33] The recent advancement in understanding of protein and AA metabolism through mTOR, the "transceptor" SLC38A9, and other upstream and downstream regulators has opened the door to studying AA and protein kinetics in the critically ill patient.

Stable isotope studies in ICU populations have yielded information that allows some early conclusions about protein kinetics. The question that often arises is how much protein can be delivered before the AA oxidation increases, essentially giving an estimate of maximal level of AA to infuse before protein synthesis is optimized. Elaborate studies help answer this question. Liebau et al,[24] using whole-body net protein synthesis, as compared with level of protein delivery, reported that protein delivery ranging from 1–2.5 g/kg/d yields better protein balance at lower levels. Interestingly, when phenylalanine (Phe) oxidation is evaluated in these studies, delivery of up to 2.5 g/kg/d showed no significant increase in AA oxidation of Phe. In 2012, Dickerson et al,[34] using nitrogen balance data in trauma patients, showed a similar level of protein was required to reach nitrogen equilibrium. In a proof-of-concept study by Liebau et al[35] and a separate study by Berg et al,[17] both of which evaluated whole-body protein kinetics in critically ill patients using two levels of enteral delivery, the higher of two levels of protein provision was tolerated and yielded a more positive total-body protein synthesis. In an attempt to further answer the question of optimal protein delivery, Ferrie et al[36] conducted a randomized prospective study of 119 ICU patients that planned to compare 0.8 g/kg/d with 1.2 g/kg/d protein delivery. The primary outcome parameter was handgrip strength. Unfortunately, the patients actually received 0.9 g/kg/d in the low-protein group, and the high-protein group received 1.1 g/kg/d. As would be expected with the near-similar protein intake level, no differences were seen between the groups.

It can be partially concluded from these studies that protein delivery in the ICU patient is safe, and dosage levels of at least 2.0 g/kg/d, and possibly even higher, can be given without harming the ICU patient. Indeed, in some studies, delivery of higher levels of protein yields a more positive whole-body protein synthesis and/or nitrogen balance.

## Protein Delivery in Specific Disease Conditions

Delivery of protein to postsurgical ICU patients and patients with cancer has recently been evaluated in several studies. A trial of protein kinetics in patients with pancreatic cancer vs

healthy controls followed whole-body protein synthetic ability, protein breakdown, and net protein balance. This study revealed that with an anabolic stimulus, the patients with cachectic pancreatic cancer could respond to anabolic stimulus despite having higher levels of protein turnover.[37] The primary outcome of protein provision in patients with cancer was to decrease the rate of protein breakdown, rather than increase stimulation of protein synthesis, while controls showed both increased synthesis and decreased breakdown.

In sepsis, the complexity of protein kinetics is at a much greater level of magnitude than the nonseptic ICU patient. Sepsis is characterized by a dysregulated inflammatory and immune response secondary to an infection that leads to organ dysfunction(s) and catabolic signaling. This dysregulation is widely variable, depending on the stage of sepsis in the patient. Significant alterations in plasma AA occur, with several AA levels being decreased while others remain unchanged or are increased.[38] Su et al[38] followed 35 patients throughout an episode of sepsis or severe sepsis, reporting a wide variation in AA levels. As the disease progressed, AA levels could be elevated or decreased with little consistency. In addition, sulfur-containing AAs tended to decrease significantly as the severity of sepsis increased.[37] Elegant studies in large animal models report increases in hepatic extraction of certain AAs (such as leucine, lysine, glutamine, and proline) with endotoxin infusion, while absorption of AAs from the gut lumen remains unchanged.[39] As can be seen by these studies, no consistent AA and protein kinetics have been established for the septic population. It does appear that protein synthetic rates are within the normal range while the degradation rates are dramatically increased.[40] The diversity of the septic etiology and the individual patient response to the septic insult make any conclusions impossible. What can be gathered from currently available literature is that protein requirements of the septic patient are elevated over the less catabolic ICU patient and that the provision of at least 2.0 g/kg/d of protein is safe. Several recent studies have shown the safety and tolerability of nutrition supplementation in the septic patient.[41–43]

## Anabolic Resistance Altering Protein Kinetics in the ICU Population

Anabolic resistance is defined by a failure of normal anabolic stimuli to induce messenger RNA (mRNA) translation of cellular protein. This phenomenon is well described in the elderly, in whom a higher level of anabolic stimulatory AA signals is required for the same degree of protein synthetic outcome.[44] Anabolic resistance is believed to be driven by an insensitivity to leucine. It has been shown that stressed ICU patients have higher free intracellular leucine levels compared with non-stressed patients.[44] Factors that are thought to be the primary drivers of anabolic resistance include splanchnic sequestration of AA after a normal protein load, decreases in AA availability to the peripheral tissue (especially muscle), and a blunted response to normal anabolic AA stimulus.[45] Other partially involved mechanisms include insulin-induced changes in microvascular blood flow and attenuated insulin-induced decreases in protein catabolism.[44] These concepts are supported by a recent clinical study by Dickerson,[46] in which a significantly higher level of protein was required for the elderly ICU trauma patients to reach the same level of nitrogen balance as younger counterparts.

## What Nutrition Interventions Work to Protect Lean Body Tissue and Protein Kinetics?

Protein supplementation consistently enhances total-body protein synthesis when given in adequate levels to overcome the anabolic levels just discussed.[44] In the non-ICU patient, pulse-dosing protein multiple times per day yields better total-body protein synthesis than does continuous feeding.[23] It has been shown that during constant infusion of AAs, an initial rapid synthetic response is noted; then, over the next 3 hours, a latency is seen and protein synthesis returns to baseline synthetic rates.[47] Specific AAs, primarily leucine and BCAA, have been consistently shown to stimulate total-body protein synthesis. Based on the work by Cerra and others,[48] formulas in the early 1980s were designed to deliver high levels of BCAA and indeed showed an increase in protein synthesis. The enthusiasm for these formulae eventually waned, as they failed to show any clinically significant outcome benefit. Other metabolic manipulations have shown benefit in supporting a net positive protein balance, either by stimulating synthesis or by inhibiting breakdown, and these include glycemic control, the use of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) to attenuate the hyperdynamic response, and methods to support a healthy and stable microbiome.[49,50]

Probably the largest contributor to maintaining lean body tissue in the ICU setting is resistance exercise or preventing the unloading of muscle. Early prospective trials, comparing nutrition supplementation with and without very modest exercise, resulted in significant improvements in strength and gait speed.[51]

Several systematic reviews have now been conducted in the critically ill population, evaluating the benefit of early ICU-based physical therapy. The outcomes are mixed, with some showing significant benefit, while others show no benefit at all.[52] Protein supplementation in conjunction with early mobilization has consistently demonstrated positive effects.[53,54] Two recent studies would challenge the results of these earlier studies. In a randomized trial evaluating protein intake and resistance training in acutely ill medical patients, no major outcome benefit was shown, although this was a very small study with several methodological flaws.[18] In another randomized clinical trial of exercise rehabilitation following critical illness,

a 12-month follow-up concluded no benefit.[55] In both these trials, compliance with the exercise portion of the study was an issue. It is possible that routine electrical stimulation of muscle while the patient is immobilized may supplant exercise, particularly because compliance with mobilization in the ICU is marginal.[56]

## How Much Is Too Much Protein or AA Supplementation in the ICU?

Protein provision appears safe in ICU patients in ranges up to 2.0 g/kg/d, with several studies reporting a range up to 2.5 g/kg/d.[15,57] Several investigators believe that even higher doses may be beneficial in some conditions.[58] Maximal synthetic rates occur with a 20- to 30-g bolus, and even higher protein levels may suppress protein breakdown. The anabolic response to higher protein levels is the sum of the fractional synthetic rate plus the associated decrease in catabolism. Verbruggen et al[59] have shown up to 3 g/kg/d is safe in adolescents. Most of these levels have been studied in healthy individuals and not critically ill patients. Caution should be employed when using higher protein levels, and monitoring for clinical response may be required. The potential for harm from excessive protein or AA is generally from the delivery of protein without adequate energy sources from carbohydrate. Azotemia will interfere with cellular protein synthesis only when the level of serum urea nitrogen becomes excessive. Clinically, serum urea nitrogen may be tempered as patients are dialyzed or given renal support therapy prior to reaching uremia-inducing levels. If imbalanced AA solutions are given, whole-body protein synthesis can be altered, but this is not a usual clinical scenario. Altered mental status can be observed from excessive AA or protein in patients with compromised hepatic function. This is believed to result from aromatic amino acids being unchallenged for central nervous system entry and serving as substrate for the formation of false neurotransmitters. In the ICU patient with intact hepatic synthetic function, this mechanism is not an issue.

## Conclusion

In conclusion, the current literature suggests that critically ill patients can use protein or AAs to a level of at least 2.0 g/kg/d (and possibly higher). The previous belief that delivery of >1.5 g/kg/d would only result in increased AA oxidation does not appear to be the case. Supplementation of either parenteral or enteral AA or protein to overcome the anabolic resistance of critical illness is beneficial, as demonstrated by whole-body protein kinetics. AA kinetics can certainly be better described with a clearer understanding of mTORC1 complex and its regulators. Generalizations regarding protein kinetics in the ICU are difficult and very dependent on the tissue being evaluated, the mitochondrial bioenergetic function, the background energy substrate, and the oxidative state of the cell. Protein kinetics also depend on the route of delivery of AA or protein and the inflammatory state of the patient. The maximum or optimal amount of AA or protein in the ICU population remains elusive, primarily due to the ICU heterogeneity of the population and the variability of disease states encountered in the ICU. One must be cautious when making firm conclusions about protein and AA delivery in the critically ill, as there are few high-quality studies of protein kinetics. In addition, clinical outcome parameters, such as mortality, length of ICU stay, days on ventilator, and quality of life, are not consistently reported. Longitudinal ICU studies describing a biologically plausible impact of protein dose, type, and timing across various disease subsets are needed.

### Statement of Authorship

R. G. Martindale, P. J. M. Weijs, J. J. Patel, and S. A. McClave equally contributed to the conception and design of the manuscript; and R. G. Martindale drafted the manuscript. All authors equally contributed to the acquisition, analysis, and interpretation of the data; critically revised the manuscript; agree to be fully accountable for ensuring the integrity and accuracy of the work; and read and approved the final manuscript.

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## Discussion

**Jan Wernerman:** We did a long-term study, starting on day 20 in the ICU, but we didn't recruit very many patients. For the first 10 days, you have a profound catabolism. If you look later on, if you compare, say, days 10–20 with days beyond 30, then this levels out, at least among the people still living in the ICU. I think that the ones who can't cope with this have probably gone to pathology. I think it's important to recognize that the information we have from short-term studies is in the early phase of critical illness and as such isn't necessarily applicable in full strength later on in the ICU. This catabolic response levels off a little bit.

I'd like to comment about correcting amino acid patterns in the ICU. There is a recent publication by a Japanese investigator, Hirosa is his name. I don't know what type of intensive care they have in Japan, but mean stay was 44 days among these patients. There you could see in the plasma amino acid pattern that most amino acids were elevated. Elevation of several individual amino acids was basically predictive of a nonfavorable outcome. So when patients are in the ICU and we can't help them, you probably see a leakage of amino acids out into the plasma, which could be a predictor for impending adverse outcome. I think it's important to provide amino acids, but it's also important that they are utilized, that they are transported into the cells.

**Stephen A. McClave:** Two areas of comments. The first is on autophagy. We keep acting like this is a big mysterious black box. Peter and I wrote a paper that said that autophagy should not direct nutrition therapy. There were a couple of points we made. First of all, the other name for autophagy is type 1 programmed cell death. Type 2 is apoptosis, and nonprogrammed cell death is necrosis. Autophagy operates very early in critical illness. There are 2 forms that work: autosomal autophagy peaks at 24 hours and it's replaced by chaperone-mediated autophagy, which involves heat shock proteins. And it's pretty much over at 48 hours. So it operates very early in critical illness and it operates in mild to moderate severity. The worse the severity of critical illness, you move beyond autophagy and get into apoptosis and quickly into necrosis. So, it's hard for me to think that autophagy should direct a lot of our thinking because I think it is operative over such a short period of time.

The question I had was on quality of protein. How big of an issue is this? Several of our speakers have commented that the quality of enteral protein is better than parenteral because the parenteral may be incomplete. They talked about the value of animal based versus plant based, which just means that dairy sources (whey and casein) may be more valuable. Do we just simply provide what formula we have and add protein supplements? How big an issue is quality of protein, and should we have a hierarchy of recommendations—this is the best, this is the worst?

**Ryan T. Hurt:** I don't know the answer to that. I do a lot of PN. If the PepT1 transporter is affected in sepsis, then PN may be better than EN. Would that not in theory be better if you're able to deliver more amino acids by the parenteral route in sepsis states where you have an absorption problem because of the PepT1 transporter issue? The quality of the amino acids we give in the PN probably is not great, as far as high levels of glycine and that sort of thing.

**Robert G. Martindale:** Peter Furst had the classic article about 10 years ago, just before he passed away actually, that looked at the ICU PN solutions. He described a whole list of things we had too much of and a list of things we don't have, like glutamine. You need glutamine and peptides. There's no citrulline, so some of our very important amino acids would not be delivered based on solubility or inability to make appropriate peptide. We overload our PN solutions with things that are rapidly soluble and stay in solution over a long period, because it has to have a long shelf life. So we end up with inappropriate nonphysiologic PN solutions. Even today in the United States we cannot give glutamine peptide if we want to.

**Ryan T. Hurt:** Shelf life in the hospital is different. If you're compounding 12he PN right there in the hospital, why is it that we have to worry about shelf life? Is it just because of the solutions you're keeping in stock? For the hospitalized patient, why can't you compound a better solution than that?

**Robert G. Martindale:** Most hospitals in this country are still in the 200- to 300-bed range. For those patients, usually the PN is not compounded. They buy premixed formulas for PN. That has to have a shelf life of months for them to keep it in stock. When you've got to have something sitting on a shelf for a couple of months, it becomes a problem for making a complete and optimally physiologic solution.

**Peter J. M. Weijs:** I'd like to comment on a few studies you mentioned, the first one being the Puthucheary study, as it made quite an impact. You mentioned that muscle wasting was still going on with sufficient protein intake. But I don't think protein intake is actually mentioned in the paper, and I don't think it is 1.4 g/kg/d either.

**Robert G. Martindale:** You are correct, but the goals were for 1.2 or 1.4 g/kg/d.

**Peter J. M. Weijs:** Okay, but in the Nicolo paper, Charlene Compher states that they actually have a low intake, like 0.6 g/kg or 60 g/d.

**Daren K. Heyland:** We wrote a letter saying that they were talking about protein intake but didn't report it in the paper. Their response actually appears in a letter 2 months later, and it is 0.67 g/kg/d. So it's low.

**Peter J. M. Weijs:** That is an important statement, because it's not muscle loss based at 1.4 g/kg/d. The second study you mentioned, the EPaNIC study, is one in which people have concluded that they should withhold feeding for 5–7 days. But it is really a study of feeding on day 1 versus day 8. It's a high-glucose diet at an early stage versus nothing for a long period of time, with very low protein intakes even at day 7. This study does not tell you anything about day 6, 5, 4, 3, 2. So we have to be careful.

The third trial I'd like to mention is the Ferrie trial. I'm really impressed by their results despite a small difference in the intervention range, 1.1 versus 0.9 g/kg/d. They measured ultrasound and handgrip strength at day 7 (not at day 0 or 1), and they didn't blind the measurements. But still the outcome was an impressive difference, higher in the 1.1 versus 0.9 groups. So, while I was impressed, I don't know what it means. It's a small study and it's maybe biased, but if we could actually reach such differences in our patients, that would be enormous.

**Robert G. Martindale:** If only they would have used 0.8 to 1.2, or even 1.2 to 2.0 g/kg/d? With the same study, if they could have used even 0.8 versus 2.0, then they might have seen a difference. They had trends, small differences they showed in handgrip strength at discharge, or at 7 days. The only one that showed a difference was at 7 days.

**Peter J. M. Weijs:** One thing that impressed me in this study was that in their high-protein group, they actually managed to be slightly lower on the energy in the first few days. Maybe this early hypocaloric diet may be more beneficial for utilization of protein in this group of patients.

**Daren K. Heyland:** Bob, I appreciate the discussion on mTOR. Sometimes I think we're a little linear in our thinking that as we give more protein, it influences protein balance, it influences muscle, and it influences functional recovery. But is it not plausible that independent of that pathway, protein amino acids may have positive effects on outcomes, because you're manipulating the machinery that is driving inflammation and organ dysfunction, which is amino acid sensitive. So by modulating mTOR, for example, I can influence outcome independent of what happens to protein balance, muscle, and outcome. Is that a plausible inference?

**Robert G. Martindale:** I think so, and I fully agree with your comments.

**Saúl J. Rugeles:** I would like to stress some points with regard to this metabolic response of the patient. The metabolic or catabolic response of the patient and oxidation of protein, it's a process necessary in the response to injury of the patient. I am not sure that we have to fight against the catabolic response but rather support the catabolic response. In line with Daren Heyland's intervention, maybe we have to measure the result of our clinical and metabolic support of the patient in terms of clinical outcomes, not whether we can improve the muscle at the end of the disease.

But the high-protein advantage was demonstrated many years ago. Drs Long and Weaver demonstrated 20 years ago that if we give more protein to the critically ill patients, the outcomes are better. But at the turn of this past century, when we shifted from PN to EN, the protein dose was lower in EN because we are using formula with a fixed amount of protein to fit our patients. And if we analyze the latest evidence in the literature, most of the studies use very low doses of protein. For example, under feeding protocols, I will discuss in my talk later, the dose of protein was about 0.2, 0.4, 0.6 g/kg/d. This is a very low dose of protein, not only for ICU patients but for every healthy person here.

Then the other side of the problem is that it is not easy to give an adequate dose of protein through the enteral route. Delivering 30 g is a problem, absorption is a problem, and utilization of the protein is another problem. I think that the way that we are talking now to think more in protein dose and less in caloric dose is the right way to approach this kind of patient.

**Frederick A. Moore:** Bob, in the study about muscle wasting, you kind of glossed over the point that 40% of the patients had an inflammatory cell infiltrate and necrosis of the muscle. And there was another recent study that came out of Canada that looked at recovery after critical illness, performing muscle biopsies at day 7 after ICU discharge and 6 months later. The same thing was seen at day 7 after ICU discharge. There's a significant amount of inflammatory cell infiltrate, which would suggest that the muscle breakdown has nothing to do with the metabolic response but rather with innate immunity attacking the muscle. You can give all the proteins you want, but you're not going to affect the innate immune response.

The second comment I wanted to make was that back when Dr Alexander wrote this paper on burned children, it was all about giving aggressive protein. I was reading that paper the other day and I might have done a miscalculation, but it looked like they were giving these kids 5 g/kg/d. The children who weren't getting it, who didn't have the great response, were getting about 2.5 g/kg. Then there was another paper by Wolfe that came out about the same time where they were taking burned adults and comparing the delivery of 1.2 g/kg versus 2.4. Dr Wolfe does these elaborate muscle breakdown synthesis studies, and he showed that there wasn't much difference between 1.2 and 2.4 g/kg/d. They've subsequently done other studies showing that when you give more protein to burn patients, you don't really get more protein synthesis. So, I'm just wondering, is there a difference between kids and adults? And what is the upper limit at which you're not getting anything more in response, other than giving protein that's not going to be used for what you want it to be used for.

**Robert G. Martindale:** First of all, there's a huge difference between kids and adults. We had some pediatrician in the audience who indicated that the amount of protein the kids require

just to maintain is about 3 g/kg. How much do you give adults? Wolfe and Deutz have recently written some articles that have discussed this very issue. By increasing protein intake, it may be that we don't get any more synthetic machinery and we don't get more positive synthesis, but we do decrease breakdown after a certain point. They're arguing now that maybe in adults, even 3 g/kg/d might be reasonable in select populations.

**Frederick A. Moore:** I want to go back to this inflammatory cell thing. The other thing I forgot to mention in that study that was out of Canada was that when they looked at 6 months at the people who didn't hypertrophy their muscle, the problem appeared to be that the muscles had a decrease in satellite cells. And at the time I read that, I didn't know what a satellite cell was. It turns out that a satellite cell is sort of a resident stem cell. We all get a certain number of these satellite cells in our lives, and when they go away, there's no more muscle regeneration. That's what happens in aging sarcopenia. It looks like that is what happens in critical illness. For some reason, we deplete satellite cells. So again, if you don't have satellite cells, you can give all the nutrition you want, but you're not going to regenerate muscle.

There are more of these recent muscle biopsy studies that really challenge our fundamental understanding of what we're trying to do. What I've come to the conclusion now is, you have this acute inflammatory process that's going on, and I don't think we're going to be able to alter that at all. You have to wait until somehow that goes away, or you've got to take it away, and then you might be able to get people to start putting more muscle on. But they have to have satellite cells to do it.

**Robert G. Martindale:** I think this is exactly the issue. I believe this is why the elderly population is really at risk. When these big operations are done on people in their 80s and 90s, if they take a second hit, they're done, as you've seen. With young 20-year-olds, we can hammer away at them all day long, we may have all kinds of problems, and yet they'll still survive in most cases. But the elderly won't survive. And we ask why is that? Isn't the physiology the same? Perfusion to the muscle is the same. Provision of adequate nutrition is the same. It's just that the ability to tolerate second hits is dramatically compromised in the elderly population.

**Daren K. Heyland:** That's the linear thinking I was referring to. This may not be only about muscle and function and outcome. There still may be a benefit to amino acids because of their influence on the amino acid–sensitive pathways that modulate inflammatory end-organ injury. I don't want to come away from this conference stuck in a linear thinking that it's just about muscle and outcome. The fact that we can influence outcome independent of those pathways is my key point.

**Stuart M. Phillips:** There has been a lot of talk around the Deutz and Wolfe commentary, and I stress the fact that it's a commentary. The evidence for that is not out there. Bob is my mentor and I know Mick very well. I've argued with them that the main point, why they see this suppression of proteolysis, it's because it is at a whole-body level. But it's actually never been demonstrated. It happens at the muscle level. A lot of those acute turnover measurements are equitably sensitive to turnover, suppression, and breakdown of rapidly turning over gut proteins. If you go back to the literature and you look at the rate at which gut protein turns over, it's 50 times faster than muscle. So all you need to do is suppress it by a small amount to see a massive impact on whole-body proteolysis. What that really means in terms of a critical care outcome I'm not really sure. They are all short-term measurements focused around a tyrosine phenylalanine whole-body turnover measurement, and they are not or ever have been shown to be related to muscle.

**Robert G. Martindale:** I couldn't agree more. It does bring up the interesting point with regard to the whole concept of gut sequestration or what happens to protein in the gut. It may be that the turnover of protein there is very, very high. And they will clearly state that this represents whole-body protein turnover. They think that's the best measure. But we should be looking at the whole body, not just the muscle. We should look at what's happening systemically.