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AN INSIDE OUT PERSPECTIVE

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Premise of Systems Microbiomics in Improving Health and Related Diagnostics for Human and Companion Animals

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Abstract

Systems microbomics — a comprehensive metabolic and microbiome phenotyping — has a high medical and public profile, as exemplified by the exponential growth of research publications and numerous lay press stories on microbiome-derived metabolites, probiotics and related topics. Research into the function of the host-microbiome interactions and the development of new microbiome-based nutritional products and therapeutics is a new horizon. The enormous diversity, functional capacity and age-associated dynamics of the host metabolome and microbiome, its association with nutrition, health maintenance and diseases ranging from localized gastroenterological disorders to inflammatory, metabolic and hepatic illnesses, make it a priority area of research and development at Nestlé and Nestlé Purina. Routine metabolome and microbiome analysis is poised to become a standard measure in following an individual's health status as well as measuring biomarkers for detecting or managing diseases.

Glossary of Abbreviations
CR: Caloric-Restricted
HDL: High-Density Lipoprotein

Introduction

Long-term restriction of energy intake without malnutrition is a robust intervention that has been shown to prolong life and delay age-related morbidity. However, modeling aging and age-related pathologies presents an analytical challenge due to the complexity of gene-nutrient and environment influences and interactions. Systems microbiomics approach was used to model serum and urinary metabolic phenotypes of caloric-restricted (CR) and pair-housed control-fed Labrador Retriever dogs. Alterations of amino acids, lipoproteins and glucose homeostasis provide further molecular evidence of the metabolic processes associated with the health benefits of long-term CR. Additionally, both aging and diet restriction altered populations of gut microbiota, manifested by variation of aromatic metabolites and aliphatic amine compounds. In summary, systems microbiomics combined with data modeling can lead to development of personalized nutrition that mimics the benefits of CR.

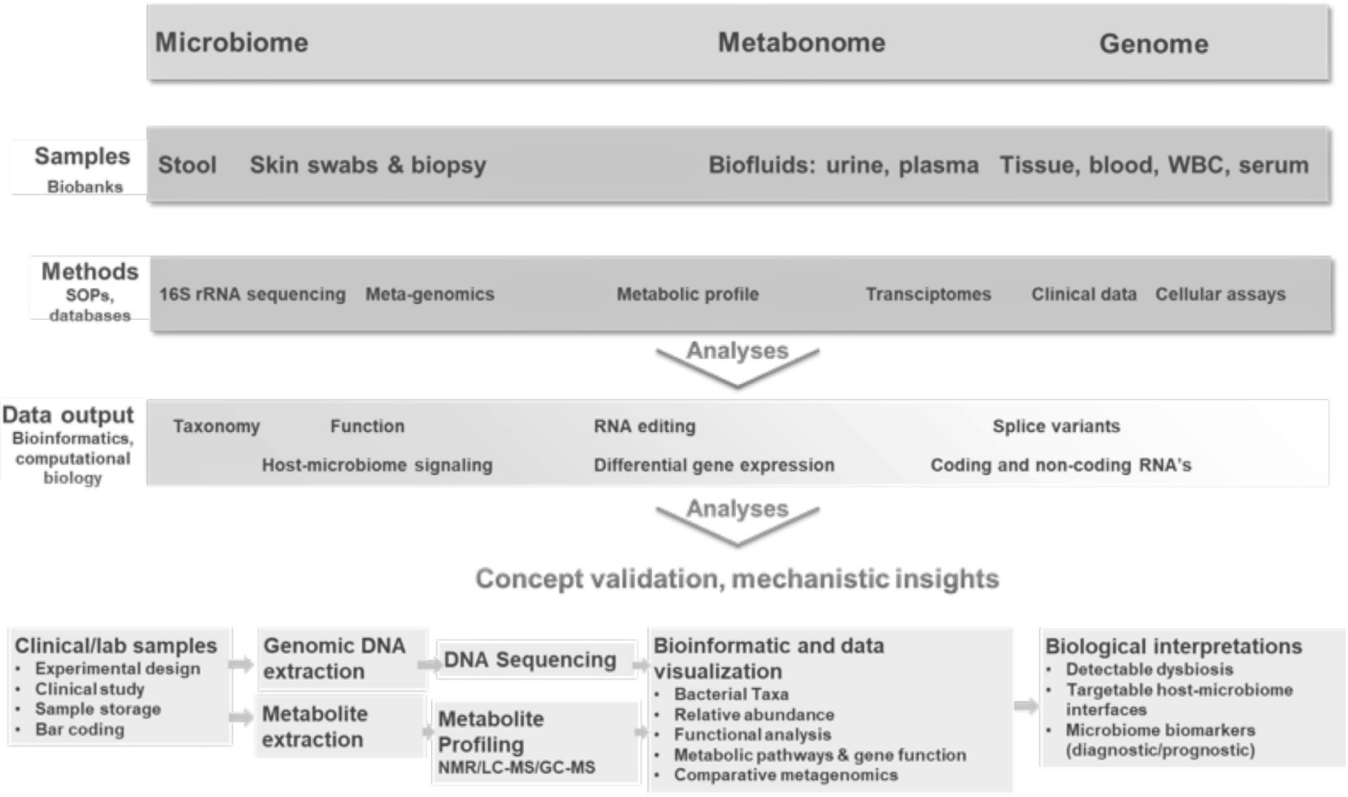
Systems Microbiomics

Scientists' interest in the human metabolome, microbiome and microbial metabolome has grown enormously in recent years. High-resolution ^{1}H NMR spectroscopy is a powerful tool for generating data on a multitude of metabolites in biofluids or tissues. Mass spectrometry when coupled to a liquid chromatography system provides a rapid platform for metabolite profiling at a concentration range of nM to pM. With the advent of ultra-performance liquid chromatograph hyphenated to a triple quad time-of-flight mass spectrometer equipped with an electrospray interface, complementary data to the ^{1}H NMR profile can be generated in 15 to 30 minutes per sample, thus enlarging the metabolite window. The acquired spectral profile of a biofluid, such as urine, plasma or saliva, reflects the metabolic status of the organism. ^{1}H NMR and/or MS spectroscopy of complex biological mixtures coupled with multivariate statistical analysis allow better visualization of the changing endogenous biological profile in response to a physiological challenge or stimulus, such as a disease process, administration of a xenobiotic, environmental stress, genetic modification, changes in nutrition, and other physiological effects.

Recent improvements in DNA sequencing, imaging, data analysis, and computing tools have begun to reveal the breadth of influence that microorganisms have on human and companion animal health. Microbiome analytics comprise standard routines for DNA/RNA preparations followed by 16S rRNA sequencing and/or metagenomics. The data is analyzed by specialized bioinformatics routines to decode genomic and microbiome profiles. Figure 1 describes the flow of the systems microbiomics including the key competencies needed.

Indigenous microbiota and its metabolic activity are essential components of the modern concept of human health, but the composition and functional characteristics of the microbiome/metabolome remain to be elucidated. Different patterns of microbial colonization or metabolic changes associated with disease states have been documented, but the patterns of microbial colonization and functional characteristics associated with health are less well-defined and vary with diet, environment and geography. Additionally, there is no widely accepted definition of a healthy microbiome or metabolome. It is important to point out that the healthy metabolic functions are preserved, even if the bugs themselves vary among otherwise healthy individuals.

Figure 1. Integrative analysis of host genome, metabolome and microbiome including a comprehensive systems microbiomics workflow.



Healthy microbiome could be described: (a) in terms of ecologic stability (i.e., the ability to resist community-structure change under stress or to rapidly return to baseline following a stress-related change), (b) by an idealized (presumably healthier) composition, or (c) by a desirable functional profile including metabolic and synthetic activity. Elucidation of the properties of a healthy metabolome and microbiota would provide a target for dietary interventions and microbial modifications aimed at sustaining health in generally healthy populations.

One major premise in the systems microbiomics research is to decipher host-microbiome metabolic, immune and neuronal signaling, thus allowing its reshaping with diet to improve health. This lends to basically three key research themes:

- Mapping of gut microbiota and metabolic status in healthy, subclinical and diseased subjects to understand its causal role in human health and the onset of disease
- Metabolome/microbiome-based predictive biomarkers of health and disease
- Modulations of gut microbial metabolites to deliver enhanced nutritional benefit

Figure 2. O-PLS-DA plots of ^{1}H NMR spectra of urine obtained from dogs fed with control and restricted diet at age of 13 weeks (A), 1.5 years old (B) and 9 years old (C).

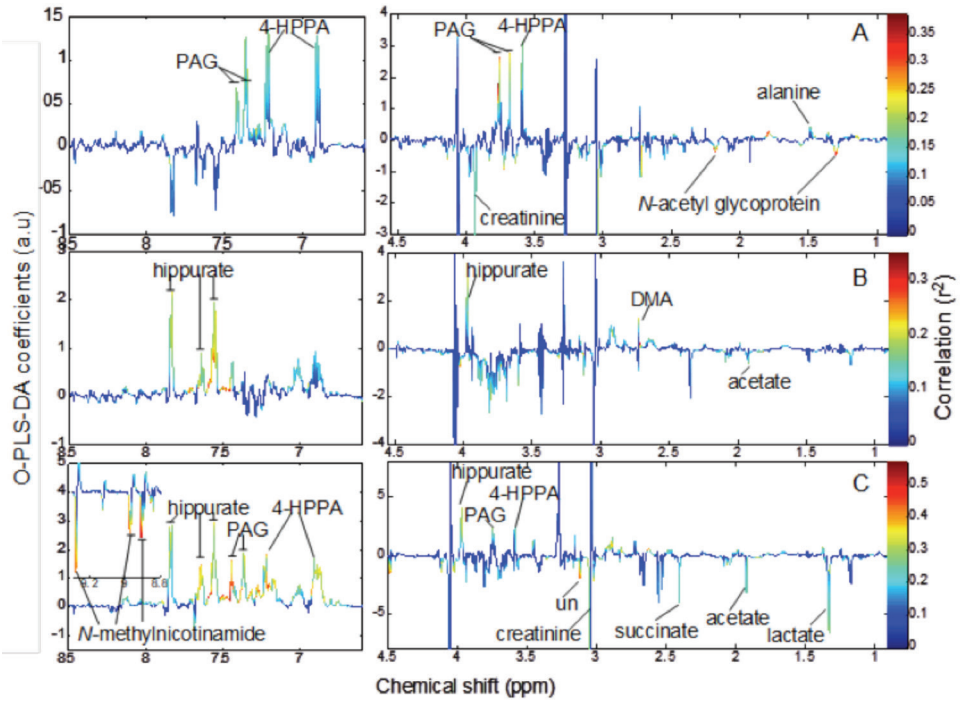
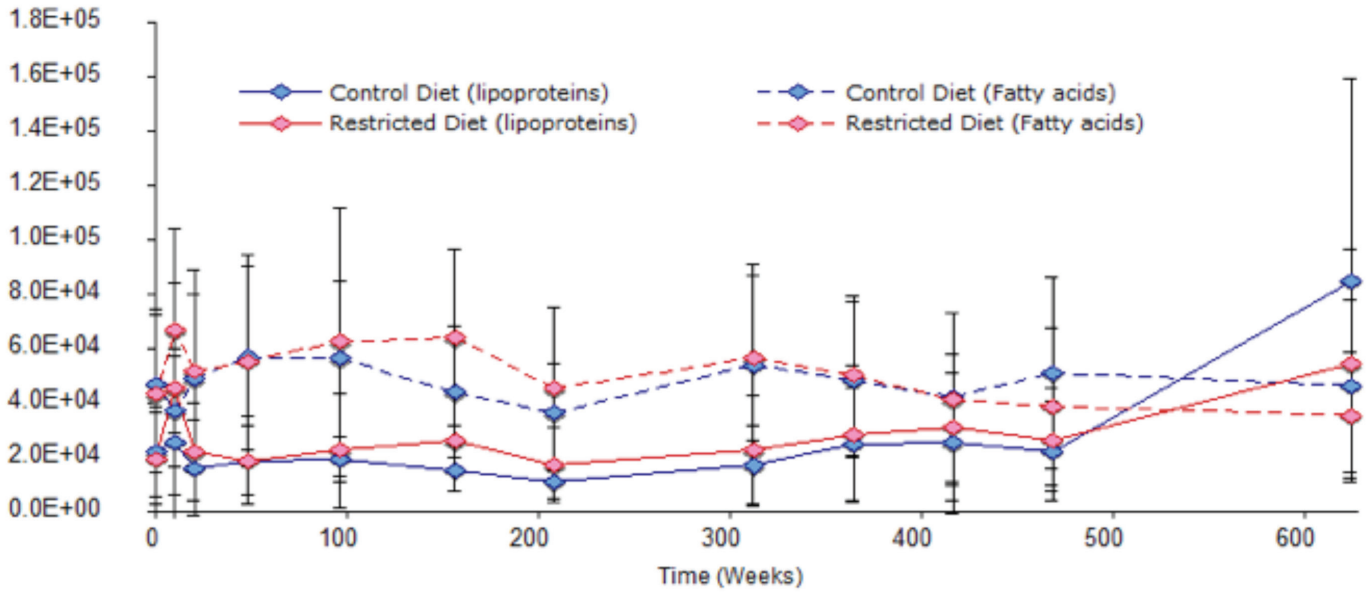


Figure 3. Blood serum metabolic profile of young and old dogs.



Proof of Concept Studies on Caloric Restriction Applying Systems Microbiomics Approach

We have applied systems microbiomics strategy to study the changes in urinary metabolic signatures for the duration of the lives of paired sibling dogs fed as controls or with 25% CR. Age was the dominating factor influencing the metabolic trajectory and was mainly associated with the increased excretion of creatinine up to adulthood, followed by a decrease in later life that occurred roughly in parallel with declining lean body composition. In addition, relatively high excretion of glycoproteins was noted in dogs at early ages. Changes in gut microbiota were associated with both aging and dietary restriction. Additional effects of dietary restriction were associated with reduced energy expenditure manifested by depleted levels of creatine, 1-methylnicotinamide, lactate, acetate, and succinate in urine of dogs fed with CR (see Figure 2). This study also has highlighted the benefits of using systems micobiomics for the detection of subtle physiological changes and dietary effects on mammalian metabolism. The role that gut microflora plays in longevity and quality-of-life responses to CR is potentially important.

^{1}H NMR of blood serum profile of the young and older dogs revealed aging metabolic phenotypes independent of diet characterized by high levels glutamine, creatinine, methylamine, dimethylamine N-oxide, and glycerophosphocholine and by decreasing levels of glycine, aspartate, creatinine, and citrate indicative of metabolic changes associated largely with muscle mass (see Figure 3). We have carried out similar CR studies in mice.

Our work demonstrates the strong potential of systems microbiomics to reveal a global snapshot of the highly dynamic and interconnected metabolic processes of various tissues of an animal, while including the often-ignored interactions with the gut microflora. This provides a valuable tool for the study of aging and its retardation by CR. Our findings provide a view of the changes in energy metabolism and consequential changes in lipoprotein metabolism associated with aging and CR. Beyond their role in lipid transport and metabolism, lipoproteins, especially high-density lipoproteins (HDL), regulate immune processes that impact the development or prevention of many aging-associated diseases.

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Notes

Notes

The Dog Aging Project: Can Old Dogs Teach Us New Tricks?

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Abstract

Aging is an extremely complex phenotype, influenced by genes, the environment and the interaction between the two. While studies from laboratory-based model organisms have taught us much about the genetic and environmental determinants of lifespan and healthspan, we have had much less success translating these findings to a "real world" population. Companion dogs offer an ideal organism in which to study aging and its determinants. Dogs vary not only in morphological and behavioral traits but also in lifespan and the effect of age on disease risk. The Dog Aging Project (DAP) will study aging and age-related disease in thousands of companion dogs throughout the country, with the goal of identifying the genetic and environmental factors that shape variation in healthspan and lifespan, and will ask whether we can increase canine healthspan through pharmacological interventions.

Glossary of Abbreviations
BMI: Body Mass Index
CCDS: Canine Cognitive Dysfunction Syndrome
CCDRS: Canine Cognitive Dysfunction Rating Scale
CCI: Canine Comorbidity Index
CFS: Canine Frality Score
CIP: Canine Inflammaging Panel
DAP: Dog Aging Project
HRQL: Health-Related Quality of Life
GWAS: Genome-Wide Association Studies
VMDB: Veterinary Medical DataBase

Introduction

Domestic dog breeds vary dramatically not only in shape, size and behavior but also in patterns of aging and age-related disease.[1] Taking advantage of that extraordinary variation, the DAP will study thousands of companion dogs to identify the genetic and environmental factors that shape lifespan and healthspan, the period of a dog's healthy lifespan.[2] The project also includes a pharmacological study to determine the potential to safely increase healthspan or lifespan in companion dogs.

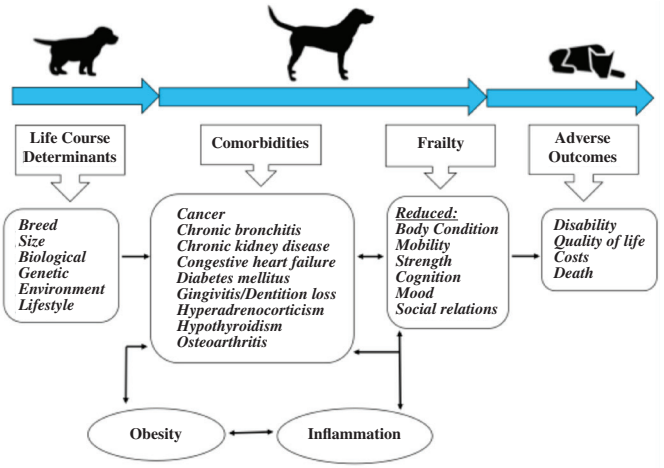
As its overarching goal, the DAP aims to identify the genetic and environmental factors that shape healthy aging and to determine the mechanisms by which they do so. However, to identify genetic or environmental determinants of healthy aging, we need to define healthy aging. In the following paper, we will first discuss ways the DAP will attempt to meet that challenge by developing a functional model of healthy aging in dogs. We then will briefly present the conceptual framework that the DAP will use to better understand the underlying causes of variation in functional aging.

A Functional Model for Measuring Healthy Aging in Dogs

Small-animal veterinary practitioners know that geriatric dogs are an increasingly important dimension of their practice, though canine aging is complex to define because dogs vary in size-based life expectancy[3] and breed-based disease risk.[1] Practitioners can readily recognize dogs that are "aging well" or "aging poorly," but such observations are challenging to document
in a manner easily understood by colleagues. By defining a canine aging phenotype, the DAP will develop immensely valuable tools to facilitate the description of, and therefore the care of, aging dogs. Additionally, shared objective descriptors will enhance veterinary research into mechanisms underlying healthy longevity in dogs. The canine aging phenotype will be described in the following ways:

1) Comorbity: This is the coexistence of two or more chronic diseases and is a common phenomenon in older people.[4,5] Strong evidence indicates that the coexistence of multiple chronic conditions increases mortality risk,[6,7] causes a decline of physical and mental functioning,[6,8] and negatively influences quality of life.[9,10] Veterinarians routinely document comorbid disease in aging dogs but do not have a comorbidity instrument to compile these measures into a unified assessment of healthy aging.
2) Frality: The concept of healthy human aging has traditionally focused on the prevention of disease and debili-

Figure 1. Integrated model for canine aging (modified from reference).[15]



tation, but expanded definitions of "active" aging require maintenance of independent physical function, cognitive ability, mental health, and well-being.[11,12] The opposite of active aging is "frailty." As described by Malmstrom,[13] models of a frailty phenotype have been developed using three domains: functional, deficit accumulation and biological.[14-20] Many components of frailty are measurable in dogs, and validating these measurements will allow early detection of the nuances of canine aging.

3) Inflammaging: An emerging theory of aging focuses on the activation of subclinical, chronic inflammation that occurs with aging, called "inflammaging."[21-23] Mounting evidence reveals that chronic oxidative and inflammatory stress mediate the aging process by damaging proteins, lipids and DNA, resulting in the age-related decline of physiological function.[24,25] The emerging concept of inflammaging will be applied to dogs, using validated clinical assays.

After refining and validating assessments of aging in companion dogs on these three separate axes — comorbidity, frailty and inflammaging — we also will investigate the genetic and environmental factors, and underlying mechanisms, that influence these aging phenotypes (Figure 1).

Comorbidity and Aging

Dogs receive sophisticated individualized medical care and may have more than one disease managed for extended periods of time. Investigation into comorbidities among dogs in the Veterinary Medical DataBase (VMDB)[1,26] dataset from U.S. veterinary teaching hospitals (VTHs) has revealed that 43.9% of dogs have three or more diagnoses at the time of death, and of these, 2.7% have 10 or more diagnoses (Figure 2A). The VetCompass database of the Royal Veterinary College in the U.K.[27] contains dogs being seen exclusively at private primary-care practices, and the records of 2,586 canine veterinary visits over three and a half years demonstrated that most dogs have only a few comorbidities, while a few dogs, even in general practice, exhibit many (Figure 2B).

Several comorbidity indices for humans exist, with diagnoses based on prevalence, chronicity and morbidity in older adults. These indices range from 5 to 20 components.[28-30] Canine-specific diseases for inclusion in the canine comorbidity index (CCI) have been selected based on these same parameters. Initial diseases for inclusion are allergic and inflammatory conditions (sites specified), cancer (any malignant neoplasm), chronic bronchitis, chronic kidney disease, cognitive dysfunction syndrome, congestive heart failure, diabetes mellitus, gastrointestinal chronic conditions, hyperadrenocorticism, hypothyroidism, obesity, osteoarthritis, periodontal disease, and seizure disorders, as well as an "other, please define"

Figure 2. Frequency of comorbidity among dogs. A) Number of diagnoses recorded for each dog at time of death in a dataset of 74,556 dogs at veterinary teaching hospitals (VTHs). Note that the Y-axis is on a log-scale, with the majority of dogs having 1-3 comorbidities. Comorbidity number at death is approximately exponentially distributed. B) Morbidity counts for dogs in general practice. As with dogs at VTHs, most dogs show a small number of comorbidities while a few experience many comorbidities.

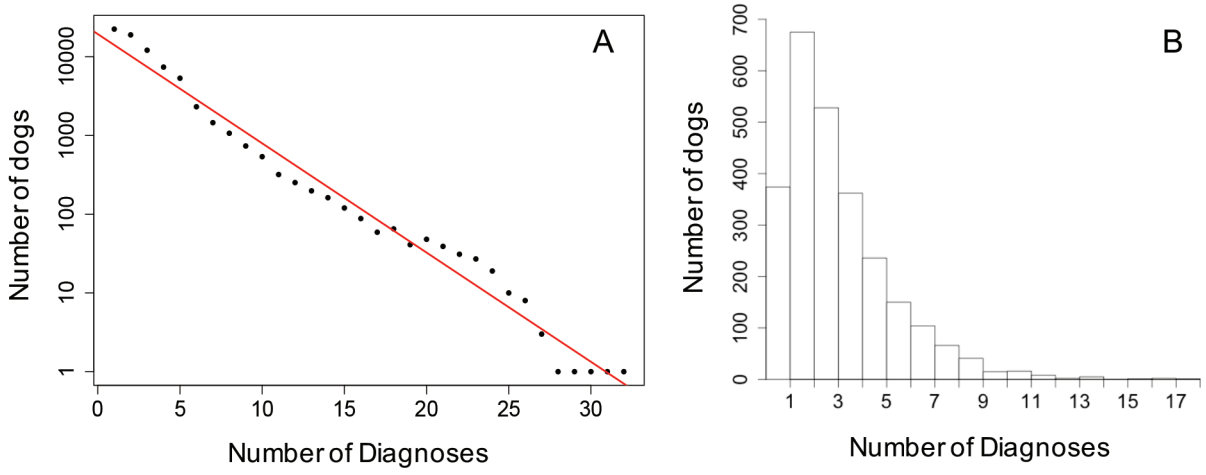
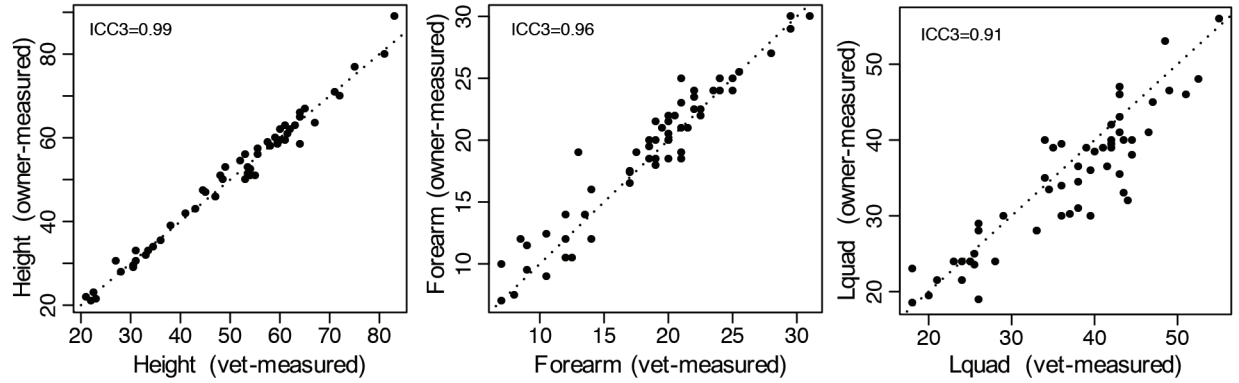


Figure 3. Morphometric values obtained from veterinarians and owners. Dasked lines indicate isometric value (vet=owner). Inset value, ICC3, refers to the intraclass coefficient for k=two judges rating each target. All ICC values are significant at P<0.001. The third panel illustrates a bias toward larger quadriceps measures from veterinarians (paired t55=3.81, P< .001).



category. Comorbidities will be collected annually on all dogs in the study, and the prospective nature of a longitudinal cohort will enable discovery of any trends in the order of accumulation of comorbidities.

Frailty

Similar to the experience of the human population, improvements in veterinary health care over recent decades have also greatly expanded the U.S. population of geriatric companion dogs. It is clear to dog owners and their veterinarians that aging dogs experience a phenomenon of frailty, including declining energy levels, limitations in mobility and changes in cognition, but this experience has been poorly described in the literature.[31-34] We will create a canine frailty score (CFS) to describe this dimension of the canine aging process. To be effectively deployed among companion dogs, new instruments to assess frailty must be simple to perform within common household or veterinary clinic conditions, painless, apply to dogs of all sizes and breed backgrounds, and result in an outcome that is easily measured.

Mobility is relevant to frailty, but dog variation in morphology adds a complicating factor in any measurement of mobility. A recent pilot study investigated dog morphometrics and mobility on a leash along a flat 10-meter distance, off leash along the same 10-meter distance at its chosen pace, and off leash up a flight of stairs. We determined that minimally trained owners could acquire similar morphologic measurements and movement times as trained staff regardless of the size, shape or breed of their dogs (Figure 3), that movement speed did vary with weight as expected, and that time to complete mobility trials varied by quartile of life expectancy regardless of size, shape or breed.

Additional frailty factors that will be measured as components of the CFS include:

- Weight Loss and Body Condition: Body weight is a standard part of the physical examination of a canine patient. Body condition score (BCS) on a nine-point scale ranging from cachexic (1) to obese (9) is also customarily recorded at each exam. Changes in these parameters can be individually tracked over time.
- Physical Activity, Behavior, Anxiety/Nervousness, and Social Avoidance: The canine cognitive dysfunction rating scale (CCDRS) is a validated instrument developed to facilitate diagnosis of canine cognitive dysfunction syndrome (CCDS), a nonspecific syndrome of senile dementia among dogs.[33,35,36] We will use CCDRS annually as a continuous measure of the range of cognitive changes that occur with aging, even those not compatible with a diagnosis of senile dementia.
- Activity Monitoring: The use of accelerometer-based activity monitors has become commonplace in dogs. We will place accelerometers on collars to be worn at scheduled intervals to develop descriptors of typical activity patterns of dogs within and across breed, age, sex, and size groups.
- Cognitive Performance: Executive functions are the higher-order processes such as inhibitory control, working memory and discrimination choices that govern goal-directed action and adaptive responses to situations. Measurement systems for these processes have been validated in companion dogs by Dognition™ and have been shown to vary across the aging trajectory.[37-43] This will be assessed annually.
- Attitude: We also will use the validated health-related quality of life (HRQL) instrument[44,45] annually in study dogs. The HRQL assesses dogs on four attitudinal axes [energetic/enthusiastic (E/E), happy/content (H/C), active/comfortable (A/C), calm/relaxed (C/R)], and has been shown to detect changes over time that parallel medical evaluations.

Inflammaging

While inflammaging is not as well-studied in canines as in humans,[46] reports of its significance in dogs have begun to appear.[47,48] Inflammatory cytokines and markers of white-blood cell activation that have proven valuable in the study of this phenomenon in humans and that are validated in the dog will be investigated as components of a canine inflammag-

ing panel (CIP).[49-60] Adipose tissue is increasingly recognized as a contributor to an inflammatory state that promotes other adverse events,[61] but measurements of adiposity are not standardized in dogs. We will attempt to develop a body mass index (BMI) calculation suitable for diverse dogs using our morphologic measurements. Lipoprotein profiling may allow for a metabolic assessment of adiposity that circumvents the challenge of diverse canine morphologies and, if so, will also be utilized.[62-65]

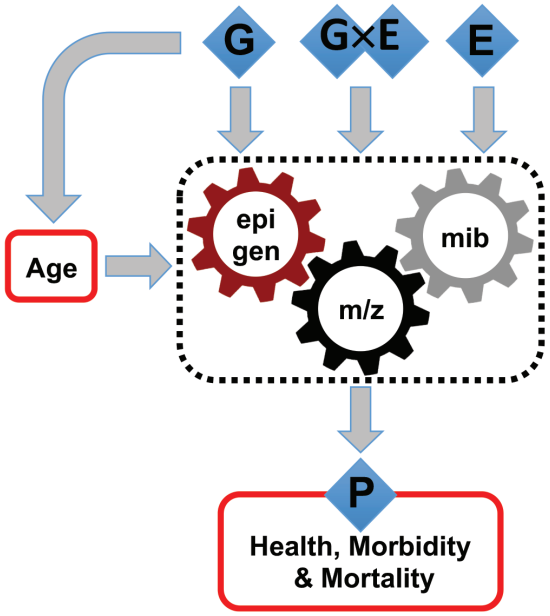
Genetic Determinants of Healthy Aging

The long-term goal is to identify the genetic and environmental determinants of healthy aging in companion dogs. In fact, age is the single greatest risk factor for many human diseases.[66] However, we have abundant evidence from lab-based studies that longevity is affected by evolutionarily conserved genetic pathways[67-73] and environmental processes.[74] Despite these advances, we are far from understanding the underlying mechanisms of these genetic pathways,[75] the extent to which they explain variation in natural populations, and the relative role of environmental variation. The DAP aims to fill this gap.

We think of the relationship between genes and environment, and the traits they influence, not in terms of mean values, but rather in terms of variances. To do this, quantitative genetics give us a single, powerful equation: P=G+E+G×E. In words, the variation in phenotype (P) is the sum of genetic variation (G), environmental variation (E), and the interaction between the two. While the equation is simple, genome-wide association studies (GWAS) that try to identify specific genes that determine the G⇢P relationship in humans typically find single-nucleotide polymorphisms that explain no more than 0.1-0.5% of the total variance.[76] This leads to the so-called "missing heritability" problem.

One potential solution lies in identifying the genetic basis not of the downstream phenotypes, like lifespan, but rather of the molecular networks that lie between genotype and phenotype — the so-called "endophenotypes." These include the transcriptome, proteome, microbiome, metabolome, and so forth. We seem to be better able to map relationships between genotype and endophenotype than between genotype and downstream phenotype. For example, metabolites, the building blocks of our physical and biochemical features, are sensitive to genetic variation, with GWAS variants accounting for up to 63% of the variance in a single metabolite.[77] As we and others have shown, the metabolome is strongly correlated with age in worms,[78,79] flies,[80,82] mice,[83-89] marmosets,[90-92] and humans.[93-95] The working model of the DAP takes advantage of the knowledge that we can gain from dogs about their genotype, their environment (e.g., air quality, social setting, activity level, etc.), and their endophenotypes, coupled with sophisticated measures of aging. The framework is illustrated in Figure 4. Importantly, this approach holds the promise of not only identifying stronger genetic and environmental signals associated with aging but also of explaining the underlying mechanisms that link genotype and environment to aging phenotype.

Figure 4. Framework illustrating the conceptual framework behind the Dog Aging Project, whereby genetic variation (G) and environmental variation (E) are causally linked to phenotypic variation (P) via diverse "endophenotypes." Shown here for illustrative purposes are the epigenome, the microbiome and the metabolome, but these are only a subset of all the molecular traits that one could measure. Note that age affects these endophenotypes and actually does so in a genotype- and environment-dependent manner.



Interventions to Improve Healthy Lifespan

The primary goal of the DAP is to understand the determinants of healthy aging in companion dogs, but we also want to know if we can delay the onset or reduce the severity of the age-related decline in vigor that affects all of us. With this in mind, the DAP has already begun safety testing of rapamycin, a drug that has been shown to be highly effective in increasing healthspan and lifespan in the laboratory setting.[96]

In mice, treatment with low doses of rapamycin not only increases lifespan but also decreases the deleterious effects of age on left ventricular heart function.[97,98] Our recent placebo-controlled pilot study in companion dogs found that rapamycin was not only safe but also that dogs on rapamycin showed improvement in multiple measures of left ventricular function.[99] The DAP will carry out the first double-blind placebo-controlled study testing the efficacy of a drug to improve healthspan outside a lab setting.

Conclusion

To develop a complete model that gives us the power to predict, diagnose, treat, and prevent age-related disease, we need one that will provide answers in years, not decades, and that can benefit from the wealth of current methods and technologies available to scientists in the 21st

century. We think the Dog Aging Project offers just such a model. Our approach will generate a tremendous amount of data (on the order of a petabyte, no pun intended, that is 10^6 gigabytes). Importantly, to maximize the outcome of this enormous effort, the DAP will be an open science initiative, with all data made publicly available, other than that needed to protect confidentiality. Scientists, veterinarians, dog owners, and the general public will be welcome to explore all that dogs have to teach us, about dogs and about ourselves.

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Notes

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Using Genomic Biology to Study Pet Aging

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Abstract

Due to improved disease management, nutrition and husbandry practices, companion animals are living longer, with seniors making up 30 to 40% of the pet population. As in humans, pet aging is accompanied by a variety of chronic disease states. Advances in genomic biology provide opportunities to increase our understanding of the aging process, identify mechanisms by which age-related illnesses occur, and design targeted prophylactics and/or therapeutics. This presentation will provide background information on the field of genomic biology, discuss recent pet genomics studies, and project what the future may hold as it pertains to veterinary medicine and pet nutrition.

Glossary of Abbreviations
HGP: Human Genome Project
mRNA: Messenger RNA
PCR: Polymerase Chain Reaction

Aging Pets

Due to improved disease management, nutrition and husbandry practices, companion animals are living longer, with seniors making up 30 to 40% of the pet population.[1] Aging is characterized by progressive organ degeneration, decline in stress response, homeostatic imbalances, and reduced immune surveillance. These physiological changes not only increase the risk of chronic disease development, it also is common to detect abnormalities in senior pets when examined physically or through standard laboratory testing (e.g., urinalysis, serum biochemistry), even when they are apparently in good health.[2] Wellness and health-care programs for senior and geriatric pets are common in veterinary hospitals. A key component of a senior care program is preventive health, including early disease detection and treatment.[3]

Aging is a multifactorial process affected by many genetic and environmental factors. Many theories have been proposed, but a few biological processes, including oxidative stress, epigenetic alterations, telomere shortening, and DNA damage, have been consistently shown to be involved and continue to be studied in humans and animal models. In addition to understanding the complicated processes involved, a key challenge in humans and pets is detecting these and other detrimental changes in a noninvasive manner so that intervention can begin early in the disease process. Genomic tools provide scientists with the opportunity to increase understanding of the aging process, identify mechanisms by which age-related illnesses occur, and design targeted prophylactics and/or therapeutics.

Genomic Biology

Nowadays, the field of genomics encompasses many things, including the ability to sequence DNA for genetic testing and measure messenger RNA (mRNA), or protein expression, and metabolite profiles (Table 1). An amazing amount of data may now be generated with automated, high-throughput sequencers and analyzed by high-powered computers, leaving human brain power as the bottleneck in the analytical system. With all the powerful tools that scientists have at their disposal, one may forget how far the field has come over the past few decades.

In the 1970s and 1980s, key developments in the genomics field included the discovery of restriction enzymes for DNA splicing, the introduction of polymerase chain reaction (PCR) and the first automated sequencers. At that time, the term "genomics" referred to the generation and analysis of information about genes and genomes.[4] Progress was certainly being made, but the term "high-throughput" was not in anybody's vocabulary. In the 1990s, the term "functional genomics" was coined and was quickly followed by many

Table 1. Common Genomic Terms

Genome	The totality of all DNA in an organism
Genomics	Study of genomes, including genome mapping and sequencing
Functional Genomics	Study of gene function
Nutritional Genomics	Study of nutrient-gene interactions
Proteomics	Study of all proteins found in a particular cell, tissue or organism
Metabolomics	Study of all metabolites found in a particular cell, tissue or organism

of the other 'omic terms used today. The 1990s also were an exciting time in the field because it is when the Human Genome Project (HGP) was launched (1990) and nearly completed. The HGP took over a decade to complete and included a sequencing battle referred to as the genome war between private industry and federally funded scientists.[5] The tools and concepts in the field have continued to evolve at a rapid pace, greatly increasing the speed and reducing the cost by which projects may be accomplished. As an example of how much things have changed, consider that while the HGP required over a decade of time, hundreds of scientists and nearly $3 billion to complete in the 1990s, the same can now be accomplished in a couple of days by a well-trained genomic biologist for less than $5,000 (*https://www.genome.gov/10001772/all-about-the--human-genome-project-hgp/*).

Even though the plug-and-play reagents, highly automated sequencers and powerful computers were not available in the 1980s and 1990s, scientists appreciated the widespread implications the field would have on medicine, agriculture and veterinary medicine.[4,6] Training as a postdoctoral researcher in functional genomics soon after the first draft version of the human genome had been published (2001), I quickly realized how powerful the genomic tools we had at the time were and imagined not only what that could mean for human medicine but also for companion animals once they were chosen for study. In regard to research, one must decide whether to focus on what is hardwired in the animal (DNA) or what may be manipulated once it is conceived (mRNA, protein, metabolites). Although genomic biology has shed a lot of light on the canine and feline genomes and what that may mean from a genetics perspective,[7] progress has been slow in regard to functional genomics and nutritional genomics, especially when it comes to aging research.

Genomics and Pet Aging

Our laboratory has published several publications on the mRNA expression profiles of young adult versus geriatric dogs, including skeletal muscle,[8] cerebral cortex,[9] adipose,[10] colonic mucosa,[11] and liver[12] tissues. Others have used genomic tools to measure mRNA expression of the prostate gland of immature, young adult dogs and geriatric dogs[13]; circulating neutrophil-related mRNA expression of growing puppies, young adult dogs and senior dogs[14]; and myocardial mRNA expression of young adult and geriatric cats.[15] The results and implications of these studies will be discussed.

Continued progress in this field is possible but will require financial resources from a variety of funding sources and teams of well-trained scientists who are passionate about companion animal health. As a newly appointed assistant professor, I was inspired by a poem written 20 years earlier by Donald Patterson, professor of medicine and medical genetics at the University of Pennsylvania and a pioneer in the field of veterinary genetics:[16]

"We'd like to explain what pathology means
In terms of what's wrong with the structure of genes
Know if a control or a structural locus
Constitutes the exact pathological focus

With the help of the enzymes that slice DNA
And cloning techniques, we now have a way
To study the actual sequence of bases
To know when those purines are not in their places"

As I prepared these proceedings, I stumbled onto a similarly written poem of my own from 2005. Being inspired and apparently having too much time on my hands and/or procrastinating on other tasks, I created a version of my own highlighting the major genomic projects and most powerful tools of the time and adding the importance that nutrition plays in chronic disease:

"It's the 21st century and we've witnessed remarkable feats
The first draft of the canine genome sequence is now complete
The 7X draft sequence is robust with very few gaps
And dogs, wolves, and a coyote are being used
to create a SNP map

The art of sequencing has been mastered;
solving genetic diseases will soon follow
But our knowledge of complex diseases
still remains quite hollow
Today's science includes nanotechniques,
microarrays, and SNP profiles
And bioinformatics techniques required
for interpreting the data we compile

For complex diseases, genetics and
environment definitely play a role
But the impact of diet on these conditions
may end up stealing the show
The search for mechanisms of disease will be
hard and long; it will not be noted for its brevity
But these endeavors will be worth it, eventually
leading to enhanced health and longevity"

While sharing this piece of work is somewhat embarrassing and is atypical for conference proceedings, I do so to make a point. Although impressive technological advancements were made from 1982 to 2005 and even more have been made since then, the goals and challenges pertaining to pet health and disease have remained the same. That is not to say that progress has not been made but speaks to the complicated nature of aging and chronic diseases and substantiates the need for more research. Given the availability of powerful molecular tools that enable high-throughput analysis of DNA, RNA, proteins, and metabolites, high-speed computers capable

of handling vast and complicated datasets, and a conceptual framework that now applies functional genomics to nutrition and health, we are living in and contributing to a historical time in science. Moving forward, genomic biology should be used effectively as an important component of research programs to understand the aging process and its relation to chronic disease states, which may contribute to early disease detection and development of prevention and treatment strategies.

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Notes

Fecal Microbiota Changes in Aging Dogs and Cats – Implications for Health and Longevity

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Key Words
Aging Microbiome
Metagenomic Profiling

Alterations in digestive function are common in elderly people and pets. Elderly people can have increased sensitivity to dietary changes and susceptibility to gastrointestinal infections. Pet owners may notice that pets which had excellent fecal quality in their younger years now have days when their fecal quality is less than ideal. Nutrient digestibility can also be impaired. Decreased fat digestibility is common in elderly cats.[1]

Early research focused on differences in gut microbiota composition between young adults and elderly.[2-6] Decreased fecal concentrations of beneficial bacteria such as bifidobacteria and increased concentrations of potentially pathogenic bacteria such as enterobacteria have been reported in humans.[2,3] Similar changes in fecal microbiota have been reported in elderly dogs. Benno[4] reported decreased fecal concentrations of bifidobacteria and lactobacilli and increased *Clostridium perfringens* in elderly dogs. Simpson[5] also noted changes in fecal bacteria in aging dogs. When compared to young adult cats fed the same diet, elderly cats[6] had lower levels of fecal bifidobacteria. Alterations in fecal microbiota in elderly humans have been correlated with inflammation and frailness.[7] Elderly people also had less diverse microbiota[7] and more individual variability.[7,8] Studies on the effects of aging on the microbiome are often complicated by differences in lifestyle and diet between elderly and younger adults with at least some of the reported differences in fecal bacteria correlated with the use of antibiotics and dietary differences.[7,8]

While early studies focused on alterations in fecal bacteria during aging, more recent studies have focused on health effects of gut bacteria in the elderly. Inflammation and immunity have been correlated with aging and microbiota in humans[9-13] and dogs[14] and may have implications for inflammatory conditions common in the elderly.[15] Probiotic supplementation can have beneficial effects on age-related changes in immune function.[13] Recent research on the gut-brain axis has highlighted potential effects of the gut microbiome on age-related neurological conditions such as Alzheimer's disease.[16] More sophisticated metagenomic profiling has illustrated the functional effects of alterations in the aging microbiome.[17] In a study with centenarians,[17] over 100 microbial genes were significantly correlated with aging. There was a loss of genes for short-chain fatty acid production and changes in saccharolytic and proteolytic genes with aging.

While there have been many studies evaluating changes in the aging microbiome, few intervention studies have been published. Cupp and colleagues at Nestlé Purina conducted a long-term intervention study with elderly cats.[18,19] The composition of the nutrient blend was based on years of preliminary research on metabolic and digestive changes during aging in cats and effects of various prebiotics on gut microbiome. Cats were fed a nutritionally complete control diet or the same diet supplemented with either an antioxidant blend or the antioxidant blend plus a fatty acid blend and prebiotic. Cats fed the prebiotic/antioxidant/fatty acid-supplemented diet lived significantly longer than cats fed the other diets and had a slower decline in several indicators of health.

As we learn more about the functions of the bacteria that reside in the digestive tract and their interactions with the host, we will better understand the influence of gut bacteria on longevity and diseases of aging. In the future, nourishing and replenishing the gut microbiota will become a conventional approach to reduce the effects of aging.

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Notes

Notes

Cellular and Functional Mechanisms Underlying Muscle Aging and Associated Diseases

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Abstract

The loss of skeletal muscle mass and function during the aging process (sarcopenia) has a major impact on muscle function and is a key component of frailty. A clear understanding of the mechanisms of sarcopenia through the identification of selective biomarkers, and thus of potential therapeutic targets, is of paramount importance in ensuring quality of life in the old age. This presentation will provide an overview of the studies associating immunohistology and omics investigations during sarcopenia and associated pathologies in humans.

Glossary of Abbreviations
ECM: Extracellular Matrix
MALDI: Matrix-Associated Laser Desorption/Ionization
MSI: Mass Spectrometry Imaging

Introduction

In the developed world, life expectancy increases at a rate of two years per decade. However, health span is not keeping pace with increasing life span. For example, in a 20-year period (from 1990 to 2010), male life expectancy increased by 4.2 years in the U.S., but healthy life expectancy lagged behind at 2.7 years.[1] Understanding the factors influencing health in old age and developing and validating interventions to combat the negative aspects of aging is therefore a major issue.

Aging affects most tissues and many physiological functions. However, one of the most dramatic effects of increasing age is the atrophy of skeletal muscle, referred to as sarcopenia, which is predictive of all-cause mortality in the elderly.[2] Sarcopenia is a universal, age-related loss of muscle mass associated with a loss of strength and function resulting in muscle weakness. Sarcopenia is a prevalent condition, as it can be detected in 13 to 24% of adults over 60 years of age and in 50% of individuals older than 80.[3] Estimates of the rate of muscle loss are 1 to 2% per year after the age of 50 years,[4] and sarcopenia can result in a loss of about 30 to 50% of the muscle mass by the age of 80 years.[5] Interindividual differences in the prevalence of sarcopenia depend not only on genetic factors but also on food habits, activity patterns and general lifestyle.

Healthy skeletal muscles are central not only for coordinated movements and postural control but also for general well-being. Hence, age-related loss in skeletal muscle contractile strength increases the risk of impaired mobility, poor balance, falls, and loss of autonomy. Skeletal muscle, which is the most abundant tissue in the adult body, also plays a central role as a reserve for energy and amino acids and is a major site of fatty-acid oxidation, carbohydrate metabolism and maintenance of heat homeostasis.[6] Hence, age-related loss of muscle mass also triggers severe metabolic side effects, including metabolic syndrome and frailty in the elderly.

Metabolic syndrome is a cluster of interrelated risk factors for cardiovascular diseases and type-2 diabetes. Metabolic syndrome occurrence strongly increases with aging, and among its components, hypertension is the most prevalent.[7] When associated with weight loss, poor physical reserves, weakness, reduced balance, and physical inactivity, sarcopenia can further result in frailty,[8] which is accompanied

by loss of independence, institutionalization and increased mortality. A clear understanding of the mechanisms of muscle aging through the identification of selective biomarkers, and thus of potential therapeutic targets, is of paramount importance in ensuring quality of life in old age.

Numerous theories have been proposed to explain sarcopenia. Obviously, muscle aging is a multifactorial phenomenon that implicates intrinsic factors such as perturbations in the endocrine system (somatopause, menopause, andropause, adrenopause), an increase in proinflammatory cytokines (IL6, TNFα) with attendant chronic inflammation (referred to as inflamm-aging),[9] motor units denervation/reinnervation,[10] decreased muscle regeneration capacity,[11,12] and increased mitochondrial reactive oxygen species produced during energy metabolism.[6] Undoubtedly, extrinsic factors such as diet and exercise, and probably other unknown mechanisms,[13] further play important roles.

To better understand the mechanisms of aging of the human skeletal muscle, we have undertaken top-down differential proteomic approaches and combined immunohistology, proteomics, transcriptomics, and mass spectrometry molecular imaging (MSI) to investigate "healthy" aging and two common age-related pathologies: metabolic syndrome and hypertension. Using these methodologies, we identified fiber-type specific alterations and several potential biomarkers of aging.

Muscle Fiber Morphometry and Chronological Aging in Men

Human skeletal muscles are of mixed fiber-type composition, as they comprise slow-oxidative (type-I) fibers, fast-oxidative glycolytic (type-IIA) fibers, fast-glycolytic (type-IIX) fibers, together with hybrid fibers.[14] Aging at the cellular level involves decline in both number (hypoplasia)[15] and size (cross-sectional atrophy) of muscle contractile cells (also named (myo)fibers). Immunohistochemical studies not only revealed the importance of aging but also of age-related pathologies, such as metabolic-syndrome, for fiber-type specific characteristics.[16] Notably, metabolic syndrome is sufficient to strongly modify the characteristics (size, mitochondrial oxidative activity, lipid droplets) of muscle fibers.

Atrophy of type-II fibers is one of the most consistent observation for chronological aging.[16,17] Altered fiber shape and/or fiber-type grouping represent the first signs of fiber disuse, cell death, denervation,[18,19] or reorganization of motor units in the old skeletal muscle.[20, 21] Another critical morphological alteration is centralization of myofiber nuclei (myonuclei).[22] Centralized myonuclei are recognized markers of regenerating fibers,[23] and in the old muscle, centralized myonuclei could also result from fiber denervation and branching,[24] or alterations in the microtubule network.[25]

Intramyocellular Lipid Droplets and Chronological Aging in Men

Skeletal muscle is a major site of fatty-acid oxidation and insulin-mediated glucose disposal, and dysregulations of lipid metabolism with accumulation or delocalization of intramyocellular lipid droplets occur in the old muscle.[16,26,27] Metabolic syndrome further alters intramyocellular lipid content and composition, and this occurs at a fiber-type specific level. Chronological aging and particularly metabolic syndrome are thus associated with an accumulation of intramyocellular lipid droplets, especially in type-I fibers. Matrix-assisted laser desorption/ionization (MALDI)-MSI was developed to characterize intramyocellular lipid profiles at the fiber-type level. Ionic maps of lipids highlighted several m/z distinctions among young men, healthy old men and old men with metabolic syndrome, which indicated that chronological aging and metabolic syndrome are associated with altered lipid composition in the human skeletal muscle.[16]

Extracellular Matrix and Chronological Aging in Men

The extracellular matrix (ECM) embedding contractile fibers is critical to maintain muscle structures and for the transfer of force from the muscle fiber out to the tendon and subsequent bone.[28,29] ECM also provides an environment in which the contractile fibers can function.[28,29] ECM further contains different types of stromal cells, such as fibroblasts, immune cells, adipocytes, and capillaries, which reciprocally are involved in the regulation of myofiber metabolism and of muscle stem (satellite) cells.[22,30]

In men we showed that healthy aging is associated with more perimysium.[31] The perimysium (surrounding bundles of myofibers) coordinates shape change during muscle contraction,[32] and more perimysium may be important to preserve muscle shape despite age-related fiber atrophy. Importantly, we also demonstrated that hypertension in old men is associated with increased endomysium (surrounding each myofiber). A greater endomysium area in hypertensive elderly subjects could contribute to alter ECM hydration and interstitial fluid pressure, which might be harmful for transcapillary exchange.[31]

Microvascularization and Chronological Aging in Men

We further investigated microvascularization because it is representative of the potential for exchange of respiratory gases, fuel and metabolites, and is thereby an important determinant of muscle functionality. Although few differences were observed in lean healthy men during chronological aging, the microvascularization was significantly altered in old men with metabolic syndrome. We identified

hypertension (a major component of the metabolic syndrome) as central for this regulation of microvascularization in the old muscle.[31] Specifically, we reported less capillary surrounding types I and II fibers, smaller length of contact between capillaries and each fiber, and reduced tortuosity of capillaries in skeletal muscle of elderly men with hypertension or metabolic syndrome. Such structural changes, together with functional changes in capillary hemodynamics,[33] could have detrimental effects on oxygen/metabolites diffusive capacity, and thereby contribute to alter muscle functionality.

Omics of Chronological Aging in Men

The overall functional, structural and biochemical alterations in muscle have been studied for chronological aging, but the detailed molecular mechanisms implicated remain to be specified. At the molecular level, the differential expression profiles of mRNAs (transcriptomes) were previously described in rodents.[34] In humans, whole-genome expression profiling[35,36] together with meta-analysis of microarray experiments[37] have been used to identify genes that change expression with chronological age in the skeletal muscle. The differential expression profiles of mRNAs constitute a first essential level of information, but analyses of the expression profile of proteins (the proteome) in aging also are required to understand the molecular mechanisms important for the muscle aging process.

In fact, unlike the genome, the proteome varies in response to many physiological or pathological factors. In addition, the proteome is orders of magnitude more complex than the transcriptome due to post-translational modifications, protein oxidation or limited protein degradation.[6] Several proteomic studies, including ours, have been conducted in rodent muscle, and profiling of chronological aging has demonstrated substantial alterations in muscle proteins involved in key metabolic pathways, myofibrillar remodelling, cytoskeleton organization, and mechanisms of cytoprotection and cytodetoxification.[38,39] However, few proteomic studies have been conducted about the chronological aging of the human muscle,[40,41] and the results were contradictory.

Differential 2D-proteomic and transcriptomic approaches were thus performed to characterize young versus healthy old men (manuscript in preparation). These investigations identified 42 proteins and 484 ARNm's as potential biomarkers. In women we assessed 2,285 spots/proteins and identified 98 potential markers for chronological aging of the skeletal muscle.[42-44] Most of the candidates partly accounted for the immunohistochemical and physiological modifications that we found associated with chronological aging and/or metabolic syndrome in men. Thus, chronological aging was associated with a decrease in glycolytic metabolism, a fast-to-slow transition and an upregulation of several proteins involved in cytoprotection/cytodetoxification and membrane repair. In elderly men, metabolic syndrome was linked with perturbations of lipid metabolism and increases in components important for proteostasis.

Conclusions

With the continuous increase in the average life expectancy, the real societal challenge is to bridge the gap between life expectancy per se and healthy life expectancy. Sarcopenia is a main component of this burden, since it triggers frailty and is responsible for a decrease or loss in mobility and independence. Important information was obtained about fiber types morphology, oxidative metabolism, lipid droplets, apoptosis, microvascularization, satellite cells, and fibrosis of the extracellular matrix. Together with state-of-the-art transcriptomic and proteomic analyses, these data are required to improve our understanding of the factors influencing sarcopenia, and they may both help identify new regulatory pathways and provide therapeutical targets.

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Notes

The Regulation of Mitochondrial Quality Control Via Autophagy and the Scope for Pharmaceutical and Nutraceutical Approaches

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Abstract
The homeostasis of eukaryotic cells relies on efficient mitochondrial function. The control of mitochondrial quality depends on the combination of distinct but interdependent mechanisms spanning biogenesis, regulation of a dynamic network, and finely tuned degradation through targeted autophagy. There is continuous evolution on the pathways orchestrating the mitochondrial response to stress signals and the organelle adaptation to quality control during acute and subtle dysfunctions. Degradation of defective mitochondria via mitophagy influences tissue homeostasis, but it remains uncharted as to how we can pharmacologically and metabolically control this mechanism. Common efforts are therefore indispensable to conceive novel approaches to design pharmaceutical and nutraceutical strategies for treating conditions associated with defective mitochondria both in human and veterinary medicine.

Glossary of Abbreviations
ATP: Adenosine Triphosphate
Drp1: Dynamin-Related Protein 1
OMM: Outer Mitochondrial Membrane
PET: Positive Emission Tomography
PMBR: Peripheral Mitochondrial Benzodiazepines Receptor
TSPO: Translocator Protein

Key Words
Mitochondria Pharmaceuticals
Autophagy Nutraceuticals

Mitochondria and Mitophagy
In mammals, mitochondria play many important roles. They produce the majority of cellular energy by coupling with unique efficiency oxygen into molecules of adenosine triphosphate (ATP). They can nonetheless act as major consumers of this key source of intracellular energy when respiratory balance in the surrounding environment does not support its coupling, such as during an absence of oxygen leading to ischemia.[1] Mitochondria also play a prominent role in controlling programmed ways of cell death by releasing death-triggering molecules from their intermembrane space or by generating toxic-free species of oxygen in consequence of the impaired respiration.[2] All of this requires constant and careful control of their function. And the catabolic process of targeted autophagy is pivotal to this controlling function.[3] Autophagy is an intracellular degradation system that delivers cytoplasmic constituents to the lysosomes.[4]

Recent progress has demonstrated that autophagy plays a wide variety of physiological and pathophysiological roles. Even though autophagy has long been considered to be a nonselective mechanism of degradation that indiscriminately eliminates cellular components, it is now clear that autophagy can instead be highly selective against subcellular organelles[5] as mitophagy, which does so against mitrochondria.[6] This organelle-specific type of autophagy was first defined by Lemasters and collaborators,[7] though as early as 1962, it was seen that lysosomes in the liver contained mitochondrial fragments.[8] In 1977, two independent research studies, one on metamorphosis in silkworms[9] and the other on the photoreceptor cells of the ground squirrel during hibernation,[10] concluded that autophagy could be selective toward mitochondria rather than other intracellular components and that once mitochondria develop functional alterations, autophagy would be activated to engulf them.

Since then pioneering studies have described the mechanisms through which the disposal of mitochondria via autophagy takes place. These have detailed genes and signaling pathways through which the selectivity of the process is exerted and preserved. Currently, mitophagy mechanisms are classified in two major types of processes: (i) Parkin-dependent and (ii) Parkin-independent.

Parkin is an E3 ubiquitin ligase identified as one of the most important players in recruiting autophagosomes to damaged

mitochondria.[11] Parkin promotes the ubiquitin-proteasome system of mitochondrial proteins degradation leading to fulfillment of the pathway, removal of the defective organelles, and quality control of the network. Loss-of-function mutations in Parkin are known to cause heritable forms of Parkinson's disease, as well as other neurodegenerative conditions such as Alzheimer's disease, amyotrophic lateral sclerosis and Huntington's disease. On the other hand, Parkin overexpression has been found in long-lived flies, suggesting a link between aging processes and life span.[12]

The Regulation of Mitophagy

The variety of recent studies on mitophagy mechanisms during aging in invertebrate and rodent models highlighted mitochondrial quality control as an attractive target in slowing down aging processes by preventing and tackling related diseases. It has therefore become of paramount importance to regulate the process via pharmacological approaches.

We described a negative regulator of the Parkin-dependent process of mitophagy: the mitochondrial 18-kDa translocator protein (TSPO).[13] TSPO, which was first discovered as a peripheral mitochondrial benzodiazepines receptor (PBR), is situated on the outer mitochondrial membrane (OMM) of mammalian cells where it lies in strict interaction with the organelle's channels. The core biochemical function of TSPO resides in the translocation of cholesterol in the mitochondria for metabolism and steroids synthesis. In the brain, TSPO is expressed in low levels at physiological conditions, but these markedly increase at sites of brain injury and inflammation as well as during aging.[15] In fact, TSPO is used as a biomarker/molecular sensor of active brain disease in both experimental animals and human studies. For over two decades TSPO ligands have been therefore used to profile expression of the protein in the brain via means of positive emission tomography (PET) to help diagnose patients affected by brain conditions.

In light of a significant clinical potential of TSPO, these ligands have been prompted for their biological efficacy in experimental models and human patients. Among these, one potent cholesterol-like TSPO ligand has been described as a neuroprotective compound.[16] Limitation of cell mitophagy by TSPO leads to incremental redox stress in cells underlying long-term damage that act therefore as a propathological factor. The dependency of TSPO activity by cholesterol pools has pointed the attention on mitophagy efficiency and regulation via dietary regimen and quality of the food. Thus it is general knowledge that activation of nonselective (macroautophagy) and selective (mitophagy) are strictly dependent on nutrient supply.

Interestingly, mitophagy could be induced under nutrient-rich conditions that end up removing redundant or dysfunctional mitochondria when general-bulk autophagy is not even activated.[11] When autophagy is induced, mitochondrial degradation does not necessarily follow. Mitophagy is the attempt to leave as much energy as possible, leaving the mitochondria therein inhibited during starvation in order to provide cells with as much energy as possible. Confirmation of this arrived when mitochondrial elongation was observed during starvation-induced autophagy in various cell models via inactivation of the dynamin-related protein 1 (Drp1), which helps with the segregation of large mitochondria into smaller ones to facilitate their removal by autophagy.[18] For the same reason we are inclined to speculate that when cholesterol metabolism is increased, such as following TSPO downregulation,[13] the consequent metabolic alterations lead to mitophagy inactivation to retain the maximal organelle capacity to deal with trafficking of the lipid.

Conclusions and Prospective

The acknowledged importance of the process in various pathological conditions calls for timely investments to unveil the interplay between diet and mitochondrial quality. This could in turn lead to the development of products to cure or prevent conditions caused by deregulated mitochondrial function. In both human and animal medicine, an increasing number of dietary supplements have become available for the prevention and treatment of diseases.

Thankfully, compounds targeting authophagy[19,20] to counteract oxidative stress[21] have emerged over the past decade. Of these, supplements of resveratrol and omega-3 fatty acids have become a paradigm example,[22] which further stimulated attention toward novel approaches, based on the utilization of naturally derived products[23,24] to regulate both general and targeted autophagy (mitophagy). Endeavors on this account must nonetheless progress in order to achieve tangible impact on both human and veterinary medicine.

Conflict of Interest

The authors declare no conflict of interest.

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Notes

The Role of n-3 PUFA on Muscle Mass and Function in Aging Humans

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Abstract

Starting in middle age, muscle mass and function decline progressively, which can affect people's mobility and independence late in life. The results from several recent studies demonstrate that dietary supplementation with fish oil-derived from n-3 polyunsaturated fatty acids (PUFA) stimulates muscle protein synthesis, improves muscle mass and function in sedentary older adults, and augments the resistance exercise training-induced increase in muscle strength in older adults. The exact mechanisms by which fish oil-derived n-3 PUFAs exert their beneficial effects on muscle mass and function remain to be elucidated.

Glossary of Abbreviations
ADP: Adenosine Diphosphate
PUFA: Polyunsaturated Fatty Acids

Introduction

Starting in late middle age, skeletal muscles atrophy progressively and muscle tissue undergoes morphological changes (e.g., infiltration with noncontractile material, such as fat and connective tissue; reduced capillary density and mitochondrial content; motor unit and neuromuscular junction remodelling), which can reduce muscles' ability to generate and maintain force and negatively affect activities of daily living (walking, climbing stairs, lifting items).[1-3] In healthy people, muscle mass and strength decline by ~0.5 to 1% and 1 to 2% per decade, respectively; periods of acute illness and chronic diseases accelerate these processes[1-3] in part as a result of the underlying disease processes that can affect muscle, but also because of the associated reduction in physical activity, which can have detrimental consequences in older adults because the resulting loss of muscle mass (~5 to 8% after only one to two weeks of reduced ambulation) and function (~10% decrease in strength after only four days of immobilization) are difficult to recover even with intense physical rehabilitation.[4-10] Although increasing protein intake is often recommended to preserve muscle mass during aging,[11,12] conclusive evidence that high-protein intake has meaningful effects on muscle mass and/or function is missing.[13-19] This is most likely due to the saturable relationship between protein intake and muscle protein synthesis after a meal, which reaches a maximum at ~20 to 30 grams,[23,24] combined with anabolic resistance of aging muscle (i.e., the inability to adequately stimulate protein synthesis and suppress protein breakdown in response to postprandial hyperaminoacidemia-hyperinsulinemia).[20-22] Recently, fish oil-derived n-3 PUFAs, i.e., eicosapentaenoic and docosahexaenoic acid, have emerged as a potential new treatment modality for the prevention and treatment of age-associated loss of muscle mass.

Effect of Fish Oil-Derived n-3 PUFA on Muscle Mass and Function

The results from epidemiological studies[25] and experiments in cell cultures and animals[26] suggest that fish oil-derived n-3 PUFA could have therapeutic effects in older adults. We[27] and another group of investigators[28] found that healthy, older women who participated in an exercise-training program and consumed 2 to 4 grams of fish oil per day for three months had greater training-induced gains in muscle strength than those who did not supplement their diet with fish oil. We also found six months of dietary supplementation with 4 grams of fish oil-derived n-3 PUFA increased muscle mass and strength in healthy, physically active but untrained older adults.[29] Daily supplementation with 1.3 grams of n-3 PUFA for 12 weeks, on the other hand, was not associated with improved muscle strength and global physical function in older adults.[30] The lack of an effect in this study was most likely due to both the low dose and short duration of the intervention, because we found significant increases in muscle mass and function after six but not three months of treatment with 4 grams of fish oil-derived n-3 PUFA per day.[29]

The mechanisms responsible for the beneficial effects of fish oil-derived n-3 PUFAs on muscle mass and function have not been fully elucidated but are likely multifactorial. We found that adding 4 grams of fish oil-derived n-3 PUFA per day for eight weeks to the diet of healthy older adults increased the acute amino acid-induced activation of the mTOR-p70s6k signaling pathway and muscle protein synthesis.[20] Others found that adding 3.9 grams of fish oil-derived n-3 PUFA to the diet of older adults augmented the acute exercise-induced increase in muscle protein synthesis.[31] The effect of fish

oil-derived n-3 PUFAs on muscle protein synthesis also has been investigated in young adults, and the results are equivocal. We found that eight weeks of fish oil-derived n-3 PUFA intake (4 grams per day) increased the rate of muscle protein synthesis during amino acid and insulin infusion in sedentary young adults.[32] On the other hand, others found no effect of eight weeks of fish oil-derived n-3 PUFA intake (5 grams per day) on the rate of muscle protein synthesis in resistance-trained young men, who consumed 30 grams of protein at rest or after a bout of resistance exercise.[33] This was likely because the high protein intake combined with regular exercise training already maximally stimulated muscle protein synthesis in this participant group.[24] Studies conducted in cell cultures, rats and patients on maintenance hemodialysis found fish oil-derived n-3 PUFA also attenuated muscle protein breakdown.[26]

Increased muscle function (strength and endurance) could be due to changes in myocytes themselves (myofiber microstructure, contractility and energy production), as well as to changes in external factors (extracellular matrix composition and function, muscle perfusion and neuromuscular function). The results from several studies suggest a coordinated response of several, or all, of these factors may be involved, but this has never been comprehensively evaluated in people. We found that fish oil-derived n-3 PUFA supplementation in healthy older adults increased the expression of genes involved in muscle mitochondrial function,[34] and others found, though not consistently, it reduces oxidant emission[31] and adenosine diphosphate (ADP) sensitivity[35] in mitochondria isolated from human muscle. Rats fed fish oil-derived n-3 PUFAs were found to use less oxygen for tension development, were able to work harder and fatigued later than those fed the control diet.[36] In healthy people participating in an exercise-training program, fish oil supplementation shortened the electromechanical delay and increased the rate of force development during maximal voluntary isometric contractions,[28] and adding fish oil to the diet of mice and rats improved their motor and sensory nerve conduction speed and protected them from developing diabetic peripheral neuropathy.[37,38] Studies conducted in rats and healthy middle-aged people found that fish-oil derived n-3 PUFAs also augment brachial artery dilation, vascular conductance and blood flow.[39,40]

Summary

Fish-oil derived n-3 PUFAs are a potential new treatment modality to prevent and reverse the age-associated loss of muscle mass and function.

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Notes

Notes

Effect of Diet on Loss and Preservation of Lean Body Mass in Aging Dogs and Cats

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Abstract

Sarcopenia may be defined as an age-related loss of lean body mass (LBM) unrelated to disease. It is a long-term process that becomes evident later in life, it has a complex and multifactorial etiology, and it increases the risks for morbidity and mortality. Dietary factors play a role in minimizing this age-related loss of LBM. Specifically, increased intake of protein and vitamin D has shown benefits. Other nutrients that appear to be important are those related to acid/base balance and inflammation. Most of the research into sarcopenia has been done in humans and rodent models, but some aspects have been confirmed in dogs or cats. More research is needed to determine optimum nutrient levels to reduce or prevent sarcopenia in aging dogs and cats.

Glossary of Abbreviations
CKD: Chronic Kidney Disease
LBM: Lean Body Mass
DHA: Docosahexaenoic Acid
eIF2α: Eukaryotic Initiative Factor 2α
EPA: Eicosapenaenoic Acid
PI3K: Phosphatidylinositol 3-Kinase
PTH: Parathyroid Hormone
RDA: Recommended Daily Allowance

Muscle and lean body mass (LBM) are determined by the balance between the rates of *de novo* protein synthesis and protein degradation. During growth, protein synthesis exceeds degradation and muscle mass increases, while in mature subjects protein synthesis and degradation are roughly equal so that muscle mass remains constant. With age this balance is lost and LBM decreases, leading to sarcopenia, which may be defined as an age-related loss of LBM unrelated to disease.

Sarcopenia is a lifelong process that becomes evident later in life, and it has a complex and multifactorial etiology.[1] In most cases, the loss of LBM is offset by an increase in fat mass, resulting in little or no change in body weight. The age-related loss of LBM occurs in all species evaluated to date, including humans, dogs and cats.[2-7]

In cachexia, a 15% reduction in LBM interferes with organic and physiological functions, while a decrease beyond 30% is usually fatal.[8] Although there is no agreed upon cutoff to define sarcopenia, one study identified sarcopenia as a 3% loss in muscle mass or a 40% loss of grip strength over a three-year timeframe.[9]

Functional measures of muscle strength are not conducted in dogs or cats, but significant loss of LBM has been demonstrated in aging dogs and cats. A cross-sectional study of 256 cats showed that apparently healthy cats lose approximately one-third of their LBM between about 10 and 15 years of age.[3] This was confirmed in a longitudinal study following mature and geriatric cats over an eight-year period, wherein mean LBM decreased by 34%.[4] In aging dogs, there was approximately a 10% loss in LBM and corresponding increase in fat mass.[6,10] These effects appeared to be more pronounced in dogs beginning at age 8 to 9 years. Lifelong limited feeding or increased dietary protein intake reduced or delayed this age-related loss of LBM.[6,10] Another longitudinal study in Labrador Retrievers confirmed loss of LBM with age and identified that the longest-lived dogs in the study experienced the slowest loss of LBM.[7] Similar results were observed in cats.[4] As with humans, loss of LBM in dogs and cats is associated with increased mortality.[4,7,10]

Etiology of Sarcopenia

The etiology of sarcopenia is multifactorial and complex. Elderly people, especially those who are sarcopenic, have a blunted anabolic response to nutritional stimuli, such as amino acids and insulin.[11] Some of the factors involved in sarcopenia include altered protein turnover with decreased muscle protein synthesis, due in part to decreased functionality of the mTOR pathway, and a relative increase in protein catabolism; chronic low-grade inflammation with increased cytokines such as TNF and IL-6; mitochondrial dysfunction; increased oxidative stress; insulin resistance; and altered neuromuscular junction structure and function.[11-13] Regardless of the mechanisms, the result is a decrease in endogenous protein synthesis, possibly coupled with increased protein catabolism, leading to decreased muscle mass and strength.

Although exercise is the most efficient therapy for managing sarcopenia, it is recognized that nutrition also plays a role in the development and management of this condition. Specifically, adequate dietary protein, specific amino acids and vitamin D have been shown to play a role. Protein supplementation coupled with exercise achieves the best results in sarcopenic people.[14] Other dietary factors that

may be important include nutrients impacting metabolism, inflammatory mediators and acid/base balance.

Role of Diet in Sarcopenia

Protein:

Insufficient protein intake can contribute to loss of LBM. Multiple studies have confirmed lower protein intakes were associated with increased risk for sarcopenia in elderly people.[15-18] Likewise, in dogs and cats, lower protein intake was associated with greater loss of LBM.[6,19-21] Dietary protein supports both endogenous protein turnover and gluconeogenesis. When dietary protein intake is inadequate, mammals will gradually deplete proteins from their LBM, particularly skeletal muscle, to support these metabolic functions.[15,21]

Aging impacts the physiological response to protein intake. Muscle protein synthesis in response to protein intake is attenuated in older people compared to younger people.[12,13] Increasing the amount of protein consumed may help to overcome this anabolic resistance. For example, muscle-protein synthesis was stimulated in young adults with <10g of whey protein, but older men required >20g whey protein to achieve similar protein synthesis.[22] It appears that the recommended daily allowance (RDA) (0.8g/kg bodyweight), which was established using data from young adult men, is not adequate to maintain nitrogen balance nor preserve muscle mass in elderly people.[12] Although not universally accepted, there is a growing consensus that older people should consume more protein. Daily intake of at least 1.0 to 1.2g/kg body weight is recommended, an increase of 50% over the RDA.[17,23-25]

The amount of protein that is "adequate" for dogs and cats also remains controversial. Traditionally, nitrogen balance studies were used to determine minimum protein requirements. However, nitrogen balance studies do not account for maintenance of muscle mass, and multiple studies have indicated that the amount of protein required to maintain LBM or protein turnover far exceeds that needed to maintain nitrogen balance.[15,21,26-30] For example, cats need only about 1.5g protein/kg body weight to maintain nitrogen (protein) balance but need over 5g protein/kg body weight to maintain LBM.[21] Dogs required about three times more protein to maintain protein/DNA ratios (an indicator of protein reserves) compared to that needed to maintain nitrogen balance, and old dogs needed 50% more protein than young dogs regardless of the measure used.[27] As in humans, greater protein intake helps reduce the age-related loss of LBM in dogs and cats.[6,16,20]

Specific types of protein and amino acids also can impact LBM. "Fast" proteins, such as whey, contribute to greater endogenous protein synthesis compared to "slow" proteins, such as casein. Whey, a soluble protein, is rapidly digested and absorbed. Casein protein clots in the stomach, delaying gastric emptying and slowing uptake of the amino acids.[31] The greatest anabolic effect of protein appears dependent on reaching a threshold concentration. Rapidly absorbed proteins achieve this threshold, while slower absorption of the same amino acids fails to achieve the threshold, resulting in less protein synthesis.[31] In healthy young adults, post-prandial protein synthesis was increased 68% by whey but only 31% by casein. Although casein protein reduced protein catabolism to a greater extent compared to whey, net protein balance was more positive for the whey protein.[31] Similar findings were observed in old rats, in elderly men and following exercise.[32,33]

Whey protein is rich in branched-chain amino acids including leucine, which is recognized to have important regulatory actions on protein turnover. Among other functions, leucine reduces proteolysis and enhances protein synthesis.[13,34] Whey protein also triggers insulin release, which promotes protein synthesis.[22,34] When the effect of whole whey protein was compared to an infusion equivalent to its essential amino-acid content, the intact whey protein resulted in greater muscle protein accrual.[35]

Based on the numerous studies showing beneficial effects from either leucine or whey protein on muscle protein synthesis, studies in sarcopenic humans have been conducted using leucine-enriched whey protein (along with vitamin D) supplements.[36,37] In the first of these multicenter trials, elderly sarcopenic subjects received a supplement containing 20g whey protein (including 3g leucine), 800IU vitamin D and a mixture of vitamins, minerals and fiber twice daily, or they received an isocaloric placebo containing carbohydrates, fat and some trace elements. The baseline protein intake in both groups averaged 1.0g protein/kg body weight daily. Over the 13-week study, the treated group showed significantly greater improvement in the chair-stand test, indicating greater strength and balance, and also in muscle mass.[36]

Another study evaluated subjects given a once daily supplement containing 22g whey protein, 9g essential amino acids, and 100IU vitamin D compared to those receiving a placebo, while both groups underwent a similar exercise program for 12 weeks.[37] The treated group showed significant improvements in muscle mass and muscle strength. In addition, the treated group showed reduced body fat, improved fat distribution, increased insulin-like growth factor, and reduced C-reactive protein, an inflammatory mediator.[37]

The amino acid lysine also may impact LBM. Studies in swine and rats show that lysine deficiency leads to increased protein degradation and decreased protein synthesis in muscle, whereas supplementation with lysine decreased muscle protein degradation.[38,39]

Since lysine is limited (low relative to requirements) in many vegetable-source proteins, supplementation may be most important for vegetarians or for those whose diets are based on vegetable-source proteins. A study in young adult dogs fed diets containing mixtures of corn gluten or poultry as the protein sources evaluated changes in LBM and body fat, and changes in the 20S proteasome of the ubiquitin proteasome pathway involved in protein catabolism.[19] While most dogs

lost LBM, those with the highest lysine intake gained LBM. In dogs fed 12% protein diets, there appeared to be an inverse linear correlation between lysine intake and LBM loss. Further, the 20S proteasome was decreased in dogs fed the high-lysine diet, suggesting a reduction in protein catabolism via this mechanism. This is consistent with pigs, where lysine-deficient diets trigger upregulation of this catabolic pathway.[39]

Similarly, in aging cats, lysine appears to protect LBM. One published study in aging cats evaluated the impact of diets containing protein ranging from 6.87 to 10.22g/100Kcal, and lysine (lysine:calorie ratio) ranging from 2.71 to 6.30 on changes in LBM. Although there were limitations to the study, it showed that increasing dietary lysine, independent of total protein, helped reduce loss of LBM in aging cats.[40]

Vitamin D:

Multiple epidemiological studies have identified an association between low serum vitamin D concentrations and an increased prevalence of sarcopenia in aging people.[9,14,41] Coupled with low vitamin D were increased concentrations of parathyroid hormone (PTH), which also has been associated with loss of muscle mass and strength.[9]

Vitamin D metabolites can influence muscle cell metabolism by mediating gene transcription as well as by other mechanisms.[9] Vitamin D affects the transcription rate of thousands of genes, including insulin receptors.[42] Activation of insulin receptors contributes to increased muscle protein synthesis, and supplemental vitamin D results in increased vitamin D receptors within muscle.[14,43] In aged rats, vitamin D deficiency reduced the rate of protein synthesis by 40% compared to vitamin-D replete rats.[42] In both rodents and humans, vitamin D deficiency induced greater body fat and intramuscular lipids, a finding linked with compromised neuromuscular function.[14] Intramuscular fat also may contribute to reduced protein synthesis via activation of eukaryotic initiation factor 2alpha (elF2α).[42] Activated elF2α inhibits initiation of protein translation and the rate of protein synthesis. Whether this specific mechanism applies to humans, dogs or cats remains to be determined.

Low serum vitamin D may impact muscle function via PTH, which can be increased due to lack of inhibition from vitamin D. PTH increases intracellular calcium concentrations, which may disrupt muscle structure or function. PTH also may stimulate release of inflammatory mediators such as IL-6. Elevated IL-6 in aging humans is associated with lower muscle mass and strength.[9] Independent of PTH, studies have shown an inverse association between serum vitamin D and IL-6 and between intramuscular vitamin D receptor density and intramuscular IL-6 in aging humans.[14]

Studies in humans evaluating vitamin D supplementation have generally yielded beneficial results with improvements in muscle strength as well as muscle mass.[14,37] These effects were primarily observed in individuals with initially low serum vitamin D concentrations, which is common in sarcopenia. Provision of vitamin D along with supplemental protein may yield the best results, but additional research is needed.[14,36,37] Currently, data on vitamin D supplementation to preserve LBM in dogs or cats is lacking.

Acid/Base Balance:

Acidosis is associated with increased protein catabolism, negative nitrogen balance and muscle protein wasting. It appears to promote muscle protein catabolism via the ubiquitin proteasome pathway and to inhibit protein synthesis via promotion of insulin resistance.[44-46] Insulin normally promotes protein synthesis, but this effect is hindered in insulin resistance. Metabolic acidosis induces insulin resistance and interferes with insulin-signaling pathways, leading to reduced phosphatidylinositol 3-kinase (PI3K) activity and increased protein degradation.[46]

The role of acidosis in LBM wasting is best recognized in patients with chronic kidney disease (CKD), but the same or similar mechanisms may play a role in other conditions, including sarcopenia.[47,48] Correction of acidosis in subjects with CKD eliminated the muscle-protein degradation and improved muscle mass.[47]

Animal proteins and cereal grains are metabolized to acidic residues, whereas fruits and vegetables are metabolized to alkaline residues, such as potassium bicarbonate. In non-CKD aging men and women, studies have established an association between greater intake of alkaline foods and greater LBM.[48,49] A small, short-term study in elderly women showed that adding potassium bicarbonate to a high-protein diet significantly reduced nitrogen excretion compared to those fed the high-protein diet alone.[49] A larger study confirmed the benefit of reducing dietary acid load on preservation of LBM in older women, but not in men.[48]

Even mild metabolic acidosis may contribute to a loss of LBM and sarcopenia.[48,50] In a study of men with CKD, in which arterial pH was adjusted by oral intake of sodium citrate/citric acid and ammonium chloride, alterations of pH within the normal range (7.37 to 7.44) induced significant differences in nitrogen balance.[50]

While serum bicarbonate may be monitored in pets, evaluation of blood pH or blood gases to quantify acid/base balance is rarely done, especially in healthy aging pets. Future research should evaluate the importance of acid/base or dietary anion gap on LBM in aging dogs and cats.

Omega-3 Fatty Acids:

Increased markers of inflammation are common in sarcopenic humans and associated with subsequent decline in muscle strength and mobility.[14] Inflammation may interfere with the mTOR signaling pathway, critical for normal protein synthesis.[11] Although not specific to sarcopenia, studies in humans have shown that consumption of fish oil, a source of the long-chain omega-3 fatty acids eicos-

apenaenoic acid (EPA) and docosahexaenoic acid (DHA), results in reduction of the inflammatory mediators C-reactive protein, IL-6 and tumor necrosis factor-alpha.[51]

Independent of circulating markers of inflammation, EPA and DHA supplementation may influence the mTOR signaling pathway to overcome age-related anabolic resistance to protein synthesis.[14] Using a hyperaminoacidemia-hyperinsulinemia clamp to study muscle-protein synthesis in healthy older humans, mTOR activation and protein synthesis were enhanced in those given fish oil over those given corn oil.[11] Observational studies showed correlations between habitual fish oil intake and greater LBM. Some, but not all, interventional studies have shown improvements in strength and muscle mass in aging humans.[14] Although fish oil can reduce inflammatory mediators in dogs and cats, there is no published data evaluating the impact of fish oil on muscle mass or function in these species.

Antioxidants:

Multiple epidemiological studies have shown associations between increased serum antioxidants or decreased markers of oxidative stress and reduced risk for sarcopenia.[14] However, the few interventional studies that have been conducted have not found benefits. On the contrary, one study actually showed a detriment from supplementing vitamins E and C, with a reduced response to exercise on muscle mass.[14]

Other Nutrients:

Reduced calorie intake or reduced digestion and metabolic efficiency can contribute to loss of weight and LBM. Although energy requirements decrease with age in most species, in cats this appears to be true only up to about 10 to 12 years of age.[3] With advancing age, geriatric feline energy requirements actually increase despite a decrease in body size. This effect appears to accelerate after approximately 13 years of age. The increased energy requirement in aging cats may be due, in part, to reduced digestive function.[3] Older cats had an average reduction in energy digestion of about 8% and in protein digestion of about 6%.[52] In other studies, 33% of healthy geriatric cats had a reduced ability to digest fat, and 20% had a reduced ability to digest protein.[3]

Concurrent with the reduced fat digestion, there is reduced absorption of numerous minerals and vitamins, including vitamin B12,[53] potentially contributing to metabolic inefficiencies. Recent epidemiological evidence in humans suggests a possible role of minerals and trace nutrients in sarcopenia. Specifically, magnesium, phosphorus, selenium, and vitamin B12 intakes were lower in the sarcopenic subjects compared to age-matched adults without sarcopenia. Serum B12 was 15% lower in the sarcopenic group compared with controls.[18,54] Given that B vitamins serve as cofactors in energy and protein metabolism, avoidance of deficiencies may be important in preserving LBM.

Conclusions

Rapidly accumulating evidence in humans and other species suggests links between dietary nutrients and preservation of LBM in aging subjects. The data suggest that protein intake should increase with aging. For aging humans, it should exceed the RDA by at least 50%. Older dogs and cats also should receive more protein compared to standard recommendations. Although dietary protein has proven to play a role in the loss of LBM that occurs in aging dogs and cats, most other nutrients have not yet been evaluated for their role in sarcopenia in pets. As loss of LBM is common in both dogs and cats, future research should focus on some of these nutrients that have been studied in other species.

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Notes

Notes

Idiopathic Chronic Enteropathy in Older Cats

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Abstract

Up to approximately 40% of cats over 10 years of age are affected by an enteropathy that causes malabsorption of a variety of nutrients and protein-losing enteropathy. These changes are commonly associated with increased serum pancreatic marker enzymes (feline trypsin-like immunoreactivity [fTLI] and feline pancreatic lipase [fPL]) reflecting probable pancreatitis. Studies of the fecal microbiome and serum metabolome have demonstrated significant relationships between the fecal microbiome and cobalamin status but not with the increased pancreatic marker enzymes. However, concentrations of serum fTLI, and especially fPL, are associated with changes in the serum metabolome, but the associations are dissimilar for these two pancreatic marker enzymes.

Glossary of Abbreviations
fPL: Feline Pancreatic Lipase
fTLI: Feline Trypsin-Like Immunoreactivity
GI: Gastrointestinal
IBD: Inflammatory Bowel Disease
ICE: Idiopathic Chronic Enteropathy

Decline in body weight is common in cats older than 11 years of age.[1] Sometimes this loss is readily attributable to apparent disease, but in many cases cats exhibit no obvious signs of illness and routine diagnostic approaches fail to reveal evidence of an underlying problem.[2,3] Energy requirements of older cats apparently do not decline as markedly as they do in dogs and humans, perhaps because physical activity does not decrease as much with age in cats. Indeed, the maintenance energy requirement of older cats may increase rather than decrease.[3,4] Although cats may be expected to regulate their energy intake to compensate for these changes to maintain body weight, this clearly is not always the case.[4,5]

It has been observed that both protein and fat digestibility decrease in many apparently normal cats after 10 years of age. While the cause of the decreases remains unclear, the changes are quite marked in some individuals and can be particularly dramatic with regard to fat digestibility.[4,5] Often these changes are not readily apparent from casual observation of feces and may only be verified if fecal fat content is quantified by appropriate analytic testing. Methods for such testing are rarely available for evaluation of veterinary patients, even at referral centers.

Whatever the explanation for weight loss and decline in nutrient digestibility in older cats, progressive decline in body weight has been reported in the two years prior to death. As cats live increasingly longer lives and receive attentive health care, this weight loss is more frequently recognized. It is often associated with a variety of seemingly unrelated diseases or laboratory abnormalities and an obvious explanation remains elusive. This article reviews what is known about common age-related changes and what may be done to halt or reverse the decline in body weight that is apparently a predictable prelude to death.[3,4,6]

Attributable Weight Loss

Well-recognized causes of weight loss in old cats include chronic renal disease, diabetes mellitus, hyperthyroidism, inflammatory bowel disease (IBD), exocrine pancreatic insufficiency, and dental problems, to name a few. Most are readily suspected and confirmed based on physical examination and routine laboratory testing. At times, selected additional testing of parameters such as serum thyroxine, trypsin-like immunoreactivity, pancreatic lipase, cobalamin, and folate, as well as orthopedic and dental radiography or gastrointestinal (GI) endoscopy and biopsy may be necessary. Despite thorough investigation, however, the underlying cause of even severe weight loss can sometimes be remarkably difficult to establish conclusively.

Unattributed Weight Loss

Subtle weight loss may not even be noted unless careful records of body weight and body condition scores are recorded over repeated veterinary examinations. Similarly, moderate increases or decreases in food or water intake will go unnoticed by many owners. Even when the most attentive owners provide the best veterinary care for their cats, a substantial proportion of senior cats will experience weight loss, despite apparently otherwise good health and no detectable change in food intake.

Evidence indicates that in these older cats with no apparent classic diseases to explain the weight loss, food digestibility declines with increased age.[3] There is a significant negative correlation between age and fat digestibility (Figure 1). Approximately 10 to 15% of mature cats (8 to 12 years of age) and 30% of geriatric cats (>12 years of age) have low fat digestibility. In some geriatric cats, fat digestibility was found to be as low as 30%, with large stools (not frank diarrhea) and low body weight as the only clinical signs.

There is a significant negative correlation between age and protein digestibility as well (Figure 2). Low protein digestibility also seems to affect mature and geriatric cats. Although the incidence of low protein digestibility is not as high as that of low fat digestibility, approximately 20% of cats older than 14 years of age show protein digestibility lower than 77%. The incidence of low fat and protein digestibility tends to occur in the same cats. A marked decline becomes particularly prevalent after around age 10 (Figures 1 and 2).

It is perhaps not surprising that these changes were correlated with several other measures of health or well-being, including serum tocopherol (vitamin E), cobalamin (vitamin B12), folate, skin thickness, body fat, and body condition score. Overall,

Figure 1. Percentage of cats with low fat digestibility by age.[3]

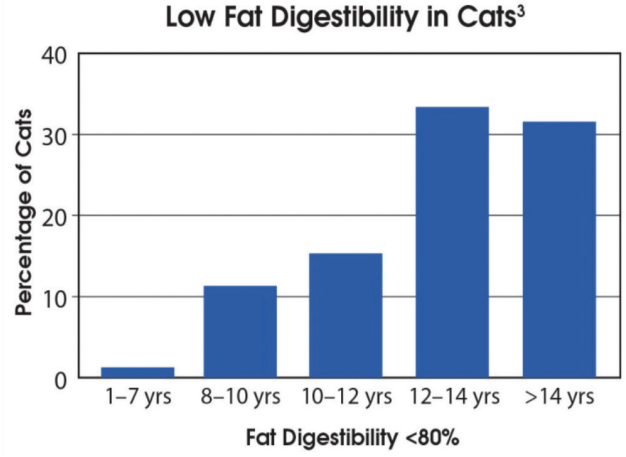


Figure 2. Percentage of cats with low protein digestibility by age.[3]

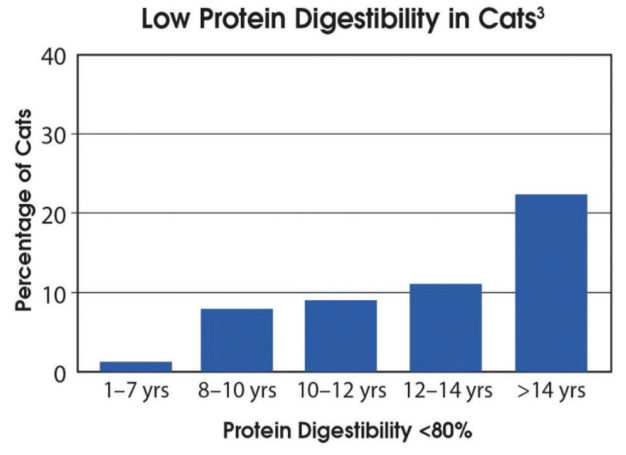


Figure 3. After 12 years of age there is a marked decline in body weight among cats. This change supplants obesity as a common life-threatening condition.[3]

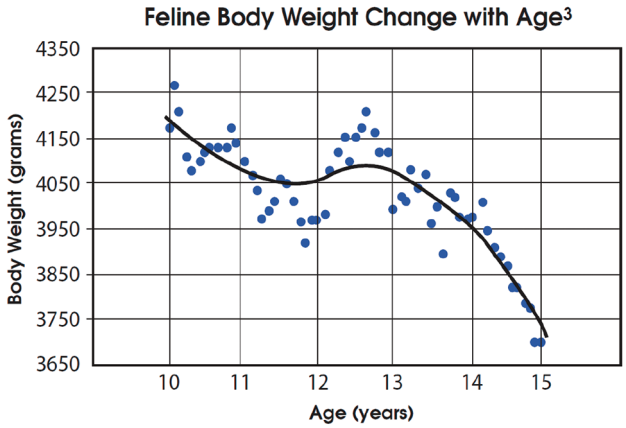


Table 1. Incidence of Feline Obesity and Underweight by Age[3]

Age Group	Body Weight (kg)	Obesity Incidence	Percent Underweight Incidence
Adult (1–7 years)	3.7 ffl 0.8	<1%	<1%
Mature (7–12 years)	4.4 ffl 1.7	28%	<1%
Geriatric (>12 years)	2.9 ffl 1.0	<1%	23%

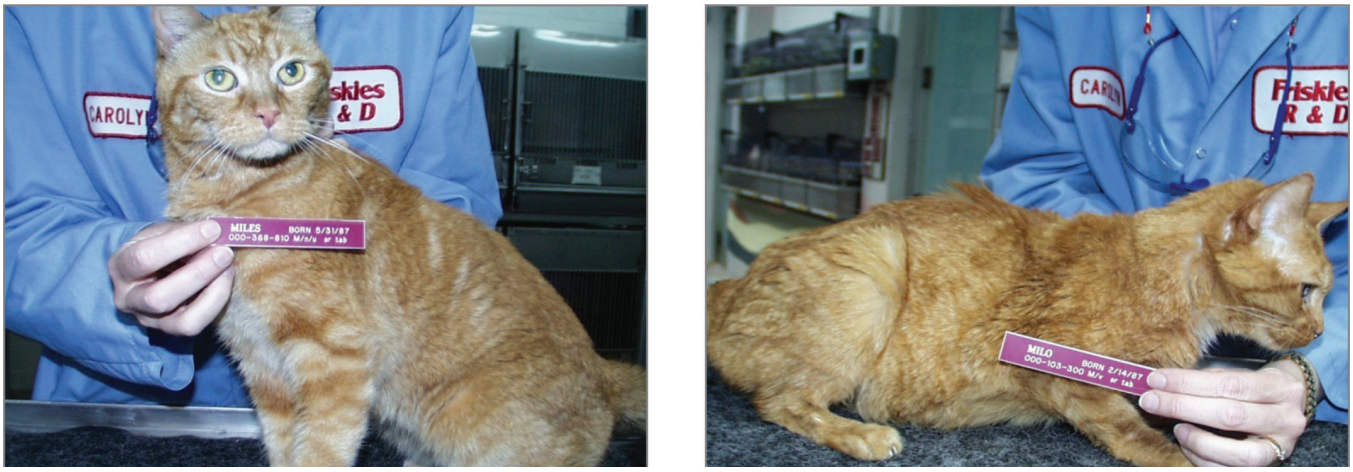
while obesity tends to be the predominant body-mass concern in cats between 7 and 12 years of age, in those older than 12 years of age, obesity is rare, and being underweight is a far greater life-threatening risk factor (Figure 3 and Table 1).[3]

Nutrient Digestibility

The cause or causes of this decline in nutrient digestibility are unknown but presumably reflect an enteropathy of some type since exocrine pancreatic function is not impaired. In some cases, this intestinal dysfunction may overlap with what is commonly loosely classified as (idiopathic) IBD. However, the term idiopathic chronic enteropathy (ICE) is preferred since this is certainly a functional disorder, while morphologic changes are notoriously difficult to quantify and standardize, and may or may not be present. Some cats may compensate for the loss in digestive function by eating more and therefore exhibit no weight loss. It is important to recognize that many cats show only subtle changes in stool characteristics (slightly larger volumes of stool with a more clay-like consistency), but not frank diarrhea, even when steatorrhea is marked.

Regardless of the precise cause(s), weight loss in otherwise healthy older cats, as well as changes in fecal characteristics, should be investigated, as should potential malabsorption. Appropriate investigation methods may include thorough physical examination, routine CBC, serum biochemistry profile, urinalysis, fecal examination, and radiographic and ultrasonographic evaluations. If

Figure 4. Compared to unaffected geriatric cats of equal age, those with poor fat digestibility due to idiopathic chronic enteropathy may exhibit a poor, unkempt-looking hair coat in addition to progressive weight loss.



nothing specific is found to explain the weight loss, then levels of serum thyroxine, feline pancreatic lipase (fPL), feline trypsin-like immunoreactivity (fTLI), and cobalamin/folate should all be determined. It is this author's recommendation that these be determined concurrently, as studies have indicated that approximately 50% of hyperthyroid cats have evidence of concurrent intestinal and/or pancreatic abnormalities, including sometimes severe hypocobalaminemia, at the time of initial diagnosis of the endocrinopathy.[7,8]

Furthermore, all abnormalities detected should be treated concurrently to optimize clinical response to treatment. Many hyperthyroid cats are appropriately diagnosed and treated, but GI signs — especially weight loss — persist despite return to the euthyroid state. Subsequent evaluation of GI function as outlined above then reveals evidence of enteric disease and cobalamin deficiency. Only when these abnormalities are appropriately treated do the cats return to optimal health.

The Diagnostic Process

Unless weight loss is extreme, many affected cats appear normal on physical examination apart from a poor hair coat and unkempt appearance in some cases (Figure 4). Determination of fecal fat (by percentage) would be desirable and may be the only way to confirm an intestinal problem in some patients. Fecal fat greater than 20% would be indicative of fat malabsorption. Unfortunately, such a test is not commercially available for pet cats. It has been reported that 100% of cats older than 7 years of age with serum tocopherol less than 5 mg/L also have low fat digestibility and that more than 90% of cats with serum cobalamin less than 100 ng/L have low fat digestibility.[3] Finding such low serum concentrations of either cobalamin or tocopherol can be the basis of inferring that a cat has low fat (and probably protein) digestibility.[3]

An immunoassay for fecal feline α1-proteinase inhibitor (which was available from the GI Laboratory at Texas A&M University until recently) showed increased results in 73% (11 of 15) of relatively mildly affected cats with early disease, indicating the presence of a component of protein-losing enteropathy in addition to malabsorption (Figure 5).[9,10] Interestingly, only two of these cats had decreased serum albumin, and the reductions were minimal; none had hypoalbuminemia and hypoglobulinemia. However, this enteric protein loss will certainly exacerbate the effects of decreased protein digestibility over time and contribute to gradual depletion of lean body mass.

Recent studies have also revealed the importance of the intestinal microflora in the cobalamin malabsorption that is so common in older cats. In 46 cats examined, serum cobalamin concentration was significantly correlated with the fecal microflora, with 12 species being positively correlated with serum cobalamin and seven species being negatively correlated.[11,12] An additional study evaluated the effectiveness of high dose oral cobalamin supplementation of 13 cats with idiopathic chronic enteropathy, as well as the

Figure 5. Fecal alpha1-proteinase inhibitor (fα1-PI) concentration was increased in 11 of 15 geriatric cats with idiopathic chronic enteropathy, indicating active protein-losing enteropathy in addition to the previously-recognized nutrient malabsorption. The shaded area indicates the reference range of fα1-PI concentration in healthy cats.

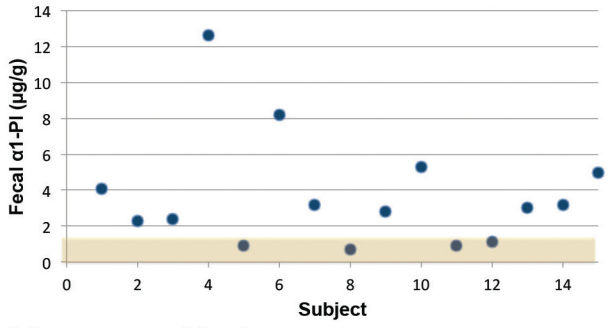
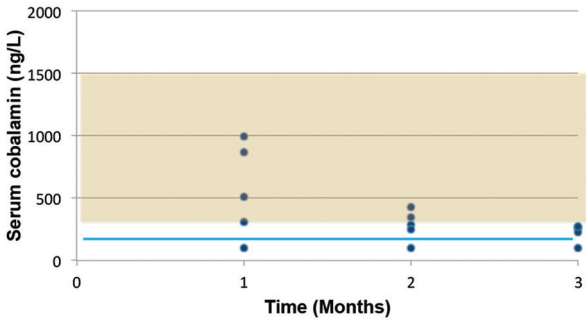


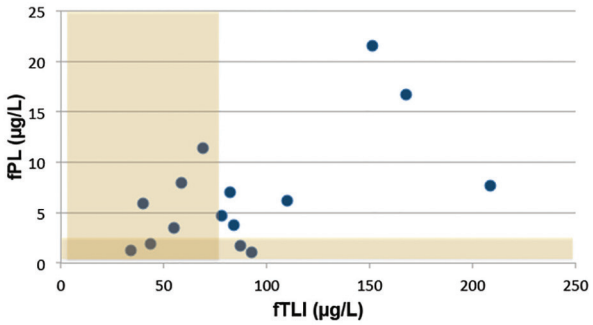
Figure 6. Serum cobalamin can decline rapidly in some affected cats if supplementation is withheld. Once hypocobalaminemia is detected, lifelong supplementation is therefore recommended, with periodic monitoring.



longevity of normal serum cobalamin concentration after withdrawal of oral supplementation. There were clear and significant differences between the fecal microflora of cats with "good" cobalamin status that responded well to oral supplementation and cats with "poor" cobalamin status that did not.[11,12] One of the latter cats developed undetectable serum cobalamin concentrations within one month of cessation of oral supplementation, while five cats had subnormal serum cobalamin within three months of cessation of supplementation (Figure 6).[12]

Finally, it should be noted that almost 90% of cats with ICE have some pancreatic involvement as reflected in increased serum fTLI and/or pancreatic lipase (Spec fPL®). These increases can be substantial in some patients (Figure 7). Given the superior sensitivity and specificity of these markers for pancreatic abnormalities compared to that of cobalamin and folate for small intestinal dysfunction, it is likely that some older cats with chronic elevations of pancreatic marker enzymes have a concurrent enteropathy that is not yet sufficiently severe or chronic to have caused changes in serum cobalamin or folate.

Figure 7. Pancreatic pathology is also present in a high proportion of geriatric cats with idiopathic chronic enteropathy, as reflected in increased serum concentrations of trypsin-like immunoreactivity (fTLI) and pancreatic lipase (Spec fPL®). The shaded areas indicate the reference ranges for each enzyme in healthy cats. Only 2 of 15 affected cats had normal values for both enzymes.



fTLI reference range: 12-82 mcg/L; fPL reference range: 0.1-3.5 µg/L

The genesis of the increases in serum pancreatic marker enzymes in these cats is not clear, but unlike the case with cobalamin our investigations have revealed little evidence of direct associations with the fecal microbiome. Utilization of metabolomic technology has indicated that several metabolites are associated with either fTLI or fPL, but of 89 associated metabolites only three were common to both marker enzymes. Most notably there were highly significant associations between increases in some serum bile acids, decreases in some serum amino acids and serum fPL.[13]

In any case, although it is not possible to differentiate the relative clinical importance of the concurrent pancreatic and intestinal abnormalities in affected cats, it is important to avoid overestimating the significance of sometimes dramatically increased pancreatic marker concentrations compared to sometimes mild-to-moderate decreased concentrations of serum cobalamin or folate; the latter abnormalities only develop secondary to severe and chronic malabsorption, whereas it is now well-established that pancreatitis, especially when chronic, can be clinically silent.

In the future, assay of enteric inflammatory markers such as fecal calprotectin might prove useful in confirming the presence of enteric disease, but the relationship of inflammation to this enteropathy currently is uncertain. Even histologic examination of intestinal biopsy specimens may not provide evidence of a conclusive diagnosis; lesions may be patchy and interpretation of biopsy findings is inherently subjective. It also is increasingly clear that in cats, as in dogs, functional problems in the intestine may not be associated with either inflammation or villous atrophy, but rather with intraluminal microbial changes and biochemical derangements in the enterocytes lining the small intestine that are not revealed by classic histologic evaluation.

Treatment

While evidence for the presence of ICE can often be obtained by the approach outlined above, in some cats despite the most thorough investigation a conclusive diagnosis is not possible, and a presumptive diagnosis of idiopathic enteropathy is the best that can be achieved. Currently, the approach to management of cats with a presumptive diagnosis is the same as those with either histolocially or functionally confirmed ICE, that is dietary change (low-carbohydrate, alternative fiber source, hydrolzyed, or novel antigen diet)), prebiotic or probiotic supplementation, correction of low serum cobalamin/folate concentrations, supplementation with vitamin E and perhaps other antioxidants, antibiotic treatment with metronidazole or tylosin (both often impractical in cats), and perhaps glucocorticoid therapy or immunomodulation with chlorambucil or cyclosporine (Table 2).[14] However, in the absence of specific laboratory abnormalities or overt clinical signs to monitor other than perhaps very slowly

Table 2. Treatment Options for Cats with Idiopathic Interopathy	
Management Approach	**Suggestions**
Dietary change	Low carbohydrate Hydrolyzed diet Alternative fiber source Novel antigen
Supplementation (May be accomplished by dietary change)	Prebiotic Probiotic Vitamin E and other antioxidants
Correction of low serum cobalamin/folate/ tocopherol concentrations	Specific parenteral or oral supplementation as appropriate
Antibiotic treatment	Metronidazole Tylosin (Both often impractical in cats and not recommended)
Enhance enterocyte function	Glucocorticoid (prednisolone)
Immunosuppression	Chlorambucil, cyclosporine, other
Treat Pancreatitis	Analgesics (buprenorphine), antiemetics (maropitant) and appetite stimulants (mirtazapine)

progressive weight loss, it is probably premature to recommend particularly aggressive treatment for these patients and a cautious, conservative approach is warranted.

As many of the observations about digestive disturbances in elderly cats are relatively new, appropriate clinical studies evaluating treatment interventions have not been performed. Dietary changes and supplements would certainly be the safest and most easily administered interventions. When specific nutrient abnormalities such as hypocobalaminemia are identified, they should be rectified. It is now clear that abnormalities in cobalamin metabolism can vary substantially between cats and that supplementation may need to be more aggressive in some individuals to maintain normal serum concentrations. Following cessation of cobalamin supplementation in five cats with idiopathic chronic enteropathy, serum cobalamin concentration fell below normal within three months and was undetectable in one cat within one month.[11,12] Lifelong supplementation is therefore required and periodic monitoring is recommended so that supplementation can be modified as needed. Daily oral supplementation is likely to be effective and can be utilized as an alternative to parenteral (subcutaneous) administration when tolerated.[15]

The effect of dietary changes has to be evaluated on an individual trial-and-error basis, which can be difficult if gradual weight loss is the only clinical sign to evaluate. Observing improvements in the newer GI disease markers such as fecal α1-proteinase inhibitor, should they become readily available, may provide objective evidence of a positive response, but the value of this approach remains to be evaluated.

Careful observation of stool characteristics may provide some evidence of improved digestibility, especially if grossly apparent abnormalities are present at the outset. If there is no apparent response to dietary change after two to four weeks, an alternative diet should be tried. This author prefers to select diet changes based on reduced carbohydrate content (generally associated with increased protein content) and/or different amounts or types of fermentable fiber. Adjusting the fat content of the diet does not appear to be particularly useful in treating feline enteropathies. Unfortunately, definitive studies in geriatric cats with malabsorption have not been done. Treatment needs to be individualized and evaluated on a trial-and-error basis.

With regard to older cats in general, evidence suggests that diet can play a role in maintaining body weight and fat mass — and prolonging life. A control diet (nutritionally complete and balanced adult cat food) supplemented with antioxidants (vitamin E and β-carotene), a blend of n-3 and n-6 fatty acids, and a prebiotic (dried chicory root) was associated with reduced decline in body weight and increased longevity (by more than one year) compared with feeding the control diet alone or the control diet supplemented with antioxidants alone.[6,16] These striking observations illustrate the potential benefit to be gained from dietary and other interventions to address the gastrointestinal changes that appear to be so common in aging cats.

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Notes

Notes

The Fountain of Age: Feeding Strategies for Senior Pets

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Abstract

As the pet population ages and life spans increase, elder pets are becoming an increasing proportion of the population. The veterinary health care team must take a proactive approach to provide nutritional assessments and individual recommendations to pets throughout their life, especially in the senior years. Nutritional needs change in healthy elder pets compared to the young adult life stage. Advancing age also is a time of higher risk for developing medical conditions. Early detection of medical problems can lead to earlier nutritional intervention to support recovery, health and quality of life. With the increased risk of health problems, some comorbidities present a nutritional conundrum. This discussion, using dogs as the primary emphasis, will outline a clinical approach of assessing each patient and prioritizing problems for nutritional care.

Glossary of Abbreviations
AA: Arachidonic Acid
AAFCO: American Association of Feed Control Officials
BCS: Body Condition Score
BW: Body Weight
DHA: Docosahexaenoic Acid
EPA: Eicosapentaenoic Acid
GI: Gastrointestinal
LBM: Lean Body Mass
MCS: Muscle Condition Score
MCT: Medium-Chain Triglycerides
MER: Maintenance Energy Requirement
OA: Osteoarthritis

Aging Is Not a Disease

Senior pets increasingly present to veterinarians for primary care and represent approximately one-third of the pet dog population.[1] Life spans are increasing and thus both the percentage and the age of elder dogs may be increasing.[2] The point at which a dog progresses from adult to a senior or geriatric life stage is variable and subjective. Dogs' life expectancies vary widely depending on breed and body size, and aging changes also are variable. Physiologic changes associated with aging may include loss of senses (hearing or vision), reduced energy requirements and lean body mass,[3,4] as well as a decline in various organ functions. The American Animal Hospital Association Senior Care Guidelines suggest that, with the exception of large-breed dogs, most dog breeds reach middle age by 7 to 8 years of age and should be considered seniors when they reach the last 25% of the predicted life span for their breed.[5] Despite this arbitrary categorization, physiologic changes may develop in middle-aged and senior dogs making them less tolerant of nutritional deficiencies or excesses. Middle-aged dogs are more vulnerable or "at risk" for age-related health problems. Middle age may bring an increasing incidence of chronic diseases, many of which can be influenced by nutritional management.[6] Thus, a vital component of preventive medical care should include a "senior" screen or health-risk assessment for early detection of health problems and adjustments to care to preventing or slowing the onset of age-related diseases. Every senior health screen should include a thorough nutritional assessment followed by an individualized nutritional recommendation.

What Is a Senior Pet Food?

Pet owners perceive that most pets, including senior dogs, are healthy and do not require a therapeutic food,[7] but they still have hundreds of pet foods to choose from. Advice and information recommending the best food is available almost anywhere, from trainers to pet food retailers and from magazines and internet sources to social media. In a survey of pet owners' opinions about nutritional requirements of senior dogs, most responded that senior dogs have different nutritional needs than adults with seniors needing lower calories, fat, sodium, protein, and carbohydrates.[8] However, it is important to remember that there is no established American Association of Feed Control Officials (AAFCO) nutrient profile for a "senior" life stage, thus the nutrient content of products marketed for senior pets varies widely. There is a wide discrepancy between perceived needs of senior pets and actual diet composition of products marketed for senior pets. This makes it even more critical for the veterinary health care team to play an active role in providing credible nutritional advice, especially for senior dogs that have unique nutritional concerns.

Performing a Nutritional Assessment

Before any diet changes are recommended, a nutritional evaluation should be performed. Each nutritional assessment and recommendation should include three components: the

patient, the diet and feeding management factors.[9] An accurate diet history is invaluable when assessing the nutritional health of the patient and will be vital to formulating an individualized diet plan. Understanding the nutritional changes that occur with aging and identifying any changes in the individual patient can help the clinician better match the appropriate food with the patient's unique needs. The patient, the food and the pet owner's feeding practices are interrelated and require reassessment. Health and nutritional status are not static especially in senior pets but rather a dynamic process worthy of continued reevaluation and treatment modifications to match the changing needs of the pet.

Patient Assessment

An initial assessment of the patient can be done quickly and uses information collected as part of a health assessment: a complete medical and diet history and a thorough physical examination and appropriate lab work (e.g., complete blood count, serum biochemical profile, urinalysis, and thyroid function [feline]). The nutritional screening process (Table 1) can quickly identify patients with "nutritional' risks. Healthy seniors, or those without identified risks, that are eating a nutritionally balanced diet, have a healthy body weight, including healthy body condition score (BCS) and muscle condition score (MCS), and are free of significant physical or laboratory abnormalities need no further evaluation at this time. A pet-specific nutrition assessment and recommendation for healthy seniors can be done quickly. Nutritional recommendations should include: the specific food that matches the pet's current nutritional needs, the amount and frequency for feeding and a monitoring plan. In many of these patients, the feeding recommendation involves little or potentially no change but should include a verification and validation for the owner that the current food and feeding plan meets the pet's needs and a documentation of the current feeding plan in the medical record.

Table 1. Intial Screen: Assessing for Nutritional Risk Factors

Nutritional Screen for Risk Factors	Require extended evaluation if (√)
HISTORY OF:	
Treats/snacks/human foods >10% intake	
Inadequate information/inappropriate feeding/food	
Consuming unconventional diets	
Previous/ongoing medical problems	
Gastrointestinal signs	
PHYSICAL EXAMINATION:	
Any abnormal BCS (≠5/9 or 3/5)	
Any MCS <3	
Unintentional weight loss or gain	
New medical condition	
Poor skin hair coat	
Dental disease	

Adapted from Table 2 , AAHA Nutrition Assessment Guidelines. The more risk factors identified, the greater the need for an in-depth nutritonal evaluation and recommendation.

Table 2. Extended Screening: Assessing senior dogs for nutritionally relevant age-related factors

Extended evaluation: Age-related diseases to evaluate in senior dogs
Abnormal Body Condition — Is this pet overweight or underweight?
Diet — Is the pet eating appropriate amounts of balanced diet? · Assess appetite and intake · Assess ability to eat; prehension, mastication swallowing for those underweight &/or poor intake · Perform oral exam — include periodontal, tonsils or any oral abnormalities · Assess sensory input; smell, vision, palatability of food. Consider palatability enhancer if necessary
Mobility and access to food and water · Is the pet able to walk, access food provided? Able to stand to eat? · Other pets or physical limitations impairing access? · Mobility and exercise — Is the pet's MCS normal (3/3)? · Presence of osteoarthritis, lameness, pain? Do these play a role in maintenance of comfort, fitness and healthy BCS? · Activity minimizes sarcopenia · Exercise and activity provide mental stimulation and environmental enrichment
Assess cognitive function · Disorientation/confusion — becomes lost or confused, fails to recognize familiar people? · Changed interactions with family members? Isolates or seeks attention less often? · Change in sleep/activity cycles? Wander or pace, sleep more in day, less at night? · Loss of house training (nonmedical reasons)
An extended evaluation is performed if more than one risk factor is identified in the nutritional screening process. This evaluation should include eating; both appetite and intake and oral exam, aspects of activity and mobility, sleep cognitive function, and behavior.

If nutritional risk factors or age-related problems are identified, an extended evaluation and management plan is indicated. This in-depth evaluation should address some common age-related diseases that may be influenced by nutritional management (Table 2):

- Weight management — achieve or maintain a healthy body weight
- Osteoarthritis
- Cognitive dysfunction
- Organ dysfunction(s)

Diet Assessment

A complete diet history is important for evaluating the pet's current nutritional status. Ideally you would like enough information to reproduce the animal's exact diet (brand and amounts eaten). The diet history should identify all snacks, treats and nutritional supplements by type and amount. The drug/supplement history should include questions about the use of food to administer medication, as it may comprise a significant portion of the pet's intake. Diet history information combined with the patient assessment provide information about the patient's daily caloric requirements and specific nutrient intake. This nutrient intake should be compared to the patient's individual needs. For example, an overweight pet with a robust appetite should not be fed a calorie-dense product. Reducing the amount of a high-calorie product to limit calorie intake could lead to deficiencies of other essential nutrients and increase hunger or undesirable food-seeking behaviors.

Feeding Management Assessment

Feeding practices and preferences influence a pet's intake. Determine whether other pets present competition or limit access to food. Determine whether food is accurately measured, how much/how often food is offered, and how much is eaten. Determine if there have been recent changes to the feeding plan and why, as well as how the pet adapted to those changes. This information will allow the veterinary team to determine the nutritional adequacy of the current diet as well as help to identify factors that could contribute potential success or problems with adherence to a new recommendation.

Reassessment and Modification of Treatment Plan

Nutritional assessment of geriatric pets is an ongoing process. Dogs experience a variable and wide variety of metabolic changes as they age. It is important to communicate and engage pet owners to create the expectation of continued reassessment and treatment modifications that accommodate the specific changes observed in each individual dog rather than adopting a "geriatric" protocol. A vigilant monitoring plan allows early detection of problems if they arise and a better opportunity to intervene or modify the pet's individualized nutritional plan to improve its health. Partner with clients to help ensure success and maintain adherence to the feeding and monitoring goals.

Effects of Aging on Nutritional Needs

Energy

Aging can result in both structural and functional changes of the gastrointestinal (GI) tract. However, no studies report clinically relevant differences in nutrient absorption between young adult and geriatric dogs.[10-11] Maintenance energy requirement (MER) is defined as the energy required to keep an animal in a "maintenance state" or maintaining a normal activity. MER varies depending on factors such as breed, health, neuter status, and age. As dogs age, MER decreases ~25%, with the greatest decrease at middle age (7 years).[12] Loss of lean body muscle (LBM) appears to be the primary factor influencing the reduction in energy requirements.[3] LBM accounts for about 96% of an animal's basal energy expenditure.[13] Aging dogs are often less active than young adults, which contributes to reduced LBM and MER. If no adjustments are made to the pet's energy intake to account for the reduction in LBM, activity and MER, then the senior pet will gain unhealthy weight and increase the risk for obesity. BCS should be closely monitored in elder dogs to prevent obesity. Unhealthy weight gain exacerbates many age-related conditions. A higher protein-to-calorie ratio diet would be beneficial to promote ideal weight maintenance in senior pets identified at risk for obesity and associated comorbidities.[14] Results from a lifetime study performed in dogs revealed lower disease incidence, later onset of disease and increased life span in calorically restricted animals. Dogs fed a 25% reduction compared to controls lived an average of 13.0 years compared with 11.2 years in the control group.[15] Thus, maintaining energy balance and avoiding unhealthy weight gain should be one of the most important health goals for senior dogs.

Water

Elder humans exhibit decreased thirst and drinking when challenged by fluid deprivation. Although unknown in dogs, a similar response is expected.[6] Thus, water intake should be monitored or ensured when elder dogs are exercising or exposed to hot environments. Senior dogs also may be at risk of dehydration if they have subclinical renal insufficiency. When a senior pet has a good appetite but water intake is suspect, add water to the food to ensure adequate intake and hydration.

Protein

Protein requirements increase with age due to increased protein turnover and reduced protein synthesis.[16,17] Healthy senior dogs do not benefit from protein restriction[18] and may be harmed by limiting dietary protein.[19] Protein restriction of seniors could be more detrimental than protein deficiency in younger animals.[20] As a general guideline for estimating daily protein needs, provide 2.55 gms protein/kg body weight (BW) or ~1 gm protein/lb BW.[8, 20,21] This level of protein intake should minimize the risk of protein deficiency. Senior dogs may need up to 50% more than this.[21] Older dogs also require fewer calories, or less food than younger dogs. Diets for older dogs should not only contain lower calories but a higher percentage of protein, or a higher protein:calorie ratio in order to meet the dog's age-related nutritional needs. Based on the diet history, assure the patient is meeting daily protein needs; ~1 gm protein/lb BW (2.55 gm/kg BW), and for cats, 2 gm/lb BW (5 gms/kg)[22-24] minimum. Food with 25% of the calories from quality protein should meet the needs of most

healthy aged dogs and minimize loss of LBM. Assess MCS to monitor LBM.

Nutritional Intervention of Selected Age-Related Diseases

Although the most common age-related conditions are best managed with a multimodal approach combining nutritional strategies, exercise or environmental enrichment and possible medical management, this discussion will focus on nutritional management.

Overweight/Obesity

Hyperadiposity, the most prevalent form of malnutrition, contributes to many of the diseases linked to obesity.[25-27] Still, pets that are overweight very often go unrecognized or may not have this health concern addressed. Based on the canine life span study,[15] which demonstrated the benefits of maintaining a lean body condition, many negative health consequences can occur with as little as 15% weight gain above healthy ideal BCS (4.5-5/9). Thus maintaining or achieving healthy weight and body condition should remain a top priority for senior pet health. Yet overweightness and obesity is still one of the most significant health problems among middle-aged and elder dogs. Monitor the pet's diet, BW, BCS, and MCS at each veterinary visit. Once excess weight is diagnosed, action should be taken to achieve healthy BW and BCS. Creating a negative energy balance promotes weight loss, but nutritional needs still must be met with fewer calories. This is best achieved by feeding foods with low-calorie density, increased protein content and increased nutrient calorie:ratio to assure adequate intake of essential nutrients while restricting calories.

Degenerative Joint Disease

Osteoarthritis (OA), the most prevalent joint disorder in dogs, affects as many as one in four dogs, with OA increasing in incidence and severity with advancing age. Being overweight or obese is recognized as a primary risk factor.[28] Poor mobility and decreased activity are both components of a frailty score in dog. Increased frailty is associated with time to death.[29] Nutritional strategies for improving geriatric health span and minimizing OA include the following:

Weight and Muscle Management: Loss of excess body weight/fat can improve clinical signs of lameness in arthritic dogs.[30] Strategies to maintain healthy BW, BCS and LBM and prevent sarcopenia should be prioritized for senior dogs. The can be achieved by selecting a complete and balanced diet that meets protein and other nutrients while also providing the amount of calories to prevent excess body fat gain. The nutritional goal is to delay the onset and prevent the progression of OA and delay frailty.

Long-Chain Omega-3 Fatty Acids (n-3): These show the greatest evidence for synovial anti-inflammatory effects[31,32] compared to other nutraceuticals. Omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compete with arachidonic acid (AA) in cell membranes to yield less inflammatory leukotrienes prostaglandins and thromboxanes, which reduce the pain of OA. Marine oils (EPA>DHA)[33] are preferred with more effective anti-inflammatory effects compared to shorter chain flax or other plant source n-3 oils. Studies have shown prefeeding an n-3 supplemented diet to dogs before cruciate ligament rupture helped reduce the severity of damage to the joint. There is currently no standard accepted dose. Veterinary diets formulated to help pets with OA have enriched concentrations of omega-3 fatty acids, EPA (EPA: 20:5, n=3) and DHA (DHA: 22:6, n=3). The therapeutic joint diets also include some combination of proteoglycan precursors (glucosamine and chondroitin sulfate) and antioxidants. Consumption of therapeutic diets may allow a reduction in NSAID use. These diets would be better-suited for pets that are not overweight, as therapeutic joint diets are not intended for weight reduction and limiting food to achieve weight loss may not only lead to nutrient deficiencies but also may not deliver the therapeutic level of supplements. For this reason, a new generation of combination diets featuring therapeutic diets formulated with a weight-loss component combined with mobility supplements is entering the market.

Cognitive Dysfunction

As many as 20 to 68% of middle-age to elder dogs are thought to experience cognitive dysfunction or behavioral changes that can manifest in varying degrees of mental decline[34] (Table 2). Nutraceuticals may have potential use both in prevention and treatment but are best when combined with environmental enrichment.[35-37]

Antioxidants: The brain is especially susceptible to free-radical damage and cognitive dysfunction. Multiple studies have shown improved clinical signs of age-related cognitive changes in dogs fed antioxidant-enriched diets or supplements.[35-37]

Medium-Chain Triglycerides: Supplementation with medium-chain triglycerides (MCT) improved cognitive performance and preserved the brain structure of elder dogs. MCT provides an alternate cerebral energy source by way of ketones without restricting dietary carbohydrate or proteins.[38-40]

Supplements Versus Enriched Diets: One caveat for the use of nutraceutical supplementation is that that they have not been adequately assessed for efficacy, optimal doses or nutrient interactions. When considering whether to select a diet containing the supplement or to prescribe a supplement, consider the nutrient composition of the "base diet." Assure that the base diet meets the macronutrient needs of the patient and then determine if it will provide an adequate dose of the intended supplement when fed to meet the energy needs of the pet. If not, it would be prudent to select a more appropriate diet and give the intended dose of supplement.

Table 3. Common Nutrient Modification Ranges for Managing Comorbidities

Nutrient Modification	Dog	Cat
Low protein	<5 gm/100 kcal	<7 gm/100 kcal
High protein	>8 gm/100 kcal	>10 gm/100 kcal
AAFCO* minimum protein requirement	2.0 gm/100 Kcal	4 gm/100 kcal
Low fat	<2.5 gm/100 kcal	<3 gm/100 kcal
High fat	>5 gm/100 kcal	>5 gm/100 kcal
Low sodium	40-120 mg/100 kcal	50-100 mg/100 kcal
AAFCO* minimum sodium requirement	20 gm/100 kcal	50 gm/100 kcal
Low phosphorus	40-120 mg/100 kcal	80-135 mg/100 kcal
AAFCO* minimum phosphorus requirement	100 mg/100 kcal	1.25 mg/100 kcal

*2016 AAFCO adult maintenance minimums
Typical nutrient ranges and AAFCO minimum levels for adult maintenance to use as reference when selecting products with nutrient modifications to either enrich or restrict a particular nutrient.

The Condundrum of Comorbidities

Making a nutritional recommendation seems straightforward when the senior pet is healthy or has only a single problem. Challenges arise when patients present with multiple seemingly competing or conflicting comorbidities such as being overweight with renal disease or cancer and pancreatitis. Except for obesity and osteoarthritis, there is little research in how to manage multiple problems. Yet in the absence of evidence, a patient must eat. A general approach is to perform a thorough nutrition assessment and first try and meet minimum nutrient requirements. If a patient is not eating enough to maintain weight, nutritional support is indicated. If the patient is eating, prioritize problems by determining which condition is progressive, impairing quality of life or imparting the poorest prognosis. Manage those aspects and when possible, address the nutrients of concern for the other conditions. Table 3 lists typical ranges of nutrient modifications to consider when managing multiple medical conditions. For example, an overweight senior cat or dog with early kidney disease may benefit from a modestly high protein, lower phosphorus diet.[41] Once a diet plan is implemented, the patient is monitored to see if the desired effect is achieved with a repeated nutritional assessment and modifications to the plan as necessary in an iterative process.

Summary

Senior pets are increasingly becoming a sizable proportion of patients seen in primary care. Therefore, a proactive approach to making nutrition recommendations to support optimal health and body condition will contribute to their health span. More frequent health screens beginning when pets are middle-aged help to improve disease surveillance, early detection and medical and nutritional intervention.

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Notes

Cachexia, Sarcopenia and Other Forms of Muscle Wasting: Common Problems of Senior and Geriatric Cats and of Cats with Endocrine Disease

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Abstract

Cachexia and sarcopenia are two important syndromes associated with muscle wasting that occur in acute and chronic disease and in aging, respectively. Our studies show that old cats, like dogs and humans, also develop muscle wasting due to sarcopenia, cachexia and metabolic diseases such as hyperthyroidism and diabetes. Of 255 clinically normal older cats evaluated, 38% had evidence of muscle wasting, with a progressive rise in prevalence as the cats' life stage advanced from mature to senior and geriatric. An even higher prevalence of muscle wasting (>75%) occurs in cats with chronic kidney disease and hyperthyroidism. More nutritional research is needed to help prevent and treat these common muscle-wasting syndromes of senior and geriatric cats.

Glossary of Abbreviations
BCS: Body Condition Score
CKD: Chronic Kidney Disease
DEXA: Dual-Energy X-Ray Absorptiometry
GH: Growth Hormone
GHS: Growth Hormone Secretagogues
IGF-1: Insulin-Like Growth Factor
ME: Metabolizable Energy
MSC: Muscle Condition Score

Introduction

Cachexia and sarcopenia are two important syndromes associated with muscle wasting that occur in acute and chronic disease and in aging, respectively.[1,2] Cachexia is a common finding in sick patients (humans, cats and dogs) characterized clinically by weight loss and muscle wasting and is associated with increased morbidity and mortality.[1-4] Sarcopenia is similar to cachexia in that it is characterized by a loss of muscle mass, but sarcopenia occurs in the absence of disease as part of the aging process.[1,2,4,5]

In addition to these two distinct syndromes, there are a variety of other causes for muscle wasting that do not neatly fit within the definitions for either cachexia or sarcopenia — such as muscle loss secondary to thyrotoxicosis.[6-9] Because of this, some experts[10,11] have proposed the more general and simple term of "muscle wasting disease" to incorporate all of these diseases or syndromes that result in a loss of muscle mass, as it is universally applicable and easily understood by the scientific community as well as the lay public.

The prevalence of muscle-wasting disease is increasing both in human and veterinary medicine, in part because of the recognition of these syndromes. Because of the high prevalence and deleterious effects of muscle wasting, a better understanding of these syndromes is critical to optimize feline (and human) care. There is great interest in the development of dietary therapy, new drugs and other treatments to combat these syndromes in people, as well as in cats and other companion animals.

Cachexia

Cachexia is a complicated metabolic syndrome related to underlying illness. It is characterized by loss of muscle mass — with or without the loss of fat mass — that is associated with anorexia, an inflammatory process, insulin resistance, and increased protein turnover.[12,13]

The syndrome of cachexia has been known for centuries. Referring to people with congestive heart failure, Hippocrates wrote that "the flesh is consumed and becomes water ... the abdomen fills with water, the feet and legs swell, the shoulders, clavicles, chest, and thighs melt away ... The illness is fatal."[14] The term cachexia has Greek roots, a combination of the words kakós (bad) and hexis (condition or appearance). Clinically, this muscle and fat wasting leads to poorer outcomes and is associated with a high mortality risk.[15]

In human patients, cachexia can develop in a variety of acute and chronic diseases, including heart failure (cardiac cachexia), cancer (cancer cachexia), chronic kidney disease (renal cachexia), and chronic obstructive pulmonary disease, as well as in patients with a variety of acute illnesses and injuries.[3,16-20] The syndrome of cachexia also appears common in cats (and dogs) with the same medical conditions.[16,21-23]

To our knowledge, an estimation of lean body mass loss as documented by muscle condition score or more specific measures of body composition has not been reported in

cats with acute or chronic diseases. However, our preliminary survey of 20 cats with chronic kidney disease (CKD, IRIS stage 2 to 3) showed that all of these cats had some degree of muscle wasting, with 18 cats (90%) having moderate-to-severe wasting. This muscle wasting of CKD-related cachexia is important since it can negatively impact survival time, at least in human patients with CKD.[24]

Factors that contribute to cachexia include anorexia and a number of associated metabolic alterations, including increased inflammatory status and increased muscle proteolysis. The weight loss associated with cachexia differs from that seen in a healthy person (or cat) who loses weight. In a healthy individual who is receiving insufficient calories to meet daily requirements, metabolic adaptations allow fat to be used as the primary fuel source, thus preserving lean body mass as much as possible. In contrast, the primary fuel source in patients with acute or chronic illness is amino acids; therefore, these patients catabolize muscle (lean body mass) and waste muscle.[3,16] Therefore, loss of lean body mass is a hallmark of cachexia, and fat is lost to a lesser degree. The loss of lean body mass has direct and deleterious effects on strength, immune function and survival.[3,16]

Total weight loss is an insensitive measure of muscle loss, so using weight loss as the sole diagnostic criteria reduces one's ability to identify cachexia until its more advanced stages. In addition, there are certain types of cachexia (cardiac cachexia with pleural effusion) in which weight loss is masked by the accumulation of fluid. Another reason for using factors other than total weight loss for a diagnosis of cachexia is that this is a gradual process. Loss of lean body mass generally develops before marked weight loss can be detected.

Therefore, we should use clinical techniques to identify lean body mass loss at an early stage, if possible, at a time when treatments are much more likely to be successful. The muscle condition score (MCS) should be evaluated in every cat at every visit, in addition to the body weight, body condition score (BCS) and diet history (see below).[25,26]

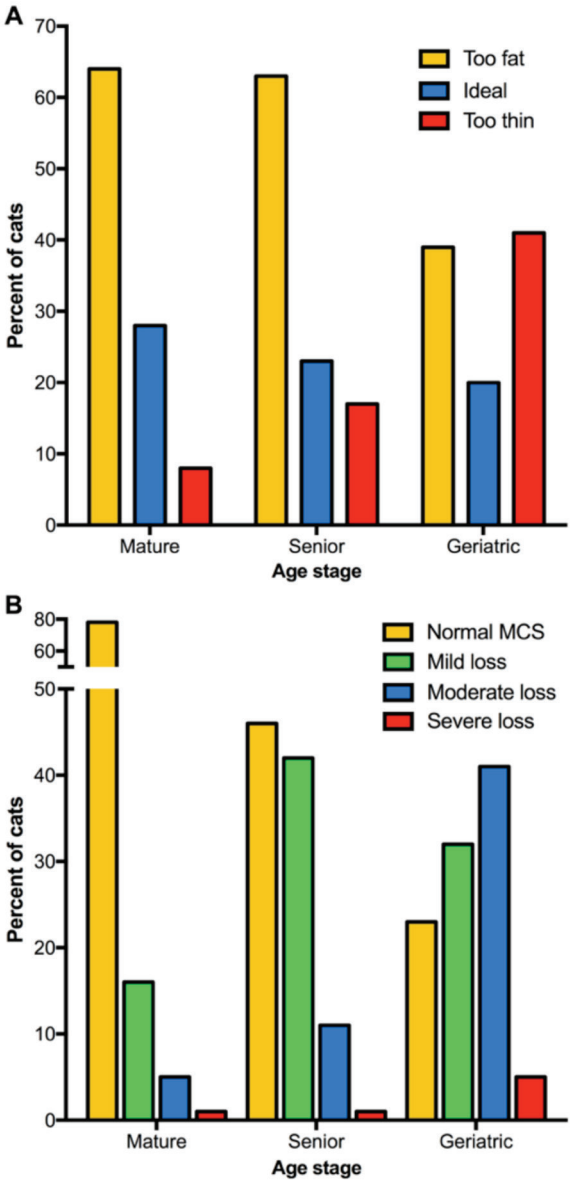
Sarcopenia

Significant loss of lean body mass can develop with aging. This loss of lean body mass — termed sarcopenia — occurs in the absence of disease, though cachexia and sarcopenia can occur concomitantly.[1,2,17,27-29] In contrast to cachexia, which has been known for centuries, the term sarcopenia (from the Greek "sarx" or flesh plus "penia" or loss) was only first proposed in 1989 to describe this age-related loss of muscle mass and strength.[30]

In people, sarcopenia actually begins early in life, with a 30% reduction in muscle mass from 30 to 80 years of age. In sarcopenia, loss of lean body mass often is generally accompanied by an increase in fat mass, then referred to as sarcopenic obesity,[31,32] so the total weight may not change or may even increase, thus masking the sarcopenia. Like cachexia, sarcopenia has important clinical implications because it is associated with physical disability, poor quality of life and increased risk of death.[33]

Few studies investigating sarcopenia have been conducted in client-owned cats, but available information shows that cats also lose lean body mass during aging.[22,34-36] In our survey of 255 cats older than 7 years of age that presented for a routine wellness examination, 96 (37.6%) cats had evidence of muscle wasting (Figure 1). As cats aged, the

Figure 1. (A) Bar graphs depicting the body condition score (BCS) of 255 clinically normal cats categorized into three groups based on their life stage (mature, senior or geriatric). As the life stage of the cats increases from mature to geriatric, notice that the prevalence of fat cats decreases while the prevalence of thin cats increases (P<0.0001).
(B) Bar graphs depicting the muscle condition score (MCS) of 255 clinically normal cats categorized into three groups based on their life stage (mature, senior or geriatric). As the life stage of the cats increases from mature to geriatric, notice that the prevalence of normal muscle mass decreases while the prevalence of moderate and severe muscle wasting increases (P<0.0001).



prevalence of muscle wasting progressively increased, rising from 22% of mature cats (7 to 10 years) to 54% of senior cats (11 to 14 years) and up to 77% of geriatric cats (≥15 years). In addition, a progressive rise in the prevalence of moderate and severe muscle wasting was noted in the cats as they aged (Figure 1A). As the cats aged and lost muscle mass, a progressive rise in the number of underweight cats also was observed (Figure 1B), in accord with previously reported studies.[22,34-36]

Metabolic Causes for Muscle Wasting

In addition to cachexia and sarcopenia, muscle wasting can develop as a result of hypermetabolic endocrine disease. In cats, common metabolic causes for muscle wasting include hyperthyroidism and diabetes mellitus, the two most common endocrine diseases of the cat.[6,8,9,37] Since many of these hyperthyroid and diabetic cats are older, sarcopenia also likely plays a role in the loss of muscle mass in these cats.

Weight loss despite an increased appetite is the classic and most common sign seen in cats with hyperthyroidism. These cats lose weight because hyperthyroidism accelerates their metabolic rate such that energy demand exceeds energy consumption. It is important to realize that hyperthyroidism is a catabolic state.[38] The progressive weight loss and muscle wasting that is characteristic of feline hyperthyroid disease is caused by increased protein catabolism leading to a negative nitrogen balance.[7,39,40]

When hyperthyroid cats first lose weight, the disorder usually can be noticed as a loss of muscle mass in the cat's lumbar paravertebral area. Despite this loss of muscle mass, most mildly hyperthyroid cats retain their abdominal adipose tissue during the initial stages of their thyroid disease and may even have a higher than ideal BCS.[9] With time, severe muscle wasting, emaciation, cachexia, and death from starvation can occur if the cat's hyperthyroidism is left untreated.

In our study of 462 untreated hyperthyroid cats, the cats' median body weight (4.36 kg) was lower than the premorbid weight (5.45 kg) recorded one to two years before diagnosis. Of the 462 cats, 35% of cats were thin or emaciated, but many more (77.3%) had loss of muscle mass. In these hyperthyroid cats, both increasing disease severity and age were associated with a lower body weight (Figure 2), as well as a higher prevalence of low BCS (thinness) and low MCS (muscle wasting) (Figure 3). In other words, severe hyperthyroidism and geriatric age both appear to contribute independently to an increased prevalence of low BCS and MCS in these cats.

After successful treatment of the hyperthyroidism with radioiodine, cats showed increases in body weight, BCS and MCS (P<0.001). However, mild-to-moderate muscle wasting persisted in 45% of treated cats (Figure 4).

In summary, most hyperthyroid cats lose body weight but maintain an ideal or overweight BCS, with only one-third being underweight. As in human hyperthyroid patients, this weight loss is associated with muscle wasting, affecting >75% of hyperthyroid cats. Successful treatment leads to weight gain and an increase of BCS in most cats, but almost half fail to regain normal muscle mass. The reason for this is not

Figure 2.* (A) Bar graphs depicting the body condition score (BCS) of 462 untreated hyperthyroid cats categorized into three equal-sized groups (n=154) of disease severity based on total T4 concentration (i.e., mild, moderate and severe disease). In each of the three groups, the percentage of hyperthyroid cats with low (too thin), ideal and high (too fat) BCS is depicted. As the severity of hyperthyroid disease increases, notice that the prevalence of too fat and ideal cats decreases while the prevalence of thin cats increases (P=0.008). (B) Bar graphs depicting the BCS of 462 untreated hyperthyroid cats categorized into three groups based on their life stage (mature, senior or geriatric). As the life stage of the cats increases from mature to geriatric, notice that the prevalence of fat cats decreases while the prevalence of thin cats increases (P=0.0087).

*Peterson ME, Castellano CA, Rishniw M. Evaluation of Body Weight, Body Condition, and Muscle Condition in Cats with Hyperthyroidism. *J Vet Intern Med*. 2016;30:1780-1789.

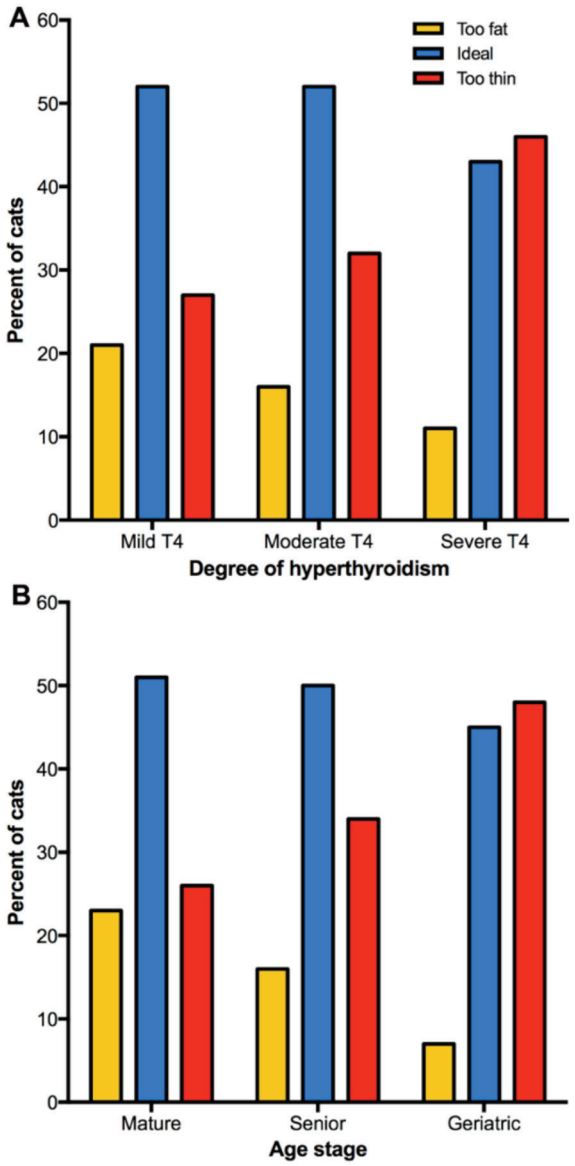


Figure 3.* (A) Bar graphs depicting the muscle condition score (MCS) of 462 untreated hyperthyroid cats categorized into three equal-sized groups (n=154) of disease severity (i.e., mild, moderate and severe disease). In each of the three groups, the percentage of hyperthyroid cats with normal muscle mass and mild, moderate and severe muscle loss is depicted. As the severity of hyperthyroid disease increases, notice that the prevalence of normal muscle mass cats decreases while the prevalence of moderate-to-severe muscle wasting increases (P=0.0002).
(B) Bar graphs depicting the MCS of 462 untreated hyperthyroid cats categorized into three groups based on their life stage (mature, senior or geriatric). As the life stage of the cats increases from mature to geriatric, notice that the prevalence of normal muscle mass decreases while the prevalence of moderate and severe muscle wasting increases (P<0.0001).

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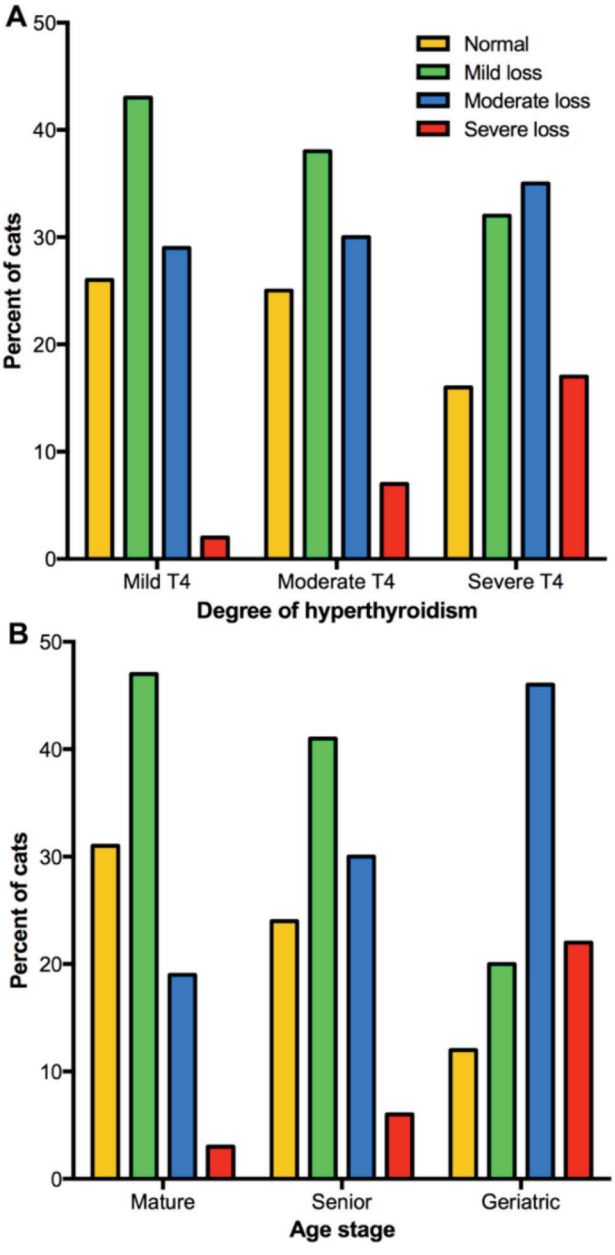


known but is likely related to concurrent sarcopenia of aging or concurrent disease and mild cachexia. In addition, some cats may not be fed diets containing enough dietary protein to help rebuild their lost muscle mass.[41]

Like hyperthyroidism, uncontrolled diabetes mellitus is a catabolic condition so though obesity predisposes the cat to becoming diabetic, loss of weight, and especially loss of lean body mass, is common in cats with diabetes. At the time of diabetes diagnosis, weight loss is reported in about 70% of cats.[42] However, cats are more often overweight or obese (40%), than they are normal weight or underweight.[42,43] Muscle wasting and poor muscle condition scores are detected in about half of cats with diabetes.[42,43]

Epidemiologic studies in cats consistently show diabetes to be a disease of senior cats. Like cats with hyperthyroidism, the typical diabetic cat is a senior, of which about 70% are more than 10 years of age at the time of diagnosis.[42,43] Therefore, because most of these cats are senior, they also are prone to developing sarcopenia of aging, as discussed earlier.

In human patients, type 2 diabetes is associated with an increased risk of concurrent sarcopenia.[44] In addition, because skeletal muscle is a primary site for insulin-mediated glucose uptake and deposition, sarcopenia, and especially sarcopenic obesity,[44] may promote insulin resistance, predisposing them to the development of type 2 diabetes and making diabetes more resistant to control.[45,46] It is not known whether the loss of muscle mass alone (sarcopenia) or combined with weight gain (sarcopenic obesity), both commonly seen in cats with diabetes, also contributes to the insulin resistance and hyperglycemia associated with the feline disorder. However, it is reasonable to assume that sarcopenia and sarcopenic obesity may do so in cats as they do in humans.

Clinical Implications for Diagnosis and Treatment of Muscle Wasting in Cats

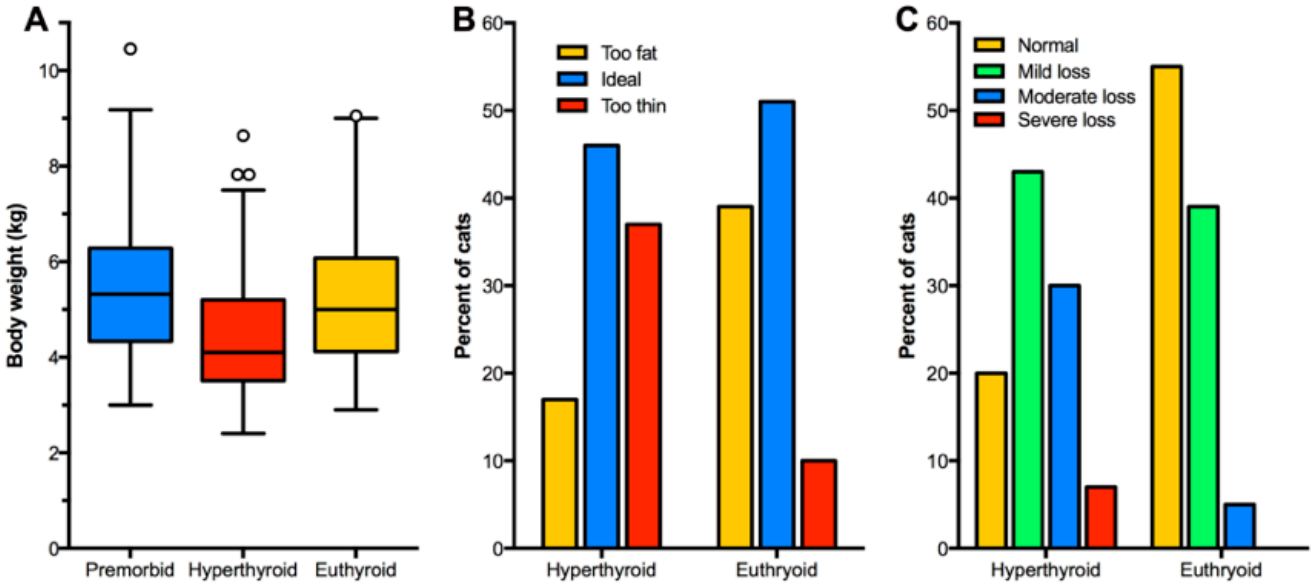
Severe weight loss and cachexia that develop in a cat with cancer, CKD or advanced heart failure are not a diagnostic dilemma. However, identification of cachexia is more difficult at an earlier stage of disease, when muscle wasting is subtler.

As one may expect, it is important to detect cachexia, sarcopenia or other causes of muscle wasting (e.g., hyperthyroidism or diabetes) in cats in its earliest stages, if possible. To achieve this, body weight, BCS and MCS should be assessed during every physical examination. The goal for BCS in a healthy adult cat should be 4 to 5 on a 9-point BCS scale. Obesity (>7/9 BCS) should be avoided.

Use of a routine MCS also is recommended as part of the routine physical examination in every cat at every visit. Muscle condition scoring systems have been developed for cats, as published by the World Small Animal Veterinary Association.[25,26] The MCS differs from the BCS in that it specifically evaluates muscle mass. Evaluation of muscle mass includes visual examination and palpation of the head, scapulae, thoracic and lumbar vertebrae, and pelvic bones.

Figure 4.* Before and after body weight, BCS and MCS of 117 cats reevaluated after successful treatment with radioiodine. (A) Box plots of premorbid, hyperthyroid and posttreatment (euthyroid) body weight in 117 cats evaluated before and after treatment with radioiodine. Notice that the cats' premorbid body weight fell significantly (P<.0001) when hyperthyroid, whereas the posttreatment euthyroid weight increased significantly (P<0.001) to levels similar to premorbid weight.
(B) Bar graphs depicting the percentage of cats with low (too thin), ideal and high (too fat) body condition scores. After treatment, notice that the prevalence of normal and overweight cats increased while underweight cats decreased (P<0.001).
(C) Bar graphs depicting the percentage of cats with normal muscle mass and mild, moderate and severe muscle wasting. After treatment, notice that the prevalence of normal muscle mass increased while mild, moderate and severe muscle wasting decreased (P<0.001). Severe muscle wasting did not persist in any of the cats after treatment.

*Peterson ME, Castellano CA, Rishniw M. Evaluation of Body Weight, Body Condition, and Muscle Condition in Cats with Hyperthyroidism. *J Vet Intern Med*. 2016;30:1780-1789.



BCS and MCS are not directly related as a cat can be obese but still have marked muscle loss, or conversely, a cat can be thin but have normal MCS.

Palpation is required for assessing both BCS and MCS, especially in longhaired cats. Consistently evaluating MCS in all cats will help to identify muscle loss at an early stage (i.e., mild) in aging or ill cats, rather than waiting until muscle loss is moderate or severe, when it may be more difficult to successfully intervene to restore lost muscle tissue.

Several other more objective techniques can be used to estimate body composition, including carcass composition analysis (not ideal for pet cats), deuterium oxide dilution, bioelectrical impedance analysis, quantitative magnetic resonance, ultrasonography, and dual-energy X-ray absorptiometry (DEXA).[47-49] Of these, quantitative assessment of muscle using a vertebral epaxial muscle score is one of the most clinically feasible methods for routine analysis of clinical patients for detecting and monitoring the severity of muscle loss in both dogs[50] and cats (Freeman, LM, personal communication).

For cats with chronic diseases in which weight and/or muscle loss is a component (e.g., CKD, hyperthyroidism, diabetes, cancer, heart disease), a variety of treatments can be used, including careful attention to diet composition.[22,41] This is particularly important because reduced total caloric and protein intake may be a contributing cause of cachexia and other types of muscle wasting in these patients.[51,52]

Dietary modification often is beneficial in improving caloric intake and quality of life for these patients, especially in the aging senior or geriatric cat. Although the optimal nutritional profile will vary depending on the individual cat, two factors — calories and protein — are critical to address for all aging cats that show muscle wasting:

(1) Caloric content of food fed: Like people, cats tend to lose weight as they age due to sarcopenia of aging. For cats that are gradually losing weight (or muscle) with aging, a more calorically dense diet should be selected to help prevent weight loss. Caloric content of commercial senior cat diets vary widely, so diets must be carefully selected for the senior cat to achieve and maintain optimal body weight and BCS.

If an aging cat is healthy, in good body condition and eating a good quality, nutritionally balanced diet, there is no reason to change foods due to advancing age alone. If the cat has one of the diseases often seen with aging (hyperthyroidism, diabetes, cancer, dental problems, CKD), adjusting the diet fed (by increasing calories or protein content) may help improve clinical signs or even slow progression of the disease. For cats with CKD, for example, reducing the phosphorus or sodium or avoiding acidic pH (as well as lower protein in advanced azotemic kidney disease) may be beneficial.

(2) Protein intake: Obligate carnivores, such as the cat, are unique in their need for large amounts of dietary protein (specifically, dispensable nitrogen), which distinguishes

them from omnivores and herbivore species.[53-55] This absolute requirement for dietary protein intake in cats is critically important when formulating a diet for cats with muscle wasting. This is especially true in hyperthyroid cats, in which protein catabolism and muscle wasting is universally present.

Assuming adequate calorie intake, protein is the primary macronutrient responsible for maintenance of muscle mass.[56-59] Restoring and preserving remaining muscle tissue in cats treated for hyperthyroidism depends on the cat consuming a diet with sufficient amounts of high-quality protein. We recommend a target of 40% or more of daily calories from protein, or greater than or equal to 12 g/100 kcal metabolizable energy (ME).[41] This higher-than-average protein level also helps restore and maintain lost muscle mass because many hyperthyroid cats develop sarcopenia as they age.

The dogma that all senior and geriatric cats should be fed reduced-energy senior diets must be questioned based on what is now known about the increasing energy requirements and nutritional needs of these cats.[34,36,56,60-62] In most non-obese senior and geriatric cats, logic dictates the use of highly digestible, energy-dense food to mitigate the decline in body weight and lean body tissue and to avoid protein/calorie malnutrition. Reducing protein intake in geriatric cats, at a time when lean tissue has been lost, is contraindicated; when deprived of adequate amounts of dietary protein, carnivores continue to break down muscle tissue to support protein turnover.[53-55] Feeding larger amounts of high-quality protein may help to restore and maintain lost muscle mass in these cats because many develop sarcopenia as they age. However, randomized clinical trials are needed to investigate the role of nutrition as a treatment for older cats with muscle wasting, as has been done for human patients.[63]

In cats with earlier stages of CKD, phosphorus should be restricted, using methods other than changing to a protein-restricted diet in order to preserve muscle mass as much as possible. In cats with later stages of azotemic CKD, however, use of a prescription kidney diet is recommended. Over-the-counter low-carbohydrate cat foods may have substantially higher phosphate levels than some of the veterinary therapeutic diets designed for diabetes. Therefore, the nutrient profile of the specific product must be obtained to determine whether the product meets the desired nutrient goals for that patient.

Potential Future Treatments for Muscle-Wasting Diseases

Because of the important implications of cachexia and sarcopenia on morbidity and mortality in human patients, there is extensive research into the prevention, diagnosis and treatment of these syndromes. In addition to nutritional modification and exercise, there is ongoing research for new and effective treatments in cachexia and sarcopenia to prevent and even reverse muscle loss.[64] Most current research is focused on drugs aimed at enhancing food intake and increasing muscle mass and function.

One promising new class of drugs is the growth hormone secretagogues (GHS) — a class of small molecule compounds discovered in the mid-1990s that stimulate the release of growth hormone (GH) and may be useful in treatment of anorexia and cachexia.[65] It was subsequently discovered that GHS compounds mimic ghrelin, the hormone that is secreted from endocrine cells in the stomach and stimulates appetite and food intake in humans.[66]

Capromorelin is an oral active ghrelin receptor agonist that mimics the action of ghrelin and acts directly on the hunger centers of the hypothalamus to stimulate appetite and enhance food consumption.[65] The FDA recently approved capromorelin (ENTYCE®, Aratana Therapeutics) as a new drug for use in dogs as a ghrelin receptor agonist. Capromorelin oral solution has been shown to increase food consumption, body weight, GH, and insulin-like growth factor 1 (IGF-1) secretion in healthy laboratory Beagle dogs.[67,68] Preliminary studies indicate that capromorelin also increases food intake and promotes weight gain in laboratory cats,[69] but only limited peer-reviewed research studies on cats have been published to date.[70]

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Notes

Hypovitaminosis D Is Associated with Negative Outcome in Dogs with Protein-Losing Enteropathy: A Retrospective Study of 43 Cases

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Abstract

Background: Hypovitaminosis D previously has been shown to be prevalent among dogs with protein-losing enteropathy (PLE). The hypothesis of this study was that low 25-hydroxyvitamin D (25(OH) D) serum concentrations could be a risk factor for a negative outcome in dogs with PLE. We collected and analyzed serum vitmain D concentrations archived at -80 degrees Celsius from 43 dogs diagnosed with PLE from 2005 to 2014. Post-diagnostic communication with the referring veterinarians allowed us to determine the outcome of the PLE dogs. Dogs that died from PLE within four months after diagnosis comprised the negative-outcome group, n=22, and dogs that were living or that died due to another disease at the end point of the study one year after diagnosis made up the good-outcome group, n=2. Serum samples taken at the time of diagnosis were analyzed for ionized calcium (iCa) concentrations and serum 25(OH)D concentration.

Results: Canine chronic enteropathy clinical activity index (CCECAI) scores, age at PLE diagnosis and iCa concentrations were not significantly different between dog groups. A significantly greater ($p<0.001$) number of PLE dogs treated with a hydrolyzed or elimination diet alone showed good outcome as compared to the PLE negative-outcome group. Median serum 25(OH)D concentration was significantly ($p=0.017$) lower in dogs with negative outcome versus PLE dogs with good outcome. Using logistic regression analysis, 25(OH) D serum concentration was shown to be a statistically significant factor for outcome determination. Cox regression analysis yielded a hazard ratio of 0.974 (95% CI 0.949, 0.999) per each nmol/l increase in serum 25(OH)D concentration.

Conclusions: Low-serum 25(OH)D concentration in PLE dogs was significantly associated with poor outcome. Further studies are required to investigate the clinical efficacy of vitamin D (cholecalciferol) as a potential therapeutic agent for dogs with PLE.

Glossary of Abbreviations
ACTH: Adrenocorticotropic Hormone
BCS: Body Condition Score
CCECAI: Canine Chronic Enteropathy Clinical Activity Index
cPLI: Canine Pancreatic Lipase Immunoreactivity
GI: Gastrointestinal Disease
HR: Hazard Ratio
IBD: Inflammatory Bowel Disease
iCa: Ionized Calcium
IL: Intestinal Lymphangiectasia
25(OH)D: 25-Hydroxyvitamin D
iCA: Ionized Calcium
PLE: Protein-Losing Enteropathy
RIA: Radioimmunoassay
TLI: Trypsin-Like Immunoreactivity

Introduction

PLE in dogs is a clinical syndrome characterized by loss of protein through the intestines.[1] There are three major causes for PLE in dogs including inflammatory bowel disease (IBD), primary intestinal lymphangiectasia (IL) and intestinal lymphoma.[1] Apart from dogs diagnosed with intestinal lymphoma, which generally show poor response to chemotherapy and short survival times, dogs with PLE secondary to IBD or primary IL have a variable prognosis.[1-5] Few reports describe prospective treatment trials of dogs with PLE since mortality is high despite intense immunosuppressive and nutritional treatment protocols.[2,3] Possible life-threatening complications include intractable diarrhea, extreme malnutrition and thromboembolic disease.[6] Risk factors associated with poor outcome have not been well-characterized in PLE dogs. Several breeds are predisposed to the development of PLE, with Yorkshire Terriers having a better outcome in some instances,[4] while in Rottweilers the disease generally carries a poor prognosis.[1] In addition, there is evidence that biomarkers, such as

serum C-reactive protein, serum canine pancreatic lipase immunoreactivity and fecal alpha-1 proteinase inhibitor concentrations, are more commonly elevated in dogs having the shortest survival times.[7,8]

Electrolyte disturbances, such as low total and ionized calcium concentrations and hypomagnesemia, also have been reported in some canine PLE cases.[9,10] It is hypothesized that the ionized hypocalcemia in IBD cases could be caused by reduced vitamin D or calcium absorption, reduced dietary intake and/or vitamin D receptor polymorphisms in impaired vitamin D metabolism.[11] Furthermore, low-serum concentrations of 25(OH)D recently have been described in dogs with chronic enteropathies,[12] and have been shown to be associated with negative outcome.[13] We, therefore, sought to investigate the presence of low iCa and 25(OH)D serum concentrations in dogs with PLE and whether these variables are associated with negative outcome.

Methods

Aim, Design and Study Setting

The aim of the current study was to assess the prevalence of decreased 25(OH)D serum concentrations in dogs with PLE caused by IBD. In addition, we investigated whether 25(OH)D could serve as a prognostic indicator of outcome.

This was a retrospective study that included 43 cases seen at the Royal Veterinary College of the University of London from 2005 to 2014.

Animals

The medical records of dogs referred to the Queen Mother Hospital for Animals (QMHA) at the Royal Veterinary College between 2005 and 2014 were reviewed retrospectively to identify dogs with a clinical diagnosis of PLE. The diagnosis of PLE was made if all of the following applied: (1) history of chronic gastrointestinal (GI) disease, including weight loss, vomiting, diarrhea, and decreased appetite; (2) panhypoproteinemia, with serum albumin less than 2.8 g/dL and serum globulin less than 2.1g/dL; reference ranges from 2.8 to 3.9 and 2.1 to 4.1g/dL, respectively; (3) diagnostic tests including performance of complete blood count, biochemistry profile, urinalysis, abdominal ultrasound, adrenocorticotropic hormone (ACTH) stimulation test, serum trypsin-like immunoreactivity (TLI), and canine pancreatic lipase immunoreactivity (cPLI) serum assays to reflect the presence or absence of primary GI disease versus extra-intestinal disease; (4) histopathological confirmation of IL or IBD with secondary IL; (5) exclusion of hepatic dysfunction by serum bile acid stimulation test; and (6) absence of proteinuria. Proteinuria was excluded in all dogs on the basis of a negative urine dipstick or a urine protein:creatinine ratio of <0.5. In addition, at the time of PLE diagnosis, all dogs had to have a CCECAI[14] recorded by the clinician, and a serum sample frozen within 30 minutes after collection and stored at -80 degrees Celsius until later analysis.

Clinical Data

Follow-up communication with referring veterinarians was made to determine post-diagnostic outcome of PLE dogs. In accordance with previoulsy published studies, dogs were divided into two groups: The first group consisted of dogs that had died from their illness or were euthanized due to intractable clinical disease within four months after diagnosis[4] (negative-outcome group), and the second group consisted of PLE dogs that were alive or had died due to non-PLE disease at least one year after diagnosis (good-outcome group). Individual treatments of dogs also were categorized into two groups: Group 1 dogs comprised those that received either an elimination diet (a commercial single-protein veterinary therapeutic diet the dog had not been given before) or a hydrolyzed diet (commercial hydrolyzed ingredient veterinary therapeutic diet) on an exclusive basis (diet group); Group 2 dogs consisted of dogs that were prescribed an elimination or hydrolyzed diet in conjunction with immunosuppressive drugs, including combination therapy with prednisolone, cyclosporine and/or azathioprine.

Measurement of Ionized Calcium (iCa) and Serum 25(OH)D Concentrations

Vitamin D status was assessed by the measurement of serum concentrations of 25-hydroxyvitamin D (25[OH]D), which is the most widely used approach to analyze whole body vitamin D status.[15] At the time of diagnosis, dogs had samples collected for biochemical and hematological analysis. Residual serum samples were then frozen at -80° Celsius within 30 minutes after collection until future analysis. Ionized calcium concentrations were measured using an ion-specific electrode, and 25(OH)D was measured using commercial radioimmunoassays (RIA) that have been validated for use in veterinary medicine.[17] Samples were shipped on dry ice to the Michigan State University Diagnostic Center for Population and Animal Health (DACPAH)[a] for batch analysis. Serum 25(OH)D and iCa concentrations previously have been shown to be stable under these conditions[16] (and have been personally communicated by DACPAH staff).

Statistical Analysis

Differences between dog groups were assessed using a Mann-Whitney U test for numerical data or Fisher's exact test for categorical data, respectively. Correlations were analyzed using Spearman's rank-order correlation tests. Breed, age, serum albumin concentrations, CCECAI scores, treatment group, iCa concentrations, and 25(OH)D concentrations were entered into a univariate logistic regression analysis. Factors that were significantly associated with outcome in the univariate logistic regression analysis were then assessed in a multivariable logistic regression. Kaplan-Meier estimator and Cox regression analyses were used to illustrate and estimate the effect of 25(OH)D serum concen-

tration on survival times after diagnosis. Hazard ratio (HR) and 95% confidence interval (CI) were reported. Statistical analyses were performed with SPSS version 22 and GraphPad Prism 7 statistical software, with $p<0.05$ considered statistically significant.

Results

Forty-three PLE dogs were included in the study with 21 dogs having good outcomes and 22 dogs having negative outcomes. In the negative-outcome group, median survival time was 19 days (range from 1 to 301 days). In the good-outcome group, 13/22 dogs were still alive at four months, while nine dogs had been euthanized due to non-PLE related illnesss. Median survival time in this latter group was 1,095 days (range from 515 to 3,130 days).

In the good-outcome group, the median age was 5.2 years (range from 1 to 11 years), with six neutered males, three intact males, nine neutered females, and three intact females making up this group. Median age in the negative-outcome group was 6.7 years (range from 0.9 to 13.7 years). Histopathology in this group was consistent with IBD in 13 dogs, IBD with IL in four dogs, and IL only present in five dogs. There was no statistically significant difference in age or breed distribution between the two PLE dog groups (p=0.35 and p=0.42, respectively). Median body condition score (BCS)[b] was not different between the two groups: the good-outcome group averaged a 4.5 BCS (range of 1 to 6), and the negative-outcome group averaged a BCS of 3.8 (range of 1 to 5), p=0.5.

The percentage of dogs receiving immunosuppressive drugs between outcome groups was significantly different, with the negative-outcome dogs receiving more immunosuppressive drugs ($p<0.001$). A greater number of dogs treated with diet alone were in the good-outcome (13/22) group versus PLE dogs in the negative-outcome group (2/21, $p<0.001$).

Median serum albumin concentration was 17g/l (reference range from 28 to 35), with no difference observed between the outcome groups (good-outcome group: median 19, range from 12 to 26; negative-outcome group: median 16, range from 10 to 27, p=0.23). Serum albumin concentration was not correlated with either iCa, 25(OH)D or CCECAI (r^2=1.15, r^2=0.21, and r^2=0.004, respectively).

The median 25(OH)D concentration was 23 nmol/L (range from 0 to 81 nmol/L, reference range from 60 to 215 nmol), being significantly lower in the negative-outcome group (16.5 nmol/L, range from 0 to 66 nmol/L) versus the good-outcome group (37 nmol/L, range from 6 to 81 nmol/L, p=0.017) (Figure 1). Hypovitaminosis D was present in 17 dogs (81%) of the good-outcome group and was not statistically different (p=0.65) than its occurrence in the 20 dogs (91%) of the negative-outcome group (reference range from 60 to 215 nmol). Higher 25(OH)D serum concentration at PLE diagnosis indicated a better prognosis for survival

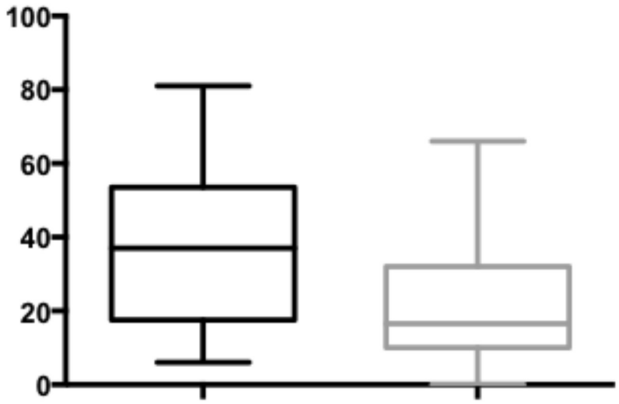

Figure 1. Box and Whisker plots representing 25(OH)D serum concentrations between protein-losing enteropathy (PLE) dogs in the poor-outcome group versus the good-outcome group. 25(OH)D serum concentration in the poor-outcome group: median 16.5 nmol/L, range from 0-66 nmol/L; good-outcome group: median 37 nmol/L, range from 6-81 nmol/L, p=0.017).

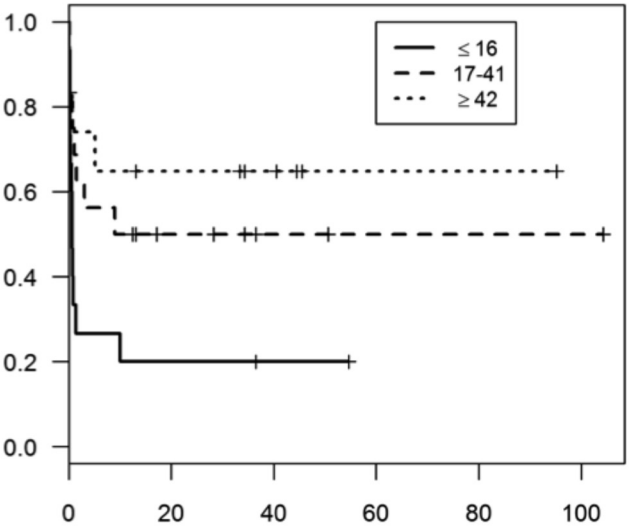

Figure 2. Kaplan-Meier curve and Cox regression using >16 nmol/l, 117-41 nmol/l, and >42 nmol/l as cutoff points for 25(OH)D serum concentration. Higher 25(OH)D serum concentration at diagnosis indicated a better survival of protein-losing enteropathy (PLE) dogs with an hazard ratio of 0.974 (95% CI 0.949, 0.999) per each nmol/l increase in vitamin D.

with a hazard ratio of 0.974 (95% CI 0.949, 0.999) for each nmol/l increase in 25(OH)D serum concentration (Figure 2).

Serum iCa concentrations were measured at the time of diagnosis in 41 of the 43 patients. The median serum iCa concentration in the combined cohorts of PLE dogs was 1.22 mmol/L (range from 0.79 to 1.45 mmol/L, (reference range from 1.25 to 1.45 mmol/L). In the good-outcome group (n=21), the median serum iCa concentration was 1.25 mmol/L (range from 0.79 to 1.35 mmol/L) with 10 dogs having iCa concentration below the reference range. In the negative-outcome group (n=20), the median serum iCa concentration was 1.18 nmol/L (range from 0.84 to 1.45 mmol/L),

with 13 dogs having iCa concentrations below the reference range. There was a moderate positive correlation between serum iCa and 25(OH)D concentrations (r=.52, p<0.0005).

The CCECAI scores between the good-outcome group versus the negative-outcome group were not statistically significant. The results for the negative-outcome group were median 8, range from 4 to 19, and for the good-outcome group were median 7, range from 4 to 13; p=0.6. There was no correlation between CCECAI scores or BCS and 25(OH)D concentrations (CCECAI: r=0.043, p=0.786; BCS: r=0.069, p=0.465). Treatment with immunosuppressive drugs and low-serum 25(OH)D concentration at diagnosis were the only factors associated with negative outcome (univariate logistic regression: p=0.006 and p=0.024, respectively). 25(OH)D serum concentration was the only significant (p=0.033) risk factor in the multivariable logistic regression analysis, with an increase of 25(OH)D level reducing the odds of having a poor outcome (odds ratio=0.96, 95% confidence interval: 0.93 to 0.997).

Discussion

Decreased iCa serum concentrations previously have been described with PLE possibly due to malabsorption of vitamin D in dogs with severe mucosal disease.[10] This study shows for the first time that low 25(OH)D serum concentrations and low iCa serum concentrations are highly prevalent in a cohort of PLE dogs and that decreased 25(OH)D serum concentrations are significantly associated with negative outcome.

There was a significant correlation between treatment group (diet versus diet + drugs) and outcome of PLE patients. The majority of patients in the good-outcome group were managed solely with nutritional therapy, while the majority of patients in the poor-outcome group were treated with diet and immunosuppressive drug protocols. The fact that BCS was not different between the PLE groups also indicates that poor nutritional status alone was not predictive of outcome. In addition, we could not find a correlation between serum albumin concentration and iCa, serum 25(OH)D concentrations or CCECAI. This indicates that loss of vitamin D-binding protein alone is probably not the sole factor for decreased serum 25(OH)D concentrations in these dogs. Furthermore, it may indicate that serum 25(OH)D concentration is an important metabolite to measure in these patients, as serum albumin alone may not be predictive for outcome.

Several studies have described dogs with GI disease, and low total and iCa serum concentrations often are prone to hypocalcemia even after clinical improvement.[9,18,19] This possibly could be due to serum vitamin D levels not being corrected and/or increased fraction of serum ionized calcium. In humans with vitamin D deficiency, survival is significantly better in patients with normal vitamin D levels compared to severely ill patients with vitamin D deficiency.[20] Future studies investigating vitamin D status in dogs should be performed using the gold standard tests as well as standard quality control schemes for laboratories, such as the vitamin D external quality assurance scheme (DEQAS).[c]

[a] Michigan State University Diagnostic Center for Population and Animal Health, Meridian Charter Township, MI
[b] WSAVA Body Condition Score for Dogs (*wsava.org/sites/default/files/Body%20condition%20score%20chart%20dogs.pdf*)
[c] *deqas.org*

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Notes

Searching for Nutrition Targets: Multi-Omics Study in Early-Stage Myxomatous Mitral Valve Disease in Dogs

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Abstract

Myxomatous mitral valve disease (MMVD), common in small breeds and older dogs, can progress to heart failure. It is of great importance to slow or prevent the progression of MMVD at its early stage to extend the longevity of the affected animals. Our goal was to identify and characterize cellular and molecular pathways that might contribute to the pathogenesis and progression of MMVD. Transcriptomics and metabolomics studies were performed using cardiac tissues and serum samples from both MMVD and control dogs. Cardiac tissues were collected from dogs humanely euthanized for reasons unrelated to our study. Our study documented numerous changes, including compromised energy metabolism, increased inflammation and oxidative stress, and altered extracellular matrix (ECM) homeostasis. Some of these changes may benefit from nutritional or medical management.

Glossary of Abbreviations
CHF: Congestive Heart Failure
DET: Differentially Expressed Transcript
ECM: Extracellular Matrix
LV: Left Ventricle
MMP: Matrix Metalloproteinase
MMVD: Myxomatous Mitral Valve Disease
MV: Mitral Valve

Introduction

MMVD, the common acquired cardiac disease in dogs, is characterized by progressive mitral degeneration, which can lead to congestive heart failure (CHF).[1,2] MMVD is common in small- to medium-sized dogs and increases with age.[3] The MMVD dogs typically experience a lengthy preclinical stage when dogs have structural heart disease but no clinical signs of CHF. Once advanced to CHF, the disease progresses much more rapidly.[4] Therefore, early intervention in the preclinical stage to slow or prevent the progression provides an opportunity to extend the life of the affected animals. A multiomics integrative study was conducted to identify molecular and metabolic pathways important for MMVD pathogenesis and progression and to generate testable hypotheses for nutritional or medical management.[5]

Metabolomics and Transcriptomics Analyses

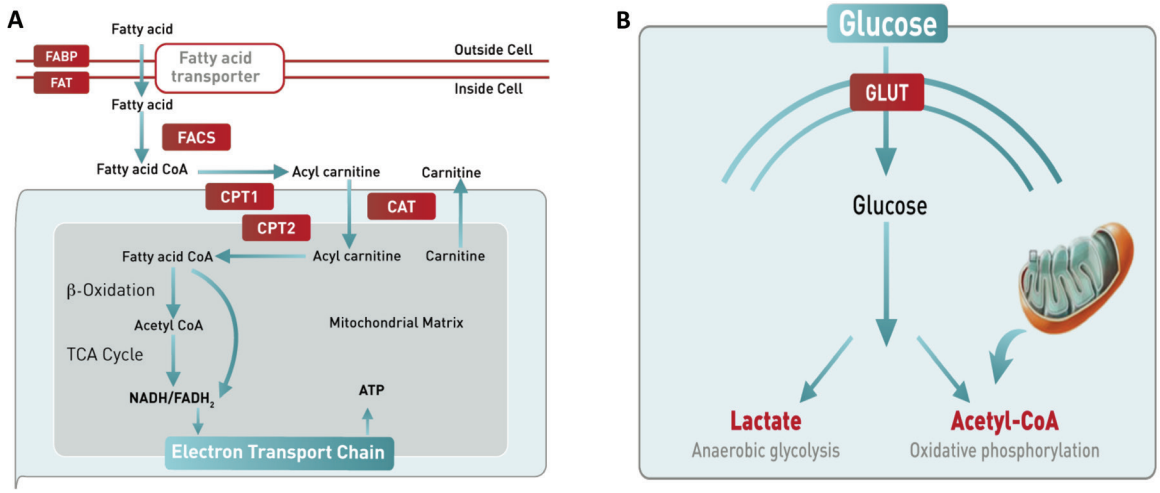
Serum samples from 18 preclinical MMVD and 11 age and sex-matched control dogs were submitted for metabolomics study. Cardiac tissues were collected from dogs humanely euthanized for reasons unrelated to this study. From those dogs, mitral valve (MV) tissues from three preclinical MMVD and three control dogs and free wall tissues of left ventricle (LV) from two preclinical MMVD and four control dogs were subject to RNA-seq transcriptomics study.

Fifty-four differentially expressed metabolites, 812 differentially expressed transcripts (DETs) from LV, and 263 DETs from MV were identified. There were 114 DETs common in LV and MV. Fifteen DETs from LV tissue were chosen for RT-qPCR validation, and 13 were confirmed.

Increases in Anaerobic Glycolysis and Decreases in Fatty-Acid Oxidation

Long-chain fatty acid (LCFA) oxidation provides more than 70% of energy for the normal adult mammalian heart.[6] Numerous changes in gene expressions related to energy metabolism pathways were observed in dogs with MMVD (Table 1).[5] The expression of three genes involved in LCFA transport to the cytoplasm, including fatty acid translocase (FAT), membrane-bound fatty acid binding protein (FABP4), and fatty acid transporter proteins (FATP6), were altered. Long-chain acyl-CoA synthetase (ACSL1 or LC-FACS), the enzyme responsible for activating LCFA to its CoA ester in the cell, was downregulated 2.6 and 3.0 folds in MV and LV of dogs with preclinical MMVD, respectively (Figure 1A). Serum concentration of deoxycarnitine, the immediate precursor of carnitine, was downregulated in MMVD dogs (Table 2), suggesting compromised fatty-acid oxidations. In addition, expression of 3-oxoacid CoA transferase 1, the rate-limiting enzyme for ketolysis and phytanoyl-CoA hydroxylase, which is important for branched-chain fatty acid oxidation, was downregulated in the mitral valve of MMVD dogs. Serum levels of three long-chain acyl carnitines also were lower in dogs with MMVD than control dogs, though the difference did not reach statistical significance. In a previous microarray gene expression study, Oyama and Chittur also observed a decreased expression in acyl-CoA synthetase in the MV of dogs with end-stage MMVD.[7]

Figure 1. Schematic representation of (A) long-chain fatty acid (LCFA) transport system in the cell membrane and mitochrondrial membrane. Fatty acid translocase (FAT), along with fatty acid-binding protein (FABP), bind LCFAs at the cell surface and transport them across the membrane. Some LCFAs also are transported by fatty acid transport proteins (FATPs). Once inside the plasma, LCFAs are activated by long-chain fatty acyl-CoA synthetase (LC-FACS or ACSL) to form acyl-CoA esters, which are converted to fatty acylcarnitine by carnitine palmitoyltransferase 1 (CPT1), also known as carnitine acyl transferase. Acylcarnitine is transported across mitochondrial membrane by carnitine/acylcarnitine transporter (CAT) for β-oxidation. (B) Glucose transport in the cell membrane. Glucose transport over the plasma membrane is facilitated by a group of membrane proteins called glucose transporters (GLUTs). Oxidation of glucose under aerobic conditions, oxidative phosphorylation, results in 32 ATP molecules per glucose molecule, while anaerobic glycolysis only generates two ATPs per glucose molecules.



In contrast, glucose uptake and anaerobic glycolysis were upregulated. Glucose transporter (GLUT) is a large family of membrane-bound proteins that facilitate the transport of glucose across the cell membrane (Figure 1B). Our gene expression study showed increased expression of GLUT3 in MV and LV and of GLUT6 in MV of MMVD dogs (Table 1). GLUT3 is a GLUT isomer with higher affinity and greater transport capacity for glucose than other isomers. In addition, serum metabolomics analysis showed lower concentration of glucose but higher lactate level in MMVD dogs compared to control dogs (Table 2). Our results suggested dogs with MMVD had compromised fatty acid oxidation and increased reliance on anaerobic glycolysis, where one glucose molecule produces only two ATPs or about 5% of its energy potential. Taken together, our study suggested energy insufficiency plays a role in the development and progression of MMVD in dogs.

Increases in Inflammation and Oxidative Stress

Glutathione S-transferases (GSTs) belong to a family of metabolic isozymes that catalyze the conjugation of reduced glutathione (GSH) to xenobiotic substrates or peroxidized lipids for the purpose of detoxification or reduction of oxidative stress. The expression of GSTP1, a GST isomer, was decreased in MMVD dogs (Table 1). Serum concentration of oxidized glutathione (GSSG) was significantly higher in MMVD dogs than in control dogs (Table 2). In addition, SIRT5, an NAD-dependent deacyclase that activates superoxide dismutase (SOD), was downregulated in MMVD dogs.[5] Previously Freeman, et al., reported that GSH:GSSG ratio and vitamin E concentration were significantly lower in dogs with CHF than in the controls.[8] Increased oxidative stress and reduced vitamin E concentrations also were reported in dogs with idiopathic dilated cardiomyopathy.[9]

In humans, impaired cardiac function was associated with elevated plasma levels of proinflammatory markers,[10,11] which decreased after treatment.[12] In dogs, increased concentration of C-reactive protein, a marker for inflammation, was associated with CHF.[13] Collectively, data from our study and others demonstrated increased inflammation and oxidative stress in dogs with MMVD.

Altered ECM Homeostasis

In the heart, dynamic homeostasis of ECM plays an important role in maintaining the structural integrity and function of normal MV.[14] The matrix metalloproteinases (MMPs) are the driving forces for ECM degradations, whereas their inhibitors, known as tissue inhibitors of MMPs (TIMPs), promote ECM synthesis.[15] Misregulation in these gene-expression programs has been implicated in the maladaptive ECM remodeling in canine MMVD.[14] In our current study, while no expression change in MMPs and TIMPs was observed in the MV tissue in MMVD dogs, greater than 100-fold increases in MMP8 and MMP9 and more than 3-fold decreases in MM11 and MMP15 were found in the LV of dogs with MMVD (Table 1). TIMP1 was upregulated by more than 50-fold in the LV, but no difference was found in MV. Interestingly, Oyama and Chittur previously documented a 4.5-fold increase in TIMP1 in the MV of end-stage MMVD dogs,[7] suggesting different regulatory programs in ECM homeostasis in dogs with early- and late-stage MMVD.

Table 1. Heat map of differentially expressed transcripts from the RNA-seq study on left ventricle and mitral valve tissues from dogs with myxomatous mitral valve disease (MMVD) and control dogs. All are significant (P<0.01) unless otherwise indicated.

Symbol	Functional Role	Mitral Valve	Left Ventricle	Description
		Fold change from control		
GLUT3	EM	7.49	16.51	Solute carrier family 2, facilitated glucose transporter member 3
GLUT6	EM	11.7	NS	Solute carrier family 2, facilitated glucose transporter member 6
ACOT6	EM	-3.33	NS	Acyl-CoA thioesterase 6
ACSL1	EM	-2.57	-2.98	Acyl-CoA synthetase long-chain family member 1
FABP4	EM	-2.91	NS	Homolog to human fatty acid binding protein 4, adipocyte
FATP6	EM	4.01	NS	Solute carrier family 27 (fatty acid transporter), member 6
PHYH	EM	-3.01	NS	Phytanoyl-CoA hydroxylase-like
OXCT1	EM	-2.5	NS	3-oxoacid CoA transferase 1
SIR5	OS	-2.31	NS	NAD-dependent protein deacylase sirtuin-5, mitochondrial
GSTP1	OS	-2.56	NS	Glutathione S-transferase pi 1
MMP8	EC	NS	162	Matrix metallopeptidase 8 (neutrophil collagenase)
MMP9	EC	NS	256	Matrix metalloproteinase-9
MMP11	EC	NS	-6.2	Matrix metallopeptidase 11 (stromelysin 3)
MMP15	EC	NS	-3.4	Matrix metallopeptidase 15 (membrane-inserted)
TIMP1	EC	NS	47.7	Tissue inhibitors of metalloproteinases -1
ADAMTS1	EC	NS	4.23	A disintegrin and metallopeptidase with thrombospondin repeats, 1
ADAMTS4	EC	7.45	13.4	A disintegrin and metallopeptidase with thrombospondin repeats, 4
ADAMTS7	EC	NS	-4.12	A disintegrin and metallopeptidase with thrombospondin repeats, 7
ADAMTS9	EC	NS	13.8	A disintegrin and metallopeptidase with thrombospondin repeats, 9

EM, extracellular matrix; OS, oxidative stress; EC, energy metabolism; NS, nonsignificance.
Red, green and gray colors indicate a significant increase, decrease and nonsignificance in gene expression, respectively. A positive number reflects increased expression in dogs with MMVD; a negative number reflects decreased expression in dogs with MMVD.
Adapted from Li, et al.[5] Permission to reproduce was obtained from Mary Ann Liebert Inc.

Table 2. Heat map of differentially expressed identifiable serum metabolites in dogs with myxomatous mitral valve disease (MMVD) and healthy controls.

RanF*	BIOCHEMICAL NAME	FC	PATHWAY	SUB PATHWAY
Y	Glutathione, oxidized	2.32‡	Amino acid	Glutathione metabolism
	Glucose	0.91§	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism
	Lactate	1.32§	Carbohydrate	Glycolysis, gluconeogenesis, pyruvate metabolism
Y	Deoxycarnitine	0.85§	Lipid	Carnitine metabolism
Y	N-acetylneuraminate	1.88‡	Carbohydrate	Aminosugar metabolism
Y	N-glycolylneuraminate	2.51‡	Xenobiotic	Food/plant component

*RanF: Y = Random Forest Analysis identified this as important for separating samples between dogs with MMVD and controls
†Fold Change (FC) in concentration of metabolites in serum samples from dogs with MMVD and controls. Red and green indicate a significantly upregulated and downregulated metabolite, respectively. A number greater than 1 reflects a higher concentration in dogs with MMVD; less than 1 reflects a lower concentration in dogs with MMVD.
‡, § Statistical significance where ‡=P < 0.01 and §=P < 0.05.
Adapted from Li et al.[5] Permission to reproduce was obtained from Mary Ann Liebert Inc.

Numerous changes in yet another ECM metalloproteinase family, A Disintegrin and Metalloprotease with Thrombospondin Repeats (ADAMTS), were observed. ADAMTS1, ADAMTS4 and ADAMTS9 were upregulated, while ADAMTS7 was downregulated in the LV of MMVD dogs (Table 1). Such changes have been implicated in LV remodeling in MMVD.[16-18] ADAMTS4 also was increased in the MV of MMVD dogs.

Histological changes in MMVD include excessive deposition of proteoglycans and abnormal fibrillary ECM organization.[14] The sialic acid family includes a group of N- or O-linked derivatives of neuraminic acid of a 9-carbon backbone. The best-known member of sialic acid family is N-acetylneuraminate. The sialic acid linkage patterns were altered in the mitral valves of pigs affected with endocardiosis.[19] Increased concentrations in serum sialic acids have been associated with heart failure.[20] Increased sialic acid metabolites, N-acetylneuraminate and N-glycolylneuraminate, were found in the serum of dogs with MMVD (Table 2),

suggesting that changes in sialic acid metabolism also may contribute to the development of MMVD in dogs.

Conclusions

Our study demonstrated numerous molecular, cellular and metabolic changes in dogs with MMVD using an integrative transcriptomics and metabolomics analysis. Our results demonstrated increased reliance of anaerobic glycolysis in the context of reduced fatty acid oxidation in dogs with MMVD. Markers of oxidative stress and inflammation also increased. Other changes included alterations in ECM homeostasis. Many of these changes may benefit from nutritional or pharmaceutical management.

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Notes

Notes

Rethinking Protein Restriction in Aging Dogs and Cats with Chronic Kidney Disease

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Glossary of Abbreviations
AAFCO: American Association of Feed Control Officials
CKD: Chronic Kidney Disease
DM: Dry Matter
DMB: Dry Matter Basis
GFR: Glomerular Filtration Rate
ME: Metabolizable Energy
Pr: Protein
Ph: Phosphorus

Renal disease is a common cause of morbidity and mortality in older dogs and cats, with the average age of dogs and cats diagnosed with renal disease being 10.2 years and 13.2 years, respectively.[1] For decades, dietary protein restriction has been a cornerstone of nutritional management of dogs and cats with chronic kidney disease (CKD) as a way to decrease production and retention of nitrogenous waste products and to reduce dietary intake of phosphorus. While clinical studies in both dogs and cats with naturally occurring CKD[2,3] clearly show the benefits of feeding therapeutic renal diets to these patients when compared to feeding a typical maintenance type diet, the renal diets and maintenance diets used in these studies varied in multiple key nutrients in the management of CKD (protein, phosphorus, omega-3 fatty acids), and therefore it is unclear which nutrient(s) resulted in the clinical benefits shown in these studies.

The majority of dogs and cats diagnosed with CKD are older animals, and there currently is a lack of consensus whether renal diets contain the optimum protein content for dogs and cats in this age range. Unfortunately, there is not a plethora of research available to help determine what optimal protein intake is in these patients, and the risks and benefits of increasing protein intake in patients with CKD must always be carefully considered before making changes to the current levels of dietary protein found in renal diets. However, there is some evidence to suggest that it may not be ideal to restrict dietary protein intake to the level that we currently do given the typical age of patients with CKD, but clearly more research is needed to determine what the appropriate level of protein intake is in these patients that maximizes benefits while minimizing risks.

Changes in Protein Metabolism as Dogs Age

The downside to reduced protein diets for the management of CKD in dogs is that this is generally a condition diagnosed in older dogs, and older dogs may actually require more dietary protein than their younger counterparts to maintain protein reserves and maximize protein turnover rates because they are less efficient in metabolizing dietary protein.[4,5] It has been shown that older dogs have less duodenal villus surface area, lower jejunal villus height and greater colonic crypt depth,[6] though there is no clear evidence that protein digestibility is adversely affected in older dogs. However, there also is an increase in protein turnover with aging in dogs, resulting in increased nitrogen excretion, as well as an age-related decline in protein synthesis.[4,7] This results in progressive sarcopenia.

In a study by Kealy,[8] 26 healthy Pointers in the age range of 7 to 9 years were assigned to diets based on gender and body weight and fed either a 16.5% or 45.6% protein diet. After two years of study, the percent of lean body mass was directionally higher and the percent of lean body fat was directionally lower in dogs on the 45.6% protein diet than in dogs on the 16.5% protein diet. The dogs fed the 16.5% protein diet had an average percent of lean body mass and body fat of 71.1 and 24.8, respectively, whereas the dogs fed the 45.6% protein diet had an average percent of lean body mass and body fat of 76.2 and 19.6, respectively. It is unknown at this time how the results of this study might have been impacted if it were done in older dogs with CKD versus healthy older dogs.

In people, loss of lean body mass that often accompanies dietary protein restriction also can result in loss of physical strength and motor coordination, as well as impaired immune function.[9,10] Loss of lean body mass also has been associated with increased rates of morbidity and mortality in people, and a similar result was observed in dogs in the Kealy study. In addition, in one study in dogs with CKD, higher body condition score, which is an assessment of percent body fat, at the time of diagnosis of CKD was significantly associated with improved survival.[11] Unfortunately, muscle condition score was not evaluated in this same study to know whether lean body mass also impacted survival.

Dietary Phosphorus Restriction Beneficial in Dogs with CKD

Two studies published in the 1990s showed that when dietary phosphorus was restricted, dietary protein restriction was not necessary in the management of CKD in dogs. One study evaluated two diets in dogs with varying levels of naturally occurring CKD.[12] In this 28-week study, 32 dogs with CKD were fed a diet consisting of 27% protein and 0.36% phosphorus dry matter (DM), while 28 dogs were fed a diet consisting of 21.5% protein and 0.38% DM. Both diets resulted in reductions in BUN and serum creatinine, however, both values were significantly lower in the higher protein diet for the majority of time points evaluated.

Another study was conducted in dogs to determine the effects of high (H) and low (L) levels of dietary phosphorus and protein on renal function and survival in adult dogs with induced CKD.[13] Forty-eight dogs divided into four diet groups (n=12) were fed one of four experimental diets for 24 months after surgical reduction of renal mass. The experimental diets contained varying levels of protein (Pr) and phosphorus (Ph) listed on a percent dry matter basis (DMB): diet 1: HPr:HPh (32% Pr, 1.4% Ph); diet 2: HPr:LPh (32% Pr, 0.4% Ph); diet 3: LPr:HPh (16% Pr, 1.4% Ph); and diet 4: LPr:LPh (16% Pr, 0.4% Ph). Diet 4 is most consistent with many commercially available veterinary therapeutic renal diets. Results showed that when renal function was reduced to the point that moderate azotemia (serum Cr: 3 to 4 mg/dl) occurs, dietary phosphorus restriction was beneficial, with a longer period of stable glomerular filtration rate (GFR) and improved survival. However, dogs fed 32% dietary protein had neither functional nor morphologic evidence of adverse effects of increased protein intake, compared to dogs fed 16% dietary protein. As a result, this study showed that survival was enhanced by phosphorus restriction but not by protein restriction.

In a multicenter clinical trial the author was involved with evaluating two diets in dogs with naturally occurring CKD, both diets were restricted in phosphorus, however, one diet was restricted in protein as well (15.28% DM), while the other diet was not restricted in protein (22.17% DM). In addition, the nonprotein restricted diet also was supplemented with fish oil, prebiotic fiber and antioxidants. When controlling for the level of azotemia at entry into the study, the higher protein diet did not result in any adverse effects on survival. In fact, in the population of dogs (n=25) enrolled by the author, survival time was a third longer in the dogs consuming the higher protein diet. Therefore, based on the three studies referenced here, it appears that dogs with CKD can be safely fed higher levels of dietary protein than what is typically used in therapeutic renal diets as long as dietary phosphorous is restricted.

Dietary Protein Intake in Cats with CKD

Unlike many therapeutic renal diets formulated for dogs with CKD where the levels of protein in the diets are less than the American Association of Feed Control Officials (AAFCO) minimum levels recommended for adult maintenance, most of the therapeutic renal diets for cats contain protein levels at or above the AAFCO-recommended minimum levels for adult maintenance. However, just like with dogs, there is no consensus on optimum protein content for cats with CKD, and there is a paucity of dietary studies in cats with spontaneous CKD to help clarify this. There also is a lack of studies in cats evaluating the effects on CKD of dietary protein versus dietary phosphorus like there is in dogs. To date, some of the studies in cats with induced CKD evaluating dietary protein have done so in conjunction with caloric intake and have not adequately assessed the effects of dietary protein versus phosphorus as the only variables.[14,15] Therefore, it is less clear what role dietary protein versus dietary phosphorus plays in cats with CKD. However, just like in dogs, CKD tends to be a disease in older cats, and cats also have a propensity to lose muscle mass with increasing age. Therefore, further assessing dietary protein versus dietary phosphorus levels is warranted to determine optimum levels of protein intake for cats with CKD.

Kirk and Hickman evaluated three levels of dietary protein intake (16%, 20%, 24% protein metabolizable energy [ME]) in cats with spontaneous CKD and in healthy control cats.[16] They concluded that the protein requirement of cats with CKD and healthy controls appears to be approximately 20% ME. A more recent study suggested that healthy adult cats may require dietary protein levels higher than those currently recommended by AAFCO.[17] As a result, there is still more research that needs to be done in older cats and those with CKD before optimum dietary protein levels can be determined.

Summary

Although there is no consensus on optimal dietary protein levels for dogs and cats with CKD, there are a limited number of clinical studies supporting the benefits of feeding diets formulated for CKD versus maintenance type diets to patients. However, there is some evidence in dogs supporting that higher levels of dietary protein can be safely fed to dogs with CKD as long as the diets are phosphorus restricted.

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Notes

Dietary Management of Bone Mineral Disturbances Associated with Chronic Kidney Disease

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Phosphate Homeostasis: New Knowledge of Regulating Factor

In the normal adult mammal that is not pregnant or lactating, the amount of phosphate absorbed from the diet is balanced each day by the amount excreted by the kidney. The control points determining the plasma phosphate concentration are: (i) the intestine, which determines the proportion of the dietary load that is absorbed following each meal; (ii) skeletal tissues, which contain bound phosphate and calcium and can release both minerals by resorbing bone or can store calcium and phosphate by increasing bone formation; and (iii) the kidney where the amount of the filtered load of phosphate, as determined by plasma phosphate concentration and glomerular filtration rate (GFR), that is reabsorbed in the proximal tubule can be regulated.

Glossary of Abbreviations
CKD: Chronic Kidney Disease
GFR: Glomerular Filtration Rate
PTH: Parathyroid Hormone

Plasma phosphate concentration is much less closely regulated than plasma-ionized calcium concentration. However, the hormones that are recognized as regulating plasma-ionized calcium concentration — parathyroid hormone (PTH) and calcitriol, in particular — also have effects on phosphate homeostasis. There always was the assumption that a phosphate-regulating hormone, a "phosphatonin," would be discovered, as it made physiological sense there should be one that would increase phosphate excretion and reduce its absorption from the gut without leading to an increased release of phosphate from bone. The main phosphatonin that has emerged is FGF-23, which appears to work in concert with α-klotho to regulate phosphate.[1]

FGF-23 is a 251 amino acid polypeptide hormone that is secreted by osteoblasts and osteocytes. The precise mechanism that regulates its secretion has not been fully characterized. Ingestion of a meal containing phosphate and presentation of a high filtered load of phosphate to the nephron both lead to increases in PTH and FGF-23 secretion. Although FGF-23 originally was thought to primarily respond to and regulate phosphate, a paradigm is appearing that suggests there needs to be a permissive level of calcium in the plasma for FGF-23 secretion to increase in response to phosphate and that there is a vitamin D responsive element on the FGF-23 gene showing that calcitriol also plays a role in regulating its synthesis. In addition, feeding a diet containing highly available phosphate leads to transient increases in serum PTH. Thus, while it would be convenient to compartmentalize and separate phosphate homeostasis from calcium homeostasis, this is not appropriate as hormones regulating calcium and phosphate interact at the level of the intestine, skeletal tissues and kidney in a complex way, integrating within a physiological system to regulate these two important minerals.

We are starting to understand some of the ways in which FGF-23 fits into this complex system. It needs α-Klotho as a co-receptor for most of its actions, though some Klotho-independent actions are emerging. Alpha-Klotho is expressed primarily in the kidney and parathyroid glands. FGF-23 binds to the FGF-1 receptor and α-Klotho to downregulate the two main sodium-linked phosphate transporters in the proximal tubule of the kidney (NPT2a and NPT2c). This reduces reabsorption of the filtered phosphate and so increases excretion of phosphate from the body at a rate that is dependent on plasma phosphate concentration and GFR. FGF-23 also inhibits phosphate absorption from the intestine indirectly by inhibiting the conversion of 25-hydroxycholecalciferol (calcidiol) to 1,25 dihydroxycholecalciferol (calcitriol). These actions of FGF-23 are through effects on both the calcitriol synthesising (CYP27B1) and catabolising (CYP24A1) enzymes. Finally, FGF-23 also inhibits phosphate resorption from bone indirectly by inhibiting parathyroid hormone secretion.

There currently are many unanswered questions in the FGF-23/α-Klotho axis. However, the discovery of the FGF-23/α-Klotho axis has changed the way we think about hyperphosphataemia and secondary renal hyperparathyroidism in chronic kidney disease (CKD). The role of more than PTH in this syndrome and the recognition that changes in bone and mineral homeostasis occur early in the course of CKD has led to use of the term "mineral and bone disturbances" to replace that of "secondary renal hyperparathyroidism." In the early stages of CKD, when phosphate homeostasis is challenged by a fall in GFR and thus there is reduced ability to excrete the phosphate load entering the body each day, increased secretion of FGF-23 is thought to be the first long-term adaptive response in human patients.[2]

In reducing phosphate absorption from the gut, increasing its excretion through the kidney without releasing phosphate and calcium from bone, increased FGF-23 seems an appropriate adaptive response in early kidney disease. In later stages of CKD, it appears that downregulation of α-Klotho expression by the kidney and parathyroid gland means this adaptive response become maladaptive, and excess FGF-23 is produced together with parathyroid hormone. Yet despite these two phophaturic hormones being secreted at ever-increasing levels, phosphate retention occurs, calcitriol deficiency worsens, and ionized calcium concentrations ultimately decrease. However, the concept that FGF-23 always increases in CKD before parathyroid hormone has been challenged,[3] suggesting that in patients with calcidiol deficiency, PTH rises before FGF-23, whereas in calcidiol-replete patients, FGF-23 rises before PTH.

Exactly at what point/stage in CKD the FGF-23/α-Klotho axis becomes maladaptive is not really clear. However, we do know that a very high circulating concentration of FGF-23 is a significant risk factor for cardiovascular complications of CKD in human patients, which is the major cause of human mortality in CKD. FGF-23 has been shown to be linked to hypertension and cardiac muscle remodelling (left ventricular hypertrophy), and α-Klotho deficiency is linked to endothelial cell dysfunction, vascular calcification and progression to end-stage kidney disease in human patients. The poor prognostic indication of whole-body phosphate overload in CKD leading to ever-increasing concentrations of serum FGF23 appears to be modified by serum magnesium. Low-serum magnesium increases the risk for adverse events related to phosphate overload, whereas if serum magnesium is maintained at normal levels, then the risk of vascular mineralization and the other adverse events resulting from phosphate overload are reduced.

How does this new knowledge impact veterinary medicine and the management of CKD in dogs and cats?

We are at our infancy of knowledge about the FGF-23/α-Klotho axis in veterinary patients with CKD. It appears that the laboratory reference range of FGF-23 in the cat is substantially higher than that found in humans. In older healthy cats fed a wide range of different diets by their owners, there was a rightward skew in this distribution, perhaps reflective of the varying phosphate intake experienced by these cats.

As has been reported in human medicine, plasma FGF-23 is increased in early stage CKD, prior to the onset of azotaemia — it is predictive on the development of azotaemic CKD[4] — and thereafter increases proportionately with the stage of CKD.[5] This is consistent with FGF-23 being renally excreted, and its clearance being dependent on GFR. However, within a given stage of CKD, if plasma phosphate was above the IRIS target range for that stage, FGF-23 was higher compared to those cases where plasma phosphate was within the target range. While PTH concentration can be elevated early in feline CKD, this is much more variable across the population of cats we have studied, and so FGF-23 elevation seems more consistently related to the stage of CKD. Thus, it appears that similar to the situation in human medicine, FGF-23 increases in CKD before PTH in many cases and certainly before plasma phosphate concentration rises, ionized calcium concentration decreases or changes in calcitriol concentration can be detected.

There is a strong indication that FGF-23 concentrations in the cat, in some way, reflect phosphate load. Dietary interventions, which reduce plasma phosphate intake, lead to reduction in plasma FGF-23 concentrations in cats both where plasma phosphate is above the IRIS target range and where plasma phosphate is within the IRIS target range for that stage. This contrasts with PTH and phosphate, which only decreased in cats that had plasma phosphate concentrations above the IRIS target range for the CKD stage before the dietary intervention was introduced.[6] These observations were made in retrospective analysis of cats transitioned onto renal diet and followed for four to eight weeks. A prospective longer term study has yielded similar findings and demonstrated that FGF-23 continues to decrease with continued dietary phosphate restriction. Over the same four-to-seven month period, both calcitriol and calcidiol tended to decrease with phosphate restriction, and baseline values were within or above the laboratory reference ranges.[7]

Finally, FGF-23 concentrations at initial presentation and diagnosis of CKD are predictive of progression of feline CKD and of all-cause mortality,[8] similar to the findings reported in human medicine. We had previously shown that plasma phosphate was predictive of progression of CKD,[9] but when FGF-23 is included in the model it comes out as the strongest independent predictor of progression and displaces phosphate and PTH from the multivariate analysis.[8]

The current knowledge concerning dogs with chronic kidney disease is at a much less advanced stage with respect to FGF-23 than the feline literature. However, the initial findings are similar to those in cats and people as one might expect.[10]

How do these new findings influence how we manage bone and mineral disorders in CKD?

The mainstay of management of CKD is to restrict phosphate intake such that daily renal phosphate excretion matches daily absorption of phosphate from the intestine and homeostasis is once again achieved. The degree of phosphate restriction required to achieve this goal is dependent on the stage of CKD, how long there has been a mismatch between intake and excretion, to name but two factors. Individual patient assessment would be ideal, tailoring the treatment to the individual. In practice we use standard clinical renal diets formulated to provide the low end of the adult cat or adult dog recommended requirements of phosphate, and

monitor the response to treatment. In late-stage patients this may not be sufficient, and phosphate binders might need to be added to the diet to further limit the availability of phosphate. The problem in doing this is that the palatability of the diet is reduced even further by the addition of phosphate binders. The evidence that formulated clinical renal diets improve survival[11] and reduce the incidence of uremic crises[12] is strong for the cat and is complemented by experimental studies.[13] Likewise, in dogs, clinical trial data demonstrate feeding of renal diets, which are restricted in phosphate, benefited survival, reduced uremic crises and slowed decline in renal function.[14] The body of experimental evidence indicating that this effect is most likely to be the result of phosphate restriction as opposed to protein restriction is much stronger in the dog than in the cat.[15,16]

However, the need to identify patients that are most likely to benefit from dietary phosphate restriction and to optimize the dietary regimen to the individual patient remains unmet in veterinary nephrology. In some cats, particularly those in early stages of CKD (IRIS stage 2), feeding a diet restricted in phosphate might lead to an increase in serum-ionized calcium concentrations. Idiopathic hypercalcaemia occurs in cats, and CKD appears to be a risk factor for its occurrence.[17] However, the relationship between the occurence of hypercalcaemia and feeding a clinical renal diet is difficult to prove. Nevertheless in our experience, in cats where hypercalcaemia is noted to occur following the introduction of a clinical renal diet, increasing the amount of phosphate in the diet often leads to resolution of the hypercalcaemia. Hypercalcaemia in response to feeding a phosphate-restricted diet has not been reported to occur in dogs and occurs in the minority of cats with CKD.

It is clear that bone and mineral disturbances are present in CKD cases in cats and probably dogs prior to plasma phosphate being elevated above the current target values. This presents a problem of identifying which cases would benefit from restricting phosphate intake. We currently use serum FGF-23 as a marker of whole-body phosphate overload and use it to monitor the response of cats that are normophosphataemic to dietary phosphate restriction. What we currently do not have is a commercial assay that can be used by general practitioners to measure FGF-23 in their clinical patients. In addition, we are not able to recommend the target concentration of FGF-23 to aim for as an indication of an adequate response to treatment. Further research is necessary to address these issues in both cats and dogs so that we have a way of providing individual treatment regimens for our patients with CKD and can optimize the degree of dietary phosphate restriction necessary for each patient we see.

Conclusion

Strong level 1 evidence supports the management of CKD in dogs and cats with dietary phosphate restriction. The new knowledge of the integrated physiological system regulating phosphate and calcium and how it is disturbed at the different stages of CKD should allow us in the future to tailor treatment to the individual patient's needs. Development of tools to enable us to do this should help advance the care we can offer our kidney disease patients.

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Notes

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Dietary Polyunsaturated Fatty Acids and Chronic Kidney Disease

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Abstract

In dogs,[1-7] and to a lesser extent in cats,[8-10] there is a rationale for the inclusion of dietary n-3 polyunsaturated fatty acids (PUFA) in IRIS chronic kidney disease (CKD) stages 2 and 3, regardless of the level of BP or UP/C. This may be accomplished using renal diets or by supplementing with additional n-3 PUFA. While reno-protective therapy is a high priority in IRIS CKD stages 2 and 3, it becomes increasingly less important in late stage 4 when the focus of therapy becomes management of the complications of uremia.

Glossary of Abbreviations
CKD: Chronic Kidney Disease
PUFA: Polyunsaturated Fatty Acids

In laboratory studies of canine kidney disease, dietary supplementation with n-3 PUFA (menhaden oil) altered the long-term course of renal injury,[1] the hemodynamic response to acute reduction in renal function,[2] and the magnitude of proteinuria. Dogs with spontaneous CKD exhibit alterations in vasoactive urinary eicosanoid excretion; these changes were interpreted to support a role for glomerular hyperfiltration in progressive canine renal injury.[6,7]

Interestingly, short-term studies in dogs with naturally occurring kidney disease indicate that supplementation with n-6 PUFA led to increased glomerular filtration rate.[6,7] However, in studies of induced kidney disease, the long-term effects of similar supplementation were deleterious, causing elevated intraglomerular pressure, increased renal eicosanoid series-2 excretion, or hastened progression of CKD.[1-4] It appears that the increase in glomerular pressure caused by n-6 PUFA supplementation may be of short-term benefit as a GFR-enhancing response in dogs with spontaneous CKD.

The effects of n-3 PUFA supplementation in feline CKD has been less well studied, though two studies in spontaneous CKD[8,9] provide indirect evidence of the benefit of supplementation with n-3 PUFA.

Based on studies to date, in which dietary supplementation with n-3 PUFA is chosen, a n-6/n-3 ratio of approximating 5:1 or a dosage of 0.25-0.50 gm/kg body weight of docosahexaenoic acid and eicosapentaenoic acid should be considered. Since PUFA within cell membranes is subject to oxidative damage, the addition of PUFA to the diet increases an animal's antioxidant (e.g., vitamin E) requirements.

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The Role of Dietary Carbohydrate in the Nutritional Management of Dogs and Cats with Cancer

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Glossary of Abbreviations
ATP: Adenosine Triphosphate

In 1956, the biochemist and Nobel laureate Otto Warburg postulated that malignant transformation in cancer cells was caused by mitochondrial dysfunction and the preferential use of glycolysis to generate energy.[1] Although we now understand that cancer is caused by numerous inherited and acquired forms of DNA damage, Warburg's observation remains accurate: Rapidly dividing malignant cells are indeed programmed to increase glucose uptake, and they use this glucose to produce essentially all of the adenosine triphosphate (ATP) they need through aerobic glycolysis, known as the Warburg effect.[2] This apparent requirement of cancer cells for large quantities of glucose has led to the recommendation that people and animals with malignant disease should be fed diets low in carbohydrate and high in fat so that their cancers can be "starved" into remission, while host tissues continue to oxidize dietary fats to supply energy. This paper will examine the objective evidence supporting the specific use of low-carbohydrate diets in dogs and cats with cancer.

Cancer Cachexia and Carbohydrate Metabolism in Dogs and Cats with Cancer

After 60 years, the Warburg effect remains an active area of scientific research and discussion. This is due at least in part to the role it may play in the development of cancer cachexia, a unique and important form of protein-calorie malnutrition that occurs in people, dogs and cats in association with malignant disease. Cancer cachexia is clinically significant because studies in multiple species have repeatedly shown that weight loss has an independent, negative impact on prognosis. Survival times in affected individuals are significantly shorter than those seen in weight-stable individuals with otherwise identical disease.[3-12]

Based on current knowledge, classic or primary cancer cachexia is best defined as a paraneoplastic syndrome in which energy metabolism is altered by systemic inflammation induced by the tumor-bearing state. Energy substrates including carbohydrate are used inefficiently, eventually resulting in loss of both skeletal muscle mass and adipose stores.[3,13-15] Accordingly, many investigators have studied energy metabolism in rodents with implanted tumors as well as in people with naturally occurring cancers in the hope of identifying precise defects that might serve as useful therapeutic targets to reverse or prevent cancer cachexia. The abnormalities found in these studies have often reflected alterations in carbohydrate metabolism consistent with the increased glucose demand and accelerated Cori cycle activity that would be predicted by the Warburg effect; glucose intolerance also is commonly identified. Specific abnormalities have included hyperlactatemia, increased rates of whole-body glucose turnover and disposal, increased rates of gluconeogenesis from lactate and amino acids, abnormal glucose tolerance curves, hyperinsulinism, and insulin resistance.[2,13,16-18]

While there is little published information regarding similar findings in the cat, some of the biochemical or metabolic abnormalities typical of the tumor-bearing state have been demonstrated in dogs with various neoplastic diseases. Significantly higher serum lactate[19,20] and insulin concentrations[20] have been documented in dogs with lymphoma as compared to normal controls, though in one study glucose tolerance curves were not different between these groups.[20] In another study, hyperlactatemia and hyperinsulinemia did not resolve when dogs with lymphoma achieved a complete clinical remission after doxorubicin chemotherapy.[21] Dogs with a variety of nonhematopoietic tumors also have been shown to have increased serum lactate and insulin concentrations after an intravenous glucose tolerance test compared to normal healthy dogs; however, once again, these abnormalities did not resolve after apparently complete surgical resection of disease in the tumor-bearing dogs.[22] Finally, dogs with osteosarcoma have been demonstrated to have increased urinary nitrogen excretion and whole-body glucose flux in the period immediately after amputation, though their whole-body protein synthetic rates are decreased.[23]

Given these findings, it is perhaps tempting to assume that a low-carbohydrate, high-fat diet might benefit dogs with various kinds of cancer. However, the available objective evidence does not support this conclusion. First, the assumption that all of the listed abnormalities must be the direct result of tumor-related alterations in carbohydrate metabolism and therefore should respond to dietary carbohydrate restriction is flawed. For instance, recent work

shows that the most common cause of hyperlactatemia in tumor-bearing dogs, including those with lymphoma, is hypoperfusion, and not underlying changes in carbohydrate metabolism.[24,25] Another important consideration is that weight loss — and therefore, by definition, cancer cachexia — is less common in dogs than it is in people with cancer.[26,27] Even though metabolic changes considered characteristic of cancer cachexia in people have been identified in dogs with spontaneous tumors,[19-23] most veterinary studies fail to demonstrate a clear step-wise association between these abnormalities, weight loss or cachexia, and clinical outcome. Thus, if abnormalities in carbohydrate metabolism are present but do not result in cancer cachexia and do not have a negative impact on prognosis, feeding a low-carbohydrate diet would not necessarily provide any benefit.

An interesting finding in several canine studies is that documented biochemical or metabolic abnormalities do not resolve after treatment of the primary tumor either with surgical resection or chemotherapy.[21,22] This suggests that the metabolic defects typical of cancer cachexia can persist even after effective antineoplastic therapy. However, this observation still does not rise to the level of objective evidence clearly supporting the use of a low-carbohydrate diet in dogs with cancer. It is not currently known whether there is a difference in outcome between dogs with metabolic alterations reflecting abnormal carbohydrate metabolism that resolve after treatment for the underlying tumor and dogs that have persistent metabolic alterations despite receiving the same anticancer therapy. A prospective, randomized study comparing uniform groups of dogs fed standardized low- versus high-carbohydrate rations would ultimately be necessary to determine if carbohydrate restriction provided any type of survival or quality of life advantage in this scenario.

Outcome of Dietary Carbohydrate Restriction in Dogs with Cancer

Another approach that might provide reliable, objective evidence supporting the use of carbohydrate restriction in dogs and cats with cancer would be to simply feed this type of diet and compare the clinical outcome to that observed in animals with the same cancer but with a higher carbohydrate intake. Such a study ideally would be prospective and randomized and would include dogs with precisely the same histologic type, stage and grade of cancer receiving exactly the same anticancer therapy with the only difference between treatment groups being diet. Further, it would be important for carbohydrate content to be the only difference between the test rations and for the diets to be fed exclusively. No studies of this type have been published. However, there is one similarly designed study that is sometimes cited by proponents of carbohydrate restriction for dogs with cancer.

Here, the impact of a test ration high in fat (58% of total calories), low in carbohydrate calories (15% of total calories), and enriched with n-3 fatty acids and arginine was investigated in dogs with lymphoma.[28] Consumption of the test diet appeared to correct some abnormalities in carbohydrate metabolism compared to the control diet, including increased lactic acid production and increased insulin secretion after glucose tolerance testing. Significantly prolonged disease-free intervals and survival times after single-agent doxorubicin chemotherapy also were observed in dogs with WHO stage IIIa lymphoma consuming the test diet. A commercial version of this ration is available containing 27% protein, 59% fat and 14% carbohydrate calories and enriched with both n-3 fatty acids (1794 mg/100 kcal) and arginine (618 mg/100 kcal).[a]

Although intriguing, the results of this study do not provide objective evidence supporting the benefit of carbohydrate restriction in dogs with lymphoma. While the formulation of the test diet with such a low-carbohydrate content certainly suggests that the investigators believed it would be advantageous, it was not their intent to examine the benefits of carbohydrate restriction. Rather, they were interested specifically in the impact of a diet enriched with n-3 fatty acids and arginine. By specific design, there was essentially no difference between their test diet and the control diet except for n-3 fatty acid and arginine content. This makes it impossible to conclude that the observed benefits had anything to do with carbohydrate restriction since all the dogs in the study consumed the same amount.

Conclusions

At this time, published studies do not provide clear, objective evidence that a carbohydrate-restricted diet will benefit most dogs and cats with cancer. While the higher caloric density and potentially increased palatability of currently available carbohydrate-restricted, high-fat commercial rations might be beneficial in individual cases where there is decreased food intake and documented weight loss, it is important to recognize that a diet change is not automatically indicated for all dogs and cats with cancer. Individualized nutritional assessment remains the best way to optimize feeding recommendations. Additional carefully constructed, randomized prospective studies are needed to better define optimal nutritional management for dogs and cats with neoplastic disease, and this includes the potential role of carbohydrate restriction.

[a] Prescription Diet® N/D® Canine, Hill's Pet Nutrition Inc., Topeka, KS.

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D-licious or D-structive?: The Impact of Vitamin D on Cancer and Its Interaction with the Microenvironment

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Abstract

Vitamin D functions as a prohormone and its active form, calcitriol (1,25-ihydroxyvitamin D), has critical functions in immune function in addition to calcium homeostasis and skeletal health. Low vitamin D concentrations (for the purpose of this review, this denotes vitamin D3) have been associated with increased risk of cancer in both people and dogs, and vitamin D supplementation *in vitro* has been shown to increase sensitivity of cancer cells to chemotherapy. Vitamin D regulates a vast array of genes and is involved in DNA repair.

Glossary of Abbreviations
iPTH: Intact Parathyroid Hormone
MAPK: Mitogen-Activated Protein Kinase
ROS: Reactive Oxygen Species
TLR: Toll-Like Receptors
VDR: Vitamin D Receptor
VDRE: Vitamin D Responsive Elements

Background

Vitamin D (throughout this text, vitamin D should be interpreted to include relevant analogues) has traditionally been considered primarily in the context of skeletal health because of its role in calcium metabolism. However, the paradigm has shifted in recent years to encompass the role of vitamin D in many cellular processes including inflammation, immune function, and the risk of various disease states such as cardiac and renal disease and cancer. Vitamins are organic compounds required in small amounts for normal physiologic function that cannot be produced by the body. While many vitamins serve as cofactors for enzymes involved in cellular functions, vitamin D exerts its effects via receptors and acts more like a hormone in its regulation of physiological processes. Vitamin D is absorbed through the gastrointestinal tract in cats and dogs in a pro form and is activated by hydroxylation of the 25th carbon in the liver and subsequently to its active form by hydroxylation of the first carbon in the kidney. Activation also can occur as a result of 1-alpha hydroxylase secretion (encoded by the gene CP27B) by macrophages, which can occur in chronic inflammatory conditions as well as in the tumor microenvironment.

Various methods have been used to assess vitamin D status. In people, the primary effect of ultraviolet light exposure is the production of vitamin D3 from its precursors, and thus proximity to the equator and other measures of UVB exposure have been used as surrogates for the health benefits of vitamin D. The primary circulating storage form of vitamin D3 is the 25-hydroxyvitaminD3, and this has been used most often for clinical research. Although some studies have measured serum 1,25-dihydroxyvitaminD3, the final step in activation to this biologically functional form can occur at the cellular level, including in the tumor microenvironment, and therefore circulating levels may not be reflective of activity in the body.

In addition to direct effects on calcium absorption, vitamin D has been shown to regulate more than 200 genes associated with over 2,700 binding sites. Binding of 1,25-vitD to the vitamin D receptor (VDR) results in translocation to vitamin D responsive elements (VDRE) on the DNA. The VDR thus acts as a transcription factor, since VDRE are short segments in the promoter region of vitamin D-responsive genes. These genes have been shown to encompass a wide variety of cellular processes including DNA repair, proliferation, extracellular matrix remodeling, and innate immunity.[1] Additional studies in healthy people have found that genes that were differentially expressed in the white blood cells of vitamin-deficient subjects normalized with vitamin D supplementation and encompassed a wide variety of cellular processes including transcriptional regulation, immune function, response to stress, and DNA repair.[2]

Effect of Vitamin D on the Immune System

Chronic inflammation has been linked to cancer development in many settings, and vitamin D plays a pivotal role. In addition, the relationship between cancer cells and the microenvironment includes local immune cells, their response to cancer cells, and the suppressive efforts of cancer cells to dampen an immune attack on cancer cells. The interaction between the vitamin D-binding receptor and the retinoid X receptor has been strongly linked with several diseases of immune dysregulation including Crohn's disease, inflammatory bowel disease and rheumatoid arthritis.[3,4]

Vitamin D and its analogues can have effects on both the innate and adaptive immune system. The response of monocytes and macrophages to infectious agents (primarily investigated with *Mycobacterium spp.*) is improved by the presence of both 25-vitD and 1,25-vitD, with upregulation of the vitamin D receptor and CP27B (vitamin D-activating enzyme). Upregulation of cathelicidin is thought to be the most likely primary mechanism of the effect of vitamin D analogues on the innate immune system. In addition, vitamin D has been associated with expression of toll-like receptors (TLR), which are expressed on macrophages and dendritic cells to recognize conserved microbial molecules. In addition, TLR9 has been implicated in the immune response to cancer, and therapies targeting this are being developed.[4] In the adaptive immune response, the VDR is expressed in activated but not in resting B lymphocytes. The complex functions of T and B lymphocytes are affected in several ways by vitamin D, which can favor a TH2 response and augment chemotaxis to (via stimulation of chemokine receptor 10 by T lymphocytes) and retention of lymphocytes in the skin. The effects must be interpreted with caution when considering cancer, as increased T regulatory cells, though beneficial in managing autoimmune disease, could also have negative implications for managing cancer.[4]

Vitamin D in Healthy and Tumor-Bearing Dogs

Compared to controls, dogs in one report with hypercalcemia and lymphoma or chronic renal failure had lower 25-vitD but similar 1,25-vitD concentrations.[5] Similarly, a cross-sectional study in Labrador Retrievers found significantly (P=0.027) lower 25-vitD levels in dogs with mast cell tumors compared to unaffected dogs, despite no difference in estimated dietary vitamin D intake. This suggests that low vitamin D is a risk factor for mast cell tumor, which is a tumor type that expresses vitamin D receptor.[6]

There is some debate regarding the optimal concentration of serum 25-vitD in dogs. With the pleiotropic effects of vitamin D, concentrations can be considered as deficient, insufficient and sufficient. One method used intact parathyroid hormone (iPTH) to define the optimal lower end of vitamin D sufficiency and found that variability in iPTH decreased when 25-vitD concentration was at or around 100 to 120 ng/mL. As with a similar study in people, this defined the lower limit of sufficiency. Some dogs in that study had values above 200 ng/mL without evidence of illness or hypercalcemia. The upper limit of adequate vitamin D is more problematic and less well-defined. Exploring the upper limit risks unacceptable toxicity and thus is less often used as an end point. Additionally, dogs with hemoabdomen (n=62) had significantly lower 25-vitD concentrations compared to healthy controls. Control dogs were younger than dogs with cancer in that study, which could impact the nutritional status and risk of cancer. Another important finding from that study was that decreasing vitamin D concentration was associated with an increasing risk of cancer with a relative risk of 2 if the concentration was less than 60 ng/mL and a risk of 3.9 when less than 40 ng/mL.[7]

Interestingly, when 25-vitD concentrations were evaluated in 20 dogs with osteosarcoma and compared to age and weight-matched controls, mean concentrations were 34.95 ng/mL and 33.85 ng/mL, respectively (P=0.784). An important limitation, however, is that based on the aforementioned study, the normal dogs should be considered insufficient at these concentrations and thus in theory could still be at risk for development of neoplasia. Additionally, a small cohort may not detect risk, whereas a larger, prospective longitudinal study would more accurately answer this question.[8]

A recent study was completed to investigate possible factors associated with 25-vitD in dogs with cancer. Three different diagnoses were included: osteosarcoma, lymphoma and mast cell tumor. The authors report an association with ionized calcium, which was directly proportional to 25-vitD in cancer patients and inversely proportional in normal dogs. Also, no patient variables apart from diagnosis were found to effect the plasma 25-vitD concentrations.[9]

Vitamin D in Cats

As with other species, vitamin D concentrations have been investigated in select circumstances to correlate with states of health and disease in cats. Low vitamin D concentrations have been described in cats with infectious and inflammatory conditions, as well as with intestinal lymphoma.[10] However unlike other species, cats have recently been shown to harbor a unique epimer (3-epi-25vitD3), which was found in lesser concentrations in rats and not at all in dogs, yet it is seen in people. The spectrum of physiologic effects of this epimer

Relative risk of the disease group compared with that of the control group

25(OH)D (ng mL^{-1})	All Cancers	HSA	Benign
<40	3.9 ($P = 0.0001$)	4.1 ($P = 0.0001$)	4.5 ($P = 0.0001$)
<60	2.0 ($P < 0.0001$)	2.2 ($P < 0.0001$)	1.5 ($P = 0.111$)
<80	1.4 ($P < 0.0001$)	1.5 ($P < 0.0001$)	1.4 ($P = 0.0001$)
<100	1.1 ($P = 0.0003$)	1.5 ($P < 0.0001$)	1.1 ($P = 0.04$)
>100	0.18 ($P = 0.08$)	0.11 ($P = 0.12$)	0.32 ($P = 0.25$)

are not fully understood, but in people it has been linked to differentiation and antiproliferation. In addition the active form of the epimer can bind to the vitamin D receptor with reduced biologic activity compared to 1,25vitD, which could suggest other regulatory effects such as competitive inhibition. Cats also are less efficient than other species in using vitamin D2 (derived from plant sources).[11]

The Role of Vitamin D in Cancer Prevention and Therapy

Along with vitamins C and E, vitamin D might have an important role in cancer prevention or in preventing cancer progression once established.[12] Many studies have supported a decreased risk of several types of cancer in people with increased UVB exposure, the primary effect of which is increased vitamin D concentrations.

In people, supplementation for patients with low vitamin D at cancer diagnosis can improve outcome for cancers of the prostate, breast, colorectal, and melanoma. The benefit is thought to be attributed at least in part to the immunomodulatory effects of vitamin D.[13] Considerations specific to cancer treatment modalities are noted below.

Surgery:

Healing after trauma or surgery for both hard (bone, e.g., fractures) and soft tissue can be improved by adequate vitamin D, though information on osseous healing is at times inconsistent and on soft tissue healing is sparse. A recent mouse model of wound healing in diabetic patients (using diabetic mice) found that vitamin D supplementation significantly accelerated wound healing, which was associated with suppression of the NF-kB inflammatory pathway.[14]

Chemotherapy:

In vitro studies have explored the expression of vitamin D receptor in cell lines from both canine and feline cancers, as well as the response to 25-vitD and 1,25-vitD. Canine transitional carcinoma cells expressed high levels of vitamin D receptor in cultures and experienced G0/G1 cell cycle arrest when treated with 1,25-vitD or an analogue, and this effect was enhanced with the addition of medium-chain triglyceride oil.[15] Canine mammary carcinoma, mastocytoma and osteosarcoma were treated with increasing doses of 1,25-vitD and increasing doses of cisplatin, and the combination resulted in synergistic antiproliferative effects.[16] Canine mast cell tumor also has been evaluated *in vitro*, and cells in cultures responded to 1,25-vitD alone or in combination with commonly used antineoplastic drugs for that cancer including CCNU, vinblastine, imatinib, and toceranib. The combination of 1,25-vitD and chemotherapy was synergistic.[17]

These *in vitro* findings were translated into *in vivo* investigations for some tumors, and responses were seen in the corresponding tumor types to both 1,25-vitD as a sole therapy and to the combination of 1,25-vitD and chemotherapy. Unfortunately, 1,25-vitD administration caused hypercalcemia in an unacceptable number of dogs, and clinical trials were discontinued in some reports. Vitamin D analogues have been and continue to be investigated in preclinical models for possible physiologic effects without toxicity due to hypercalcemia.

Radiation Therapy:

Vitamin D has been investigated in a canine transitional cell carcinoma cell line. Exposure to calcitriol and other analogues increased catalase expression and activity, and increased mitogen-activated protein kinase (MAPK) signaling.[18] Catalase is a major detoxifying intracellular enzyme, responsible for harvesting reactive oxygen species (ROS), and ROS are the primary method by which low linear energy transfer radiation (photons) indirectly cause DNA damage. By this reasoning, vitamin D might act as a radioprotectant, which would be beneficial if critical tissues surrounding a tumor could be preferentially protected during radiation therapy but would be deleterious if the tumor was also protected. This also must be balanced with the concurrent impact of vitamin D on the local immune system, which plays a role in tumor homeostasis, and the response to an inflammatory microenvironment, which can occur with radiation therapy.

One study in people undergoing external beam radiotherapy for cancer near the rectum demonstrated significantly worse radiation-induced proctitis after five weeks of radiation therapy (50 Gy) in patients who were deficient in circulating 25-vitD compared to those with higher concentrations (independent of possible confounding factors, odds ratio=3, P=0.013).[19]

In the complex environment of cancer, vitamin D has pleiotropic effects. VDR and VDR-vitD axis depletion have been shown to decrease BRCA1 recruitment to sites of DNA damage and double strand breaks. Although improved DNA repair would be expected to lead to radiation resistance, oncogene-induced senescence contributes to progressive genomic instability by downregulating both repair mechanisms and VDR, and improved vitamin D-mediated DNA repair could paradoxically mitigate the emergence of more aggressive populations of cells within a tumor.[20]

Summary

The effect of vitamin D on multiple physiologic and cellular processes is clear and creates a landscape of complicated and sometimes conflicting effects that warrant further study, especially in the context of cancer. It is likely that vitamin D and its analogues play important roles in cancer prevention and treatment, with ongoing and future efforts designed to achieve improved outcome with minimal toxicity.

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Effect of Omega-3 Polyunsaturated Fatty Acids in Humans, Dogs and Cats with Cancer

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Glossary of Abbreviations
DHA: Docosahexaenoic Acid
EPA: Eicosapentaenoic Acid
PUFA: Polyunsaturated Fatty Acid
SNP: Single Nucleotide Polymorphism

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in certain fish oils and represent the active forms of the omega-3 polyunsaturated fatty acid (PUFA) family. Although studies using cell cultures, animal cancer models, and epidemiological and clinical trials in humans have provided evidence to support the use of EPA and DHA in the prevention and treatment of cancer, some studies still report inconsistent results.

The metabolism of PUFAs is complex and controlled by enzymes that are highly polymorphic and map to a genomic region frequently associated with cancer in humans.[1] Single nucleotide polymorphisms (SNPs) in the genes involved in PUFA metabolism help to explain 28% and 12% of the variance in plasma levels of arachidonic acid and linoleic acid, respectively.[2] Therefore, genetic heterogeneity in human cancer patients can result in differences in PUFA metabolism, and this may help to explain the inconsistent results between dietary PUFA intake and cancer in human population studies. Therefore, future studies aim to focus on gene-nutrient associations between SNPs and PUFAs in humans with cancer. The findings from such studies might allow for the identification of individual cancer patients with altered PUFA metabolism that may benefit from personalized diets.

Only a limited number of clinical trials on the effects of dietary omega-3 PUFAs in dogs with cancer have been published. One randomized, double-blinded, placebo-controlled clinical trial investigated a diet high in EPA and DHA on outcomes in dogs with cancer; 32 dogs with stage III or stage IV lymphoma were randomized to receive either a diet supplemented with menhaden fish oil and arginine or an identical diet supplemented with soybean oil. Dogs fed the experimental diet had significantly higher mean serum DHA and EPA compared to controls. Increasing serum DHA concentrations were associated with a longer disease-free interval and survival time for dogs with stage III lymphoma fed the experimental diet.[3] Unfortunately, the design of this study was not ideal, as the potential benefits of arginine cannot be separated from those of omega-3 PUFAs. Also, the post-hoc subgroup analysis and the method of initial staging of lymphoma in the dogs have been criticized.

In a second clinical trial, 12 dogs with malignant carcinoma of the nasal cavity were randomized to receive either dietary menhaden oil or soybean oil (control) and then received radiation therapy.[4] Dogs that were fed menhaden oil had significantly higher plasma concentration of DHA and EPA and significantly decreased tissue inflammatory eicosanoids compared with controls. Increased plasma DHA also was significantly associated with decreased matrix metalloproteinases. Although the dose of fish oil used in these two clinical trials was relatively high, a separate study showed that hemograms and serum biochemical profiles were not adversely affected by fish oil supplemented foods in dogs with lymphoma or hemangiosarcoma.[5]

Unfortunately, no clinical trials assessing the effects of dietary omega-3 PUFAs in cats with cancer have been published. Due to the concern for platelet dysfunction and prolonged bleeding times in cats receiving dietary omega-3 fatty acid supplementation,[6] as well as the absence of information on the long-term safety of omega-3 PUFAs in cats and the lack of a defined safe upper limit per the National Research Council, further studies may be needed in this species before their efficacy in cancer can be definitively determined.

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Appendix
2018 CAN Summit
Gerontology: An Inside Out Perspective

Premise of Systems Microbiomics in Improving Health and Related Diagnostics for Human and Companion Animals

Sunil Kochhar, PhD,
Nestlé Research Center

Dr. Sunil Kochhar is the microbiome research program manager at the Nestlé Research Center in Lausanne Switzerland. He specializes in the implementation of new technologies and competencies for basic research and developing new processes designed to make food more natural and flavorful, which involves working with a multidisciplinary team of scientists, engineers, technicians/operators, analytical chemists, and biochemists for the successful industrialization of products. Dr. Kochhar is the author of more than 120 articles in prominent international scientific journals, and he has written 16 patents and presented research findings at more than 100 international conferences. Currently, he is leading an initiative to assess the role of the human microbiome in developing novel solutions to improve the health of humans and companion animals. His research focus is applying state-of-the art omics to elaborate biomarkers/predictors for health-related disorders, thus allowing the possibility for appropriate nutritional interventions at an early stage. He is also a visiting professor at the Imperial College London.

The Dog Aging Project: Can Old Dogs Teach Us New Tricks?

Daniel E.L. Promislow, DPhil,
University of Washington

Dr. Daniel Promislow began working on the evolution of aging over 30 years ago as a graduate student at the University of Oxford. Following postdoctoral work in France and Canada, he spent 18 years in the Department of Genetics at the University of Georgia. In 2013, he moved to the University of Washington, where he currently is a professor in the Departments of Biology and Pathology. Throughout his career, Dr. Promislow has focused on the biology of aging and age-related disease. In particular, his work addresses the challenge of understanding how genes and the environment shape patterns of aging in natural populations, using theoretical models, epidemiological analysis and empirical studies. His laboratory is funded by grants from the National Institutes of Health, the National Science Foundation and the Glenn Foundation for Medical Research. About 10 years ago, Dr. Promislow began epidemiological studies of aging in companion dogs. He is especially interested in studying the ways an organism's biology can be measured through high-throughput molecular biology, particularly the "epigenome," the "microbiome" and the "metabolome," and when combined with statistical models of network interactions can help us to better understand how genetic variation gives rise to variations in behavior, morphology and ultimately life span and the risks of age-related disease. As co-director and principal investigator of the Dog Aging Project, Dr. Promislow leads a large interdisciplinary team creating a nationwide long-term study of aging in 10,000 companion dogs, with a goal of understanding how genes and the environment shape healthy aging.

Using Genomic Biology to Study Pet Aging

Kelly S. Swanson, PhD,
University of Illinois

Dr. Kelly Swanson is the Kraft Heinz Company Endowed Professor in Human Nutrition at the University of Illinois at Urbana-Champaign. He received a bachelor's degree in animal and range sciences from North Dakota State University in 1997 and a master's and doctorate degrees in nutritional sciences at the University of Illinois at Urbana-Champaign in 1999 and 2002, respectively. His laboratory studies the effects of nutritional intervention on health outcomes, identifying mechanisms by which nutrients impact gene expression and host physiology, with primary emphasis on gastrointestinal health and obesity. His research addresses basic and applied target areas, including rodent models, dogs, cats, and humans. Over the past decade, Dr. Swanson has established an internationally recognized research program, highlighted by over $12 million in research support, over 100 invited lectures in 11 countries around the world, and over 150 peer-reviewed publications. He has received 12 research and teaching awards, including those from the American Society for Nutrition and the American Society of Animal Science. Thus far, Dr. Swanson has trained 26 graduate students and postdoctoral fellows, hosted 14 international visiting scholars and mentored 26 undergraduate research projects. He teaches three nutrition courses annually to undergraduate, graduate and veterinary students, and has been elected 17 times to the "List of Teachers Ranked as Excellent by Their Students." Finally, Dr. Swanson serves on many committees at the departmental, college and campus levels, including being vice chair of the Institutional Animal Care and Use Committee.

Fecal Microbiota Changes in Aging Dogs and Cats — Implications for Health and Longevity

Gail L. Czarnecki-Maulden, PhD,
Nestlé Research Center

Dr. Gail Czarnecki-Maulden is a senior research nutritionist at the Nestlé Research Center in St. Louis. She received a doctorate degree in animal nutrition from the University of Illinois. Before joining Nestlé, Dr. Czarnecki-Maulden was associate professor of companion animal nutrition at the University of Illinois. She is a member of the National Academy of Science Board on Agriculture and Natural Resources and the Association of American Feed Control Officials (AAFCO) Dog and Cat Nutrient Profiles Subcommittee, which sets nutrient standards for dog and cat foods in the U.S. She has served as a member of the Scientific Advisory Board of the International Probiotics Association, the Division of Nutritional Sciences External Advisory Board of the University of Illinois, and the National Academy of Sciences/National Research Committee on Evaluating the Safety of Dietary Supplements for Horses, Dogs and Cats. Dr. Czarnecki-Maulden has published over 60 articles and abstracts on pet nutrition based on her research related to the effect of nutrition on gastrointestinal health. In her personal life, she is actively involved in therapy dog visits and dog training. She is a certified dog trainer and a licensed Family Paws parent educator, as well as a member of the board of directors of the Greater St. Louis Training Club and Havanese Rescue.

Cellular and Functional Mechanisms Underlying Muscle Aging and Associated Diseases

Daniel Béchet, PhD, Unité de Nutrition
Humaine and Clermont Université

Dr. Daniel Béchet received a doctorate degree from Bristol University in the U.K. He currently is research director in the Department of Human Nutrition of INRA, CRNH and Auvergne University. He is a research member of UMR1019 and has explored the mechanisms regulating lysosomal autophagy and proteasome-ubiquitin proteolytic systems that are central for muscle atrophy. Dr. Béchet contributed to the identification of the primary amino acid sequence and gene structure of lysosomal cathepsins and provided the first evidence that lysosomal proteolysis plays a significant role in the physiopathology of skeletal muscle. He also demonstrated the role of a major signaling pathway (PIK3C3-beclin) in regulating autophagy in muscle. Since 2005, Dr. Béchet has developed an expertise in sarcopenia and associated chronic diseases and largely contributed to settle high-throughput proteomics, transcriptomics and molecular imaging analyses of sarcopenia in unique cohorts of elderly subjects. Using these methodologies, his group identified fiber type-specific alterations and many potential biomarkers of muscle chronological aging and age-related pathologies.

The Regulation of Mitochondrial Quality Control Via Autophagy and the Scope of Pharmaceutical and Nutraceutical Approaches

Michelangelo Campanella, Pharm D, PhD,
MRPharmS, PGCAP, FHEA, FRSB,
University of London and
University of College London

Dr. Michelangelo Campanella is a native of Italy who moved to the U.K. 10 years ago to be the Embo/Marie Curie postdoctoral research fellow. He currently leads a research unit at the Royal Veterinary College that is affiliated with the University of College London's Consortium for Mitochondrial Research. Dr. Campanella is internationally recognized as an expert in the field of mitochondrial cell biology and pharmacology, and his research focuses on quality-control mechanisms in mammals and organism models, particularly on those underlying cell pathology and inflammation. His scientific breakthroughs relate to the hidden pathways of the homeostatic mitochondrial function and their pharmacological regulation. Dr. Campanella has received several awards in research and is a member of various editorial boards of scientific journals.

The Role of n-3 PUFA on Muscle Mass and Function in Aging Humans

Bettina Mittendorfer, PhD,
Washington University

Dr. Bettina Mittendorfer is professor of medicine and nutritional sciences at Washington University School of Medicine in St. Louis, where she also directs the Nutrition and Obesity Research Center Clinical Science Research Core and the Clinical Translational Research Unit. She has a long-standing interest in integrated physiology research with a focus on nutrition in the context of age-related sarcopenia, obesity and cardiometabolic diseases. Since 1995, Dr. Mittendorfer has been conducting complex, interdisciplinary clinical and translational studies to evaluate the effect of diet, physical activity and pharmacological interventions on body composition, physical function substrate metabolism and associated cellular signaling, which have resulted in more than 100 peer-reviewed original research papers.

Effect of Diet on Loss and Preservation of Lean Body Mass in Aging Dogs and Cats

Dottie Laflamme, DVM, PhD, DACVN

Dr. Dottie Laflamme received a Master of Science degree in nutrition, then her veterinary degree from the University of Georgia. She went on to complete a PhD in nutrition and physiology, and her clinical nutrition residency as the ALPO postdoctoral fellow in clinical nutrition, also at the University of Georgia. She is board-certified in veterinary nutrition and a past president of the American College of Veterinary Nutrition. While working for Purina (first Ralston Purina and then Nestlé Purina) in the Research & Development Department

for 25 years, until her retirement in 2015, Dr. Laflamme served in numerous research and management positions. During her tenure, her research focused on therapeutic nutrition, especially in obesity management and geriatric nutrition. Dr. Laflamme is an author of over 250 scientific and technical publications and has been a speaker at a number of veterinary, research and continuing education programs worldwide. She introduced and organized the former Purina Nutrition Forum as well as the current Companion Animal Nutrition (CAN) Summit. Dr. Laflamme currently serves as a consultant to The Purina Institute.

Idiopathic Chronic Enteropathy in Older Cats

David A. Williams, MA VetMB, PhD, DACVIM-SAIM, DECVIM-CA, University of Illinois

Dr. David Williams received his veterinary degree from the University of Cambridge and his PhD from the University of Liverpool, where he first developed the canine trypsin-like immunoreactivity (cTLI) assay. He completed an internship and residency at the University of Pennsylvania and has held faculty positions at the University of Florida, Kansas State University, Purdue University, Texas A&M University, and the University of Illinois at Urbana-Champaign. While at the University of Florida, Dr. Williams founded the GI Lab in 1985, when he introduced the TLI assay for use in dogs to the U.S. His research has focused on the development and application of new tests for gastrointestinal diseases, particularly those affecting the pancreas (TLI, pancreatic lipase) and small intestine (cobalamin, folate) of dogs and cats. His current research is directed at the relationships between the intestinal microbiome and metabolomics changes elsewhere in the body in aging cats with idiopathic chronic enteropathy and in both dogs and cats with exocrine pancreatic insufficiency. Dr. Williams continues to work as a consultant with the GI Lab at Texas A&M University, providing telephone consultations regarding management of patients diagnosed using the GI Lab services.

The Fountain of Age: Feeding Strategies for Senior Pets

Julie A. Churchill, DVM, PhD, DACVN, University of Minnesota

Dr. Julie Churchill received her undergraduate and veterinary degrees from Michigan State University, and then completed a small-animal internship in medicine and surgery at the University of Georgia. Following this, she completed combined residencies and a doctorate degree in small-animal internal medicine and clinical nutrition at the University of Minnesota. In the final years of her graduate work, Dr. Churchill developed a small-animal clinical nutrition service at the University of Minnesota Veterinary Medical Center that became financially self-sustaining within five years. She continued at the University of Minnesota in a newly created faculty position. Dr. Churchill is board-certified by the American College of Veterinary Nutrition and a member of the American Academy of Veterinary Nutrition. At the University of Minnesota, she currently is associate medical director for Specialty, Primary and Urgent Care Services, associate clinical professor and director of the Nutrition Service at the Veterinary Medical Center. Dr. Churchill is passionate about all aspects of small-animal clinical nutrition and works to improve client communication to successfully integrate nutrition into the care of every patient. Among her contributions, she served on the task force to develop the American Animal Hospital Association guidelines for weight management, and she currently serves on the board of the Pet Nutrition Alliance (PNA) as president elect, as well as the educational tools committee of PNA, which is working to develop a "Go To" website for credible nutritional information for veterinary practice teams and consumers. Dr. Churchill also is a member of the board of the Association for Pet Obesity Prevention.

Cachexia, Sarcopenia and Other Forms of Muscle Wasting: Common Problems of Senior and Geriatric Cats and of Cats with Endocrine Disease

Mark E. Peterson, DVM, DACVIM, Animal Endocrine Clinic

Dr. Mark Peterson was awarded a Doctor of Veterinary Medicine degree with high distinction from the University of Minnesota in 1976. He then moved to New York City, where he completed an internship and medical residency at The Animal Medical Center, followed by a postdoctoral fellowship in endocrinology and nuclear medicine at The New York Hospital-Cornell Medical Center. Dr. Peterson became board-certified from the American College of Veterinary Internal Medicine in 1981. He served as head of the Division of Endocrinology in the Department of Medicine at The Animal Medical Center for over 30 years. In addition, Dr. Peterson has held faculty appointments as professor of medicine at the School of Veterinary Medicine at the University of Pennsylvania (1996-2000), associate professor of radiology at the Weill Medical College of Cornell University (1983-2005), and assistant professor of medicine at the New York State College of Veterinary Medicine-Cornell University (1982-1988), where he has been adjunct professor of medicine since 2015. In 2009, Dr. Peterson opened the Animal Endocrine Clinic, a specialty referral hospital devoted exclusively to the diagnosis and treatment of cats and dogs with endocrine disease. Over the past 40 years, his clinical and research focus has been on advancing understanding of naturally occurring endocrine disorders of the cat and dog, especially hyper- and hypothyroidism, diabetes mellitus, calcium disorders, and adrenal disease. Dr. Peterson has published more than 600 journal articles, book chapters and research abstracts, and has given more than 600 lecture presentations at veterinary colleges and scientific seminars both in the U.S. and abroad.

Hypovitaminosis D Is Associated with Negative Outcome in Dogs with Protein-Losing Enteropathy: A Retrospective Study of 43 Cases

Karin Allenspach, Dr.med.vet, PhD, DECVM-CA,
Iowa State University

Dr. Karin Allenspach received her veterinary degree from the University of Zurich in Switzerland. She did an internship in small-animal emergency medicine and critical care at Tufts University and a residency in small-animal internal medicine at the University of Pennsylvania. She received a PhD in veterinary immunology from the University of Bern in Switzerland for her work on canine chronic enteropathies. Dr. Allenspach is a board-certified internist and currently is professor of internal medicine and translational health at Iowa State University.

Searching for Nutritional Targets: Multi-Omics Study in Early-Stage Myxomatous Mitral Valve Disease in Dogs

Johnny Li, PhD,
Nestlé Research Center

Dr. Johnny Li is a senior research scientist at the Nestlé Research Center in St. Louis. He earned his bachelor's degree in biochemistry from Xiamen University in China, a master's degree in computer science from Columbia University in New York City, and a doctorate degree in molecular biology and genetics from the University of Texas at Austin. In his PhD thesis work, he studied molecular signaling and cellular communication in the developing Drosophila compound eyes. Dr. Li started his computational biology training with Prof. Bill Noble at Columbia University. In 2002, Dr. Li moved to Raritan, New Jersey, to work as a bioinformatics postdoctoral fellow in the Research & Development Department of Johnson & Johnson Pharmaceuticals, where he worked with scientists from various therapeutic teams on high-throughput big data. In 2004, he joined Nestlé Purina as a scientist, where his work focuses on molecular mechanism and nutrigenomics studies in canine and feline heart health as well as microbiome research in pet obesity.

Rethinking Protein Restriction in Aging Dogs and Cats with Chronic Kidney Disease

Sherry L. Sanderson,
DVM, PhD, DACVIM, DACVN,
University of Georgia

Dr. Sherry Sanderson received her veterinary degree in 1990 from the University of Minnesota College of Veterinary Medicine in St. Paul. She then completed a one-year year rotating internship in small-animal medicine and surgery at Oklahoma State University College of Veterinary Medicine. After this, she returned to the University of Minnesota to complete a combined graduate program and dual residency in small-animal internal medicine and small-animal clinical nutrition. Dr. Sanderson is board-certified by the American College of Veterinary Internal Medicine and the American College of Veterinary Nutrition. She currently is an associate professor at the University of Georgia College of Veterinary Medicine, where she received the Zoetis Distinguished Veterinary Teacher Award in 2013 and was honored as a Veterinary Medicine Outstanding Teaching Faculty in 2014. Dr. Sanderson has published over 70 manuscripts, book chapters and research abstracts, and her research interests include using nutritional management for the prevention and treatment of diseases in dogs and cats, particularly related to urology, nephrology, obesity, prebiotics, probiotics, and the interaction of carnitine and taurine in canine dilated cardiomyopathy. She also focuses on research related to the human animal bond.

Dietary Management of Bone Mineral Disturbances Associated with Chronic Kidney Disease

Jonathan Elliott, VetMB, PhD,
Cert SAC, DECVPT, FHEA, MRCVS,
Royal Veterinary College

Dr. Jonathan Elliott is professor of veterinary clinical pharmacology and vice principal for research and innovation at the Royal Veterinary College. He serves as a member of the college's senior management team with responsibility for all aspects of research strategy including ensuring academic research outputs have impact. He received his veterinary degree from the University of Cambridge. He also holds a doctorate degree in vascular biology and completed postgraduate clinical training at the University of Pennsylvania. He joined the Royal Veterinary College in 1990 as a lecturer in veterinary pharmacology and developed research interests in feline kidney disease and hypertension and equine laminitis. His research has resulted in numerous awards recognizing the impact of his work on clinical practice. They include the Pfizer Academic Award in 1998, BSAVA Amoroso Award in 2001, Petplan Charitable Trust Award in 2005, and ESVNU Scientific Award in 2007. Dr. Elliott is a board member of the International Renal Interest Society. He served as a member of the U.K. Government's Veterinary Products Committee from 2001 to 2009 and was junior vice president of the European College of Veterinary Pharmacology and Toxicology from 2015 to 2018. He has published more than 180 international peer-reviewed papers and reviews and has supervised 28 doctorate students to the completion of their degrees.

Dietary Polyunsaturated Fatty Acids and Chronic Kidney Disease

Scott A. Brown, VMD, PhD, DACVIM (Internal Medicine), University of Georgia

Dr. Scott Brown received his veterinary degree in 1982 from the University of Pennsylvania. He is board-certified in internal medicine and currently serves as associate dean for academic affairs and is the Josiah Meigs Distinguished Professor in the Departments of Small Animal Medicine &

Surgery and Physiology & Pharmacology in the College of Veterinary Medicine at the University of Georgia. Dr. Brown has published over 200 research articles, abstracts and book chapters on topics related to nephrology in general and canine nephrology in particular. His work has been supported by more than $12 million in extramural grant support. Dr. Brown has been recognized for excellence in research and teaching, having received numerous national awards including the AVMA Excellence in Research Award, the Royal Canin Award and the National Norden Distinguished Teacher Award.

The Role of Dietary Carbohydrate in the Nutritional Management of Dogs and Cats with Cancer

Glenna E. Mauldin, DVM, MS, DACVIM (Oncology), DACVN, PetCure Oncology

Dr. Glenna Mauldin graduated from the Western College of Veterinary Medicine in Saskatoon, Canada, in 1985. She completed an internship in 1986 and medical oncology residency in 1988 at The Animal Medical Center in New York City. In 1991, she became a Diplomate of the American College of Veterinary Internal Medicine (Oncology). Dr. Mauldin went on to receive a Master of Science degree in nutrition in 1995 from Cornell University and became a Diplomate of the American College of Veterinary Nutrition in 2004. She served as a staff veterinarian in oncology and nutrition at The Animal Medical Center from 1990 to 1997, as an associate professor of veterinary oncology at Louisiana State University from 1998 to 2007, and as a consulting veterinarian in medical oncology and nutrition at the Western Veterinary Specialist and Emergency Centre in Calgary, Canada, from 2007 to 2017. Dr. Mauldin currently is director of clinical research and a consultant in medical oncology and nutrition for PetCure Oncology, and she also serves as a sessional instructor and clinical instructor at the University of Calgary. She was president of the American College of Veterinary Internal Medicine (Oncology) from 2004 to 2007 and a member at large for the American College of Veterinary Nutrition from 2007 to 2010. Dr. Mauldin is the author of more than 75 scientific articles, book chapters and abstracts, and lectures frequently at national and international meetings.

D-licious or D-structive?: The Impact of Vitamin D on Cancer and Its Interaction with the Microenvironment

Kim A. Selting, DVM, MS, DACVIM (Oncology), DACVR (Radiation Oncology), University of Illinois

A native of Colorado, Dr. Kim Selting completed her undergraduate and veterinary studies at Colorado State University (CSU). Following a one-year rotating small-animal internship in medicine and surgery at The Animal Medical Center in New York City, she moved to St. Louis, where she spent one year in emergency work and three years in small-animal private practice. Dr. Selting then returned to Colorado to purse a residency in medical oncology at the CSU Animal Cancer Center. At the completion of this training, she had earned a master's degree and was board-certified by the American College of Veterinary Internal Medicine in the specialty of oncology. Dr. Selting then joined the faculty at the University of Missouri from 2002 to 2017. In 2013, she completed a nonconforming residency in radiation oncology and achieved board certification by the American College of Veterinary Radiology. In July 2017, Dr. Selting accepted a position as associate professor at the University of Illinois to develop a radiation therapy program. Her clinical and research interests include biomarkers of cancer (specifically cardiac troponin I, thymidine kinase, vitamin D, and exosomes) and chemotherapy toxicity, novel chemotherapy drugs and treatments, novel radiation techniques including radioisotope therapy for cancer with a focus on osteosarcoma, and the effects of radiation on the tumor microenvironment. Dr. Selting is president of the Veterinary Cancer Society, past president of the Veterinary Cooperative Oncology Group, past member-at-large for the Veterinary Cancer Society, and past chair of the Oncology Certifying Examination Committee.

Effect of Omega-3 Polyunsaturated Fatty Acids in Humans, Dogs and Cats with Cancer

Aarti Kathrani, BVetMed (Hons), PhD, DACVIM, DACVN, MRCVS, University of Bristol

Dr. Aarti Kathrani graduated in 2006 from the Royal Veterinary College in London and then completed a rotating small-animal medicine and surgery internship in 2007 at the Queen Mother Hospital for Animals at the Royal Veterinary College. She received a doctorate degree in canine inflammatory bowel disease in 2011 from the Royal Veterinary College, followed by completing a three-year residency program in small-animal internal medicine in 2014 at Cornell University and becoming board-certified in small-animal internal medicine. She completed a two-year residency program in small-animal clinical nutrition in 2016 at the University of California-Davis, and then became board-certified in small-animal nutrition. Dr. Kathrani currently is a senior lecturer in small-animal medicine at the University of Bristol in the U.K.

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