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Protein at the Speed of Life

Kevin Burke Miller, PhD
Minneapolis, MN
E-mail: kevin-1.miller@hotmail.com

Abstract

Dietary proteins are necessary for growth, maintenance and repair of the body. The basic protein requirements for maintenance in healthy populations are fairly well understood. However, age and illness may alter protein needs. Protein sources can be selected to better suit the needs of these populations to promote health. The structure of some proteins common in animal feedstuffs (e.g., animal byproducts) renders the proteins less readily bioavailable, but proteins can be modified to increase their utilization. The rate of dietary protein utilization by tissues, especially in the periphery, is linked to bioavailability at digestion, efficacy of amino acid signaling, and presence in the plasma for incorporation into tissues. There may be some advantages of providing proteins with different rates of absorption while optimizing the amino acid profile to deliver targeted benefits.

Glossary of Abbreviations

AAA: Aromatic Amino Acids
BCAA: Branched-Chain Amino Acids
eIF-4G: Eukaryotic Initiation Factors 4G
mTOR: Mammalian Target of Rapamycin
RDA: Recommended Daily Allowance
TAA: Total Amino Acid

Introduction

The role of dietary proteins and amino acids is multifaceted. Protein nutrition is often associated only with provision of the total protein content in the diet. The profiles of the constituent amino acids are overlooked. However, many health benefits may be obtained through the judicious selection of protein sources based on their amino acid composition as well as their form, i.e., intact versus modified proteins.

Protein Requirements with Age and Illness

Monogastric animals have dietary requirements not only for protein, but for the constituent indispensable amino acids. The relative proportion required for each of the indispensable amino acids is somewhat similar among humans, dogs and cats (Figure 1). Because of these similarities, sources of dietary protein that will meet the basic requirements for adult humans often meet the requirements for dogs and cats. Notable exceptions include methionine and taurine for cats and phenylalanine for dogs.

The protein and amino acid requirements can vary with age and health status.[3] Considerable research has been conducted in humans to assess the protein needs of the aging population. The Food and Nutrition Board's recommended daily allowance (RDA) for protein increases between childhood and adulthood, but remains constant between adults and the elderly. There is research, based on nitrogen balance, that suggests the requirements of elderly may indeed be similar to that of younger adults,[4] but increasing protein intake may be advantageous to attenuate the decline in skeletal muscle and thus contribute to improved functionality.[5]

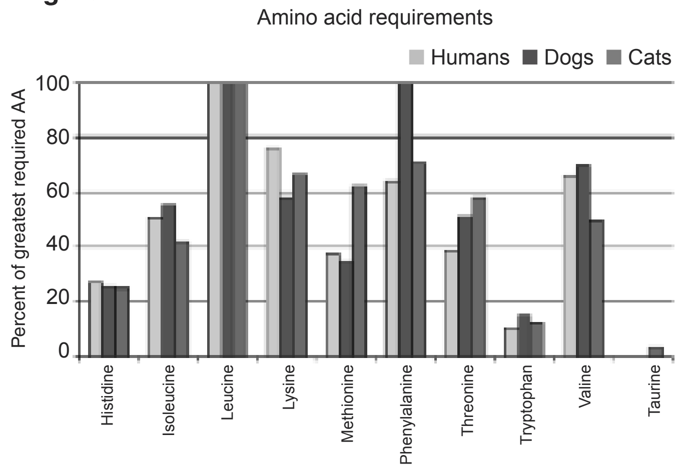
Despite a general agreement that some benefits can be derived from increased protein intake, the general statement that protein requirements increase with age does not necessarily reflect the fact that one protein source may offer different benefits than another. For example, Williams et al.[6] evaluated whole body protein turnover in young adult and geriatric dogs by feeding three levels of crude protein (16, 24 or 32%; chicken byproduct meal and corn protein). In the aged dogs, protein synthesis was greater with each of the three increasing dietary protein levels, but it was also found that protein degradation was equally increased (Figure 2). Therefore, overall whole body protein was not improved by increasing dietary protein despite strong stimulus of anabolism with increasing protein intakes. This raises the question whether it is the amount of protein delivered in the diet or the source of protein, including amino acid profile and digestibility, that plays a significant role in protein accretion.

Amino Acid Profiles of Dietary Protein

Dietary proteins are not created equal. The ratio of the indispensable to dispensable amino acids can vary widely between sources. Protein isolated from wheat typically contains only one-quarter indispensable amino acids in contrast to the whey fraction of milk protein that is often more than one-half indispensable amino acids. However, the value of protein is not solely dependent upon the ratio of amino acids. Wheat protein isolate may be low in indispensable amino acids, more specifically, it is low in the branched-chain amino acids (BCAA) (leucine, valine and isoleucine), but is abundant (~40%) in the amino acid L-glutamine. Glutamine is used as a fuel by rapidly proliferating cells, including intestinal epithelial and immune cells. This example serves to illustrate the importance of matching the strengths of a protein source with the intended benefits sought.

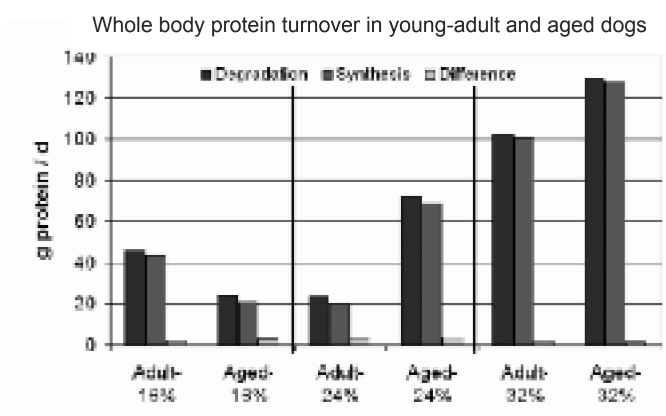
Physiological stresses may alter the needs for specific amino acids. It has been demonstrated that health status is reflected in the circulating amino acid profile. Chan et al.[7] recently compared amino acid concentrations in critically ill dogs to

Figure 1



The amino acid requirements for humans, dogs, and cats as a percentage of their highest required amino acid, leucine. Data from the Institute of Medicine of the National Academies, 2005, and National Research Council, 1985.

Figure 2



Whole body protein turnover in young-adult and older dogs fed one of three concentrations of dietary protein is basically unchanged despite increases in protein synthesis.

their healthy controls to evaluate the relationships among plasma amino acids, illness severity and clinical outcome. It was found that critically ill dogs had significantly lower concentrations of certain amino acids, including arginine, methionine, proline and serine, but significantly higher concentrations of lysine and phenylalanine (Table 1). This pattern resulted in a significantly lower Fischer ratio (molar ratio of BCAAs to aromatic amino acids [AAA]) in the critically ill group. Concentrations of arginine, isoleucine, leucine, serine, valine, total BCAA, and the Fischer ratio were significantly higher in survivors compared with non-survivors (all measures $P \leq 0.04$).

Because illness influences the circulating tissue amino acid profile, it would be reasonable to expect that the selective supplementation of specific amino acids would improve outcomes. Likewise, the provision of proteins that are enriched in one or more desirable amino acids is a topic under exploration. Currently, some of the amino acids most commonly used in clinical nutrition include glutamine for intestinal cell integrity; arginine for immune cell proliferation and insulin secretion; cysteine for synthesis of glutathione; and leucine for the stimulation of protein synthesis. Because leucine is of particular interest for the maintenance of skeletal muscle in an aging population, it will be explored more closely below.

Protein Digestibility

The benefits derived from dietary protein are not solely dependent upon the amino acid profile. The physical properties of a protein are also critical to the benefits it may provide. A casual review of the literature describing protein bioavailability reveals comparisons between sources common in the human diet, including egg, whey, casein, and soy. However, some common dietary proteins are limited in their nutritional value when used as the sole protein source. This limitation may be the result of the physical properties of the protein or even anti-nutritional factors naturally occurring in the source.

Maize is a common foodstuff in domestic animal diets. The protein fraction of maize is comprised of zein and glutelin proteins, which are relatively insoluble in their raw form. However, early research on the bioavailability of corn protein reported that heating did not improve digestibility or biological value.[8] The insoluble nature of zein can reduce the overall bioavailability of corn protein because of resistance to enzymatic degradation. In contrast, the proteins found in the whey fraction of milk (globular proteins: beta-lactoglobulin and alpha-lactalbumin) are highly soluble in the acid environment of the stomach. Because these proteins are suspended in the liquid fraction of the gastric contents, they are rapidly transported to the small intestine for hydrolysis and absorption. The rate at which these proteins exit the stomach and are then broken into easily absorbed peptides and amino acids is often described as being "fast" or "slow." Some approximate absorption rates of various protein sources are shown in Table 2.

Proteins and Amino Acids in Musculoskeletal Health

The combination of whey's rapid gastric emptying rate and high ratio of indispensable to dispensable amino acids results in the rapid appearance of amino acids in plasma, such as the beneficial amino acid leucine. The increase in the concentration of plasma leucine can act as a signal to stimulate protein synthesis (Figure 3) through activation of the mammalian target of rapamycin (mTOR) and/or the eukaryotic initiation

Table 1. Comparison between critically ill and healthy control dogs and among different subgroups of diseases within the critically ill group.

	Controls	All Critically Ill	*P*-Value[a]	Sepsis[b]	Pancreatitis[b]	Traum[ab]
n	24	48		23	14	11
Arginine	117.7	64.0	< 0.001	64.3	68.5	61.6
Glutamic acid	46.2	42.7	0.07	44.5a	49.5a	28.5b
Isoleucine	65.3	74.7	0.50	67.1a	101.0b	85.7a,b
Leucine	155.1	176.5	0.46	158.1	201.4	173.5
Lysine	163.6	186.9	0.02	168.0	232.5	202.5
Methionine	67.5	44.7	< 0.001	44.0	57.2	36.7
Phenylalanine	56.1	103.2	< 0.001	98.0	111.3	107.2
Proline	107.7	45.4	< 0.001	45.5	51.6	37.0
Serine	128.9	88.1	0.001	89.3	109.3	62.7
BCAA	407.1	465.2	0.24	425.4	538.7	465.1
Fischer ratio	3.9	3.1	0.001	2.9	3.6	3.0

Data are presented as median. Amino acid concentrations are in nmol/mL. BCAA, branched chain amino acid.
[a]Comparison of all critically ill dogs (n = 48) to healthy controls (n = 24).
[b]Comparison of values among different disease groups of critically ill dogs (i.e., sepsis versus pancreatitis versus trauma).
Data within a row with different superscript letters are significantly different.

Table 2: Approximate rates of absorption associated with common dietary proteins.

Protein	Absorption Rate (g/hour)
Whey Isolate	9.0
Soy Protein Isolate	4.0
Total Milk Protein	3.5
Pea Protein	3.5
Cooked Egg Protein	2.9
Raw Egg Protein	1.4

factors (eIF-4G). Leucine has been reported to stimulate protein synthesis while acting both independently of insulin[9] and with insulin.[10] The differences in the results were hypothesized to be the result of leucine's transient rise in the plasma. Following absorption, the circulating amino acids are taken up from the plasma and incorporated into tissues or oxidized. Therefore, a drawback of rapid protein digestion and availability is that the elevation of the amino acids in plasma is relatively transient.

The Rise and Fall of Amino Acids

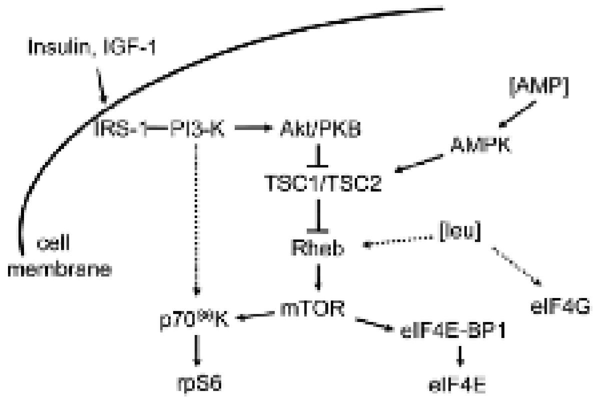
The concept of "protein speed" has been predominantly driven by performance nutrition. In the quest for optimal muscle mass and function, the dietary proteins are being researched to determine whether we can unlock more of their potential. The published literature focuses on the comparison between the two milk protein fractions, casein and whey. It is recognized that both protein fractions play an important role in recovery following bouts of exercise or stress. However, these comparisons and benefits may also hold true for other sources of dietary protein and physiological stresses, including aging and disease.

The comparison of amino acid concentration in the plasma among different dietary protein sources suggests that each has distinct advantages and disadvantages according to the rate of gastric emptying, absorption and amino acid profile. Lacroix et al.[12] evaluated three protein fractions: total milk protein, micellar casein and milk soluble proteins (i.e., native whey proteins isolated directly from milk) in healthy adult humans.

The BCAA concentration in those fed the milk soluble protein isolate quickly rose within the first hour after ingestion, but returned to baseline within four hours (Figure 4). The total amino acid (TAA) concentration in those subjects fed milk soluble protein isolate rose similarly to the previously reported BCAAs, but the TAA concentration dropped below baseline concentrations three to four hours after ingestion (Figure 5). The total milk protein, which is a naturally occurring combination of casein and whey proteins, did not induce a rise in BCAA or TAA concentrations to the extent of the milk soluble protein isolate, but it did maintain a consistent amino acid concentration for several hours.

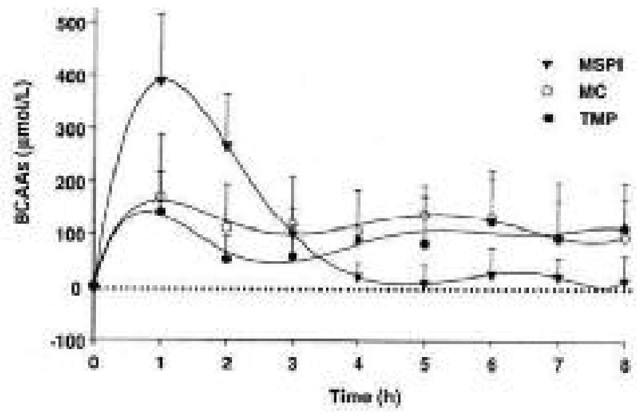
The likely reason the blend did not better replicate an average profile between the soluble milk protein isolate and casein is related to the relative proportion of each fraction in the total protein. The ratio of casein to whey protein in cow milk is approximately 80:20. As a result of this study, it is hypothesized that customizing blends of proteins to elevate plasma amino

Figure 3



The metabolic signal cascade integrating anabolic effects of growth factors, cellular energy status, and intracellular leucine (leu) to regulate protein translation. (Norton and Layman, 2006.)

Figure 4



The transient nature of the branched-chain amino acids from intact proteins (total milk protein) and its protein fractions (micellar casein and milk soluble protein isolate).

acid above a threshold concentration for amino acids necessary to optimally stimulate protein synthesis and also maintain this concentration for a longer duration of anabolism may be possible.

Protein Hydrolysates: Built for Speed

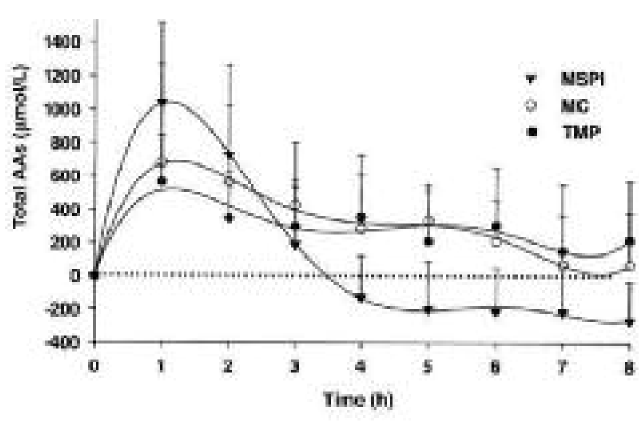
Protein type directly influences both the rate of absorption and rate of incorporation into tissue. The nutritional value of some protein types is reduced because of limitations caused by low bioavailability. Methods to overcome these limitations often include cooking and hydrolysis. Hydrolysis of proteins, typically through enzymatic activity (e.g., alcalase), has been used to modify proteins and aid digestion, especially if conditions of malabsorption are present. Not only are protein hydrolysates reported to be more easily digested and absorbed from the gut than intact proteins, hydrolysates also promote amino acid availability and improve muscle protein synthetic response.[13]

The rate of protein absorption is influenced by the degree of hydrolysis; even "slow proteins," such as casein, can, in effect, become "fast proteins." Koopman et al.[13] fed 10 elderly men either intact or hydrolyzed casein (13C-phenylalanine-labeled). The appearance rate of the 13C-label was nearly 30% faster following ingestion of the hydrolyzed casein as compared to intact casein. Plasma amino acid concentrations were increased (25 to 50%), and splanchnic extraction was lower following the ingestion of hydrolyzed versus intact casein.

Lastly, a trend (P = 0.1) for greater muscle protein synthesis rates in the hydrolyzed versus intact casein group was reported. These data suggest that high-quality slow proteins have all the potential benefits for maintenance of muscle as fast proteins, including accelerated protein digestion and absorption, with processing. In dogs, hydrolysis of soy protein also was shown to increase the rate of protein absorption,[14] suggesting that these effects apply across protein sources and across species. Therefore, hydrolysis of proteins may be a method to promote incorporation of dietary amino acids into skeletal muscle protein. In addition, hydrolysis also may improve the nutritional value of some lower "quality" proteins that are readily available.

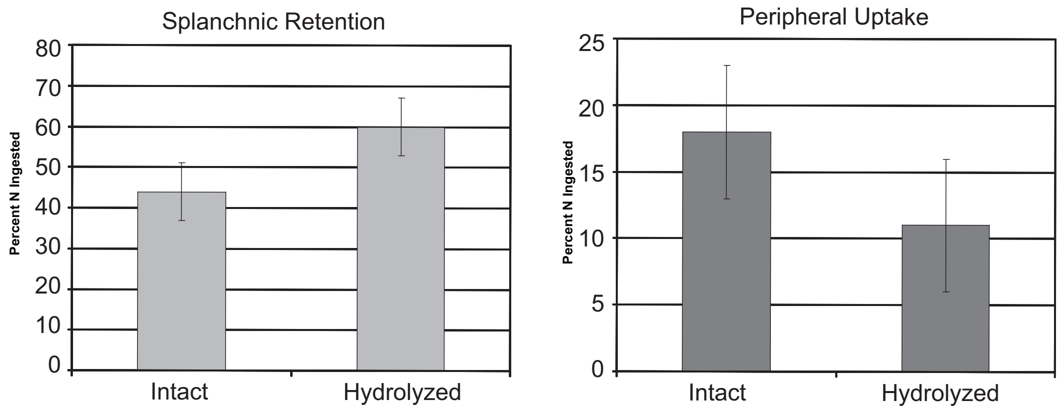
Deglaire et al.[15] recently reported that fast proteins may contribute less to peripheral protein anabolism as a result of greater splanchnic extraction than slow proteins. In healthy men consuming isotope-labeled intact or hydrolyzed casein protein, it was shown that hydrolyzed casein was absorbed faster than intact casein, which promoted both an early and stronger rise in plasma amino acids. However, this increase was associated with both an elevated anabolic and catabolic effect that reduced peripheral amino acid availability (Figure 6). Although not addressed by the authors, the results may

Figure 5



The rapid rise and fall of total amino acids concentration in the serum to sub-baseline concentrations in subjects fed milk soluble protein isolate (e.g., whey fraction).

Figure 6

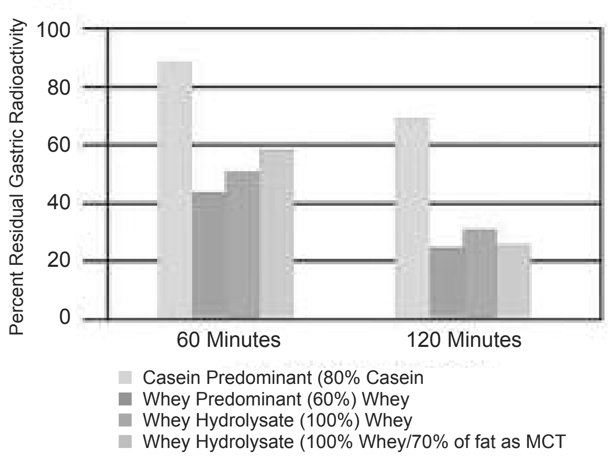


Nitrogen retention in the splanchnic tissues and peripheral tissues following ingestion of intact or hydrolyzed casein protein by healthy adults.

support the hypothesis that blends of fast and slow proteins are better utilized to deliver benefits including skeletal muscle anabolism. As previously described, the initial hyperamino-acidemia promoted by fast proteins may be helpful to reach a threshold for maximal stimulation of the cellular machinery for protein anabolism, but the slow proteins are likely necessary to provide a steady flow of amino acids for incorporation into tissues.

Protein speed is not only “fast” or “slow” but rather encompasses a range of different rates. The effect of whey hydrolysate (fast), intact casein (slow) and intact soy proteins (moderate) (balanced for equal indispensable amino acids) on muscle protein synthesis in healthy men was recently reported.[16] Ingestion of the fast protein whey resulted in greater indispensable amino acid concentrations (including BCAAs) in the circulation. The difference in protein synthesis following ingestion of whey versus casein was 93%, but protein synthesis with whey was only 18% greater than soy protein. This data suggests that soy proteins, even intact soy proteins, are relatively quickly absorbed. The advantages for protein synthesis were thus attributed to the rate of protein digestion and absorption. To suggest that differences in protein synthesis between whey versus casein and soy were the result of specific amino acids, such as leucine, are difficult to support as soy contains less leucine than casein.

Figure 7



The effect of hydrolysis of whey protein on gastric emptying.

Because whey proteins are readily digested and absorbed, it is not clear whether the additional step of hydrolysis would significantly increase absorption and offer a specific advantage in a healthy population. The differences in gastric emptying rates between intact and hydrolyzed whey have been previously reported. The residual amount of intact and hydrolyzed whey protein that remained after two hours suggests that their rates of digestion were not different (Figure 7). However, hydrolysis of fast proteins can still be advantageous when considering diets for those with compromised digestive/absorptive capacity as a result of illness or disease.

Conclusion

Sources of dietary protein have unique health benefits that are determined by properties including their respective bioavailability, rate of absorption and amino acid profile. The protein sources that are rapidly emptied from the stomach promote a condition of hyperaminoacidemia that can be useful to promote anabolism. The benefit of anabolism is especially strong when the amino acids that are elevated include a high percentage of leucine. However, it has been illustrated that the “fast” proteins are either incorporated into tissues or rapidly oxidized, which results in a transient benefit. In order to maintain protein synthesis with dietary proteins, it is recommended to explore protein blends. Blends of fast and slow proteins with complementary amino acid profiles targeted at delivering a specific health benefit may be useful in the maintenance of muscle mass in aging and disease.

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Q&A Discussion

Comment: Dr. Joe Millward, University of Surrey: Whey protein does have a very big profile. If you go into any gym, nine out of 10 supplements available are whey protein. And it's quite interesting to see where a lot of the hype has come from. It may well be that there are some very specific things about whey, and one of the possibilities that I have heard raised recently is that there may well be some sequences of whey protein that have absorbed as polypeptides and that are bioactive. I want to really make two points. First, on the issue of splanchnic uptake having an effect on the supply of amino acids to the periphery, the database that this effect changes with aging is more or less completely absent. You also mentioned one study that showed a decrease in peripheral utilization with fast proteins. What actually happens is the branch-chain dehydrogenase is very concentration-dependent so that the increased excursion of plasma and cellular branch-chain amino acids after whey compared with after casein increases the oxidation of the branched-chain amino acids. And, one of the consequence of this is, although you may well get a stimulation of protein synthesis because of the leucine effect, the actual available branch-chain amino acids for utilization is much less. So the overall effect of fast proteins needs to be judged, rather critically, in terms of what are the parameters that you are looking at. And ultimately, if you are going to talk about utilization, then utilization is the difference between what gets deposited and what gets oxidized. And fast proteins get oxidized more rapidly than slow proteins.

Q: Dr. Bob Backus, University of Missouri: I have a comment on the practicality of all this. Interesting idea, but as you know, pet foods deal with a matrix rather than single proteins. Fat varies, which affects gastric emptying and no doubt affects protein digestion. Another effect that I really came to appreciate is the processing effect and with the issue with taurine [where processing can reduce the bioavailability of taurine]. Now, as I recall, whey protein is fairly high in cysteine, which can form cross-links in a matrix. It may work great in a purified diet where there's no processing, but I wonder about the effects of processing. Now, my question: What is the energy ratio with respect to protein and fat? Does the portion of fat differ between fast versus slow proteins?

A: Dr. Miller: I think processing can be an issue, especially in regard to the human supplement industry that we have now. Whey protein may be very beneficial toward an aging human population if we're looking at just trying to increase protein synthesis. But trying to deliver large amounts of whey protein to an aging individual is incredibly complicated. As you mentioned, thermal processing causes the proteins to coagulate. It's very difficult therefore to be able to deliver that in any way that's easy to use by the consumer. And that's really what it comes down to in the end.

Q: Dr. Joe Wakshlag, Cornell University: You actually got me a little excited because we are always talking about supplementing our performance animals and with supplements we don't have to worry too much about a matrix. I think these have some implications for the performance arena. But I wonder if you can clarify the relative role of branched-chain amino acids, especially leucine. Does it really have that proanabolic effect on skeletal muscle once you put it in the system? What are your opinions on that?

A: Dr. Miller: I think Bob Wolfe, who was here earlier, is probably one of the best published on that topic. Leucine has been used as a supplement, and they have seen increases in protein synthesis. But I think the point that we have reiterated a few times is even though you may be increasing the amount of phenylalanine that's being taken up across the muscle, are we actually seeing anything that's significant? Are bodybuilders going to be able to associate their increase of maybe 7 grams of leucine with an accretion of muscle protein? And I think that's very difficult for us to do at this time.

Q: Dr. Brian Zanghi, Nestlé Purina Research: Dr. Rennie's group did a rodent study comparing isonitrogenous whey protein with, I think, soy protein. What they were seeing was that lean mass accretion was the same. But when they looked at protein expression, the phosphorylation of mTOR was increased over control in the soy diet but much higher with the whey protein. They attributed it to the elevated level of branched-chain amino acids. So, why can't you see the whole phenotypic response in lean muscle? I think that maybe there's a threshold that it hadn't gone over.

A: Dr. Miller: The work that I was involved in was actually in a cancer cachexia model with a tumor burden, so therefore it may not be applicable to other models because the pathways may be dissimilar. However, in our studies, each of the branched-chain amino acids was separately fed on top of the casein in standard AIN-93 diets. We saw there was actually better maintenance of muscle mass with those that were fed the leucine and valine, but not with isoleucine. And I agree, I think there's some evidence to show that it can have a benefit, but it's very difficult to demonstrate repeatedly.