From the Purina Companion Animal Nutrition Summit: *Focus on Gerontology*; Clearwater Beach, FL; March 26-27, 2010.

Dietary Protein Consumption in the Healthy Aging Companion Animal

Joseph J. Wakshlag, MS, DVM, PhD, DACVN
Cornell University College of Veterinary Medicine
Department of Clinical Sciences
Ithaca, NY
E-mail: jw37@cornell.edu

Abstract

The amount of dietary protein needed to optimize lean body maintenance has been an elusive topic in companion animal medicine. Work in humans and dogs suggests that there is at least a 50% increase in the dietary protein requirement in elderly dogs and potentially even more in those that are exhibiting age-induced sarcopenia, resulting in an adequate protein intake of 5.4 g/kg body weight. Cats, as true carnivores, may accrete lean body mass due to dietary protein well beyond the threshold for dogs, with assumed needs of at least 6 g/kg body weight if not more for healthy elderly adult cats.

Glossary of Abbreviations

AAFCO: Association of American Feed Control Officials
AKT: Protein Kinase B
BCAA: Branched-Chain Amino Acids
BIA: Bioelectrical Impedance Analysis
BW: Body Weight
CT: Computed Tomography
DEXA: Dual X-Ray Absorptiometry
DM: Dry Matter
EAA: Essential Amino Acids
eIF4E: Eukaryotic Initiation Factor 4E
FOXO: Forkhead Box O
IGF1: Insulin-Like Growth Factor 1
IL-8: Interleukins 8
IK-15: Interleukins 15
LBM: Lean Body Mass
MAP: Mitrogen Activated Protein
ME: Metabolizable Energy
MRI: Magnetic Resonance Imaging
mTor: Mammalian Target of Rapamycin
Nfκβ: Nuclear Factor κβ
NRC: National Research Council
PI3 Kinase: Phospho-Inositol 3 (PI3) Kinase
p31: Proteasome p31
PCR: Polymerase Chain Reaction

Goals of Protein Consumption

In veterinary medicine the goal of optimizing lean body mass (LBM) encompasses palliative treatment of chronic degenerative diseases, as well as optimal performance in the athletic and show arena. From a health perspective, LBM is adversely affected by multiple disease processes including renal failure, neoplasia, endocrinopathies (diabetes, hyperthyroidism and hyperadrenocorticism) and potentially chronic inflammation. All these maladies can alter LBM, often causing a shift toward catabolism of skeletal muscle, rather than anabolism, resulting in a gradual loss in LBM.[1,2] In the show and athletic arena, the implications are subtle with incorporation of diet strategies and training regimens that have the potential of enhancing skeletal muscle mass depending on the breed intended for show or the athletic event performed. The balance between skeletal muscle synthesis and degradation is primarily genetically controlled, but small changes may be achievable through alteration of diet or physical activity.[3,4]

The study of aging has revealed a number of changes in skeletal muscle from a biochemical perspective. Most of the age-related changes that occur have been studied in rodent models and humans, with sparse evidence in companion animals. In the past 20 years there has been implementation of more sophisticated mechanisms for measuring lean versus fat mass in experimental and clinical medicine, including dual X-ray absorptiometry (DEXA), bioelectrical impedance analysis (BIA), regional and whole-body computed tomography (CT), and magnetic resonance imaging (MRI).[5,6]

In companion animals, methods such as DEXA and BIA have been used; however, there are drawbacks to each procedure as both methods require sedation and rely on appropriate hydration and positioning.[7,8] Additionally, the use of BIA relies on precise body measurements, complex computer software and averaging of numerous measurements.[8] CT and MRI technologies have not been explored to assess lean body mass in companion animal medicine, as DEXA may be equally useful and is more accepted in the veterinary literature.[8-10] Traditionally, the evaluation of LBM has been related to overall fat mass in "before" and "after" weight-loss intervention studies in populations of dogs or cats where protein consumption may be important for maintenance of lean body mass.[11-13]

These findings in obesity underscore the importance of diet as it relates to LBM maintenance. It remains poorly understood in companion animal medicine how diet influences maintenance of LBM in aging dogs and cats. Age-induced sarcopenia is a burgeoning area of research in human medicine, as sarco-

penia plays a significant role in quality of life.[14] In veterinary medicine the idea of sarcopenia does exist since we have all seen the geriatric dog that loses weight after the age of 10 or the cat that loses weight but does not have a disease process that explains the phenomenon. The greater prevalence of this phenomenon in cats may be due to their different metabolic processes since they are "true carnivores" with higher overall protein requirements and a reduced inability to downregulate hepatic transaminase/gluconeogenic activity.[15,16] Molecular evidence in humans and rodents has provided a greater understanding of aging muscle that may be applied to cats and dogs, with subtle differences in cats due to their status as true carnivores.

The Biochemistry of Aging Muscle

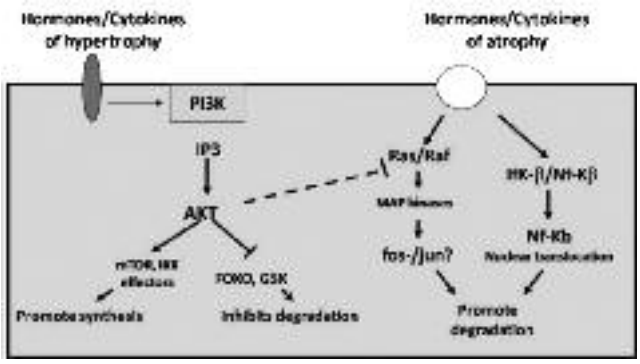
There are many theories related to the mechanism of sarcopenia, and the most attractive hypothesis revolves around the "mitochondrial theory of aging." As mitochondrial DNA mutations occur with age, mitochondrial function is diminished. This results in skeletal muscle myofiber dysfunction leading to an imbalance in muscle myofiber apoptosis and satellite cell regeneration.[17] However, there are a number of hormones and/or cytokines that have the ability to induce myofibril machinery synthesis or degradation that can also play a role. These endocrine/cytokine mediators and their effects on hypertrophy and atrophy have been shown to influence LBM in rodent models and primary culture systems.[1,18]

Hypertrophy and Atrophy

From a veterinary perspective, muscle hypertrophy has been a phenotype that is desired in agriculture and performance arenas. Selective breeding has perpetuated genetic mutations, such as the myostatin gene that makes the Belgian Blue cow "double muscled" for production and the Whippet run faster, that have become fully understood only in the past 10 years.[3,19] Myostatin's function is related to developmental hypertrophy mechanisms causing excessive satellite cell maturation and enhanced fiber size.[20,21] However, for animals without this desired mutation, other stimuli including insulin, insulin-like growth factors, androgens, cytokine/myokines, and serum amino acids are continually fluctuating to maintain balance between synthesis and degradation in the fully functional myofiber.[22,23] If chronic inflammation becomes part of the equation, then cytokines like tumor necrosis factor-α can tip the balance toward degradation leading to mild LBM loss.[18]

The molecular machinery involved in promotion of myofibril synthesis has been elucidated with a simplistic view depicted in Figure 1. Insulin signaling and other growth factors cause activation of heterodimeric or homodimeric receptors, which cause activation of phospho-inositol 3 (PI3) kinase. Once activated, PI3 kinase can activate protein kinase B, also known as AKT, which signals the mammalian target of rapamycin (mTor), which then activates transcriptional regulators s6 kinase and eukaryotic initiation factor 4E (eIF4E) binding protein to induce myofibril protein synthesis.[22,24,25] The phosphorylation of AKT is involved in the inactivation of forkhead box O (FOXO) transcription factors preventing nuclear transcriptional activity and inhibition of the mitrogen activated protein (MAP) kinases, stopping the synthesis of certain aspects of the proteolytic machinery. This system is constantly in flux and is thought to be part of the mechanism of LBM demise in diseases of LBM wasting, such as diabetes.[2,25]

Figure 1

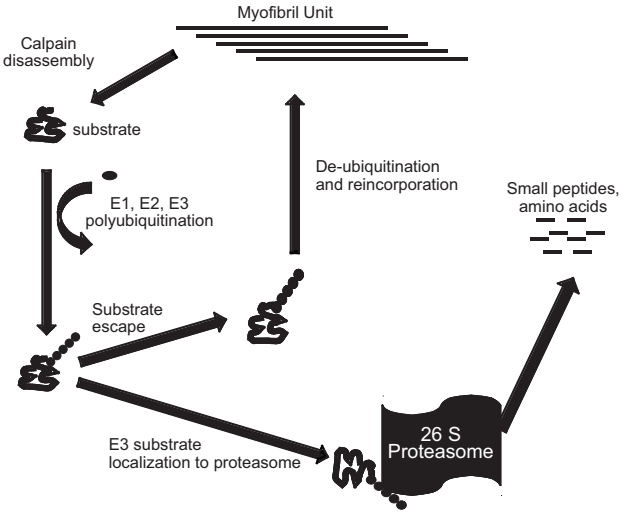


Depiction of the signaling events and signaling proteins of interest in hypertrophy and atrophy in skeletal muscle.

The degradation of myofibril proteins is equally complex as three proteolytic systems are involved in the destruction of a myofibrillar unit. A majority of the myofibril proteins are thought to undergo a distinct process of degradation that involves disassembly of the myofibril unit (dissociation of the myosin and actin from titin in **Z** bands). Disassembly involves either the caspase system, which is usually activated in disease states, or typical turnover involving the calpain system.[26] Two major calpains are thought to be involved; calpain M and calpain μ. These calcium-activated proteolytic enzymes liberate the myofibril proteins from titin, and this dissociation is a signal for the ubiquitin proteasome pathway to initiate the degradation process.[27] Once recognized by the ubiquitin-proteasome pathway, the protein is then degraded into small fragments that are further proteolyzed into amino acids or into small peptides for introduction to major histocompatability complexes that will be introduced to the cell surface, much like typical processing of viral and bacterial proteins for stimulation of the immune system.[1]

The ubiquitin-proteasome pathway has received a lot of attention due to its complexity and some highly specific skeletal muscle enzymes in this pathway (see Figure 2). Initially, when a protein-like myosin is liberated from the myofibrillar unit and recognized by a ubiquitin 3 ligase, the ubiquitin 1 and ubiquitin 2 ligases will polymerize multiple small ubiquitin proteins to specific lysine residues of the substrate protein to

Figure 2



Depiction of ubiquitin-proteasome dependant proteolysis of myofibril substrates.

create a polyubiquitin chain in a specific orientation. This polyubiquitin chain is then localized to the proteasome. The importance of this step cannot be understated, as two very important ubiquitin 3 ligase components specific to skeletal muscle, called MURF and atrogin, are highly upregulated during atrophy.[28] The ubiquitin enzymes are constantly competing against deubiquitination enzymes. Hence, if a substrate-like myosin is not ubiquitinated, theoretically it may be reincorporated into the myofibrillar unit and stall atrophy.[27] Once the polyubiquitinated substrate reaches the proteasome (a 33-subunit proteolytic complex), it is destined for degradation. The capping structure recognizes ubiquitinated substrate, while the core complex performs proteolysis.

The upregulation of this system is triggered by a variety of cellular signals or lack of signals. For example, a decrease in insulin will cause diminished AKT phosphorylation, therefore FOXO nuclear signaling proteins will cause transcription of ubiquitin 3 ligases MURF and atrogin.[2,28] Besides growth factors and hormones, cytokines have also been associated with stimulation of the nuclear factor κβ (Nfκβ), which has the ability to upregulate the ubiquitin proteasome proteolytic machinery as part of the LBM changes associated with chronic inflammation, and may also play a role in aging muscle due to the imbalanced activation of this pathway.[2,18]

Exercise and Cytokines

Exercise causes a complex interplay of signaling events, leading to enhanced skeletal muscle myofibril deposition, which involves mechanoreceptor stimulation, but also relies on increased insulin-like growth factor 1 (IGF-1) expression, decreased myostatin synthesis, and enhanced satellite cell differentiation signals post-exercise, tipping the balance toward anabolism and regeneration.[24] Exercise also enhances insulin-independent glucose transport and improved insulin sensitivity, promoting the hypertrophic pathways of PI3 Kinase/AKT signaling, leading to myofibril protein synthesis.[22] This has been the argument for exercise-induced hypertrophy, but we must remember that without counter-regulatory compensation of enhanced proteolysis, we would get uncontrolled growth. This principle was re-enforced with some recent work showing that, during active training of hunting Pointers, there was a net increase in the ubiquitin-proteasome proteolytic machinery from pretraining to peak training, likely due to enhanced synthesis and degradation of myofibril proteins.[29]

Apparently, the only type of exercise that can retard the sarcopenia of aging is concentric resistance exercise and not eccentric exercise, e.g., walking.[24] Concentric exercise includes weight training or isometric activities. In companion animals this type of exercise is not traditionally used in physical therapy regimens for elderly sarcopenic animals. Water resistance activities and use of specialized "backpacks" or specially designed attachable weights during certain activities may be a desirable approach during canine rehabilitation in elderly patients.

Cytokines must be mentioned again, but in this context they are termed "myokines," since exercising skeletal muscle may be releasing these myokines in a paracrine fashion to effect myofibril homeostasis.[30] Interleukins 8 (IL-8) and interleukins 15 (IL-15) seem to be highly expressed post-exercise. The function of IL-8 is not well defined, but some evidence suggests that it may be involved in helping regulate neovasculogenesis in skeletal muscle.[30] IL-15 has received considerable attention due to its anabolic capabilities through abrogation of myofibril proteolysis by the ubiquitin proteasome system during concentric exercise. IL-15 may also be involved in decreasing mature myofiber apoptosis as well as maintaining the myofiber in a hypertrophic state.[30,31] Additionally, IL-15 causes lipolysis in adipose tissue, inducing mobilization of fat stores and causing an increase in skeletal muscle lipolysis for energy. This may be a direct effect on these tissues, or it may be due in part to an increase in the release of adiponectin from fat tissue.[31]

Surprisingly, recent findings from our lab showed a modest decrease in serum IL-15 after a 350-mile race in sled dogs (unpublished data), which may seem counterintuitive. However, these dogs typically lose weight during endurance distance racing,[32] and mild muscle atrophy ensues due to the caloric needs during endurance racing, which may be the nidus for this mild decrease in IL-15. Regardless, the implication for IL-15 in muscle tissue maintenance and adipose tissue metabolism, particularly in age-related sarcopenia, suggests this may be an interesting pharmacologic target for improving LBM.

Dietary Protein and LBM in Dogs and Cats

Numerous studies have addressed the use of dietary protein to ameliorate the sarcopenia of aging in humans.[33] In companion animals there have been few studies investigating the use of enhanced dietary protein in the amelioration of LBM loss other than those centered around obesity and the maintenance of LBM during weight loss, which seems to be a successful strategy.[12,13] We are still trying to fully understand protein requirements in companion animals as they relate to LBM. For the past four decades research has revolved around nitrogen retention as the primarily means of determining protein requirements in dogs, which has led to the National Research Council (NRC) recommendations of a minimum of 1.2 g/kg body weight (BW) in adult dogs in appropriate body condition.[34]

These numbers are based on net nitrogen intake versus nitrogen excretion to establish concentrations needed for a net balance of zero in experimental settings for companion animals. What remains vague is whether the nitrogen contributing to the net balance equaling zero results in any changes in LBM since many of these studies rarely incorporated any measure of lean mass. It can be argued that there may be loss of LBM during slight protein deficiency while still meeting caloric requirements and achieving nitrogen balance. One of the reasons for variations in the apparent lower limit of protein requirement from different studies is the differences in protein quality, digestibility and quantity tested. For example, in some laboratory studies, dogs and cats may consume casein, soy or other single protein source diets, or purified diets, which may not be reflective of what companion animals are eating; therefore, there may be slight differences in the apparent dietary protein requirement.[16,24,35]

This concept of availability and source is highlighted in a study by Mauldin and colleagues wherein isocaloric parenteral nutrition was delivered to Beagles for an entire week to meet their maintenance energy requirements with either 0, 1.36 or 2.04 g/kg BW from an amino acid solution, hence meeting the 1986 NRC recommendations for all amino acids.[36] In both amino acid treatments there was some weight loss, but loss was worse in the no intravenous protein group. Regression analysis of this data suggested that 2.3 g/kg/day of protein, provided intravenously, would have been needed to maintain positive nitrogen balance. Whether this would have resulted in lean mass retention is unknown. This concentration of protein does not take into account potential losses due to digestion and enterocyte metabolism, thereby potentially increasing the amount of dietary protein needed during normal consumption. This raises the debate whether higher protein diets can subtly enhance the total LBM, and when switched to lower protein diets, there may be a catabolic shift that causes a decrease in LBM, while nitrogen balance equilibrates quickly, but at the expense of small amounts of LBM.

A partial answer comes from a study where adult dogs were transitioned from a 24% protein metabolizable energy (ME) diet, which supplied approximately 5 g/kg BW, to either 12% protein ME or 28% protein ME diet.[35] A modest decrease in DEXA-analyzed LBM in the 12% group was observed after 10 weeks on the diet. More interestingly, as the quality of the protein source changed in the four separate 12% ME (2.5 g/kg BW) diets from chicken based to noncomplemented corn gluten based, there was a progressive increased loss of LBM suggesting that amino acid imbalance or deficiency causes an exacerbation of LBM loss. In the 28% ME protein groups, the only group to show positive LBM gains was the group receiving the 100% chicken-based diet.

This increase in LBM seemed to correspond with increased muscle calpastatin (calcium regulating protein involved in inhibition of calpain induced proteolysis), and the 12% ME diets showed an incremental decrease in proteasome p31 regulatory subunit.[35,37] This decrease in p31 as well as the loss in LBM showed a significant linear effect suggesting that as the quality of protein became more imbalanced (insufficient lysine), the catabolic machinery decreased. This may seem counterintuitive, but the proteolytic components were examined 10 weeks after initiation of diets. Hence, LBM has likely equilibrated, and the proteolytic rate and synthetic rate have achieved balance; therefore, as synthesis decreases, so does the proteolytic machinery.[35] Unfortunately, the relative synthetic rate was not assessed in these dogs, which would have been equally, if not more, interesting.

The previously mentioned studies as well as a study by Wannemaker et al. suggest that the intake of protein (high quality) to maintain LBM is between 2.5 g/kg to 3.75 g/kg BW in adult dogs.[38] However, elderly canines required a greater amount of dietary protein (> 3.75 g/kg BW) to even approach repletion of reserves. Examination of leucine kinetics showed that elderly dog skeletal muscle does not synthesize protein at the same rate as young adult dogs and did not reach a plateau even at the highest protein concentration (3.75 g/kg BW), suggesting that elderly dogs may need even higher concentrations.

Enhancing protein intake in dogs is further supported by recommendations in elderly humans where it has been postulated based on experimental evidence that the typical 0.8 g/kg BW needed for the average human over 19 years of age should be increased to 1.0 to 1.3 g/kg BW in elderly humans at risk for sarcopenia, roughly a 50% increase.[33] If dogs are similar to elderly humans by needing up to 50% more dietary protein, this would be roughly 5.4 g/kg BW of a highly digestible, high-quality protein source. From a commercial standpoint, the Association of American Animal Feed Control Officials (AAFCO) guidelines state that a minimal protein requirement for adult dogs should be 18% dry matter (DM) protein in a 4 kcal/g diet, or 5.1 g/100 Kcal ME.

AAFCO does not provide separate guidelines for geriatric animals. If a low protein geriatric diet is chosen for the average

10 kg dog eating up to 200 grams/day, this translates into 36 g/day or 3.6 g/kg BW, which is close to the lower limit for appropriate intake established by Wannemakers and colleagues. This does not account for the potential decrease in maintenance energy requirements in geriatric dogs, which might have this same 10 kg dog consuming as little as 100 grams a day, underscoring the importance of using higher protein diets (24 to 30% DM) to maintain LBM in muscle-wasted elderly dogs.

The only evidence of the effects of dietary protein on LBM in aging dogs is from Kealy and colleagues.[39] They studied the effect of dietary protein in 8-year-old Pointers fed either a 16.5% protein ME or a 45% ME protein diet over a two-year period. The low protein group lost approximately 6.2% lean mass and increased in fat mass. The group of Pointers on a 45% protein diet exhibited a smaller change in LBM with only a 3.5% decrease, however, the initial diet fed was not reported, which may have influenced these findings. Although this work is not a definitive study to suggest a minimum concentration needed in the elderly canine, it does suggest that more work is needed in this area to address this conundrum, particularly in the lean mass-wasted elderly canine population. Pending no health implications due to high protein consumption, an elderly dog undergoing lean mass loss with age should get at least 5.5 g/kg BW.

Cats, as true carnivores, have higher dietary protein requirements. These requirements have been well-studied, particularly during growth, due to the work of Rogers, Morris and their colleagues at the University California-Davis.[15,34] The intricate differences in amino acid metabolism of cats results in a higher basal protein requirement, exceeding dogs and rats by nearly threefold. Once again, much like in dogs, there is relatively little information regarding the dietary needs of adult cats for maximal LBM. It has been established that there is a minimal need of around 2.5 g/kg BW to maintain nitrogen balance, but not for optimizing LBM.[16,34]

In the author's opinion the idea of sarcopenia in cats has a far greater implication than in dogs considering the aging cat can undergo lean body changes more readily, particularly in elderly cats greater than 12 years old. This change has been noted in large colonies of cats where LBM has been shown to decrease in conjunction with changes in protein digestibility, further emphasizing the importance of quality and quantity of protein in aging cats.[40] In veterinary medicine, this is extremely important since owners' decisions regarding euthanasia may take the "emaciated appearance" of the cat into consideration.

There have only been two studies examining the protein requirements in adult cats to maintain LBM.[41,42] In the first study, cats were fed nearly isocaloric diets with poultry, soy, fish and crystalline amino acids to meet amino acid requirements at 22%, 28% and 36% on a DM basis. All cats maintained a positive nitrogen balance after the two-month dietary trial, but only the cats on 36% DM protein managed to maintain their LBM, while, on average, the cats on the 28% and 22% protein diets lost LBM.[41] The original diet consumed before the dietary trials was a 36% DM diet when baseline assessments were made; therefore, it's evident that decreasing the amount of dietary protein has a negative effect on LBM. This higher dietary protein intake equates to an average of 5.2 g/kg BW, suggesting minimally a 100% increase in protein intake is needed to optimally support LBM above the amount needed to maintain nitrogen balance.

The second study examined the effects of two isocaloric diets that were approximately 4 kcal/g and designed somewhat similarly to the diets in the previous study but based primarily on animal proteins. These cats were previously fed a 36% DM protein diet and were switched to diets at 30% DM and 53% DM protein in a crossover design. Interestingly, when cats were on the 30% protein diet, they lost about 1.2% LBM, and the cats on the 53% protein diet averaged an accumulation of 4.2% LBM.[42] This change in LBM is not surprising as nitrogen balance studies in cats have shown nitrogen retention and oxidation of protein for energy in cats as dietary protein is increased, and a distinct plateau in nitrogen retention is not always observed.[16,34] Therefore, the importance and amount of protein intake in cats for maximal retention of LBM has yet to be defined, but it is clear that some cats may benefit from high-protein diets well beyond the NRC requirement and feeding normal healthy cats above 6 g/kg BW may actually be ideal when age-induced sarcopenia is considered clinically.

Diet and Exercise: Are All Protein Sources Created Equal in the Aging Patient?

The use of whey protein has become popularized in the human athletic arena particularly for building lean tissue mass. Numerous studies have shown that athletes undergoing resistance training gain more LBM when supplementing this protein either pre-exercise or post-exercise.[43] This is primarily due to the rapid digestion and elevation of serum essential amino acids (EAA) compared to comparable proteins like casein, which are digested slower and do not cause high serum EAA. Additionally, whey protein has a high essential amino acid content compared to other protein sources and a high branched-chain amino acid (BCAA) content (25%), adding to its proanabolic attributes.[43] Current evidence suggests that this rapid elevation in serum EAA causes a burst in muscle protein synthesis and a diminished or unaltered proteolytic response.[44-47]

There have been equivocal results regarding the use of protein to maintain LBM in the elderly, which may be due to the prosynthetic properties being severely blunted compared to younger adults or athletes, so there is debate as to the amount of supplementatal protein needed.[33,44] To further confound these studies is the fact that timing of supplementation (during or between meals) and amounts often differ across studies; therefore, it is evident that more work needs to be done with

standardization of supplementation regimens.[33,44-47]

When using essential amino acids, the BCAAs may be somewhat unique in that they can preferentially bypass splanchnic metabolism for delivery to skeletal muscle where metabolism (gluconeogenic) or incorporation into protein becomes preferential.[48] If protein intake is sufficient, much of the BCAA pool reaches skeletal muscle, where the most abundant BCAA, leucine, may have independent cell-signaling capabilities to augment protein synthesis. It has been shown *in vitro* and *in vivo* that leucine supplementation not only enhances insulin signaling but also independently will promote protein synthesis in skeletal muscle.[49,50,51] Most recent clinical research into the use of BCAA enrichment, similar to essential amino acid supplementation, seems to show a positive correlation between retention or stimulation of LBM, but a recent study using leucine in an elderly population showed no effect on leg mass or strength.[50-52]

As previously stated, these discrepancies may be related to dosages, timing of supplementation, basal energy intake, and basal protein intake. More often than not, interventions that preserve LBM or promote small gains show no appreciable gains in strength,[47,52] which is the final clinical outcome of importance to the client or patient. As work in human sarcopenia is continually unfolding, it remains hard to translate these findings to dogs and cats as the protein requirements differ dramatically, and the incidence of sarcopenia in dogs and cats may differ as well. Sophisticated tools such as easily accessible and rapid screening using DEXA and CT will make this type of research easier to perform in companion animals. Now that many of the pertinent hormones and signaling proteins are known in the dog and cat, these clinical imaging endeavors can be coupled with similar small needle biopsy techniques used in people to perform quantitative polymerase chain reaction (PCR) and phospho-protein assays to better understand the milleu of events occurring in sarcopenic companion animals, resulting in better treatment options.

References

1. Mitch WE, Goldberg AL. Mechanisms of muscle wasting. *New Eng J Med*. 1996;335:1897-1905.

2. Szewczyk NJ, Jacobson LA. Signal-transduction networks and the regulation of muscle protein degradation. *Int J Biochem Cell Bio*. 2005; 37:1997-2001.

3. Mosher DS, Quignon P, Bustamante CD, et al. A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genetics*. 2007;3:779-786.

4. Knoopman R, vanLoon LJC. Aging, exercise and protein metabolism. *J App Phys*. 2009;105:2040-2048.

5. Muller MJ, Bosy-Westphal A, Kutzner D, Heller M. Metabolically active components of fat-free mass and resting energy expenditure in humans: recent lessons from imaging technologies. *Obesity Rev*. 2002;3:113-122.

6. Lee SY, Gallagher D. Assessment methods in human body composition. *Curr Opin Clin Nutr Metab Care*. 2008;11:566-572.

7. Elliott DA, Backus RC, Van Loan MD, Rogers QR. Extracellular water and total body water estimated by multifrequency bioelectrical impedance analysis in healthy cats: a cross-validation study. *J Nutr*. 2002;132:1760S-1762S.

8. Son HR, d'Avignon DA, Laflamme DP. Comparison of dual-energy x-ray absorptiomentry and measurement of total body water content by deuterium oxide dilution for estimating body composition in dogs. *Am J Vet Res*. 1998;59:529-532.

9. Freeman LM, Kehayais JJ, Roubenoff R. Use of dual-energy x-ray absorptiometry to measure lean body mass, body fat and bone mineral density in dogs and cats. *J Vet Intern Med*. 1996; 10:99-100.

10. Mawby DI, Bartges JW, d'Avignon A, et al. Comparison of various methods for estimating body fat in dogs. *J Am Anim Hosp Assoc*. 2004;109-114.

11. German AJ, Holden S, Bissot T, et al. Changes in body composition during weight loss in obese client-owner cats: loss of lean tissue correlated with overall percentages of weight loss. *J Fel Med Surg*. 2008;10:452-459.

12. German AJ, Holden SL, Bissot T, et al. A high protein high fiber diet improves weight loss in obese dogs. *Vet J*. 2009;180: 114-118.

13. Vasconcellos RS, Borges NC, Goncalves KNV, et al. Protein intake during weight loss influences the energy required for weight loss and maintenance in cats. *J Nutr*. 2009;139:855-890.

14. Van Kan, A. Epidemiology and consequences of sarcopenia. *J Nutr Health Aging*. 2009;13:708-712.

15. Morris JP. Idiosyncratic nutrient requirements of cats appear to be diet-induced evolutionary adaptations. *Nutr Res Rev*. 2002;15:153-168.

16. Green AS, Ramsey JJ, Villaverde C, et al. Cats are able to adapt protein oxidation to protein intake provided their requirement for dietary protein is met. *J Nut*. 2008;1053-1060.

17. Carmeli E, Coleman R, Reznick AZ. The biochemistry of aging muscle. *Experiment Gerentol*. 2002;37:477-489.

18. Pajak B, Orzechowska S, Pijet B, et al. Crossroads of cytokine signaling—the chase to stop muscle cachexia. *J Physiol Pharmacol*. 2008;59:251-264.

19. McPherron AC, Lee SJ. Double muscling in cattle due to mutations in the myostatin gene. *Proc Natl Acad Sci*. 1997;94:12457-12461.

20. Kollias HD, McDermott JC. Transforming growth factor-β and myostatin signaling in skeletal muscle. *J Appl Phys*. 2008;104:579-587.

21. Bradley L, Yaworsky PJ, Walsh FS. Myostatin as a therapeutic target for musculoskeletal disease. *Cell Mole Life Sc*. 2008;2119-2124.

22. Spangenburg EE. Changes in muscle mass with mechanical load: possible cellular mechanisms. *Appl Physiol Nutr Metab*. 2009;34:328-335.

23. Solomon AM, Bouloux PMG. Modifying muscle mass — the endocrine perspective. *J Endocrinology*. 2006;191:349-360.

24. Sakamoto K, Goodyear LJ. Intracellular signaling in contracting skeletal muscle. *J Appl Phys*. 2002;93:369-383.

25. Tisdale MJ. Is there a common mechanism linking muscle wasting in various disease types? *Curr Opin Support Palliat Care*. 2007;1:287-292.

26. Bartoli M, Richard I. Calpains in muscle wasting. *Int J Biochem Cell Bio*. 2005; 37:2115-2133.

27. Hasselgren PO, Menconi MJ, Fareed MU, et al. Novel aspects on the regulation of muscle wasting in sepsis. *Int J Biochem Cell Biol*. 2005;37:2156-2168.

28. Murton AJ, Constantin D, Greenhaff PL. The involvement of the ubiquitin proteasome system in human skeletal muscle remodeling and atrophy. *Biochim Biophys Acta*. 2008;1782:730-43.

29. Wakshlag JJ, Kallfelz FA, Barr SC, et al. Effects of exercise on canine skeletal muscle proteolysis: an investigation of the ubiquitin-proteasome pathway and other metabolic markers. *Vet Ther*. 2002;3:215-225.

30. Klarlund B, Akertrom TCA, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Phys*. 2007;103: 1093-1098.

31. Argiles JM, Lopez-Soriano FJ, Busquets S. Therapuetic potential of interleukin-15: a myokine involved in muscle wasting and adiposity. *Drug Disc Today*. 2009:14;208-213.

32. Hinchcliff KW, Shaw LC, Vukich NS, Schmidt KE. Effect of distance traveled and speed of racing on body weight and serum enzyme activity of sled dogs competing in a long-distance race. *J Am Vet Med Assoc*. 1998;213:639-644.

33. Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. *Clin Nutr*. 2008;27:675-684.

34. Rogers Q. Protein and amino acids. In Beitz DC (ed): *Nutrient Requirements of Dogs and Cats*. National Academy Press, Washington DC. 2006:111-144.

35. Wakshlag JJ, Barr SC, Ordway GA, et al. Effect of dietary protein on lean body wasting in dogs: correlation between loss of lean mass and markers of proteasome-dependent proteolysis. *J Appl Anim Phys Nutr*. 2003;87:408-420.

36. Mauldin GE, Reynolds AJ, Mauldin N, Kallfelz FA. Nitrogen balance in clinically normal dogs receiving parenteral nutrition solutions. *Am J Vet Res*. 2001;62:912-920.

37. Helman EE, Longerhan EH, Davenport GM, Longerhan SM. Effect of dietary protein on calpastatin in canine skeletal muscle. *J Anim Sci*. 2003:81:2199-2105.

38 Wannemakers RW, McCoy JR. Determination of optimal dietary protein requirements in young and old dogs. *J Nutr*. 1966;88:66-74.

39. Kealy RD. Factors influencing lean body mass in aging dogs. *Compendium*. 1999; 21:34-37.

40. Perez-Camargo G. Cat Nutrition: What is new in the old? *Compendium*. 2004;26:5-14.

41. Hannah SS, LaFlamme DP. Effect of dietary protein on nitrogen balance and lean body mass in cats. *Vet Clin Nutr*. 1996;3:30.

42. Nguyen P, Lerray V, Dumon H, et al. High protein intake affects lean body mass but not energy expenditure in non-obese neutered cats. *J Nutr*. 2004;134:2084S-2086S.

43. Cribb PJ, Hayes A. Effect of whey protein isolate on strength, body condition and muscle hypertrophy during resistance training. *Curr Opin Clin Nutr Metab Care*. 2008;11:40-44.

44. Paddon-Jones D, Short KR, Campbell WW, et al. Role of dietary protein in the sarcopenia of aging. *Am J Clin Nutr*. 2008;87:1562S-1566S.

45. Volipi E, Kobayashi H, Sheffield-Moore M, et al. Essential amino acids are primarily responsible for the amino acid stimulation of muscle protein anabolism in healthy elderly adults. *Am J Clin Nutr*. 2003;78:250-258.

46. Sebastiano SB, Gazzaruso C, Bonacasa R, et al. Nutritional supplements with oral amino acid mixtures increases whole-body lean mass and insulin sensitivity in elderly subjects with sarcopenia. *Am J Cardiol*. 2008;101:69E-77E.

47. Paddon-Jones D, Sheffield-Moore M, Urban RJ, et al. Essential amino acid and carbohydrate supplementation ameliorates muscle protein loss in humans during 28 days of bed rest. *J Clin Endocrinol Metab*. 2004;89:4351-4358.

48. Harris RA, Joshi M, Jejuna NH, Obayashi M. Overview of the molecular and biochemical basis of branched chain amino acid catabolism. *J Nutr*. 2005;135:1527S-1530S.

49. Anthony JC, Anthony TG, Kimball SR, Jefferson LS. Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. *J Nutr*. 2001;13:856S-860S.

50. Katsafanas CS, Kobayashi H, Sheffield-Moore M, et al. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids. *Am J Physiol Endocrine Metab*. 2006;291:E381-E387.

51. Riau I, Balage M, Some C, et al. Leucine supplementation improves muscle protein synthesis in elderly men independent of hyperaminoacidemia. *J Physiol*. 2006;575:305-315.

52. Verhoeven S, Vanschonbeek K, Verdijk LB, et al. Long-term leucine supplementation does not increase muscle mass or strength in healthy elderly men. *Am J Clin Nutr*. 2009;89:1-8.

Q&A Discussion

Q: Dr. Claudia Kirk, University of Tennessee: You made reference to the Wannamacher (1966) study in your talk. In that study, they used casein, and at the very low end of the protein concentrations that were fed, some of the sulphur amino acids as well as arginine would be deficient. Then, how do we interpret those data in light of incomplete protein sources?

A: Dr. Wakshlag: That is a huge conundrum because a lot of the data that's been generated has used casein or soy-based diets, so you have a completely different amino acid profile than what is found in whey, or the proteins in dog and cat food. So, can you extrapolate any of that data and say that it truly does mean that we need this amount of protein? I agree that we do have some areas of potential data deficiencies.

Q: Dr. Robert Wolfe, University of Arkansas: My question relates to obesity in cats, and if cats are getting obese with old age, and if that's a problem. One of the things that I was kind of surprised by when I got into this research in geriatrics in the last few years is the fact that geriatricians stop worrying about being overweight after the age of 65 and really don't recommend weight loss in the elderly under almost any circumstance. Under certain circumstances, for example, congestive heart failure, obesity or at least being overweight seems to actually be beneficial in providing protection. So, in cats, is it a problem if they're getting fat, is it something you want to do something about, or is this something that is a protective mechanism?

A: Dr. Wakshlag: In the cat world we have a huge problem with type 2 diabetes just like in people. In the veterinary arena, we're always worried more about things like diabetes than sarcopenia, even though I think maybe we should be worrying just as much about sarcopenia. Owners will make an end-of-life decision based on their cat's unthrifty appearance. I think the main point is that it really comes down to screening the geriatric patient to make sure we don't have all these other problems and then making your decision about whether this slightly plump cat really needs a weight-reduction plan. In cats that do need to lose weight, I think we have enough data to say that their protein needs to be increased.