

Nestlé PURINA

Companion Animal Nutrition Summit

Focus on Gerontology 2010

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Clearwater Beach, Florida

Preface

Gerontology is the study of the elderly, and of the aging process itself; the branch of science that deals with the problems of the aged. It may be distinguished from geriatrics, which is the study of the diseases of the elderly. Gerontology covers the social, psychological and biological aspects of aging. Further, it deals with helping aging subjects live healthier for longer, or prolonging healthspan. Nestlé Purina PetCare has long pursued research to find better ways to prolong the healthspan of dogs and cats. Thus, it is highly appropriate that this first Nestlé Purina Companion Animal Nutrition Summit is focused on gerontology. This conference provides an opportunity to look at specific topics in depth, and readers are encouraged to read all articles on a given topic, as there may be contrasting viewpoints presented.

While gerontology is more encompassing than geriatrics, it certainly does include a focus on the diseases of aging. Preventing, delaying or treating them is an important part of gerontology. The interface between nutrition and gerontology is absolutely critical. No lifestage is so diverse as the "geriatric" lifestage. While obesity remains an issue, cachexia, sarcopenia and underweight conditions are common, especially in the truly aged.

Sarcopenia, the decline in lean body mass most often recognized as a decrease in muscle mass and strength, is a common feature of aging in most species. It is a major determinant of impairment and disability in humans, and heralds impending mortality in dogs and cats. Several papers in these proceedings address this issue, including theories on mechanisms, impact and prevention or treatment. One of the challenges with studying sarcopenia is that it develops very slowly, over many years, so recognizing treatment effects in relatively short-term studies can be difficult.[1]

Despite this challenge, some studies have found ways to decrease the loss or even increase lean body mass. The most common approaches have involved administration of hormones, increases in weight-bearing exercise, and nutrition including feeding management. For example, when elderly humans were provided 80% of their daily protein intake during one meal a day, rather than split among four meals, they had a more positive nitrogen balance and reduced loss of lean mass.[1] Amino acid supplements plus exercise resulted in a synergistic effect to increase muscle mass.[2]

In dogs, increased dietary protein intake reduced the loss of lean mass compared to those on minimal protein intake.[3] A loss of lean body mass is associated with increased morbidity and mortality, so it is hoped that reversing sarcopenia can reduce the associated morbidity, although this has not yet been confirmed. Nevertheless, there is evidence in dogs, and now in cats, that shows that preservation of body weight and lean body mass is associated with a longer life and healthspan.[4,5]

A common problem among aged populations is cognitive decline. For example, 28% of owners of 11- to 12-year-old dogs reported at least one sign consistent with cognitive decline, and this percentage increased to 68% among 15- to 16-year old dogs.[6] The severity of cognitive decline may range from minimal changes to severe dementia. The similarities between human and pet cognitive decline are addressed in these proceedings, demonstrating that what is learned in one species may be applicable to others.[7]

Prevention of cognitive decline in pets focuses on environmental enrichment and mental stimulation, while management might entail pharmaceutical and nutritional care. In addition to potential benefits from antioxidants and long-chain omega-3 fatty acids, alternative energy sources may prove beneficial to offset cognitive decline. While glucose is generally believed to be the primary energy source of neurons in the brain and central nervous system, glucose metabolism becomes less efficient with aging and alternate sources can support the high energy requirements of the brain. For example, fatty acids from medium-chain triglycerides (MCT) readily cross the blood-brain barrier, and can provide up to 20% of the energy used in normal brain tissue.[8]

In addition, research in dogs showed that MCT supplementation results in an increase in omega-3 fatty acids in brain phospholipids, a benefit that may help offset the age-related decline in these fatty acids.[8] Whether because of changes in fatty acids or its use as an energy source, MCTs have been shown to reduce signs of cognitive dysfunction in a subset of humans with mild Alzheimer's disease.[8] In aging dogs fed diets containing MCTs, cognitive impairment was significantly reduced as shown by superior performance on a number of cognitive tests, compared to control dogs.[9]

One two-day conference is insufficient to cover all of gerontology in depth. Rather, the focus of this two-day conference was selected to address two important and common problems in aging populations: cognitive disorders and morbidity associated with sarcopenia. Both conditions are common among pets and people, so it is fitting that experts in human and veterinary medicine and nutrition were among the speakers. Excellent speakers, with deep knowledge about their topics, are essential to a good conference. But, the magic of small conferences is the more comprehensive input that comes from the audience. In this case, the audience itself represented experts from around the globe with experience in geriatric

veterinary medicine and veterinary nutrition. Discussion was encouraged, and highlights of that discussion are captured in the final proceedings from that conference.

This proceedings book is being published in digital format only. There are several reasons for this. One is that it allows the book to be accessed by more people, more easily. Digital books are the wave of the future, and this book leverages this current technology. Perhaps most important, digital publishing is easier on the environment. Nestlé Purina is dedicated to finding ever better ways to promote a cleaner, healthier environment, and this proceedings is consistent with that design. We at Nestlé Purina PetCare hope that the readers of these proceedings agree that this is a worthy pursuit.

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Declining Physiological Reserves: Defining Aging

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Aging is the sum of the deleterious effects of time upon the cellular function, microanatomy and physiology of each body system. These biological aging changes manifest in progressive deteriorations in physical condition, organ function, mental function, and immune response, but not necessarily correlating with the patient's actual chronological age. How old an organism is in actual time is referred to as "chronological" aging and should be distinguished from "biological" aging," the relative functional age of each of an individual's diverse organ systems. Because there is little correlation between biological and chronological age, each animal must still be evaluated as an individual.

Glossary of Abbreviations
ALP: Akaline Phosophatase
ALT: Alanine Aminotransferase
ATP: Adenosine Triphosphate
BMR: Basal Metabolic Rate
GI: Gastrointestinal
GTT: Gamma Glutamyl Transferase
NH: Nodular Hyperplasia
NSAIDS: Nonsteroidal Anti-Inflammatory Drugs

Aging is not a specific disease, but rather a complex process influenced by genetics, environment, stress and nutrition. These factors affect progressive irreversible degenerative changes of all body tissues. Aging in dogs and cats is associated with gradual and progressive deterioration in the delicate body systems that eventually results in decreased physiological functions.

As the functional organ reserves are gradually lost, the long-term result is a physiological decline of the major organ systems leading to an altered response to stressors, infections and various drugs. At some stage in the progressive decline (these "benchmark tipping points"), all physiological reserves are exhausted resulting in overt changes in diagnostic screening tests, biochemical parameters, and/or the onset of clinical symptoms of age-related disease.

Unfortunately the organ system changes are often subtle or undetected until the patient is stressed by illness, hospitalization, medications or general anesthesia. Increasingly, those changes are routinely identified on senior wellness screening tests further validating the value of such testing protocols.

Although these processes influence one another, physiological decline does occur independent of disease. Age-related changes are further impacted by those diseases commonly seen in elderly patients. Over the past decade, the veterinary profession has attempted to make clear distinctions between the process of aging and various age-related diseases. However, the dichotomy between aging and age-related disease continues to be somewhat ambiguous and blurred.

In biology, senescence is the state or process of aging. After a period of renewal (maintenance years), senescence is characterized by animals' declining physiological reserves that hamper their ability to respond to stress and maintain homeostatic balance, thus resulting in increased susceptibility to disease. The older patient is more likely to have multiple marginal organ system dysfunction and significantly less functional reserve capacity than the younger patient.

The physiological decline and lack of reserves are responsible for the deterioration in physical condition, organ function, sensory function, mental function, and immunity. The rate of the physiological decline and lack of reserves varies among species, breeds and even littermates. Individuals of the same chronological age may experience these alterations differently, i.e., for some, the level of decline may be rapid and dramatic; for others, the changes may be much less significant.

Using the patient's age as a benchmark of collective decline is appropriate. However, because each organ has a different rate of biological aging, any critical assessment of a patient's overall health status should be based on a complete health screening of each specific organ function, if possible.

With the large variability in those age-dependent physiological changes in patients, the only way to be certain about various organ functions is to measure them whenever possible. With the various diagnostic screening tools currently available, the physiological decline can be accurately assessed usually before clinical symptoms appear. Specific aging changes are often identified during a comprehensive history or an age-specific physical examination, or uncovered on a biochemical screening profile or other advanced diagnostic technique. Accurately assessing the degree of physiological decline of each organ system is critical in developing an appropriate senior wellness program, proper nutritional strategies, correct drug selection/dosage, and suitable anesthetic protocol.

The process of aging is complex and based on the widely accepted "theories of aging," initiated by a combination of genetic, biological and/or environmental factors all contribut-

ing to the progressive regression called aging. However, it is the declining physiological reserves that define our medical approach to the geriatric patient.

Decisions regarding specific drug therapies, anesthetic protocols, pain management strategies, and quality of life issues hinge on the variety of declining physiological "benchmarks." A clear understanding of the level of physiological decline of each organ system dictates how a specific patient is best managed.

Assessment of the level of physiological decline in each organ system requires diagnostic evaluations. The interpretation of a patient's urinalysis, hematology and biochemistry panels results in data used to aid in the diagnosis, prognosis and treatment of various conditions. This data combined with appropriate imaging techniques and other advanced diagnostics presumably will facilitate early identification of both physiological decline and various pathological states.

The generalized changes associated with aging include dryness of all tissues, progressive degeneration of organ function, tissue hypoxia, cellular membrane alterations, decreased enzyme systems, decreased immune competence, and definite personality alterations.[1,2]

Thermoregulation

Effective thermoregulation (heating and cooling) is deceased in the aging dog. Their decreased ability to pant and decreased cardiac output combined with ineffective vasodilatation make older pets more prone to heat stroke. Older patients are more susceptible to cold ambient temperatures. This age-related cold intolerance is attributed to decreased basal metabolic rate, decreased cardiac output, and decreases in peripheral vaso-constriction, often combined with less subcutaneous fat in the very old pet.[1] The resulting response to "cool ambient temperatures" may manifest as behavioral issues including hiding, shivering/trembling, sleep-cycle disturbances, and/or a reluctance to go outside for elimination. On a more critical note, the older patient is more susceptible to anesthesia-induced hypothermia, which can produce arrhythmias, decreased coagulation and increased risk of post-operative infections.[3] The inferences are comfort issues and anesthesia risks.

Metabolism

As animals age, their basal metabolic rate (BMR) decreases. The consequences include weight gain if there is not a corresponding decrease in caloric intake.[4,5] With a decreased BMR, less metabolic heat is generated resulting in a "cold intolerant" patient. Another impediment is decreased cell turnover in the gastrointestinal (GI) tract making the patient more susceptible to erosion and ulceration. The inference is weight control.

Integument

The integumentary aging changes are the most obvious to the owner. The hair undergoes some degree of pigment loss (graying), obvious follicular atrophy (coat thinning), plus decreased sebum production (dry, scaly skin and coat).[6] The decrease in both quantity and quality of the sebum makes the skin and coat dry, flaky and dull. The nails become longer, more brittle, and usually thicker as a result of decreased exercise and decreased blood flow to the digits. The skin undergoes dermal epidermal atrophy and may develop areas of hyperkeratosis on the nasal and footpads. The inferences are mainly dietary and cosmetic.

Cardiovascular Decline

Functional reserve is reduced with age due to myocardial fibrosis and free wall thickening. These changes reduce efficiency, ventricular filling and cardiac output.[7] Regional and organ blood flow also decreases.[2,3] To compensate for a decreased cardiac output, older patients primarily increase the stroke volume mainly through increased preload and increased atrial kick.[3] Chronic valvular disease (valvular fibrosis/endocardiosis) resulting in valvular incompetence is the most common heart condition of older dogs.[8] The inference is cardiac disease, dietary, anesthesia, and the potential consequences of decreased blood flow to the brain and kidneys.

Pulmonary Decline

Mechanically the patient loses thoracic compliance, develops progressive atrophy of the diaphragm and intercostals muscles, and loses alveolar elasticity. The result is a decrease in arterial oxygen concentration. While difficult to quantify, it is very important in general anesthesia.

Renal Decline

The renal system has aging structural changes that may not be clinically evident. Uncovering chronic progressive renal disease is often difficult in the very early stages. A loss of 50% of functional nephrons is not unusual in older animals; however, that degree of nephron loss cannot be easily detected.[9] Proper assessment of urinary system function involves an accurate history (estimated water consumption/urine output when possible), a complete physical examination, complete urinalysis, and serum chemistries. Depending on the specific case and initial laboratory findings, microbial cultures and imaging may also be indicated.

There are specific physiological/biochemical benchmarks of the declining physiology associated with the progressive nephron loss. The aging kidneys have a decreased renal blood flow, decreased glomerular filtration rate and decreasing ability to concentrate the urine.[2,10] These physiological benchmarks may be picked up on a biochemical profile (BUN, serum creatinine and serum phosphorous elevations) and urinalysis in the later stages. With the large variability in age-dependent changes in renal function, the only sure way of determining

the animal's renal function is to measure them. However, an age–dependent dosage reduction should be anticipated for all drugs that are eliminated by the kidneys.[11] The inferences are primarily chronic renal dysfunction, dietary, anesthesia, and drug elimination.

Hepatic Decline

Geriatric patients can have a decrease in liver mass of up to 50%, which leads to decreases in liver function and available hepatic enzymes for metabolism and detoxification.[1,2,12] The age-related decreases in cardiac output result in decreased blood flow to the liver with subsequent decreases in coagulation factors, plasma proteins and serum glucose. Fatty infiltration of the hepatocytes and nodular hyperplasia (NH) are the two most common age-related lesions in the canine liver.[13] Each could be causes for the mild elevations in serum alkaline phosphatase (ALP) commonly found in older dogs.

The liver performs a wide variety of different and seemingly unrelated functions. It is important in plasma protein synthesis (including coagulation factors), carbohydrate (glucose) metabolism, lipid metabolism, bilirubin metabolism, BUN formation, bile synthesis, plus detoxification of various substances. Biochemical profiling of the liver entails assessments of the liver enzymes, i.e., serum alanine aminotransferase (ALT), ALP and serum gamma glutamyl transferase (GTT), and the various (indirect/secondary) liver function tests, i.e., BUN, glucose, total protein, cholesterol, and fibrinogen. Often there is no direct correlation between liver function, the degree of enzyme elevation, and the prognosis. Pre/post prandial bile acids are a commonly used liver function test. The early inferences are drug elimination including NSAIDs and dietary selection.

Skeletal Muscle Atrophy

Progressive age-related skeletal muscle atrophy is a common finding in older dogs. It is the result of a generalized decrease in the number of muscle cells, i.e., muscle fiber fibrosis, combined with a decreased sensitivity to adenosine triphosphate (ATP). On physical examination, atrophy of the semimembranosus, semitendenosis biceps femoris and quadriceps is most obvious. The consequences of this muscle atrophy include inactivity and hind limb locomotion issues. The inference is quality of life issues and dietary selection.

Table 1. Prevalence of Sensory Decline in Dogs[16]

• Visual Impairment
– 41% of dogs >12 Years Old
– 68% of dogs > 16 Years Old
• Hearing Impairment
– 48% of dogs >12 Years Old
– 97% of dogs > 16 Years Old

Gastrointestinal Decline

Impaired swallowing, decreased gastrointestinal motility, decreased gastric acid secretions, decreases in digestive enzymes, and decreased absorptive capacity may be found in the older patient.[2,11,14,15] The normal loss of olfactory neurons combined with the loss of taste buds decrease the palatability of most foods. A picky appetite may result.[2] A lack of sufficient saliva to aid in swallowing dry food also can contribute to a deceased appetite in the "healthy" older pet.[1] The inferences are dietary, GI ulcerations and partial inappetence.

Sensory Decline

Decreases in hearing and vision are common age-related problems that increase in frequency with aging (Table 1). Changes in vision and hearing are referred to as sensory dysfunctions and often result in changes in behavior patterns.[16] The inferences are quality of life issues.

Hearing: The loss of hearing in older dogs and some cats is well-recognized.[17] Hearing loss associated with decreased sound wave conduction from the external ear to the cochlea (conduction deafness) can be helped with amplification. Dogs with fibrotic or ruptured tympanums fit into this category. Neurogenic deafness (sensorineural), i.e., specific loss of nerve function, is the most common cause of deafness in older dogs.[18] Amplification will not help this type of deafness. However, the use of a high-frequency dog whistle is a temporary solution until the higher frequency sound recognition is lost.

Vision: The normal aging changes in the lens called nucleus or lenticular sclerosis should be differentiated from opacities of the lens/capsule (cataracts). Lenticular sclerosis is never a cause for visual impairment. All cataracts should be staged to better assess the cataracts' impact on the patient's vision. If the problem is retinal, then cataract extraction would obviously not benefit the patient.

Central Nervous System Decline

Older animals undergo somewhat predictable personality changes with aging.[2] Most older pets need increasing amounts of attention and are exceedingly more jealous of new housemates and visitors. They are more irritable and less tolerant with once-endured actions of housemates, owners and visitors. Elderly pets are less mentally alert plus they sleep a larger percentage of time although the actual sleep patterns may be significantly altered. Most animals exhibit varying degrees of cognitive decline with age. Declines in the cognitive abilities of memory, learning, perception, and/or awareness are hallmarks of the progressive process.[16,19] For some patients, it is a significant issue resulting in quality of life issues and progressively unacceptable behaviors.

Declining Immunity

Immunosenescence refers to the gradual deterioration of

the immune system brought on by natural age advancement. It involves both the host's capacity to respond to infections and the development of long-term immune memory, especially by vaccination.[20] Decreases in both cellular and humoral immunity are closely associated with the aging process and should be considered a major contributory factor to the increased frequency of morbidity and mortality among older pets. The progressive decrease in the patient's immune status explains the increased prevalence of tumor growth and infection rates in geriatric patients. The inference is vaccine-induced immunity plus the routine use of immunostimulants.

Our goals should be to optimize the quality of life for the older pet, using preventive health care strategies combined with state-of-the-art diagnostics and therapeutics. Historically, veterinarians have only reacted to those diseases and age-related problems in elderly pets. We need to refocus our efforts on a proactive approach to older patients, and thus, not waiting until overt disease is present. The program should emphasize slowing the aging process, implementing steps for prevention and early detection of age-related diseases, plus vital client education programs.

Considerable research in the area of interrupting the aging process is ongoing in both humans and animals. Technologies such as gene splicing might someday allow each of us to live longer, more productive lives. Until then, decreasing known risk factors, along with implementing regular exercise programs, premium senior diets, and antioxidant supplements, are generally recognized as valuable anti-aging strategies.

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Q&A Discussion

Q: Dr. Joe Millward, University of Surrey: I was interested in what you said in relation to sensory decline and the eye. In the case of the aging human, there's a lot of interest in age-related macular degeneration. The growing research base suggests that it is a nutritionally sensitive disease with a growing evidence base for very long-chain omega-3 fatty acids and also some of the carotenoids. I wondered what the research base is like in the case of companion animals.

A: Dr. Fortney: Since dogs do not have an anatomical macula, they do not experience macular degeneration. However, the question whether nutritional deficiencies may be a cause for some of the visual impairments we see in dogs is a good one. Unfortunately, I do not know the answer to that question.

Q: Dr. Barb Kitchell, Michigan State University: The oncology literature is obviously gerontology literature, and one of the things that is addressed a lot in that aspect is some of the cellular changes that take place around being unable to balance redox, free radical scavenging, DNA repair and things like that. Do you know, is there any validity to trying to do something with antioxidants from a nutritional standpoint in trying to mitigate some of these age-related changes?

A: Dr. Fortney: Yes, I routinely prescribe antioxidants to all my senior patients, whether it's in a dietary form or as a supplement. I think there's enough scientific evidence that it may help slow the aging process. I'm all in on that one.

Q: Dr. Joe Wakshlag, Cornell University: I'd like to comment on Barb's question. Within the oncology literature, which I refer to all the time, is a study from Purdue looking at traditional cell carcinoma in Scottish Terriers. From that data, the incidence of that disease is lower when those dogs are put on some sort of vegetable matter as a treat or part of their diet, something that is missing in a lot of dog foods today. So, is that something we should be addressing as a dietary factor that needs to be put into foods to essentially mitigate those kinds of problems?

Dr. Barb Kitchell: And just to come back with that a little bit, you are referring to natural products, dietary sources of antioxidants versus supplements. One of the biggest problems we have in the oncology community is these patients who come in with like 27,000 supplements and who knows what anything is actually doing. So, it becomes a bit of a conundrum for us because some of our therapeutics are redox-dependent in their anti-cancer effect, but we have these patients who are on massive levels of various antioxidants, and what does that do? The literature is very unconvincing in terms of antioxidant effect in patients with cancer, or at least mixed in terms of antioxidant effect and outcomes from either radiation or chemotherapy.

A: Dr. Fortney: I think there are two pieces of business here: One is therapeutic use of antioxidants and the other strategy is preventive, trying to slow the routine aging process by using the antioxidants. I'm a firm believer that dietary antioxidants are the best way to go.

Dr. Joe Wakshlag: Getting back to Joe Millward's question with reference to the human diet, we talk about antioxidant consumption being deficient in one individual and very high in another. A lot of dog foods tend to be made with mixed tocopherols, which are natural antioxidants, as well. So I would say that a lot of dogs are getting this wonderful antioxidant dose at least with tocopherols. So, is that enough? I don't know, but it does point out a very different way of feeding in the human world and the canine world.

Feline Decline in Key Physiological Reserves: Implications for Mortality

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Abstract

Adult cats undergo changes in body weight and tissue reserves during the course of their lives. These changes are predictable trends that form distinctive life stages: adulthood (1 to 7 years), mature (7 to 12 years) and geriatric (over 12 years). During adulthood cats gain body weight slowly, leading to a mature life stage with high risk of obesity. In the geriatric life stage, cats suffer progressive losses of weight, fat and lean tissue reserves. Data records show that cats associated with longer life had a mature life stage with lower body condition scores than the population mean. The strategy for longevity should target ideal body weight and provide nutrition that evolves to manage specific trends during different life stages.

Glossary of Abbreviations
ANOVA: Analysis of Variance
BCS: Body Condition Score
BW: Body Weight
CR: Caloric Restriction
CRF: Chronic Renal Failure
DEXA: Dual Energy X-Ray Absorption
MER: Maintenance Energy Requirements
VMDB: Veterinary Medical Database (Purdue University)

Introduction

In this presentation, we share data from Nestlé Purina studies collected over several years. Specifically, these data include body weight (BW), body condition scores (BCS), water balance, food intake, maintenance energy requirements (MER), and body composition measured by dual energy X-ray absorption (DEXA). The trends found in these data are used to substantiate the notion of distinctive physiological life stages of fully grown cats. The particularities found for each specific life stage are discussed, as well as the risks that might affect the health status of the aging cat. Finally, the information is used to propose life-stage specific nutrition to address the particular needs of each life stage and help promote ideal body weight in pets.

Cats

Data presented is from our colonies of cats. We care for cats in our colonies over their entire natural life, unless they are adopted as pets. Our aim is that our cats are true representatives of the general pet cat population. This is for scientific reasons, i.e., to ensure that our studies on nutrition and behavior are meaningful and in line with our company values and passion for pets. Our cat colonies represent the pet population in gender balance and age span. We also work to create a homelike, enriched environment in terms of space, temperature, access to windows and natural light, access to toys, and opportunities for interaction with other cats and pet care personnel.

The majority of our cats have a job: They decide which pet products they like better, and thus are offered choices of the majority of pet foods available in the market. Our veterinarians focus on preventive medicine (vaccinations, worming, periodic dental and health examinations) to ensure a healthy colony. Our cats enjoy at least the same standard of veterinary care available to most house pets.

Adult Cat Life Stages
Adult: 1 to 7 Years of Age
Mature: 7 to 12 Years of Age
Geratric: Over 12 Years of Age

This paper presents historical data from two unrelated cat colonies. There are probably a limited number of cat colonies in the world that maintain a pet-like environment and compile years of data on BW, BCS, body composition, clinical histories, and food intakes. Hence, these data can be of significant value to understand the life stages of the species. The data coming from the two different colonies are not combined, but compared with one another to see if observed trends in one data set are reproducible in the other.

Cat Life Stages

The first step to study cats' life stages was to look at BW records. A plot of cats' BW data from one colony (Figure 1) shows trends with age in fully grown cats (1 to 21 years). One way to look at the data is to fit a nonlinear regression model to two tendencies: Cats increased in BW steadily between 1 and 9 years following the equation BW (kg) = 3.5 + (0.1 x years), and then, after age 9 years, BW decreased following the equation BW (kg) = 6.6 - (0.2 x years).

Figure 1: Body Weight (g) of cats (n = 235) by age (1 to 21 years).

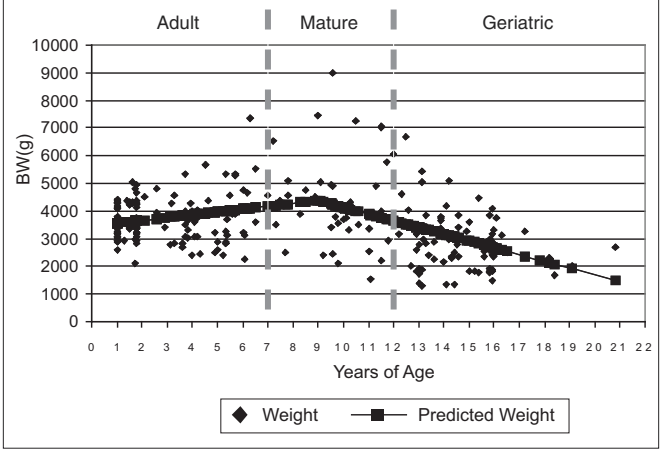


Table 1. Comparison of BW, obesity incidence (BW > 6 kg) and underweight incidence (BW < 2 kg) in different age groups.

Age (yrs)	BW (kg)	Obesity Incidence	Underweight Incidence
1 to 7	3.7 ± 0.8[a]	1/114[a]	0/114[a]
7 to 12	4.4 ± 1.7[b]	6/39[b]	1/39[a]
Over 12	2.9 ± 1.0[c]	1/82[a]	19/82[b]

From these analyses it could be suggested that something changes around age 9, and hence, a cat's life stages could be defined as "pre-9" and "post-9." However, this segmentation creates two groups with great variability that actually do not differ in BW. The post-9 group contains some of the heaviest and some of the thinnest individuals in the colony. It would be impossible to formulate diets suitable for all cats in such a heterogeneous group. Age 9 is an inflexion point, rather than an actual limit between two life stages.

In order to better define groups with greater differences and less within-group variability, the data can be grouped according to the following age periods: 1 to 7 years (n=114), 7 to 12 years (n=39), and over 12 years (n=82). Table 1 shows results from analysis of variance (ANOVA) where BW (kg) of the three age groups are all significantly different.

The incidence of obesity and underweight of these age groups was compared, based on some arbitrary definitions: Cats were considered obese if their weight was greater than 50% over the mean BW and were underweight if less than 50% of the mean BW. As the mean BW of the cats was close to 4 kg, cats weighing over 6 kg (4 kg + 50% mean BW) were considered obese, and those weighing less than 2 kg (4 kg - 50% mean BW) were underweight. Fisher's exact test showed a higher ($P<0.001$) incidence of obesity in the group 7 to 12 years and a higher ($P<0.001$) incidence of underweight cats in the group over 12 years.

The BW records of an unrelated cat colony (Figure 2) show similar trends. Mean BW for this colony was 4.039 kg. BW increased with age up to an inflexion point around 9 years, and then decreased. The incidence of cats with a BW over 6 kg was higher in the 7- to 12-year group. The incidence of cats with a BW below 2 kg was highest in cats older than 12 years. Hence, we assume that these BW trends are a real reflection of the cat life changes.

Besides the BW records, we used BCS scores data available from one colony to look at the condition of each cat. BCS was assessed systematically by trained personnel according to charts, using a 9-point BCS system where 1 is severely underweight, 9 is morbidly obese, and 5 is ideal. The advantage of BCS is that it is independent of the size of the frame of the cat, and it is a better reflection of its real condition. Two cats of identical BW could have different body conditions, with one large frame cat having low BCS and the smaller frame cat having high BCS.

Figure 3 shows the average BCS of a cat colony plotted by cats' ages. A very similar pattern to BW can be observed: The population means plotted with age shows a tendency to obesity (BCS>7) in the 7- to 12-age period. The mean BCS shows a progressive decline for geriatric cats (over 12 years).

The increased risk for obesity at age 7 to 12 was investigated further using DEXA. Obesity, defined in this case as more than 25% body fat, was again highest (one-third of cats) during the 7- to 12-year period (Figure 4). These body composition data substantiate the need for nutritional management for mature cats (7 to 12 years of age) to address the risk of obesity.

Figure 2: BW data plot by age from another cat colony (n = 581).

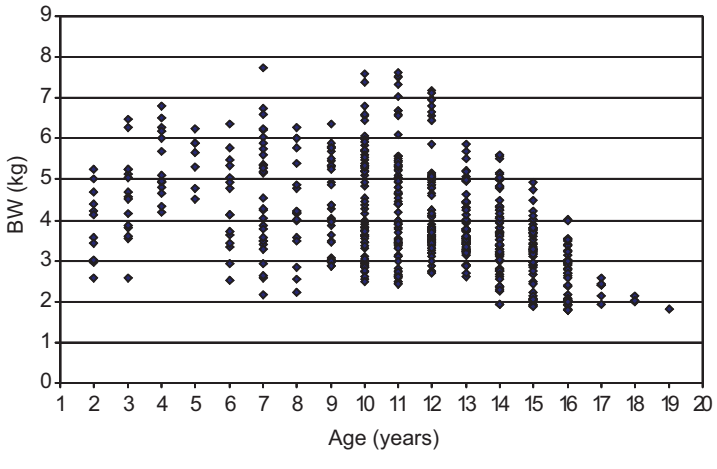


Figure 3: Mean BCS (range 1 to 9, ideal = 5, obese > 7) data plotted versus age (n = 631).

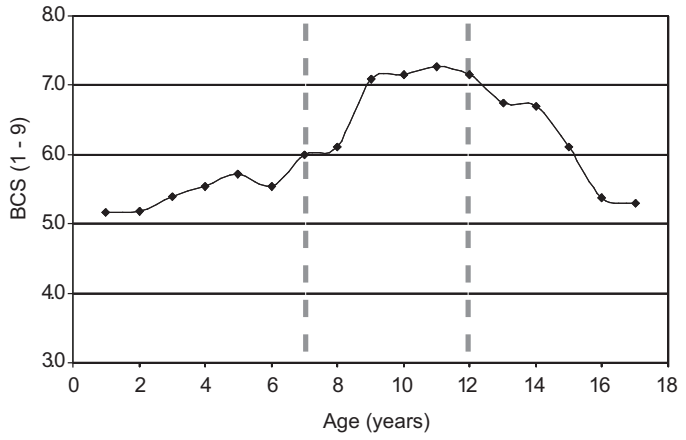
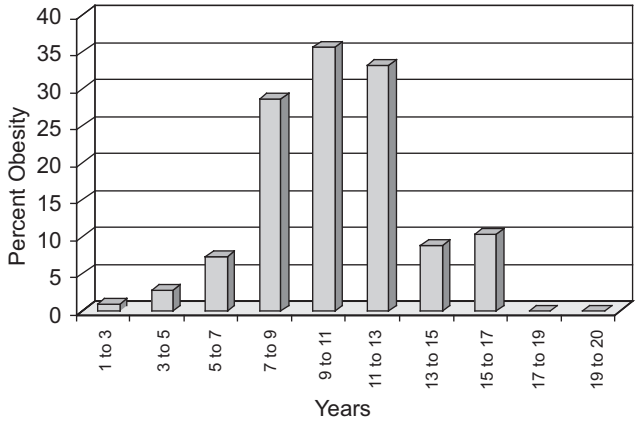


Figure 4: Incidence (%) of Obesity in cats (n = 256) by age (from 1 to 20 years). Obesity defined as more than 25% body fat.



Similarities found between the two unrelated cat colonies, and using different techniques like BW, BCS and DEXA, are unlikely to be a coincidence. The available data indicates that cats over 1 year of age undergo three distinctive life stages: adulthood, 1 to 7 years, during which cats are more likely to show ideal BCS but with a tendency to progressive BW increase with age; mature, 7 to 12 years, with a high risk of obesity; and geriatric, over 12 years, when BW and BCS tend to decrease progressively. These life stages allow diet products and recommendations to be tailored to the specific tendencies of the cats' age groups.

There could be some parallels drawn between the life stages of the cat and humans: 7 years of age in the cat is equivalent to 45 years in humans, and 12 years in the cat is equivalent to 65 years in humans.[1] The definition of mature cat as equivalent to 45 to 65 in human years, and geriatric cat defined as equivalent to over 65 in human years, could be used to make these life stage concepts more anthropomorphic. In general terms, humans have a tendency toward higher BW during maturity (45 to 65 years) than they had during early adulthood (i.e., at 25 years), and very elderly people tend to become progressively thinner or even frail, consistent with what is seen in cats.

Changes Along Life Stages and Decline in Physiological Reserves

DEXA measurements provide a valuable noninvasive tool to study changes in body composition. Percent fat and percent lean data from 256 adult cats, grouped in two-year age intervals, are shown in Figure 5, with scales on the left and right sides respectively. The overlapping of percent fat and lean tissue shows how the trends in both tissues mirror each other. Important shifts in body composition occur around the ages of 7 and 12 years, which are indicative of differences between life stages.

During the adulthood life stage (1 to 7 years), cats showed a mean of 10% body fat and 87% lean tissue. Toward the end of adulthood and during the mature life stage (7 to 12 years), cats have an increased percentage of fat, reaching a mean of approximately 18% by 9 years of age.

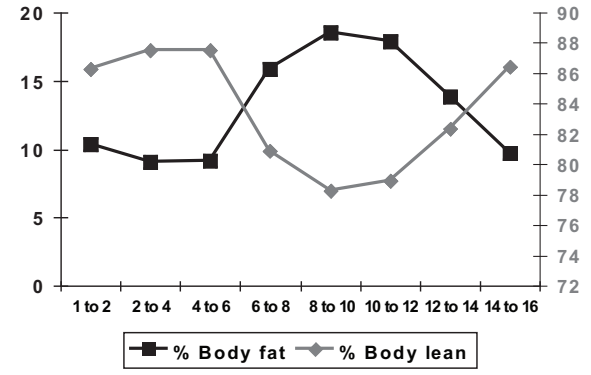
Mean percent body fat values in cats drop progressively after around 12 years of age (Figure 7). Percent lean tissue appears to increase in geriatric cats, but as they suffer progressive BW loss, the absolute values of lean tissue decrease (Figure 6). Lean tissue mean values drop dramatically after 12 years, and by age 15, geriatric cats have a mean lean tissue weight under 2 kg, one-third less than the mean during maturity (around 3 kg).

A longitudinal model analyses applied to data after 12 years of age estimated that geriatric cats lose in excess of 100 grams lean tissue per year. Lean tissue is an indication of muscle mass and is likely to affect the appearance and the capacity for activity of the cat. Reduced lean tissue and body fat can certainly contribute to the frail look of the geriatric cat.

Declining trends in the BW, BCS, fat reserves and lean tissue in the geriatric life stage seem consistent. We also know that the incidence of terminal diseases is most prevalent in this life stage. It is reasonable to question if there is an association between BW changes and disease.

To try to answer this question we used clinical histories from our colony archives to identify the primary causes of death in our colony cats. The data were grouped into those that died from cancer (n=26), chronic renal failure (CRF) (n=50), or hyperthyroidism (n=17). The remaining natural causes were combined into a fourth group (n=165). Then we tracked the BW records of each individual during the four years prior to their death. Figure 8 shows these historical BW data plotted by quarter (every three months) for a total of 16

Figure 5: Mean percentage body fat and lean tissue by age in cats (n = 256).



quarters. The BW from the last quarter prior to death was discarded as it was highly influenced by parenteral fluid treatments in the care of these cats.

In order to ensure that our colony data was representative of the pet population, the incidence of these diseases in the colony was compared to the general feline patient population using data from the Veterinary Medical Database (VMDB) at Purdue University collected over the same time period (1995 to 2001). No significant differences were found in the prevalence rates of cancer, CRF or hyperthyroidism between the Nestlé Purina colony and the U.S. pet cat population.

Two-stage nonlinear regression was used to fit the data, as shown in Figure 8. It was assumed the cats' BW remained constant and then at the point when BW losses start (inflection point), a quadratic model was fitted to allow for an increasing amount of BW loss as they approach death. The inflection point in BW for cats that died of cancer, renal failure and hyperthyroidism was at quarter 10 prior to death (equivalent to 2.5 years). The group of cats dying from other causes had an earlier inflection point at 15 quarters prior to death (equivalent to 3.75 years).

The decline in BW in the second year prior to death was over 6% for cancer, CRF and hyperthyroidism (Table 2). During the last year of life, the average percent of BW loss was over 10% for all four groups. The average age at death did not differ significantly among cancer (13.5 ± 2.3 years), CRF (13.0 ± 3.9 years) and hyperthyroidism (14.3 ± 1.9 years), but the cats from other causes of death died significantly earlier (12.4 ± 3.5 years) than the cats that died from hyperthyroidism. Over the four years prior to death, cats that died of renal failure lost significantly more body weight than the cats in the "other" diseases group.

All studied disease groups have a progressive decline in BW during a period of over two years prior to death. Hence, when it comes to decline in BW, the type of terminal disease of the cat does not seem to be a factor that makes the individual differ greatly from the main trend seen in the cat "other" population. We do not believe it is possible to single out any particular disease type as responsible for the body mass decline seen in the geriatric period. Data seems to indicate that weight loss in geriatric cats cannot be explained by one particular terminal disease.

Water Turnover in Different Cat Life Stages

The decline in lean tissue in cats during their geriatric years needs further exploration in relation to their protein turnover and nitrogen balance. Another likely implication of the progressive loss of lean tissue is the reduction in the total amount of water in the body. This could make geriatric cats more prone to dehydration or less likely to recover from it.

We conducted studies comparing water balance between adult cats (1 to 7 years) and geriatric cats (> 12 years) fed the same canned diet. All cats in these studies had normal markers of renal function (BUN 11.7-33.3 mg/dl and creatinine 0.5-1.8 mg/dl). In the studies, water balance was defined as the relationship between water intake and water losses over a period of three weeks while maintaining BW. Water intake

Figure 6: Fat Content (grams) of cats (n = 631) plotted by age (years).

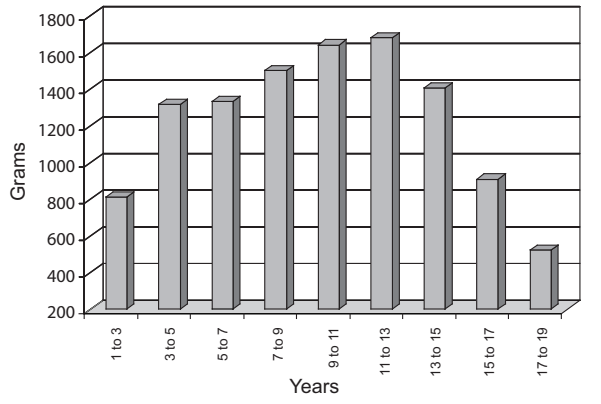


Figure 7: Lean Tissue Content (grams) of cats (n = 256) plotted by age (years).

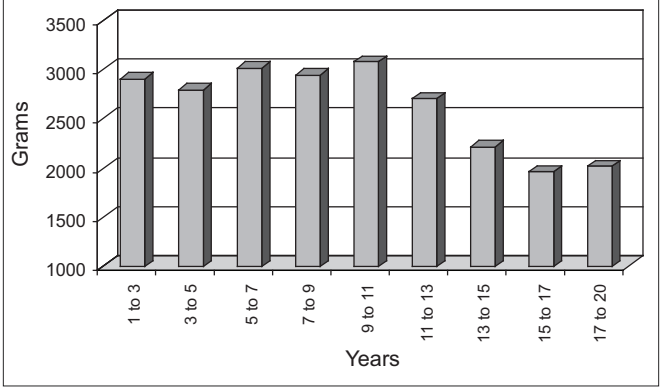


Figure 8: Mean BW (g) in the last four years prior to death by quarter (3 months) in colony cats (n = 258).

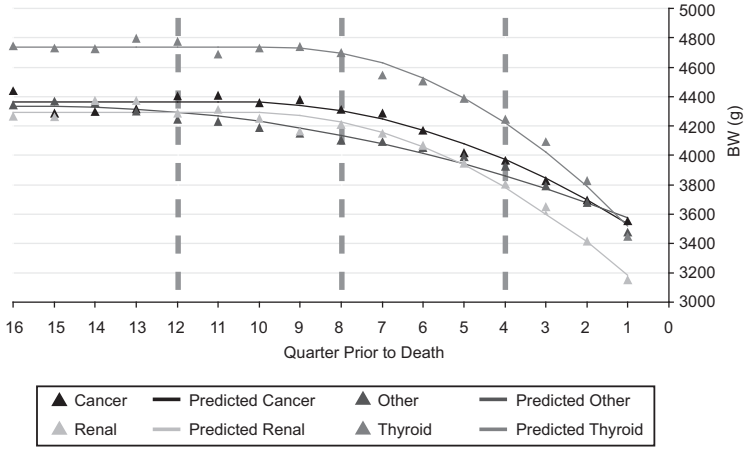


Table 2. Changes in % BW in the four years prior to death, by type of terminal disease.

Death Type	Percent BW change pre-death				Average Age at Death
	4th year	3rd year	2nd year	last year	
Cancer	-2,74	-0.59	-6.88	-10.34	13.5±2.3a,b
Others	-0.76	-2.28	-2.72	-11.38	12.4±3.5a
Thyroid	1.04	-0.77	-6.66	-18.85	14.3±1.9b
CRF	2.54	-2.82	-6.34	-17.35	13.0±3.9a,b

included both the moisture of the food ingested and the amount of drinking water consumed. Water losses were the sum of fecal moisture plus urinary volume. Some other water losses (salivation and respiration water losses) could not be accounted for, but it was assumed that there were no significant differences between adult and geriatric cats as they all shared the same colony environmental conditions (temperature and relative humidity).

As shown in Figure 9, there were neither significant differences in the water ingested with the food nor in the drinking water consumed between the two age groups. Likewise, no differences were observed in fecal moisture losses, but the urinary volumes were significantly higher ($P<0.05$) in the geriatric cats. As a consequence of the urinary volume differences, geriatric cats have higher total water losses ($P<0.05$) than adult cats, possibly due to decreased ability to concentrate urine even when there is no other evidence of renal insufficiency. Small but continuous water losses could predispose geriatric cats to a negative water balance. If we consider water as an essential nutrient, it would be advisable to encourage higher intake particularly in the geriatric cat.

Importance of the Mature Life Stage on Longevity

It is clear that BW, BCS, fat and lean tissue decline in cats during their last stage of life. The speed of the decline seems to be a predictor of time of death. In view of this, we wonder what would be the best strategy for a cat in the mature life stage to try to extend its life. Should the cat aim at starting the geriatric life stage at the highest BW possible to allow for a more prolonged decline over coming years? This approach would appear to contradict caloric restriction (CR). CR has been shown to extend life in mice, dogs and other species in a consistent manner, although data in cats is lacking.

Given the unavailability of data on CR in cats, we attempted to approach this question by evaluating the association between longevity and body composition during the cats' mature years. We identified a small number of cats that had lived longer than the colony average (around 14 years). With data records available during their mature years (n=2 at 8 years, n=4 at 9 years, n=5 at 10 years, n=3 at 11 years, and n=7 at 12 years), we compared (Figure 10) their BCS data records against the average of the colony at mature years. Cats that lived over 14 years (14 +) showed lower mean BCS than the colony average during the mature life stage (7 to 12 years). Regression analyses for BCS of 14+ survivors showed significantly different slopes ($P=0.0245$) from the rest of the colony. The 14+ group maintained a more constant BCS average (around 6.5) while the non-survivors increased average BCS during maturity (>7).

Limited DEXA data of 14+ survivors were available to compare body composition at maturity. Regression analyses comparing percentage fat of 14+ survivors versus colony during mature years showed no significant difference. However, averages of percentage fat were around 3% lower during maturity for 14+ survivors than for the colony (Figure 11). Likewise, the 14+ survivors were more likely to have a higher percentage of lean tissue (3% more) than the colony.

We have to be cautious as we do not know yet the longevity of all the cats contributing to the data in the colony. The database will continue to grow with time and as better data mining tools become available. However, we found significant indications that those cats that lived over 14 years had a lower BCS during their mature period, and preliminary indications that they also had lesser percentage of fat and leaner body composition than the colony average.

To answer the question regarding "which is the best strategy during the mature life stage for a cat to live longer," there seems to be some evidence to support that it is best to maintain BCS closer to ideal. "Piling up fat reserves" during maturity, prior to geriatric years, does not seem to be associated with longevity. An obese or overweight mature period may overload body organs and systems (e.g., cardiovascular, joints, hepatic, pan-

Figure 9: Water Balance (g/day/kg BW) differences between adult (1 to 7 years) and geriatric cats (> 12 years) fed canned food.

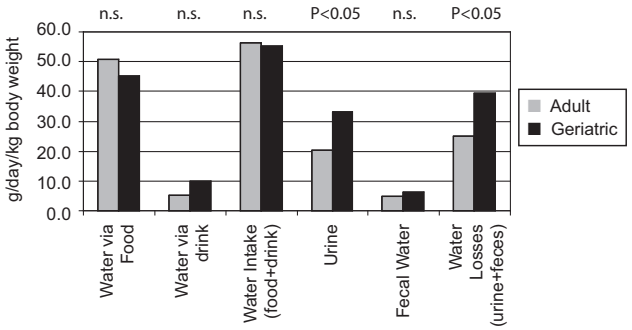
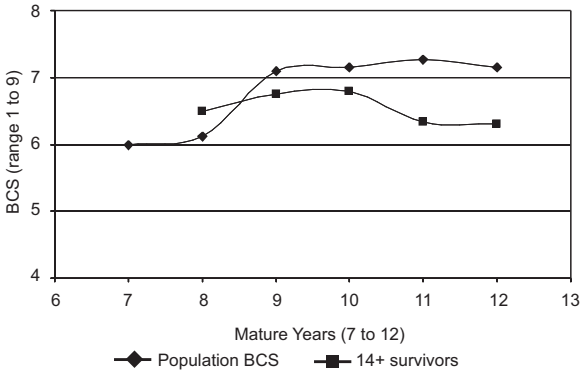


Figure 10: Comparison of BCS means during the mature life stage between the colony and cats that have lived over 14 years.



creatic) and predispose the body for a harder and steeper "fall" in the geriatric years, contributing to a shorter life. This is aligned with CR findings in other species.

The maintenance of ideal BCS and high percentage of lean tissue could help promote healthy activity levels, more interaction with the environment and the pet owner, and a better quality of life. Hence, particular attention must be paid to the mature life stage as this is the period when cats are more likely to have excess BW.

Matching Nutrition to Cat Life Stages

Over the years, we have conducted a number of studies evaluating the MER of adult cats. Generally, MER decreased in mature cats, compared to younger adult cats.[4] However, MER was found to increase in cats older than 10 to 12 years of age.[5,6] This increase was not linear, increasing more dramatically between 12 and 15 years of age, as shown in Figure 12.

It is not easy to identify the cause of the decreasing trends in BW, BCS, body fat and lean tissue in geriatric cats at a time when cats tend to eat more. An increase in energy requirements due to higher activity levels in geriatric years is unlikely. Perhaps metabolic efficiencies might be reduced in some organs. One organ that has been shown to decrease in efficiency is the digestive system.[7,8] Digestion data from several Nestlé Purina colonies were evaluated to test this theory and identified that one-third of geriatric cats have compromised fat digestibility. In some geriatric cats, fat digestibility was found to be as low as 30% with no apparent health problem.[9,10] As fat is the most energy-dense macronutrient, impaired ability to digest fat could contribute, at least in part, to the changes in BW and body composition observed in geriatric cats.

The ability to digest protein is also compromised in many geriatric cats. After the age of 14 years, one-fifth of geriatric cats have reduced ability to digest protein.[9,10] Reduced protein digestibility in geriatric cats seems to occur in parallel with reduction of lean tissue and it might predispose them to negative nitrogen balance.

Figure 11: Comparison of Percent fat between 14 + survivors versus the colony average during the mature years.

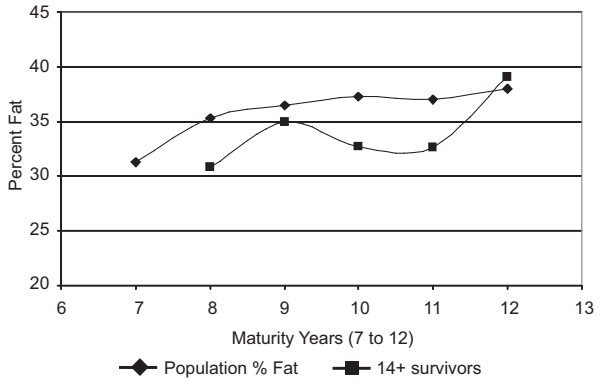
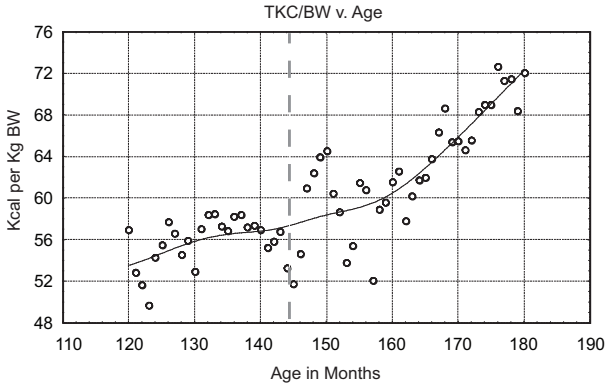


Figure 12: Daily energy requirements (kcal per kg BW) with age (months).



General Nutritional Recommendations for Cat Life Stages

The most sensible approach seems to be to try to maintain ideal body condition during all life stages. Obesity has been linked to increased risk of hepatic lipidosis, glucose intolerance and musculoskeletal problems in cats. It would seem logical to use diets of moderate energy density that could help reduce the risk of weight gain during the mature life stage.

Although moderation of calorie intake may be suitable for mature cats, it does not appear to match the needs of geriatric cats. On the contrary, it would seem more logical to use highly digestible, energy-dense food for geriatric cats in an attempt to slow down the decline in BW and lean body tissue. Protein reduction for geriatric life stage, at a time when lean tissue is lost, seems contraindicated. Geriatric cats seem to have nutritional requirements closer to kittens than to mature adult cats.

Of course, the "average" cat does not exist; it is just a statistical calculation. Dietary management must take into account the individual BCS and MER, and the presence of diseases. A diet must provide all required nutrients and the energy to maintain ideal BW. It must also be palatable to ensure intake. Fecal consistency plays a part in ensuring water balance, which can be compromised in geriatric cats. The diet could be used as a vehicle to aid water intake for the geriatric cat.

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Q&A Discussion

Q: Dr. Margarethe Hoenig, University of Illinois: I was wondering if you could elaborate on how these cats were fed throughout that time. Was it group feeding? Were they separated during feeding? What happened?

A: Dr. Pérez-Camargo: Thank you for the question. That's something that I forgot to mention, and it's very relevant. These cats live in social rooms with around 15 to 20 cats per group, and they are used in our palatability panels. So their job is basically to choose between "I like this today better than that," or the other way around. They have access to food most of the day, and they have access to a variety of products in the market, so they see nearly everything in the marketplace. They have personalized access to their own feeding boxes, gaining access via a chip in their collar. The computer recognizes that cat and allows the cat to enter into that feeder and access the feeder however many times a day as he or she wants. Cats tend to prefer feeding multiple times per day.

Q: Dr. Aulus Carciofi, Sao Paulo State University: When you measured energy data in cats and concluded that they have increased requirements, did you take into consideration that old cats have lower digestibility?

A: Dr. Pérez-Camargo: No, the data was based on actual energy intake, so part of the increased requirement may be related to decreased digestive function. The impact of age on digestion will be better explained by my colleague, Avi Patil, later in the conference, and how that will impact the energy requirements of the cat. But, I would dare to say that the impact of the decreasing digestibility can be so extreme that the cats do not manage to compensate by increasing their intake.

Q: Dr. Joe Millward, University of Surrey: Let me ask you about this phenomenon of weight loss in the geriatric period. I guess what interests me is to what extent it is a function of disease development. It wasn't clear whether you were saying that the weight loss was in cats that were developing chronic illness as opposed to a physiological decline that is, for example, comparable to sarcopenia in humans where you can have muscle wasting in perfectly healthy individuals. Are your geriatric cats basically sick cats or cats that are physiologically unable to maintain their lean body mass?

A: Dr. Pérez-Camargo: Your question is a very good one. For most of these cats we could detect illness probably around one year prior to death if they had chronic renal failure or hyperthyroidism. We monitor the cats regularly, particularly after the age of 7 to 9, in order to detect some of their diseases early, but the fact is that they began to lose body weight before we knew they were sick. And because there currently are no sensitive early markers, we don't actually know if cats have subclinical disease until they show some biochemical marker or clinical sign of disease.

Q: Geraldine Blanchard, Animal Nutrition, France: Your data showed that the energy requirements per kilogram body weight increases with age in cats. However, since that is based on actual body weight, which was already lower in these cats, their total calorie requirements may not be different, still 50 something calories per kilogram of ideal body weight. I wonder how many of these cats were neutered?

A: Dr. Pérez-Camargo: Most of them would have been spayed or neutered. We tend to keep males intact only for reproduction, and females are kept intact if they will be used in the breeding colony but only until they are around 4 to 6 years of age. Once they are no longer needed for breeding, they are spayed to reduce uterine problems. By the time they get to maturity around 9 years of age, they all are neutered.

Q: Dr. Stan Marks, University of California-Davis: You eloquently stated that cats that maintain the more optimal BCS at maturity, live longer, more than 14 years compared to cats that are obese at maturity. Did your data show any association between obesity and a terminal disease, like cancer for example, in those cats?

A: Dr. Pérez-Camargo: Unfortunately, our data does not provide an answer to that question.

Protein for Optimal Health in Aging Humans: Current Controversies

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Abstract

Although protein requirements as measured by nitrogen balance do not change with age, the elderly are at the greatest risk of deficiency (much more so than children). There is great interest whether the quantity and quality of their protein intakes can influence their quality of life in terms of overall mobility and general well-being. This presentation will attempt to make sense of an often diverse and complicated literature.

Glossary of Abbreviations

aLM: Appendicular Lean Mass
BMD: Bone Mineral Density
BMI: Body Mass Index
IGF-1: Insulin-Like Growth Factor 1
MD: Metabolic Demand
N: Nitrogen
PE Requirement: Protein-Energy Ratio (Ratio of requirements for protein expressed as energy)
PPU: Postprandial Protein Utilization
RCT: Randomized Controlled Trials
WHO: World Health Organization

Introduction

In discussing optimal intakes of protein in the human diet more than 10 years ago,[1] I noted that "progress is slow in defining quantifiable indicators of adequacy other than balance and growth" and that for the elderly "there is no evidence of an increased requirement or benefit from increased intakes, except possibly for bone health." Elsewhere in discussing sarcopenia in the context of aging and protein requirements,[2] I had argued that "the main determinant appears to be the decline in resistance-type physical activities," and "information on the extent of any nutritional influence on this decline is not currently available."

Since writing this, a new report on protein requirements has been published by WHO,[3] which has reviewed both the protein requirements and the beneficial and adverse influences of protein intakes on health. Important new research findings have been published leading to considerable advocacy that protein intakes above the recommended dietary allowances may benefit the elderly population.[4] My task here is to examine the justification of this advocacy in the context of the science base.

Protein Homeostasis and Aging

We can ask whether there are any *a priori* reasons to expect a change in the protein requirement with age based on our understanding of age-related changes in protein turnover and homeostasis. In fact, although a change with age in protein turnover would be clearly important in relation to the organism's ability to remodel itself, any such change need not necessarily relate to the dietary protein requirement. The protein requirement is best described in terms of metabolic demand (MD), a function of postabsorptive net catabolism, and efficiency of dietary protein utilization, which can influence the response to protein feeding.[3,5] Changes that occur with age in these aspects of protein homeostasis are the important question.

We need to examine within the daily cycle of feeding and fasting, the changes in protein synthesis, proteolysis and amino-acid oxidation that mediate fed-state gains and postabsorptive losses of body protein. Even from this more specific standpoint, it is still a difficult question because in subjects in overall nitrogen balance, the amplitude of fed-state anabolism and postabsorptive net catabolism is a function of the habitual protein intake.[5] This means there is no single measure in terms of protein synthesis, proteolysis or amino-acid oxidation that is completely independent of habitual protein intake that can be examined as a function of age, and this partly explains the divergence of reported findings.[2]

Key Words

Aging
Amino Acids
Bone Health
Muscle
Protein Intakes
Protein Requirements
Protein Synthesis
Sarcopenia

What we do know is that for metabolic demand, i.e, the magnitude of postabsorptive losses, there is a small but significant fall with age as indicated in ^{13}C leucine kinetic studies of leucine oxidation[2,6,7] (see Table 1). This occurs because of a greater decline in postabsorptive proteolysis (per unit of FFM) compared with protein synthesis.[2] Not only is there a small decline in the capacity of the organism to remodel itself, but

Table 1. Changes with Age in Metabolic Demands, Efficiency of Protein Utilization and the Apparent Protein Requirement for Nitrogen Balance

		Metabolic Demand (g protein/kg per d)†		PPU‡		Apparent Protein Requirement (g protein/kg per d)§	
	n	Mean	SD	Mean	SD	Mean	SD
Young Adult (F)	5	0·83	0·14a	0·99	0·07a	0·79	0·15a
Young Adult (M)	5	0·90	0·06a	1·05	0·10a	0·80	0·07a
Middle-Aged (M)	5	0·87	0·18a	1·01	0·05a	0·79	0·18a
Elderly (F)	5	0·52	0·14b	0·92	0·16a	0·57	0·20b
Elderly (M)	5	0·58	0·16c	1·05	0·13a	0·53	0·15b
All	5	0·74	0·21	1·00	0·12a	0·70	0·20

PPU, postprandial protein utilization; F, female; M, male.
Studies involved changes in N balance calculated from [1-13C] leucine balance during the transition from a low- to a high-protein intake in subjects fed repeated small milk-based meals.[5]
a,b,c Mean values within a column with unlike superscript letters were significantly different (P <0·05).
† Calculated from postabsorptive leucine losses scaled to 24 h assuming that leucine oxidation represents an equivalent loss of tissue protein nitrogen at 4·77mg leucine/g N and that the total amino acid–N conversion factor is 7·31.
‡ Fractional efficiency of protein utilization (utilization/intake) calculated from leucine balance/leucine intake.
§ Apparent dietary requirement for daily balance, calculated as the metabolic demand/PPU.

also an increasing restraint in postabsorptive proteolysis that limits net catabolism in the period after the protein intake from food has been absorbed and utilized. Whether these changes reflect an adaptive fall with age mediated by lower habitual protein intakes or a fundamental feature of aging is currently unknown, but they do mean that this component of the protein requirement is lower rather than higher in the elderly.

As for the response to feeding, this is much more difficult to assess and few reports have quantified this. In multilevel nitrogen (N) balance studies, the slope of the regression of N balance on protein intake is a measure of the efficiency of protein utilization, and the recent N balance studies of younger and older men and women[8] indicated no significant age (or gender) effects, especially when calculated on the basis of fat-free mass. However, as argued elsewhere[5] in N balance studies, the apparent efficiency of protein utilization (the slope of balance versus intake) at 50% or less is much lower than would be expected with the high-quality protein sources that are usually used in the balance studies.

The most likely explanation of this is incomplete adaptation to the low-protein diets fed in these multilevel N-balance studies that results in a lower utilization than occurs when adaptation is complete.[5] However, when studied with ^{13}C leucine balance in the fasted and fed state with milk or animal-sourced food-derived protein meals, no change with age in efficiency of protein utilization is observed[2,5,6] (see Table 1). The efficiency of protein utilization measured as N gain/N intake calculated from leucine gain/leucine intake is close to 100% efficient, i.e., postprandial protein utilization (PPU) is close to 1 in all age groups. These studies suggest a fall in age in the protein requirement, at least as measured in the laboratory under standardized conditions.

Many will find these results surprising, especially the lack of change in postprandial protein utilization, in light of experimental studies of the stimulation of protein synthesis in skeletal muscle by amino acids. We showed some three decades ago[9] that human muscle protein synthesis is stimulated 1.5 to 2 fold by a protein meal, and this effect is now known to be mediated by a combination of amino acids[10,11] and insulin.[14] In the case of amino acids, it appears that leucine, especially in the extracellular compartment, plays an important role in initiating a signaling cascade[12,13] activating protein synthesis, while the insulin response to feeding mediates a fall in proteolysis.[14]

Importantly, in elderly men there appears to be an anabolic resistance, a feature of aging in which amino acid supply is less able to elevate muscle protein synthesis,[15] and insulin is less able to decrease proteolysis of muscle protein[14] under conditions in which it would be expected that muscle anabolic processes would be stimulated, e.g., with feeding and after exercise. Anabolic resistance appears to be induced by decreased physical activity[16] so that age and decreased physical activity have a double-edged effect in decreasing the anabolic processes of muscle maintenance.

What is not known is the practical implication of this anabolic resistance within the daily gains and losses of tissue protein in muscle and elsewhere. On one hand, the rate of muscle wasting in the elderly exhibiting sarcopenia must be very slow on a daily basis because the deficit of muscle mass develops slowly over many years,[17] so that it is unlikely to be detectable within the overall whole body anabolic response to eating studied in Table 1.

On the other hand, the maximization of meal protein utilization for the elderly may require higher meal protein intakes than younger subjects. To some extent this has been examined in the daily feeding pattern of large meals versus multiple small meals.[18] If there is an anabolic resistance, then it might be assumed that the greater postprandial hyperaminoacidemia after a large protein meal would be more effective in mediating protein deposition than several smaller meals. This has been tested in meal feeding studies in subjects adapted over 14 days to specific meal size regimes.[18] When the protein intake was

mainly limited to one large meal, i.e., 80% of the daily protein intake fed at midday rather than spread throughout four meals, subjects did exhibit a more positive nitrogen balance and a better maintenance of fat-free mass.

Another reported change with age in amino acid kinetics that has been suggested to influence protein utilization relates to the splanchnic sequestration of dietary amino acids (the first pass effect) during the absorption of a protein meal. While the first pass effect is generally only important in relation to the interpretation of tracer-kinetic studies of amino acid and protein turnover, because there are suggestions that this splanchnic uptake increases with age,[19] it is often included as one of the features of aging that could impair dietary protein supply to the peripheral tissues, especially muscle.

However, it is not clear whether this is a real effect of aging or a consequence of confounding in the original studies because the elderly subjects were mainly overweight and obese compared with the younger subjects. Thus, the main correlate of splanchnic extraction in this study was body mass index (BMI), which indicates that as the relative splanchnic mass increases with BMI, so does apparent splanchnic extraction. This is unlikely to be an age-related change in protein and amino-acid metabolism that is relevant to the issue of protein requirements.

Measurement of Protein Requirements of the Elderly

In the new WHO report,[3] the protein requirement for adults was derived from a meta-analysis of nitrogen balance studies in healthy adults,[20] which identified no significant age effects but included only one relevant study. It also accepted the view of a rigorous reassessment of other reports of reanalysis and aggregation of earlier nitrogen balance data and all other available data[21] that no convincing evidence exists for a change in the protein requirement with age. This conclusion was confirmed by the recent nitrogen balance studies of younger and older men and women,[8] in which linear regression of the three protein intakes on nitrogen balance indicated protein intakes for nitrogen equilibrium of 0.59 g/kg/d, with no significant age or sex effects, especially when calculated on the basis of fat-free mass. This requirement value is within the range of the average requirement proposed in the new report, i.e., 0.66 g/kg/d.

The new WHO report[3] makes the point that because individuals consume food rather than individual nutrients there has been increasing interest in deriving food-based dietary requirements. Furthermore, because food intakes are largely driven by the organism's needs for energy, the expression of nutrient requirements as nutrient-energy ratios becomes the important descriptor of the dietary requirements for that nutrient.[22] Thus, the protein:energy ratio (protein calories/total calories), or PE requirement, the ratio of requirements for protein and for energy, identifies the required protein density of the diet that when consumed to energy needs will satisfy the protein requirements of an individual or population group.

Protein requirements for all ages are currently defined in terms of a fixed function of body weight. In contrast, energy requirements vary according to lifestyle (i.e., physical activity) and basal metabolic rate, itself a variable in relation to age, size and gender. This means that the PE requirement varies markedly with age and size (increasing as the BMR/kg falls), with gender (being higher for women than men because of the lower BMR/kg in women), and especially with lifestyle (falling as physical activity increases).

Thus, in contrast to protein requirements per kg, which fall markedly from infants to adults, the relationships between energy requirements and age, size, gender and activity protein result in a somewhat counterintuitive set of values for the PE requirement, which are lowest for preschool children and highest for elderly, large, inactive women. In other words, the very high energy requirements (per kg) of the child are satisfied by consuming large amounts of food that need only contain relatively low concentrations of protein to supply the protein requirement. In contrast, the much lower energy requirement (per kg) of an elderly, inactive woman is satisfied by smaller amounts of food that must therefore contain a relatively higher protein concentration.

While there are considerable theoretical difficulties in identifying reference or safe PE requirement values,[22] as shown in Table 2 from the new WHO report,[3] the PE requirement values for sedentary elderly women at 70 kg are more than twice the value for 5-year-old girls, i.e., 0.085 compared to 0.039 for mean PE requirement values and 0.106 compared to 0.052 for the reference (safe) PE requirement values. Although this may be surprising, compared with the PE ratio of an average adult omnivore diet, (e.g., PE ratio = 0.15) breast milk, with a PE ratio of 0.06, is a low-protein food. In practice this means that for any diet thought to be low in protein, it is the inactive elderly who are most likely to be at risk of protein deficiency, although the dietary PE value would have to be less than 0.11 (11% protein calories).

Protein Intakes

For the elderly, like the general adult population, surveys of protein intakes show that, with few exceptions, most mixed diets consumed to appetite provide intakes well above the requirement. This is certainly the case in the United Kingdom elderly population as shown in Figure 1. A wide overall range of protein intakes, up to >2 g/kgld, mainly reflects relative intakes of meat and other animal source foods.[23] Most of the population (80%) have intakes between 0.83 and 1.44 with a mean of 1.14 g/kgld. Because almost everyone has an intake above the mean protein requirement, prevalence of deficiency (intakes< requirement) is very low; the actual value, calculated with the algorithm reported in the WHO/FAO protein report,[3]

Table 2. Changes With Age in the Mean and Safe Protein:Energy Ratio of the Requirements[3]

Physical Activity Level Age (y)	Mean Protein:Energy Ratio[a]						Safe Protein:Energy Ratio[b]					
	Males			Females			Males			Females		
	1.55 Light	1.75 Moderate	2.2 Heavy	1.55 Light	1.75 Moderate	2.2 Heavy	1.55 Light	1.75 Moderate	2.2 Heavy	1.55 Light	1.75 Moderate	2.2 Heavy
0.5		0.056			0.056			0.078			0.076	
2.5		0.036			0.039			0.05			0.053	
5		0.036			0.039			0.05			0.052	
10	0.054	0.046	0.04	0.059	0.05	0.043	0.074	0.062	0.054	0.081	0.068	0.059
15	0.061	0.052	0.045	0.068	0.06	0.05	0.084	0.071	0.062	0.093	0.082	0.069
Adults at 70 kg Body Weight												
18–29	0.068	0.06	0.048	0.068	0.069	0.055	0.094	0.083	0.067	0.108	0.096	0.076
30–59	0.071	0.063	0.05	0.071	0.074	0.059	0.098	0.087	0.069	0.117	0.103	0.082
>60	0.085	0.075	0.06	0.085	0.082	0.065	0.117	0.104	0.083	0.128	0.113	0.09
Adults at 50 kg Body Weight												
18–29	0.059	0.052	0.041	0.059	0.061	0.049	0.081	0.072	0.057	0.096	0.085	0.068
30–59	0.059	0.052	0.041	0.059	0.06	0.048	0.081	0.072	0.057	0.094	0.083	0.066
>60	0.073	0.064	0.051	0.073	0.068	0.054	0.1	0.089	0.071	0.106	0.094	0.075

[a]Calculated from the values for protein and energy requirements as (protein (g/kg) × 16.7) /energy (kJ/kg).
[b]Safe protein:energy ratio for an individual calculated from the values for protein and energy requirements.[3] These values are generally similar to calculations assuming a reference protein requirement = mean +3SD for adults and mean +3-4SD for infants and children.

is 2.7%, an acceptable level. Even after adjusting for <100% digestibility for the plant protein component, prevalence of deficiency is unlikely to warrant concern[23] especially when judged in the context of the adaptive metabolic demand model for the protein requirement.[5]

Protein Intakes and Disease and Disability in the Elderly

Because there is no objective measure of protein status, "deficiency" as calculated above is defined as a statistical construct, i.e., a measure of prevalence of intakes<requirement. The unambiguous identification of disease risk in relation to protein intakes or an optimal protein requirement is exceedingly difficult,[1,4] and in the review of the subject within the recent protein requirements report,[3] few specific benefits of higher-than-average protein intakes were identified. In fact, populations with lower protein intakes associated with low meat diets exhibit generally similar or even lower rates of morbidity and mortality compared with meat eaters.[24,25]

The issue whether variation in protein intakes toward marginal intakes is detrimental in elderly people consuming self-selected diets was addressed some years ago[26] with measurements of dietary intakes, plasma protein and arm muscle area for 691 men and women aged 60 to 98 years old consuming on average 1.04 g protein/kg. Some 12 to 15% of subjects had protein intakes < 0.8 g/kg, but clear, overt protein deficiency was not observed as far as serum albumin, triceps skin-fold thicknesses and transferrin concentrations, with no evidence that lower intakes of protein in the group adversely influenced any of these variables. Indeed, both arm circumference and a "nutritional index" score calculated from albumin, triceps skin-fold thicknesses and transferrin concentrations were inversely correlated with protein intakes, implying at the very least no deleterious effect of consuming protein at the lower end of the observed range.

Another important study[27] reported actual N balances for elderly people (70 to 86 years old) who were either housebound, consuming low energy and consequent low-protein intakes (recorded values 0.67g/kg/d), or healthy (70 to 86 years old) with higher protein intakes (0.97 g/kg/d). While the housebound subjects were mostly in negative balance and the healthy were at zero balance, there was no indication that protein intake determined balance with no correlation between protein intake and balance in either group over a wide range of intakes: 24-79 g protein/d in the housebound and 35-92 g protein/d in the healthy group.

Furthermore, at the same intakes, housebound subjects tended to be in negative balance whereas the healthy subjects were in positive balance. The immobility and/or illness or the lower energy intake of the housebound subjects accounted for the negative nitrogen balance. Notwithstanding the limitations of the N balance measurements, these data do not support any effect of protein intake on N balance over a range of intakes as wide as that likely to be observed in a free-living population.

Thus, these two studies point to free-living elderly individuals being able to adapt to protein intakes over a wide range, with no benefit from higher protein intakes, at least in terms of either biochemical indicators or measured N-balance.

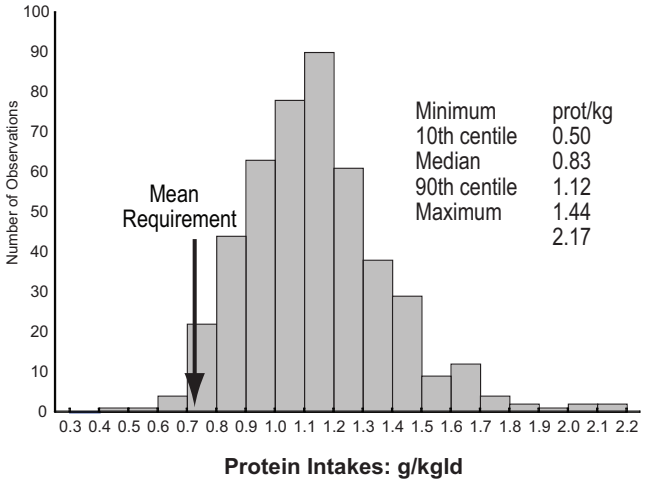
Protein Intakes and Sarcopenia

A major driver of the current advocacy of higher protein intakes for the elderly is the suggestion that higher protein intakes will protect against sarcopenia.[28] Sarcopenia, the decline in skeletal muscle strength and mass with advancing age, is a major determinant of impairment and disability. This is because skeletal muscle strength correlates with walking speed, balance, time to rise from a chair, ability to climb stairs, incidence of falls, and survival rates[29] and predicts physical dysfunction.[30]

Because it has been known for many years that strength training is quite effective in restoring age-related losses in muscle mass and strength function,[31,32] it is generally assumed that the main determinant of sarcopenia appears to be the decline in resistance-type physical activities.[33-37] It has been reported that a lifetime of intense aerobic physical activity has little impact on the decline in mass and strength,[38] and even in extremely fit elderly subjects or endurance-trained master athletes,[39] sarcopenia occurs. However, in the MINOS study,[17] low physical activity at work was identified as a risk factor for sarcopenia, and it has recently been demonstrated that muscle mass and strength in the elderly does respond to aerobic exercise.[40] This tends to suggest that sarcopenia is related to and can be reversed by all types of muscle activity.

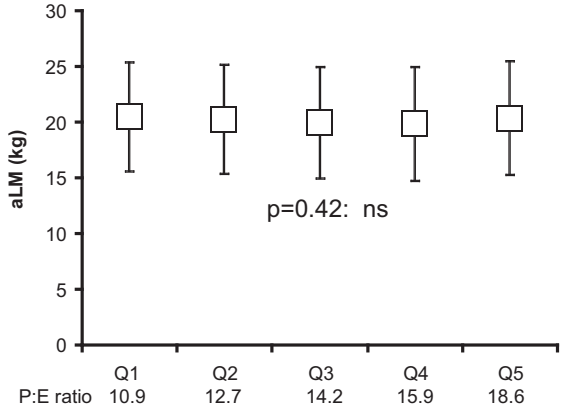
Nevertheless, it must be assumed that an adequate dietary protein intake is also a prerequisite for healthy aging, and it is clearly important to identify the extent of any nutritional influence on this decline in relation to both muscle strength and the metabolic implications of sarcopenia. There are two ways to establish the likely influence of protein intakes on sarcopenia, namely epidemiology and randomized controlled trials (RCTs). As far as epidemiology is concerned, the fact that sarcopenia occurs in the extremely fit implies that it is highly unlikely that a simple nutritional etiology, especially inadequate protein, would be identifiable. This is because high-intensity physical activity will promote increased energy demands and associated increased food intakes.

Figure 1: Distribution of Protein Intakes in the UK Elderly



Source: UK National Diet and Nutrition Survey[72] trimmed for under-reporters (i.e., energy intake <1.35 x estimated BMR).

Figure 2: Appendicular LM (kg) By Quintiles of Protein Intakes



Source: Health ABC study[44] at baseline: n=2066 mean age 74.5.

It can be assumed therefore that such populations will exhibit protein intakes at the upper end of the normal range. It is not surprising therefore to find that cross-sectional studies of sarcopenia and protein intakes have generally failed to show any association.[17,41-43] However, a recent large community-based longitudinal study of the loss of appendicular lean mass (aLM) conducted over three years (n=2066) in men and women aged 75,[44] showed that the aLM loss was greater in the lowest compared with the highest quintile of protein intake. This suggests that dietary protein may be a modifiable risk factor for sarcopenia in older adults and that higher intakes afford some protection.

In fact, some caution is needed in the interpretation of this study. First, there was no relationship between aLM and protein intake at baseline when the dietary data was collected (see Figure 2). This means that, as others have found, on a cross-sectional basis for the men and women studied in the Health ABC study, dietary protein intake was not identified as a causal factor for sarcopenia. Second, the positive protein intake-aLM relationship during the three-year longitudinal phase of the study was only observed for those who either lost or gained weight. The loss of aLM in the otherwise weight-stable subjects, half of the entire cohort, was not related to protein intake. This means that the relationship observed for the whole cohort is unlikely to be a simple consequence of

higher dietary protein intakes reducing sarcopenia.

As for RCTs of protein intakes and sarcopenia, given the slow rate of its development over many years,[17] RCTs for the prevention of sarcopenia are impractical. However, there is extensive literature on the influence of dietary protein on the efficacy of strength training. In one early study it was shown that muscle hypertrophy and strength gains can be achieved in elderly subjects even on a protein intake at the RDA level (0.8 g/kgld), which represented a reduced protein intake for the subjects.[45] According to a recent review,[32] "most of the limited research suggests that resistance training induced improvements in body composition, muscle strength and size, and physical functioning are not enhanced when older people who habitually consume adequate protein (modestly above the RDA) increase their protein intake by either increasing the ingestion of higher-protein foods or consuming protein-enriched nutritional supplements."

On the basis of a review of interventions for sarcopenia and muscle weakness in older people,[47] a recent task force on sarcopenia[46] concluded "it is not clear if protein supplementation in the absence of malnutrition enhances muscle mass and muscle strength, as protein supplementation alone or in association with physical training has proved unsuccessful." It would appear therefore that dietary protein intakes have little influence on the development of sarcopenia or the effectiveness of its treatment with resistance exercise.

Protein Intakes and Bone Health

Bone health and osteoporosis is clearly an important issue for the quality of life in the elderly. In men, osteoporosis and sarcopenia appear to go together, although this doesn't occur in women because bone mineral content is to a considerable extent a function of fat mass and associated oestrogen status, which tends to overwhelm any relationship between muscle mass and bone health.[48]

Dietary protein has been associated with both positive and negative influences on bone. There is a requirement for amino-acid precursors from dietary protein to maintain bone structure, and, in addition, the anabolic drive of amino acids on the organism includes an influence on bone, mediated in part through the stimulation of growth factors such as insulin-like growth factor I (IGF-I).[49] IGF-1 has been suggested to increase bone mass by increasing osteoblast activity, and may also increase the mineralization of bone matrix[50] in part by increasing calcium absorption.[51] Therefore, an inadequate anabolic drive due to insufficient dietary protein[52] may decrease bone strength through adverse changes in bone microarchitecture.[53] This indicates a need for adequate protein intakes for both the elderly and the general population to help optimize bone health. However, the balance between beneficial and detrimental influences of dietary protein on bone health is a long-standing debate.[3,54-56]

Dietary protein is a major contributor to acid production[57] as a result of the oxidation of the sulphur amino acids, and declining pH values influence the balance between osteoblastic and osteoclastic activity[58] and increase urinary calcium excretion.[59] The key question is the balance between these effects. High animal protein intakes have been associated with high bone fracture rates in cross-cultural studies,[60] but within populations there is little evidence for this. In fact, our recent systematic review and meta-analysis of dietary protein and bone health found only benefit or no influence.[61] Thus, we found that in a pooled analysis of cross-sectional surveys of the relation between protein intake and bone mineral density (BMD) or bone mineral content at the main clinically relevant sites, there was a beneficial influence with protein intake explaining 1 to 2% of BMD. Similarly a meta-analysis of randomized placebo-controlled trials indicated a significant positive influence of protein supplementation on lumbar spine BMD. However, there is no evidence that higher protein intakes reduced the relative risk of hip fractures at least in terms of studies published to date.

Protein Intakes and Cardiovascular Disease

There is a complex relationship between protein intake and cardiovascular disease that has yet to be fully resolved.[3] On the one hand, animal studies point to animal protein intakes having hypercholesterolaemic and atherogenic influences, but no such influences are observed in humans. Indeed, human studies have suggested higher protein intakes to be beneficial for the heart although no consensus has been reached about the causality or mechanisms of such associations. One possibility is a protective influence of protein intake on hypertension with inverse relationships between protein intake and blood pressure identified in several cross-sectional studies, the most recent being the INTERMAP study.[62]

A meta-analysis of studies up to 2002[63] indicated a convincing cross-sectional inverse association between dietary protein intake and blood pressure although longitudinal studies of intakes or changes in protein intake in relation to change in blood pressure or incidence of hypertension have been inconclusive. Elliott[64] cautions about over-interpretation of these studies, many of which involve secondary analyses and could be subject to various sources of bias. Certainly to date, there is no convincing body of evidence from intervention studies and no clear underlying mechanisms. Thus, while it can safely be assumed that high protein intakes are not likely to be damaging for cardiovascular health, any benefit must remain uncertain.

Conclusions

After reviewing the literature on protein intakes and health, WHO[3] concluded on a cautious note: "Current knowledge of the relationship between protein intake and health is insufficient to enable clear recommendations about either optimal

intakes for long-term health or to define a safe upper limit." Since then, although the expanding evidence base is certainly weighted toward benefit of higher protein intakes rather than harm, it does not contain the clear evidence from properly conducted randomized controlled trials that would warrant any change in this conclusion.

The only report of a successful protein-related intervention in the elderly that improved lean tissue mass is a double-blinded control trial in 78 elderly men and women (76 years old) given for one year daily an amino-acid cocktail containing arginine, lysine and the leucine metabolite β-hydroxy-β-methylbutyrate (BHMB).[65] This cocktail had been shown previously to improve functionality, strength, fat-free mass, and protein synthesis after 12 weeks,[66] and in the yearlong study it increased lean tissue mass. However, somewhat disappointingly, this time no change in strength was observed raising questions about the physiological significance of the increases in lean tissue mass.

In terms of protein intake related to resistance exercise and sarcopenia, a recent review concluded "research has not identified a synergistic effect of protein supplementation and resistance exercise in aging populations,"[67] and this was echoed in a more recent review.[68] A subsequent report reinforced that modestly increasing protein intake (from 0.9 to 1.2 g/kgld) predominantly from eggs had no influence on the gain in muscle induced by resistance training in older people.[69] In terms of optimal intakes for bone health, while the new analysis of the protein intake bone health literature points toward a benefit of protein, as yet fracture prevention has not been identified.

What has emerged in relation to maintaining muscle and bone health in the elderly is a clear benefit from adequate vitamin D. Not only has the importance for bone health of much higher levels of plasma 25-hydroxy vitamin D than are currently observed been unequivocally demonstrated in most populations with limited sunshine exposure, but an important role in maintaining muscle strength in older adults is now clear, resulting in less falls and thereby contributing to less fractures.[70,71] Muscle weakness is a prominent feature of the clinical syndrome of vitamin D deficiency, and vitamin D and its receptor are important for normal skeletal muscle development and in optimizing muscle strength and performance, with many supplementation trials of vitamin D in older adults showing a reduction in the risk of falls and improvements in tests of muscle performance.[70]

The most recent meta-analysis of double blind RCTs with supplemental vitamin D confirms a dose-dependent benefit on fall prevention in women, although not in men.[71] This is not to underestimate the importance of an adequate protein intake for the elderly, especially for sedentary populations with only modest energy needs. For these in particular, diets with adequate protein are necessary to enable current requirements to be met. For those who are active with higher energy needs and consuming higher food intakes, most balanced diets will provide more than enough protein.

The recent task force on sarcopenia[46] summarized its view on nutrition as follows: "A well-balanced diet, with adequate amounts of essential minerals, fatty acids and amino acids, together with an active and healthy lifestyle with regular periods of aerobic and resistance training, would be a correct life-course approach toward reducing the prevalence of sarcopenia and other chronic diseases in future elderly generations." As recently discussed,[73] my own highly speculative view is that sarcopenia results from reduced tension on muscle as bones slightly shorten with age. Thus, the key to health and active longevity may be sufficient appropriate exercise and healthy eating to ensure adequate intakes of protein and most other key nutrients to maintain muscle and bone strength and mobility. The demand for animal protein will no doubt continue to grow in the emerging economies, because meat is a preferred food in most societies. How much protein is needed will certainly continue to be debated, but whether global demands can be met is another story.[23]

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Q&A Discussion

Q: Dr. Dottie Laflamme, Nestlé Purina PetCare: So much of the data on protein requirements are based on nitrogen balance evidence. And there are some individuals who would say that nitrogen balance is probably not an appropriate measure of optimum protein requirements because you can have a loss of lean body mass and you can have a reduction in protein turnover and still maintain nitrogen balance. So I'd like to invite you to give your opinion on if you think that nitrogen balance truly is a sufficient measure, or if in the long term, we should be looking at something else?

A: Dr. Millward: It would be very nice to be looking at something else because nitrogen balance is not a measure of optimal protein intakes. What nitrogen balance does, in theory, is what any nutrient-balance study does. It tells you whether the organism is gaining or losing that nutrient. There are problems with nitrogen balance. Nitrogen balance measures the minimum protein requirements, and that has to be very clear. That is the minimum intake to maintain people in nitrogen equilibrium. There are many secondary problems with nitrogen balance in terms of whether it actually does that. There can be problems with methodology and with unmeasured nitrogen loss. But nitrogen balance should never be claimed to be a measure of optimum status. And there isn't a single measurement that can give you optimum status, because if it's bone health, if it's sarcopenia, if it's whatever it

is, then there has to be an evidence base that builds up sufficient quantitative data between intake and outcome that enables you to make sensible evidence-based conclusions about what intake would be appropriate. And I don't think there is an evidence base that allows us to do that.

Q: Dr. Bob Backus, University of Missouri: Is there any data on whether people eating more protein live longer?

A: Dr. Millward: I know of none. I think that whole area is problematic. I know we've got this huge animal database and primate database for energy and longevity and there is supposed to be a little bit of human data that supports that, which I'm quite skeptical of. Increased physical activity is supposed to give us better prolonged health, which, of course, would involve increased intakes. But as far as protein intake, it's actually not a question that we can answer now, I don't think.

Sarcopenia in Aging: Implications of the Age-Related Loss of Lean Body Mass

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Abstract

Loss of muscle mass and function is inherent to aging. Severe muscle loss is termed "sarcopenia." Loss of muscle strength and function not only decreases mobility and quality of life, but also is related to numerous unfavorable health outcomes. Hormonal, exercise and nutritional therapy can all play a role in lessening the rate of muscle loss with aging.

Glossary of Abbreviations
ATP: Adenosine Triphosphate
COPD: Chronic Obstructive Pulmonary Disease
RDA: Recommended Daily Allowance
REE: Resting Energy Expenditure

Introduction

Sarcopenia of aging refers to severe loss of muscle mass associated with aging. A proposed definition of sarcopenia is a reduction in lean body mass to less than two standard deviations below the norm for young individuals of comparable body mass index. A corresponding loss of muscle strength and functional capacity is implicit in the definition of sarcopenia. Most importantly, the loss of muscle mass and strength with aging is a continuum, and it appears that, with rare exceptions, no one is exempt from this response. A wide variety of studies performed in healthy elderly subjects indicate losses in strength of 30% or more by the seventh decade of life as compared to young adult strength[1] (Table 1). Maintenance of muscle mass, strength and metabolic function is key to the quality of life with advancing age, and recent evidence suggests that even morbidity and mortality are related to the maintenance of these factors at a reasonable level.

Is Adequate Muscle Mass and Strength Important for Health?

The role of muscle in performing activities of daily living, as well as recreational activities, is well-known. The metabolic role is much less appreciated. Muscle plays a central role in whole body protein metabolism and is important in maintaining normal plasma glucose concentrations.[2]

All body protein is in a constant state of turnover, i.e., synthesis and breakdown. Thus, the extent of gain or loss of protein in a tissue or organ at any point in time is determined by the balance between protein synthesis and breakdown. Amino acids from protein breakdown can be reutilized within a tissue for protein synthesis, but only very unusual circumstances would allow a sufficient reutilization of amino acids within a tissue to enable complete balance between synthesis and breakdown. This is because there is generally a certain amount of oxidation of amino acids within tissues and some efflux into the blood where amino acids can transit to the liver for metabolism and excretion.

A catabolic state results in any tissue when the synthesis of protein is less than breakdown. A catabolic state will result in loss of tissue protein at a rate dependent on the rate of protein turnover and the extent of imbalance between protein synthesis and breakdown. A catabolic response can be counted only by sufficient uptake of amino acids from plasma to balance the amount of amino acids lost from the intracellular pool, through either oxidation or outward transport. However, in the fasted state there is no absorption from the intestines to supply amino acids to the blood. Consequently, amino acids must be released into the blood from tissues that can afford a transient loss of protein to provide necessary precursors for synthesis in other tissues whose protein supply is indispensable. In the fasted state, muscle is the only tissue in the body with adequate protein reserves that tissue protein catabolism can be sustained for a significant length of time without significantly affecting health.

Consequently, in fasted conditions there is a net breakdown of muscle protein in order to provide amino acids to the blood so that tissues whose protein content cannot be markedly labile, such as skin, heart and brain, can derive precursors to maintain protein balance even in the absence of food intake. The loss of muscle protein in the fasted state is reversed in the fed state, so that if adequate protein is eaten, there will be no net change in muscle protein content over the course of the day. In contrast, tissues that maintain their protein mass in the absence of food

Table 1. Age-Related Changes in Knee Extensor Strength[1]

Study	Gender	Age/Decade	Testing Condition	% of Young Adult Strength
Larsson et al.	M	7th	Isometric	75
Murray et al.	M	8-9th	Isometric	55
Murray et al.	F	8-9th	Isometric	63
Young et al.	F	8th	Isometric	65
Young et al.	M	7th	Isometric	61
Overend et al.	M	7-8th	Isometric	76
Ivey et al.	M	7-8th	Isometric	76
	F	7-8th	Isometric	75
Poulin et al.	M	7-8th	Isokinetic (90°/s)	
			Concentric	68
			Eccentric	81
			Isokinetic (180°/s)	
			Concentric	69
			Eccentric	98
Vandervoort et al.	F	7-8th	Isokinetic (90°/s)	
			Concentric	50
			Eccentric	64
Lynch et al.	M	8th	Isokinetic (30°/s)	
			Concentric	65
			Eccentric	67
	F		Concentric	69
			Eccentric	73

absorption usually do not require the deposition of excess protein in the fed state.

The clinical and nutritional implications of the role of muscle protein in overall whole body protein metabolism are twofold. Adequate muscle mass is needed to maintain circulating plasma amino acid concentrations to support protein synthesis during periods when food is not being absorbed or protein from vital tissues will be lost, and repletion of muscle protein is the primary metabolic role of absorbed amino acids resulting from the digestion of dietary protein. In stressed states, such as the response to infection or inflammation, the demand for amino acids from muscle protein breakdown is increased because of increased requirements for protein synthesis that may be required for enhanced immune function, wound healing and other responses.

At the same time, the response to stress that enhances the rate of muscle protein breakdown desensitizes the muscle to the normal anabolic effect of ingested protein.[3] Consequently, muscle degradation in stressed states can be quite rapid and is termed "cachexia," which is distinct from sarcopenia.[4] An elderly individual who has adequate muscle protein for normal life may acutely lose enough muscle protein in the cachectic state that it is not possible to maintain adequate fasting amino acid levels, with catastrophic results as protein is lost from essential tissues. For this reason, clinical responses to the rapid loss of muscle mass in elderly is often manifested in a "threshold" response, with little adverse effect noted until muscle mass becomes less than the necessary threshold, at which point survival is directly affected.

Relation of Muscle Mass to Health Outcomes in Elderly

Maintenance or enhancement of muscle mass is important in optimizing health outcomes in aging individuals. Numerous examples have been provided in recent literature.[5-7] The relation between thigh muscle cross-sectional area and mortality in patients with chronic obstructive pulmonary disease (COPD) is an excellent example. In the study by Marquis et al.,[5] patients with COPD were categorized according to severity of disease and extent of muscle loss. There was minimal effect from the severity of disease on the probability of surviving five years in patients in whom muscle mass (as reflected by thigh muscle cross-sectional area) was essentially maintained, but in individuals with severe disease the loss of muscle mass had a direct impact on survival. Individuals with severe disease and a low muscle mass had less than 40% probability of surviving five years, whereas five-year survival was greater than 80% in individuals with the same classification of disease with minimal muscle loss.

The same pattern of response is observed in terms of the amount of body protein and the recurrence of lung cancer.[6] Relapse-free survival over a five-year follow-up was found to be greater than 40% in individuals whose body protein increased by more than 5%. Survival dropped to 30% after two years if there was less than a 5% change in total body protein over that time, and if total body protein dropped more than 5%, there was no survival beyond one year. Similarly, the relative risk of death in patients with end-stage renal disease has been reported to be directly related to the amount of muscle mass.[6]

It is impossible to know the extent to which the loss of muscle mass directly contributed to death in these patient groups, as opposed to the possibility that loss of muscle reflected the severity of the general stress state induced by the disease. However, the relation between survival and muscle mass in a variety of clinical situations is consistent with the central role of muscle protein in overall whole body protein metabolism discussed above.

Not only is muscle mass related to survival in a number of clinical states that affect the elderly, muscle strength is also related to health outcome measures. Regardless of the level of cardiovascular fitness, muscular strength was found to be related to all-cause mortality in a study involving almost 9,000 men.[7] In the same study, it was found that muscular strength highly predicted all-cause mortality and especially the cancer mortality rate in men over 60 years of age, but not in men younger than 60.[7] This result probably reflects the fact that as men age, the loss of muscle mass and strength decline closer to the critical threshold level, so there is less ability to compensate for a cachectic state than would be the case in a younger man.

Muscle and Diabetes

Muscle and liver are the principal sites of clearance of glucose from the blood as a result of insulin action. Depending on the increase in insulin concentration and the amount of glucose available, the muscle may be the primary site of glucose clearance. DeFronzo et al.[8] measured the arterial-venous balance of glucose during the euglycemic-hyperinsulinemic clamp procedure in order to quantify the amount of muscle glucose uptake in normal and diabetic subjects. They found that the reduction in whole body glucose uptake in diabetics could almost entirely be explained by reduced clearance of glucose by muscle.[8]

This response is more reflective of the "quality" of the muscle rather than the muscle mass per se. The difference in muscle glucose uptake between groups persisted when normalized for leg weight. Recent studies suggest the difference in muscle quality between diabetics and normal individuals is directly related to the accumulation of lipid in the muscle in diabetics.[9] The extent of lipid accumulation is also increased in elderly,[10] and increased muscle lipid probably explains the high incidence of insulin resistance in this group of individuals. The extent of insulin resistance is correlated to the amount of intramuscular lipid in elderly.[10]

Muscle and Osteoporosis

Bone health is determined by a variety of factors. The mechanical force on bone is essential for optimal bone health. Muscle contraction is essential for the generation of mechanical force on bone. For that reason, it would be expected that there would be a correlation between muscle mass or strength and parameters of bone health. It is thus not surprising that grip strength correlates with bone area, mineral density and mineral content of bone.[11] Thus, exercise and diets that affect muscle likely affect bone health in a corresponding, albeit indirect, manner.

Muscle and Energy Balance

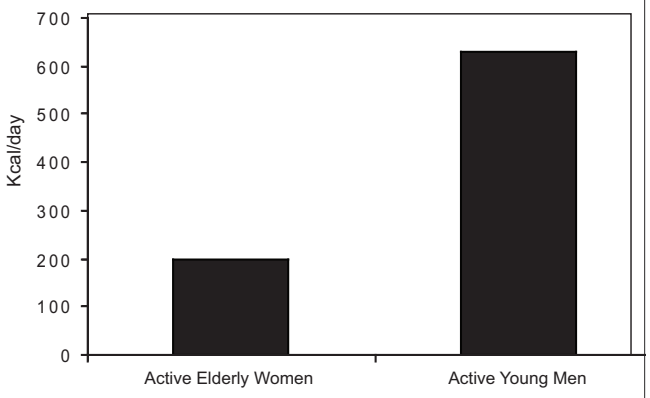
Gains and losses of body mass are responses to imbalances in energy expenditure and energy intake. Weight-loss programs traditionally focus on modifying energy intake, but it is also possible to modulate energy expenditure. The three major components of energy expenditure, with their approximate contribution to overall energy expenditure, are as follows: physical activity (~20%), diet-induced thermogensis (~15%), and resting energy expenditure (REE) (~65%). The physical activity component can be modified with exercise programs, but the practical difficulty in maintaining long-term changes in energy expenditure by changes in voluntary physical activity is well documented. Furthermore, even if a good activity program is followed, energy expenditure is elevated only while the activity is being performed. Small changes in the REE component, on the other hand, can have significant impact because of the fact that changes would last 24 hours per day. In this regard, muscle mass can affect energy balance.

Muscle protein is in a constant state of turnover, meaning that the protein is constantly being synthesized and broken down. There is an energy cost of protein synthesis in terms of the amount of high-energy bonds in the form of adenosine triphosphate (ATP) that is required for the synthesis of new protein. The amount of energy required to maintain muscle protein turnover is a significant component of REE that is directly related to the amount of muscle mass. The energy cost of muscle protein synthesis in average elderly women is approximately 400 kcal per day less than in young men (Figure 1), based entirely on the difference in average muscle mass difference.[2] This reflects the fact that every 10 kg in muscle mass equals approximately 150 kcal per day in REE. Since 1 kg of fat stores approximately 7,000 kcal, then every 10 kg of muscle translates to +/- 7.1 kg fat mass per year, assuming everything else is constant. A difference of 10 kg of muscle mass between young and elderly is reasonable. Consequently, a decrease in muscle mass can explain to some extent the lower resting energy expenditure in elderly as compared to young individuals

Solutions to Muscle Loss

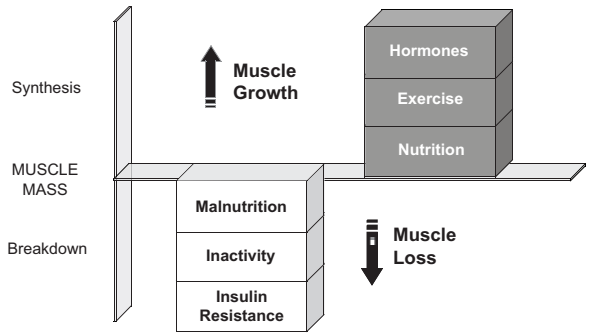
The principal causes of muscle loss with aging are malnutrition, inactivity and insulin resistance. The metabolic basis for reversing these responses is a stimulation of muscle protein synthesis and/or slowing of muscle protein breakdown (Figure 2). While either stimulating synthesis or slowing breakdown can theoretically affect net gain or loss of muscle protein equivalently, it appears that stimulating synthesis is

Figure 1



Calculated energy expenditure due to maintenance of the basal rate of muscle protein synthesis in active elderly women versus young, health men. The difference in energy expenditure is due to the greater muscle mass in young men.[2]

Figure 2



Principal causes of net muscle protein breakdown with aging, and potential categories of solutions.

the most effective therapeutic approach. The reason that stimulating synthesis is more beneficial than slowing breakdown is that stimulation of synthesis increases muscle function in addition to increasing mass. The reason that increased synthesis improves muscle function is that newer muscle protein fibers contract more effectively at the single fiber level.[12] This translates to a significant positive correlation between the *in vivo* rate of muscle protein synthesis and muscle strength in older individuals.[13]

Hormonal Therapy

The most common hormonal approach to maintaining or increasing muscle mass is to administer testosterone and/or growth hormone. Testosterone is a potent stimulator of net muscle protein synthesis, and six months of testosterone therapy in hypogonadal men resulted in significant increases in total and leg lean body mass as well as the maximal amount of weight that can be lifted by the leg.[14] Whereas these results are encouraging, one must always be concerned about potential long-term adverse of hormonal therapy, as exemplified by the reports of problems stemming from long-term estrogen replacement therapy.

Whereas the balance between the beneficial effects of testosterone and potential (as-of-yet unidentified) negative effects is still under review, studies of growth hormone therapy in elderly have failed to show a beneficial effect in terms of stimulating muscle protein synthesis.[15] It seems likely that by the time an individual has reached 65 years of age or older, the normal physiological role of growth hormone has diminished greatly, and therefore responsiveness to exogenous administration is minimal. It is not known if there are adverse effects of chronic growth hormone administration.

The Role of Exercise

A number of studies have documented the role of exercise for improving strength in elderly. Frontera et al. were at the forefront in this area, with a study of the response to weightlifting in men ranging from 60 to 72 years of age.[16] They showed that a 12-week training program could double strength.[16] Numerous other studies have also shown that resistance exercise can reverse the effects of sarcopenia, provided the intensity is sufficient. The challenge with exercise is not determining if it works, but how to get elderly individuals to perform high-intensity resistance exercise on a regular basis for the rest of their lives.

Can Optimal Nutrition Improve Muscle Mass and Function in Elderly?

Approaching the treatment of muscle loss with aging through nutrition has the appeal that physician supervision is not required (unlike hormonal therapy), and in contrast to the performance of exercise, people are driven to eat every day. However, controversy persists on the optimal approach to nutrition in elderly. In this regard, it is not possible to rely on national guidelines such as the National Academy of Sciences Dietary Recommended Intakes (DRIs), USDA dietary guidelines, and derivatives such as the "food pyramid" when determining optimal intake for elderly.

The guidelines for macronutrients are based on data from young, healthy individuals and does not account for possible changes that may occur with advancing age. Further, "requirements" are just that, generally defined based on avoiding deficiencies. In the case of elderly, it is important to identify the optimal intake of macronutrients, rather than an amount necessary to avoid deficiencies. Nutritional studies, as well as national guidelines, have generally not targeted the identification of optimal levels of macronutrient intake.

Within the limitations discussed above, it is possible to identify dietary approaches to minimize muscle loss with

aging. Dietary protein is the most important dietary nutrient with regard to muscle protein. It is clear from a number of studies that increasing the dietary intake of protein above the recommended dietary allowance (RDA) of 0.8 g protein/kg/day increases muscle mass and strength in older individuals.[17] However, protein intake generally drops with advancing age. Over 30% of Americans over the age of 65 years eat less than 0.8 g/kg/day. Reasons for decreased protein intake in the elderly can include decreased appetite, difficulty chewing, altered taste, and the generally higher cost of foods rich in protein. Consequently, it may be most advantageous to specifically target muscle with the key dietary components that stimulate muscle protein synthesis.

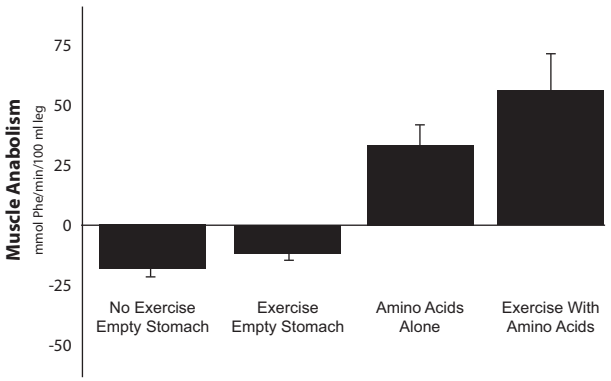
An increase in circulating amino acid levels is a potent stimulator of muscle protein synthesis. The magnitude and duration of stimulation of muscle protein synthesis by amino acids is dependent on the extent of increase in amino acid levels and the profile of the plasma amino acids. In particular, it is the amount and profile of essential amino acids that determine the amount of stimulation of muscle protein synthesis.[18] In the correct formulation and amount, a dietary supplement of essential amino acids can acutely stimulate muscle protein synthesis sufficiently so that repeated doses over a period of time will translate to improved muscle mass and function. For example, elderly individuals were maintained at complete bed rest for 10 days. All food intakes were tightly controlled, and activity was controlled by strict bed rest. Subjects were given capsules containing either a placebo or a mixture of essential amino acids in a double blind, randomized fashion. Supplementation with essential amino acids prevented the drop in the rate of muscle protein synthesis that normally occurred with bed rest. Correspondingly, the reductions in strength and muscular function that normally occurred with bed rest were also prevented by supplementation.[19]

Nutrition and Exercise

In the postabsorptive state, there is a net breakdown of muscle protein that supplies amino acid precursors for proteins to be produced elsewhere in the body. Resistance exercise alone causes an acceleration of protein turnover, meaning that both rates of synthesis and breakdown occur, but the net balance of protein in muscle remains negative. Ingestion of amino acids causes a stimulation of muscle protein synthesis that puts the muscle into an anabolic state, meaning that the rate of synthesis exceeds the rate of breakdown and muscle mass increases.

When amino acids are given in conjunction with exercise, the anabolic action of amino acids and exercise is synergistic, meaning that the response to amino acids plus exercise is greater than the sum of the response to both individual treatments (Figure 3). These results highlight the importance of optimal nutrition in amplifying the beneficial effect of other therapies. In the case of exercise, it appears that exercise "primes" the muscle to respond to the anabolic action of amino acids. Expressed differently, once the muscle is activated to increase the rate of synthesis, there must be adequate precursors available for that activation to be reflected in an actual increase in the amount of protein produced. This same rationale explains the interaction between hormonal stimulation of muscle protein synthesis and the extent of increased protein actually produced.[20]

Figure 3



Principal causes of net muscle protein breakdown with aging, and potential categories of solutions.

Summary and Conclusions

1. Loss of muscle mass and function is inherent to aging.
2. Severe muscle loss is termed sarcopenia.
3. Loss of muscle strength and function not only decreases mobility and quality of life, but also is related to numerous unfavorable health outcomes.
4. Hormonal, exercise and nutritional therapy can all play a role in lessening the rate of muscle loss with exercise.

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Q&A Discussion

Q: Dr. Joe Wakshlag, Cornell University: There's been a lot of debate about timing of the amino acids supplementation, whether it's best with a meal or between meals. Can you comment on this from your experience?

A: Dr. Wolfe: The composition affects the response. There is a threshold of the amount of leucine necessary to stimulate an anabolic response. If there is not enough protein in the meal to achieve a great enough increase in leucine, then one would expect a very modest response of muscle protein synthesis. In fact, traditional meal replacement elicits a minimal response of muscle protein synthesis in the elderly. This explains why traditional supplementation has minimal effect in the elderly. Providing a supplement between meals is theoretically the optimal approach because this should minimize the time muscle is in a catabolic state, which occurs in the post-absorptive state. However, a controlled study done over a prolonged period of time assessing whether a supplement is more effective when provided with meals or between meals has not been done.

Q: Dr. Jane Armstrong, University of Minnesota: I think you can appreciate that measuring muscle strength in dogs and cats is a challenge. I wondered, other than grip strength, if you have any suggestions or experience from perhaps muscle

strength evaluations in individuals who are not necessarily cooperative, such as dementia patients, pediatrics, anything along that line.

A: Dr. Wolfe: There actually is a test in humans where the ulnar nerve is stimulated and the strength of the contraction of the thumb is quantified. This has been used in the intensive care unit for people who are unconscious and has been shown to be a useful predictor of nutritional status. It may be possible to use a similar approach in animals. Gerontologists have developed functional tests in elderly that translate to activities of daily living that are apparently reliable predictors of functional outcomes. Perhaps functional tests, even if just observational, might be developed for animals that would be able to predict outcome.

Q: Dr. Margarethe Hoenig, University of Illinois: You talked about hormonal therapies for men, but you did not present any studies on women. Is there any evidence that hormonal therapy has any influence?

A: Dr. Wolfe: Studies with estrogen therapy have been limited by the NIH. There is a synthetic steroid called oxandrolone that theoretically has none of the androgenizing effects of testosterone, which we found to be quite useful in promoting muscle protein synthesis in both boys and girls, without apparent adverse effects. I am not aware of a comparable hormonal treatment study in older women.

Q: Dr. Joe Millward, University of Surrey: Can I ask you about the relationship between muscle mass and strength? We have information showing that individuals can maintain quite high VO2 maxes, but nevertheless still lose absolute strength because of a loss of absolute muscle mass. So clearly there are very big differences in the quality of muscle in terms of the vascularization, the ability to deliver oxygen to get aerobic work done in muscle. But things like grip strength and all of these are to do with anaerobic force transduction. My question refers to your statement that there is a disconnect between the loss of muscle mass measured with DEXA (dual energy X-ray absorptiometry) and called sarcopenia, and actual functional impairment. How much do we know about that? Are there measurements that you can make with imaging, for example, that inform on the likely muscle strength?

A: Dr. Wolfe: On the one hand we know there is a relation between strength and muscle mass. Certainly in competitive weight lifting, heavyweights lift the most weight. The relationship between muscle mass and strength may not be as great in the elderly. Several studies have shown that strength per muscle mass decreases with advancing age. Further, with nutritional supplementation studies during bed rest, we observed functional changes in the absence of measurable changes in mass. Also, neural innervation of the muscle fibers is decreased with inactivity, which is common in the elderly. This may be a factor in muscle recruitment to perform a task, irrespective of muscle mass. The possibility that the rate of muscle protein turnover itself is a factor is appealing. For example, in our bed rest study, the improved muscle fiber function at the single fiber level with EAA supplementation corresponded with the higher 24-hour protein turnover rate. Of course, we do not know that the accelerated rate of protein turnover was the cause of the improved muscle fiber function. It is possible that accelerated turnover involves selective breakdown of defective fibers, meaning that a high turnover rate would result in better functioning muscle fibers, irrespective of size. In addition, with the elderly population there are further factors affecting function, including balance and joint pain. So, clearly, function and muscle mass have some relation, but they're not necessarily directly related, particularly in the elderly. The point that I wanted to make was that in the elderly you can see changes in functionality that don't correspond to changes in muscle mass.

Dietary Protein Consumption in the Healthy Aging Companion Animal

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Abstract

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

Glossary of Abbreviations

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

Goals of Protein Consumption

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

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

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

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

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

The Biochemistry of Aging Muscle

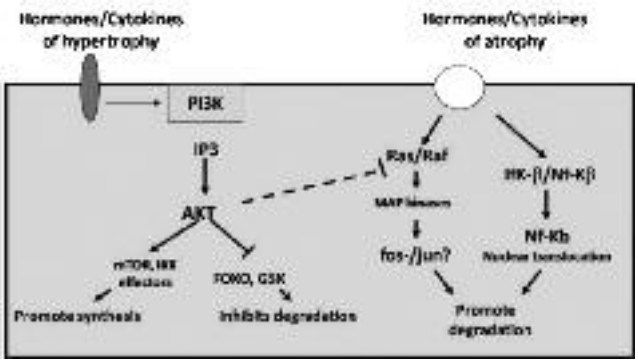
There are many theories related to the mechanism of sarcopenia, and the most attractive hypothesis revolves around the "mitochondrial theory of aging." As mitochondrial DNA mutations occur with age, mitochondrial function is diminished. This results in skeletal muscle myofiber dysfunction leading to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

Hypertrophy and Atrophy

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

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

Figure 1

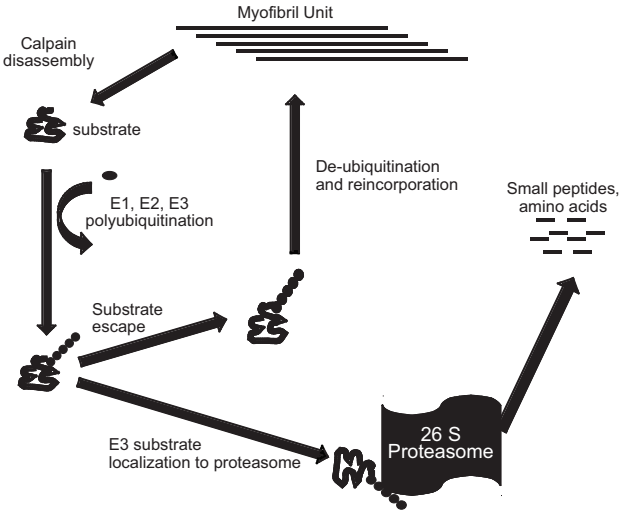


Depiction of the signaling events and signaling proteins of interest in hypertrophy and atrophy in skeletal muscle.

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

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

Figure 2



Depiction of ubiquitin-proteasome dependant proteolysis of myofibril substrates.

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

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

Exercise and Cytokines

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

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

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

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

Dietary Protein and LBM in Dogs and Cats

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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Q&A Discussion

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

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

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

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

Effect of Diet and Body Composition on Life Span in Aging Cats

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Abstract

A longitudinal study found that a diet containing supplemental antioxidants, polyunsaturated fatty acids and a prebiotic increased longevity and improved the health of senior cats. Body weight and body composition data from the study were evaluated to assess possible associations of these parameters with longevity. Body weights along with all body composition parameters measured by DEXA were significantly related to survival, confirming the hypothesis that loss of body mass is a risk factor for mortality in aging cats. Cats eating the supplemented diet better maintained body weight and body composition over time. Nutrition can play a role in delaying age-related changes in body weight and body composition in senior cats.

Glossary of Abbreviations
BCS: Body Condition Score
BMC: Bone Mineral Content
BMD: Bone Mineral Density
BMI: Body Mass Index
BW: Body Weight
DEXA: Dual-Energy X-Ray Absorptiometry
LBM: Lean Body Mass
PUFAs: Polyunsaturated Fatty Acids

Introduction

In all mammals, aging is associated with changes in body composition, declining organ functions and other metabolic changes. Sarcopenia, an age-related loss of muscle and lean body mass, is common in aging humans, cats and other species. Nutrition may play an important role in delaying such changes or preventing their progression.[1,2]

Cross-sectional studies in cats[3] have documented that aging adult cats fall into two categories based on metabolic differences. In middle-aged cat populations (7 to 11 years of age), average energy requirements are reduced while the prevalence of obesity is increased. In geriatric cat populations (ages 12 years and above), the reverse is true in that average energy requirements increase while the prevalence of obesity greatly decreases. A limitation of cross-sectional studies is that they do not follow changes in individual cats over time. Thus, it is not known if the reduced prevalence of obesity is due to loss of weight and body condition in cats with time, or if it is due to attrition reflecting early mortality in obese cats.

Previously, we conducted a study to evaluate the effect of dietary management on life span in aging cats.[4,5] The study tested the hypothesis that supplemental antioxidants alone or combining antioxidants with added polyunsaturated fatty acids (PUFAs) and a prebiotic fiber could measurably benefit the health and longevity of aging cats. Among the three diets tested, the one containing a combination of supplemental antioxidants, a prebiotic and a blend of omega-3 and omega-6 fatty acids increased longevity and improved health in senior cats.

Data from that longitudinal study was evaluated to determine changes within individual cats in body composition over time. The objective was to determine if any of these changes in body composition may be associated with or predict morbidity or mortality in aging cats.

Materials and Methods

The dataset evaluated for this paper was derived from a long-term feeding study initiated with 90 healthy mixed-breed cats, conducted at our Pet Center for nutritional studies. Healthy cats between the ages of 7 and 17 years were selected for the study. Cats were distributed equally among three dietary treatment groups, controlling for age, body condition score (based on 5-point scale), and gender. Groups of cats were assigned to one of three diets: Diet 1 Control (basal diet of nutritionally complete and balanced adult cat food); Diet 2 (basal diet with added antioxidant vitamins E and β-carotene); and Diet 3 (basal diet with added antioxidants, dried whole chicory root as a source of prebiotic, and a blend of supplemental n-6 and n-3 fatty acids). The diets were fed *ad libitum* as the exclusive source of nutrition for the remaining natural lifetime of each cat. Typical nutrient comparisons were previously reported.[4]

Clinical Observations

Health monitoring and medical treatments of all cats were carried out according to established colony veterinary standards throughout the trial, and veterinary personnel were blinded to dietary treatment groups. In addition to physical examinations and routine blood sampling (serum biochemistry and hematology), measures of body weight and body composition by dual-energy X-ray absorptiometry (DEXA) were taken for all

cats at study initiation and regular intervals throughout the study. Food consumption was measured daily during the study, and body weights were assessed weekly.

Statistical Analysis

Analysis of variance was used to compare initial parameters across groups to confirm that randomization was effective in producing balance at baseline (t_0) in the three study groups.[5]

Survival analyses were performed to compare the three diets for the age at which the cats died of natural causes (Age at Death) and the number of days the cats were alive (Days on Trial). For Age at Death, a Kaplan-Meier nonparametric analysis was performed.[6] For Days on Trial, a Cox's proportional hazard model was used to compare the survival rates of the three diets (pair-wise comparison).[6] Hazard ratios along with their 95% confidence intervals were estimated. Because there was a wide range of ages of the cats at trial initiation, the initial age of the cat was used as a covariate in the model.

Analysis of measured health parameters was performed by a longitudinal analysis.[7] The longitudinal model allowed for each animal's trend to be considered over time and an average trend or slope predicted for each group. Where appropriate, a quadratic effect was included in the longitudinal model.

To determine if any of the measured parameters were related to survival, a Cox's proportional hazard model was performed using the measured parameter as a time-varying covariate. The Cox's model evaluates whether an increase or decrease of the parameter is associated with an increased or decreased rate of survival. The hazard ratio indicates what percent increase or decrease in "hazard of dying" is associated with each unit of measure for that parameter. Using the formula, (1.00 – Hazard Ratio) x 100, gives the correlation with survival.

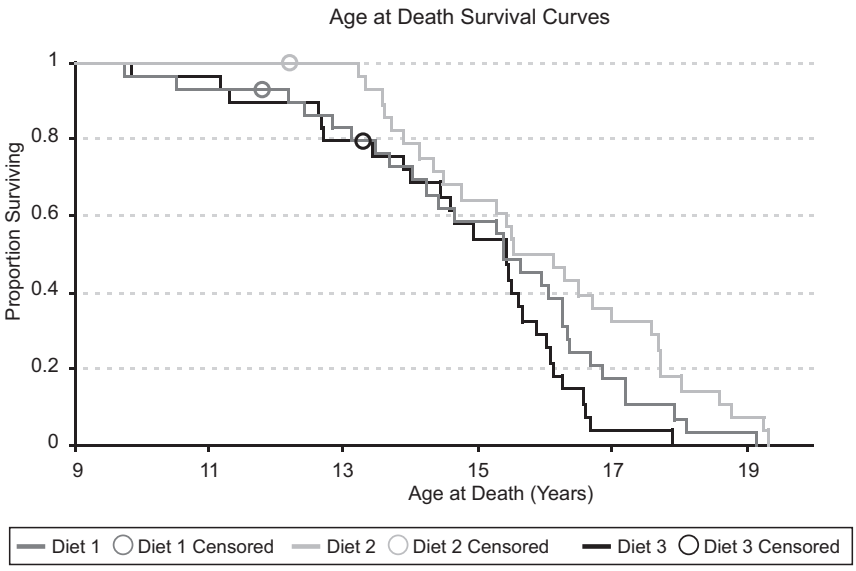
Longitudinal DEXA data for lean body mass (LBM) and fat mass in grams also were evaluated retrospectively from the time of death for the entire population of cats to determine how changes in these measures might be predictive of death. For fat mass, a segmented regression model was used, where the best fit for the period closest to death was a quadratic model. For LBM, the best fit for the data was found to be a longitudinal linear regression model.

All statistical calculations were performed using SAS.[8] Statistical significance is at the $P < 0.05$ level unless otherwise stated.

Table 1. Age at Death (Nonparametric)

Diet	Mean Age at Death (Years)	P Value	
1	14.72	Diet 1 vs Diet 2	0.1429
2	15.09	Diet 1 vs Diet 3	0.0074
3	16.00	Diet 2 vs Diet 3	0.1121

Figure 1: Age at Death



Results

Longevity

For the survival analysis, two cats were removed from trial within the first six months for poor food consumption and were not included. There were 88 total cats in the final data set — 85 that died, and 3 that were removed for poor food consumption before they died (but after they had been on trial for over six months). These last three cats that did not complete the trial were considered censored in the statistical analysis.

As previously reported, cats fed Diet 3 lived significantly longer than cats fed Diet 1.[4,5] Using nonparametric analysis for Age at Death, cats fed Diet 3 lived approximately 1.3 years longer, on average, than cats on Diet 1 (Table 1). Figure 1 shows the Kaplan-Meier curves for Age at Death.

The Cox's proportional hazard regression analysis using initial age as a covariate also showed a significant difference between Diets 1 and 3 for Days on Trial (Table 2). The hazard ratio of Diet 1 versus Diet 3 was 0.418, meaning that the hazard of dying for the cats on Diet 3 was only 42% of the hazard of dying for the cats on Diet 1. There were no significant differences between Diets 1 and 2 or between Diets 2 and 3.

Body Weight

All three diet groups lost weight over time, but cats fed

Table 2. Days on Trial, Cox Regression (Initial Age as Covariate)

Variabe	df	P Value	Hazard Ratio	95% CI for Hazard Ratio
Diet 1 vs Diet 2	1	0.3487	0.763	0.433-1.344
Diet 1 vs Diet 3	1	0.0038	0.418	0.231-0.754
Diet 2 vs Diet 3	1	0.2722	0.741	0.433-1.266

Diet 3 lost less than cats on Diets 1 and 2 (Figure 2). The Cox's proportional hazard model for Diets 1 and 3, using body weight (BW) as a time-varying covariate, showed a significant relationship between BW and survival (Table 3). Using the hazard ratios in Table 3, for every 1 kg increase in BW, there was a 64% increased chance of survival, or decreased "hazard of dying."

Table 3. Survival Analysis with Time-Varying Covariate, Diet 1 Versus Diet 3

Parameter	Hazard Ratio	P Value	Survival Correlation
Body Weight	0.364	<0.0001	64% increase/kg
Body Condition Score	0.117	<0.0001	88% increase/BCS unit
DEXA Lean g	0.998	<0.0001	0.2% increase/gram
DEXA Fat g	0.996	<0.0001	0.4% increase/gram
DEXA BMD	0.00002	0.0045	100% increase/g/cm2
DEXA BMC	0.985	0.0161	1% increase/gram
DEXA % Lean	1.118	0.0002	12% decrease/%
DEXA % Fat	0.876	<0.0001	12% increase/%
DEXA % Bone	2.35	<0.0001	135% decrease/%
Vitamin E	0.946	0.0357	5% increase/mg/L

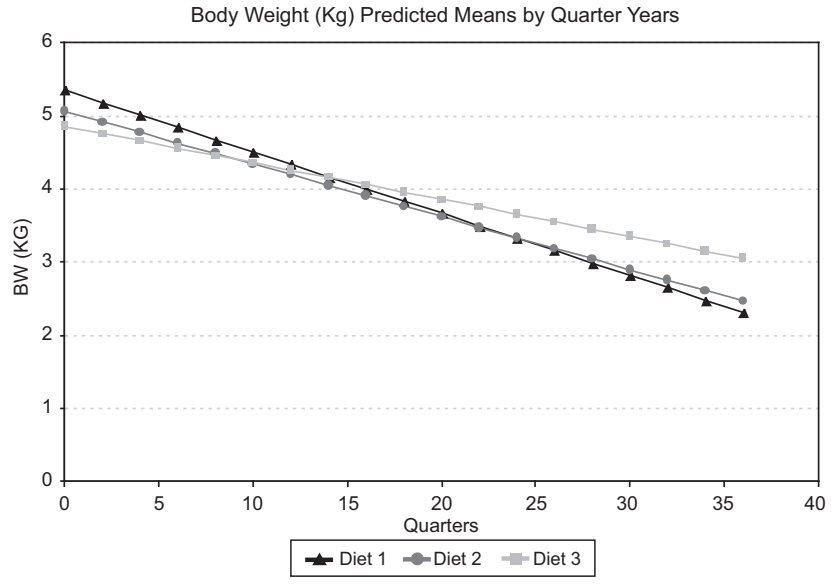
Body Composition

Consistent with body weight, body condition score (BCS) decreased significantly over time for all dietary groups. Although there were no statistical differences among diets, there was a significant relationship between BCS and survival (Table 3). For every 1 point increase in BCS, there was an 88% increased chance of survival.

Both grams and percentage of body fat, as well as grams of LBM, bone mineral density (BMD) and bone mineral content (BMC), showed significant decreases over time for all dietary treatment groups, while percent of LBM and percent of bone increased over time. In general, cats fed Diet 3 showed less change over time than the other diets in the longitudinal analysis of these parameters. There was a trend for differences between Diets 1 and 3 over time for percent of LBM ($P < 0.10$; Figure 3), percent of fat ($P < 0.10$; Figure 4), BMD, BMC, and percent of bone ($P < 0.10$).

The Cox's proportional hazard model for Diets 1 and 3, using LBM (in grams and percent), fat (in grams and percent), BMD, BMC, and BCS as time varying covariates, showed significant relationships between these parameters and survival (Table 3). LBM in grams, fat in grams and percent, BMD, BMC, and BCS were all positively correlated with survival with hazard ratios less than 1, indicating that higher levels of these parameters are associated with a decreased hazard of dying. For example, for every 1 gram increase in LBM, there was a 0.2% increased chance of survival, and for every 1% increase in percent of body fat, there was a 12% increased chance of survival. Percent of LBM and percent of bone were negatively correlated with survival with hazard ratios greater than 1, indicating that higher levels of these parameters are associated with an increased hazard of dying. For example, for every 1% increase in percent of lean, there was a 12% decreased chance of survival.

Figure 2: Body Weight Predicted Means



Mean predicted LBM (gm) and fat mass are shown for all cats by months prior to death in Figures 5 and 6. A longitudinal linear regression model best fit the LBM data, and the prediction line shows a general progressive decrease in average LBM as the cat nears the end of its life span (Figure 5).

The fat mass in grams was transformed into natural logarithms to account for extreme observations. A segmented regression model was used, with a quadratic model best fitting the period closest to death, as shown by the predicted average line (Figure 6). This analysis estimated that cats start losing fat around 37 months prior to death, and that the decline showed an increasing amount of fat loss toward the end of life. The overall longi-

Figure 3: Percent of LBM Predicted Means

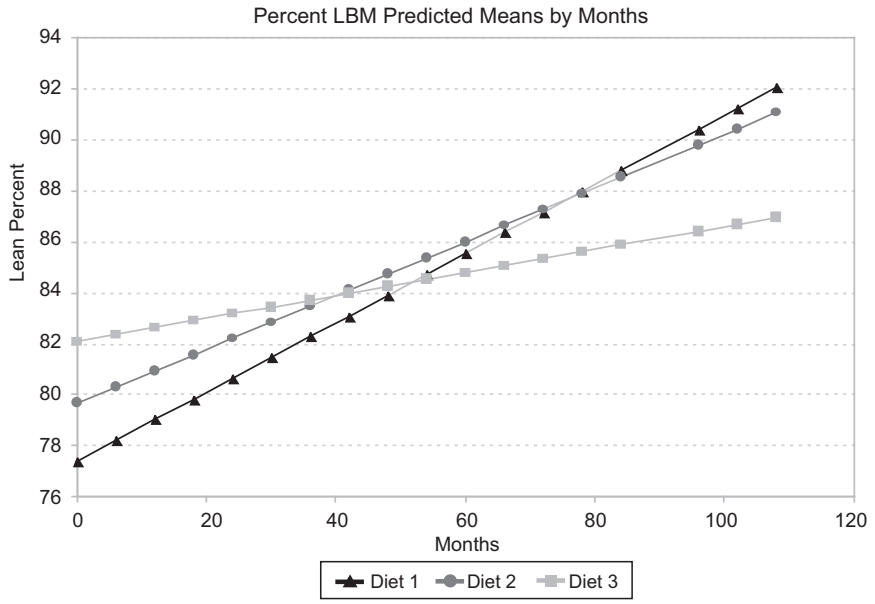
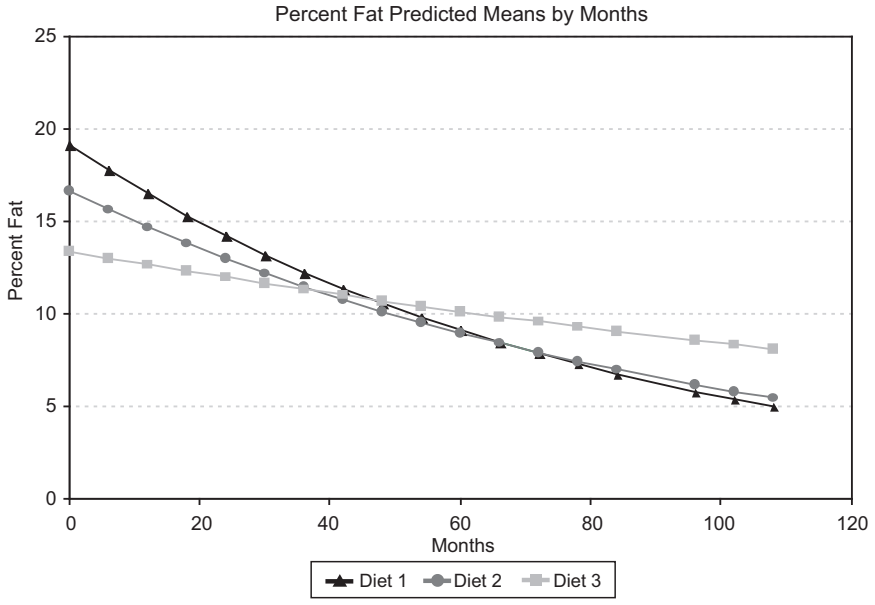


Figure 4: Percent Fat Predicted Means



tudinal patterns of mean predicted LBM (gm) and fat mass are shown together in Figure 7.

Discussion

Prior cross-sectional studies evaluating populations of cats suggested that loss of body weight in aging cats was a risk factor for mortality,[3] and it was reported that extremely lean old cats had a significantly higher risk of death compared with cats in optimal body condition.[9] In the current study evaluating aging cats longitudinally, this observation was not only confirmed, but also the conclusions can now be expanded. According to this data, aging cats that lose excess body weight and body condition (fat or lean) have a significantly greater risk for earlier mortality.

Across most human age groups, a higher body mass index (BMI) is associated with increased morbidity and mortality.[10-12] Staying lean is widely recommended to decrease and delay disease incidence and extend longevity. In many species, including dogs,[13] maintaining a lean body condition through lifelong caloric restriction has been shown to increase longevity.

However, this relationship between leanness and longevity is not evident in older adults or those with chronic disease.[14] Instead, a low body mass index in very old humans is associated with an increased risk of morbidity and mortality.[10,15,16] With the highest mortality observed for the very obese and very thin, the result is a U-shaped curve for the relationship between BMI and risk of death or disease by age group.[15,16] Higher BMI and lean mass have been associated with increased longevity in these elderly age groups.[14,17,18] Higher fat mass was also associated with significantly lower mortality rates in human hemodialysis patients.[19] Based on the results presented here, an increase in longevity from higher levels of lean and fat tissue may be true for aging cats as well.

In this study, all parameters measured by DEXA, as well as body weights and BCS, showed a significant relationship with survival. LBM (in grams), fat mass (in grams and percent), bone mineral density and bone mineral content, as well as BCS, were all positively correlated with survival, indicating that higher levels of these parameters were associated with a decreased risk of dying.

The negative correlation between percent of LBM and survival and the positive correlation between body fat and survival observed in this study might be misleading if applied to the wrong population. These cats were in normal to lean body condition. The cats with the highest percent of LBM in this study had very low levels of body fat, and due to overall body weight loss, their percent of LBM was increased despite

a significant reduction in total grams of lean. Substantially decreased absolute values of lean and fat tissue more clearly reflect the actual changes that are occurring in these elderly cats, with as much as one-third less lean tissue compared to what is measured during young adulthood.[25] A higher percent of LBM and bone relative to lower percent of fat mass previously has been described in our historical research colony data in cats toward the end of their life spans.[3] Slowing down this loss or change in relative amounts of these body tissues could theoretically help extend life expectancy.

It is interesting that the overall longitudinal data on LBM (in grams) showed a steady linear decline prior to death while fat mass (in grams) remained constant until approximately three years prior to death, then increasingly declined toward the end of life. This pattern closely follows that seen in body weights of our colony cats that were tracked in the four years preceding their death[20] and likely reflects the onset of chronic disease and more rapid utilization of body tissue reserves during disease states.

Sarcopenia is common in aging humans and is recognized as a significant contributor to disability and mortality in geriatric people.[2,21] There are many causes of sarcopenia, including age-related decreases in hormones, inefficiencies in protein synthesis, increased pro-inflammatory cytokines and oxidative stress.[2,21] In addition, dietary factors can contribute to sarcopenia. Inadequate intake of energy or protein can contribute to gradual loss of lean body mass.[2]

Figure 5: Mean Predicted LBM by Months Prior to Death

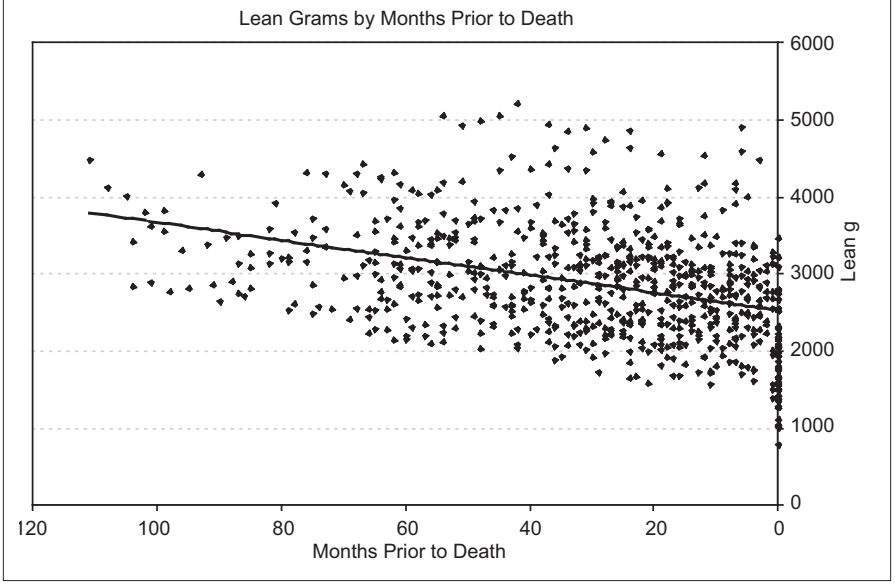
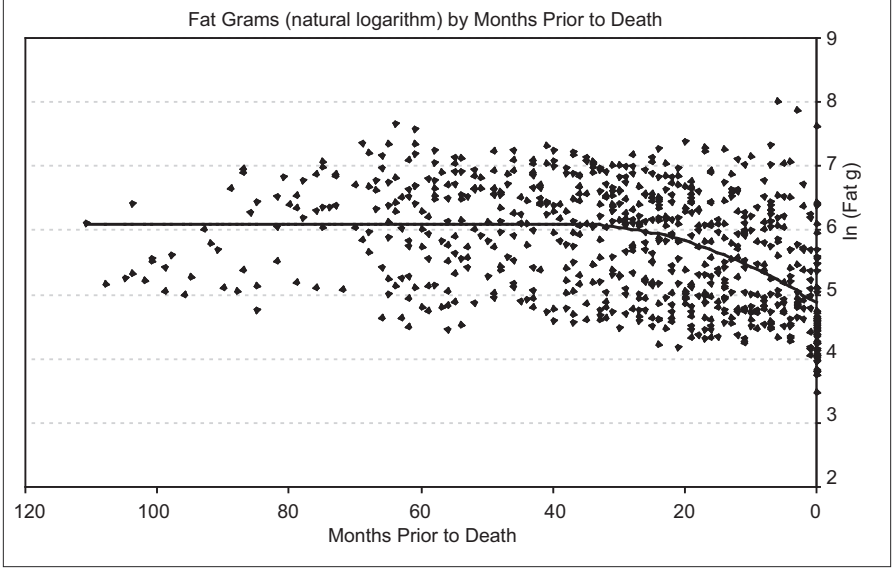


Figure 6: Mean Predicted Fat Mass by Months Prior to Death



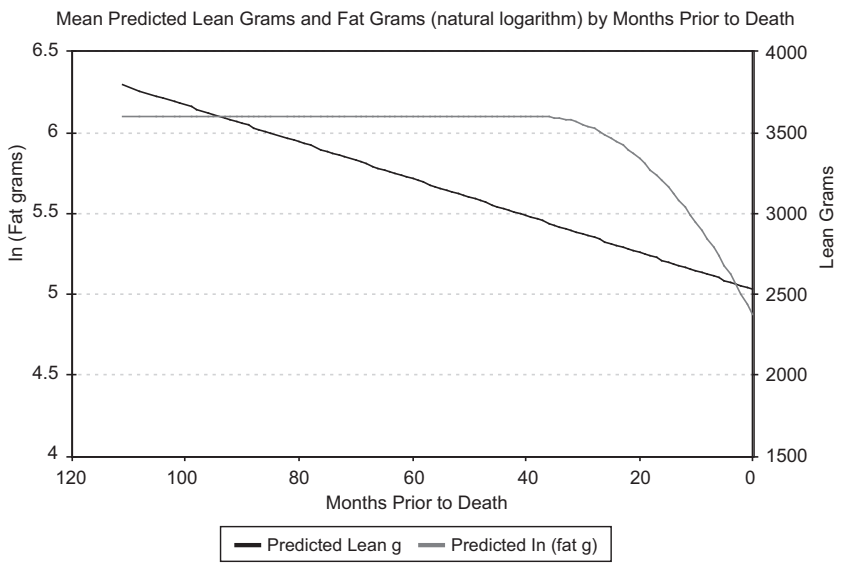
Increased dietary protein has been proposed as a means of preserving LBM in older dogs.[22] Old dogs require more protein to achieve better protein turnover, a process that decreases with increasing age.[23,24] Sufficient protein turnover is important for preserving immune competence in the older dog and will certainly have an effect on morbidity and mortality.[22,25]

Cats have high protein requirements to maintain their LBM, and we previously reported cross-sectional population data showing that body weight, LBM and fat mass all decline in cats over the age of 12 years, particularly in the last one to two years of life.[3,20] In this study, where differences in body composition and life span developed longitudinally among different dietary treatments, protein and caloric density of the diets were similar across all three diets. Food consumption, while initially similar, increased in those diet groups that lost the most body weight.[4] Hence, inadequate protein or calories does not explain the loss of lean and fat mass.

Neither inflammatory cytokines nor markers of oxidative stress were measured in this study. However, serum vitamin E levels were increased to a significantly greater extent in the Diet 3 cats,[5] and this is the group that maintained body weight and body composition to a greater extent.

There is a considerable body of research on the role vitamin E plays in protecting against oxidative stress in aging.[26,27] It has been hypothesized that vitamin E status may alter health and quality of life during aging through effects on immune function

Figure 7: Mean Predicted LBM and Fat Mass Prior to Death



and inflammatory pathways.[28] These authors reported that elderly humans with poorer physical and mental health status had lower circulating concentrations of alpha-tocopherol and increased concentrations of inflammatory markers. Inflammation does appear to be a fundamental characteristic of aging, and vitamin E has been shown to exert anti-inflammatory actions.[29,30] Low concentration of vitamin E also was found to be significantly associated with decline in physical function in elderly adults.[31]

It is possible that the sarcopenia and fat loss in the cats in this study were related to oxidative stress, and that the diet that maintained higher serum vitamin E levels helped maintain body tissue reserves and contributed to an overall increase in longevity.

Conclusions

Data from this longitudinal study confirm observations from previous cross-sectional studies that weight loss is a risk factor for mortality in aging cats. Additionally, cats that lose body fat, lean and bone mass are at greater risk for earlier mortality. In this study, all body composition parameters measured as well as body weight were significantly related to survival. A retrospective longitudinal analysis of lean and fat mass indicated that, on average, LBM (in grams) declines at a steady rate for many years prior to death, while fat mass (in grams) is maintained until around three years before death, then increasingly declines.

The data also suggests that nutrition can play a role in delaying these age-related changes in body weight and body composition. Higher body weights and better maintenance of lean and fat mass may extend life by providing increased nutritional reserves in elderly cats. The diet containing supplemental antioxidants, a prebiotic and polyunsaturated fatty acids better maintained body weight and body composition in the senior cats in this long-term study, and this likely contributed significantly to their increased longevity.

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Q&A Discussion

Q: Dr. Joe Millward, University of Surrey: One of my own pet hypotheses is that sarcopenia is a consequence of the shortening of the bones that accompanies demineralization. So you get some osteoporosis and the bones shorten a little bit and that takes tension off muscles, and muscles atrophy as a result of that. How good is your data on the interaction between loss of bone and loss of muscle, and have you looked at that?

A: Dr. Cupp: We haven't looked a lot at the bone data. Osteo-

porosis hasn't been as much of an issue in pets as it has in humans. And it's one of the things that DEXA obviously measures. We did see the correlation with survival, but we haven't looked at any great length at that data at this point.

Q: Dr. Bill Milgram, CanCog: I'd like some clarification on your cat study and the impact of the antioxidants. Since the cats were at different ages at the start of the study, was the effect different for cats that were on the study longer?

A: Dr. Cupp: The study used 90 cats ages 7 to 17 at the start. The cats were blocked by age into three groups aged 7 to 9, 10 to 12, and 13 and above, with 30 cats per group. These groups were then blocked by age, sex, body condition and hematology into groups of 10. So, each age group was equally represented among the three diets. Unfortunately, the groups were not large enough to provide statistical power to determine if the dietary effect differed among the different age groups within a dietary treatment.

Q: Dr. Bill Milgram, CanCog: My second question is if you followed individual animals with respect to weight loss as opposed to lumping the data together of all the animals at a given point in time. And I'll tell you what I'm thinking of. The population data suggests a nice linear relationship, but if you look at an individual cat, I wouldn't be surprised to see the weight relatively stable rather than a sharp drop.

A: Dr. Cupp: Yes, that's correct. The data showed that lean mass tended to decrease slowly over time, while body weight was fairly stable until near the end of life, in the last couple years of life.

Protein at the Speed of Life

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Abstract

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

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

Introduction

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

Protein Requirements with Age and Illness

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

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

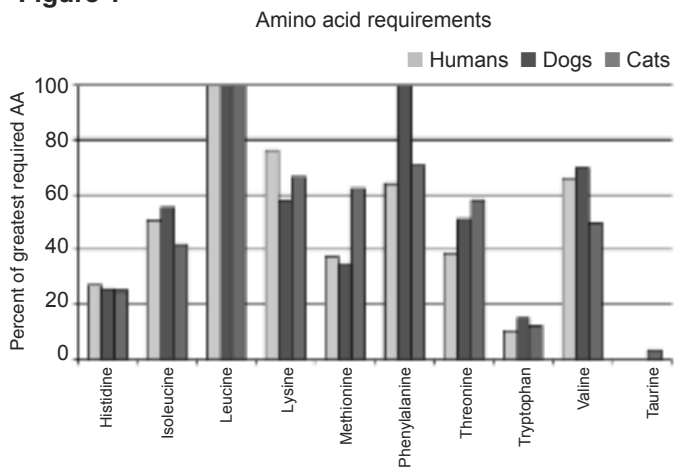
Despite a general agreement that some benefits can be derived from increased protein intake, the general statement that protein requirements increase with age does not necessarily reflect the fact that one protein source may offer different benefits than another. For example, Williams et al.[6] evaluated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

Amino Acid Profiles of Dietary Protein

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

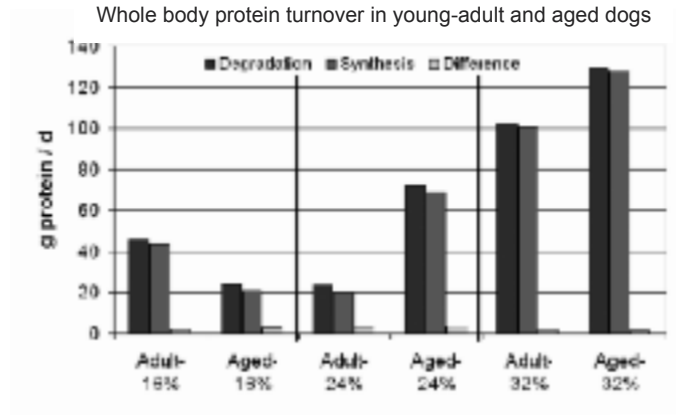
Physiological stresses may alter the needs for specific amino acids. It has been demonstrated that health status is reflected in the circulating amino acid profile. Chan et al.[7] recently compared amino acid concentrations in critically ill dogs to

Figure 1



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

Figure 2



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

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

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

Protein Digestibility

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

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

Proteins and Amino Acids in Musculoskeletal Health

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

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

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

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

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

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

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

The Rise and Fall of Amino Acids

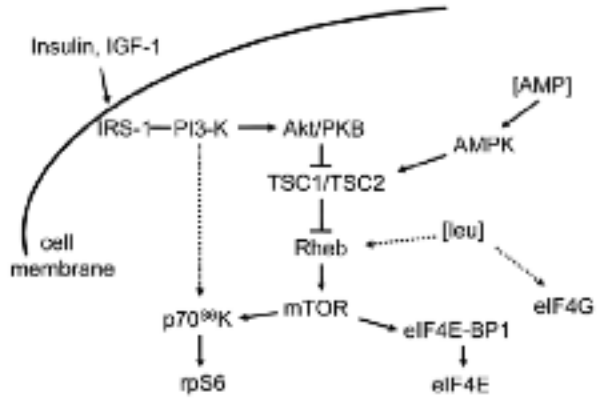
The concept of "protein speed" has been predominantly driven by performance nutrition. In the quest for optimal muscle mass and function, the dietary proteins are being researched to determine whether we can unlock more of their potential. The published literature focuses on the comparison between the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

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

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

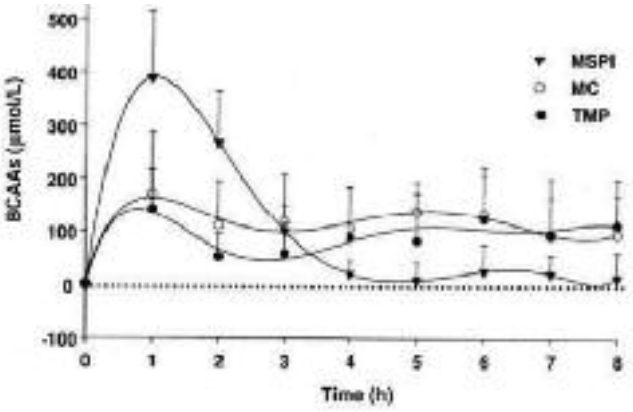
The likely reason the blend did not better replicate an average profile between the soluble milk protein isolate and casein is related to the relative proportion of each fraction in the total protein. The ratio of casein to whey protein in cow milk is approximately 80:20. As a result of this study, it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

Figure 3



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

Figure 4



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

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

Protein Hydrolysates: Built for Speed

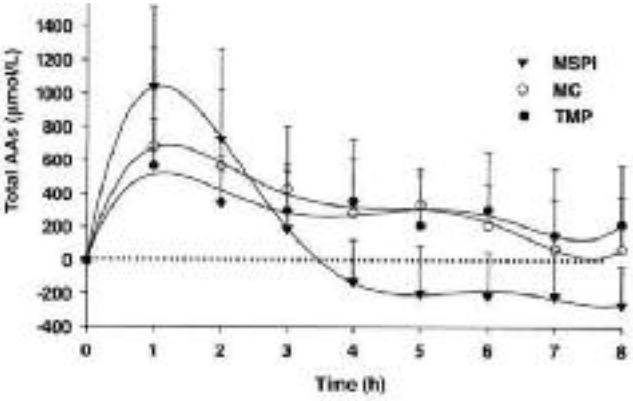
Protein type directly influences both the rate of absorption and rate of incorporation into tissue. The nutritional value of some protein types is reduced because of limitations caused by low bioavailability. Methods to overcome these limitations often include cooking and hydrolysis. Hydrolysis of proteins,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

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

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

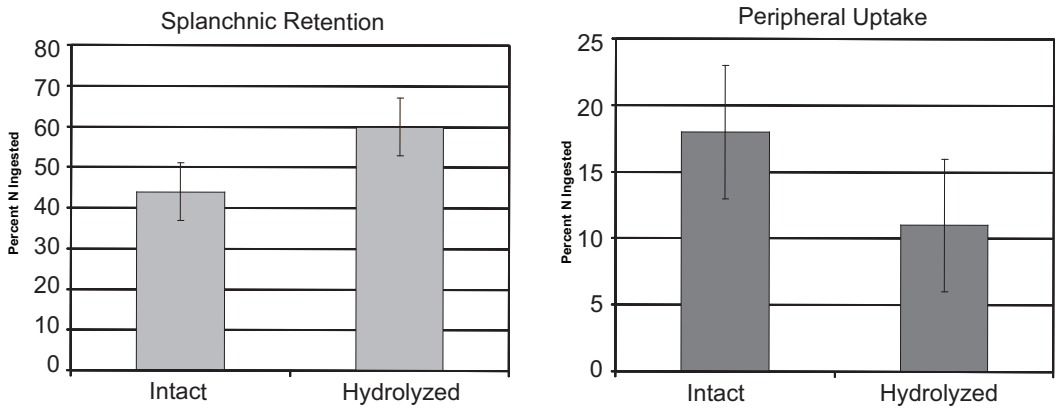
Deglaire et al.[15] recently reported that fast proteins may contribute less to peripheral protein anabolism as a result of greater splanchnic extraction than slow proteins. In healthy men consuming isotope-labeled intact or hydrolyzed casein protein, it was shown that hydrolyzed casein was absorbed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

Figure 5



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

Figure 6



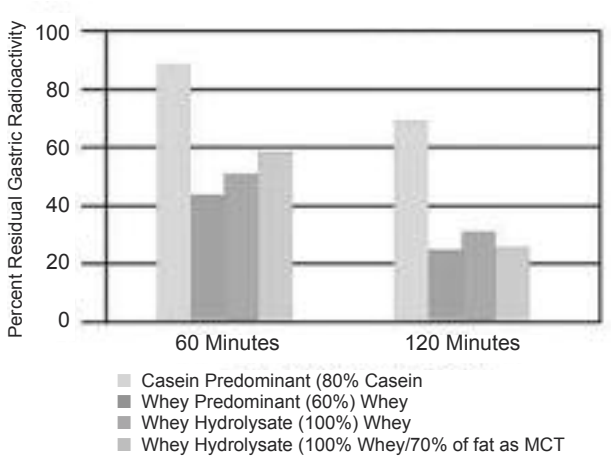
Nitrogen retention in the splanchnic tissues and peripheral tissues following ingestion of intact or hydrolyzed casein protein by healthy adults.

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

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

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

Figure 7



The effect of hydrolysis of whey protein on gastric emptying.

Conclusion

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

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Q&A Discussion

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

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

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

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

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

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

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

Feeding the Aging Heart

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Abstract

More than 10% of all dogs and cats have cardiac disease that, especially in dogs, becomes increasingly common with age. However, even in the absence of obvious disease, changes in cardiac function occur with aging. Nutrition plays an important role in the management of cardiac disease and also may help to optimize cardiac function.

Glossary of Abbreviations

ACE: Angiotensin-Converting Enzyme
AAFCO: Association of American Feed Control Officials
ATE: Arterial Thromboembolism
CHF: Congestive Heart Failure
CVD: Chronic Valvular Disease
DCM: Dilated Cardiomyopathy
DHA: Docosahexaenoic Acid
EPA: Eicosapentaenoic Acid
HCM: Hypertrophic Cardiomyopathy
IL-1: Interleukin-1β
ISACHC: International Small Animal Cardiac Health Council
TNF: Tumor Necrosis Factor-α

Cardiac Disease in Dogs and Cats

Cardiac disease is one of the most common disorders in both dogs and cats. Approximately 11% of dogs have cardiac disease;[1] 95% of these dogs have adult-onset (acquired) cardiac disease. For dogs with acquired disease, the majority have endocardiosis (commonly referred to as mitral regurgitation due to chronic valvular disease or [CVD]), with smaller numbers having dilated cardiomyopathy (DCM) or the less common pericardial disease, endocarditis, primary arrhythmias, or heartworm disease. Small- to medium-sized dog breeds are predisposed to CVD, while DCM is the most common cause of congestive heart failure (CHF) in large-breed dogs.

Hypertrophic cardiomyopathy (HCM) currently is the most common form of cardiac disease in cats, but other forms of cardiomyopathy (i.e., dilated, restrictive or unclassified) and other diseases (e.g., heartworm disease, pericardial disease) also can occur. Cardiac disease often is perceived as a relatively uncommon disease in cats, but results of recent studies suggest that up to 16% of apparently healthy cats may have cardiomyopathy.[2,3] Thus, cardiac disease appears to be a very common disease in the feline population and, regardless of the cause, often leads to CHF, arterial thromboembolism (ATE), syncope or sudden death.

Age-Associated Cardiac Changes

In both dogs and cats, most cardiac disease occurs more commonly in older animals. However, even in the absence of disease, the aging heart undergoes a variety of changes that affect the susceptibility to disease and response to stress, as well as contributing to functional deficits. Changes that have been identified in aging are listed in Table 1 and include cardiac hypertrophy, altered vascular resistance and abnormal endothelial function.[4] Note that these changes have been most often studied in rodents or humans; much research needs to be done in the aging canine and feline heart.

Treatment of Cardiac Disease

Few of the common cardiac diseases in dogs and cats currently are easily corrected. Pacemaker implantation can be successfully accomplished in animals with severe bradycardia, certain pericardial diseases are much improved following surgery, and surgical replacement or repair of the mitral valve is possible where cardiopulmonary bypass is available. However, the vast majority of the common cardiac diseases in dogs and cats do not have a surgical solution, yet these diseases can often be successfully managed medically. Medical therapy of cardiac disease has improved in recent years, with newer and more effective drugs, but medical therapy still is only palliative. Goals of medical care include controlling clinical signs, slowing the progression of disease, and improving quality of life. Maintaining good quality of life is particularly important in dogs and cats, as owners often prefer quality of life to "quantity" of life.

Careful attention to the diet of animals with cardiac disease is a key component of optimal medical treatment of these patients. In the past, the goal of nutritional management for animals with cardiac disease was purely symptomatic and focused mostly on sodium restriction. This was primarily due to the limited number of medications available for treatment, and in that situation, sodium restriction was beneficial for

Table 1

Cardiac Changes	Vascular Changes
Structural • Increased o Heart weight o Cardiomyocyte size o Collagen o Inflammatory mediators (e.g., cytokines, reactive oxygen species) • Decreased o Cardiomyocyte number o Functional atrial pacemaker cells o Mitochondrial function	*Structural* • Increased o Vascular stiffness o Arterial wall thickness o Total peripheral resistance o Endothelial permeability • Decreased o Endothelial nitric oxide o Elasticity and distensibility of the vasculature
Functional • Increased o End-diastolic filling o Contraction duration • Decreased o Early diastolic filling o Responsiveness to β-adrenergic stimulation (chronotropic and inotropic) o Peak cardiac output with exercise o Lusitropic function o ATP production o Energy reserve	*Functional* • Increased o Blood pressure • Decreased o Endothelial function o β-adrenergic-mediated vasodilation

Adapted from: Ferrari, et al. *J Appl Physiol*. 2003;95:2591-2597.[4]

reducing fluid accumulation in animals with CHF. The development of more effective medications has made severe sodium restriction less important in most animals with cardiac disease. Current goals for the nutritional management of animals with cardiac disease are to maintain optimal body condition, avoid nutritional deficiencies and excesses, and to gain potential benefits from pharmacologic doses of certain nutrients. Integrating nutrition into the care of animals with cardiac disease may reduce the number or doses of medications an animal requires, reduce complications, improve quality of life, and may slow the progression of the disease.

Optimal Body Condition

Cardiac cachexia is the loss of lean body mass that occurs in CHF. In healthy animals, weight loss is associated primarily with reductions in fat, and lean tissue is relatively spared. However, weight loss in CHF comes from the metabolically active lean body mass. Cachexia has important detrimental effects in the cardiac patient and is an independent risk factor for mortality. One study found that 50% of dogs with CHF had some degree of cachexia.[5]

The deleterious effects of cachexia and the role of body weight and body composition in heart failure have been emerging. While obesity is a risk factor for development of heart disease in people, obesity may actually be associated with a protective effect once heart failure is present. This is known as the obesity paradox. A recent large meta-analysis on body condition in people with heart failure concluded that obesity and being overweight were associated with lower all-cause and cardiovascular mortality and that underweight patients consistently had a higher risk[6] of death. Given the adverse effects associated with cachexia, the association between obesity and improved survival in heart failure appears to be due to a lack of cachexia, rather than obesity per se. This is likely due to the increased reserve of lean body mass in overweight and obese people. The obesity paradox also has been demonstrated in dogs and cats with heart failure, and recent studies suggest a "U-shaped" curve with the worst survival for those with the lowest and highest weights.[7] These data emphasize the importance of avoiding weight (and muscle) loss, as well as severe obesity, in the patient with heart failure.

Cardiac cachexia is a multifactorial syndrome with contributing factors including reduced or altered appetite, increased energy requirements, and increased production of inflammatory

cytokines (e.g., tumor necrosis factor-α [TNF], interleukin-1β [IL-1]). These cytokines cause anorexia, increased energy requirements and loss of lean body mass. Omega-3 fatty acids reduce inflammatory mediators, including cytokines. Fish oil, which is high in omega-3 fatty acids, decreases cachexia, and, in some dogs with CHF-induced anorexia, improves food intake.[5]

Nutritional Excesses

Sodium

Sodium excretion is reduced in cardiac disease, so sodium restriction is recommended for dogs and cats with heart disease. However, dietary sodium restriction can further activate the renin-angiotensin-aldosterone system so the authors do not recommend severe sodium restriction in early heart disease. For animals with asymptomatic cardiac disease ISACHC (International Small Animal Cardiac Health Council) Stage 1a and 1b,<100 mg sodium/100 kcals in the diet is recommended. For animals in ISACHC Stage 2, <80 mg sodium/100 kcal is recommended, and for those in ISACHC Stages 3a and 3b, <50 mg sodium/100 kcals is recommended. However, further research is needed to develop optimal recommendations for dose and timing of sodium restriction in animals with cardiac disease.

Potassium

Angiotensin-converting enzyme (ACE) inhibitors are commonly used in animals with cardiac disease. These drugs cause increased serum potassium and a small proportion of animals develop hyperkalemia. Spironolactone, now used in some dogs and cats with CHF, is an aldosterone antagonist and a potassium-sparing diuretic. Some animals receiving ACE inhibitors or spironolactone can develop hyperkalemia. As some commercial cardiac diets contain increased potassium concentrations to counteract the theoretical potassium loss due to diuretics, these diets can contribute to hyperkalemia. Therefore, monitoring serum potassium and consideration of the dietary potassium content is recommended.

Nutritional Deficiencies/Nutritional Pharmacology

Protein and Amino Acids

Protein

The authors strongly recommend avoiding restriction of dietary protein intake in dogs and cats with cardiac disease, unless warranted by concurrent disease, as dietary protein restriction can contribute to loss of lean body mass. Dietary protein restriction could occur unintentionally in an animal with cardiac disease by recommending a renal diet. In addition, some of the commercial cardiac diets are very low in protein. Reduced protein diets should be avoided in all animals with cardiac disease unless severe renal dysfunction is present, and dietary protein should be fed to at least meet Association of American Feed Control Officials (AAFCO) minimums for adult dogs (5.1 gm/100 kcal) or cats (6.5 gm/100 kcals).

Taurine

There has been a dramatic reduction in the incidence of feline dilated cardiomyopathy (DCM) since the late 1980s when increased dietary supplementation of taurine was instituted in commercial pet foods. Most current cases of feline DCM are unrelated to taurine deficiency, but taurine deficiency should be suspected in all cases of feline DCM, especially in cats fed a poor quality, homemade, vegetarian, or otherwise unbalanced diet.

Taurine deficiency is now suspected in some cases of canine DCM. Unlike cats, most dogs should be able to synthesize adequate amounts of taurine and so are not thought to require dietary taurine. Most dogs with DCM do not have taurine deficiency, but low taurine concentrations have been found in some dogs with DCM. Low blood taurine concentrations are most commonly reported for American Cocker Spaniels, and large-breed dogs such as Golden Retrievers, Labrador Retrievers, Newfoundlands, Portuguese Water Dogs, and Irish Wolfhounds.

Taurine deficiency in dogs may be related to dietary factors as it appears to be associated more commonly with certain lamb meal and rice-based diets or high-fiber diets, and has been induced by feeding a low-protein, low-taurine diet long term to dogs. Taurine deficiency also may be the result of increased renal or fecal loss of taurine, or other metabolic defects present in certain breeds. Taurine supplementation may be beneficial in some dogs with taurine deficiency, but even in dogs that respond, the response is typically not as dramatic as in taurine-deficient cats with DCM. Further research is required to determine the role of taurine in canine DCM. In addition to the potential for correcting a deficiency, some of the potential benefits of taurine may be due to its positive inotropic effects or role in calcium regulation in the myocardium.

Fat

Omega-3 Fatty Acids

The omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are normally present in low concentrations in the diet and subsequently low levels are in cell membranes. However, dietary and membrane concentrations can be increased by a food or supplement enriched in omega-3 fatty acids. Dogs with CHF have a relative deficiency of EPA and DHA compared to unaffected dogs, and fish oil supplementation normalizes these abnormalities.

Omega-3 fatty acids also have a number of other effects that may be beneficial in animals with cardiac disease, including a reduction in inflammatory mediators (e.g., eicosanoids, TNF, IL-1). In addition, omega-3 fatty acids have antiarrhythmic effects. Although an optimal dose of omega-3 fatty acids has not been determined, the authors currently recommend a dosage of fish oil to provide 40 mg/kg EPA and 25 mg/kg DHA for dogs and cats with cardiac disease. Omega-3 fatty acids appear to be particularly useful in animals with anorexia or cachexia but also may be beneficial in animals with less advanced cardiac disease

(e.g., DCM, CVD, HCM).

A few specially designed therapeutic diets contain high levels of omega-3 fatty acids, but for most animals, supplementation will be necessary to achieve a sufficiently high omega-3 fatty acid dose. When recommending a supplement, it is important to know the exact amount of EPA and DHA in the specific brand fish oil since supplements vary widely. The most common formulation of fish oil, however, is one gram capsules that contain approximately 180 mg EPA and 120 mg DHA. At this concentration, fish oil can be administered at a dose of one capsule per 10 pounds of body weight to achieve the authors' recommended EPA and DHA dosage.

Fish oil supplements should contain vitamin E as an antioxidant, but other nutrients should not be included to avoid toxicities. Similarly, cod liver oil should not be used to provide omega-3 fatty acids because it contains high levels of vitamins A and D, which can result in toxicity. Flaxseed or flaxseed oil also should not be used because its omega-3 fatty acids cannot be efficiently converted to EPA and DHA in dogs (and particularly in cats).

Vitamins

B Vitamins

Anorexia and increased urinary loss of water-soluble vitamins associated with diuretic use could predispose animals with CHF to low B vitamin concentrations. Thiamine deficiency is known to be a cause of cardiomyopathy and a consequence of CHF in people, but there has been little investigation into the role of B vitamins in animals with cardiac disease.

Minerals

Magnesium

Magnesium plays an important role in normal cardiac function. Animals with CHF may be at increased risk for magnesium deficiency due to diuretics and other cardiac medications. Hypomagnesemia can increase the risk of arrhythmias, decrease cardiac contractility, cause muscle weakness, contribute to renal potassium loss, and can potentiate the adverse effects of certain cardiac medications. Hypomagnesemia has not been a consistent finding in studies of animals with heart disease but this may be because serum magnesium concentration is a poor indicator of total body stores. Monitoring magnesium status in animals with CHF can identify those that would benefit from supplementation.

Other Nutrients

L-Carnitine

L-carnitine is critical for fatty-acid metabolism and energy production and is concentrated in skeletal and cardiac muscle. While carnitine deficiency is associated with primary myocardial disease in a number of species, including a family of Boxer dogs, L-carnitine supplementation may have benefits even if a deficiency is not present by improving myocardial energy production.

Table 2. Treats for Dogs with Heart Disease

Acceptable Treats and Foods That Can Be Used to Increase Palatability

Note: All foods in this list should be prepared without salt.

- Pasta
- Rice (plain white or brown rice, not flavored rice)
- Honey
- Maple syrup
- Low-sodium cheese
- Lean meats, cooked (chicken, turkey, beef, or fish) – not sandwich meats/cold cuts
- Eggs, cooked
- Homemade soup — not canned soups
- Low-salt breakfast cereal (such as Frosted Mini-Wheats) — the label should read "this is a low-sodium food"
- Fresh vegetables/fruit (such as carrots, green beans, apple, orange, banana [avoid grapes])
- Dog treats that are low in sodium:
  Purina® Alpo® Variety Snaps Treats;
  Purina Veterinary Diets® Lite Snackers;
  Iams® Adult Original Formula Small Biscuits;
  Science Diet® Simple Essentials Training Treats™;
  Stewart® Fiber Formula® Medium Dog Biscuits

Foods to Avoid

- Fatty foods (meat trimmings, cream, ice cream)
- Baby food
- Pickled foods
- Bread
- Pizza
- Condiments (ketchup, soy sauce, barbeque sauce, etc.)
- Sandwich meats/cold cuts (ham, corned beef, salami, sausages, bacon, hot dogs)
- Most cheeses, including "squirtable" cheeses (unless specifically labeled as "low sodium")
- Processed foods (such as potato mixes, rice mixes, macaroni and cheese)
- Canned vegetables (unless "no salt added")
- Potato chips, packaged popcorn, crackers and other snack foods
- Soups (unless homemade without salt)
- Most dog biscuits and other dog treats

Coenzyme Q10

Coenzyme Q10 also is a co-factor required for energy production. In addition, it has antioxidant properties. There are many anecdotal reports of benefits to coenzyme Q10 supplementation, but controlled prospective studies are necessary to accurately judge the efficacy of this product. Most human studies of coenzyme Q10 supplementation have not been well-controlled and results are conflicting. Possible reasons

Tips for Administering Medications

Foods commonly used to administer medications can provide a large amount of additional sodium in your dog's diet. Better ways clients can give medications include:

- Have a doctor or technician teach them how to give medications without using food
- Insert medications into one of the following foods:
  - o Fruit (for example, banana, orange, melon [avoid grapes])
  - o Low-sodium cheese
  - o Low-sodium canned pet food
  - o Peanut butter (labeled as "no salt added")
  - o Home-cooked meat such as chicken or hamburger (without salt), not lunch meats

for the reported benefits of supplementation include correction of a deficiency, improved myocardial metabolic efficiency, or increased antioxidant protection.

Antioxidants

Reactive oxygen species are a normal byproduct of oxygen metabolism and are typically adequately compensated through the production of endogenous antioxidants. Normally, there should be a balance between oxidants and antioxidants, but dogs with CHF have been shown to have an imbalance between oxidant production and antioxidant protection. Antioxidants are produced endogenously but also can be supplied exogenously with either enzymatic antioxidants (e.g., superoxide dismutase, catalase, glutathione peroxidase) or oxidant quenchers (e.g., vitamin C, vitamin E or glutathione). While dietary antioxidants have been shown to increase antioxidants and to reduce oxidants, clinical benefits of antioxidant supplementation in animals with cardiac disease have not yet been demonstrated.

Clinical Issues

The process of choosing an appropriate diet for an animal with cardiac disease involves examining the patient, the diet, and the owner's feeding practices and considering all the issues at hand. It is important to assess all these factors to determine which diet or diets might best suit an individual patient.

The Patient

In general, the nutrients of concern in cardiac patients are calories, sodium and chloride, protein, potassium, and magnesium. However, patients with cardiac disease vary tremendously in terms of their clinical signs, laboratory parameters, and food preferences, and these all affect diet selection. For example, dogs with asymptomatic heart disease require less severe sodium restriction than those with CHF. Thin cats require a more calorically dense diet than would a normal or overweight cat. Laboratory results (e.g., hypokalemia versus hyperkalemia) and concurrent diseases also influence diet choice. For animals with acute CHF, dietary changes should be avoided until the patient is stabilized. Once the animal is home and stabilized on medications, a gradual change to a new diet can be made. Forced dietary changes when the animal is sick can induce food aversions.

The Diet

Based on these and other patient parameters, a diet or diets can be matched to the individual patient. For an animal with cardiac disease without CHF (i.e., an asymptomatic dog with CVD or cat with HCM), the authors recommend only mild sodium restriction and counseling the owner to avoid diets high in sodium, and treats or table food high in sodium. Most owners need very specific instructions regarding which foods are appropriate. When CHF first arises, additional sodium restriction is recommended, but attention to providing adequate protein and optimal levels of other nutrients of concern also is important.

As CHF becomes more severe, more severe sodium restriction may allow lower dosages of diuretics to be used to control clinical signs. Careful selection is important to achieve not only the desired sodium level, but also appropriate levels of protein, potassium, magnesium and other nutrients. Above all, the diet must be palatable enough that the animal will willingly eat it to aid in maintaining optimal weight.

There is usually not a single "best" diet for any patient. The authors typically select several diets that are appropriate for an individual patient based on the patient, diet and feeding practices. These diets are offered as choices for the owner and for the pet so they can determine the one that works best. Having multiple appropriate dietary choices is particularly beneficial for animals with more advanced CHF, in which a cyclical or selective loss of appetite is common.

Feeding Practices

While it is important to find a diet or diets that the individual animal likes and will willingly eat to maintain optimal body condition, it also is necessary to meet the owner's expectations in terms of diet. A pet's quality of life is of tremendous importance to owners of pets with cardiac disease so providing diets that are palatable and readily eaten is critical. Also, be sure to address not only the pet food but also the treats, table food and foods used to administer medication.

Most dogs and many cats receive treats, so it is important to specifically discuss treats with the owner. Most owners are unaware of treats that would be contraindicated (e.g., high salt treats or table food). The author typically provides a list of foods that are appropriate and foods to avoid as treats to assist the owner in wise selection (see Table 2).

In addition, most dog owners (and many cat owners) give medications with "people food." Including this information in

the overall diet plan is important to achieve success with nutritional modification. The type of food preferred also varies between owners (and pets, e.g., canned versus dry versus homemade) and must be taken into consideration. Finally, cost preferences should be considered as veterinary therapeutic diets may be out of the price range for long-term use by some owners. In these cases, lower-priced alternatives should be offered.

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For Further Reading

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Q&A Discussion

Q: Dr. Margie Scherk, Vancouver: Can you clarify in the U-shaped curve showing survival versus body weight in the cats, was the weight taken at the time of diagnosis or during progression of disease?

A: Dr. Freeman: The curve of survival in the cats was their weight or their body condition at the time of diagnosis of heart failure — not just heart disease but heart failure. We also did look at changes in weight over time in both feline and canine patients. And, in dogs, it was significant. Dogs that gained weight actually did better than if they lost weight. But we did not find that in the cats, in terms of the change in the weight.

Q: Dr. Bob Backus, University of Missouri: I have a quick question on magnesium that you have listed as a nutrient to consider. How do you assess status? You mentioned, and I think a lot of us believe, that serum is not a very accurate indicator of status. Do you have any suggestions on how we should assess magnesium status?

A: Dr. Freeman: We measure total magnesium in our hospital, but we also measure ionized magnesium. You are absolutely right that serum magnesium is a poor indicator of the patient's status. Certainly, if they are low on total magnesium, that is something to pay attention to, but I think that ionized magnesium is a little bit more accurate indicator, so we would keep track of that as well. We also start looking a little bit more critically if the animal is having arrhythmias or other issues that may be the result of low magnesium status. And then the other thing is looking at the diet. If they are also on a low magnesium diet or on a lot of diuretics, I may have a higher index of suspicion for magnesium depletion.

Addressing Age-Related Changes in Feline Digestion

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Abstract

Body weight decline is widespread among cats older than 11 years. A large number of such cats do not show obvious signs of illness. Digestibility data from a large population of cats maintained in different colonies showed that approximately 30% of cats older than 11 years had an impaired ability to digest nutrients, more specifically fat and protein. There is a significant negative correlation between age and nutrient digestibility in senior cats. Decreased digestibility of nutrients over the long term may lead to negative energy and nitrogen balance. This may contribute to loss in body weight and lean body mass. Data also shows that reduction in digestibility may lead to a significant decline in circulating levels of critical nutrients such as vitamins E and B12. It is important to monitor body weight of senior cats. If weight loss is observed, it is critical to provide highly palatable, digestible, energy-dense food.

Glossary of Abbreviations
AAFCO: Association of American Feed Control Officials
DEXA: Dual Energy X-Ray Absorptiometry
GI: Gastrointestinal
MER: Maintenance Energy Requirements
PLI: Pancreatic Lipase Immunoreactivity

Introduction

Aging is defined as the progressive changes that occur after maturity in various organs, leading to a decrease in their functional ability.[1] Like humans, average life span of pets is increasing due to advances in medicine, nutrition and effective control of infectious diseases. Over the past 10 years there has been a 15% increase in cats over 10 years of age, and cats over 15 years of age have increased from 5 to 14%.[2] Gunn-Moore[3] suggested four life stages in cats based on physical and metabolic changes occurring with age, beginning with young (birth to 1 year), progressing to adult (1 to 7 years), followed by maturity (7 to 11 years), and finally a senior or geriatric stage (>11 years). Therefore, in feeding cats, the nutritional goals are to slow or prevent the progression of metabolic changes associated with aging, enhance quality of life, and, if possible, increase life expectancy.

The objective of this presentation is to discuss age-related changes in nutrient digestion, specifically fat and protein digestibility and how these changes with aging may influence certain health measures. The health implications of reduced fat and protein digestibility in geriatric cats are discussed. A method for identifying cats with poor nutrient digestion in clinical situations is suggested. Postmortem data of cats that were identified as suffering from poor nutrient digestion are presented, and finally, potential strategies for dietary management of geriatric cats suffering from reduced digestive capacity are recommended.

Data Origin

The data presented are from Nestlé Purina PetCare (NPPC) cat colonies. NPPC cats are cared for with the objective that they are true representatives of the general cat population. Those that are not adopted into private homes are cared for during their entire natural lives in NPPC colonies. A home-like environment is provided, including regular play and interaction with humans and other cats. Development of social bonds with pet care personnel is encouraged. Health of the colony cats is maintained by in-house veterinarians with a focus on preventive medicine to ensure a healthy colony.

Nutrient Digestibility — Age

The nutrient digestibility data presented relates to apparent or total tract nutrient digestibility. Digestibility tests were performed using standard Association of American Feed Control Officials (AAFCO) digestibility protocol.

Fat digestibility in healthy adult cats in our colony averages 90 to 95%. Fat digestibility less than 80% is considered low, as it is more than two standard deviations below our colony average. Protein digestibility in healthy adult cats in our colony averages 85 to 90%. Protein digestibility less than 77% is considered low, as it is more than two standard deviations below our colony average. Fat or protein digestibility below 80% and 77%, respectively, may lead to significant negative health consequences over time.

Figure 1 shows historical fat digestibility data in cats with age ranging from 8 to18 years. There was a significant ($P<0.0001$) negative correlation ($r= -0.76$) between age and fat digestibility. The incidence of low fat digestibility increases with age, affecting approximately 10 to 15% of mature cats (8 to 12 years of age) and 30% of the geriatric cats (>12 years of age). In some geriatric cats, fat digestibility was found to be as low as 30% with large stools and low body weight as the only clinical signs.

Figure 1: Fat digestibility (%) of cats by age (n=208).

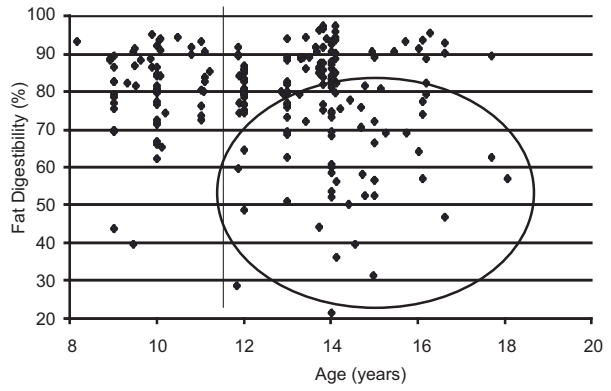


Figure 2: Protein digestibility (%) of cats by age (n=208).

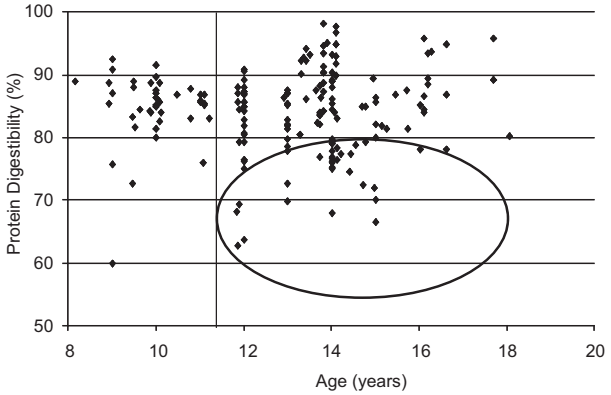


Figure 2 shows historical protein digestibility data in cats with age ranging from 8 to 18 years. There was a significant ($P<0.0001$) negative ($r= -0.66$) correlation between age and protein digestibility. Low protein digestibility also seems to affect mature and geriatric cats. Although the incidence of low protein digestibility is not as high as low fat digestibility, approximately 20% of cats older than 14 years show protein digestibility lower than 77%. The incidence of low fat and protein digestibility tends to occur in the same cats.

Nutrient Digestibility and Age Across Multiple Colonies

Nutrient digestibility tests were conducted in 140 cats with age ranging from 8 to 18 years and given the same fish-based wet diet, and in 48 cats fed a corn and poultry meal-based dry diet across four different colonies to ensure that these conditions were not specific to one colony. Digestibility tests in all four colonies used the standard AAFCO digestibility protocol. Diet and stool analysis were conducted at the same laboratory in order to remove analytical variation. Additionally, 12 cats with age ranging from 1 to 7 years with normal fat digestibility were also fed the same diets in each colony.

Cats across four different colonies showed similar results. The incidence of low fat digestibility increased with advancing age. Approximately 10 to 15% of cats aged 8 to 12 years had low fat digestibility and over 30% cats aged over 12 years had low fat digestibility. Young cats (1 to 7 years) had a very low incidence of reduced fat digestibility.

Similar to fat digestibility, cats across four different colonies showed low protein digestibility. The incidence of reduced protein digestibility is not as dramatic as low fat digestibility, but there is a gradual increased incidence of reduced protein digestibility with advancing age.

Nutrient digestibility declines with age in several mammalian species. Digestibility studies with dogs reported little evidence of an age-related decline in digestive efficiency.[4,5,6,7]

Figure 3: Percent of cats with low fat digestibility (pooled data from four colonies).

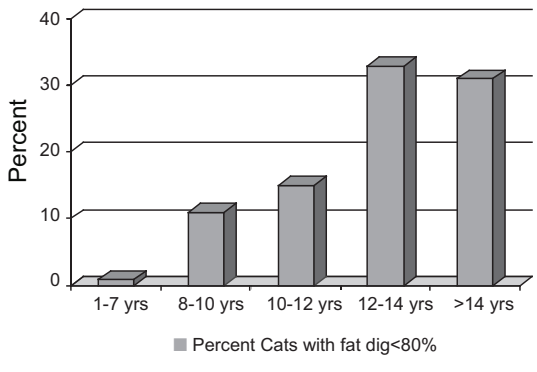
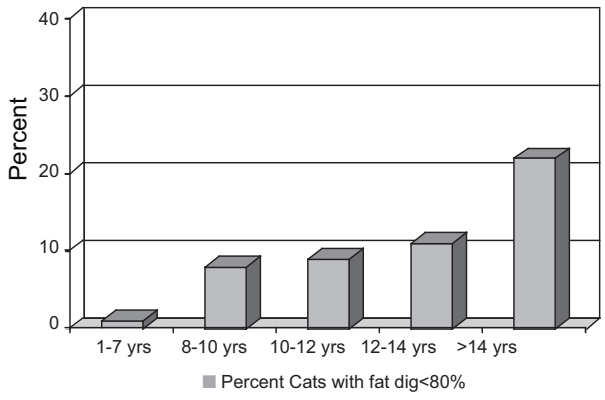


Figure 4: Percent of cats with low protein digestibility (pooled data from four colonies).



Very little information is available in the literature regarding the effect of age on digestive function in cats. Taylor et al. [8] reported a significant decrease in nutrient digestibility with age in cats.

Senior Cats — Obesity or Underweight

Many studies have been conducted in cats to address the prevention of obesity. However, very little attention has been given to address the opposite problem of the skinny, frail cat with low body weight and poor body condition. A significant number of geriatric cats may suffer from this condition.

Perez-Camargo[9] reported historical NPPC colony body weight data in 235 cats. Cats were divided into three age groups: adult (1 to 7 years, n=114), mature (7 to 12 years, n=39), and geriatric (>12 years, n=82). The mean body weight of cats was approximately 4 kg. A cat was considered obese if the body weight was 50% greater than the mean body weight, and was considered underweight if the body weight was less than 50% of the mean body weight. The data showed that the incidence of obesity happened mainly during the mature stage, while underweight condition was most prevalent in the geriatric life stage. In the mature life stage, 28% of cats (11/39) were found to be obese and only one cat was underweight. The trend was almost reversed during the geriatric life stage. In geriatric cats, 23% of cats (19/82) were found to be underweight and only one cat was obese.

Table 1. Incidence of obesity and underweight by age.

Age group	BW (kg)	Obesity Incidence	Percent Underweight Incidence
Adult (1-7 years)	3.7 ± 0.8 a	<1%	<1%
Mature (7-12 years)	4.4 ± 1.7 b	28%	<1%
Geriatric (>12 years)	2.9 ± 1.0 c	<1%	23%

Harper[10] reported retrospective body weight data of 53 healthy cats over 11 years of age. Between 5 to 8 years of age, 50% of cats gained body weight, while 35% maintained weight, and 15% lost more than 10% body weight. From 8 to 11 years of age, 20% gained weight, 50% maintained weight, and 30% lost weight. Data from these studies indicate that in senior cats (> 11 years of age), weight maintenance or loss is much more common than weight gain.

In humans and dogs, age-related declines in maintenance energy requirements (MER) are related to decreased physical activity, with a smaller contribution from decreased basal metabolic rate. In contrast to humans or dogs, cats do not appear to exhibit an age-related decline in MER.[11]

In a long-term feeding study[12] of cats ranging in age from 10 to 15 years, cats were fed fish-based wet foods. Only data from cats that were able to maintain body weight were considered. Total calorie intake/kg BW in cats increased with advancing age to maintain body weight. It is possible that the increase in total calorie consumption in these cats could be to compensate for some decrease in digestive capacity. Despite this compensation by some cats, there were several cats over the age of 13 that were not able to maintain body weight. No cat over the age of 15 was used in the study as none was able to maintain body weight. When body weight data for all cats was presented in relation to age there was a significant decline in body weight with advancing age (Figure 5).

In some instances, an underweight condition or weight loss is related to specific diseases; however, reduced ability to digest fat and protein may contribute to the development of weight loss and underweight conditions even in otherwise apparently healthy cats. Perez-Camargo[9] reported average body weight change of 258 cats during the four years prior to their death. Body weight declined gradually during the geriatric life stage, however, there was a dramatic decrease during the last year of a cat's life regardless of the cause of death.

Fat is the most energy-dense macronutrient and an impaired ability to digest fat could contribute, at least in part, to the negative changes in BW and body condition in geriatric cats. It is likely that the onset of reduced fat digestibility is gradual, but over the long term may contribute negatively to energy balance in a large number of geriatric cats.

Nutrient Digestibility Affects Other Health Measures

A study was conducted to evaluate the relationship between fat and protein digestibility with several health measures. Seventy senior cats (7 to 17 years) were fed a standard wet food until their natural death. Digestibility testing was conducted every six months. Blood samples for serum vitamin E, vitamin B12, folate, and pancreatic lipase immunoreactivity (PLI); body condition scores (5-point scale); body composition by dual energy X-ray absorptiometry (DEXA); and skinfold thickness measurements were taken on the same cats when digestibility was determined.

Correlation analysis was performed on the data between fat and protein digestibility and the other variables. To calculate correlations, analysis of covariance was used wherein fat or protein digestibility was the dependent variable and the independent variables were time, cats and each of the other variables.

Table 2 indicates that several health measures were correlated with fat digestibility. The relationship was significant ($P<0.05$) in all cases, but the correlation values showed variability among the different parameters. A strong positive relationship existed between fat digestibility and vitamin E. This was not unexpected as vitamin E is fat soluble, and in cats exhibiting low fat digestibility, the serum vitamin E levels were generally lower. In some cats with poor fat digestibility, the serum vitamin E levels were below 5 mg/L. Inadequate vita-

Table 2. Important correlations of fat digestibility and health measures.

Correlated with Fat Digestibility	Correlation	P-Value
Age (years)	-0.76	<0.0001
Vitamin E (mg/L)	0.65	<0.0001
Vitamin B12 (ng/L)	0.60	0.0001
PLI (ug/L)	-0.52	0.0422
Skin Thickness (mm)	0.36	0.0229
Body Condition Score	0.46	0.0001
Tissue Fat by DEXA (%)	0.43	0.0027

Table 3. Correlations of protein digestibility and health measures.

Correlated with Protein Digestibility	Correlation	P-Value
Age (years)	-0.66	0.0003
Vitamin E (mg/L)	0.60	<0.0001
Vitamin B12 (ng/L)	0.63	0.0002
Lean Body Mass by DEXA (g)	0.49	0.0069
Body Condition Score	0.47	0.0001
Folate (ug/L)	0.42	0.0767
Tissue Fat by DEXA (%)	0.35	0.0385

min E uptake can result in health problems including steatitis.[13] Gershoff and Norkin[14] reported serum vitamin E levels of 0.3-5.0 mg/L in cats fed a vitamin E deficient diet. Thus, the low fat digestibility condition observed in some cats may lower serum vitamin E levels similar to those produced in cats given vitamin E deficient diets. Other fat soluble vitamins (A, D, K) were not measured, but it is possible that a similar relationship existed between fat digestibility and other fat soluble vitamins.

Vitamin B12 also showed a strong positive correlation with fat and protein digestibility, with some cats showing levels below 200ng/L. Simpson[15] reported subnormal concentrations of vitamin B12 in plasma of cats exhibiting weight loss due to various reasons such as diarrhea, anorexia, thickened intestines and vomiting.

Body condition score, tissue fat and skin thickness were positively correlated with fat digestibility. Most cats with low fat digestibility showed a body condition score below 2.5 (on a 5-point scale) and a skin thickness below 2 mm.

Table 3 shows correlations of several health measures with protein digestibility. Vitamins E and B12 showed correlations similar to those that were observed with fat digestibility. There was also a positive correlation with lean body mass and body condition score. Perez-Camargo[9] reported body composition changes with age in 256 cats. During the adult life stage, average body fat was 10%, peaked to 18% during the mature life stage, and then progressively dropped after 12 years of age. Lean body mass (grams) was highest in the adult life stage, declined gradually with age, and at age 15, cats had lean body mass 30% less than the average lean body mass of healthy adult cats. Decreases in both body fat and lean body mass with advancing age may contribute to the frail look of many geriatric cats. Reduced protein digestibility with age may contribute to predisposing cats over age 12 to have a negative nitrogen balance and loss of lean body mass. The correlation data presented above suggests that some of the health changes in geriatric cats may be attributed to reduced digestive capacity that occurs with advancing age.

Identifying Cats with Reduced Digestive Capacity

Digestibility testing often requires lengthy and complicated procedures that are difficult or inconvenient, particularly in clinical situations. Therefore, the correlation data was further examined to potentially develop a quick screening method for identifying cats with reduced fat and protein digestibility in clinical situations. Serum vitamin E and vitamin B12 were shown to have a strong inverse association with reduced digestive function. Based on the literature and internal colony data, abnormal levels were determined for each measure. Table 4 shows the percent of cats with abnormal levels of these parameters that also had low fat (<80%) and low protein (<77%) digestibility.

These data show how blood measures can be used to identify cats suffering from poor digestive capacity. For example, 100% of cats over 7 years of age that had serum vitamin E level below 5 mg/L also had low fat digestibility, and 80% of cats showed low protein digestibility when their serum vitamin E levels were below 5 mg/L. This data were obtained in a study with senior cats (n=70) age 7 to17 years fed standard wet cat food. These quick diagnostic methods are important for a veterinary practitioner. If a cat presents

Figure 5: Body weight change with age.

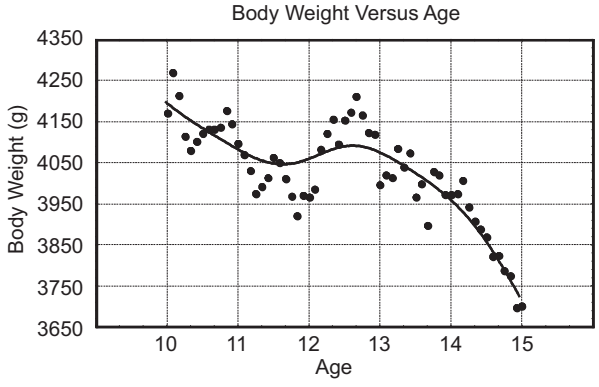


Table 4. Examples of abnormal levels of parameters and percent of cats with low digestibility.

Parameter	Abnormal levels	Percent with low (<80%) fat digestibility	Percent with low protein (<77%) digestibility
Vitamin E	< 5 mg/L	100	80
Vitamin B12	< 100 ng/L	92	67

with low body weight or condition and no specific disease is diagnosed, the practitioner can have a blood sample analyzed for the measures mentioned above to determine if the cat may be suffering from poor fat and/or protein digestion. Furthermore, once the cat is put on a specific dietary regimen or treatment, timely analysis of these measures may show if the cat is improving.

Pathologies in Low Fat Digestibility Cats

Primary cause of death and pathologies at necropsy were examined in senior (7 to17 years) cats (n=69) fed standard wet cat food. In this study, fat digestibility was determined within six months prior to death. Thirty-six cats showed low fat digestibility and 33 cats showed normal fat digestibility when determined within six months prior to death. High incidence of low fat digestibility in these cats was possibly due to the advanced age when the digestibility was determined.

Figure 5 shows the primary cause of death in these cats, determined by both clinical diagnosis before death and necropsy results. Thirty-nine percent of low and normal fat digestibility cats died of cancer; however, 70% of low fat digestibility cats developed gastrointestinal (GI) related cancer as compared to only 23% of normal fat digestibility cats that had GI-related cancer. Incidence of other GI disease such as enteritis and enterocolitis was also higher in cats with lower than normal fat digestibility.

Although a single primary cause of death was determined for each cat, many cats suffered from multiple pathologies observed at necropsy or on histopathology. Figure 7 shows the incidence of pathologies that were reported from the necropsy and histopathology, and is expressed on a percent basis. Data shows that cats suffering from low fat digestibility had a high prevalence of most pathologies compared to cats with normal fat digestibility. It is likely that cats with low fat digestibility may have suffered from multiple pathologies. The prevalence of renal, cancer and GI related pathologies were higher in cats with low fat digestibility than cats with normal fat digestibility.

There are a number of pathologies that could predispose cats to poor digestibility of fats and protein. Malabsorption and maldigestion can occur from almost any diffuse disease of the intestine. Pathologies may originate in the pancreas, liver and/or intestines. Pancreatitis is recognized as the most common disorder of the exocrine pancreas in cats.[16] Despite this, very little is known about its etiology, and its diagnosis remains challenging. Early necropsy studies reported a prevalence of feline pancreatitis from 0.6% to 2.4%[17,18]; however, in a more recent study,[19] histopatholgic examination of pancreatic tissue from 115 healthy and diseased cats showed inflammation of pancreas present in 67% of cats evaluated. Pathologies associated with the pancreas may account for some cases of reduced fat digestibility. Diffuse intestinal diseases, such as intestinal lymphoma, small intestinal bacterial overgrowth, inflammatory bowel disease, and liver disease, may also lead to reduced nutrient absorption in the small intestine. In addition to the disease conditions, age-related changes in digestive physiology, hormones and gut microbiata may directly or indirectly reduce the digestive capacity.[20]

In the same pool of low and normal fat digestibility cats, we compared mean predicted fat mass (g) and lean body mass (g) for cats prior to death. A longitudinal linear regression model best fit the lean body mass data, and the prediction line showed a general progressive decrease in average lean body mass as the cat neared the end of its life span for both groups of cats.

Figure 6: Primary cause of death in low and normal fat digestibility cats.

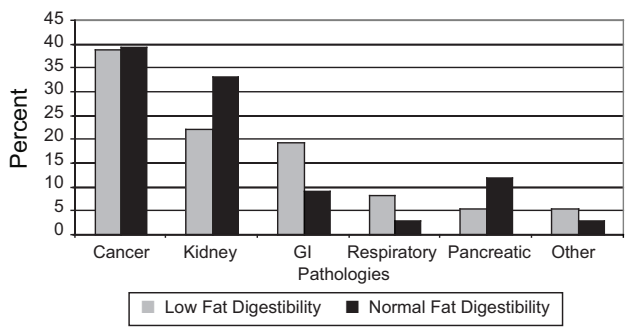


Figure 7. Percent of pathologies at necropsy — Low and normal fat digestibility cats.

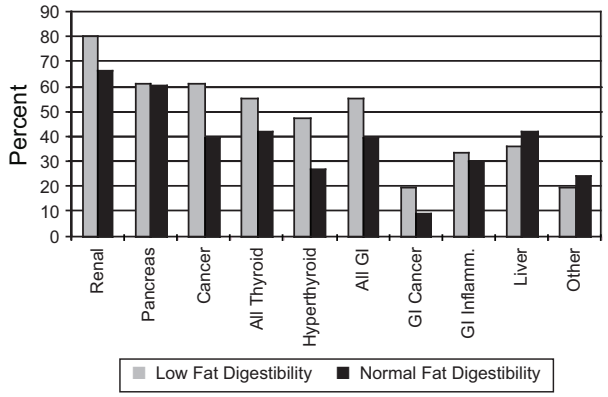
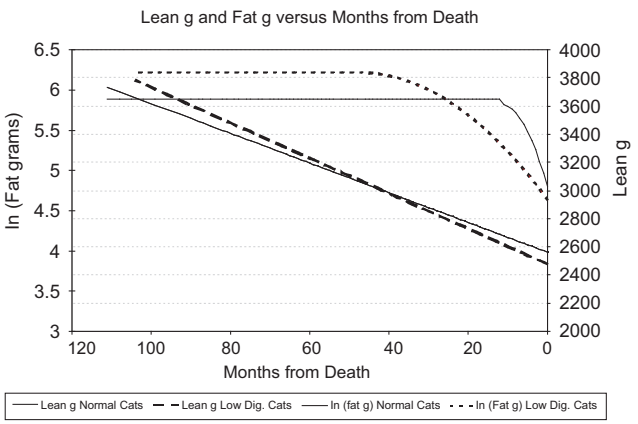


Figure 8. Lean and fat (g) change prior to death.



The fat mass was transformed into natural logarithms to account for extreme observations. A segmented regression model was used, with a quadratic model best fitting the period closest to death. There was a significant difference between the models for low and normal fat digestibility cats. The analysis estimated that cats with low fat digestibility start losing body fat 47 months prior to death much as compared to the cats with normal fat digestibility that start losing body fat 12 months prior to death (Figure 8).

Age-related changes in fecal microflora were studied in 115 cats with ages ranging from 1 to 16 years.[21] Fecal *bifidobacteria* and *lactobacilli* were lower and fecal *Clostridium* perfringens were higher in mature and geriatric cats than in young and adult cats. The relationship between gut microbiata and nutrient digestion in geriatric cats needs to be studied further. The precise etiology of reduction in nutrient digestibilities in geriatric cats is unknown, and it is likely that there is more than one cause.

Nutritional Recommendations for Cats with Impaired Digestion

Cats that develop age-related weight loss may show decreased muscle mass, increased urine volume, increased stool volume, thin skin, and rough hair coat. It is critical to monitor weight on a regular basis in aging cats. Evaluating percent weight change, rather than just absolute weight, may highlight small but important changes in body weight. Regular physical examination and routine blood work also are important.

Humans and dogs consistently show an age-related decline in MER by approximately 20%, whereas cats reportedly exhibit no such age-related MER decline[11]; therefore, unless undesirable weight gain is observed, energy provided to geriatric cats should not be reduced. Although some cats can compensate for lower digestive capacity via increasing food intake, many cats cannot and are susceptible to body weight loss.

In geriatric cats, especially with those starting to lose weight, the major goal is to maintain body weight and body condition by frequent access to highly palatable, highly digestible, nutrient dense foods. In geriatric cats, there is increased risk of losing sense of smell. Therefore, different flavors and textures should be tried to determine which flavors and textures the cat prefers. Dry foods with high energy density, excellent palatability and high digestibility may be used if the cat is able to chew and consume dry food. Canned foods are usually highly palatable and have the advantage of increasing water intake, but are lower in caloric density on a volume basis. Adding warm water to dry food may increase acceptance for some cats and may be easier to chew. Providing food multiple times during the day will help increase food consumption. If the cat continues to lose body weight, and no underlying disease can be identified, then critical care diets with the highest caloric density may be tried.

Further work is needed to identify specific causes of the reduction in digestive capacity that occurs in a large percentage of geriatric cats. It is important to develop a targeted nutritional solution for the skinny condition seen in this population of senior cats.

Acknowledgements

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Q&A Discussion

Q: Dr. Jane Armstrong, University of Minnesota: I wonder if the rather marked incidence of fat digestibility changes are indicative of early pathology that we might consider a specific marker or indication of a variety of disease processes, or how much is reflective of physiologic decline in things that would not cross over into where we could diagnose it as a specific disease process. So, I'm wondering if perhaps some of the cats had very marked declines in PLI (Pancreatic Lipase Immunoreactivity), and if you did any interventions? Did you supplement pancreatic enzymes in any cats that were particularly low or use prebiotics or intervene at all in these cats?

A: Dr. Patil: These cats were on a long-term feeding study so we were not able to do any kind of dietary intervention. They stayed on whatever diet they were getting during that study, but they were treated if they had evidence or clinical signs of disease, such as pancreatic diseases. I might also mention that some of the PLIs didn't always necessarily correlate with pathologies.

Q: Dr. David Williams, University of Illinois: It was very, very interesting information. Several years ago I presented an abstract at a Nestlé Purina Nutrition Forum showing that among cats with signs of GI disease, 30% that were greater than 10 years of age had low serum cobalamins as opposed to 10% in young cats, which is a remarkable resemblance to some of the data you just presented. My observation is that human beings who present with cobalamin deficiency or pernicious anemia have GI signs that are completely reversed when they are supplemented with cobalamin. So I wonder, but maybe you have answered the question already, did you take any of these cats and replace the cobalamin deficiency? Because it is possible that, as in human beings, a lot of the GI signs you are looking at are secondary to cobalamin deficiency.

A: Dr. Carolyn Cupp, Nestlé Purina Research: Providing supplemental cobalamin would have been considered an interventional therapy in the long-term study and would have invalidated the study, so we couldn't do it on those cats. But in cats that were not on that study, yes, we have tried cobalamin therapy. We were not able to see a normalization of GI function.

Comment: Dr. David Williams, University of Illinois: As a clinical observation, when pet cats are tested for voluminous pale yellow stools, most people think it is pancreatic insufficiency. Maybe 5% of the time it is, but most often it is small intestinal disease associated with severe cobalamin insufficiency. I don't have any figures, but many of those cats respond remarkably well to just cobalamin injections.

Q: Dr. Richard Hill, University of Florida: I wondered whether you had any data on carbohydrate digestibility in these older cats, and whether you could possibly use carbohydrates as a calorie source?

A: Dr. Patil: We did not collect data on carbohydrate digestibility because most of these studies were done using wet cat food. There is very little carbohydrate in wet cat food.

Comment: Dr. Aulus Carciofi, University Sao Paulo: In response to Dr. Hill's question, we have some old cats in which we detected low digestive function, and we tested starch digestibility. It is also reduced in these animals. Also we tried to improve the fat digestibility in these cats adding lecithin to the diet. Maybe if the problem was emulsification we could see some increase in digestibility adding lecithin to the diet. This didn't work. So the problem in fat digestibility is maybe related to enzymes or some other problem.

Q: Dr. Tom Schermerhorn, Kansas State University: I have just a point of clarification and then a question. With regard to the data on body weight changes with age, as I understand it, it was cross-sectional data of a kennel or cattery, rather than profiles of individual cats' journey through life. So some of the decrease in obesity in old age could simply reflect mortality related with obesity at a certain point. And if that's true, I guess the other question is whether age is a disease or not? I am having a hard time trying to find the line between when these cats are just aging and they become sick. So I think it would be really interesting to see when the veterinarians caring for these cats identified those fading cats as being clinically ill and they had a diagnosis attached to them, rather than just an old cat. I wonder if you have any data on that.

A: Dr. Patil: We do not have that data. However, I showed you one controlled study where we measured digestibility in 140 cats. Each of those cats was screened for any kind of illness prior to the study, so they had at least no diagnosed disease at that point when we did the study. And that population of cats showed a significant decrease in fat digestibility with age.

Comment: Dr. Gerardo Perez-Camargo, Nestlé Purina Research: I would just like to clarify one issue, as we may have confused the audience with multiple data sets from our facility. Earlier, I presented data that was cross-sectional. We looked at body weights across populations of cats, and we identified a decrease in average body weight in cats over 12 years of age. My colleague, Dr. Carolyn Cupp, presented data from a longitudinal study of 90 cats. So her data reflects, as Tom described, profiles of individual cats' journey through life. So we looked both ways with similar results.

Dietary Effects on Gastrointestinal Microbiota of Aging Dogs: Potential Tools to Health Improvement

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Abstract

The relationship among nutrition, gut physiology and microbiology, and host immunology were reviewed in the context of the aging dog and cat. The dynamic interrelationship among these three areas opens opportunities to improve gut health and immunological status of aging dogs by diet formulation. Dietary ingredients, processing and nutritional composition, and mainly some special carbohydrates, like prebiotics, can be potential tools to access gut and health changes in geriatric dogs.

Glossary of Abbreviations
FOS: Fructoligosaccharides
GALT: Gut-Associated Lymphoid Tissue
GIT: Gastrointestinal Tract
IgA: Immunoglobulin A
IgG: Immunoglobulin G
MOS: Mannanoligosaccharides
SCFA: Short-Chain Fatty Acids
YCW: Yeast Cell Wall

Introduction

Aging could be defined as the progressive changes that occur after maturity in various organs, leading to a decrease in their functional ability. The aging process is affected by alterations in physiological systems and metabolic processes. Unfortunately, these alterations are not well-defined in pet animals. Although the terms "old," "senior" and "geriatric" often are used interchangeably, they do have distinct definitions. The term "senior" refers to the functionality of an animal. An animal is considered to be senior when it decreases activity, gains or loses weight, and develops other age-related physical and behavioral changes. Conversely, the term "geriatric" refers to the chronological age of the animal. Generally, large- and giant-breed dogs are considered geriatric at 5 years of age, whereas small- or medium-breed dogs and cats are not considered geriatric until 7 or more years of age.[1]

Geriatric dogs and cats are not recognized to have specific nutrient requirements that clearly differentiate them from mature animals in maintenance.[1] Therefore, geriatric nutrition of dogs and cats is more a good sense approach that includes several aspects of the diet such as palatability, physical form, apprehension and chewing, nutrient composition, energy density, digestibility, and especially the use of nutrients and ingredients to promote health and well-being. This nutritional intervention should aim at prolonging the length and quality of life and delaying the onset of geriatric dysfunction and disease states. To accomplish these goals, it is important to understand age-related changes. Defining such mechanisms lays important groundwork for devising interventions that might further prolong the length of life for which an individual may remain clinically healthy (the "health span").[2]

One nutritional approach in geriatric nutrition focuses on the gut. The two main functions of the gastrointestinal tract (GIT) are the digestion and absorption of nutrients and body protection. Beyond the well-known function to provide nutrients to the organism, GIT is a very active immunological organ that has a complex structure and several specialized cell types that play an important role in protecting the body form the external environment.[3,4] As an active organ, GIT has a high nutrient demand, utilizing a significant amount of the energy, protein and amino acids required by the animal.[5,6]

Thus, an adequate nutrient supply to GIT is important to support good organ development and function. The main source of nutrients for the intestinal mucosa is from the gut lumen, with the blood nutrient supply assuming lesser importance. These compounds absorbed form the lumen come from dietary ingredients or are produced and released by intestinal microbiota. It is well-recognized that intestinal microorganisms play an essential role in digestion and gut health.[1] Several microbial byproducts from organic matter utilization, like amines and short-chain fatty acids, have important specific functions in enterocytes and colonocytes. Considering this, the dynamic interrelationship among nutrition, immunology and gut microbiology opens opportunities to improve gut health and the immunological status of aging dogs by diet formulation.

Age-Related Changes in the Gastrointestinal Function and Metabolism of Dogs and Cats

Studies on age-related physiological alterations in dogs and cats have become more frequent in the last two decades, perhaps as a result of the recent increase in life expectancy of these animals. Aging brings with it physiological changes. Some changes are obvious, such as whitening of hair, a general decline in body and coat condition, and failing senses (sight and hearing). Other changes are less obvious, however, and these include alterations in the physiology of the digestive tract, immune system, kidneys, and other organs. Pets, like people, do not age consistently, and chronological age does not always match physiological age. Although many pets remain active and youthful well into their teens, some dogs start to slow down and may show signs of aging beginning as early as 5 or 6 years of age.[7,8]

Gut physiology and function are altered during the aging process, which is often accompanied by an increased incidence of gastrointestinal infections. With increasing age, several gastrointestinal dysfunctions can manifest in human beings, including slowing of intestinal transit times, decreasing organ reserves, alterations in enzyme activity, impaired circulation, and reduced bile and pancreatic secretions.[9,10] It is not certain if these alterations occur in dogs or cats. In old cats, alterations in GIT function are mainly related to a decrease in digestibility of nutrients such as protein, fat and starch.[11,12] On the other hand, advanced age generally does not reduce apparent nutrient digestibility in dogs.[13] Other age-related changes in cats and dogs include an increased incidence of periodontal disease, difficulty in apprehension and chewing, and increases in diarrhea, vomiting and regurgitation.[14]

Reduced digestibility in old cats does not seem to be related to the duration of gastric emptying nor to intestinal transit time, as no differences were found between the passage of ingested foods through the intestinal tract of aging cats compared to younger cats.[15] Morphological changes in the intestine do not seem to be the cause of decreased nutrient digestibility in humans.[16] However, this does not appear to have been studied in cats; therefore, this hypothesis cannot be rejected as a cause of reduced digestibility in this specie. The consequences of reduced digestibility in aging cats are not precisely known. The reduced digestibility may contribute to decreased fat mass, lean mass and body weight of aging felines.[17] This effect is further reinforced by a lack of change in voluntary eating behavior with aging.[18,19] Thus, the maintenance of food ingestion habits, associated with a reduced utilization of ingested food, would result in lower uptake of bioavailable nutrients.

The concept of "gut health" is complex and broadly defined. According to Conway,[20] three major components of "gut health" can be considered: diet, intestinal mucosa and intestinal microbiota. Intestinal mucosa morphology changes according to nutrition, stress, aging, and/or disease. These changes may affect the physiology of the intestine, influencing nutrient absorption and metabolism. Studying the age and diet effects, Kuzmuk et al.[21] observed morphological differences in young (1.2 years) and old (12.1 years) dogs fed either a plant- or animal product-based diet. Jejunal villus height increased in young dogs consuming the plant product-based diet compared with both young dogs consuming an animal product-based diet and old dogs consuming either a plant product- or animal product-based diet. Colonic crypt depth also was greater in old dogs as compared with young ones.

Fermentable carbohydrates may be considered an important part of "gut nutrition" in old age. They include some types of fiber, resistant starch, non-starch polysaccharides such as mannanoligosaccharides, fructooligosaccharides, stachyose and raffinose, and nonabsorbed sugars that reach the colon and are suitable of bacterial fermentation. They allow an adequate organic matter supply for the large intestine.[22] Bacterial fermentation of these compounds results in short-chain fatty acid (SCFA) production and pH reduction, which could modify the composition and metabolic activity of the intestinal bacteria,[23] which in turn could reduce the quantity of nitrogenous waste materials entering the bloodstream. SCFAs, especially butyric acid, are important energy sources for the colonocytes,[1,24] lead to suitable ion absorption, and act in intestinal blood flow and peristalsis. In a study with dogs, fermentable fiber promoted better development of colon mucosa, greater relationship between colon volume and surface, and improved the histological mucosa structure.[25] Thus, whereas non-fermentable fiber acts as bulk, fermentable fiber plays other important physiological roles.

Although not the focus of this review, it is important to consider that GIT has a high metabolic rate and represents a high demand for nutrients delivered by dietary ingredients. The intestinal mucosa has the highest rates of proliferation and cell renewal throughout the body, and this process can utilize from 10 to 20% of the energy and up to 50% of the daily protein requirement.[26] Protein, arginine, glutamate, glutamine, glutathione, glycine, histidine, vitamin A, zinc, and fatty acids are among nutrients that are key for the intestinal mucosa[27] and that must be adequately supplied by diet to ensure the intestine develops adequately its functions of digestion and protection.

Alterations in nutrient profile and dietary fermentation patterns as related to old age have not been well studied in companion animals. It is possible that by manipulating nutrient composition, and gut microbial composition, activity and fermentation byproduct formation, the gastrointestinal health of old companion animals could be enhanced, ameliorating some of the consequences of old age and promoting health and well-being.

Gut Microbiota

The GIT of dogs and cats presents a microbial colonization pattern similar to that of other mammals. At birth, the intestines

are sterile but are rapidly colonized by environmental bacteria. The colonization process follows successive populations' changes affected by age, health status, diet, and environment.[28,29] The normal intestinal microbiota plays an important role in host digestion and metabolism, and provides a natural defense mechanism against invading pathogens.[1]

Although the microbiota in adults has been studied, less is known about the changes that occur with aging.[3,30] These may have important consequences in senior pets, especially in those receiving antibiotic therapy and that are most susceptible to intestinal dysbiosis. Intestinal microbiota of young adult dogs seems to comprise large numbers of *bacteroides, bifidobacteria, lactobacilli*, and *anaerobic cocci*, while older animals harbor greater numbers of *clostridia* and *enterococci*.[32] However, some studies have found conflicting results, such as increasing *lactobacilli* and *bacteroides* in old dogs.[33]

The causes of changes in the microbiota with age are still unclear. A number of physiological and immunological changes occur in the body with advancing age. Hopkins et al.[32] suggested that some bacterial strains could take advantage of new ecological niches, thereby inducing a shift in the composition of the gut microbiota. It has been proposed that reduced adhesion to the mucosa may be a factor involved in the decreasing colonization of old subjects by certain species of *bifidobacteria*.[34] These bacterial community shifts in the large bowel may have great effects on host physiology and metabolism, aspects that need further studies.

Dogs and Cats Immunosenescence and Inflammageing

Immunosenescence may be defined as the multifactorial complex of changes that occur in the immune system of elderly individuals that predispose them to increased morbidity and mortality due to infection and age-related diseases.[2] Changes in immune status are considered major contributing factors toward morbidity and mortality in aging humans. In many other species, this age-related remodeling of the immune system has also been recognized and commonly involves the deterioration of some aspects of immunity accompanied by the enhancement of others. Such changes may leave aged individuals susceptible to infection and are possibly related to the increased incidents of cancer observed in the elderly of all species.[35]

Inflammageing is a term that refers to changes in the balance of pro-inflammatory and anti-inflammatory agents produced in the elderly.[36] During a lifetime of constant antigenic challenges, the adaptations of the animal to produce efficient inflammatory responses can confer high resistance to infectious diseases, but also an increased susceptibility to inflammation-based diseases later in life. On the other side, low inflammatory responses, while rendering the subject more susceptible to infectious diseases, can confer a survival advantage in old age. A large part of the aging phenotype, including immunosenescence, is explained by an imbalance between inflammatory and anti-inflammatory networks, which results in the low-grade chronic pro-inflammatory status called inflammageing.[36] For successful longevity, an individual must therefore find the means of reducing the impact of pro-inflammatory factors while still maintaining the essential aspects of protective immunity and preventing the emergence of deleterious (e.g., auto reactive) immunity.

Mounting evidence for prolongation of the canine and feline health span through nutritional intervention is becoming available.[37,38,39] Pet food manufacturers have been active in the investigation of immunosenescence and inflammageing in the dog and cat, with a view to formulating supplemented specialized diets that may slow these processes.

The effects of the aging process on dogs' and cats' immune system were recently reviewed by Day.[2] Briefly, these include an age-related decrease in the proliferative response of blood mononuclear cells to mitogens; a decline in the numbers of peripheral blood lymphocytes, B-cells, T-cells, and relative percentage of CD4+ T-cells (helper); and an increase in the relative percentage of CD8+ T-cells (natural killer) resulting in a decreased ratio CD4+:CD8+ cells. Phenotypic alteration is accompanied by functional changes, such as reduced ability to respond to stimulation by nonspecific mitogens, relative changes in the balance of Th1 versus Th2 CD4+ T-cell activity, and reduced delayed type hypersensitivity response to mitogens. Although there is general agreement on age-related changes to immune status, contradictory results have been produced by different research groups that can be attributed to some extent to the use of different techniques or different subject selection criteria.[35]

These changes in peripheral blood lymphocytes seem to occur also within the intestinal lamina propria of the aging dog with reduced T cell numbers and lower proliferative activity of intestinal cell populations.[2,40] The GIT is the largest lymphatic cell-bearing organ, playing a major role in both local and systemic immunity, including blocking pathogens, modulation of immune response and oral tolerance.[40] As a result, these immunological consequences of aging are likely to have a health impact in these animals. The mucosal immune system of GIT in dogs consists of organized lymphoid structures, including Peyer's patches, mesenteric lymph nodes, and the intestinal lamina propria.[41] The latter is populated by cell types including T and B lymphocytes, macrophages, mast cells, dendritic cells, neutrophils, and eosinophils.[42] The gut-associated lymphoid tissue (GALT) performs the following activities: a) capture, processing and presentation of ingested antigens; b) local antibody production, especially immunoglobulin A (IgA); and c) activation of immune-mediated responses, particularly those mediated by cytotoxic T cells CD8+ or NK (natural killer cells) and macrophages.

It is speculated that these gut-associated immunological

changes may be caused by alterations in intestinal microbiota that are reported in aging dogs.[2] The increased numbers of aerobic and anaerobic bacteria in fecal samples, especially of *clostridia* from old dogs compared with young dogs, could be one of the causative factors of these immune changes. The possible use of fermentable carbohydrates, prebiotics and probiotics to induce a favorable shift in gut microbial population and bacterial end products formation in old animals is an interesting avenue of research, with interesting prospects of alleviating the adverse effect of immunosenescence in companion animals.

Potential Tools to Health Improvement Through Exchange in Gut Microbiota

Companion animal health is linked to many factors, not least being the maintenance of gut function and environment. The last decade has provided useful research into this area, allowing nutritionists to use commercial products to help establish and maintain the gut environment.

A key factor in animal health is the status of *eubiosis*, i.e., the establishment and maintenance of a stable and healthy microbiota in the digestive tract. *Eubiosis* can be broken down into several areas: digestion of nutrients, vitamin synthesis, stimulation of the immune system, protection/strengthening of mucosa as a barrier to invasion, and antagonistic effects against pathogenic microorganisms.[43] At any life stage those would be key points to maintain health, but those considerations should be mostly important during the time that animals may be more susceptible to disbiosis and other manifestations of GIT dysfunction — as in old age.

The mechanisms by which the normal microbiota helps the host animal's health include lowering the colonic pH and producing SCFAs. Low pH is inhibitory to the growth of many pathogenic bacteria and may reduce the intestinal absorption of potentially toxic compounds, such as ammonia. The SCFAs produced are rapidly absorbed from the intestinal lumen, with 95 to 99% being absorbed before reaching the distal colon. Besides being the primary energy source for colonocytes, SCFAs promote local mucosal health and integrity by stimulating proliferation, maturation and differentiation of colonocytes in the crypts, and facilitating the normal secretory and absorptive functions of the colon. They also stimulate protein synthesis and mucin production, assuring the integrity and effectiveness of the physical barrier.[1,44] Moreover, butyrate may also inhibit the development of malignant colonic cells, but this effect is still controversial and seems to depend on the joint presence of both butyrate and fish oil.[45]

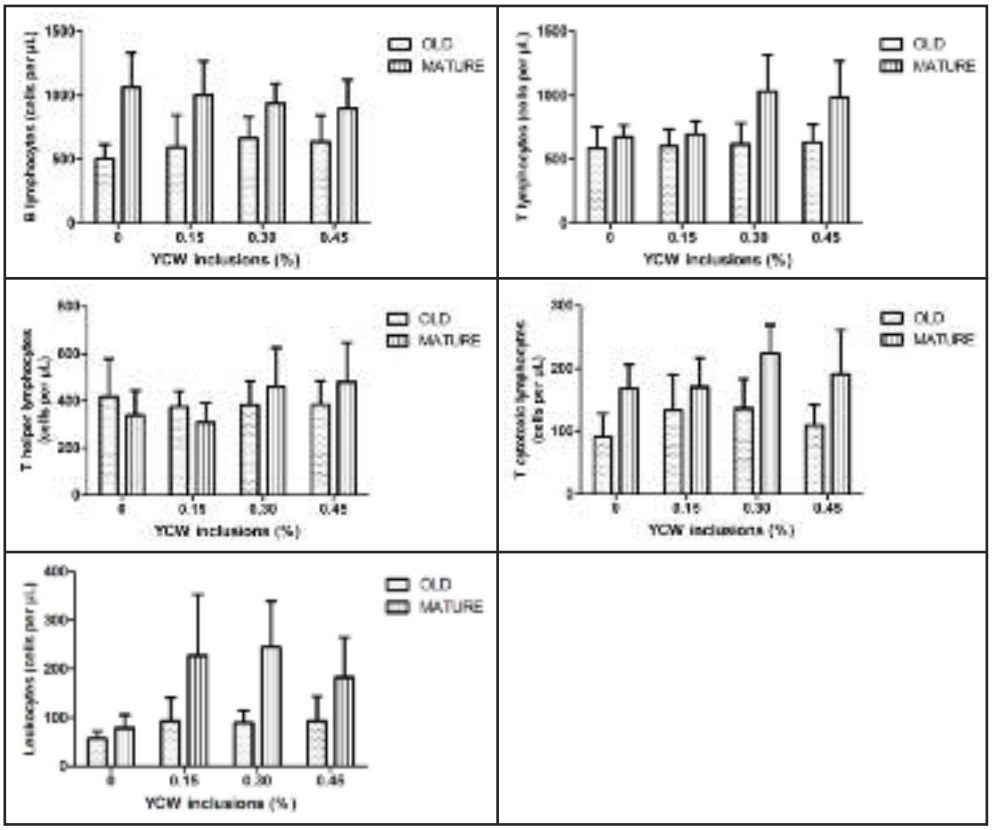
In vitro and *in vivo* studies show that end products of fermentation produced by colonic bacteria depend largely on the chemical composition of the digesta reaching the large bowel, especially protein and undigested carbohydrates. The amount produced and the ratio of individual SCFAs (e.g., acetate, propionate and butyrate) and lactic acid vary, depending on substrate and colonic microbial populations. Microbial fermentation of undigested amino acids results in the production of several putrefactive compounds. These include ammonia, which results from the deamination of amino acids, phenols, indoles (products of aromatic amine decarboxylation), branched-chain fatty acids (derived from branched-chain amino acid catabolism), and several biogenic amines, such as putrescine, cadaverine, histamine, pheniletilamine, and others. These protein catabolites not only result in fecal odor, but also can be toxic at high concentrations.[21]

Of special interest for the formulation of pet food and specific veterinary diets is the use of special ingredients that can influence the composition and metabolic activity of the intestinal microbiota, promoting the maintenance of the eubiosis status.[46] Noteworthy among these compounds are the prebiotics. A prebiotic is "a selectively fermented ingredient that allows specific changes both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health."[47] Because of their chemical structure, these compounds are not absorbed in the upper part of the GIT or hydrolyzed by digestive enzymes,[48] reaching relatively intact into the colon. Modification by prebiotics of the composition of the colonic microbiota leads to the predominance of a few of the potentially health-promoting bacteria, especially, but not exclusively, *lactobacilli* and *bifidobacteria*.[47] Basically, two mechanisms of microbiota alteration by prebiotic use are proposed: nutrient supply for desirable bacteria and competitive exclusion. Prebiotics can also modify the metabolic activity of the colonic microbiota, reducing the concentration of undesirable compounds like ammonia, biogenic amines, indoles, fenoles,[30] even without changes in fecal bacterial counts.[50]

Prebiotic use in companion animal nutrition was recently reviewed.[51] In comparison to other species, little information is available in dogs and especially in cats. There are still controversies about the effectiveness of these compounds,[52] with conflicting results among studies. One important consideration about prebiotic action is the strong influence of basal diet; diet type, diet nutritional composition and ingredient quality also influence the composition of colon microbiota and bacterial end product formation,[53] pointing, perhaps, to the need for prebiotic application in select dietary situations.

Among the prebiotics, fructoligosaccharides (FOS) are the most studied in dogs and cats. They can be used to alleviate small intestinal bacterial overgrowth,[54] to promote a reduction in *clostridia*, an increase in *bifdobacteria* and *lactobacilli* populations,[55] and to reduce the concentrations of protein catabolites produced in the colon. FOS is a readily available energy source for gut microbiota, reducing the bacterial fermentation of protein to supply energy and increasing the incorporation of N-containing substances into bacterial protein. High doses, however, can result in soft feces production and reduced nutrient digestibility.

Figure 1: Lymphocytes subset consentrations in peripheral blood of mature (4 years old) and old (10 years old) Beagle dogs, after consumption of diets with different inclusions of yeast cell wall (YCW).



Another prebiotic evaluated in dogs is the mannanoligosaccharide (MOS). MOS can be isolated from yeast cell wall (YCW), from which the mannan fraction can be as much as 31%, making YCW a potential prebiotic source for pet food. The consulted literature assigns to YCW the ability to change beneficially the intestinal microbial counts and/or their metabolic activity.[46] Moreover, there are reports of immunomodulatory effects for this prebiotic, including increased concentrations of IgA, immunoglobulin G (IgG) and plasma lymphocytes.[51] Mucus secretion by intestinal goblet cells also appears to increase in diets supplemented with YCW, strengthening the defense barrier in the gut. MOS has the ability to agglutinate *Escherichia coli* and *Salmonella* strains that possess mannose-specific fimbriae (Type-1 adhesins), reducing the intestinal binding and colonization of these bacteria.[56] However, MOS has other mechanisms for beneficial change of enteric microbiota, since its addition to diet can also reduce *Clostridium perfringens* counts in dog feces,[52] and *clostridia* species do not possess mannose-specific fimbriae.

In vitro studies suggest that MOS is moderately fermentable by canine and feline microbiota,[57] being a source of energy to lactate-producing bacteria. This explains the reduced fecal pH and fecal ammonia excretion verified in dogs, improving indices of colonic health.[46] MOS is a surface carbohydrate of yeasts, being detected by the animal immune system through mannan-binding lectin receptors in macrophages. These receptors recognize cell wall compounds of pathogens, including many bacteria and some viruses, and can result in opsonization and activation of the complement cascade.[58]

In an study conducted by Gomes et al. (unpublished data)[59] to evaluate the prebiotic potential of the YCW for dogs, four isonutrient kibble diets were used with inclusions of 0%, 0.15%, 0.30% and 0.45% of YCW. Eight Beagle dogs were used, divided in two groups; four mature animals (4 years old), and four old dogs (10 years old). The experiment followed a two 4x4 Latin square design, one Latin square for mature and the other for the old dogs. In each period an adaptation phase of 15 days preceded a five-day total feces collection for digestibility trial and one-day collection of fresh fecal samples for bacterial enumeration, pH measurement and determination of short-chain fatty acids and bioactive amines concentration. On day 21, blood samples were collected for immunophenotypic quantification of lymphocyte subsets through flow cytometry. The data were evaluated using Proc GLM of SAS; means were compared by Tukey test with polynomial and orthogonal contrasts ($p<0.1$).

Nutrient digestibility and metabolizable energy did not vary between diets, showing no effects of YCW ($p>0.05$) and also no effect of age ($p>0.05$). Yeast cell wall supplementation did not result in differences in fecal counts (log of CFU per g of feces DM) of total aerobes, total anaerobes, *E.coli, Clostridium spp, Lactobacillus spp*, and *bifdobacterium spp* ($p>0.10$). An age effect on fecal bacteria counts was not verified; only a tendency for total aerobe increases in old dogs ($p=0.15$) was found.

Some indices of bacterial metabolic activity, on the other hand, changed. The inclusion of YCW resulted in a linear increase in fecal concentration of butyrate (mMol/kg DM; $p=0.055$), and in reductions of fecal concentrations of some bioactive amines (tyramine, histamine, phenylethylamine, and tryptamine). These alterations suggest that YCW may improve

gut health, reducing the formation of toxic compounds delivered during protein fermentation and increasing butyrate supply to colonic mucosa. An age effect on microbial degradation products was also verified. Older dogs presented lower fecal concentrations of butyrate ($p=0.01$), histamine ($p=0.04$), agmatine ($p<0.01$), and spermine ($p=0.01$), and higher fecal pH ($p=0.03$). These findings suggest alteration in bacterial metabolic activity and end product formation, with a decrease in colonic fermentation with aging.

Dogs showed a linear increase in T lymphocyte subset concentration (cells/ L; $p=0.1$) and a higher number of B lymphocytes ($p=0.05$) with YCW addition, evidencing immune stimulation of the animals. Compared to younger adult dogs, older dogs showed a decrease of T lymphocytes ($p=0.01$), T-cytotoxic lymphocytes ($p<0.01$), and B lymphocytes ($p<0.01$) concentrations. Although the evaluation of the two groups together demonstrated an YCW effect, adult dogs exhibited a more exuberant change in peripheral lymphocyte subsets than the old dogs to this prebiotic (Figure 1). Unfortunately, the small number of dogs in each group (only four) does not allow an adequate statistical comparison of these findings, but one possible implication is that higher doses of oligosaccharides are required for senior animals due to the reduced fermentation activity in their large bowel.

Conclusion

The importance of nutrition in old animals lies with the aim of preventing or slowing the progression of the metabolic changes that follow the aging process. Research into the desirable dietary characteristics of the foods for old dogs and cats is needed, opening opportunities to improve health and well-being. The comprehension of the underling mechanisms that result in gut microbial population and/or metabolic activity alterations in old age, their consequences to host immune status, and how to use the diet to favorably manipulate gut microbiota of old dogs and cats might allow the development of dietary tools for health promotion in these animals.

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Q&A Discussion

Q: Dr. Avi Patil, Nestlé Purina Research: In your study you included beat pulp, which is a soluble fiber, in your diets. Do you think that if you did not have beet pulp in the diet, you would have had a stronger response in your study?

A: Dr. Carciofi: Yes. What we provided was a minimum, about 2%, fiber in the diets. Now, we are working on adding soluble and nonfermentable fibers derived from sugar cane. Certainly the base diet will influence the outcomes. This is why it is difficult to compare results across studies. For example, we had a study that included 15% soybean meal as a protein source in the diet. Soybean meal contains various nondigestible sugars, stachyose and raffinose. We did not find any benefit from the addition of prebiotics to this base diet because there already were plenty of fermentable carbohydrates.

Q: Dr. Richard Hill, University of Florida: What was the water content of the food?

A: Dr. Carciofi: I did not show the results, but the water did not have an effect.

Day One, General Discussion

Kaplan-Meier Curves

Dr. Margie Scherk, Vancouver: This question is for Dr. Carolyn Cupp. With respect to the Kaplan-Meier curves that you showed from your study of the 90 cats, the curves for all three diets seem to come together around age 15 or 16 or so, and then they split apart again. I was wondering if you had an explanation for that? And also, did you notice any change in all causes of death across the three diet groups?

Dr. Carolyn Cupp, Nestlé Purina Research: Regarding the Kaplan-Meier data, I don't know that I have an explanation for why that happened. We presented preliminary data maybe three or four years ago on the pathology from the cats that had died, and I have started to look at the final data set. We appear to still see the same pattern. There did appear to be about half the number of cats on diet three, the supplemented diet with lymphocytic-plasmacytic inflammation of the GI tract and about half the number of hyperthyroid cats, compared to diet one cats. I don't know if it's significant because usually we see multiple diseases in each cat when they die. Some cats have renal and thyroid disease, and others have GI disease and cancer. So we just tabulated them. We're looking at that. Numerically there may be a reduction in renal disease as well.

Benefits of High-Protein Diets for Senior Dogs

Dr. Bill Milgram, CanCog: I was under the impression, before I came to this meeting, that senior dog food should have reduced protein. But coming from this meeting, I'm wondering whether it should be high protein. The question for discussion is should there be protein supplementation in food for senior animals?

Dr. Dottie Laflamme, Nestlé Purina Research: I will start since I've been a strong proponent for many years that restricting protein in senior dogs is the wrong approach. The evidence that strongly supports that senior dogs need more protein is limited. There's just not that much data, period. But what is out there? The Wannamacher study, the McCoy work, the work from the 1950s and 1960s that showed that senior dogs need about 50% more protein than young dogs in order to maintain protein turnover, in order to maintain lean body mass, in order to maintain normal immune function and response to stress. That work from the '50s and '60s was published and then tended to be ignored because of fear that protein causes kidney damage. Later, Dr. David Kronfeld was the big champion of increasing protein for dogs, and, of course, even though he was a strong proponent of it, he was not really successful in convincing the world of it. There's a little bit of evidence published by Dr. Dick Kealy on changes in lean body mass with age in dogs. His work showed that dogs, like cats, lose lean body mass with age and that by providing higher protein, you can help to offset that. The other work that Dr. Kevin Miller presented today from, I believe it was an Auburn study feeding 16, 24 and 32% protein, showed some changes in protein turnover. Not necessarily changes in accretion. If we go back to the McCoy and Wannamacher work, protein turnover per se is important because that helps support immune function even if it's not changing the actual protein reserves. So the point I would say is that while there's not a whole lot of evidence that dogs need more protein, unless I've missed something, there's no evidence that they need less.

Dr. Steve Ettinger, California Animal Hospital: I'm old enough to remember David Kronfeld and those arguments. In fact, for many years in practice I always told clients they should supplement their dogs' food with cooked egg every day. Just because of David's argument. So there are two things that I would ask: One is how much of all this does just the lowered sodium have to do with the benefit of the aged or elderly type diet? Might reduced sodium itself be enough? Then the second question I would ask is: The one group that seems to have published papers suggesting that there was a benefit to protein restriction is the Minnesota group, so how does that fit into the overall picture?

Dr. Dottie Laflamme, Nestlé Purina Research: Just to clarify, are you saying that they published data that seems to suggest a benefit to protein restriction in dogs with renal failure or in otherwise healthy dogs? In renal failure, OK, now that's a different scenario. You are talking about dogs that have existing chronic renal failure and there is certainly evidence that phosphorous- and protein-restricted diets are beneficial, although Del Finco's work really raises the question whether it is protein at all or simply the fact that protein and phosphorous go hand in hand. For renal diets, protein may be restricted to achieve phosphorous restriction or to control uremia in dogs with damaged kidneys. As far as restricting protein in order to protect kidneys from damage, unless I'm mistaken, there's no evidence of that either.

Dr. Fran Kallfelz, Cornell University: I think Steve has sort of hit the nail on the head, and you have also, Dottie. Like you, I have been of the opinion that older dogs need more protein, not less. And I think the idea of lowering protein in the senior diets came from the idea that non-protein-losing renal failure

was of significant incidence in older dogs, and perhaps the completely erroneous position that high protein might cause it. But I think from some of the data that was presented here today, as well as other data that I've read, there's just no evidence that non-protein losing renal failure is a major cause of mortality in older dogs. So, I think we've all been kind of sucked into something here, for years and years that we're finally, thankfully, getting out from.

Dr. Julie Churchill, University of Minnesota: My PhD thesis work at the University of Minnesota was in nutritional effects of renal aging in geriatric dogs, using the uninephectomy model. Dr. Del Finco (University of Georgia) did a similar study about the same time. Both of us were looking at what would have been similar to a "typical senior diet" at about 18% protein compared to 35% or 36% protein. In my study with uninephrectomized geriatric dogs, there was no clinical evidence of renal dysfunction during the study. The dogs were non-azotemic with normal urine concentrating ability. There was no difference, no beneficial effect at all from restricting protein. We did nitrogen balance studies that certainly could be criticized. Nitrogen balance in the low protein group was zero, with positive nitrogen in the higher protein group. We did histomophometric analysis and, again, no difference in any of the histomorphic measurements. No evidence of hypertension, any of those things.

Dr. Steve Ettinger, California Animal Hospital: So now, you're coming into my field of cardiology. If we believe that once renal failure develops then protein becomes "poisonous," then the question is where in that spectrum of the normal dog that is becoming an aging mammal that is moving toward renal failure do we look for protein restriction to make the difference? I made the comment about cardiology because, you know, one of my interests is biomarkers as they relate to identifying when things are occurring in the heart, before the damage occurs, before the modeling occurs. So, if indeed there really is this protein effect on the kidneys, then at what point would you want to not administer additional protein because you may be causing a toxic effect?

Dr. Julie Churchill, University of Minnesota: Well, I can tell you that my dogs were very old at the time of death. They were 14 and 15 years old, so I think that in a normal, aging healthy dog, the kidneys outlive the dog. Also, I asked Dr. Liz Lund who was working with Jane Armstrong in the National Companion Animal Study, and she helped me pull from the data the prevalence of kidney disease in geriatric dogs. For all causes of renal disease, the prevalence rate was less than 2.7%. So, again, I just don't think it's a disease of commonality.

Dr. Steve Ettinger, California Animal Hospital: This is just an interesting comment. Our hospital was actually involved in the original work with (Purina Veterinary Diets) EN, CV and NF. And the study director, Dr. Morris Cover, wanted to know when and why we couldn't get these dogs that are going into kidney disease and find them and treat them for a year or so? And my comment was, I don't believe in practice that you see those dogs. And yet everybody talks about all these dogs that are going into renal failure. But in clinical practice, you don't see them. You see normal dogs and then one day they come in with vomiting and polyuria or polydipsia, and they go into renal failure.

Dr. Joe Wakshlag, Cornell University: Being fairly fresh out of private practice, I think it's almost an overdiagnosis. I've got so many records that say old dog in renal failure because the BUN was 50 and that dog ate a steak that morning. And so, I think as you say, it's prevalent, but I think it's busy veterinarians saying, "Oh well, it might be renal failure,"so bang, you've got to put him on something low protein, when really that dog's a little dehydrated and kidney disease is overdiagnosed.

Dr. Dennis Chew, The Ohio State University: I want to address something. You said that veterinarians don't see these kinds of dogs. You see them every day. You don't diagnose them because you don't look at urine, and you don't look for diseases that are emerging. This is happening in every practice, in everybody's visits when they're coming in. You should look at urine. You look at urine proteins. You do specific tests to look for declining kidney function. It's a very rampant thing. I'd also like to add in, I agree that protein restriction is extremely overrated in dogs that have chronic kidney disease. There is almost no information to suggest that the protein restriction does anything for you. If the patient is severely azotemic, it might reduce some of the morbidity, but it doesn't do anything to save the kidney. And there's been some very elegant work that has separated the effects of phosphorous restriction from protein restriction. And it's the phosphorous restriction that saves the kidney and saves the animal's body; it's not the protein restriction. And there is some evidence in dogs, the Bovee work from many years ago, showing that really high-protein supplementation doesn't do anything to harm the patient that has significant underlying chronic kidney disease.

High-Protein Diets for Senior Cats

Dr. Esther Plantinga, Utrecht University: We have been only talking about dogs, and I was curious what your opinion is about high-protein diets for geriatric cats.

Dr. Claudia Kirk, University of Tennessee: I'll make two comments just because some people kind of know from whence I hail, and that is I personally think that dogs and cats do better on a high-protein diet. Certainly cats have a much higher

proportion of renal failure, but so as long as you're screening the animal appropriately, I think that it's not a problem to feed high-protein diets, and cats usually have better appetites and better lean body mass. My concern is I don't like the Wannamacher study. I think it is a poor study because it's basically using deficient proteins once you get down to the lower levels. So that's a personal bias. As we agreed, there are no other studies out there. I just don't like any study overinterpreted. So you can hear my bias and my questions on that study. In cats that we've looked at with DEXA, and I think some of the studies that Margarethe Hoenig and others have done, looking at low-carbohydrate, high-protein diets, lean body mass tends to be higher with animals fed high-protein diets. So if it's lean body mass that's important for longevity and better quality of life, then it would certainly support a much higher protein intake in the aging cats.

Aging Feline Study

Dr. Bob Backus, University of Missouri: I just want to ask Dr. Cupp a question about the feline aging study. Was the protein energy ratio the same in diet three? And was the energy and protein intake the same across the diets? The reason that I'm asking this is because we know that in most species, energy restriction prolongs life. Was there any indication that those cats in diet three ate less protein and less energy?

Dr. Carolyn Cupp, Nestlé Purina Research: Diet three, with chicory, had a little more fat added, but all diets were similar in energy and protein content. The cats were offered the same amount of calories across the groups, and actual intake did not differ between groups.

Optimal Body Condition Scores

Dr. Kathy Michel, University of Pennsylvania: My question is for Dottie Laflamme. Dottie, I need you to clarify something. I'm a little confused on some of the data both Carolyn and Dr. Perez-Camargo presented, because I'm remembering back to the study that you did using DEXA and body composition to validate the body conditioning systems. I thought that the optimal body condition scores, four to five in the dog and five in the cat, were in the 20% body fat ranges. And the cats in these studies seem to have a lot less fat.

Dr. Dottie Laflamme, Nestlé Purina Research: I think Carolyn made a good point in introducing her study that none of her cats were obese. And some of the conclusions from her study, that increasing body fat or maintaining body fat was beneficial, were because she was working almost exclusively on the left-hand side of that U-shaped curve with cats on the normal to low side. The studies were not dealing with the right-hand side where the obese animals are, where increasing body fat can be detrimental. That was a very good pickup on your part, Kathy, that she was looking at body fat percentages in the 20 to 25% or less, with body condition scores in the 3, 4 and 5 range, maybe pushing 6, but not the 7, 8, 9 like you would see in middle-aged cats in practice. This is the geriatric, skinny cat that we're dealing with.

Modeling Human Cognitive Aging in the Beagle Dog

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Summary

This paper summarizes what we have learned in the past 20 years about canine cognitive abilities, how these abilities change over time, and how canine cognition compares with human cognition. We have developed a battery of objective neuropsychological tasks to assess several cognitive domains. With respect to learning ability, dogs are proficient in tasks requiring associative learning and acquisition of simple relational rules. We've also examined performance on several tasks that assess executive function. Dogs perform well on two of these — reversal learning and selective attention — and poorly on others — notably tasks aimed at concept learning. On tasks designed to assess working memory, dogs require extensive training and show erratic performance in visual object recognition, but show much higher levels of performance on tasks assessing visuospatial memory. Most of our research has used laboratory-housed Beagle dogs. We have now extended this work to the clinic and have obtained similar data from pet dogs.

Cognitive abilities change with age in a manner that varies with cognitive domain. Tasks that involve complex learning are more sensitive to age than tasks solved more easily. These findings parallel the kinds of age-dependent cognitive changes that are known to occur in humans. There also are notable individual differences, particularly in performance on specific tasks. Some dogs can be characterized as successful agers, while others show a moderate impairment, which may correspond to a human condition known as mild cognitive impairment (MCI). Still other dogs develop more severe impairment, which may correspond to human dementia. These results model many aspects of human cognition, including the cognitive changes associated with human aging and the development of dementia.

Glossary of Abbreviations
DNMP: Delayed Non-Matching-to-Position Task
DNMS: Delayed Non-Matched to Sample
MCI: Mild Cognitive Impairment
SLL: Spatial List Learning Task
TGTA: Toronto General Test Apparatus

Why Study Cognitive Aging in Dogs?

Over the past 20 years, our research program at the University of Toronto, and later at CanCog Technologies, has focused on understanding the process of cognitive aging in the Beagle dog and participating in developing interventions to counteract cognitive decline. Our interest was based on both a desire to understand and characterize canine aging per se and the potential value of the dog as a model of human aging and dementia.

Canine aging represents a uniquely important area of study because of the multiple roles that dogs have in human society, which includes companion animals, guide dogs, military working dogs and odor detection dogs. All these functions are sensitive to the cognitive status of the dog. Furthermore, clinicians have now identified a cognitive dysfunction syndrome in pet dogs that becomes progressively more severe with increased age.[1,2]

With respect to human aging, in some individuals, progressive cognitive decline is a prominent feature that can have devastating effects on quality of life and also impose a significant economic cost to society as a whole. Animal models provide a tool to better understand aging processes and develop interventions. Until recently, cognitive aging models were largely limited to rodents and primates.

Over the past 20 years, much of our work has been focused on understanding canine cognitive abilities and the link among cognition, age and brain changes. The rationale for the specific approach we've taken was based on both parallels between canine and human aging and on practical considerations. When compared to humans, the key features of the canine model include, but are not limited to, similarities in brain pathologies and similarities in the process of cognitive decline. There is now a large body of research on neurobiological changes associated with aging in the dog. We have known for some time that the aged canine brain develops diffuse amyloid plaques similar to the senile plaques seen in Alzheimer's patients.[3] More recent work has established that, like in the human brain, canine plaques contain pathological deposits of beta amyloid protein, following a biochemical pathway

virtually identical to that seen in humans.[4] Brain aging in the dog is accompanied by structural changes associated with neuronal loss, including cortical atrophy and loss of white matter[5] and again, like the human brain, can develop cerebrovascular pathology and oxidative damage, processes that model events associated with human brain aging.[6]

This article focuses primarily on the work we've done in characterizing cognition and age-dependent cognitive decline in the Beagle dog. Because a major goal has been to utilize the dog as a model of human aging, we'll start with a brief overview of human cognitive aging. We next describe our work in characterizing canine cognition in the laboratory setting, examining how cognition changes with age and comparing the performance of dogs and humans on similar tasks. We'll also briefly discuss some new work involving development of cognitive assessment technologies for use in clinical settings. We conclude by identifying new target areas for future research.

Features of Human Cognitive Aging

Before we can effectively utilize an animal model of human cognitive aging, we must first identify what aspects of human cognitive aging we hope to model. We can identify three main features: domain specificity; individual differences; and age-associated neuropathology.

Domain Specificity

Human cognition encompasses a set of separate functions or domains, which are at least partially independent. These functional domains include executive functions (higher level functions that control other cognitive processes), visuospatial function, memory, learning of new information (episodic memory), language, and psychomotor function (link between cognitive control and motor function). Evidence of independence is supported by individual differences showing subjects can excel in one domain but do poorly in another, by data obtained from brain imaging studies that highlight differences in underlying brain circuitry, and by evidence showing that processes, such as aging, can be accompanied by greater deterioration in some functions than others. Thus, digit span, which assesses our ability to hold information into memory for a short-time period shows minimal age differences.[7] By contrast, we tend to process information more slowly, have greater difficulty in recalling previous events (episodic memory), and are slower in acquiring new information as we age.

Individual Differences in Impact of Age on Cognitive Abilities

In "successful agers," age-associated cognitive changes are relatively small and have little or no impact on quality of life, although we're apt to complain about memory lapses.[8] Successful agers are distinguished from subjects showing mild cognitive impairment and from a third group that develop dementia. The diagnostic label of MCI covers a wide range of individuals who show some degree of cognitive impairment but are not demented and can function within society. Dementia refers to a more global impairment in more than one cognitive domain that severely impacts the overall quality of life.

The existence of different subtypes of MCI provides further evidence of functional specificity. One distinction is between two primary clinical subtypes, based on whether they show a predominant memory disorder. Amnestic MCI is associated with memory disorders; non-amnestic MCI does not have a memory component. Within each of these systems, we can further distinguish subjects that show impairment in only one domain from those that show impairments in multiple domains.[9]

Link to Age-Dependent Brain Pathology

Human cognitive decline is associated with changes in the functioning of brain circuitry and with distinct lesions in the nervous system that disrupt normal functioning of nerve cells. Two such lesions, neurofibrillary tangles, resulting from the accumulation of intracellular hyperphosphorylated tao deposits, and neuritic plaques, linked to accumulation of extraceullar amyloid deposition, are widely thought to be linked to age-dependent cognitive decline, and more specifically to Alzheimer's disease.

Procedures Used in Canine Cognitive Assessment

When we started our examination of canine cognitive aging, previous research had shown that aged dogs develop similar brain pathologies to those seen in aged humans, but there was no work looking at the effects of age on cognition. As a starting point, we were guided by previous research on non-human primates in which neuropsychological strategies had been used to develop cognitive assessment measures and to use these measures to study cognitive aging. This strategy entailed developing tasks that could be linked to known brain circuitry and to distinct functional cognitive domains. Our goal was to develop comparable protocols for use with the dog. We subsequently extended this work to develop novel tasks that were uniquely suited for testing dogs.

General Methods

Toronto General Test Apparatus (TGTA)

Most of our cognitive assessment protocols have utilized a standardized test apparatus that was modified from a testing apparatus used in assessment of primates (called the Wisconsin General Testing Apparatus). The canine version, which we've called the Toronto General Test Apparatus (TGTA), consists of an enclosed chamber with height-adjustable stainless steel bars at the front.[10] The experimenter is separated from the dog by a screen with a one-way mirror and hinged door that can be raised by the experimenter. The apparatus also consists of a sliding Plexiglas tray with three or four parallel food wells

that can be covered with a variety of stimulus objects and presented to the animal by the experimenter. The current version of the test apparatus has a number of small modifications to reduce distractions and to simplify the presentation of the tray (see Figure 1). When the experimenter now slides the tray toward the animal, it automatically opens a screen to permit a response by the subject.

Setting up Different Tasks

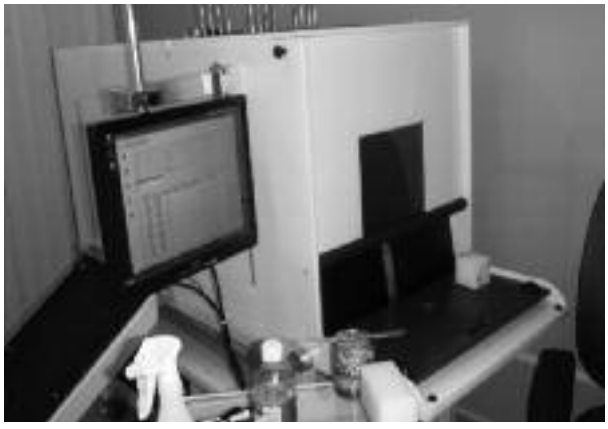
We've developed a standardized training protocol that we use in all our dogs. The dogs are initially trained to find food rewards in open food wells upon presentation of the tray. They then are taught to displace a single object covering a food well to obtain a food reward when the tray containing the object is presented by the experimenter. After learning to respond to a single object, the dogs are repeatedly presented with two distinct objects, only one of which is associated with a food reward. This task, known as object discrimination learning, provides an initial measure of dogs' learning ability.

Dedicated software is used to control timing and randomization procedures, to indicate stimuli and reward locations, to store responses, latencies and comments, and to generate and store back-up electronic files at the end of a cognitive test session. The TGTA and software allowed us to develop a variety of different test protocols, which were designed to provide assessment of domain specific cognitive functions. Figure 2 illustrates examples of how we set up different test protocols.

Learning Versus Performance Test Protocols

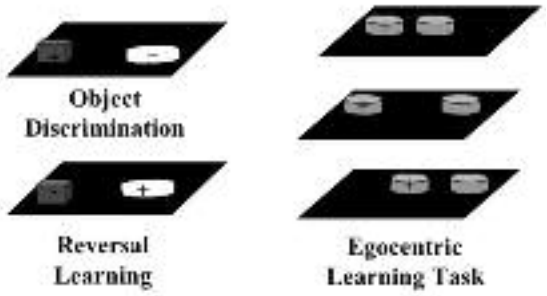
The tasks we've used fit into either of two kinds of test protocols. For the first, the animals are naïve to the task and are tested for their ability to learn a rule. The second involves testing the animals on a familiar task, which can be used to assess specific functional abilities such as memory, attention and perceptual ability.

Figure 1



TGTA: This shows the current version of the test apparatus used for cognitive assessment of canines. The supplies on the table are part of the standard equipment used in cognitive testing.

Figure 2



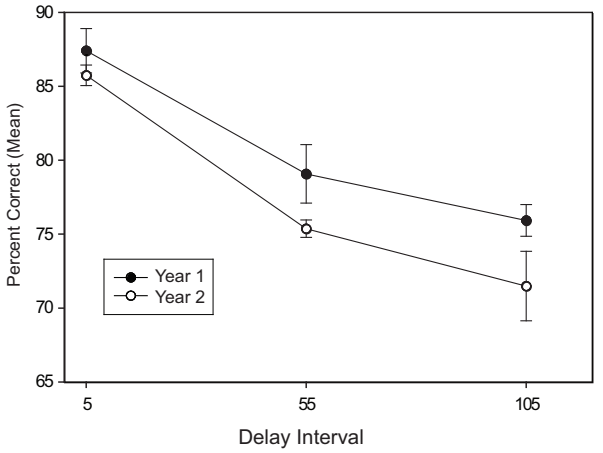
Test paradigms used in assessing discrimination and reversal learning (left panel) and egocentric spatial learning (right panel). In discrimination learning protocol, subjects are presented with two objects, one of which is associated with reward and the other with non-reward. In the reversal task, the associations are switched so that the object initially associated with reward is now associated with non-reward and vice versa. In the egocentric task, subjects are presented with two identical objects using three food wells and are always rewarded for responding to the object closest to their left (or right). Thus, the center well is the correct response when the alternative is to the animal's right, and the incorrect response when the alternative is to the animal's left.

Learning Protocol: In training animals on a new task, our standard training entails testing animals repeatedly, once daily over a fixed block of trials until the animals either achieve a predetermined criterion level of performance or unsuccessfully complete a maximum number of test sessions. Thus, we customarily set an upper limit on the number of days that an animal will be tested before concluding that the animal is unable to learn the task. Our learning criterion typically requires that the subjects first demonstrate performance accuracy of at least 80%, and second, that they can maintain a 70% level of performance over subsequent testing.

One task we used frequently is called the delayed non-matching-to-position task (DNMP) and requires the subjects to first learn a specific rule about the location of food and to then hold in memory the location of a test object for a variable amount of time. The task consists of two components: a sample component, in which a stimulus is presented at one of three locations, and a test component, in which identical stimuli are presented at two locations, including the location of the sample. To learn the task, the animals must learn to respond to the new location on the test trial.

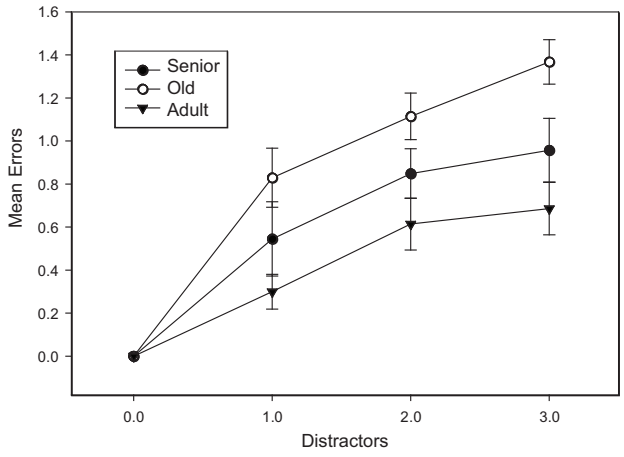
Assessment of Working Memory, Perceptual Abilities and Attention: In the second type of protocol, the animals are tested on a task they have previously learned, but the test conditions are modified to allow assessment of specific cognitive domains. For example, after an animal has learned the DNMP, the time

Figure 3



Performance on the DNMP as a function of delay between presentation of sample and test stimuli. The above data were obtained from the same group of highly experienced six animals one year apart. The results show that accuracy of performance falls off as a function of delay. The performance decrement in the second test year is probably linked to the fact that the animals are one year older.

Figure 4



Performance of adult (3 to 4 years of age), old (8 to 9 years of age) and senior (>10 years of age) dogs on the attention task. Subjects were presented with 0 to 3 distracters. Subjects were tested over seven days, with each condition occurring three times per day. Y-axis indicated mean errors, out of a maximum of eight. Increasing the number of distracters was particularly disruptive for senior dogs.

interval (delay) between presentation of sample and presentation of the test stimuli can be increased, which increases the memory demands required of the animal. The longer the delay, the longer the information must be held in memory and the poorer the animal is likely to perform (see Figure 3).

Cognitive Tests and Capabilities

Task Acquisition

Table 1 summarizes the tasks and cognitive domains that we've studied thus far and what is known about age and species differences. Table 1 reveals that Beagle dogs are capable of learning a wide variety of tasks, which includes associative learning, more complex rule-based learning and psychomotor learning (skill learning). Associative learning requires animals to learn that two events are related and can be assessed using discrimination learning tasks in which subjects are trained to associate a specific object with reward (discrimination learning) and positional learning, in which dogs learn to respond to objects based on location (e.g., egocentric discrimination). Dogs also are capable of more complex learning, including the DNMP (described previously) and utilizing the location of an external cue to determine the location of reward (landmark discrimination). Finally, we've found that dogs can rapidly learn to acquire specific skills (psychomotor learning) in which they learn to perform a new motor response to obtain a reward. We can assess this with a reaching task in which dogs are required to use their paws to pull a coaster toward them, a response that's typically outside their normal behavioral repertoire.

We've also found that dogs can perform at high levels on a set of tasks designed to assess executive function, which includes reversal learning and a new test we've developed to assess selective attention. In reversal learning tasks, dogs are first taught a discrimination learning task, to respond selectively to one of two objects or an object in a specific location. Next, in the reversal phase, the relationship between the objects or location and reward are switched, and the animals must learn to respond to the previously unrewarded object or location. Although most dogs can learn the reversal task, it usually takes about twice as many training trials as does the original discrimination task. In the selective attention task, the animals are presented with a correct object and from 0 to 3 incorrect objects, all of which are identical and serve as distracters. The task requirement is to ignore the incorrect objects and, as Figure 4 shows, we observe that the greater the number of distracters, the greater the number of errors.

A second subset of tasks can be learned by only a small proportion of animals. There are two examples. The first is the delayed-non-matching-to-sample (DNMS), which is intended to provide a measure of recognition memory. The task entails presenting dogs with a single object and allowing them to respond to obtain reward. They are then presented with two objects, the sample object and a second novel object, which is now associated with reward. Thus, this task requires them to remember what the sample object was and then to respond to the novel object. When we initially attempted to train dogs on this task, they were able to perform at better than chance, but they were not able to achieve the learning criterion.[10] We subsequently found that learning could be improved if the

objects were first presented to the dog at its near point; when the distance was closer, the dogs probably had difficulty focusing.[14] Nevertheless, even under optimal conditions the task is difficult and only a small proportion of dogs are able to master it.

The second is called spatial list learning task (SLL) and involves three phases. In the first, the dog is presented with a single object in one of three locations. In the second, the dog is presented with two identical objects: one in a new location (the correct response) and one in the same location. On the third trial, the dog is presented with three of the identical objects and must respond to the location that was not used in either of the first two trials. Performance varies with age and memory demands, with aged dogs doing much more poorly than young dogs.

Cognitive Limitations

A third category of tasks are ones for which we have not been able to convincingly demonstrate learning. These include both true oddity task and conditional discrimination tasks. The oddity task involves presenting dogs with three objects, two of which are identical and the correct response is to select the

Table 1. Summary of tasks used in canine cognitive assessment, domain associated with task, effects of age and comparison with humans (N/A indicates that comparable data is not available).

Task	Cognitive Domains	Performance of Adult Dogs	Age Effects in Dogs	Normal Humans	Dementia
Discrimination Learning[10,11]	Associative Learning	Acceptable, show learning set	Varies with similarity of objects	Use rule based strategy	Similar to dogs
Reversal Learning[10,11]	Executive Function	Acceptable	Declines with age	No reversal learning effect	Similar data to dogs
DNMP Acquisition[12,13]	Complex Learning	Acceptable	Strongly Correlated	Moderately difficult	Very difficult task
DNMP – Performance[13]	Episodic Working Memory	Generally Excellent	Greater individual differences	Parallels work with humans	N/A
DNMS – Acquisition[10,14]	Recognition memory	Very difficult task	Highly Signficant	Easy task	Difficult
Spatial List Learning[15]	Episodic Working Memory	Very difficult task	Age-Dependent Deficit	No Data	N/A
Landmark Discrimination[16]	Allocentric Spatial	Requires special training	Age-Dependent Deficit	N/A	N/A
Attention Task[17]	Selective Attention	Acceptable	Highly sensitive to age	Similar age-effects in humans	N/A
Concurrent Discrimination (unpublished)	Episodic memory	Yes – varies with difficulty	N/A	N/A	N/A
Oddity (unpublished)	Concept Formation	Difficult or impossible	Difficult or impossible	Very easy task	N/A
Conditional Discrimination (unpublished)	Relational Strategy	Difficult or impossible	N/A	N/A	N/A
Motor Reaching Task (unpublished)	Psychomotor Function	Large majority learn rapidly Some	Small age deficit	Varies with task difficulty	N/A
Concept Extraction[18]	Executive function (Concept Formation)	Show simple concept abstraction	Slower learning in aged dogs	N/A	N/A
Learning Set Performance[19]	Acquisition of Higher Level Rules	Successfully show learning sets	N/A	N/A	N/A

odd object. In the true oddity task, each set of three objects are presented only once. Thus, accurate performance requires the animals to learn the oddity concept. We have shown that dogs can only learn to select the odd object, if they are repeatedly presented with the same three objects,[20] but they are unable to learn the oddity concept when unique object sets are used.

For conditional discrimination, the animals are presented with a choice of two objects, with the correct response being contingent on another event. When we originally set this up, the animals were presented with identical objects in the left and right food well, and one of two objects in the center food well. The rule was to respond to the left when the center object was a circle and to respond to the right when the center object was a triangle. We've been unable to demonstrate learning, despite giving dogs up to 1,000 training trials.

These results suggest that dogs are incapable of acquiring higher level concepts. On the other hand, we have found that dogs are capable of distinguishing objects based on size and of responding based on relative size, thus demonstrating concept abstraction.[18]

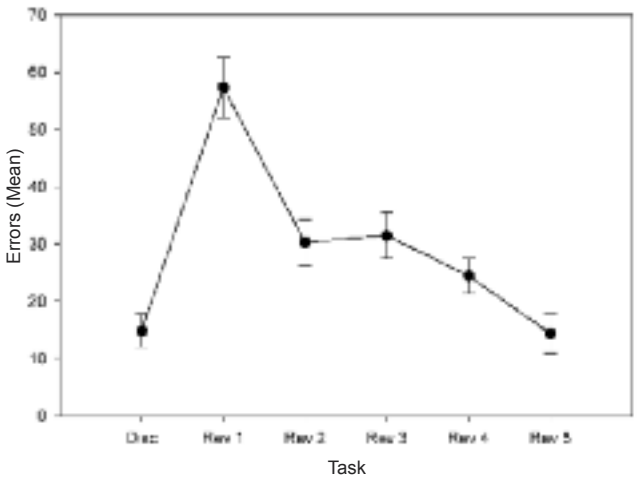
Role of Experience

Previous experience is important in two respects. First, animals that have had considerable cognitive experience perform better overall than animals that have had little experience. This is important when looking at age differences in which age-differences are seen most clearly if animals are highly experienced.[21] Thus, experienced young animals learn faster than experienced old animals. Inexperienced young and old animals, by contrast, show a much smaller age-related difference.

One of the reasons that experience is important is because dogs are able to learn general rules that can be used in solving new cognitive protocols. We have known for some time that when dogs are repeatedly tested on the same type of problem, they show progressively better performance and may achieve mastery of the problem.[18] In some cases they become sufficiently proficient to be able to learn the correct response in a single trial. This is an example of the development of a learning set and is illustrated in Figure 5.

Experience also differentiates animals with respect to accumulated knowledge. Dogs that are well-trained on the DNMP at a young age, for example, are apt to perform at reasonably high levels when tested later in life — even at an age when learning the task is very difficult.

Figure 5



Development of a learning set. Beagle dogs were first tested on an egocentric discrimination and subsequently on a series of egocentric reversal tasks. The group showed progressively better performance over successive reversal and eventually learned reversal more rapidly than initial discrimination. Moreover, some animals reach a stage of proficiency in which they can learn the reversal task in a single trial.

Domain Specificity

Several lines of evidence indicate that the tasks we've developed span functionally distinct cognitive domains. First, there are clear differences in the effect of age on task performance, which we've already alluded to and will discuss in more detail in the following section. Second, specific interventions have been found to have different effects on some tasks but not on others. For example, adrafinil, a highly effective stimulant, was found to significantly improve performance on discrimination learning tasks[20] but to impair performance on DNMP.[21]

Another way of demonstrating domain specificity is by testing a group of animals on a battery of tasks and calculating the correlations between tests. Table 2, for example, is based on data from 36 animals, 8 to 15.2 years of age, tested on DNMP at 20 and 90 seconds, a discrimination learning task and two tests of selective attention. The DNMP at 20 and 90 seconds are highly correlated with each other (see Figure 6). At a 20-second delay, the DNMP is weakly correlated with discrimination learning and the "same" attention task. At the high delay, the correlation is even lower. These results suggest that the cognitive processes underlying DNMP performance, discrimination learning and selective attention must be at least partially distinct.

We've also examined performance of the tasks combined by converting data into Z scores. The results reveal relatively small groups of high-performing and low-performing animals, and a larger group performing closer to average, suggesting a distinction between dogs that show global cognitive impairment and dogs that show more selective deficits to specific cognitive domains.

Table 2. Correlation matrix between four cognitive assessment measures and age.

	AGE	DNMP 20 (Accuracy)	DNMP 90 (Accuracy)	DISCR (Errors)	ATTENT SAME	ATTENT DIFF
AGE	1.00	-0.12	0.12	0.41	0.26	0.07
DNMP 20 (% correct)	-0.12	1.00	0.86	-0.23	-0.19	0.01
DNMP 90	0.12	0.86	1.00	-0.08	-0.08	-0.06
DISCR (errors)	0.41	-0.23	-0.08	1.00	0.71	0.16
ATTENT SAME (errors)	0.26	-0.19	-0.08	0.71	1.00	0.38
ATTENT DIFF (errors)	0.07	0.01	-0.06	0.16	0.38	1.00

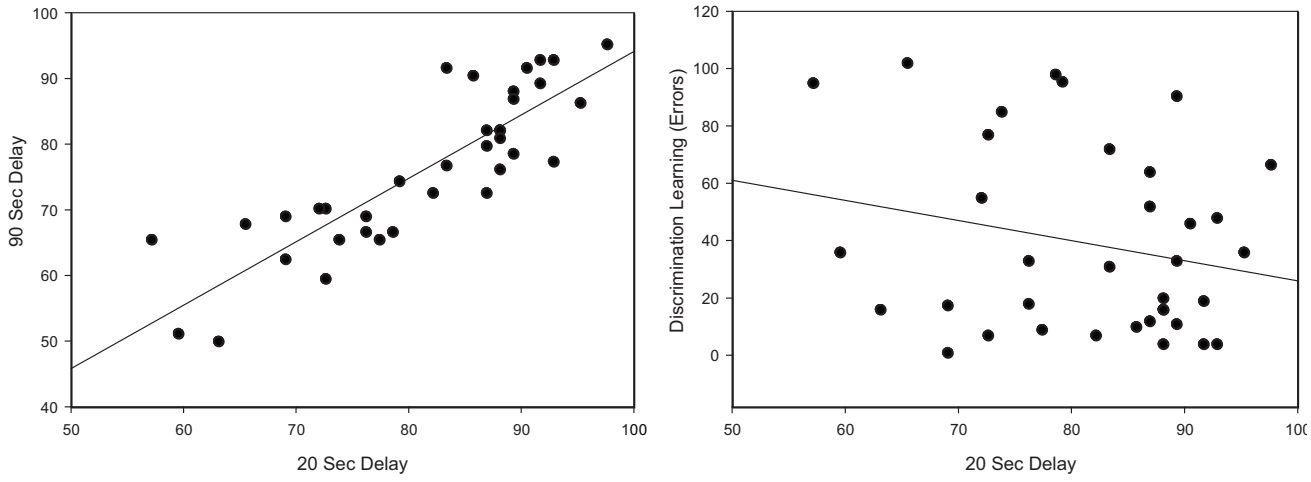
Individual Differences and Changes Associated with Age

We have completed a substantial amount of work dealing with cognitive changes associated with age. We typically begin to see impairment in Beagles as young as 5 years of age, depending on task and previous experience. The differential sensitivity to task is summarized in Table 1.

Simple discrimination learning shows relatively little change with age — dogs generally perform quite well at learning to distinguish objects that differ in more than one dimension (e.g., size, shape and color). Performance, in general, deteriorates when dogs are required to discriminate more similar objects. For example, we see significant age differences when dogs are tested on a size discrimination learning task, in which the dogs are asked to discriminate between two red blocks that differ in size, but not in color or shape.[11] Age differences also are enhanced when dogs are tested on an oddity discrimination task in which they are required to learn to select the odd object when the animals are given a choice of responding to one of three, with two being identical and incorrect.[22] This is a discrimination learning problem, rather than a concept learning problem, because the dogs are repeatedly tested with the same object set.

Reversal learning tasks also tend to show greater age sensitivity than discrimination learning,[10,11] indicating an executive function deficit. Greater age differences are seen in more complex learning in which animals are required to learn a more general rule. Figure 7 illustrates the effect of age on

Figure 6



Acquisition of DNMP as a Function of Age Range. Scatter plots showing relationship between performance on the DNMP at 20-second delay with a highly correlated task (performance at 90-second delay – top panel) and with performance on a discrimination learning task (bottom panel). Note that although the correlation with discrimination learning is low, the highest performing dogs tended to acquire the discrimination learning task more rapidly.

acquisition of DNMP, a task that shows marked age sensitivity. Note also that the spread of scores increases with age. This is a reflection of individual differences among aged groups, which can be subdivided into dogs that show successful aging and are high performing, dogs that show impairment, and dogs that differ from young dogs by more than two standard deviations and may be classified as demented.[23]

The DNMP also can be used to demonstrate age differences in memory capacity. When training experience is equated between young and old animals, the young animals perform more accurately and show greater improvement with practice. However, with extended practice a subset of old animals are able to show further improvement. A second subset, aged impaired animals, show substantial impairment at long delays.[24]

Clinical Assessment of Cognitive Ability in Companion Animals

Although the number of aged pet dogs is huge, the total population of aged laboratory-housed dogs is small. Furthermore, restricting our sample to Beagle dogs neglects the huge potential diversity that exists among different breeds. To increase the available population of dogs and to obtain evidence about the effect of breed, we have recently developed a portable cognitive test apparatus and accompanying protocol for use in the clinical setting. This project was initially designed and carried out by Dr. Paolo Mongillo over the course of a year sabbatical taken at CanCog in Toronto. Preliminary data were first presented in 2009 by Dr. Mongillo at the annual Meeting on Canine Cognition and Aging in Niagara-On-the-Lake, Ontario. The test box was similar to our standard test apparatus with a few modifications to increase its portability and to enable testing of larger breeds.

The test procedure was also modified to allow training on several tasks within a short time frame. We therefore used a less stringent learning criterion, in which subjects were deemed to have learned when they obtained a string of five consecutive correct responses. Thus far, we've obtained complete data on four tasks from 11 pet dogs and 8 laboratory-housed Beagles: reward approach learning; object approach learning; discrimination learning; and reversal learning. As shown in Figure 8, these data show very similar results from pet and laboratory-housed dogs, and validates the use of this technique for objectively assessing cognition in clinical trials.

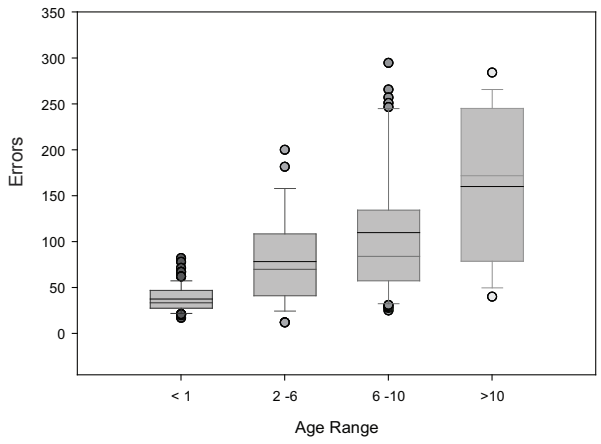
Cognitive Assessment in Human Populations Using Parallel Testing Protocols

One of our goals in examining dogs as a potential model for human cognitive decline is to develop tasks that are translational, which require age-related task differences to predict how different human populations will respond. To advance this goal, we have developed a battery of test protocols that we can use to assess human subjects, and we have used these to obtain preliminary data from various human populations including Down syndrome,[25] children with Fragile X disorder,[26] autistic children, aged populations,[27] and patients with dementia. The specific tests were set up in a manner that paralleled the setup used in canine assessment. Thus, we developed a specialized test apparatus (Figure 9) and dedicated software based on the canine model adapted for testing humans. The learning criterion we used was the same as used in the clinical testing of pet dogs. That is, a subject was deemed to have learned a task if it obtained five consecutive correct responses.

Figure 10 shows data we've obtained from several different populations of human subjects on an object discrimination and reversal learning task. The left-hand panel illustrates that performance is impacted by age and that the tasks are also sensitive to cognitive impairment. One notable result was that both the oldest of the normals (> 70) and Alzheimer's patients performed more poorly on the reversal task than the original discrimination, illustrating a reversal learning deficit consistent with that seen in dogs. Younger human subjects, by contrast, learn the reversal task more quickly than the original discrimination. The right-hand panel demonstrates that these tasks can also be used to detect developmental abnormalities, as evidenced by the extremely poor performance of the Fragile X group on the reversal learning task.

Figure 11 compares groups of humans on two tasks, DNMP and DNMS, and for comparative purposes shows how dogs perform on the same two tasks. Note that DNMS is acquired more rapidly than DNMP by all groups of human subjects,

Figure 7



A box and whisker plot showing acquisition of DNMP task as a function of age group. The Y-axis represents errors to achieve a criterion level of performance at a delay of 5 seconds. The boundaries of the box represent the 25th and 75th percentiles. The whiskers indicate the 10th and 90th percentiles. The solid line shows the median, and the fainter line shows the mean. Note that both mean errors and variability (spread) increase as function of age.

while for dogs, it's the opposite. These species differences probably reflect the importance of object recognition to the human and accompanying specializations in the human brain. Overall, these results suggest that the tasks we've developed in dogs can also be used in assessment of human subjects and that they can be used to detect abnormalities. Moreover, human subjects showing cognitive impairment or dementia are more impaired on DNMP than DNMS, suggesting that the canine DNMP, in particular, can serve as a transformative model for human assessment.

General Discussion

The research we have completed thus far has accomplished several goals. First, it's provided us with an extensive database to better understand the cognitive capabilities of the dog. Second, it's provided several age-validated model systems that can be used to assess the effectiveness of potential interventions and hopefully further our ability to develop treatments for dementia and Alzheimer's disease. Finally, the work has helped us understand how cognitive capabilities change over the course of aging.

What Have We Learned About the Cognitive Capabilities of the Dog and the Effects of Age?

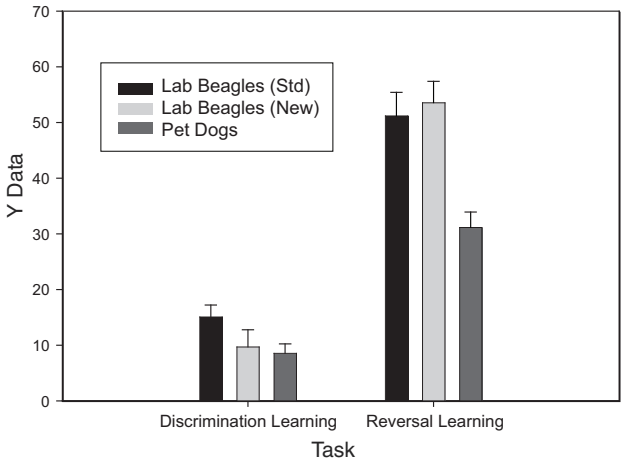
Cognitive Capabilities of Dogs

There is an accumulating literature on what dogs are and are not capable of doing. Most of this work, however, has been done with small sample sizes and is subject to alternate interpretations based on the dogs' ability to utilize cues associated with the tester. Our focus, therefore, has only been on the work that's been done by our group. We've discovered that the cognitive capabilities of dogs are extensive but are also limited by their sensory and motor capabilities. While dogs are capable of learning a DNMS task, it is a very difficult task, particularly for aged dogs. We suspect that the difficulty of this task reflects limitations in the dogs' visual system, which is better designed to detect movement and location than to detect complex shapes and objects. This suggestion is consistent with evidence that dogs are more capable of learning a recognition.

A second limiting aspect is the amount of information that dogs are required to retain. One example that illustrates this is the concurrent discrimination task. When we first tried to test dogs on this protocol, we used 10 different object pairs and did not find any dog that learned all the pairings. To the contrary, it was not clear whether the dogs were able to learn much of anything as there was simply too much information. We have now retested a group of animals with only three object pairs, and this made the task solvable; seven of eight animals were able to learn all the problems over a 37-day test period.

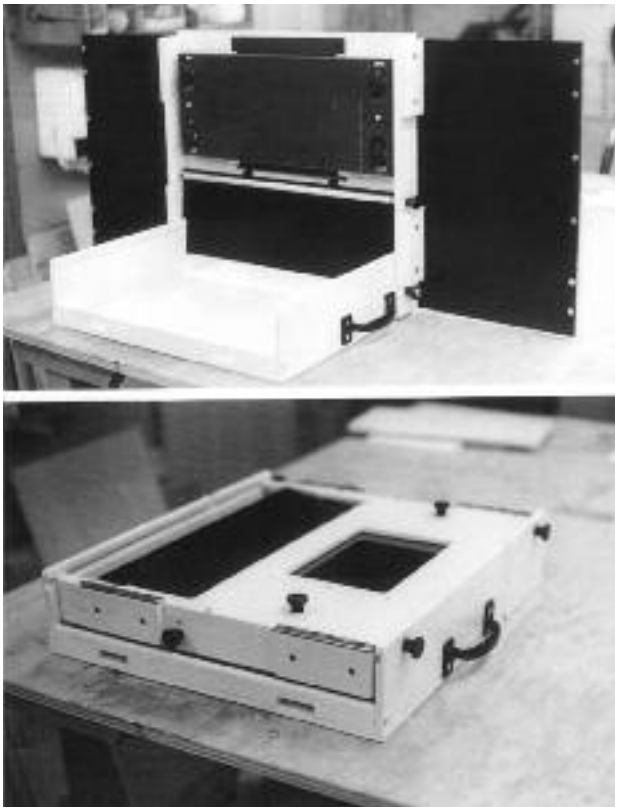
A third limiting factor is the nature of the problem that the animal is asked to solve. Dogs appear to be incapable of learning tasks if the rule is abstract and requires the animals to learn a novel concept, such as oddity or conditional respond-

Figure 8



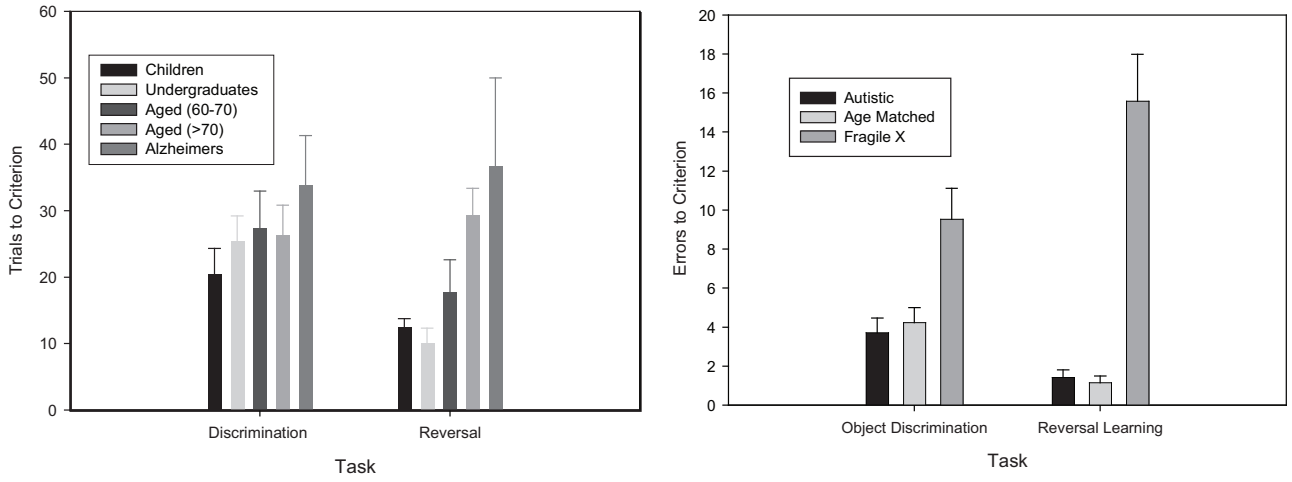
Discrimination and reversal learning in pet and laboratory dogs. This figure compares laboratory Beagle dogs trained on a discrimination and reversal learning task using our standard training procedures with laboratory and pet dogs trained using modified test procedures.

Figure 9



Portable cognitive test apparatus used in assessment of human cognition, which was modeled after the apparatus used in assessment of dogs.

Figure 10



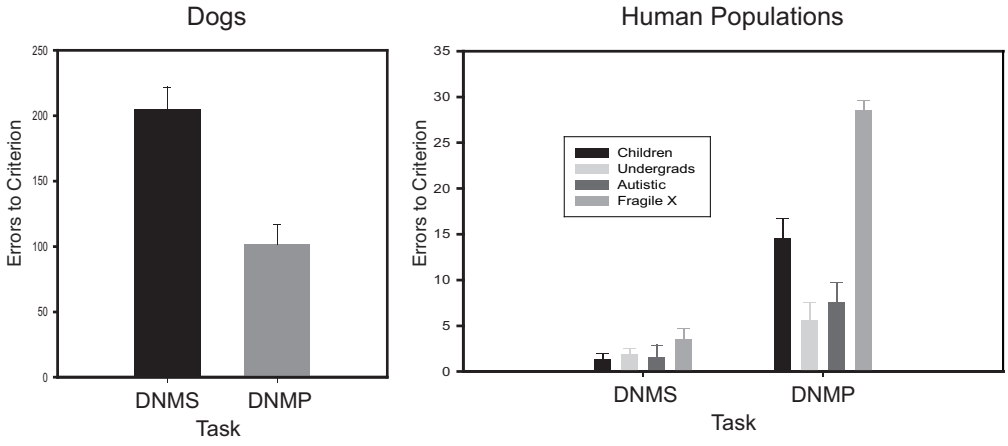
Trials to criterion on an object discrimination and reversal learning task for groups of human subjects that differ in age and level of cognitive function.

ing. This conclusion, however, may be constrained by the specific task or modality. Pietrzykowska and Soltysik[29] were able to train dogs on an auditory task in which they were presented with two tones and were required to make a particular response to the second tone only if it was the same as the first. They were able to solve the task, suggesting the ability to learn the concept of "sameness." However, the same dogs were unable to learn the same type of task when presented with photic stimuli.[30]

Distinction Between Fluid and Crystallized Intelligence

Another key factor determining the performance of dogs is their previous cognitive test history; dogs show the ability to benefit from experience. Psychologists studying human cognition have found it useful to distinguish between knowledge that we've acquired, which is referred to as crystallized intelligence, and the ability to think logically and solve problems in novel situations, which is referred to as fluid intelligence. The distinction can also be made for the dog, but it seems clear that their level of fluid intelligence is far below that of the human. Thus, their inability to learn oddity or conditional discrimination tasks indicates limited fluid intelligence. By contrast, once dogs have had experience learning discrimination and reversal problems, they are able to solve subsequent problems more rapidly. Further evidence that dogs have high levels of crystallized intelligence is demonstrated by the fact that once dogs are trained on DNMP, they retain it and are able to perform at reasonably high levels up until they reach advanced age; this is despite the fact

Figure 11



DNMP and DNMS learning in human and canine groups. The left panel shows that dogs make many more errors in learning DNMS than in learning DNMP. In this experiment, the animals were given a maximum of 400 trials to learn each of the tasks, and the majority of dogs failed. The left-hand side compares four groups of human subjects – undergraduate students, autistic children, age-matched controls, and children with Fragile X syndrome. All the human groups showed very rapid learning of DNMS. All the groups learned DNMP more slowly, and there were much greater group differences.

that aged dogs are generally unable to learn the task or do so only after extensive training.

Use of the Dog as a Model of Human Cognitive Aging

A major goal of our research program with dogs was to develop assessment protocols that could be used to predict how human subjects would respond to therapeutic interventions designed to improve cognitive function. Evidence supporting the use of the dog includes similarities in brain pathology associated with aging and general parallels in the process of cognitive decline.

On one hand, individual tasks are not always going to be comparable. We've seen that dogs perform significantly better on memory tests of visuospatial function than object recognition, while the opposite is true for humans. This difference probably relates to differences in the functioning of the canine and human visual system. On the other hand, there also are qualitative differences between human and canine cognitive capabilities, as evidenced by the inability of dogs to acquire concepts, such as oddity, that are easily demonstrated in humans.

Perhaps the more interesting comparisons between dogs and humans are those linked to neurobiological disorders, including developmental disorders associated with pathological brain aging. For example, dogs, aged humans, children with Fragile X disorder, and patients with dementia all show reversal learning deficits, which is indicative of executive function deficits and suggest that dogs can model both human developmental disorders and pathological cognitive aging.

Future Directions

There are two new research areas that we hope to pursue in future studies — breed differences and social cognition. Breed differences in canine intelligence is of general interest but has not yet been systematically evaluated using objective techniques.[31] We hope to increasingly participate in clinical assessment of canine cognition, with the goal of obtaining a large database of cognitive performance on our tasks as an outcome of clinical testing.

Regarding social cognition, this relates directly to the nature of interactions between dogs and other dogs, particularly between dogs and humans, and is an active area of investigation. One aspect that has not yet been critically examined, however, is how social cognition is affected by age. We're planning collaborations with Dr. Lisa Lit to investigate social cognitive interactive behaviors in dogs. This includes the possibility of using the dog as a model for social behaviors relevant to disorders such as autism.

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Q&A Discussion

Q: Dr. Barb Kitchell, Michigan State University: How did you control for olfactory cues in the way that the objects were placed or positioned for the dogs, since they are so much more acute in the olfactory sense?

A: Dr. Milgram: What we did, what we do routinely, is we have a coaster and an object placed on top of the coaster. On the bottom of the coaster we stick food so that the amount of food between the two wells is equivalent. They can't get at it because if they knock the coaster down it's under the coaster, not in the well. And to demonstrate that we have controlled for olfactory cues, we've done further work and have shown that dogs actually are unable to learn if the odor is hidden. What some of the dogs did, and what we also had to control for, was that some dogs would lick one coaster and use that as a cue. The only way we could control that was wiping off

the top of the coaster, which we do after every trial. So basically we masked the odor by presenting the food in both wells.

Q: Dr. Ake Headhammer, Sweden: I'm glad to see that you are interested in going on to breed differences. I wonder if you have been thinking of comparing Beagle dogs selected for laboratory use compared to Beagles selected for hunting. Have you seen any differences between genders?

A: Dr. Milgram: We have never seen gender differences. And we've looked really hard. As far as using dogs that are bred for hunting, we hadn't really planned to do a direct comparison. When we started this research in the '90s, our old dogs were all retired hunters. We now get the dogs from commercial breeders, so we've actually had a lot of experience with both retired hunters and those bred for laboratory use. And basically we've never seen any real differences between them, and that's sort of interesting because these dogs would have had a lot of experience in the real world.

Recognizing and Managing Cognitive Dysfunction in Dogs

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Abstract

With increasing age, some dogs develop a neurodegenerative disease that is commonly referred to as canine cognitive dysfunction syndrome (CDS). Diagnosis of CDS can be clinical or based on laboratory tests. The main behavioral changes associated with CDS are disorientation, altered interactions with people or other animals, sleep-wake cycle alterations, housesoiling, and changes in activity level. Ruling out medical conditions that can cause similar changes in behavior is important when performing a clinical diagnosis. Management of CDS includes dietary and pharmacological intervention. Dietary treatment of CDS has been based on the use of antioxidants and mitochondrial co-factors, and recent work has shown that long-term supplementation with medium-chain triglycerides can improve cognitive function in aged dogs. CDS must be considered an animal welfare issue, and the implications of this are discussed in this paper.

Glossary of Abbreviations
CDS: Cognitive Dysfunction Syndrome
CRF: Corticotropin-Releasing Factor
CRH: Corticotropin-Releasing Hormone
GAS: General Adaptation Syndrome
HPA: Hypothalamic-Pituitary-Adrenal Axis
SA: Sympatho-Adrenomedullary

Introduction

With increasing age, some dogs develop a neurodegenerative disease that is characterized by a gradual decline in cognitive function and is commonly referred to as canine cognitive dysfunction syndrome (CDS).[1] Interest in CDS has rapidly grown as it has been realized that CDS has many similarities with Alzheimer's disease in humans. Clinically, CDS may cause disorientation, altered interactions with people or other animals, alterations in the sleep-wake cycle, changes in activity level and housesoiling, among other signs.[2] The objectives of this paper are to: 1) briefly review the diagnosis as well as the dietary and pharmacological treatment of CDS, and 2) discuss the animal welfare implications of CDS.

Recognizing CDS

Diagnosis of CDS can be clinical or neuropsychological, e.g., based on laboratory tests. Apart from the clinical signs mentioned earlier, CDS may cause other signs, including an increase in anxiety, one of the main underlying factors of many behavioral problems in dogs.

Prevalence of CDS increases with age. According to one study[3] of 180 dogs, 28% of owners of 11- to 12-year-old dogs reported at least one clinical sign consistent with CDS, and this percentage increased up to 68% with owners of 15- to 16-year-old dogs.

Ruling out medical and behavioral conditions that can cause changes in behavior in geriatric dogs is important when performing a clinical diagnosis. For example, aging may lead to changes in the hierarchical relationship between dogs living in the same household, and this, in turn, may cause aggression. Also, animals that have impaired senses, physical debilitation or painful conditions may become more aggressive.[4] Other diseases that should be considered as differential diagnoses include renal and hepatic diseases, diabetes insipidus, Cushing's syndrome, diabetes mellitus, pancreatitis, cardiovascular and respiratory disease, and urinary incontinence.[4]

What is DISHA?
The acronym DISHA is frequently used to describe the main behavioral changes associated with CDS: disorientation, altered interactions with people or other animals, sleep-wake cycle alterations, housesoiling, and changes in activity level.[2]

Clinical diagnosis is based on the owners' report and therefore may be subjective and not very sensitive. A number of laboratory tests that measure the learning abilities and the spatial memory of dogs have been developed and provide valuable information on cognitive impairment and response to treatment, as recently reviewed.[5]

Managing CDS

Management of CDS includes dietary and pharmacological intervention. Additionally, changes in the environment may be extremely helpful and are dealt with in a different section of this paper.

Dietary Treatment

Dietary treatment of CDS has been based on the use of antioxidants and mitochondrial co-factors that may decrease the deleterious effects of free radicals. There is ample evidence suggesting that free radicals play an important role in aging; the brain is particularly susceptible to the effects of free radicals, as it has a high rate of oxidative metabolism, a high content of lipids and a limited ability for regeneration.[6] It has been shown that antioxidants improve the performance of aged rodents,[6] and several studies show that an antioxidant-enriched diet improves cognitive performance in senior dogs.[7]

Recent work has shown that long-term supplementation with medium-chain triglycerides can improve cognitive function in aged dogs. The underlying mechanism appears to be an increase in the circulating levels of ketones, which provide the brain with an alternative energy source.

Pharmacological Treatment

Selegiline, which is a selective inhibitor of monoamine oxidase B, is the first therapeutic agent approved for the treatment of CDS in dogs. The mechanisms by which selegiline has a positive effect on dogs with CDS are not clear. However, at least the following effects may have a role: an increase in dopamine activity in the cortex and hippocampus, a decrease in free radical load in the brain, and a neuroprotective effect on dopaminergic, noradrenergic and cholinergic neurons. The recommended dose is 0.5-1 mg/Kg daily in the morning, and if there is no significant improvement after one month, the dose can be increased for another month.[5,8]

CDS as a Welfare Problem

What is Animal Welfare?

Before discussing whether and why CDS is an animal welfare problem, it is useful to provide a short overview of our current understanding of the basic principles of animal welfare. Definitions of animal welfare can be grouped into three main approaches: a "feeling-based" approach, a "functioning-based" approach, and a third set of approaches in which welfare is measured by assessing whether the animal can live according to its inherent "nature."[9]

According to the "feeling-based" approach, animal welfare involves the subjective feelings of animals, so that welfare will be reduced by negative subjective states, such as pain and fear, and improved by positive states. The task for science, therefore, is to study the subjective experiences of animals.[9] The main problem with this approach, however, is that subjective experiences cannot be measured directly. This raises a long-standing debate whether trying to study them falls within the realm of science. A thorough discussion of the arguments involved in this debate is beyond the scope of this paper, and the reader is referred to a review by Dawkins[10] and references therein for further information.

Many scientists agree that although an animal's experiences of suffering are the defining traits of its welfare, such experiences are difficult to assess, whereas measures based on biological functioning and the animal's ability to cope with the environment provide relevant information. Indeed, one of the most frequently cited definitions of animal welfare is that the welfare of an individual is its state as regards its attempts to cope with its environment.[11] This definition refers to both how much has to be done in order to cope with the environment and the extent to which coping attempts are succeeding. Impaired life expectancy and reduced ability to grow or reproduce are examples of indications of failure to cope. Attempts to cope include emergence physiological responses and a variety of behavioral changes. All these measures can be integrated in an assessment of welfare that is objective and independent of moral considerations.[12]

It is important to emphasize that although the different approaches to animal welfare do not always give rise to the same conclusions, very often they do. In particular, research indicates that the feeling-based and functioning-based interpretations often correspond.[9] Indeed, pleasant and unpleasant feelings — including suffering — are part of the experience of an animal when it attempts to cope with its environment.[12] According to Dawkins,[10] suffering occurs when the animal is unable to perform those behaviors that would allow it to cope with its environment.

In 1993, the United Kingdom Farm Animal Welfare Council proposed the so-called Five Freedoms,[13] which have become a widely used framework to define and assess welfare. Although primarily developed for farm animals, the five freedoms can also be used in companion animals. The five freedoms are:

1. Freedom from thirst, hunger and malnutrition — by ready access to fresh water and a diet to maintain full health and vigor.
2. Freedom from discomfort — by providing a suitable environment including shelter and a comfortable resting area.
3. Freedom from pain, injury and disease — by prevention or rapid diagnosis and treatment.
4. Freedom to express normal behavior — by providing sufficient space, proper facilities and company of the animals' own kind.
5. Freedom from fear and distress — by ensuring conditions that avoid mental suffering.

The five freedoms combine elements from the three approaches to welfare explained above and are a very useful framework to identify the main welfare problems in a given production system and also to use as a starting point to select the main welfare indicators.

Why Is CDS a Welfare Problem?

Many of the behavioral changes and medical conditions associated with aging may impair the welfare of the animal as defined by the five freedoms. For example, several diseases may result in pain and/or interfere with the expression of normal behavior. However, as for CDS in particular, its main effect on welfare is that, as already mentioned, it may cause an increase in anxiety and stress, particularly when animals are exposed to novelty.

In 1929, Cannon described stress as the sympatho-adrenomedullary (SA) system's attempt to regulate homeostasis when threatened by a variety of aversive stimuli.[14] Later, Selye conducted some of his classic studies on the response of the hypothalamic-pituitary-adrenal (HPA) axis to noxious stimuli; Selye suggested that the organism reacted in a nonspecific manner to a wide variety of aversive stimuli, and this stress reaction was termed "general adaptation syndrome."[14] More recently, Mason[15] suggested that the psychological component of the aversive stimuli is the main determinant of the stress response. For example, animals that could control and/or predict the occurrence of an electric shock showed less pronounced stress responses than counterparts with no control or warning signals.[16,17] Most researchers agree that the animal's appraisal of the situation is a major determinant of the stress response.[18]

Current research on stress biology has addressed the role of the brain.[19] Several areas of the brain are involved in the organization of responses to aversive or threatening stimuli, and these areas interact extensively. Neurons in the hypothalamus, for example, are sensitive to internal physicochemical stimuli and to external physical and psychosocial stimuli.[20] To a great extent the stress response is mediated by the hormone CRF (corticotrophin-releasing factor) that is secreted mainly by the paraventricular nucleus of the hypothalamus.[21] For example, behavioral stressors have the potential to severely reduce feed intake in animals. Although the mechanisms underlying the effect of stress on feed intake are not completely understood, corticotrophin-releasing hormone, which plays a key role in the stress response, appears to have a significant effect on appetite. It has been shown that intracerebroventricular administration of CRH reduces feed intake in a variety of animals.[22]

Managing CDS as a Welfare Problem

When considering CDS as a welfare problem, two main issues arise. First, it is important to provide animals that suffer cognitive impairment with an environment that does not lead to unnecessary stress. In particular, avoiding sudden changes in the animal's routine and allowing it to have control over its environment seem particularly important.

Second, the possibility to express normal behavior patterns has positive effects on the health and welfare of animals. Environmental enrichment is widely used in both captive wild animals and domestic animals to improve their welfare. Environmental enrichment techniques for animals in captivity follow one or more of the following guiding principles: (a) increasing control or contingency between animal action and environmental reaction; (b) presenting cognitive challenges such as learning what a trainer is requesting or solving a problem; (c) meeting specific behavioral needs such as need for shelter/hiding or foraging; (d) providing an environment in which exploration is stimulated and rewarded; and (e) stimulating social interaction.[23] There is ample evidence showing that environmental enrichment has positive effects on welfare, and in most cases, it causes a decrease in stress, either in baseline level responses or in responsiveness to acute stressors.[24] In aged dogs, environmental enrichment has positive effects on cognitive performance, and these are more pronounced when dietary treatment and environmental enrichment are combined.[7]

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Q&A Discussion

Q: Dr. Stan Marks, University of California-Davis: Can you expound on the topic of hearing impairment in geriatric dogs that may contribute to cognitive dysfunction? When working these patients up, we talk about the medical approach to ruling out organic disease, liver, kidney, etc. However no mention was made of hearing impairment that we see so commonly in our geriatric patient population, and as it is in people, this is an important aspect of the senile dementia deterioration/depression that we see in human patients. So, I was wondering if you could comment further on the role of hearing impairment and how that should be evaluated in these patients.

A: Dr. Manteca: You are absolutely right. As I said, we have to think about behavior as something that results from the function of the brain, but the brain is responding to a variety of stimuli in the environment, and obviously the dog responds to olfactory, visual, auditory stimuli and many others. So any impairment in the senses, including in sight or in hearing, may lead to behavioral changes that may contribute to that pattern of altered behavior in the geriatric dog. The other thing that I think is very important is that any sense impairment may contribute very much to a perceived sense of lacking control and predictability. So, I think that checking whether the dog

is hearing well, checking whether the dog is seeing well, must be a fundamental part of the medical workup. And when that it not the case, when there is a hearing impairment, we have to try to provide mechanisms that may replace sound stimulus, for example, by olfactory or tactile stimulus so that the dog may somehow recover that sense of control.

Q: Dr. Dottie Laflamme, Nestlé Purina Research: I have heard that as hearing loss progresses in senior dogs, the lower frequencies are lost first and the higher frequencies are retained. And I've heard suggestions from trainers and behaviorists that using whistles and so forth is a way to work with the senior dog and provide appropriate stimuli in a way to train them during the phase when their hearing is going down but is not quite gone. And I'd like to ask you and maybe others in the audience who have experience in that regard to comment.

A: Dr. Manteca: What I can contribute to that is that the hearing ability of the dog is very different from ours in the sense that the dog is more sensitive to higher frequencies than we are. So when we compare the two audiograms, the human and the dog, the dog would be toward the right-handed side of the diagram in the sense that they hear higher frequency sounds better than we. So if we assume that when the animals partially lose their hearing ability, it is logical to assume that since they are more sensible to higher frequency sounds, they may still retain the ability to respond to some of those sounds. So, I am not convinced that this has been explored in a scientifically controlled way, how the aging dogs respond to different types of sounds, but considering that they are more receptive to high frequency sounds, I think that would be worth exploring.

Dr. Gary Landsberg, Moderator: One element that I would comment on is that in some cases of cognitive dysfunction, you will sometimes notice that the hearing and visual response to stimuli sometimes seem to actually improve with the treatment of cognitive dysfunction, meaning it's more of a central or cortical problem. These animals that apparently couldn't hear before, after treatment of the cognitive components sometimes seem to start "beginning to hear again" or beginning to respond to stimuli again. So remember that hearing also can be central or cortical rather than just the specific sensory organ itself.

Dr. Karen Overall, University of Pennsylvania: In addition to what Gary just said about animals beginning to show the behavioral effects of being able to hear after they're not distressed, I think that we have to realize how distress factors into how they process all sorts of information, whether it's visual or olfactory or auditory. Once they become distressed, they can't learn about their environment. I'll explain why when I give my talk after lunch. Once that happens, they begin to lose parts of the information chain that gives them the predictability. So if you can both treat that anxiety and treat the cause of the dysfunction, you see a whole host of effects including, oddly enough, increased olfactory ability. And it's interesting to see that happen. As far as the range of the spectrum, because they hear at the frequency that Xavier said so much better than we do, they actually lose the very end of that relatively early on. And I have to tell you, our ability to assess hearing in dogs is pathetic. We really need to be concentrating on not only how well they hear but also how well they process information, and measure it instead of measuring deafness. Our work has shown that the reactivity and the kind of reactivity you have to noise is heritable. All the genes that we've so far got localized that are overrepresented in dogs that have noise reactivity are all involved, with one exception, in how well you process information. So this is a cognitive issue. And when we look at them losing that first end, when they realize that other dogs in the house are hearing things they don't hear, they become distressed and then they also clip off that other end. And one of the things we can do to ameliorate this early is not just whistles, but there are now vibrating collars, that vibrate in a variety of ways, so that a dog gets to be included in things and gets told to attend to something. I believe this is important because they are so social and so cognitive, I believe that as in humans, deafness is far more isolating than blindness. It's your early warning system that keeps you alive.

Dr. Richard Hill, University of Florida: I guess as an internist I'm a little bit uncomfortable about a diagnosis of exclusion that your approach seems to be. The clinical approach is really to rule out everything else and then, if we have these signs, therefore it must be cognitive dysfunction. In a diagnosis of exclusion you're really depending on your ability as a diagnostician in many, many fields. I always get a little worried that maybe we're not picking up that they are in pain, that they've got hearing impairment, whatever. So my question is whether there is any test we can run that really shows that they do have cognitive dysfunction?

Dr. Manteca: Yes, I think you are absolutely right. I am as uncomfortable as you with any diagnostic approach that is based on ruling out everything else because we never rule out everything else. So, this approach is fraught with difficulties. Dr. Milgram has explained some tests that can be used in a clinical setting. The portable equipment he has been referring is being developed and those tests will show that the dog has a cognitive deficit. But, as far as I know, we don't have any practical test that would tell us that those cognitive changes are due to the sort of brain pathological changes that we associate with cognitive dysfunction syndrome. So I would say that this is the best we have for the moment.

Dr. Bill Milgram, CanCog: The situation in dogs, by the way, isn't necessarily that different from the situation with humans. In human subjects the classification of dementia and different kinds of dementia is sort of an ongoing area. And I think from a critical perspective, the one thing that you can probably be confident in, based upon the work we've done, is if your dog is beyond a certain age, it's going to be showing cognitive impairment. But to be able to get it more refined, as in humans where we try to distinguish between people who are demented and people who show mild cognitive impairment, that involves a lot of work and there's often an invisible line. It is probably the case with dogs as well. There may be other diagnostics in the future that would be useful. There might be, for example, an analysis of certain types of metabolites in cerebrospinal fluid. I think it is something that will require more than just behavior to come up with a more confident diagnosis. And you can be confident that all dogs show cognitive impairment, but the extent of the impairment is, at this stage, something I think is very, very difficult to quantify.

Cognitive Dysfunction in Cats: Clinical Assessment and Management

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Key Points

- Increasing numbers of cats are living to old age, and they commonly develop behavioral changes.
- The behavioral changes reported most frequently are loss of litter box training and crying loudly at night.
- The most typical causes of these problems are cognitive dysfunction syndrome (CDS), osteoarthritis, systemic hypertension (commonly secondary to chronic kidney disease or hyperthyroidism), hyperthyroidism (even without hypertension), deafness, and brain tumors.
- Almost one-third of cats 11- to 14-years of age develop at least one geriatric-onset behavior problem that appears to relate to CDS, increasing to over 50 percent for cats 15 years of age or older.

Glossary of Abbreviations

CDS: Cognitive Dysfunction Syndrome
CKD: Chronic Kidney Disease
CT: Computed Tomography
DM: Diabetes Mellitus
ECG: Electrocardiogram
FeLV: Feline Lukemia Virus
FIP: Feline Infectious Peritonitis
FIV: Feline Immunodeficiency Virus
FLUTD: Feline Lower Urinary Tract Disease
GI: Gastrointestinal
MRI: Magnetic Resonance Imaging
OA: Osteoarthritis
UTI: Urinary Tract Infection

Introduction

With improvements in nutrition and veterinary medicine, the life expectancy of pet cats is increasing. The number of aging cats seen by veterinarians has increased considerably in recent years. For example, there has been a 15% increase in cats over 10 years of age seen at veterinary clinics in the United States.[1] Currently, it is estimated that there are over 2.5 million senior cats in the United Kingdom,[2] while between one-third and one-half of pet cats in the United States are 7 years of age or older.[3] Hence, good management of these individuals is becoming an increasingly important consideration for small animal veterinary practitioners.

Unfortunately, accompanying this growing geriatric population are increasing numbers of pet cats with signs of altered behavior and apparent senility. Behavior changes may result from many different disorders (Figure 1) including systemic illness, organic brain disease, true behavioral problems, or cognitive dysfunction syndrome. Diagnosis involves a full investigation looking for underlying illness (Figure 2) and assessment for behavioral problems. Once these have been ruled out, CDS should be considered, although ante-mortem this is a diagnosis of exclusion. The most commonly seen behavioral changes include spatial or temporal disorientation, altered interaction with the family, changes in sleep/wake cycles, housesoiling with inappropriate urination/defecation, changes in activity, and/or inappropriate vocalization (often displayed as loud crying at night) (Figure 3).

Potential Causes of Behavioral Changes in Geriatric Cats

Perhaps the most common causes of behavioral changes in older cats are CDS, osteoarthritis (OA), systemic hypertension, hyperthyroidism, deafness, and brain tumors. Much has been written elsewhere about the diagnosis and treatment of other potential causes of behavioral disorders in old cats so this paper will concentrate on CDS.

Cognitive Dysfunction Syndrome

Cognitive dysfunction syndrome is the term applied to age-related deterioration of cognitive abilities, characterized by behavioral changes (Figure 3), where no medical cause can be found.[4-7] A survey looking at older cats (7- to 11-years of age) revealed that 36% of owners reported behavioral problems in their cats, and this increased with age to 88% in cats between 16 to 19 years of age.[8] A more recent study suggests that 28% of

Figure 1: Potential Causes of Behavioral Changes in Geriatric Cats

- Cognitive Dysfunction Syndrome (CDS)
- Osteoarthritis (OA)*
- Systemic Hypertension (high blood pressure may either be primary or secondary to hyperthyroidism, chronic kidney disease or possibly, diabetes mellitus, acromegaly or hyperadrenocorticism)
- Hyperthyroidism
- Chronic Kidney Disease (CKD)
- Diabetes Mellitus (DM)
- Urinary Tract Infection (UTI)
- Gastrointestinal (GI) Disease
- Liver Disease (hepatic encephalopathy)
- Reduced Vision or Hearing
- Brain Tumors (e.g., meningioma, lymphoma)
- Neurological Defects (either sensory or motor deficits)
- Infectious Disease (e.g., FIV, FeLV, toxoplasmosis, FIP)
- Pain and/or Inflammation in General (e.g., dental or periodontal disease)
- True Behavioral Problems, Stress

* The importance of OA should not be overlooked.[29] Radiographic evidence of degenerative joint disease is present in 70 to 90% of cats over 10 years of age.[30-32] Associated pain and/or dysfunction can result in reduced activity and mobility, aggression, altered interactions with the family, and/or loss of litter box training. Owners can help their arthritic cats by adjusting their house; for example, by moving food and water bowls to lower surfaces, adding ramps to allow easier access to favored sleeping areas, providing deep comfortable bedding that will support and protect the cat's joints (heated beds can be particularly soothing), and placing low-sided litter boxes within easy reach of the cat.

pet cats aged 11- to 14-years develop at least one geriatric-onset behavior problem that appears to relate to CDS, and this increases to over 50% for cats 15 years of age or older. Excessive vocalization and aimless activity are the most common problems in this older age group.[7,9]

The cause of the syndrome is still unknown, but 1) compromised cerebral blood flow, and 2) chronic free-radical damage are both believed to be important.[7] Numerous vascular changes can occur in the brains of old cats, including a decrease in cerebral blood flow, the presence of small hemorrhages around the blood vessels, and a form of arteriosclerosis.[6,10] In addition, the brain of an elderly cat also may be subject to compromised blood flow and hypoxia due to heart disease, anemia, blood-clotting defects or hypertension.

Chronic free-radical damage also can occur as cats age. A small amount of the oxygen that is used by cells in energy production is normally converted to free radicals. As cells age they become less efficient, producing less energy and more free radicals. (As a simile think of increasing emissions as a car engine ages and becomes less efficient.) Normally these free radicals are removed by the body's natural antioxidant defenses, including a number of special enzymes and free radical scavengers, such as vitamins A, C and E. The balance between the production and removal of free radicals can be upset by disease, age and stress. An excess of free radicals can lead to damage, and the brain is particularly susceptible because it has a high fat content, a high demand for oxygen, and a limited ability to repair.[6,11]

Ultimately, chronic damage can eventually lead to disease processes similar to those seen in humans suffering from Alzheimer's disease, with alteration of proteins within nerve cells (e.g., tau hyperphosphorylation) and deposition of protein plaques outside the nerve cells (made from β-amyloid protein). In humans and dogs, genetics, diet and lifestyle choices have all been found to influence the prevalence and pattern of neuropathological changes (particularly β-amyloid plaques) and the nature of the cognitive dysfunction. While these relationships have still to be determined in cats, it is likely that they will be similar.

Diagnosis and Management of Older Cats with Behavioral Disorders

Gaining a correct diagnosis involves a full investigation (Figure 2). Unfortunately, the diagnosis and management of older cats is often complicated by the concurrent presence of multiple interacting disease processes. In some cases, interacting conditions may worsen clinical signs. For example, OA, chronic kidney disease (CKD) or other causes of polyuria, plus or minus increased fecal urgency with chronic gastrointestinal (GI) disease, or difficult defecation with constipation may each exacerbate apparent loss of litter box training. Concurrent hyperthyroidism and diabetes mellitus (DM) can be very confusing as the clinical signs can be similar and because each condition can affect laboratory findings for the other. For example, DM may suppress the serum thyroxin concentration to within the reference range,[12,13] while the increased protein turnover associated with hyperthyroidism can reduce the serum fructosamine to a lower level than would be expected in a cat with uncomplicated DM.[14,15] In some cases, the treatment of one disease may worsen another. For example, treatment of hyperthyroidism can unmask the severity of CKD.[16] Prompt and full investigation is therefore essential if management is to be effective.

It is not always easy for owners to recognize signs of ill health in their cat as they often do not know what signs to look for. Veterinarians need to educate owners as to what they should monitor and encourage them to report any changes in their cat. Owners need to understand that the changes they see are not "normal" and that they may represent the presence of treatable disease. Owners need to monitor their older cats for changes in food and water consumption, body weight, production of urine and feces, and behavior.

The implementation of Senior Health Care Clinics can be

beneficial. While the clinics do need to be tailored to individual cats, in general they should include regular and thorough physical examinations including assessment of body weight, calculation of percentage change in body weight, body condition score, systemic blood pressure, and retinal examination. Ideally, the evaluation also should include in-practice mobility assessment plus full orthopedic and neurological examinations, which can be challenging to perform in cats as they need time to relax and move about on their own volition, preferably on a floor surface that gives them sufficient grip without catching their nails. A blood sample should be collected for biochemical screening, thyroxin concentration and hematology and, where appropriate, serological testing for feline lukemia virus (FeLV) and/or feline immunodeficiency virus (FIV). A urine sample should undergo routine analysis, urine protein to creatinine ratio and, wherever possible, bacterial culture. Initially, most cats will only need to attend a clinic once or twice a year. However, those cats showing significant aging changes may need to attend more frequently for repeated reassessment, monitoring and treatment.

Management of Cats with CDS

While there are no published studies relating to the treatment of cats with CDS, it is possible to consider potential treatment options by extrapolation from studies of humans with Alzheimer's disease and dogs with CDS. Potential interventions include dietary modification, environmental management and drug therapies.[17]

Dietary Modification and Environmental Management

Diets enriched with antioxidants and other supportive compounds (e.g., vitamin E, beta carotene and essential fatty acids) are believed to reduce oxidative damage, thus reducing β-amyloid production and improving cognitive function. In humans, studies have shown that high intake of fruits, vegetables, vitamins E and/or C, folate and/or B12 may improve cognition. In addition, alpha-lipoic acid and l-carnitine enhance mitochondrial function, and omega-3 fatty acids promote cell membrane health and have, in humans, been found to be beneficial in the treatment of dementia. Unfortunately, excessive intake of some of these compounds can be harmful. In general, combinations of these compounds are believed to work best.

There have been a number of studies investigating the potential benefit of various supplements in dogs with CDS.[11,17-19] For example, a study of dogs over 6 years of age, when given a supplement containing omega-3 fish oils, vitamins E and C, L-carnitine, alpha-lipoic acid, coenyzyme Q, phosphotidylserine and selenium (this supplement is sold in the United Kingdon as Aktivait® from VetPlus) over a two-month period resulted in significant improvements in signs of disorientation, social interaction, and housesoiling.[20] Unfortunately, a different formula is needed for cats as alpha-lipoic

Figure 2: Investigation of Behavioral Changes in Older Cats Should Include:

- Full history, including the possibility of previous trauma (which may have led to OA), any potential exposure to toxins or drugs, and any recent environmental changes (in the household, family members, diet, etc.). Asking specific questions about alterations in the cat's behavior can help determine how the cat has changed (see below Mobility/Cognitive Dysfunction Questionnaire).
- Full physical examination (including body weight, calculation of percentage change in body weight, body condition score, and retinal examination).
- Assess systemic blood pressure (this is important as hypertension occurs commonly in older cats and produces many of the same signs as CDS).
- Mobility assessment, plus neurological and orthopedic examinations, which can be challenging in some cats.
- Assess hematology and serum biochemistry, including thyroxin level.
- Urine analysis (including urine protein to creatinine ratio and bacterial culture).

Further investigation may include:

- Where appropriate, serological testing for FeLV, FIV, toxoplasmosis or FIP.
- Thoracic, abdominal or skeletal radiography, abdominal ultrasound examination, electrocardiogram (ECG), echocardiography, intestinal endoscopy/exploratory laparotomy, and biopsy collection, as indicated from initial findings.
- Head computed tomography (CT) or magnetic resonance imaging (MRI) testing.

Mobility/Cognitive Dysfunction Questionnaire*

My cat …	Yes	Maybe	No
Is less willing to jump up or down	❑	❑	❑
Will only jump up or down from lower heights	❑	❑	❑
Shows signs of being stiff at times	❑	❑	❑
Is less agile than previously	❑	❑	❑
Shows signs of lameness or limping	❑	❑	❑
Has difficulty getting in or out of the cat flap	❑	❑	❑
Has difficulty going up or down stairs	❑	❑	❑
Cries when picked up	❑	❑	❑
Has more accidents outside the litter tray	❑	❑	❑
Spends less time grooming	❑	❑	❑
Is more reluctant to interact with me	❑	❑	❑
Plays less with other animals or toys	❑	❑	❑
Sleeps more and/or is less active	❑	❑	❑
Cries out loudly for no apparent reason	❑	❑	❑
Appears forgetful	❑	❑	❑

*Ensure there have been no environmental reasons for the change(s). It can be difficult to differentiate between many of the changes caused by CDS and/or other behavioral/neurological diseases in old cats, and those caused by OA. In addition, it is not unusual for an individual cat to have multiple interacting conditions.

Figure 3: Common Behavioral Changes That Can Be Seen in Older Cats

- Spatial disorientation or confusion, e.g., getting trapped in corners or forgetting the location of the litter box (housesoiling is the most common reason for referral of old cats to behavioralists).
- Altered social relationships with owners or other pets in the household, e.g., increased attention seeking or aggression.
- Altered behavioral responses, e.g., increased irritability or anxiety or decreased response to stimuli.
- Changes in sleep/wake patterns.
- Inappropriate vocalization, e.g., loud crying at night.
- Altered learning and memory, such as forgetting commands or breaking housetraining.
- Changes in activity, e.g., aimless wandering or pacing, or reduced activity.
- Altered interest in food, either increased or, more typically, decreased.
- Decreased grooming.
- Temporal disorientation, e.g., forgetting they have just been fed.

acid is toxic in this species,[21] so products containing it should not be given. While the new feline-safe version of Aktivait is on the market, trials in cats still need to determine its efficacy.

Environmental enrichment can lead to an increase in nerve growth factors, the growth and survival of nerves, and an increase in cognitive function. The combination of environmental stimulation (e.g., toys, company, interaction and food hunting games) and a diet enriched with antioxidants is believed to have a synergistic action in improving cognitive function. In aged dogs, a two-year study on the use of an antioxidant-enriched diet (e.g., vitamins E and C, selenium, fruit and vegetable extract [beta carotene, other carotenoids, flavinoids]), mitochondrial cofactors (dl-lipoic acid and l-carnitine), and essential fatty acids (omega-3 fatty acids), plus environmental enrichment (e.g., toys, kennel mate, walks, and cognitive experience testing) revealed rapid (two to eight weeks into treatment) and significant improvements in learning and memory. Interestingly, while there was no reversal of existing pathology, the antioxidants did appear to prevent the deposition of more β-amyloid while the environmental enrichment did not.[22,23]

While a similar study showing improvement of CDS in cats in response to dietary supplementation is not yet available, a five-year study feeding healthy old cats (7- to 17-years-old; n=90) a diet supplemented with antioxidants (vitamin E and β-carotene), essential fatty acids (omega-3 and -6 fatty acids) and dried whole chicory root (which contains the prebiotic inulin to modify intestinal flora) resulted in the supplemented cats living significantly longer (and more healthily) than the unsupplemented ones.[24] Another diet, designed for cats with OA, is supplemented with a mixture of antioxidants (e.g., vitamins C and E, and beta carotene), essential fatty acids, chondroprotectants (e.g., methionine, glycosaminoglycans, glucosamine and chondroitin sulphate), and L-carnitine and lysine. In a two-month study in which this diet was fed to 75 cats 12 years of age or older that were not selected for signs of CDS (or OA), owner-completed questionaires indicated >70% improved in one or more signs of cognitive function (and >50% improved in one or more signs of mobility).[25]

Unfortunately, once cats develop significant clinical signs of CDS, instigating environmental change can actually have a negative effect. This is because affected cats often become stressed and cope poorly with change whether in their environment, their daily routine, their diet, or the members of the household. The cat's response to this stress is to show more obvious signs of CDS (e.g., anorexia, hiding, and/or upset of toileting habits).[26] For these cats, whenever possible, change should be kept to a minimum; when it cannot be avoided, change should be made slowly and with much reassurance. Some cats may be so easily disorientated and cope so poorly with change that they may benefit from having their area of access reduced in size to a single room containing everything they need, i.e., the key resources for cats: food, water, litter box, resting places, somewhere to hide and/or some way of escaping, and companionship (as dictated by the particular needs of the individual cat). This core territory can then be kept safe and constant. Environmental application of synthetic feline appeasement pheromone (Feliway®; Ceva) can also help in reducing feline anxiety.

Potential Drug Therapies

There are a growing number of possible drug options for Alzheimer's disease. These include various cholinesterase inhibitors (to increase the availability of acetyl choline at the neuronal synapses), selegiline (to manipulate the monoaminergic system), antioxidants (e.g., vitamin E), and non-steroidal anti-inflammatory drugs (to reduce neuronal damage). While there are no drugs licensed for the treatment of CDS in cats, a number of drugs have been used "off label."[6,17,27,28] These include selegiline (Selgian®; Ceva: Anipryl®; Pfizer: suggested dose 0.25 to 1.0 mg/kg PO q24h), propentofylline (Vivitonin®; Intervet: suggested dose 12.5 mg/cat PO q24h), and nicergoline (Fitergol®; Merial: suggested dose quarter of a 5mg q24h), all of which have been used in cats with varying degrees of success. For example, a small open trial using selegiline showed a positive effect,[17] and the American Association of Feline Practitioners supports the use of this drug for the treatment of CDS. Other drugs that have been used to treat particular signs of CDS in cats include anxiolytic drugs, such as a number of nutraceuticals (e.g., Zylkène®; Intervet Schering

Case Report — "Sally"

History: "Sally," a 16-year-old neutered female domestic shorthaired cat was presented with a two-week history of crying loudly at night and a six-month history of urinating around the house, which was now occurring with increasing frequency. However, she was still defecating in her litter box. Sally had always had a "picky" appetite, but her owner reported that she had become very fussy with her food, had lost weight, and stopping grooming. Overall, they felt Sally had "aged" considerably in the last two years. Sally was an indoor/outdoor cat, the only pet in the household, and was fed dry and wet cat food.

Physical Examination: Sally was bright and alert, but thin (body condition score 2 to 3 of 9). Her coat was ill-kept and matted, and she appeared slightly dehydrated. Her heart rate was 190 beats per minute, with a grade II of VI systolic murmur, loudest over the sternum, and occasional gallop sounds. Her respiratory rate was 40 breaths per minute. Her left thyroid gland felt slightly enlarged, and there was considerable bony enlargement of both elbows and stifles (consistent with OA).

Q.What is the major problem list for this case?

- 1) inappropriate urination; 2) night crying; 3) tachycardia, cardiac murmur and occasional gallop sound; 4) OA.

Q. What are the major differentials for the problems?

- Inappropriate urination – 1) feline lower urinary tract disease (FLUTD); 2) polyuria/polydipsia (e.g., CKD, DM, hyperthyroidism, liver disease, hypercalcaemia, etc.); 3) neuromuscular/orthopedic disease (e.g., OA); 4) CNS/ behavioral problems (see Figure 1).
- Night crying (see Figure 1).
- Tachycardia, cardiac murmur and occasional gallop sound — Primary cardiac disease (which is unlikely in a cat of this age); secondary cardiac disease (e.g., due to hyperthyroidism, hypertension, CKD, DM, etc.).
- OA — Idiopathic or secondary to trauma, infection, obesity or developmental defects.

Q. What is your diagnostic plan?

See Figure 2, including ECG, echocardiography, chest radiography, and head MRI.

Results:

Serum Biochemistry:		**Reference Range:**
Albumin	28 (2.8)	28-39g/l (2.8-3.9g/dL)
Globulin	32 (3.2)	23-50g/l (2.3-5.0g/dL)
ALT	64	15-60u/l
ALP	112	10-100u/l
Bile acids	8.0	(0.0-7.0 umol/l)
Creatinine	180 (2.0)	140-177umol/l (1.6-2.0mg/dL)
Urea	11.2 (31.4)	5.5-10.5mmol/l (15.4-29.4mg/dL)
Glucose	7.6 (138)	3.3-5.0mmol/l (60-90mg/dL)
Ca	2.2 (8.8)	2.1-2.9mmol/l (8.4-11.6mg/dL)
PO_4	2.5 (7.7)	1.4-2.5mmol/l (4.3-7.7mg/dL)
K	4.0	4.0-5.0mmol/l (mEq/L)
Na	148	145-156mmol/l (mEq/L)
Thyroxin	60 (4.7)	19-65nmol/l (1.5-5.0ug/dL)

Systolic BP:	150 120-180 mmHg
FeLV/FIV Tests:	Negative
Urine: (collected by cystocentesis)	SG 1.035 (ref. >1.035), pH 7.8, glucose negative, ketones negative, protein positive, sterile
Hematology:	Unremarkable
Thoracic Radiographs, Abdominal Ultrasound and Head MRI:	Unremarkable
Echocardiography:	Moderate cardiac hypertrophy with a basal septal bulge
ECG:	Tall QRS complexes

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Q. What is your interpretation of these findings?

- **Marginal renal insufficiency —** Slightly increased serum urea and creatinine concentrations (in a cat that has very little muscle mass and has not been fed for 12 hours), with a urine SG just within normal limits (but she is slightly dehydrated and a reasonable proportion of her diet consists of dry cat food so her urine SG should be higher than this).
- **Possible early hyperthyroidism —** Serum thyroxin is at the top of the reference range, but Sally is an old, ill cat who might be expected to show thyroxin suppression; there are also slight increases in her liver enzymes and bile acid concentration.
- **Stress —** Slight increase in blood glucose concentration.
- **Increase in urine pH —** This can be caused by stress (hyperventilation), diet, urease-producing UTI, old urine, etc.

Case Report — "Sally," continued

- Moderate cardiac hypertrophy – This could indicate either primary cardiac disease or (perhaps more likely in a cat of this age) cardiac disease secondary to hyperthyroidism, hypertension, CKD, DM, etc.

Q. What is your diagnosis?

- CDS, OA (elbows + hips), moderate cardiac hypertrophy, marginal renal insufficiency, and possible early hyperthyroidism.

Q. How would you manage this case?

- **CDS —** Environmental modification, diet change or supplementation, drugs?
- **OA —** Environmental modification, diet change or supplementation, NSAIDs?
- **Monitor cardiac hypertrophy**
- **Regularly reassess —** Monitor renal function, blood pressure, serum thyroxin concentration, etc.

Follow-Up: Sally was initially managed with environmental modification. This involved ensuring that she had easy access to all her key resources (food, water, litter box, resting places, hiding places/escapes routes, and company). Her food and water bowls were moved to lower surfaces, ramps were added to allow easier access to favored sleeping areas, a deep comfortable heated bed was added, and a large low-sided litter box was placed within easy reach. These changes were made gradually. It was hoped that they would help Sally's CDS and OA, and the newly added litter box meant any polyuria caused by the early CKD had less chance of resulting in peruria. Sally's food was slowly changed to a feline OA diet containing a mixture of antioxidants (e.g., vitamins C and E, and beta carotene), essential fatty acids, chondroprotectants (e.g., methionine, glycosaminoglycans, glucosamine and chondroitin sulphate), and L-carnitine (as its formulation was considered beneficial to both her OA and CDS). Together these changes resulted in a significant improvement that was noted within a month of instigating the changes. Sally cried less at night, had no further episodes of periuria, and ate better.

Six months after the initial investigation Sally was reported to be doing well, but still crying at night, which her owner felt was due to progression of the OA as the vocalization appeared to occur when Sally was changing position during sleep or when she was leaving her bed. Full reassessment, including repeated assessment of serum biochemistry, urine specific gravity and systemic blood pressure revealed little change. Sally was started on a two-week trial of low-dose meloxicam® (0.01 mg/kg PO q24 hours). At Sally's reassessment two weeks later, her owner reported that the night crying had almost completely resolved. Repeat serum biochemistry and urine analysis showed no worsening of kidney function. It was recommended that Sally's owner should monitor Sally's behavior and appetite closely and only give the meloxicam if Sally was first willing to eat her food. To date, Sally has been on this regimen for nearly six months and continues to do well, with regular full checkups scheduled every three to four months.

Further information for owners of cats with geriatric diseases can be found on the FAB Website (*www.fabcats.org*). Books written to help owners of cats with CKD, FLUTD, hyperthyroidism or blind cats are available at *www.catprofessional.com*.

Plough), buspirone and benzodiazepines (e.g., diazepam, although hepatotoxicity is a particular risk with this drug), or antidepressants (that lack anticholinergic effects) such as fluoxetine.

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Q&A Discussion

Q: Dr. Esther Plantinga, Utrecht University: I saw your advice to use NSAIDs in this particular case, but this cat had moderate renal insufficiency. Normally, veterinarians are reluctant to use NSAIDs with moderate renal insufficiency. What is your opinion about that? When is it an advantage to use them, and when do you try not to?

A: Dr. Gunn-Moore: I use a lot of them in elderly cats despite the fact that some of the elderly cats will have apparent reasons for never going anywhere near them with a non-steroidal. But it's about weighing the risks, using the safest non-steroidal you can, and making sure that the owners understand the risks. And I tend to do a pain trial first using buprenorphine, but getting buprenorphine into some of these older cats on a daily basis isn't easy. We've also used tramadol, etc., but I really like meloxicam, providing their kidneys are stable. And, as we heard yesterday, there's a much bigger risk of GI ulceration, etc., in old cats. This particular cat was only an IRIS stage 2 and stable with a sensible owner. And so, I said, "Let's try the meloxicam; let's see how it works." But she's not allowed any meloxicam unless she eats all her breakfast. So the moment she won't eat, she doesn't get dosed because she could be hydrated, her stomach's hurting her, etc. And also, we're using tiny drops, so literally a drop per cat per day. So, it's tiny doses, and that's often enough. If some days she's much worse perhaps buprenorphine or tramadol is added on those days, so it's balanced. Other people are using other analgesics; people are using things like gabapentin, things as well. But I'm very comfortable with non-steroidals, particularly meloxicam, if the owners understand the potential risk,s and we keep a very good assessment. We regularly recheck ureas, creatinines, that sort of thing. Thank you for that question because it's always so important to the whole health of the older cat.

Q: Dr. Steve Ettinger, California Animal Hospital: I think we all understand the need to give things to clients to give their pets, but I'm a little bit bothered about the lack of evidence-based medicine on many of the medications that you're suggesting. The fact that those medications are apparently only legal in a very narrow area of countries and have never been taken anyplace else because I think it's pretty clear that they would never be approved anyplace else. And I guess I'm just a little bit bothered by that because, you know, it goes back to the whole holistic approach. "Give this, give that, give a little bit of this." It's just bothersome to me when we start doing that and passing it around at meetings to veterinarians as if these products really are effective.

A: Dr. Gunn-Moore: As I said, we have no good data. I completely agree with you, and I have been campaigning to try and get some of these studies done. We desperately need them, and then we can make more educated comments. Yes, there is often a feeling that you need to do something, but it's also getting the owner to look at the whole picture. And so much can be done by helping these animals, by making a good investigation and finding out what other things may be wrong. There maybe is a bit more science base but also all the environmental stuff. So, that's why I said we have no data on any of those drugs. I mean it really is frustrating. I quite agree.

Dr. Gary Landsberg, Moderator: If I could make a comment. That doesn't dismiss the need for welfare and treatment of these animals. Dr. Ettinger was talking in part about safety. We're not talking about safety of these products that are licensed for cats anyway, such as the diet. And I have a partner who's a dermatologist and know quite a bit about the dermatology that they practice, and I'll defer to any dermatologist in the room. But they'll feed something like JD occasionally for skin problems to get the extra fatty acids into that skin in a way that's in the diet. So these things are being done if there's logic and science to them and as long as they're not, as Dr. Ettinger suggested, harmful for the pet.

Q: Dr. Gary Pan, Nestlé Purina Research: Are you aware of any risk factors that increase risk of cognitive disorder syndrome in cats?

A: Dr. Gunn-Moore: Again, data is so limited because we still don't have a way to diagnose it premortem. Following on post-mortems, we can look back retrospectively and try and find some of the factors, for example, the slide of tau phosphorylation I showed you. We found that in aged cats, but they were also cats that had had seizures. And in humans, we know you get tau phosphorylation as a response to seizure activity. So we are just at the start of the discipline in cats. There's a little bit more on dogs, which my eminent colleague commented on, but at the moment it certainly is purely the anecdotal stuff like uncontrolled high blood pressure, kidney disease, that sort of thing. Interestingly, in Britain, we still see cats with dysautonomia, and some of the cats that we got through the dysautonomia, they've then gone on to develop dementia at quite young ages. And when they had their post-mortems done there was quite a lot of vacuolization in those brains. I think there are so many different insults that can lead to a damaged brain so cognitive dysfunction will probably end up being a dump diagnosis just the same way inflammatory bowel disease is. We can't properly diagnose that either. But we need a lot more work on cognitive dysfunction.

Brain Energy Metabolism and Effects of Aging: Do We Become What We Eat?

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Dogs as potential models for human cognitive change, and the benefits to dogs from what we have learned about humans: Although rats and mice are more commonly used, the domestic dog may be a better model for complex genetic traits such as those involving behavioral disorders, including brain aging.[1] The dog has several important advantages over rodents as a model for complex behaviors, among which is the shared evolutionary history of dogs and humans emphasized here. In addition, the enthusiasm and close cooperation of dog owners and breeders facilitate an ongoing interest in canine genetics within the dog community and provide access to needed samples. Finally, breeds were developed through selection for specific types of tasks or work and most extant breeds are less than 150 years old, further reducing heterogeneity.[2]

Glossary of Abbreviations

ALA: Alpha-Linoleic Acid
APOE: Apolipoprotein E
APP: Amyloid Precursor Protein
ARA: Arachidonic Acid
ATP: Adenosine Triphosphate
8-OHB: Beta-Hydroxybutyrate
BDNF: Brain-Derived Neuotrophic Factor
cAMP: Cyclic AMP/Cyclic Adenosine Monophosphase
CD: Cognitive Dysfunction
CDS: Cognitive Dysfunction Syndrome
CREB: Cytosolic Response Element Binding Protein
DHA: Docosahexanoic Acid
E-LTP: Early-Phase LTP (Protein Independent)
EPA: Eicosapentaenoic Acid
EPSP: Excitatory Post-Synaptic Potential
L-LTP: Late-Phase LTP (Protein Dependent)
LTM: Long-Term Memory
LTP: Long-Term Potentiation
MCT: Medium-Chain Triglycerides
NMRS: Nuclear Resonance Spectroscopy
PUFAs: Polyunstaturated Fatty Acids
RNA: Ribonucleic Acid
STM: Short-Term Memory
TCA Cycle: Tricarboxylic Acid Cycle (Krebs Cycle)
trkB: Tyrosine Kinase B

Recent advances in canine genomics have potentially facilitated efforts to map genes for complex behaviors,[3,4] including some of those potentially involved in information processing and age-related effects on this. The work of Lindblad-Toh and colleagues is remarkable for its demonstration of extensive synteny — basically, exact similarity —- between the canine and human genomes, as well as the finding that, despite being more distantly related to humans than rodents,[5] the dog shows more nucleotide homology with humans than do rodents. Again, such patterns are likely the result of a co-evolutionary process that still may be ongoing.

Recent data indicate that dogs are significantly more comparable to humans than are chimpanzees and wolves with regard to the complex social cognition involved in understanding long-distance signals that indicate where food is hidden. Dogs are further able to communicate this information to other dogs.[6-10] Dogs appear to have the ability to "fast map" — to make deductions about object class and name without having learned them — and to communicate this ability to humans.[11]

Also, like humans, dogs suffer from what we recognize as maladaptive anxiety, which interferes with normal functioning and which was selected against during the co-evolution of dogs and humans.[12] Finally, when examining the rates of gene expression mutations in regional brain tissue, the only species studied to date that has comparable rates to those found for humans is the domestic dog.[13] Such data, when

taken together, strongly suggest that dogs can be excellent models for human brain aging, and that any data that accrue from studies of human brain function may be relevant for understanding canine brain function. Such syntenic patterns open an array of treatment and mechanistic modalities for those interested in brain aging.

Brain aging as a special case — newer challenges and findings: Given the previous evolutionary discussion, no one should doubt that canine brains age, and when they do, many of the dimensions of canine brain aging resemble those seen in humans. While humans are afflicted by numerous tauopathies, each of which may have defining cognitive and/or anatomical dimensions, emphasis in canines has been placed on a relatively nonspecific diagnosis of canine cognitive dysfunction (CD), sometimes also called cognitive dysfunction syndrome (CDS)[14].

In dogs, CD is usually diagnosed because of a history of disorientation, alterations in social/interactive behaviors, changes in locomotor behavior and sleep cycles, and what is often called "loss of housetraining." In early onset cognitive dysfunction, animals may have only slightly altered sleep cycles and appear more anxious. Alterations in social/interactive behaviors may manifest early in the condition as an increased neediness but change to a form of aloof disengagement in social interactions with all species.

We have chosen to treat such conditions with medications designed to address anxiety, panic and depression[15] because of the changes such medications cause at the receptor level. Oxidative damage to receptor systems has been at the core of much research on cascades that become damaged once free radicals are involved.[16] However, such treatments neglect other aspects that may affect how well learning occurs and how cognition can occur, including those aspects in providing power to the learning process.

Review of what happens in the dog's brain when it learns something: Behaviors are reinforced or learned best if every time they occur they are rewarded. At the cellular level, repeated reinforcement ensures better, more numerous and more efficient connections between neurons.[17,18] Stimulation is induced when a neurochemical in a synapse triggers a receptor to engage it. This stimulation of the receptor engages second messenger systems in the post-synaptic cell, usually cyclic AMP/cylic adenosine monophosphate (cAMP). The result is cellular memory or long-term potentiation (LTP). By itself, this initial process represents early-phase LTP (E-LTP) and short-term memory (STM). The process is short-lasting and RNA and protein-synthesis independent, and the result does not persist or become self-potentiating unless the stimulus is consolidated into late-phase LTP (L-LTP), which is a more permanent form.[19] E-LTP can be induced by a single train of stimuli in either the hippocampus or the lateral amygdala.

In contrast, L-LTP and long-term memory (LTM) require repeated stimulation of cAMP, induction of cAMP response element binding protein (CREB, a nuclear transcription factor), and are long-lasting processes, dependent on RNA transcription and protein synthesis.[19] When stimulation continues, brain-derived neuotrophic factor (BDNF) enhances neurotransmission and potentiates what is called activity-dependent plasticity at synapses (e.g., learning), particularly in the region of the brain most involved in learning, the hippocampus. This effect can also occur in the lateral amygdala and is one modality postulated to be involved in learned or conditioned contextual fear[19] (see Figure 1).

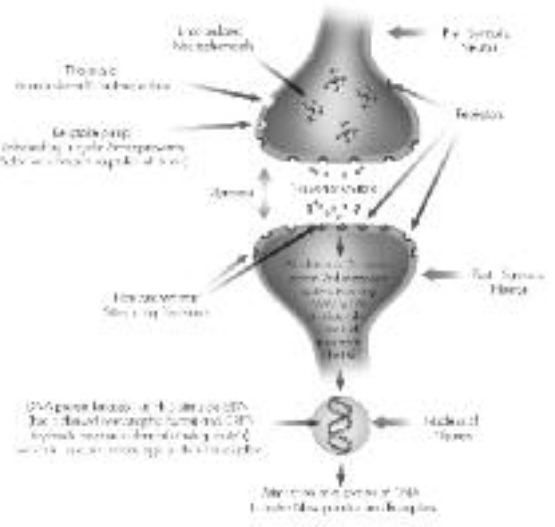

Figure 1: The stimulation of second messenger systems as a result of neurotransmission ultimately produces increases in BDNF and CREB, which are responsible for new protein formation as one mechanism by which molecular memory is produced. Note that many psychotropic medications affect this process, as may diet. All these activities require energy to be executed and completed.

Courtesy of Nestlé Purina PetCare Europe, Vevey, Switzerland.

What does the brain use for energy: Diet can affect behavior through chemical interactions between amino acids and by altering brain energy sources, allowing alterations in use of resources. Energy sources for the brain can actually be variable, and lactate, acetate and pyruvic acid are now considered viable energy sources in addition to what traditionally has been considered the main energy source, glucose.

Energy sources in the brain: Glucose is considered the common brain energy currency, but it is not stored. The stored form of glucose is glycogen. Glycogen is found mainly in astrocytes, and the amount of glycogen available is affected by glucose concentration and neurotransmitter presence and function.[20] During hypoglycemia, glycogen is converted to lactate via pyruvate (glucose → pyruvate → lactate). The lactate is then

transferred to adjacent neurons. This conversion and transfer allow the neurons to use a source of aerobic fuel.

Glycolysis can also be anerobic and is faster at producing energy than is oxidative phosphorylation.[21] In fact, glycolysis makes pyruvate faster than it can be oxidized: By converting glucose to lactate, adenosine triphosphate (ATP) is made twice as fast than would be the case were glucose oxidized completely.[21]

Lactate:

The use of lactate in hypoglycemic events can extend axon functions for 20+ minutes.[20] This conversion of astrocyte glycogen to lactate also occurs during periods of intense neural activity, demonstrating the role of astrocytes as bankers of energy-conversion compounds.

Lactate is the preferred energy source for the human brain, after glucose,[22] and there is no reason to assume that this may not also be an important pattern in dogs. The majority of lactate used as an energy source is thought to come from glycogenic processes because most lactate itself is too large a molecular to pass through the blood-brain barrier. However, blood lactate has been measured in oxidized form and may be a source of some energy for brain tissue.[22] In fact, some astrocytes appear to "prefer" to process glucose glycolytically into lactate.[23] Lactate can then be converted into pyruvate and enter the tricarboxylic acid (TCA) cycle, providing energy in the form of ATP.

Medium-Chain Triglycerides (MCT):

Ketone bodies and fatty acids have been proposed as alternate energy sources (see Figure 2) because of their modulating effects on hypoglycemia. In particular, 8-hydroxybutyrate (8-OHB) may be useful[24] for protecting hippocampal neurons from toxicity. In a placebo-controlled, double-blind study, Reger et al.[24] found that mildly impaired Alzheimer's disease patients who were supplemented with MCT showed improvement in a number of pretreatment versus posttreatment cognitive test measures, and that such improvement correlated with 8-OHB increases. It should be noted that this result depended on whether there was an apolipoprotein E (APOE) genotype: Only patients without an APOE-epsilon4 allele responded to acute elevation of 8-OHB.

Fatty acid oxidation in the brain has been studied in rats using nuclear magnetic resonance spectroscopy (NMRS). One of the MCTs, octanoate, is thought to comprise up to 13% of the free fatty acid pool in humans. Because it readily crosses the blood-brain barrier, it's been studied in a variety of clinical and experimental settings. In a labeling study in rats subjected to NMRS, octanoate could contribute 20% of brain energy in an intact, physiological system.[25] The mechanism for this was likely incorporation into both glucose and ketones, and secondary effects on the metabolism of the excitatory neurotransmitter, glutamate.

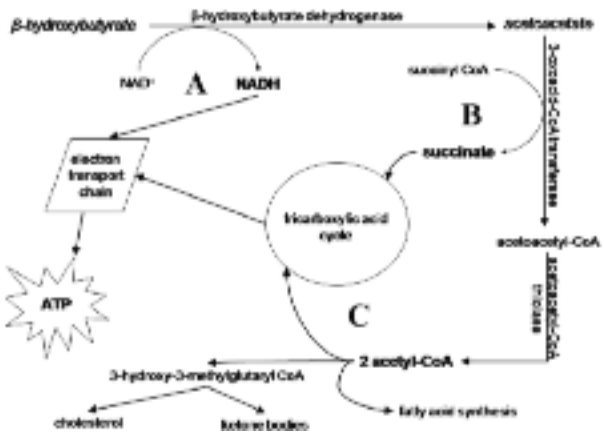

Figure 2: In this illustration showing ketone body catabolism, ATP is generated three ways. (1) NADH is produced from the dehydrogenation of 8-OHB, making electrons available for the electron transport chain (pathway labeled A). (2) CoA is transferred to acetoacetate, forming succinate, which then enters the TCA cycle (pathway labeled B). (3) Ketone bodies are converted to acetyl CoA, which then can also enter the TCA cycle (pathway labeled C). Source: Studzinski et al. *Brain Res.* 2008;1226:212.

In a study of 8 Beagles (4 control, 4 treatment) from 9- to 11-years of age, supplementation with MCT at a dosage of 2 g/kg/day resulted in improved mitochondrial function that was most pronounced in the parietal lobe.[26] Steady state levels of amyloid precursor protein (APP) also decreased in the parietal lobe after short-term supplementation leading the authors to conclude that short-term MCT supplementation can improve brain energy metabolism and also decrease APP levels in old dogs.

Taha et al.[27] have postulated that age-related cognitive decline in dogs may be associated with decreases in omega-3 PUFAs in the brain. Because MCT increase fatty acid oxidation, they may increase omega-3 polyunsaturated fatty acids (PUFAs) in the brain via metabolism of adipose tissue. In a two-month study of 8 Beagles (4 control, 4 treatment) fed an MCT-enriched diet, enrichment was shown to result in increases in brain phospholipid and total lipid concentrations.[27]

Factors Affecting Oxidative Stress — Roles for Neurotransmitters:

One of the major foci of age- and illness-related changes is the effect of a cumulative burden of oxidative stress over time. Increased oxidative stress is one of the most common topics examined in brain aging, and it appears to affect all major classes of molecules involved in neurotransmission. Development of oxidative stress may not be independent of energy source or use. Interestingly, intermittent fasting has been reported to induce the production of BDNF,[28] which is associated with neurogenesis and molecular learning and memory, particularly in the hippocampus. Increases in BDNF affect numerous

signaling pathways involving tyrosine kinase B (trkB), which may directly or indirectly affect regional brain metabolism and function.

Astrocytes are responsible for de novo synthesis of two neurotransmitters: glutamate and D-serine.[29] Glutamate, the excitatory neurotransmitter that is responsible for an estimated 85% of synaptic activity, appears to also be essential in metabolic activity of the brain. Glutamate may be responsible for energy regulation by affecting neurovascular exchange.[23] Glutamate has as its signaling targets the synapse, astrocytes and intra-parenchymal capillaries.

In normal brain function, glutamate affects signaling by altering flow of calcium and sodium ions: post-synaptically it modifies the permeability of NMDA receptors to sodium and calcium, and the AMPA receptors to sodium, and presynaptically it affects NMDA receptors and metabotropic receptors via calcium. This interaction is what causes an excitatory postsynaptic potential (EPSP). Glutamate activity is also thought to be involved in pathological conditions where excitatory sensitivity has been implicated (e.g., strokes, impulsively aggressive states, cortical and hippocampal epileptogenic activity). In both normal and pathological conditions, glutamate's main effect is on excitability and synaptic plasticity.

Glutamate also affects astrocytes, which are non-neuronal cells.[23] Glutamate transporters appear to use the sodium gradient to facilitate glutamate uptake by astrocytes. Recent anatomical studies show that astrocytic processes ensheath intraparenchymal capillaries and synapses, and that many of these processes have receptors and reuptake sites for neurotransmitters. It is these findings that allow glutamate to act as a metabolic intermediary. In short, glutamate stimulates the conversion of glucose into lactate in astrocytes.

Interestingly, many pathways that affect glycolysis for brain energy are also adversely affected at some point by oxidative change. Many of these effects may be modulated by antioxidant or co-factor treatment, coupled with active behavioral interventions/enrichment. Alpha-enolase interconverts 2-phosphoglycerate and phosphoenolpyruvate. Alpha-enolase has been shown to be altered in canine models of neurodegenerative disorders and responds to treatment with antioxidants, mitochondrial co-factors (lipoic acid) and behavioral/ social/cognitive enrichment.[30] Decreased oxidation of alpha-enolase and GAPDH could improve glycolytic function, with a resultant increase in ATP production. Together, these alterations appear to lead to neuronal recovery and improved cognitive function in the canine model of human brain aging.[30]

In a study of gene expression in brains of old dogs, the expression of genes involved in neurochemical signaling and synaptic transmission was decreased.[31] Particularly affected were levels of growth and transmission factors already discussed, including BDNF and trkB. These factors did not respond to antioxidant diet supplementation. Interestingly, in the same study, compounds like glutathione S-transferase — responders to oxidative stress — were also decreased in geriatric dogs. Such findings show the ultimate interrelatedness of available brain energy, neurotransmission and neuroregulator function and structural changes in aging dogs.

Structural components of neuronal membranes that may be important for use and transport of energy in the brain: Arachidonic acid (ARA), docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) are long-chain PUFAs that are essential for developing and maintaining the integrity of cells of the brain's membranes. These PUFAs are related by their synthetic sequence: linoleic acid (18:2 n-6) becomes ARA (20:4 n-6), which becomes docosapentanoic acid (22:5 n-6). Elongation of alpha-linoleic acid (ALA) by desaturation produces EPA (20:5 n-3), which can then be metabolized to DHA (22:6 n-3).[32]

All these PUFAs are essential for early brain development. ARA is thought to especially maintain hippocampal cell membrane fluidity and protect cells in the hippocampus from oxidative stress. The hippocampus is one of the main areas involved in LTP, a form of molecular learning, and is one of the main regions where associational learning takes place.

DHA may encourage development-stage specific associational learning, although the data are mixed. Supplementation with DHA and EPA affect concentrations of these substances in rat brains, but their distribution is not uniform. Diets deficient in ALA especially cause decreases of DHA in the frontal cortex — the part of the brain responsible for complex learning and integration of information and executive function. In dogs, low concentations of DHA during gestation and/or lactation depress the retinal sensitivity of puppies, which can have profound and complex behavioral outcomes. The current data support the need for DHA for optimal neurological development in puppies, and there are hints that it may improve both early- and long-term cognitive abilities, but the data are scant.

There has been some suggestion that PUFAs are also important in some canine behavioral conditions. In a study of German Shepherd Dogs with a history of aggressive behavior, aggressive dogs showed a significantly lower concentration of DHA (22:6 n-3) and a higher omega-6/omega-3 ratio when compared to unaffected dogs.[33] Plasma concentrations of ARA (20:4 n-6) and EPA (20:5 n-3) did not differ. These same animals showed reduced levels of cholesterol compared to control dogs. Similar nonspecific findings regarding cholesterol have been reported for aggressive dogs.[34] It is important to realize that the characterization of "aggression" in these studies is variable, and that such correlations say nothing about cause. Such findings could be the outcome of aberrant neurochemical function. However, one of the main roles of PUFAs appears to be maintenance of membrane fluidity and protection from oxidative stress, espe-

cially in the part of the brain essential to associational learning, the hippocampus.

Finally, in humans, the brain contains 600 g lipid/kg, with approximately equal amounts of ARA and DHA. It's been postulated that a dietary intake of 6 to 12% protein comprised of Rift Valley lake fish and shellfish provided sufficient DHA and ARA that allowed the early hominoid cerebral cortex to grow disproportionately without requiring an increase in body mass.[35] Any putative effects of these PUFAs on cognitive abilities are likely routed in this evolutionary history. Interestingly, PUFA levels in brains of young versus geriatric dogs, when measured, have not been shown to be different,[31] but effects of varying amounts in different regions of the brain (e.g., the hippocampus, which is key to learning, and the frontal cortex, which is involved in learning and essential for executive function or application of that learning) in older animals has not been studied.

Summary

Our brains are doubtless shaped by what we eat, and so our dogs' brains are shaped by what we choose for them to eat. It would surprise no evolutionary biologist that alternative brain energy pathways exist and that they maintain healthy and active brain function and neurotransmission. But the effects of ebb and flow of food on these effects may suggest that our culture — and our dogs' culture — of constantly available food, rather than constantly available cognitive stimulation, has not adequately considered the extent to which this is a strategy that has not been chosen by the shared dog-human evolutionary history. Exploration of effects of diet on canine cognition and recovery from the dreaded effects of aging could enhance our understanding of the shared development of dogs and humans as interdependent, potentially co-evolved species.

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Q&A Discussion

Q: Dr. Margie Scherk, Vancouver: If lactate is a brain energy source, why is it then whenever I work out and I've got lactic acidosis happening that I feel dense as two bricks?

A: Dr. Overall: It depends on where the lactate is because you can also kill off muscle cells and heart cells and do other things with it. The other thing is, you know we actually infused lactate into a series of dogs because it's used as a test for panic disorder in human psychiatric patients and found out that you get different behavioral and physiological responses depending on what your diagnosis is. So, what you are actually experiencing postexercise is the primary muscle response, the secondary response to actually depleting that energy. And there may be secondary damage, especially if you have CPK leakage. Oddly enough, that's what we found out with dogs that were noise-phobic. We didn't have to subject them to noise, we just gave them intravenous lactate, and these dogs leaked CPK out of their muscles, like the second coming. The dogs got separation anxiety, and the normal dogs did not.

Circadian Biorhythms of Sleep/Wake and Activity/Rest Cycles In Adult and Aged Dogs

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Introduction

Circadian biorhythms occur in many different organisms from prokaryotes to higher mammals, and nearly all physiological and behavioral processes in animals possess daily variations that are controlled by circadian timing.[1] The sleep/wake cycle in animals is the most obvious and easily observable circadian biorhythm and is an indispensable function of a healthy life. Chronic phase shifts in the biological clock cause circadian disruption that can lead to increased mortality,[2] increased risk for cancer,[3] sleep disorders,[4,5] and various other conditions or diseases.[6]

Glossary of Abbreviations
AD: Alzheimer's Disease
CSF: Cerebrospinal Fluid
EEG: Electroencephalography
EMG: Electromyography
EOG: Electro-Oculography
NREM: Non-Rapid Eye Movement
PSG: Polysomnography
REM: Rapid Eye Movement
SCN: Suprachiasmatic Nuclei
SWS: Slow Wave Sleep

Sleep physiology and activity/rest patterns have been widely studied in humans and rodents to characterize the biology of sleep. This has led to the current understanding that sleep is not a passive resting state but a dynamic physiology that is controlled by very active neurological processes. Sleep frequency by phase also is dynamic, as it varies between species. Sleep duration and timing relative to day or night also varies in animals.[7] Humans are typically monophasic sleepers, in that sleep is consolidated into a single main block of time and aligned with the dark phase of the day. In contrast, nearly all other animals, including cats and dogs, are polyphasic sleepers that experience multiple bouts of sleep or inactivity over the course of a 24-hour period.

The chronobiology of sleep has been well-documented in humans and rodents over the past several decades, and most recently the literature on chronobiology has been extensively reviewed.[8-14] In addition, multiple authors have discussed topics relating to circadian rhythm sleep disorders.[4,5,15,16] Yet, despite the importance of sleep rhythms in sustaining health, and compared to the extensive examination of sleep in people, only an initial understanding of the chronobiology of sleep exists in dogs[17-20] and cats.[21-24] These data provide an initial characterization of circadian sleep/wake patterns, with very little understanding of how it is influenced by aging.

Growing evidence suggests that age-related changes in sleep in people are linked to disruptions in mechanisms underlying circadian rhythm generation.[25-27] Circadian rhythm disruption also is linked to cognitive dysfunction. Thus, age-related sleep changes tend to be exaggerated in dementias, such as Alzheimer's disease,[28,29] and can be manifested as either hyperactivity or hypoactivity.[30] In dogs, as in people, declines in cognitive health can also be manifested with advancing age.[31] The relationship between behavioral activity patterns and canine cognitive impairment, as well as how age-related changes are involved or related, have only been initially explored in dogs.[32] The present review examines the existing literature concerning the circadian rhythms of "normal" (non-narcolepsy models) sleep/wake and activity/rest cycles in dogs, along with the related physiological aspects of sleep, while also examining the effects of aging.

Circadian Pacemaker and Circadian Rhythm Regulation

In mammals, circadian biorhythms can be endogenous, as controlled by the circadian pacemaker (i.e., sleep/wake cycle), or be a consequence of the animal alternating between sleep and wakefulness (i.e., food-finding behavior).[12] Endogenously controlled circadian biorhythms in mammals originate from the circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus.[33] The SCN is entrained by light or dark cues received as efferent nerve signals from nonvisual photoreceptor cells present in the retina. In the dog, the retinohypothalamic tract has been delineated, in which the transmission of chemical signals occurs from retinal ganglionic cells through the optic nerve to SCN in the medial hypothalamus, which is dorsal to the optic chiasm and ventral to the paraventricular nucleus.[34,35]

Photo entrainment of the SCN increases the length of the day-night cycle to be slightly more than 24 hours in length.[14] Consequently, the circadian pacemaker acts as a biological clock, which is intrinsically active and synchronized by the prevailing photoperiod to facilitate the regulation of behavioral and physiological responses of the animal to periods of

wakefulness and rest. In this way, hormonal secretions, motor activity and other bodily functions are regularly adjusted to be in appropriate timing with a 24-hour period of light and darkness, which contributes to maintaining a homeostatic system. Although the circadian pacemaker is the primary regulator of the sleep/wake cycle, activity during wakefulness and rest during periods of sleep can be influenced by many other factors including environmental light, temperature, other animals, hunger, and age.

Modulation of Melatonin Through the SCN

One of the ways that the SCN regulates circadian physiological processes is through conveying the photoperiodic signaling that is received from the retina to the pineal gland for the nocturnal release of melatonin. Melatonin is the chemical messenger to the rest of the body concerning information regarding night length.[36] The neural tract that connects the photosignal to the pineal gland for melatonin synthesis and secretory activity is described in detail by others, and has been demonstrated to be a function of noradrenaline release from postganglionic sympathetic nerves that terminate at the pineal gland.[37,38]

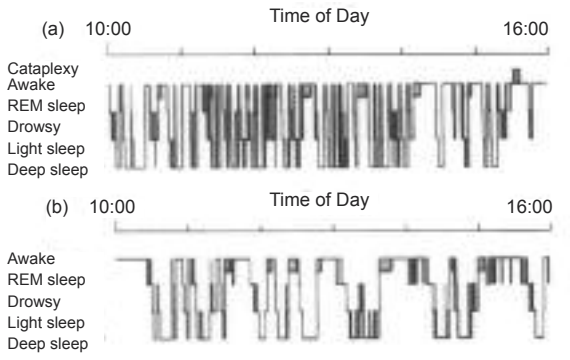
It is well-established that blood melatonin levels oscillate with a circadian pattern in mammals, in which peak levels occur during the night phase.[36-39] Several studies in dogs have demonstrated that serum[35,40,41] and cerebrospinal fluid (CSF)[34] melatonin levels exhibit a circadian rhythm with a nighttime rise, with peak nighttime concentrations in jugular blood ranging from approximately 6 to 25 pg/mL.[35-41] This nighttime rise conveys light/dark photoperiodicity to the rest of the body to synchronize the circadian clock. However, the nighttime rise is dependent on continued darkness, as light exposure after dark disturbs the melatonin rhythm by inhibiting synthesis and release in addition to causing chronodisruption.[42]

In humans, the nocturnal rise is associated with an increased sleep propensity by enhancing the amplitude of the circadian clock oscillations via the melatonin receptor, MT1, in the SCN.[43] However, in nocturnal mammals like rodents, the nighttime rise in melatonin is associated with increasing locomotor activity and wakefulness.[44] Therefore, the role of melatonin in regulating sleep/wake cycle is not a function of inducing sleep, but reinforces melatonin's role as an endogenous zeitgeber ("time-giver" or synchronizer) and modulator of circadian biorhythms. In this way, sleep/wake cycles and circadian behavioral patterns (i.e., activity, rest) are appropriately synchronized with the day/night cycle. A great deal of research has been conducted on melatonin's role in regulating circadian rhythms, as recently reviewed by others.[10,43,46]

Sleep Physiology and Sleep-Wake Patterns in Dogs

Sleep physiology has been researched extensively in people and a variety of animals. Consequently, normal sleep patterns, along with rapid eye movement (REM) and non-REM (NREM), also called slow wave sleep (SWS), sleep states have been well-defined. Regardless of species, all the different mammals studied experience both REM and SWS sleep. SWS is further defined as stages 1 through 4 with distinctions based on various patterns of brain activity, as determined by electroencephalography (EEG).[9,10]

Figure 1



Hypnograms of (a) a narcoleptic dog and (b) a healthy control dog. Recordings were obtained from cortical electroencephalography (EEG) and neck muscle electromyography (EMG) monitoring.[65] Sleep was scored on the basis of EEG and EMG signals, and each 30-second epoch was classified as awake, drowsy (stage 1 SWS), light sleep (stage 2 SWS), deep sleep (stages 3+4 SWS), or rapid eye movement (REM) sleep (or cataplexy for the narcoleptic dog).[66] Used with permission from *Veterinary Record*. Tonokura, et al. 2007;161:375-380.

SWS stages and REM sleep are best determined and studied by polysomnography, which allows for the simultaneous measurement of three physiological variables: central nervous activity of the brain (EEG); movement of the eye (electrooculography [EOG]); and/or skeletal muscle tissue for muscle tone (electromyography [EMG]). Polysomnography (PSG) is considered the "gold standard" in determining sleep/wake status.

Domesticated dogs possess a clear diurnal activity pattern[17,20,32,47] and have sleep-wake cycles comparable to human sleep. The similarity is reflected in the dog's ability to transition between the different sleep states of REM and SWS sleep.[17-20,47-49] Sleep state transition has been observed using the polysomnography methodology, and the large amount of electrogram data is summarized to generate a hypnogram that outlines the transitioning between wakefulness and the various sleep stages. A schematic representation of a healthy dog hypnogram, and one from a narcoleptic dog, is provided to illustrate daytime sleep stages and the obvious differences in daytime sleep/wake patterns associated with narcolepsy (Figure 1).

The relative proportions of different sleep and wake states observed in dogs, as determined over a 24- or 48-hour period,

Table 1. Summarization of studies that evaluated the percent (mean values) of recording time spent in different states of sleep or wakefulness.

	Citations					
	18	**17**	**47**	**19**	**20**	**49**
Recording Timeframe	48 hours	24 hours	24 hours	24 hours	24 hours	12 hours[1]
Wakefulness	42.6	44	52.7	46.2	54.4	39[2]
Light Sleep (Stage 1+2 SWS)	18.4	21	20.7	16.7	17.0	
Deep Sleep (Stage 3+4 SWS)	28.0	23	14.4	23.6	15.6	42[2,3]
REM Sleep	11.1	12	12.1	13.5	13.0	20[2]

[1]Data from Takeuchi and Harada, 2002, was representative of recordings collected during the night phase; [2]percent values are estimates based on reading the data published in bar graphs; and [3]data was published as total slow wave sleep.

are generally similar across multiple studies (Table 1). These studies revealed that dogs spent between 42 and 54% of a 24-hour period in the waking state, approximately 17 to 21% in stage 1 ("drowsy" state) and 2 SWS (light SWS), 15 to 28% in stage 3 and 4 SWS (deep SWS), and 11 to 13.5% in REM sleep. These studies also calculated REM sleep cycles and duration, which were relatively consistent across studies and demonstrated that dogs have a polycyclic sleep/wake cycle in which they frequently transition in and out of sleep multiple times through the night.

Lucas and co-workers (1977) observed that dogs experience REM sleep approximately two times for every sleep cycle lasting about 83 minutes. Several studies also provide evidence that the NREM-REM cycle is relatively short, ranging from 15 to 30 minutes compared to humans who typically have NREM-REM cycles that predictably last 90 to 120 minutes.[8,50] Transition into REM sleep for dogs was often preceded by a brief episode of deep SWS. Then REM sleep was followed by awakening or a "drowsy" state (stage 1 SWS), which led to the dogs sleeping again.[17,19]

In addition to dogs having a shorter NREM-REM cycle, the proportion of wakefulness experienced during the sleep cycle is 8 to 10 times greater than humans, who experience less than 5% of the sleep cycle in wakefulness.[51] Lucas and co-workers (1977) also compared their dog findings to sleep-wake observations in the cat[23] and noted that dogs experience more frequent interruptions of alert and drowsy states within a sleep cycle compared to cats, especially during the 10-minute interval preceding REM onset. These researchers hypothesized that this frequent waking behavior may be part of an innate behavioral mechanism to periodically scan the environment possibly relating to self-protection and/or feeding.

Day-Night Locomotor Activity in Dogs to Assess Activity/Rest Cycles

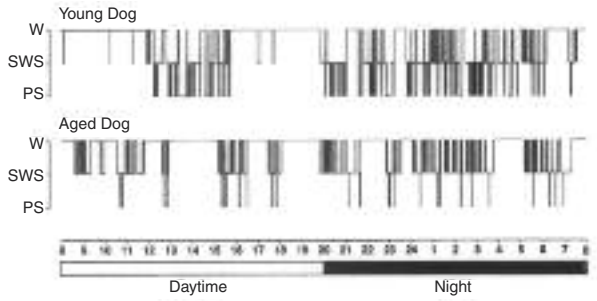
Recording and evaluating locomotor activity in dogs has been one way of assessing day/night behavioral patterns. Activity pattern alignment in animals defines the classification of nocturnal or diurnal, and activity rhythm is largely influenced by feeding or seeking food, but many factors are involved. These can include temperature, environment, breeding, predation, season, presence of other animals, and/or other factors. In dogs, the alignment of sleep time with a diurnal or nocturnal behavior pattern differs between kennel/home-living pets and wild/feral canids. Dogs living in a kennel or home-based environment consistently possess a diurnal sleep pattern in which most activity occurs during the daytime and aligns with the pet owner's routine.[32,47,52,53,55,56] In contrast to domesticated dogs, wolves and foxes were observed to generally sleep more during the daytime hours and have increased activity at night. In addition, feral dogs exhibit a similar increase in nighttime activity.[54]

PSG wakefulness of dogs predominates during the light phase of a light/dark cycle, as wakefulness comprises 63 to 70% of the daytime readings[20,47] compared to 40 to 42% during the nighttime phase. Similarly, 60 to 75% of total locomotor activity, as determined with the use of ambulatory activity monitoring, occurred during the 12-hour daytime period.[32,55,56] The time in which the highest level of activity occurred during the daylight phase can vary, as some studies observed the highest level of locomotor activity during the first six hours of light,[55,56] whereas others have noted activity was greatest at midday (after 12 p.m.).[32] These differences were likely influenced by feeding frequency (twice[55,56] daily versus once[32] daily) and/or time of feeding relative to light onset.

Sleep and Aging Data

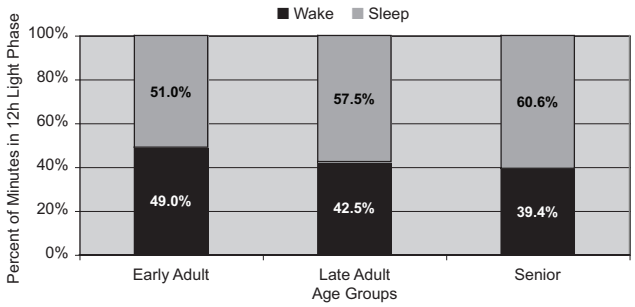
With increasing age, humans develop chronic problems associated with sleep, such as frequent nighttime waking, increased incidence of early-morning awakenings, and increased daytime naps.[16,27] In general, there is awareness by dog owners that activity declines with advancing age.[57-59] However, declining activity can be influenced by various age-related factors (i.e., loss of vision, arthritis, excess weight and/or others). Since changes in locomotor activity and sleep/wake patterns have been observed in people with advancing age, a more refined

Figure 2



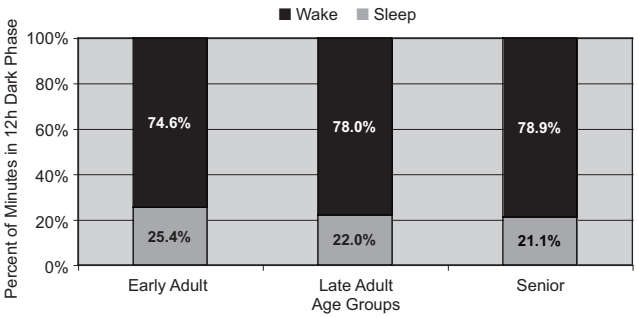
Hypnograms of (a) young (3 to 4 years old) and (b) aged (16 to 18 years) dogs. Recordings were obtained from cortical electroencephalography (EEG) and neck muscle electromyography (EMG) monitoring.[65] Sleep was scored on the basis of EEG and EMG signals, and each 15-second epoch was classified as wakefulness (W), slow wave sleep (SWS), paradoxical sleep (PS or REM sleep).[47] Used with permission from *Behavioral Brain Research*. Takeuchi, Harada. 2002;136:193-199.

Figure 3



Mean proportion of estimated wakefulness and sleep minutes within the 12-hour light phase (7 a.m. to 7 p.m.) from adult Beagle dogs of different age groups. Activity monitors were worn on collars to record data over five days to generate a daily mean for each dog. Age groups were: early adult dogs (1.5 to 4.5 years of age, n=8), late adult dogs (7 to 9 years of age, n=8), senior dogs (11 to14 years of age, n=7). Light phase sleep time marginally (p=0.09) increased by 75 minutes in senior dogs.

Figure 4



Mean proportion of estimated wakefulness and sleep minutes within the 12-hour dark phase (7 p.m. to 7 a.m.) from adult Beagle dogs of different age groups. Activity monitors were worn on collars to record data over five days to generate a daily mean for each dog. Age groups were: early adult dogs (1.5 to 4.5 years of age, n=8), late adult dogs (7 to 9 years of age, n=8), senior dogs (11 to14 years of age, n=7). Dark phase sleep time marginally (p=0.06) increased by 31 minutes in senior dogs.

characterization of rest/activity rhythms of different age dogs would contribute to a more comprehensive understanding of advancing age on behavioral activity in dogs.

An initial study assessing day/night locomotor activity in senior dogs compared to young dogs was conducted to determine if activity/rest patterns were altered with age.[32] They observed that total activity differed with age, with senior dogs exhibiting less activity compared to young, adult dogs. This difference in activity was a result of reduced activity during the day, as nighttime activity was not different with age. More recent research has indicated that senior dogs (11 to 14 years) demonstrate reduced activity during both daytime (51%) and nighttime (55%) compared to young dogs (1.5 to 4.5 years).[56]

As in humans, changes in sleep/wake rhythms occur in the dog with advancing age.[49] PSG monitoring of very old dogs (16 to 18 years) indicated a significant increase in the amount of daytime SWS sleep and a significant decrease in the amount of REM sleep during both the daytime and nighttime. These researchers observed that the senior dogs exhibited fragmented sleep over the course of light phase with no peak sleep time, whereas peak sleep was observed in the young dogs from 12:00 to 16:00 hour (Figure 2). In addition, wakefulness tended to increase during the night (42 versus 55 wake bouts, young versus senior, respectively). Ultimately, these observations were consistent with changes observed in older people and changes in sleep/wake rhythms reported in narcoleptic dogs.[60]

Recently, age-related changes in actigraphy-generated sleep variables have been observed in dogs by subjecting the activity count data to a sleep/wake scoring threshold algorithm set to 80 counts/epoch.[61] This study revealed that senior dogs (11 to 14 years) were estimated to sleep 75 more minutes during the 12-hour light phase (Figure 3) and 31 more minutes during the dark phase (Figure 4) compared to young adult dogs (1.5 to 4.5 years). Further analysis revealed that the increase in daytime sleep was from an increase in the number of daytime nap bouts (40 versus 31) and not an increase in nap-bout duration. In contrast to the PSG study, senior dogs did not experience an increase in nighttime wake bouts. In fact, nighttime wake bouts quantitatively decreased with age (21 versus 17, young versus senior, respectively), which corresponds to the increase in total nighttime sleep minutes. There are many factors that can be contributing to the inconsistency between

these two studies, not the least of which could be the dogs used in the studies, the different ages of the senior dogs, and also the settings applied to analyze the actigraphy data.

As mentioned previously, activity onset in senior dogs was delayed 2 hours after lights on and 1.5 hours after activity onset in young dogs.[32] This observation that activity onset was significantly delayed in senior dogs is contrary to the increased incidence of early morning awakenings observed in humans. In contrast, recent data from our lab indicates that dogs can exhibit an earlier morning awakening, as initial activity was calculated to occur approximately 30 minutes or 1 hour prior to lights on, depending on the method of activity onset determination (unpublished data). However, the activity onset did not differ with age. Siwak[32] indicated that the initial bout of activity was defined as the first duration of activity lasting at least 30 minutes. It is possible that if the initial bout of activity was of shorter duration, activity onset might be different. The discrepancy between studies may also be related to feeding time, as dogs monitored in our lab were fed twice daily at 8 a.m. and 8 p.m., whereas Siwak and coworkers (2003) had a once a day feeding regimen.

Activity Rhythms and Cognition

In addition to evaluating the day/night activity patterns of senior dogs, Siwak and co-workers[32,62] evaluated senior dogs that were determined to be cognitively impaired, as determined by neuropsychological testing that evaluated memory and executive function. Cognitively impaired individuals, like those with Alzheimer's disease (AD), have disrupted circadian rhythms and experience significantly more daytime sleep and disrupted nocturnal sleep.[63] In the Siwak studies,[32,62] the impaired senior dogs exhibited more daytime activity compared to the unimpaired senior dogs, and similar daytime activity to the young dogs. The impaired dogs also experienced a delay in peak activity compared to the other dogs, which the authors suggested was similar to the phase delay observed in AD patients.[30,64] Satlin (1991) observed that some AD patients are referred to as "pacers" because they exhibit a higher level of activity than cognitively healthy individuals and AD patients that are "non-pacers." It will be interesting to determine if dogs diagnosed with severe canine cognitive dysfunction also tend to exhibit either hyperactive or hypoactive behavioral patterns relative to healthy aged dogs.

Summary

This review has compiled and described the rhythms of sleep/wake cycles and activity/rest patterns in dogs, along with some physiological aspects of circadian regulation. In doing so, an attempt has been made to not only consolidate the existing canine literature on this topic, but also to indicate that relatively little work has been conducted with dogs to examine the use of actigraphy as a means of assessing sleep physiology. The assessment of sleep/wake rhythms with actigraphy could be an important tool in continuing to explore the physiology of sleep or related circadian biorhythms, yet the methodology still has limitations and more characterization is required.

Clearly, the domesticated dog possesses diurnal behavioral attributes and polyphasic sleep. The activity/rest patterns are the most prominent rhythm in the dog and provide an indication of the animal's biological clock. The physiological characteristics of sleep in dogs are similar to those observed in other species, particularly with respect to the SCN and circadian regulation by melatonin.

One feature of activity rhythms and physiological aspects of sleep that is common between species is the influence of age. The etiology of age-related changes in activity can be a manifestation of many factors, and can be a problem for older dogs as a significant reduction in physical activity can exacerbate the age-related decline in lean muscle tissue. People can see firsthand that sleep patterns and aging impact the lives of their pets in similar ways as it does themselves or others. However, it is not clear what interventions may provide support for age-related changes in sleep patterns, particularly since this issue has not been thoroughly resolved in human medicine. Yet, further examination of healthy activity/rest patterns and the corresponding effects of aging on sleep variables in dogs may eventually be extended into the clinic by providing some perspective of the aging process or cognitive status to the clinician and the pet owner.

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Enhancing Cognitive Functions in Old Dogs: A New Nutritional Approach

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Abstract

This study was designed to determine whether dietary supplementation with medium-chain triglycerides (MCT) can improve cognitive function in aged dogs by providing the brain with ketones as an alternative energy source. Aged dogs were randomized into two groups based on baseline cognitive tests and were maintained on either a control diet or an MCT diet for eight months. During the feeding trial, dogs were tested on a battery of cognitive test protocols to assess learning ability, memory and attention. Dogs fed the MCT diet showed significantly better performance on most of the cognitive tests than the control dogs.

Glossary of Abbreviations

AD: Alzheimer's Disease
ANOVA: Analysis of Variance
ATP: Adenosine Triphosphate
BHB: Beta-Hydroxybutyrate
CBC: Complete Blood Count
DNMP: Delayed Non-Matching-to-Position Task
MCT: Medium-Chain Triglycerides
MER: Maintenance Energy Requirements
PUFAs: Polyunsaturated Fatty Acids

Introduction

Decline in brain energy metabolism is a common feature associated with aging in animals including rats,[1] dogs,[2] humans,[3] and monkeys,[4] and may be partially responsible for age-dependent cognitive decline. For example, Milgram[5] reported that cognitive function decreased significantly in middle-aged Beagle dogs compared to young dogs, while a separate study indicated that cerebral glucose metabolism was significantly reduced in middle-aged Beagle dogs compared to 1-year-old dogs.[2] Interestingly, human studies showed that cerebral glucose metabolism was significantly lower in elderly patients with Alzheimer's disease (AD) compared to healthy elderly control subjects [6] or patients with mild cognitive impairment.[7] These data suggest that the reduction in brain glucose metabolism is a common feature associated with aging, that the process is progressive, starting around middle age, and that the decline in glucose metabolism may, at least partially, contribute to the cognitive decline associated with aging.

The brain accounts for only about 2 to 3% of body weight, but consumes 25% of whole body glucose utilization.[8] A tight coupling exists between neuronal activity and cerebral glucose utilization, and sustaining increased neuronal activity usually depends on increased adenosine triphosphate (ATP) production from glucose metabolism.[9] Although the brain utilizes glucose as the main energy source, the brain can utilize alternative energy sources to compensate for an insufficient supply of glucose. For example, ketone bodies (acetoacetate and β-hydroxybutyrate) are a natural endogenous energy source mainly produced by the liver from mobilization of endogenous body fat and utilized by extrahepatic tissues (brain, heart, kidney, muscle, etc). In fact, the brain is able to metabolize ketone bodies as energy substrates under the conditions of starvation or high fat diet.[10]

Henderson[11] proposed that dietary supplementation with MCTs can increase blood and brain levels of ketone bodies without the need for restricting dietary carbohydrates. MCTs are readily digested, and the resulting medium-chain fatty acids are converted to ketone bodies by the liver and, to a lesser extent, by astrocytes in the brain. The ketone bodies can then be used by neurons as an alternative energy source when there is a decline in brain glucose metabolism.

Reger et al.[12] reported that MCT supplementation to patients with Alzheimer's disease resulted in an improvement of cognitive function in a subset of subjects that were negative for the apopolipoprotein E ε4 allele, and that cognitive improvement correlated positively with blood levels of beta-hydroxybutyrate. More recently, Page et al.[13] found that hypoglycemia impaired cognitive function and that dietary supplementation of MCTs increased blood level of ketone bodies and reversed cognitive impairment caused by hypoglycemia in intensively treated type 1 diabetic patients. These findings provide further evidence that deficiency in the brain energy supply results in cognitive impairment, and MCTs can provide the human brain with ketone bodies as an alternative energy source to compensate for the deficiency of glucose supply.

The objective of this study was to determine whether dietary MCT supplementation could improve cognition in aged dogs.

To that end, cognition was measured by a battery of cognitive tests that assessed learning ability, visuospatial function and attention in aging dogs fed either a control diet or one containing 5.5% MCTs during an eight-month feeding trial.

Materials and Methods

Dogs and Diets

Twenty-four Beagle dogs (10 males and 14 females) of 7.5 to 11.6 (mean age±SD: 9.79±0.84) years of age were recruited in the study. The study was approved by the CanCog Technologies Institutional Animal Care Committee. During the baseline phase, all dogs were tested on a variable version of the delayed non-matching-to-position (DNMP) task,[14] a size discrimination learning task and a size discrimination reversal task.[15,16] Performance of the dogs on these three tests was ranked, and the ranking was used to assign dogs to two cognitively equivalent groups. One of the groups was then fed a standard control diet and the other a similar diet modified to contain 5.5% MCTs for eight months. Both diets, manufactured by Nestlé Purina PetCare (St. Louis, MO), were isoenergetic and contained the same levels of protein, fat and carbohydrates (Table 1). The dogs were offered food once daily for about an hour and fed to meet their maintenance energy requirements (MER) estimated by the formula "MER = 110 kcal/day * (BW0.75)."[17] Dogs had free access to water.

Cognitive Test Apparatus and Test Criterion

The cognitive testing apparatus consisted of a wooden box approximately 0.609 m × 1.15 m × 1.08 m.[18] The dogs were in the test apparatus only during the cognitive tests. The animal care technician was separated from the dog by a plastic partition containing a one-way mirror and a hinged door. The food reward tray was made of Plexiglas and contained either three or four equally spaced food wells, depending on the task. The food reward was approximately one gram of Purina® Pro Plan® brand Adult Chicken & Rice Entrée (wet dog food, manufactured by Nestlé Purina PetCare).

To pass one test and move on to the next test, dogs had to complete a two-stage criterion. The first stage was successfully completed when the dogs responded correctly in at least 9 of 10 trials or in 8 of 10 trials over two consecutive days. The second criterion stage was achieved when the dogs responded correctly in more than 70% of the total trials over three consecutive days.

Table 1. Nutrient Composition of Diets*

	Control	MCT
Nutrient Composition (% as fed)		
Moisture	7.41	7.10
Ash	6.32	6.24
Crude Protein	32.80	33.10
Crude Fat	18.5	18.8
Crude Fiber	2.86	2.47
Linoleic Acid (% of total fat)	10.1	10.2
Caprylic Acid (% of total fat)	<0.10	24.1
Capric Acid (% of total fat)	<0.01	1.33
Energy Content		
Calculated ME§(kJ/g)	17.86	18.06

*The table is modified from the original published in a previous paper.[22]
§Calculated based on the predictive equation for metabolizable energy in dog foods.[28]

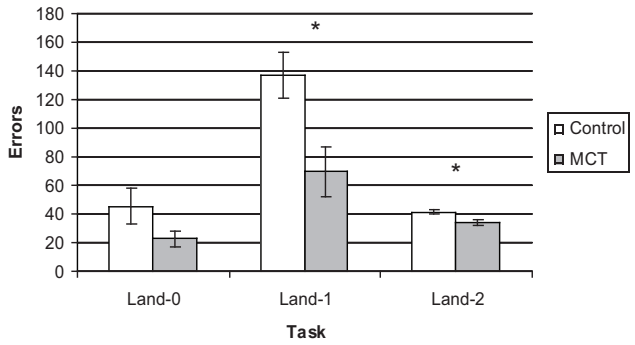
Cognitive Test Protocols and Schedule

After a one-week wash-in, all dogs were tested on a landmark protocol for up to 92 days. All the dogs were subject to an egocentric protocol test, starting on day 100 after the initiation of the study. The variable object oddity task protocol began on day 190 and continued for 35 days. During the whole study, dogs were tested once daily on one cognitive task at a time using a test schedule similar to previous studies.[19,20]

Dogs' learning ability and visuospatial function was assessed using a landmark discrimination protocol. The details of the landmark test protocol have been previously published.[21,22] Briefly, the landmark discrimination protocol included three separate tasks (land-0, land-1 and land-2), which are designed to assess allocentric spatial ability and require dogs to use external landmarks to localize objects in space. In all landmark protocols, dogs had to use a yellow peg (2 cm x 2 cm x 9 cm) as the external landmark to find the food reward. In land-0 test, two identical white coasters were used to cover food wells and the yellow peg was attached to one of the coasters. For land-1 test, the landmark was moved 1 cm medially and diagonally away from the edge of the coaster. For land-2 test, the landmark was moved 2 cm medially and diagonally away from the edge of the coaster. On each trial, the animal care technician placed the food reward in either the left or right food well and positioned the landmark accordingly. Dogs were able to obtain the food reward if they displaced the coaster attached to or closer to the yellow peg. In this and subsequent cognitive tasks, food inaccessible to the dogs was placed in the bottom of the coaster or objects associated with nonreward food well(s) in order to prevent the dogs from responding based on olfactory cues.

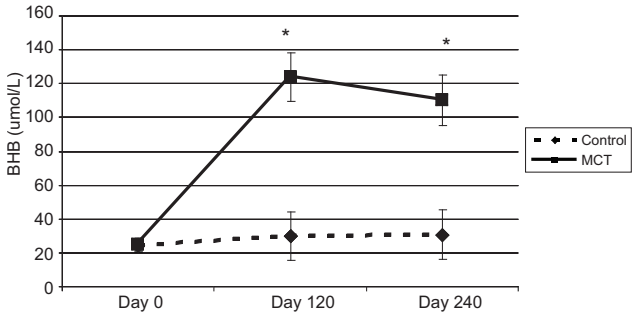
The egocentric protocol evaluates the animals' egocentric spatial learning ability to use a body-centered coordinate system to locate objects in space. The egocentric reversal protocol provides an additional measure of flexibility and executive function.[19] These protocols have been described in previous publications.[19,22] Briefly, the protocol first evaluated the ability of the dog to selectively respond to an object based on prox-

Figure 1



Effects of dietary MCT supplementation on blood BHB. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is adapted from the data presented in a previous publication.[22]

Figure 2



Effects of dietary MCT supplementation on dogs' performance in the landmark tests. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is adapted from the data presented in a previous publication.[22]

imity of the dog to its left or right side, and second, to reverse its original response. The egocentric protocol included three phases: a preference phase, an acquisition phase and a reversal phase. During the preference phase, the dog was presented with 10 discrete trials with identical objects covering both food wells containing food rewards over a single test day. The side chosen most frequently was selected as the preferred side and assigned to be the correct side for the acquisition phase. For dogs that responded five times to each side, a coin toss was used to determine the preferred side. All dogs were given two reversal tests (reversal 1 and reversal 2). The reversal phase was initiated on the day following completion of the acquisition phase. The reversal test procedure was identical to that of the acquisition phase except that the rewarded side was switched to the opposite side. Thus, if the dog's preferred side was right in the acquisition phase, the side closest to its left was rewarded in reversal 1 testing. Dogs that passed reversal 1 testing moved on to reversal 2 testing, which was identical to reversal 1 testing except that the rewarded side was switched to the opposite side of the reversal 1 testing.

The oddity discrimination protocol was designed to assess attention of the dogs. The details of the protocol were published in a previous paper.[22] Briefly, the task had three phases: acquisition, same distractor and different distractor. During the acquisition phase, the dog had to learn to selectively respond to a particular object to obtain a food reward. For the same and different distractor phases, dogs were presented with one, two, three or four objects, including the object they had been trained to respond to during the acquisition phase. The number of distractors varied from 0 to 3. Once a dog passed the acquisition phase, the dog moved on to the distractor phases. The first 7 sessions (1 to 7) had the same distractor phase, with the same distractors as in the acquisition phase. Sessions 8 to 14 covered the different distractor phase, with the reward object remaining unchanged but using new distractors.

Body Weight, Clinical Chemistry, Complete Blood Count (CBC) and Blood Ketone Bodies

Baseline blood samples were collected for measurements of beta-hydroxybutyrate (BHB), complete blood counts (CBC), and clinical chemistry. These measures were repeated after four and eight months of the feeding trial. Blood BHB samples were collected two hours after feeding. Samples for clinical chemistry, CBC and BHB were sent to Advance Vet Lab (Mississauga, Ontario) for analyses. Body weight was recorded at two-week intervals.

Statistical Analysis

Errors were used as the dependent measure and group comparisons were made using both Student's t-test and repeated measure analysis of variance (ANOVA). Values are means ± SEM except the cognitive data in the figures.

Results

Effect on Body Weight and Blood Levels of BHB, CBC and Clinical Chemistry

The MCT diet significantly increased blood ketone bodies as measured by BHB under fed conditions (Figure 1). Body weight did not change in either group. All the CBC and clinical chemistry parameters were within normal ranges for both groups at baseline and throughout the study (data not shown).

Effect on Performance of the Landmark Test.

The effects of MCT diet on landmark test were presented in Figure 2. The two groups were compared using repeated measure ANOVA, and the results showed that the MCT group differed significantly from controls, making fewer errors on the land-1 task (p=0.02), but not on the land-0 task (p=0.08).

The average days for the dogs fed the MCT diet to reach the test criterion in land-0 and land-1 tests were 15 days and 31.67 days after the initiation of the feeding trial, respectively. The average days for the dogs fed the control diet to reach the test criterion in land-0 and land-1 tests were 20.5 days and 50.17 days after the initiation of the feeding trial, respectively. Eleven dogs from each group completed the land-2 task. The groups were compared with Student's t-test, and the results showed that the MCT group made fewer errors than dogs in the control group on the land-2 task (p=0.0364).

Effect on Performance of the Egocentric Test

Multiple comparisons were performed to analyze the group effect (Fischer's least significant difference), and the results showed that the MCT diet significantly improved egocentric reversal 2 task (p=0.03, Figure 3). Dogs fed the MCT diet tended to make fewer errors to reach criterion in both acquisition and reversal 1 tests compared with control dogs, but the difference failed to reach statistical significance (data not shown).

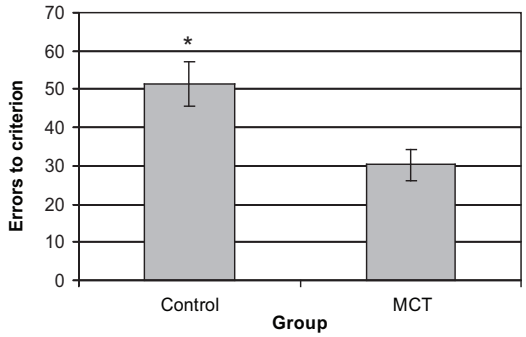
Effect on Performance of the Variable Object Oddity Test

Results from the acquisition phase of the oddity task showed that the dogs fed the MCT diet committed fewer errors than controls, but the differences were not significant ($p > 0.05$). During the zero distractor phase, all dogs performed at a very high level of accuracy leaving no room for improvement, and the data from this phase were not used in the statistical analysis. The data from 1-, 2-, and 3-distractor conditions were analyzed, and the results showed that dogs fed the MCT diet made significantly fewer errors per 168 trials than the control dogs (Figure 4).

Discussion

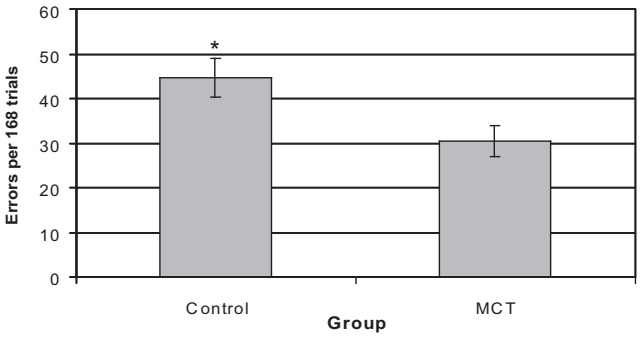
The objective of the present study was to evaluate whether dietary supplementation with MCTs can enhance cognitive function in aged dogs. Cognitive protocols designed to assess object discrimination learning and allocentric spatial ability showed improved function in the MCT-supplemented dogs. The average days for the dogs fed the MCT diet to reach the test criterion in land-0 and land-1 tests were 15 days and 31.67 days after the initiation of the feeding trial, respectively. These data indicated that on the average, the MCT supplementation tended to improve spatial learning and memory within two weeks after initiation of MCT supplementation and significantly improved spatial learning and memory in old dogs within one month after beginning supplementation. Dietary MCTs also significantly improved egocentric reversal learning and oddity task evaluations compared to the control dogs, indicating that the dietary MCT supplementation significantly enhanced spatial memory, executive functions and concept learning in old dogs, as well as improving attention ability. These results in dogs are comparable to those observed in humans.[12,13]

Figure 3



Effects of dietary MCT supplementation on dogs' performance in the egocentric reversal 2 task. The performance was expressed as error to criterion. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is modified from the original presented in a previous publication.[22]

Figure 4



Effects of dietary MCT supplementation on dogs' performance in the oddity attention task. The performance was expressed as errors per 168 trials. The data are means ± SE, n = 11 for the control group, n = 9 for the MCT group. *Mean values significantly differed (P<0.05).

Blood levels of BHB at baseline, 120 and 240 days after initiation of the feeding trial were measured to determine the effects of MCT supplementation on the levels of ketone bodies in old dogs. Dogs fed the MCT diet had higher blood ketones, under fed conditions, confirming the ability of dietary MCTs to increase blood ketone levels under fed conditions and without restricting dietary carbohydrates. The resulting blood levels of ketone bodies were well-tolerated by dogs and were about four times lower than the levels of ketone bodies (0.5 mmol/L) in fasted dogs.[23]

All parameters of CBC and blood biochemistry were within normal physiological ranges at the end of the study in all dogs, indicating that the MCT diet was suitable to maintain the health of the dogs. In addition, an independent safety study confirmed the safety of 15% dietary MCT in dogs.[24]

Normally most of the ATP production in the neurons comes from glucose metabolism, and neurons maintain a tight coupling

between neuronal activity and cerebral glucose utilization. Increased neuronal activity usually requires increased glucose metabolism for more ATP production.[9] Since the brains of old animals have a reduced ability to metabolize blood glucose, they are not able to increase ATP production high enough to support increased neuronal activity, which may, at least partially, contribute to the decline in cognitive function in old animals. Reduced brain glucose metabolism is also observed in elderly humans[3]; it is highly possible that dietary MCT supplementation may be able to improve brain function in elderly people with and without dementia symptoms.

In addition to providing an alternative energy source, dietary MCT may have other beneficial effects on brain function. For instance, poor cognitive function is associated with loss of polyunsaturated fatty acids (PUFAs) in the brains of old animals.[25] Animal studies showed that dietary MCT resulted in enhanced concentrations of PUFAs in the brains of rats[26] and dogs.[27] These data suggest that dietary MCT may indirectly enhance brain function by increasing brain levels of PUFAs.

In summary, this study shows that dietary MCT supplementation can significantly increase blood ketone body concentrations under fed conditions and improve cognitive function in old healthy dogs. The MCT diet had no adverse effects on CBC and blood chemistry.

Acknowledgements

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Q&A Discussion

Q: Dr. Wouter Hendriks, Utrecht University: Can you elaborate a little bit on the allocation procedure of the dogs to the various treatments? Were the dogs equally intelligent in both groups before you started?

A: Dr. Pan: Yes, at the beginning we used two tests based on cognition, DNMP and the size discrimination test, then we randomized based on cognition to two groups, so they have equal cognitive functions between two groups at the beginning.

Q: Dr. Esther Plantinga, Utrecht University: I was wondering how do the data of the MCT-supplemented old dogs compare to young dogs relative to their cognitive function.

A: Dr. Pan: The MCT helped improve cognition, but not close to the young dogs, maybe 70% or 60% of young dogs' performance. The older dogs fed MCTs were intermediate between the young dogs and the untreated control dogs.

Q: Dr. Ernie Ward, Seaside Animal Care: I have a different interest in MCTs from an athletic performance standpoint. But in humans we've known that MCTs have some effects, and I've got a couple of little follow-up points to this. No. 1, the MCTs can suppress appetite. While this mechanism isn't totally understood, did you see that in any of your dogs?

A: Dr. Pan: No. On the contrary, study dogs actually ate more in the MCT group compared to the control group. They did not gain weight but actually ate more to maintain body weight.

Q: Dr. Ernie Ward, Seaside Animal Care: In humans it's been reported MCTs can cause nausea and GI discomfort. Did you see any of that?

A: Dr. Pan: Not in our study, and in addition, we actually sponsored a second study to look at safety and other parameters. They found that even with 15% MCT in their diet, there was no impact on all those parameters. That study has already been published.

Q: Dr. Ernie Ward, Seaside Animal Care: From a manufacturing standpoint, many dry dog foods are going through an extrusion process, in which they are flooded by thermal stress heat. And an issue with MCTs in humans is that it oxidizes when you start getting at high temperatures, above about 150 degrees. So how did you accommodate for this?

A: Dr. Pan: We have a patent on this to incorporate MCTs into dry food. Our food scientists figured out a way to put in and without oxidation and without influencing palatability. We use a natural source of MCTs from coconut oil.

Dr. Dottie Laflamme, Nestlé Purina Research: Ernie, to your point, one of the things that people often don't understand about commercial pet food extrusion is that it's not actually a high-temperature cooking process. It's actually a very short-term, relatively low-temperature process. It's about 120 to 140 degrees centigrade. So, it's not like the fryer stuff where you have really overcooked, rancid fat. And some people just don't understand that aspect of commercial pet food.

Q: Dr. Richard Hill, University of Florida: My question was going to be how you controlled for energy intake. You substituted MCTs with triglycerides, is that right? Or what was your substitution?

A: Dr. Pan: We reduced the beef tallow in exchange for MCTs.

Q: Dr. Richard Hill, University of Florida: Well, of course, beef tallow contains more calories per gram than MCTs do, and I believe MCTS increase the metabolic rate a bit. So you have to eat more of it, so they would have to eat more. So you fed to maintain body weight, presumably. This means your treatment dogs consumed two things more as a consequence. One would be protein, and the other one would be more glycerol, which would be a source of glucose. So how do you distinguish between your effect from giving more glucose versus glycerol and giving more protein?

A: Dr. Pan: We actually measured the blood glucose in our dogs. There are no differences between two groups in the blood glucose. But, I don't think the major benefit would be from the glucose. We think the major benefit is from ketone bodies produced from the MCTs. The whole purpose is to give the MCTs because the neurons are less capable of metabolizing glucose. So even if we increase the glucose intake a little bit, I don't think it will make a difference because the blood glucose level didn't change that much. I think the major benefit still comes from the ketone body production because we actually see the correlation between the blood ketone body concentration and performance in the dogs.

Q: Dr. Richard Hill, University of Florida: Well, certainly you had more beta-hydroxybutyrate, but that could be due to increased production or it could be due to reduced utilization. And, so there may be two aspects to that.

A: Dr. Pan: Yes, it's possible. I think the glucose also plays an important role. Actually, maybe 80% of the energy soup comes from glucose, maybe only 20% from ketone bodies. So we are not denying that glucose is an important part of this benefit, but the MCT makes the difference because we saw the correlation between the ketone body concentration and cognition in the model.

Q: Dr. Aulus Carciofi, University Sao Paulo: I have no doubts about this study. I would like your comments about when we put a dog in a weight loss program; we see that they become more alert. We see improvement in behavior. I understand that this improvement in behavior may have several causes, but could you comment about the possibility that some of these improvements in alertness and the behavior of the dog could be attributed to better hydroxybutyrate and more ketones in the blood of these dogs?

A: Dr. Pan: Yes, it could be, but when you have weight loss, you usually see improvement in glucose tolerance. What happens is chronically obese dogs usually have a glucose intolerance, not only for muscle, liver, but I think the brain also has problems using glucose. When you have weight loss, you not only increase ketone bodies, you also improve the glucose sensitivity in different tissues that actually helps the brain. Actually, some people suggest that dementia is a type 3 diabetes condition, so you can imagine if you induce weight loss for the obese dogs, definitely you will see the benefit.

Q: Dr. Bob Backus, University of Missouri: I just have two questions. One is how close was the control diet to the test diet? Was it just the fat substitution? The other was sometime back I remember reading that humans may be unique in the brain being able to utilize ketone bodies for energy. Is there work to show that the dog can substitute ketone bodies for glucose like the human?

A: Dr. Pan: The only difference between the diets was we substituted some beef tallow with the MCTs. I haven't seen any *in vivo* study for dogs. But today, people look at dog milk because they have MCTs in the milk. Dogs are born to have the ability to metabolize MCTs and ketone bodies. So my speculation is that dogs must be able to shift metabolism from glucose to ketone bodies to sustain the brain functions to keep them alive. Bill, do you know any studies to confirm the dogs actually metabolize ketone bodies?

Dr. Bill Milgram. CanCog: I'm not sure if there have been any studies. Before we started this work, there was a myth that dogs don't even create ketone bodies. And we were told by members of the faculty at Guelph University that this couldn't possibly work because they don't produce ketones. And so the first step was to show that they produce ketones. There, obviously the beta-hydroxybutyrate is removed from the blood, but the actual mechanistic work hasn't been done. It hasn't been done with people either. It's a very difficult thing to do.

Dr. Richard Hill, University of Florida: It's some time since I read it and I don't read Dutch, so I only read the English version, but De Bruijne looked at starvation and ketone turnover

in dogs and found it twice the rate of that in people. And it actually didn't increase much with starvation.

Dr. Joe Millward, University of Surrey: Just a quick comment about the uniqueness of humans in using ketones in the brain. It is my understanding that a lot of work on the regulation of the interaction between glucose and ketones in the brain was done in rats. There was some nice work done in the 1970s showing that ketones specifically blocked the oxidation of glucose in rat brains, so it's not unique to humans. I've always worked on the assumption that all or most species can utilize ketones.

General Discussion

Cognitive Function & Antioxidants

Dr. Joe Millward, University of Surrey: It is my understanding that work on antioxidants more or less dropped out of human studies, and the main focus for cognition is on B12 and DHA. And the B12 issue is becoming more and more prominent. Given the problems of digestive function in the case of the cats that were discussed this morning, then surely that would have a profound effect on B12 uptake. It's now known that there are very big differences in the bioavailability of B12 from different food sources with the possibility that it binds to collagen in meat and is very poorly available. The Norwegians have got a lot of data on milk consumption and B12 status. I think that Rosenberg has published data showing that B12 deficiency is much more common when appropriate indicators are used and that meat consumption in the elderly has very little impact on B12 status. So given the potential for hypomethylation in the brain and all of that, then I'm very surprised that it just hasn't been discussed.

Dr. David Williams, University of Illinois: I have been measuring cobalamin in cats for 15 or 20 years now, and I've been recommending replacement therapy. And many owners, even though the cat is no longer deficient because we've rectified the blood levels and the methylmalonic acids are back to normal, they will continue to give cobalamin injections. The feedback from the owners is that it really makes a difference in their animal's behavior. They act younger; they just generally do better. It is anecdotal, and I've only heard this from cat owners, so it may or may not be true. Also you mentioned that neuropsychiatric problems in older human beings with cobalamin deficiency are very well-recognized. So it probably is a component of some of the cognitive dysfunction. And one final point. I forgot that in our study we did measure body weight. We gave 20 cobalamin-deficient cats weekly injections of cobalamin, and over four weeks, those cats gained either half a kilogram or half a pound. Either way, it was really quite a striking increase in body weight.

Activity & Learning in Aging Animals

Dr. Gary Landsberg, North Toronto Animal Clinic: There seemed to be disconnects between a couple of lectures when Bill was giving his talk on the learning deficits that go on in the dogs in the laboratory and when Xavier talked about the clinical signs in pet dogs. They seemed to be two different topics. Brian brought up one study that Christina [deRivera] did with the sleep/wake cycle alterations in these dogs with the poor scores. Remember, they were the old dogs that Gary mentioned that did poorly on their learning scores and on their memory scores. Those old, aged dogs with learning deficits also had, in similar studies, alterations in exploring novel toys, alterations in interactions with people, with unfamiliar people. So, in the laboratory, there were some clinical changes in some of these dogs and the way they explored and interacted and in their activity levels. And you saw increased activity in these demented dogs, is that correct? Increased aimless activity, I should say.

Dr. Bill Milgram, CanCog: The activity changes that we reported were either of two. Either the animals were couch potatoes, or they showed sort of a random circling where they were really hyperactive, but they would not explore the objects. And it was sort of striking. We would put them in a room, and they would circle round and round and round the room. The reason that was interesting to us was that it completely paralleled what we see in patients with Alzheimer's disease, where some of them are wanderers. And if you ever watch them it's remarkable, they are constant moving. Typically they will pick things up, put them in their pockets and walk out the door, but they don't really know what they are doing. And there was sort of that parallel between what we saw in the dogs, in the two different kinds of cognitively impaired dogs. The problem with this work is that you're dealing with relatively small numbers, so that in order to get a really large sample, we needed to have a much larger population of aged impaired dogs so that we could see a good division.

Dr. Gary Landsberg, North Toronto Animal Clinic: And then you see these repetitive pacing, repetitive licking, repetitive activity, older dogs with dementia. You see the older dogs that are less interested in exploring novel toys, new environment. And so there were some clinical signs in the laboratory dogs is the point I am getting at. These were published and are in the literature in the laboratory animals and correlate to some of the clinical signs we're seeing in clinical cases, so just trying to show the link between these populations and studies.

Dr. Bill Milgram, CanCog: I'm not sure if this is going to turn out or not, but we have some very preliminary observations with cats. I know these will make some of you very happy. Simply looking at the behavior of old cats, it looks like it will be a lot more informative than for dogs if you put them in a room, look at them and observe what they do. And you're looking at your really old cats. They seem like they're going to be fascinating, but we still have a lot of analysis to do.