LESS IS MORE
Advancing Life Through Diet Restriction

THE PURINA PET INSTITUTE SYMPOSIUM

September 20–21, 2002
Hyatt Regency Union Station
St. Louis, Missouri, USA
# TABLE OF CONTENTS

## General Sessions
- **Dietary Restriction and Aging: An Historical Overview** / Richard Weindruch, PhD .................................................. 7
- **Effects of Diet Restriction on Life Span and Age-Related Changes in Dogs: Experimental Design** / Richard D. Kealy, PhD, and Dennis F. Lawler, DVM .................. 11
- **Diet Restriction and Longevity: Chronic Diseases and Causes of Mortality** / Dennis F. Lawler, DVM, Richard H. Evans, DVM, MS, Richard D. Kealy, PhD, Jay M. Harrison, MS, MA, and Brian T. Larson, PhD .................. 13
- **Influences of Limit-Feeding and Aging on Antioxidant Status of Pair-Fed Labrador Retrievers** / Howard D. Stowe, DVM, PhD, Diplomate ACVN, Richard D. Kealy, PhD, and Dennis F. Lawler, DVM .................. 14
- **A Longitudinal Study of Immunosenescence: Does Diet Restriction Ameliorate the Aging Process in Dogs?** / Elizabeth H. Greeley, PhD, Joan M. Ballam, MS, Jay M. Harrison, MS, MA, Richard D. Kealy, PhD, Dennis F. Lawler, DVM, and Mariangela Segre, DSc .................. 15
- **Diet Restriction and Osteoarthritis in Animals** / George Lust, PhD .................. 16
- **Effects of Restricted Feeding on Onset, Incidence, and Severity of Hip Dysplasia and Osteoarthritis in Dogs: Diagnostic, Therapeutic, and Genetic Ramifications** / Gail K. Smith, VMD, PhD, Diplomate ACVS, Darryl N. Biery, DVM, Richard D. Kealy, PhD, and Dennis F. Lawler, DVM .................. 21
- **Clinical Significance of Osteoarthritis and Hip Dysplasia Findings in the Restricted Feeding Trial** / Gail K. Smith, VMD, PhD, Diplomate ACVS, Darryl N. Biery, DVM, Richard D. Kealy, PhD, and Dennis F. Lawler, DVM .................. 27

## Osteoarthritis
- **Diet Restriction, Carbohydrate Metabolism, and the Retardation of Senescence** / Edward J. Masoro, PhD .................. 29
- **Diet Restriction and Aging: Canine Carbohydrate and Lipid Metabolism** / Brian T. Larson, PhD, Dennis F. Lawler, DVM, Jay M. Harrison, MS, MA, and Richard D. Kealy, PhD .................. 34
- **Life is Shorter if You Eat Dessert First: Clinical Implications of the Purina 448 Study** / Deborah S. Greco, DVM, PhD, Diplomate ACVIM .................. 35

## Carbohydrate Metabolism
- **The Aging Cardiovascular System: Alterations Induced by Dietary Restriction** / Jeremiah T. Herlihy, PhD .................. 39
- **Electrocardiography in a Study of Diet Restriction and Aging in Labrador Retrievers** / Walter E. Weirich, DVM, PhD .................. 53
- **Systemic Arterial Blood Pressure: The Silent Killer that Never Should Be!** / Robert L. Hamlin, DVM, PhD, Diplomate ACVIM .................. 54

## Body Composition
- **Estimated Body Composition Values of Control-Fed Versus Restricted-Fed Labrador Retrievers in a Life Span Study** / Richard D. Kealy, PhD, Dennis F. Lawler, DVM, Brian T. Larson, PhD, and Edward C. Hume, BS .................. 55
- **Body Composition and Dietary Restriction in Rhesus Macaques** / Joseph W. Kemnitz, PhD, and Ricki J. Colman, PhD .................. 56
- **Controlling Body Composition: Who, When, and How** / D. P. Laflamme, DVM, PhD, Diplomate ACVN .................. 61
Brief Calorie Restriction Leads to Enhanced Insulin Signaling in Skeletal Muscle / Gregory D. Cartee, PhD, Carrie E. McCurdy, BS, Robert T. Davidson, PhD, and Edward B. Arias, PhD .......................................................... 65

Calorie Restriction Provides Insights into Insulin Signaling in Skeletal Muscle / Heidi K. Ortmeyer, PhD, Noni L. Bodkin, PhD, and Barbara C. Hansen, PhD ................................................................. 66

Effects of Calorie Restriction in Long-Lived, Growth Hormone (GH)-Resistant GHR/GHBP-Knockout (KO) Mice / Michael Bonkowski, MS, Khalid Al-Regaiey, DVM, and Andrzej Bartke, PhD ................................................................. 67

Age-Related Changes in Canine CD8 Memory Cells: A Longitudinal Aging/Dietary Restriction Study / Elizabeth H. Greeley, PhD, Joan M. Ballam, MS, Jay M. Harrison, MS, MA, Richard D. Kealy, PhD, Dennis F. Lawler, DVM, and Mariangela Segre, DSc ................................................................. 68

Inhibition of Th-2 Cytokines and Prolongation of Life Span of Lupus Disease Prone Mice by a Combination of Dietary n-3 Fatty Acids and Food Restriction / Gabriel Fernandes, PhD, Christopher Jolly, PhD, Richard Lawrence, PhD, and Dongxu Sun, MD, PhD ................................................................. 69

Energy Requirements of Old Cats / Gerardo Pérez-Camargo, MR CVS, PhD, and Robert Rudnick, MS ................................................................. 70

A Gene Expression Signature for Delayed Aging in Mice / Richard A. Miller, MD, PhD, Yayi Chang, BS, Andrzej T. Galecki, MD, PhD, Khalid Al-Regaiey, DVM, John J. Kopchick, PhD, and Andrzej Bartke, PhD ................................................................. 71

Comparison of Indirect Calorimetry Methods in Dogs: Open Flow Cage Versus Mask / Sharon A. Center, DVM, Diplomate ACVIM, Karen L. Warner, AS, LVT, and D.P. Carey, DVM ................................................................. 72
<table>
<thead>
<tr>
<th>Article Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Evaluation of the Effect of Limited Food Consumption on Radiographic Evidence of Osteoarthritis in Dogs</td>
<td>Richard D. Kealy, PhD, Dennis F. Lawler, DVM, Joan M. Ballam, MS, George Lust, PhD, Darryl N. Biery, DVM, Gail K. Smith, VMD, PhD, and Sandra L. Mantz</td>
<td>105</td>
</tr>
<tr>
<td>Effects of Diet Restriction on Life Span and Age-Related Changes in Dogs</td>
<td>Richard D. Kealy, PhD, Dennis F. Lawler, DVM, Joan M. Ballam, MS, Sandra L. Mantz, Darryl N. Biery, DVM, Elizabeth H. Greeley, PhD, George Lust, PhD, Mariangela Segre, DSc, Gail K. Smith, VMD, PhD, and Howard D. Stowe, DVM, PhD</td>
<td>110</td>
</tr>
</tbody>
</table>

**NESTLÉ PURINA BODY CONDITION SYSTEMS**

- Canine Body Condition System .......................................................... 119
- Feline Body Condition System .......................................................... 120
THE EARLY YEARS OF DIETARY RESTRICTION RESEARCH

It has been known for more than 60 years that dietary restriction (DR), if properly executed to avoid malnutrition, will greatly extend the maximum life span of laboratory rodents and decrease the incidence of cancer and other late-life diseases. This field began with observations published in 1935 from McCay et al. on rats whose growth was stunted by DR, which as they aged appeared much younger than age-matched, ad libitum-fed animals and lived far longer, too. Next, studies were published by Tannenbaum in the 1940s and 1950s on reduced spontaneous and induced cancer incidence in calorie-restricted mice. In the 1960s and 1970s, some of the most important work was published by Ross, who described longevity and spontaneous cancer patterns in rats on various DR regimens. Thus, the initial phase of DR research was focused on its effects on survival and cancer, but this began to broaden in the early 1970s with studies on the effects of DR on immune system aging in mice initiated by Walford et al. and Good et al.

During most of that period, these impressive actions of DR were regarded more as an interesting curiosity than as a unique model system for investigating the biology of decelerated aging. Interest in DR-induced phenomena increased markedly in the mid-1970s, and even more so thereafter, due in part to increased focus on the investigation of biological aging in the United States and other developed countries as well as the widening appreciation of the striking efficacy of DR to retard a broad spectrum of age-associated biological and pathological changes. Notable here are the efforts of Masoro’s group, which began in the late 1970s to characterize multiple responses of male Fischer 344 rats to DR.

THE 1980s

This was a decade of rapidly increasing interest in and research funding for DR. The National Institute on Aging (NIA) established a colony of some 30,000 rats and mice, which were either fed normally or subjected to DR, in order to support study by NIA-funded grantee laboratories to establish the validity and usefulness of potential biomarkers of aging. The 1980s also brought substantial progress on three other fronts:

1. characterizing the effects of DR on age-sensitive physiological and pathological processes in laboratory rodents;
2. studies in rodents on the mechanisms underlying aging retardation by DR; and
3. initiating long-term trials of DR in monkeys at the NIA and at the University of Wisconsin–Madison.
In 1988, this author and his mentor, Dr. Roy Walford, published a book which reviewed all published data on DR. As discussed therein, it is noteworthy that life-span extension by DR has also been observed in fish, spiders, *Daphnia* (water-fleas), and other non-rodent species which suggests a broad relationship between caloric intake and the aging process. Four other books on DR were published between 1989 and 1995.


**The 1990s and Beyond**

Over the last 12 years, there has been continuing emphasis on investigating underlying mechanisms of aging retardation in laboratory rodents and, quite recently, in simpler animal models such as yeast, nematodes, and fruit flies. In rodents, there is accumulating evidence to suggest that age-associated increases in oxidative stress and damage of mitochondrial origin may represent a primary aging process in postmitotic tissues, which is attenuated by DR. Our recent experiments in mouse skeletal muscle and brain using microarrays to conduct gene expression profiling support this notion and reveal two types of effects of DR on gene expression:

1. prevention of age-associated changes, and
2. shifting the expression level of genes that do not change with aging (transcriptional reprogramming).

A new area of inquiry is the attempt to develop drug or nutrient interventions that may mimic the actions of DR. A challenge in that regard is that the current “gold standard” of life extension for screening interventions to retard the aging process has some major shortcomings: the assay requires ~4 years to complete and provides imprecise data on the rate of aging in individual organ systems (e.g., did the candidate mimetic retard aging in the heart?). Importantly, advances in genomics provide exciting new opportunities and have created the new field of nutrigenomics, which can be applied to this problem. Our data strongly suggest that gene expression profiles (which consist of hundreds of markers of aging at the transcriptional level) can be used to determine the biological age of a tissue and thereby allow one to determine whether interventions can retard aging on an organ-specific basis. Further, one can quantify the ability of a candidate mimetic to induce the transcriptional reprogramming that is associated with DR.

Will DR retard the aging process in primates? The recent discoveries by Nestlé Purina scientists that DR in dogs increases average life span and health span represent an important extension to a larger mammalian species. The observations from the studies in rhesus monkeys subjected to DR and limited human epidemiologic data support the notion of human translatability. Compared with age-matched, normally fed controls, rhesus monkeys on DR for over a decade show increased insulin sensitivity, favorable changes in plasma lipid profiles, and reduced free radical damage in skeletal muscle. Definitive data on longevity and disease patterns in these populations will require another 15 to 20 years of study. The NIA will soon launch DR studies in nonobese people to explore the extent to which people can adhere to a targeted 20% to 30% lowering of caloric intake. However, whether or not DR retards human aging, the prolongation of the health span and life span of rodents by DR has major implications for many disciplines, and raises important questions about the desirability of ad libitum feeding.
ACKNOWLEDGMENT

The author thanks Jennifer Christensen and Roger Klopp for their contributions to his research program. This author’s research is supported by the National Institutes of Health (P01 AG–11915, R01 AG-18922). This is Publication No. 02-05 from the Veterans Administration Geriatric Research Education and Clinical Center, Madison, Wisconsin.

REFERENCES


EFFECTS OF DIET RESTRICTION ON LIFE SPAN AND AGE-RELATED CHANGES IN DOGS: EXPERIMENTAL DESIGN

Richard D. Kealy, PhD, and Dennis F. Lawler, DVM
Pet Nutrition Research Department, Nestlé Purina PetCare Company, St. Louis, Missouri

ABSTRACT:

This study was conducted with 48 Labrador retrievers from 7 litters, in a paired feeding design. The dogs were paired at age 6 weeks by gender and body weight within litter, and assigned at random to dietary treatment. At age 8 weeks, one pair-mate began ad libitum (AL) feeding, while the other (restricted-fed) pair-mate received a quantity equal to 75% of the amount of food that its counterpart consumed the previous day. When the dogs were age 3.25 years, two adjustments were incorporated into the feeding protocol. All dogs were switched from the growth formula (27% protein) to an adult formula (21% protein), and the amount of food was reduced and fed at a constant amount to prevent insidious development of obesity in the dogs that were fed AL. This was done by estimating the ideal body weight for each dog that was fed AL, based on skeletal size in reference to other dogs of the same breed. This group of dogs then was fed 62.1 kcal of metabolizable energy (ME) per kg of estimated ideal body weight (maintenance requirement for large breed dogs), while each of their respective restricted pair-mates received 25% less. After 3.25 years, the group that was fed AL was referred to as the control-fed group. The dogs were weighed weekly as pups, periodically as adolescents, and then weekly as adults. They were evaluated for body condition annually from age 6 years, using a 9-point scale ranging from 1 (emaciated) to 9 (severely obese), to assess degree of leanness/fatness. Amounts of lean, fat, and bone mass were estimated annually from age 6 years, using a Lunar Model DPX alpha dual-energy x-ray absorptiometer (DEXA). Analyses of serum were done annually for glucose, cholesterol, and triglycerides, using a Ciba-Corning Blood Chemistry Analyzer, Model 550 Express. Serum triiodothyronine (T3) analyses were done annually from age 4 years by radioimmunoassay. Samples were obtained by jugular venipuncture after overnight fast. Intravenous glucose tolerance tests (IVGTT) were done annually from age 9 years, by injection of 2.0 mL/kg body weight of 50% glucose solution, with samples obtained by jugular venipuncture at 0, 5, 30, 45, 60 and 120 minutes. Plasma insulin was estimated by radioimmunoassay of the same samples. The dogs were monitored daily throughout life for signs of illness or abnormality. When necessary, appropriate therapeutic measures consistent with established colony protocols were taken under supervision of the attending veterinarian. Health management and euthanasia protocols were pre-established for the entire facility. Similar conditions among dogs were managed as uniformly as possible. Dietary treatments were not adjusted because of illness, and choices of therapeutic measures were not influenced by dietary treatment. Most mortality (46 of 48) occurred by euthanasia, according to the dictates of humane treatment. Humane euthanasia was carried out only after extensive diagnostic evaluation, careful monitoring and assessment of...
response to treatment, serial evaluations of clinical condition, and consideration of prognosis, according to the practices established for the entire colony. Response variables were examined with a mixed-effects analysis of variance model for a repeated measures design. Feeding regimen, age, and their interaction were considered to be the fixed effects of interest. Random effects accounted for variation among litters, pairs within litters, and their interaction with age. The repeated measures aspect of the design was addressed by assigning blocks to individual dogs. The Wilcoxon signed rank test for paired data was used to evaluate differences in median life span. The paired Prentice-Wilcoxon test was used to evaluate differences in median time to treatment for osteoarthritis and other chronic conditions.
DIET RESTRICTION AND LONGEVITY: CHRONIC DISEASES AND CAUSES OF MORTALITY

Dennis F. Lawler, DVM,1 Richard H. Evans, DVM, MS,2 Richard D. Kealy, PhD,1 Jay M. Harrison, MS, MA,3 and Brian T. Larson, PhD1

1Pet Nutrition Research Department, 2Statistical Services Department, Nestlé Purina PetCare Company, St. Louis, Missouri; and 3Veterinary Pathology Services, Aliso Viejo, California

ABSTRACT:

Labrador retriever dogs (48) were studied lifetime to assess effects of diet restriction on longevity and physiological parameters. Restricted-fed (RF) dogs received 75% of the same diet consumed by control-fed (CF) pair-mates. Median life span of RF dogs (13.0 years) was greater ($P < 0.01$) than CF dogs (11.2 years). Chronic diseases included osteoarthritis (43 by radiographic diagnosis); malignant neoplasia (21 tumors, 17 dogs); benign mammary gland neoplasia (35 tumors, 12 dogs); benign non-mammary neoplasia (7 tumors, 6 dogs); recurring skin disease (19); liver disease (11); cystic endometrial hyperplasia, pyometra, or recurring severe pseudopregnancy (11); hypothyroidism (4); and seizures (4). Primary causes of mortality concentrated in musculoskeletal (26) and digestive (12) systems; neoplastic disease of these (4) and other body systems (7) accounted for 11 mortalities. Among 26 total mortalities from musculoskeletal causes, 12 were RF dogs (mean 13.1 years at death) and 14 were CF dogs (mean 10.6 years at death). Hazard of death from musculoskeletal diseases differed between RF and CF dogs ($P=0.002$). Twelve dogs died from digestive system diseases, including 7 RF (mean 10.9 years at death) and 5 CF (mean 10.7 years at death, $P > 0.05$). Eleven dogs died from neoplastic diseases, including 5 RF (mean 11.6 years at death) and 6 CF (mean 9.7 years at death, $P > 0.05$). Neoplastic diseases were not independent of musculoskeletal and digestive systems mortalities, but a numerical trend was found for neoplastic causes of mortality. Restricted feeding influenced median life span, but mortality causes generally were similar by body systems.
ABSTRACT:

Twenty-four littermate pairs of 8-week-old Labrador retrievers were assigned to an experiment in 1987 to evaluate biological markers of aging in dogs and determine the effects of limit-feeding (75% of control-fed pair) on the quality and span of their lives. Between 1992 and the end of the study, the antioxidant status of these dogs was monitored by annual clinical assays of serum, plasma, or whole blood for retinol, retinyl palmitate, vitamin E, selenium, copper, ceruloplasmin, ascorbic acid, uric acid, total peroxyl-radical trapping activity, glutathione peroxidase, superoxide dismutase, and catalase. For the period covered by this report (ages 5 through 10 years), limit-feeding reduced serum retinol ($P < 0.05$), vitamin E ($P < 0.05$), ceruloplasmin ($P < 0.01$), and copper ($P < 0.01$). Aging was associated with decreases ($P < 0.01$) in serum retinyl palmitate, serum vitamin E, serum selenium, and whole blood catalase, and with increases in serum retinol ($P < 0.01$), serum copper ($P < 0.01$), plasma uric acid ($P < 0.05$), and whole blood glutathione peroxidase ($P < 0.01$). Female dogs were found to have lower ($P < 0.05$) serum retinyl palmitate, ceruloplasmin, and copper than male dogs. Litter effects ($P < 0.01$), presumably reflecting genetic influences, were observed for serum vitamin E, serum copper, plasma uric acid, and whole blood glutathione peroxidase. The limit-feeding effects on serum retinol, vitamin E, and copper can reflect the reduced daily per-dog intake of each of these nutrients, compared with control-fed dogs. This indicates, in the case of the serum retinol and copper, that these two variables in Labrador retrievers are not as homeostatically regulated by hepatic storage as is reported for other species. The decreases in retinyl palmitate, vitamin E, and selenium with aging may signal factors especially important in canine geriatric diets. The lower serum retinol, retinyl palmitate, and copper values in females versus males might be expected to negatively affect pregnancy and/or lactation. These antioxidant profiles provide some new and contemporary clinical reference data which can enhance our understanding of canine nutrition, have practical application in formulating canine diets, and help reveal how limit-feeding has a positive effect on the life span of dogs.*

*See Kealy RD, Lawler DE, Ballam JM, et al. Effects of diet restriction on life span and age-related changes in dogs. *JAVMA* 2002;220:1315-1320, which is reprinted with permission in Appendix I in these proceedings.
A LONGITUDINAL STUDY OF IMMUNOSENESCENCE: DOES DIET RESTRICTION AMELIORATE THE AGING PROCESS IN DOGS?

Elizabeth H. Greeley, PhD,1 Joan M. Ballam, MS,2 Jay M. Harrison, MS, MA,2 Richard D. Kealy, PhD,3 Dennis F. Lawler, DVM,4 and Mariangela Segre, DSc1

1Department of Veterinary Pathobiology, University of Illinois, Urbana, Illinois; and
2Nestlé Ralston Purina Co., St. Louis, Missouri

ABSTRACT:
The effects of dietary restriction (DR) on the canine immune system have been investigated in a longitudinal study. A group of Labrador retrievers (30 females, 18 males), divided into age- and gender-matched pairs, were maintained on a diet restriction protocol from age 8 weeks until death. The restricted-fed (RF) dog received 75% of the total food consumed by its control-fed (CF) pair-mate. The immune status of these dogs was evaluated at established time intervals using a battery of immune parameters including lymphoproliferative responses to the mitogens concanavalin A (Con A), phytohemagglutinin (PHA), and pokeweed mitogen (PWM); natural killer (NK) cell activity; white blood cell (WBC) counts and lymphocyte subset distributions; and neutrophil phagocytic activity. Data were evaluated as a function of age, diet, and gender. Lymphoproliferative responses to all three mitogens declined with age. Diet-restricted dogs exhibited a slower rate of decline, an effect that was more pronounced in females. Age-related alterations of lymphocyte subset distribution in peripheral blood included decreases in CD4-cell and B-cell percentages, with RF dogs demonstrating lower B-cell percentages than CF dogs. Age-related increases in T-cell percentages were observed overall, but the rate of increase in RF females was not significant. Age-related increases in CD8 T-cell percentages were also observed. The absolute numbers of all monitored lymphocyte subsets declined with age; RF dogs had lower numbers of B cells than their CF pair-mates. Diet restriction slowed the rate of decline for CD4, CD8, and total T-cell numbers. Natural killer cell activity declined with age, but was not affected by diet; gender differences were observed with males having higher levels of activity. The phagocytic capacity of neutrophils was not significantly affected by age, diet, or gender. Diet restriction was indeed beneficial in retarding several age-related immune changes including the declines in lymphoproliferative responses and numbers of CD4, CD8, and total T cells, and the increase in T-cell percentages. Correlation of immune findings with decreased risk of death revealed that an immune profile of high lymphoproliferative responses, a high percentage and number of CD8 cells, and high numbers of T cells favored increased survival. Three of these predictors of increased survival were positively affected by diet restriction. This study seems to suggest that the female immune system derives greater benefit from dietary restriction than the male immune system.
**WHAT IS OSTEOARTHRITIS?**

Osteoarthritis (OA) is a disease of diarthrodial joints characterized by pain, stiffness, and limitation of motion. At present the terms osteoarthritis, osteoarthrosis, and degenerative joint disease are used interchangeably. When the underlying predisposing factor is unknown OA is referred to as primary or idiopathic, but in the vast majority of cases a pathogenic cause can be identified and then it is called secondary. It can occur as a result of trauma, in association with malformations in the region of the joint, or as part of the aging process. Osteoarthritis most often is diagnosed in the older animals; however, it can appear at any age. The disease can affect a single joint or several joints. The large weight-bearing joints of the hip, stifles, shoulder, elbow, or ankle are frequently affected, and the joints of the spine (vertebrae) and extremities are often abnormal. Although multiple joints may be diseased, pain and disability are usually greatest in one joint. Osteoarthritis is one of the most prevalent causes of disability in humans, and it occurs in primates. It is a serious veterinary medical problem in animals and is commonly diagnosed in dogs, horses, and pigs, and has been well described in mice, guinea pigs, and chickens (Table 1).

Joint disease that results from trauma clearly is not inherited, but other cases have a strong genetic association. Osteoarthritis is often observed in littermates and in the progeny of affected parents, and environmental effects such as time, physical activity, and nutrition influence the expression of disease. Injuries or malformations that cause joints to become unstable or alter their weight-bearing function promote the development of OA. Hip dysplasia is a frequent predisposing condition in humans and in dogs. Other causes of OA include misalignment of the kneecap and unfused bones in joints. Separation of articular cartilage from the subchondral bone, a condition called osteochondrosis, results in OA in shoulder, stifles, elbow, and hock joints and is observed in rapidly growing animals.

An early sign of OA is an animal’s reluctance to ambulate normally. Less weight is put onto affected limbs and animals may limp or appear stiff. Rising from a lying position is difficult and an animal may whine or snap when an affected joint is manipulated. Intense activity can aggravate the pain in joints and thereby reveal signs of disease in animals that otherwise appear normal. The pain often is made worse by cold or a change in the weather.

**DIAGNOSIS AND SIGNS OF DISEASE**

Observation can raise suspicions of OA, but diagnosis is done by radiographic examination of the affected joint. Evidence on an x-ray photograph includes joint capsule
thickening, mineral deposits in tissues surrounding the joint, osteophytes at the edge of the joint, subchondral bone sclerosis, and joint space narrowing signaling loss of cartilage. Synovial fluid can also be examined to provide more information. Disease-free joints contain only a small amount of clear viscous fluid. An increased amount or discoloration of the fluid, or an increase in its content of discarded synovial cells suggests disease. Osteoarthritic joints characteristically do not have elevated numbers of white blood cells.

The progression of OA is similar in most animals. The articular cartilage that covers the ends of bones is affected early. Cartilage functions to distribute loads over a wide area and also provides low-friction surfaces for movement of joints and any impairment in this tissue leads to disease. The normally smooth and elastic cartilage becomes soft, then rough, and often is worn away exposing underlying bone. Capsule and ligaments become stretched and enlarged and even muscles in the region of a joint are weakened. Opposing bones articulate abnormally causing pressure, deformation, and spurs called osteophytes and inflammation and further injury. Movement becomes impaired and painful.

SOURCES OF PAIN

The principal symptom of OA is pain which arises after joint movement and is relieved by rest. Pain arises from noncartilaginous intra-articular tissues and periarticular structures and not from the cartilage itself; it is aneural. Pain emanates from the periosteum, the osteophytes, trabecular microfractures, subchondral bone exposed by eburnation, ligamentous and capsular distention, and synovitis.

OVERWEIGHT AND DIET RESTRICTION

The role of obesity in the pathogenesis of OA is complex and is of great interest for scientists, clinicians, clients, and the affected animals. Obesity in animals with OA may be a consequence of reduced activity because of joint pain, but studies have suggested that it is likely that obesity has a direct role in the initiation and progression of OA. For example, weight change was directly associated with OA risk, and weight loss was correlated to a reduced risk for OA. In addition to the biomechanical effects of extra weight as the cause of OA, a metabolic role for biochemical constituents has been proposed and a number of hormones and other biochemicals have been implicated in OA development (e.g., lipids, insulin-like growth factor [IGF-1], insulin, as well as sex hormones). Definitive progress in this area has been slow, but many studies are ongoing and further research will help clarify the pertinent interrelationships between altered body metabolism and the pathogenesis of OA.

A survey of the nutritional literature identifies numerous studies on diet and weight reduction in humans and also in experimental animal models such as mice. However, there is a paucity of reports of
controlled studies on diet restriction and OA development in animals. A few good studies do exist and results of four of these are summarized in Table 2.

Three species, dogs, guinea pigs and chickens, were fed reduced quantities of their respective control diets and weight loss was recorded and signs of OA were analyzed. A diet reduction of 25% in growing dogs (up to 5 years) genetically at risk for canine hip dysplasia substantially lowered the frequency and severity of hip joint OA as diagnosed radiographically. In another study, overweight adult dogs were put on a reducing diet that resulted in an average weight loss of 14% (in 10–19 weeks). Symptoms of hip joint OA in these dogs were reduced clinically as assessed by increased mobility and range of motion in the hip joint. Apparently weight loss in the adult dogs compared with controls reduced the stress on their weight bearing joint, easing pain and reducing symptoms of the disease. A third study utilized guinea pigs, and a 28% reduction in weight as compared with controls resulted in an impressive average 40% reduction of pathology in the OA of stifles in guinea pigs as assessed by histologic examination of stifle joint tissue. The fourth report concerned breeder fowl. Even chickens fed a smaller quantity of food that resulted in a 10% weight reduction had the evidence for OA of hock joints reduced. Concomitantly the content of proteoglycan in the articular cartilage of the hock joint in these chickens was observed to be at disease-free levels, whereas control-fed OA chicken cartilage had diminished proteoglycan content. Loss of proteoglycans from the articular cartilage of OA joints is a characteristic feature commonly observed in all species that have been examined. Thus, the results reviewed in Table 2 clearly substantiate that diet restriction in three species of animals with OA leads to improved joint function as assessed clinically, radiographically, and histologically. The data suggested that in the case of the young growing dogs development of OA of the hip even has been prevented as disclosed by a lower frequency of OA in the hip joints of the diet-restricted dogs.

The progression of OA in hip joints of two groups dogs that were genetically predisposed for canine hip dysplasia is presented in Table 3. The cardinal feature of hip dysplasia is displacement of the femoral head away from the acetabulum as observed on a pelvic radiograph. With few exceptions OA is the invariant

### Table 2. Diet Restriction and Osteoarthritis in Three Species

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>WEIGHT REDUCTION (%)</th>
<th>JOINTS IMPROVED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growing Dogs</td>
<td>23%</td>
<td>OA in hip and other joints: dramatic radiograph improvement in frequency and severity</td>
</tr>
<tr>
<td>Adult obese dogs</td>
<td>14%</td>
<td>OA in hip; improved mobility (clinical)</td>
</tr>
<tr>
<td>Guinea pigs</td>
<td>28%</td>
<td>Knee OA improved 40% (pathology)</td>
</tr>
<tr>
<td>Chickens</td>
<td>10%</td>
<td>Hock OA and proteoglycan content improved</td>
</tr>
</tbody>
</table>

### Table 3. Occurrence of Hip Osteoarthritis in Dogs with Age

<table>
<thead>
<tr>
<th></th>
<th>2 YEARS HIP DYSPLASIA</th>
<th>PERCENT AFFECTED DOGS AT:</th>
<th>8 YEARS OA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 YEARS OA</td>
<td>5 YEARS OA</td>
<td>8 YEARS OA</td>
</tr>
<tr>
<td>Control-fed dogs</td>
<td>67%</td>
<td>33%</td>
<td>53%</td>
</tr>
<tr>
<td>Restricted-fed dogs</td>
<td>29%</td>
<td>4%</td>
<td>13%</td>
</tr>
<tr>
<td>Percentage change&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57%</td>
<td>88%</td>
<td>75%</td>
</tr>
</tbody>
</table>

<sup>a</sup>25% Diet Restriction Study (Nestlé Purina Co.) included 24 dogs in each group. Osteoarthritis was diagnosed radiographically as described in References 5, 10, and 11.

<sup>b</sup>Percent change = difference between control-fed and restricted-fed divided by control-fed, times 100.
consequence of the trait called canine hip dysplasia. Results were that 67% of the control-fed dogs had evidence for hip dysplasia at 2 years of age. Strikingly different, only 29% of the dogs in the restricted-fed group had hip dysplasia. Thus the results in Table 3 suggest that the overall chondro-osseous conformation of hip joints in the restricted-fed group was better, or more normal, i.e., less disconformity, than for the control group. Also at 2 years of age, 33% of control-fed dogs actually had hip OA (e.g., osteophytes and other bone changes in the femoral head and acetabulum), whereas only 4% of the restricted-fed dogs had definitive radiographic signs of OA. At 5 and 8 years of age 52% and 71% of control-fed dogs, respectively, had hip OA, but only 13% and 15% of restricted-fed dogs, respectively, had OA. These results suggest that the restricted diet effects ameliorate the underlying joint conformation (displacement, i.e., the dysplastic trait) by 57%, but an additional improvement resulted for signs of OA because the radiographic evidence for OA specifically was reduced even more. The underlying chondro-osseous joint instability is the trait called hip dysplasia, but other factors including time and physical activity interacting with the genotype of susceptible dogs lead from joint instability to overt OA in dogs with hip dysplasia.

Some of the data from the lifetime diet restriction study in dogs that also have relevance to the status of OA in these dogs are presented in Table 4. A 25% restriction in food consumption resulted in a mean 22% reduction in weight over the lifetime for the restricted dogs when compared with the control group of dogs. As reported previously, the 50% survival age was extended by 1.8 years in the restricted dogs and the corresponding 10% survival age was extended about 1 year. The severity of OA was evaluated when the dogs were 8 years old in hip, shoulder, elbow, and stifle joints. Eighty-six percent of the dogs in the control-fed group at 8 years of age had two or more joints with radiographic evidence of OA. In marked contrast, the restricted-fed group had only 24% of the dogs with OA in two or more joints. This represented a reduction of 72% in the joint pathology that was attributable to diet reduction. Of significance was that the radiographic improvements in the restricted-fed group in fact were consistent with the clinical behavior of the dogs. The lessened joint pathology in each dog was translated into a meaningful clinical observation in that the age at which 50% of the dogs required treatment for OA was delayed to 12.6 years when compared with control-fed dogs that needed OA therapy at a mean age of 9.8 years. Taken together these results provide compelling evidence that diet restriction by 25% resulted in biological and clinically meaningful improvements in joint function in these dogs.

**SUMMARY AND CONCLUSIONS**

Obesity is a common form of malnutrition particularly among elderly humans, but animals of all ages are similarly affected, including dogs, pigs, and some rodents. Excessive weight gain is associated with
decreased exercise and a decline in caloric needs for weight maintenance. Overweight animals are at increased risk for cardiac disease, diabetes, and for OA. Weight loss mitigates these conditions, but it has some potentially adverse effects as well, such as excessive loss of lean body mass, possible electrolyte imbalance, and liver abnormalities including elevated bile acids in blood. The adverse effects of dieting usually are considered not severe enough to contraindicate a reducing diet for weight loss.

The data reviewed here lend strong support to the concept that reduced food consumption resulting in a reduction in body weight with its concomitant altered body metabolism is clearly beneficial to lessen the severity of OA and even prevent its occurrence if fed to young, growing animals. This effect was substantiated in dogs, guinea pigs, and chickens. The lessening of the signs of OA, either clinically or pathologically, was achieved by reducing diets of both long and short duration and in growing as well as in adult animals. Restricted food consumption resulted in a striking amelioration of OA.

REFERENCES

Osteoarthritis (OA) is a common, often-debilitating disease of both animals and humans. In dogs a highly prevalent form of OA, known as canine hip dysplasia (CHD), is capable of causing pain and dysfunction in affected animals. The prevalence of CHD is as high as 75% in some breeds of dogs. Canine hip dysplasia is a developmental disease recognized to have complex inheritance, a so-called polygenic disease. It is understood that the incidence and severity of such genetic diseases can be influenced considerably by environmental factors,\(^1\) meaning any "nongenetic" factor, such as diet, lifestyle, housing, or trauma. Dogs that develop CHD are born with apparently normal hip joints, but after they have reached a few weeks of age the earliest signs, such as hip joint laxity, can be demonstrated by necropsy studies.\(^1,5,6\)

It is well accepted that hip laxity plays a prominent role in the pathogenesis of CHD.\(^7\) Initial phenotypic expression of CHD is recognized radiographically as femoral head subluxation, often followed by progressive degenerative joint disease (DJD). Femoral head subluxation, subjectively scored on the hip-extended radiograph, and the distraction index, directly measured on the distraction radiograph, are two types of passive hip laxity. They are static phenomena. Osteoarthritis, however, is thought to be caused by functional hip laxity, the dynamic phenomenon associated with the femoral head slipping partially out of the acetabulum during weight bearing.\(^5\) It has been shown that joints with excessive passive hip laxity are susceptible to traumatic subluxation of the femoral head.\(^8\) Tight joints are not. Hip joint laxity and femoral head subluxation, if functional, produce pathologic consequences that include abnormal loading during weight bearing causing cartilage damage and bony remodeling. The end result of this self-perpetuating cycle of abnormal loading and remodeling is osteoarthritis, characterized by synovitis, increased joint fluid volume, joint cartilage erosion, elongation and edema of the round ligament, thickening of the joint capsule, and osteophyte formation.\(^6,7,10\)

Degenerative changes develop in joints of many dogs as they age; however, more severely affected dogs will show radiographic signs of OA well before the geriatric period, often by 1 year of age or younger. Joint laxity associated with CHD is the most important risk factor for OA in canine coxofemoral joints.\(^9\) Trauma or metabolic dysfunctions are also thought to affect disease expression.\(^6,10\) One theory of pathogenesis for OA includes the idea that excessive body weight leads to mechanical stress on joints, facilitating the transformation of passive hip laxity to functional hip laxity, thereby initiating OA. Excessive body weight has been documented as a risk factor for OA development in humans, guinea pigs, mice, and dogs.\(^9,11-16\)
The studies summarized here were conducted to evaluate the effect of food restriction on development of hip joint laxity during growth and subsequent occurrence of OA in coxofemoral joints during adulthood. In addition, the effect of food restriction on development of OA in joints other than hips was evaluated.

EFFECT OF LIMITED FOOD CONSUMPTION ON HIP DYSPLASIA IN GROWING DOGS

In this study, 17 48 8-week-old Labrador retriever puppies from seven litters were allotted by pairing to two groups of 24 dogs each. This pairing created two groups of genetically similar dogs. One group was fed ad libitum (full fed) and each member of the other group (restricted) was given 25% less of the same food given to the full-fed pair mate. Radiography of the hip joints was done when the dogs were ages 30, 42, 54, 78, and 104 weeks. Subluxation was measured using the Norberg angle on radiographs made with the dog in the standard, hip-extended position. The same radiographs were evaluated for evidence of CHD by independent, blinded radiologists, one using the Swedish scoring system and the other, the Orthopedic Foundation for Animals (OFA) scoring system.

Independent of the age at which radiography was performed, hip joint quality was better in the diet-restricted dogs, as a group. Radiographs taken when dogs were age 104 weeks revealed less subluxation and less OA in restricted-fed dogs, irrespective of whether the Norberg angle, Swedish scoring system, or OFA scoring system was utilized. Using the Swedish method, CHD at 2 years of age was diagnosed in 5 of 24 restricted dogs and 18 of 24 full-fed dogs. Using the OFA method, CHD was diagnosed in 7 of 24 restricted dogs and 16 of 24 full-fed dogs. These findings support a clinical recommendation to avoid excessive food intake in growing dogs, particularly in breeds prone to CHD.

EFFECT OF LIMITED FOOD CONSUMPTION OVER 5 YEARS ON COXOFEMORAL JOINT OSTEOARTHRITIS

The same Labrador retrievers from the growth study were continued on test for an additional 3 years and additional radiographic evaluations were recorded yearly. At 3.25 years of age two adjustments were made in the feeding protocol. All dogs were switched from a 27% protein puppy growth formula to a 21% protein adult formula, and the amount of food was reduced to prevent insidious development of obesity in full-fed dogs. We estimated the ideal body weight for each full-fed dog on the basis of skeletal size in reference to other dogs of the same breed. These dogs then were fed 62.1 kcal metabolizable energy/kg of estimated body weight. Restricted dogs continued to receive 75% of the amount fed to the corresponding full-fed pair-mate, and thus the intake relationship between the two groups remained the same as prior to the diet adjustment.

Radiographic evaluations of coxofemoral joints for frequency and severity of OA were done at yearly intervals up to age 5 years. Three investigators evaluated each radiograph without knowledge of group assignments, using a scoring system based on radiographic features of OA. The median value obtained by the three investigators was assigned as the score for each dog.
Frequency and severity of OA were greater in full-fed dogs. By age 52 weeks, differences between the groups for frequency and severity of OA were statistically significant. The prevalence of DJD in hip joints increased through age 5 years. By age 5 years, 12 of 23 full-fed dogs and 3 of 23 restricted dogs had radiographic signs of OA. Body weight correlated significantly with OA scores at age 5 years.

Dogs varied considerably with respect to individual maintenance energy requirements, and so it is not feasible to specify a universal energy intake to achieve the benefits observed in the restricted dogs. It is recommended, however, that growing puppies and adult dogs be fed throughout their lives to maintain lean body conformation to minimize the development of DJD with advancing age.

**EFFECT OF LIMITED FOOD CONSUMPTION ON MULTIPLE JOINT OSTEOARTHRITIS**

In this study, 24 restricted dogs continued to receive 75% of the amount fed to the corresponding full-fed pair-mate through age 8 years. Hip, shoulder, elbow, and stifle joints were evaluated radiographically at this time. Radiographs were evaluated by one radiologist without knowledge of dietary treatment.

Osteoarthritis affecting multiple joints was significantly more common in full-fed dogs. Ten of 22 (45%) full-fed dogs had OA in two different joints, and seven (29%) had OA in three joints, whereas only one of 21 (5%) restricted dogs had DJD in two joints, and one (5%) had OA in three joints. Five restricted dogs had no evidence of OA in any evaluated joint, while only two full-fed dogs lacked evidence of OA in the evaluated joints.

Theoretically development of OA at a primary site such as the hip could produce aberrant compensatory biomechanical forces acting on other joints; the altered weight bearing and ambulation leading to multiple joint OA. In our study, hip joints were not always affected first; some dogs had radiographic evidence of shoulder or elbow lesions without hip joint involvement. Thus, alternatively it may be hypothesized that OA has a systemic cause, with variable expression in different joints. This might explain in part the development of OA in joints that are not subject to large forces associated with weight-bearing, such as lumbar intervertebral joints in dogs and joints in the skeleton of sharks. Alternatively, a humoral substance from an affected joint may affect tissues in other joints. This concept is supported by the recent finding that extract of human arthritic bone tissue induced abnormalities in disease-free articular cartilage. Additional support for the concept of variability in tissue susceptibility is derived from a recent report that shoulder joints in young adult dogs at high risk for CHD have histopathologic articular cartilage abnormalities similar to those found in the corresponding hip joints. Yet another hypothesis might be that OA among different joints represents phenomena that are not interrelated or interdependent.

**ABSTRACT (UNPUBLISHED DATA): EFFECT OF RESTRICTED FEEDING AND AGE ON HIP OSTEOARTHRITIS AND HIP SCORE: A LIFE-LONG STUDY IN LABRADOR RETRIEVERS**

It is generally agreed that there has been slow progress in reducing the incidence of CHD by selective breeding of normal dogs. The conventional diagnosis of CHD has been based on subjective radiographic findings of subluxation of the coxofemoral joint, or secondary OA as seen on evaluation of the hip-extended, ventrodorsal radiographic view of the pelvis. In the USA this analysis is performed by the OFA after dogs are 2 years of age. In much of Europe a similar analysis is made after 1 year of age. In the published reports cited above it was shown that the condition of excessive hip laxity (Norberg angle measured from the hip-extended radiograph) can be marginally reduced by caloric intake and this tightening of the joint prevents or delays the expression of OA in some dogs predisposed to the condition. It has been generally assumed that the subjective scoring of hip phenotype at 1 or 2 years of age accurately reflects the true phenotype of the dog. No lifelong studies have been conducted to document the accuracy of the one- or two-year...
evaluation to predict the end-of-life hip phenotype. It was the purpose of the present investigation to test the influence of food restriction on hip phenotype and to compare end-of-life hip phenotypes with OFA scores, PennHIP scores, and OA scores at 2 years of age.

Materials and Methods. Forty-eight 8-week-old Labrador retriever puppies from seven litters were allotted by pairing to two groups of 24 dogs each. One group was fed ad libitum (full-fed) and each member of the other group (limit-fed) was given 25% less of the same food given to the full-fed pair-mate. Radiography (ventrodorsal, hip-extended) of the hip joints was done when the dogs were ages 30 and 54 weeks and yearly thereafter for life. Subluxation was measured using the Norberg angle on radiographs made with the dog in the standard, hip-extended position. The same radiographs were evaluated for evidence of CHD and OA by a board-certified radiologist using the scoring system of the OFA. At 2 years of age the hips were scored using the PennHIP method.

Results. Limit-feeding had a profound positive effect on the hip phenotype of Labrador retrievers. Limit-fed dogs had significantly lower incidence and severity of CHD and OA compared with full-fed pair-mates. This health benefit continued for the life of the dogs. In the pooled sample of 48 dogs, the prevalence of hip OA increased linearly throughout the study, from 15% at 2 years of age to 67% at end-of-life. For the full-fed dogs, end-of-life OA prevalence was 83% and for the limit-fed dogs, 50%. At 2 years of age, OFA-type scoring found 19 of the 48 dogs in the study to be “dysplastic” while 29 dogs were scored as “normal.” The 19 dysplastic dogs remained dysplastic for life, with OFA scores increasing in severity for many of the dogs. However, of the 29 dogs scored “normal,” 16 (55%) were scored dysplastic by end-of-life, representing a 55% false-negative rate of diagnosis. PennHIP results showed that all the dogs included in this investigation were susceptible to OA (distraction index [DI] >0.36, range 0.36–0.92). Kaplan-Meier curves of disease-free interval showed that for dogs with a low DI the onset of OA was much later in life than dogs with a large DI. For dogs with DI ≤0.4 the median disease-free interval was 12 years of age compared with dogs with DI >0.6, whose median disease-free interval was only 3 years of age.

Discussion. Results of this lifelong study showed that by keeping dogs lean the onset of OA was delayed and its severity and prevalence was reduced significantly. In addition, OA prevalence in other joints of the lean dogs was decreased. Such findings are both statistically and clinically significant. The linear increase in OA incidence over the life of these Labrador retrievers refutes popular dogma that holds that hip OA occurs either early in life, in the case of dysplastic dogs, or much later in the geriatric years in the case of “old age OA.” The principal risk factor, if not the cause, for the development of hip OA has been shown to be joint laxity irrespective of age. In the hip-extended view this laxity is underestimated and often masked completely, leading to false-negative diagnoses. This results in some dogs appearing phenotypically normal although they are genotypically abnormal. Evidence for this impression derives from the continued high frequency of CHD in many breeds of dogs despite systematic mating of normal parents. The life-long study reported here provides additional evidence. Hip phenotypes in this sample were much worse at the end of life than at 2 years of age. The “normal” designation of hips at 2 years of age was wrong more than it was right (55% false-negative rate). The dogs in this study derived from lines of dogs with hip dysplasia and therefore there was a high probability to express OA. Dogs that were permitted to become overweight (mean body condition scores of 6) expressed OA much more, 83%, than those kept lean (OA 50%) (mean body condition score of 4). The PennHIP distraction index indicated that all dogs in this study (at 2 years of age) were susceptible to OA and therefore genotypically abnormal. This predicted susceptibility was borne out by the observed pattern of OA incidence later in life. In this regard the distraction index was not influenced by the effects of the diet. This finding represents an extremely desirable characteristic of a genetic test.
REFERENCES


The studies abstracted in the preceding article in these proceedings have profound clinical significance for the practicing veterinarian, orthopaedic surgeon, dog breeder, and pet owner: Dogs suspected to be susceptible to canine hip dysplasia (CHD) should be kept lean for life and dogs selected for breeding should have hip evaluation at regular intervals throughout life. Although the studies were performed in one breed of dog, the Labrador retriever, it is reasonable to consider the results clinically applicable to similar breeds, such as other retriever breeds and even Rottweilers and German Shepherd dog breeds. This opinion finds support from studies that have shown body weight to be a risk factor for hip osteoarthritis (OA).1,2 Owners of dogs of OA-susceptible breeds should strive to keep body condition at score 5 or below (on a scale of 1 to 9 where 9 represents extreme obesity). For tight-hipped breeds of dogs known to not be susceptible to hip OA, such as performance greyhounds and Borzois, it is unnecessary to adhere to these dietary recommendations. However, as will be shown in other parts of this symposium, keeping body condition scores at 5 or below has other health benefits unrelated to OA.

The foregoing studies demonstrated that controlling body weight delayed, lessened the severity, or prevented the development of OA in some dogs. Consistent with these radiographic findings was a parallel benefit in clinical signs within this pool of dogs. Dogs that were kept lean required less pain medication (nonsteroidal anti-inflammatory drugs) starting later in life than dogs in the full-fed group. The mean therapy-free interval for the lean dogs with OA was 10.3 years, and for the restricted-fed dogs, 13.3 years ($P < 0.01$).2 Further support for this experimental finding was published recently in studies showing that reducing body weight and body score in obese dogs with clinical signs of hip dysplasia resulted in a substantial improvement in clinical lameness1 and in gait5 (as demonstrated by force plate testing). These findings add to the growing pool of data in humans showing similar beneficial effects of weight reduction in alleviating the discomfort of OA.

Hip dysplasia has long been understood to be a disease of complex inheritance. Environmental factors can influence the expression of diseases of complex inheritance and in this investigation, food consumption and body weight were found to be potent factors. Keeping dogs lean certainly did not change the genes of dogs predisposed to hip dysplasia. Rather, leanness was shown to delay or prevent the expression of radiographic signs of CHD in dogs prone to CHD, likely by antagonizing the conversion of passive hip laxity to functional hip laxity that ultimately leads to OA. Some would argue that such environmental manipulation is contraindicated because it “masks” the disease phenotype, thereby confounding diagnosis and related breeding recommendations.

Dr. Gail Smith received his VMD from the University of Pennsylvania, completed orthopaedic surgical residency training, and was awarded a PhD in engineering. He is currently Professor of Orthopaedic Surgery and Chairman of the Department of Clinical Studies at the University of Pennsylvania School of Veterinary Medicine. Dr. Smith has published more than 150 research articles, book chapters, and proceedings. Dr. Smith’s research interests have focused on orthopaedic problems of the spine, the knee and the hip. A clinically compelling product of his research is a stress-radiographic diagnostic method capable of predicting the susceptibility to hip dysplasia in dogs as young as 16 weeks of age. Dr. Smith founded PennHIP®, a cooperative scientific initiative representing multiple collaborative centers in North America and Canada, trained to test the new hip dysplasia diagnostic technology. Research continues to support the role of the PennHIP phenotype to effectively select breeding stock to reduce the frequency of this most-prevalent osteoarthritic disease.
Others, however, would counter that for the comfort of the dog, one should invoke all known environmental measures to delay the onset or lessen the severity of CHD. It is conceivable if the dogs in this study were stressed even more (environmentally) or if they had lived longer, that all of them might have expressed OA. Clearly a diagnostic test for hip dysplasia that is not confounded by environmental factors is needed. The measure of such a test is embodied in the concept of “heritability” and the higher the estimate of heritability the better. Comparative studies estimating the heritability of hip phenotype have shown the distraction index (DI) to have higher heritability than subjective hip scores. It is encouraging that in this study the PennHIP distraction index was not confounded by the diet and that, consistent with observed lifelong hip phenotypes, the distraction index put all of the dogs into the OA-susceptible category. These findings support the view that the estimate of heritability for the distraction index is likely higher than the subjective hip score.

Longitudinal studies, such as this one, are essential to understanding the true biological behavior of a disease as complex as CHD. To the authors’ knowledge no similar studies have been published. Of particular interest and importance is the observation that OA prevalence and OFA score increased linearly long after 2 years of age, the accepted convention for phenotypic expression. This understanding raises questions about the diagnostic accuracy of the standard radiographic method of hip scoring, which is usually performed shortly after 2 years of age. For scoring methods performed at 1 year of age, the diagnostic error would likely be even larger. Hitherto unappreciated, these studies draw critical attention to the magnitude of change in hip score with aging. Of dogs graded normal by OFA-type scoring at 2 years of age, 55% became dysplastic by the end of life. Conventional subjective hip scoring at 2 years of age, therefore, underestimates the frequency of hip dysplasia in dogs and this observation provides partial explanation for the recognized slow progress in reducing the frequency and severity of CHD by selective breeding. There currently is no requirement for dogs that are OFA certified at 2 years of age to undergo repeat evaluations to validate the 2-year score. Minimally, this new data warrants a strong clinical recommendation for hip films well beyond 2 years of age, and in the case of breeding dogs, hips should be evaluated at regular intervals for life.

REFERENCES

Studies with laboratory rodents have convincingly demonstrated that the reduction of caloric intake is the nutritional factor primarily responsible for the life-extending and anti-aging actions of diet restriction and this has resulted in gerontologists more frequently using the term caloric restriction (CR) rather than diet restriction when referring to this anti-aging phenomenon. These findings also logically led most gerontologists to the conclusion that a reduction in metabolic rate was the basis of the life-extending effect of CR. This concept has required revision, however, in light of the findings of Duffy et al with mice and McCarter and Palmer with rats showing that CR can increase life span without decreasing metabolic rate per unit of lean body mass or per unit of "metabolic mass."

CALORIC RESTRICTION AND CARBOHYDRATE METABOLISM IN RODENTS

One line of thinking led to the hypothesis that CR has its life-prolonging effect by altering the characteristics of carbohydrate fuel use. The fact that hyperglycemia and hyperinsulinemia damage mammalian organisms and that this damage has some resemblance to what occurs in senescence are the triggers that led to this hypothesis.

Early Studies

Even before this hypothesis was proposed, the effect of CR on carbohydrate metabolism was the subject of considerable study. Long-term CR was found to decrease the fasting plasma levels of glucose and insulin in mice and rats. In addition, Reaven and Reaven made a single determination of plasma insulin between 1400 and 1600 hours in 3- and 12-month-old rats and found that animals on a reduced caloric intake have lower levels of plasma insulin. CR also was found to decrease the postprandial level of plasma insulin in rats.

Ivy et al studied the effect of insulin on glucose utilization in anesthetized 12- to 13-month-old rats fasted for 4 hours using a method involving continuous infusion of epinephrine, propranolol, glucose, and insulin. They found that rats on a low caloric intake were less insulin resistant than those on a higher caloric intake. To the contrary, Kalant et al used the same technique to measure the effect of insulin on glucose utilization in anesthetized 4-, 12-, 18-, and 24-month-old rats that had been fasted overnight and found that long-term CR decreased the ability of insulin to stimulate glucose utilization. Ivy et al measured glucose utilization by rat hindlimb preparations; they found that preparations from rats on long-term CR had an increased ability to utilize glucose.

These early studies are difficult to interpret in regard to the anti-aging action of CR because they do not provide information on carbohydrate metabolism and insulin.
action under conditions similar to those in which animals in longevity studies spend their lifetime. Rather these measurements were made on rats and mice that were either fasted or anesthetized or surgically traumatized or subjected to a combination of these stressors.

**Lifetime Longitudinal Study of Carbohydrate Metabolism in Rats**

A longitudinal study of male F344 rats was carried out in my laboratory with the aim of determining plasma glucose and insulin levels and glucose utilization over the lifetime under usual daily living conditions. The study included a detailed analysis of the circadian pattern of plasma glucose concentration and the measurement of plasma insulin concentration at the daily maximum and minimum glucose concentrations. Throughout most of the day, plasma glucose concentration in the rats on CR was significantly lower than that of the rats fed ad libitum. Only during the 2 to 3 hours following receipt of their daily allotment of food did the plasma level of the CR rats approach that of those fed ad libitum. This circadian pattern of plasma glucose concentration continued throughout the life span of the ad libitum-fed rats. (Of course, because the rats on CR lived much longer than those fed ad libitum, there was a considerable period of the life of the CR rats during which there were no ad libitum-fed rats of the same age alive for comparison.)

There were some age-associated quantitative differences; the mean 24-hour difference in plasma glucose concentration ranged from 13 to 21 mg/dL with greatest difference occurring in rats in 9- to 13-month age range and the smallest difference in rats in the 21- to 25-month age range. The average difference in the 24-hour mean plasma glucose concentration between the two groups over the lifetime of the ad libitum-fed rats was 12%. Plasma insulin levels were measured during the daily periods of the maximum and minimum plasma glucose levels; at all ages in common, the plasma insulin concentration was markedly lower at both times of day in the CR rats compared with those fed ad libitum. The difference, which varied with the age of the rat and the time of day, ranged from 45% to 80% lower concentrations in the CR rats.

McCarter and Palmer had measured the oxygen consumption of male rats throughout the life span in their home cages under what was their usual living conditions and found that over most of the life span, the rate of oxygen consumption per kilogram of “metabolic mass” was the same for ad libitum-fed and CR rats. They also found that over the lifetime of these rats that the respiratory quotient (RQ) averaged 0.89 for both groups of rats, which is the expected value if the fuel mixture eaten is the same as the fuel mixture utilized. This finding was not surprising because, for most of their lives, the rats are in a near steady state in regard to body mass and body composition. Based on this information and on the plasma glucose insulin levels in our longitudinal study, it is evident that the rate of glucose fuel use per kilogram of “metabolic mass” is the same for rats on the CR regimen and those fed ad libitum even though our study showed that CR rats maintain lower plasma glucose levels and markedly lower plasma insulin levels. We decided that this information was so important that it should be confirmed with a group of rats in which the oxygen consumption, RQ, and plasma glucose and insulin levels were measured simultaneously. Male F344 rats aged 8 to 10 months fed ad libitum or on a CR regimen from 6 weeks of age were used. The two dietary groups had a similar daily RQ and rate of oxygen consumption per kilogram “metabolic mass.” During the 2-hour peak period of plasma glucose concentration, CR rats had an oxygen consumption of 0.94 L/kg “metabolic mass,” a RQ of 0.93, plasma glucose of 155 mg/dL, and plasma insulin of 4.4 ng/mL, while the ad libitum-fed rats had an oxygen consumption of 0.92 L/kg “metabolic mass,” a RQ of 0.90, plasma glucose of 159 mg/dL, and plasma insulin of 8.1 ng/mL. Based on the sum total of these findings, our conclusion is that CR either increases glucose effectiveness or insulin sensitivity, or both.
In Vitro Studies on Glucose Effectiveness and Insulin Sensitivity

McCarter et al\textsuperscript{12} used the rat epitroclearis muscle to determine the effect of CR on glucose effectiveness. They found that muscle preparations from rats on long-term CR exhibited increased glucose effectiveness. Using the same rat skeletal muscle preparation, Cartee et al\textsuperscript{13} found that CR enhances insulin stimulation of glucose transport. CR also enhances insulin’s ability to promote glucose metabolism by isolated adipocytes.\textsuperscript{14}

In Vivo Studies on Glucose Metabolism by Specific Tissue and Organ Sites

It is important that the effect of CR and insulin action on carbohydrate metabolism be determined in specific tissues and organs as they function in the intact organism. Escriva et al\textsuperscript{15} did the pioneering work in this regard. They used the 2-deoxyglucose method and studied glucose uptake by various skeletal muscle and adipose tissue sites in anesthetized 70-day-old Wistar rats under basal and hyperinsulinemic conditions. They found that CR increased insulin-mediated glucose uptake at all these sites.

Wetter et al\textsuperscript{16} also used the 2-deoxyglucose method but made a major advancement by carrying out their study on 11- to 12-month-old (F344 × BN)\textsuperscript{F1} rats under as usual living conditions as possible. They found that although CR maintained low levels of plasma insulin, it did not affect glucose uptake by cerebellum, lung, kidney, soleus muscle, and diaphragm and it enhanced glucose uptake by brown adipose tissue, white adipose tissue, and the epitroclearis, plantaris, and gastrocnemius muscles.

Role of Altered Carbohydrate Metabolism in Anti-Aging Action of Caloric Restriction

Initial attention focused on the potential role of the lifetime lowering of plasma glucose levels as a factor in the life-prolonging action of CR. The reasons for this focus were two. First, hyperglycemia results in functional and morphologic deteriorations similar to those of senescence.\textsuperscript{17} Second, Cerami\textsuperscript{18} proposed that nonenzymatic glycation and subsequent reactions involving proteins and nucleic acids are causally involved in senescence and that increasing levels of plasma glucose concentration promote their formation. CR has been found to decrease the extent of glycation of proteins and the age-associated increase in advanced glycation end-products in rodents.\textsuperscript{19,20} It still remains to be established, however, that glycation and subsequent reactions play a major role in aging.

Recently, the focus has shifted to the potential role of the marked reduction in plasma insulin levels in CR rodents as the factor most responsible for the anti-aging action of CR. One reason for this focus is that long-term hyperinsulinemia causes premature senescence-like damage.\textsuperscript{21} The main reason, however, is the recent research on genetic manipulations that extend the life span of the nematode Caenorhabditis elegans and the fruit fly Drosophila melanogaster. Loss of function mutations in genes coding for components of the insulin-signaling pathway of this nematode species\textsuperscript{22} and this fruit fly species\textsuperscript{23} extend the life span of these invertebrate animal models. It has been suggested that there is an evolutionary link between the effects of these loss of function mutations in these invertebrate animal models and the life extending effect of CR in rodent models.\textsuperscript{24} My initial reaction was negative to the hypothesis that these findings on nematodes and fruit flies relate to the life-extending action of CR in rats and mice because CR does not decrease but rather enhances the insulin signaling system in the skeletal muscle and adipose tissue of rodents. The recently reported findings of Wolkow et al,\textsuperscript{25} however, made me reconsider this hypothesis. These investigators restored insulin signaling in specific tissues of a nematode strain that has a mutation resulting in a loss of function in insulin signaling and found that restoring insulin signaling in the nervous system of these animals eliminated the increased longevity but that restoring it in muscle and intestine did not. Indeed, if CR does not enhance or attenuate insulin signaling in the central nervous system of rodents, the sustained low levels of circulating plasma insulin may have the same effect on insulin signaling in the...
central nervous system of rodents as that of the loss of function mutations in *C. elegans*. Although the proponents of the hypothesis that insulin signaling is a universal component of aging in all animal species seem overly exuberant given the current state of knowledge, the concept is certainly worthy of further study.

**CALORIC RESTRICTION AND CARBOHYDRATE METABOLISM IN RHESUS MONKEYS**

Three studies are ongoing on the effect of diet restriction on aging of rhesus monkeys; these studies are being conducted at the National Institute on Aging, the University of Maryland, and the University of Wisconsin. As yet, none has provided definitive evidence that CR increases the length of life of these monkeys but they have shown that many of the phenotypic effects of CR in rodents also occur in rhesus monkeys. Long-term CR has been found to decrease fasting plasma glucose levels in two of these studies but not in one of the studies. CR decreased fasting plasma insulin levels and increased insulin sensitivity in all three studies. In all three studies, however, insulin sensitivity was determined in fasted monkeys under light anesthesia. Thus, as of now, there is no information on the effects of CR on carbohydrate metabolism and insulin action in rhesus monkeys under usual living conditions.

**REFERENCES**


**ABSTRACT:**

Labrador retrievers (48) were used to assess diet restriction effects on carbohydrate and lipid metabolism. Restricted-fed (RF) dogs were fed 75% of the same diet consumed by control-fed (CF) pair-mates. An intravenous glucose tolerance test (IVGTT) was performed annually for years 9 to 12. Dietary treatment, age, and interactions were fixed effects. Statistical procedures used when appropriate included mixed-model, repeated-measures ANOVA; least-squares means; Tukey’s multiple comparison; paired t-test; Spearman rank correlation; and Cox proportional hazards regression. The following IVGTT indicators improved \((P < 0.001)\) in RF dogs compared with CF dogs: glucose and insulin means, peaks, \(\Delta\)'s, return-to-basal times, areas-under-curve; glucose decline rate; and insulin sensitivity overall average and for each year 9, 10, and 11. The following IVGTT indicators were not significantly different \((P > 0.05)\) for RF versus CF dogs: insulin decline rate; year 12 insulin sensitivity; and insulinogenic index. Peak and \(\Delta\) glucose were positively correlated and insulin sensitivity negatively correlated with body condition score (BCS), fat percentage and mass, and body weight. Serum cholesterol and triglyceride were not remarkably correlated with glucose/insulin indices nor associated with survival. Median survival time tended to be greater with lower basal glucose and insulin \((P = 0.065, P = 0.096)\). Time-to-first osteoarthritis treatment or death was greater in dogs with lower basal glucose and higher insulin sensitivity \((P = 0.021, P = 0.023)\); however, diet restriction explained most of the relationship variation. Higher insulinogenic indices were associated with greater median survival \((P = 0.053)\) and those with higher insulin sensitivity had less \((P = 0.018)\) hazard of dying or receiving chronic disease treatment. These insulin indices added more information than diet restriction alone \((P = 0.057, P = 0.055)\). Lifelong diet-restricted glucose disposal efficiency and insulin response were associated with increased quality and quantity of life.
“Life is short, eat dessert first” — Anonymous

We live to eat and eat to live. When food is plentiful, we store “fuel” as fat. Glucose is absorbed from carbohydrate sources in food and insulin is secreted to allow storage of glucose as fat and glycogen. During periods of starvation, however, our metabolic machinery changes from “storage phase” to “starvation phase.” Insulin secretion is diminished and fat deposits are used as an energy source to conserve glucose for insulin-independent organs such as the brain. Do we have the mechanisms for preventing obesity and its many detrimental effects? Does body composition—lean versus fat mass—make a difference? Is the onset of disease heralded by the onset of perturbations in carbohydrate metabolism? What are the lessons learned from the Purina 448 study? This article will address the clinical consequences of the Purina 448 study for today’s veterinary practitioner.

OBESITY

Humans are not the only species beset by the problem of obesity. It is estimated that approximately 35% to 40% of cats, 25% to 35% of dogs, and more than 50% of humans are obese.1-3 In humans, and probably in cats, obesity is a precursor to diabetes mellitus (type 2). Obese cats are four times more likely to develop diabetes, five times more likely to develop lameness, and three times more likely to have non-allergic skin conditions compared with cats of optimal body condition.4 To say that obesity in the pet population is a problem is a gross understatement. Until only recently, however, veterinarians had no real “smoking gun” to convince owners to watch their pet’s weight.

THE SET POINT

Studies have shown that weight loss and neuroendocrine mechanisms revolve around a body set point. Perturbations from the “normal” set point, which may be genetically determined, result in weight gain and redistribution of body mass (lean versus fat). This set point can be altered by changes in diet caloric content and composition (protein versus carbohydrate). Studies in humans have shown that subjects consuming a high-glycemic index diet for 9 days have higher levels of serum leptin, a greater decrease in resting energy expenditure (REE), and more negative nitrogen balance than those consuming a low-glycemic index diet.3

Obese diabetic cats fed a restricted carbohydrate diet lost body fat (based on dual energy X-ray absorbiometry), gained lean body mass, became normoglycemic, and lost insulin dependence.5 Overweight dogs fed 20% calories from protein lost twice as much lean body mass as overweight dogs fed 30% or 39% calories from protein.6
Obese cats fed 35% metabolizable energy as protein showed weight loss consisting of 20% as lean body mass (LBM) and 79% as fat; however, the cats fed 45% energy as protein lost only 11% as LBM and 88% as fat. Clearly, shifting to a diet which is lower in carbohydrates, lower in glycemic index, and higher in protein has a beneficial effect on metabolism leading to maintenance of lean body mass and loss of body fat. Finally, as shown in the Purina 448 study, dogs fed ad libitum had a higher percentage of body fat, perturbations in carbohydrate metabolism, and significantly shorter life span than dogs fed a restricted-calorie diet.

CLINICAL IMPLICATIONS

One of the problems associated with obesity management is the lack of an objective, practical method for accurately assessing body composition (LBM and percent body fat). The most useful research tool for assessment of body composition is dual energy x-ray absorptiometry. Most veterinarians, however, do not have access to such tools. Therefore, body condition score systems have been developed for both cats and dogs and correlated with laboratory methods such as DEXA. The nine-point system developed by Laflamme provides a simple, repeatable method of assessing body composition in small animals. (See Appendix II at end of these proceedings for the canine and feline body condition score charts.)

One of the easiest and most practical methods is to take a digital or Polaroid photograph (head on and profile view) of the dog or cat on a yearly basis. Photographic images can be imported into the animal's medical record thus providing serial images of body condition over the years. Body weight and body condition scores should be recorded on a yearly, if not quarterly, basis. Dogs and cats with heavy haircoats should be palpated carefully to assess changes in body condition which may not be visually apparent.

Changes in insulin, glucose, and thyroid metabolism were evident in the Purina 448 study. Controlled-feeding dogs had higher fasted serum insulin concentrations and greater area under the curve (AUC) for glucose after an intravenous glucose tolerance test (IVGTT) than restricted-feeding dogs. From a practical standpoint, IVGTTs are not easily performed in a practice setting for routine annual examinations. However, a serial record of annual fasting blood glucose values for an individual animal may be very illuminating. Particularly in cats, because the risk of type 2 diabetes is so great, annual fasting and postprandial blood glucose values might be of assistance in identifying early type 2 diabetic cats. Dietary intervention at this point could prevent the development of insulin dependence.

Another parameter that might be beneficial, particularly in cats, is serum fructosamine. Fructosamine is albumin and other serum proteins which have become glycosylated by a nonenzymatic process. Increased serum fructosamine values may presage the onset of overt diabetes mellitus in cats and dogs. Again, serial fructosamine values on an annual basis may provide a more accurate reflection of normal versus abnormal for a particular patient.

Changes in serum triiodothyronine (T3) concentrations were also noted in the Purina 448 study; in fact, higher serum triiodothyronine (TT3) were evident in the controlled-feeding group. Higher serum TT3 has been shown to be a consequence of over-feeding because of an increase in deiodination of thyroxine (T4) to T3. In contrast, carbohydrate restriction and weight reduction cause a decrease in serum T3 concentrations. This brings up the question of whether serial measurements of serum T3 and T4 might be beneficial in monitoring and prevention of obesity in pet animals.

The problem with this strategy, particularly in dogs, is the tendency to over-diagnose thyroid disorders. Thyroid metabolism is complex and the finding of low serum T4 in an otherwise healthy animal may not be of much importance. In dogs, if thyroid hormone is low, endogenous thyroid-stimulating hormone (eTSH) concentrations should be measured. The findings of a high serum eTSH combined with a low TT4 will ensure
against the misdiagnosis of hypothyroidism. Serial measurement of T4 and T3, while not detrimental, probably do not provide any additional information for the management and prevention of obesity. Obviously, hypothyroid dogs should have these parameters measured on an annual basis.

**STRATEGIES TO COPE WITH OBESITY**

In a recent article examining factors involved in feline obesity, the top four main factors contributing to body condition were neuter status, age, frequency of treat feeding, and ad libitum feeding.1 Because neutering has health benefits and prevents pet overpopulation, it seems unlikely that current recommendations for neutering will change. Age cannot be eliminated as a factor, although increased vigilance in middle age seems appropriate as this age group is most at risk for obesity.

Treat feeding is a source of calories and a known contributor to obesity in small animals. If animals are fed a diet that satisfies them, or treats that satisfy their hunger, overeating will become less of an issue. In cats and dogs, owners should provide a food with adequate protein for maintenance of lean body mass and in the case of cats, enough fat to provide satiety. In dogs, higher protein levels will maintain body mass, even during calorie restriction for weight loss. In cats, the author has had success in reducing body fat in both diabetic and nondiabetic cats using a high protein, low carbohydrate canned formulations such Purina DM® Diet (Nestlé Purina PetCare Company, St. Louis, MO).5

Just as in humans, portion size should be regulated. A true measuring cup of dry food is a small amount. Clients should not use a jumbo sized cup or giant scoop to measure dry food amounts. Clients need to realize how much food they are feeding—a “Big Gulp” cup contains about four actual cups of food. Portions may be more accurately assessed using canned formulations. The expense of canned foods also helps limit over-feeding and waste of excess food. In the author’s experience, cats are more satisfied with foods that are higher in fat and protein and lower in carbohydrate content. This “satiety” factor seems to help limit over-eating in this species.

The most obvious change that veterinarians can promote is a change in feeding regimens and the easiest change to implement is to eliminate free-choice or ad libitum feeding. Ad libitum feeding (which is not available in nature, by the way) has been shown to increase the incidence of osteoarthritis in young growing dogs and increase obesity in cats.1,12 Veterinarians can now cite the Purina 448 study which showed that ad libitum feeding has detrimental effects—namely, a decrease in life span!8

**CONCLUSIONS**

If you told your clients there was a way to prolong their dog or cat’s life by almost 20% would they listen to you? The answer is a resounding “yes.” It is up to us, as veterinary professionals, to bring the enlightening information of the Purina 448 study to the pet-owning public.

**REFERENCES**


Physiologic deterioration and a rise in pathology of organs and organ systems characterize aging. Dietary restriction (DR) retards the age-related physiologic decline and inhibits the appearance of pathology associated with aging. The task of this report is to review the effects of DR on the aging cardiovascular system in all species other than the dog. At first sight, the scope of the review seems exceptionally broad. In reality, however, virtually all the available data are confined to the rat or mouse. These species have relatively short life spans, can be used in cross-sectional as well as longitudinal studies, and allow for invasive measurements. Studies utilizing these species are less expensive to complete than those involving larger species such as humans or nonhuman primates. Moreover, food intake is more easily controlled in the rodent, thereby minimizing a large source of variability in the experimental conditions. Finally, because of the size differential, the rat is preferred over the mouse when functional measurements are made on the cardiovascular system. Therefore, with the exception of the studies performed by Purina on dogs, most of the available data on the effects of DR on the aging cardiovascular system are confined to rats.

**BODY WEIGHT/HEART WEIGHT**

Heart weight increases with age in both ad libitum-fed (AL) and calorie-restricted (DR) rats.\(^1,2\) At all ages, the heart weights of the DR rats are less than those of the AL animals.\(^1,2\) In contrast, the heart weight to body weight ratio of AL rats decreases from 6 to 12 months of age, while the ratio for DR hearts increases with age such that the ratio for the DR hearts is always higher than that of the AL rats (Table 1). This difference may indicate that the heart is spared the loss in mass with DR at the expense of other organs. The functional ramifications of the elevated heart weight/body weight ratio are not known. It would be interesting to determine whether the apparent cardiac hypertrophy seen with DR parallels that seen in trained athletes where an elevated stroke volume preserves cardiac output at a lower heart rate. In fact, DR is associated with lower heart rates in rats (see below). Not all studies report an increase in the

<table>
<thead>
<tr>
<th>AGE (MONTHS)</th>
<th>BODY WEIGHT (g)</th>
<th>HEART WEIGHT (mg)</th>
<th>HEART WEIGHT/BODY WEIGHT (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL DR</td>
<td>AL DR</td>
<td>AL DR</td>
</tr>
<tr>
<td>6</td>
<td>342±8 224±2</td>
<td>895±17 659±9</td>
<td>2.62±0.03 2.94±0.06</td>
</tr>
<tr>
<td>12</td>
<td>485±20 273±2</td>
<td>1148±61 813±15</td>
<td>2.36±0.04 2.98±0.06</td>
</tr>
<tr>
<td>18</td>
<td>552±14 305±3</td>
<td>1314±24 938±31</td>
<td>2.39±0.06 3.07±0.10</td>
</tr>
<tr>
<td>24</td>
<td>530±11 321±3</td>
<td>1291±17 995±21</td>
<td>2.45±0.06 3.10±0.06</td>
</tr>
</tbody>
</table>

AL = fed ad libitum; DR = diet restricted.

**TABLE 1. Body Weight and Heart Weight**

\[\text{AL} = \text{fed ad libitum; DR} = \text{diet restricted.}\]
heart weight/body weight ratio. Dietary restriction for shorter durations (5 to 6 weeks) does not elevate the heart weight/body weight ratios for young adult, female, Sprague-Dawley rats, although the body weights and left ventricular weights decreased upon DR.3–6

CARDIOMYOPATHY

The age-related increase in heart mass is often associated with the appearance of cardiomyopathy involving loss of cardiocytes, hypertrophy of surviving cardiocytes, and infiltration of fibrotic tissue.7–11 Early studies by Berg and Simms7,8 indicated that DR drastically reduced the age-related cardiomyopathy in Sprague-Dawley rats. Similar protective effects of DR were observed in the Fischer 344 male rat subjected to 40% DR,11 even when the DR was initiated during young adulthood.12 DR was also effective in ameliorating the appearance of cardiomyopathy in the SHR and WKY rat models.13 This study is extremely interesting in that DR exerted its anti-aging actions (increased life span, decreased incidence and severity of pathologic lesions) without altering the rise in blood pressure seen in the control SHR rats! Thus, DR prevented cardiac and renal degeneration that was associated with high blood pressure without affecting the blood pressure itself.13 The mechanism by which DR bestows protection against end-organ damage in the face of genetically determined pathology is not known. Given the clinical importance of hypertension in developed countries, it would seem important to explore and expand the findings obtained in Lloyd’s study.13

CORONARY CIRCULATION

Concurrent alterations beset the coronary circulation where aging leads to increases in minimal coronary vascular resistance and decreases in coronary flow reserve.9,10,14,15 These age-related functional changes in the coronary circulation are thought to contribute to the structural and functional alterations observed in the aging heart.14 In fact, Vandewoude and Buysens16 suggested that vascular deficiency, ultimately leading to ischemia and cell loss, might contribute to the physiologic deterioration seen in the aging heart. They also noted15 that extreme malnutrition led to regression of age-related morphologic changes in the vasculature, thereby offering some protection to the aging cardiocyte by enhancing metabolic exchange. A fortuitous observation made in our laboratory was that isolated, perfused hearts from 14-month-old Fischer 344 male rats subjected to 40% DR exhibited higher coronary conductance than control hearts.16 This observation was extended throughout the life span as shown in Figure 1.17 Because the degree of coronary tone present in the various age and diet groups during perfusion was not assessed, it is not certain whether the higher

![FIGURE 1. Effects of age and DR on the coronary conductance of isolated hearts from Fischer 344 rats. Ad libitum–fed controls (AL) are denoted by open circles; dietary-restricted (DR) rats are denoted by closed circles. Circles and bars represent the means ± SEM for 5 hearts in each group. * Represents a significant (P< 0.05) difference from the AL group of the same age. Drawn from data obtained from Reference 17.](image-url)
the enhanced coronary conductance seen in DR hearts may contribute to the lower occurrence of age-related cardiomyopathies, as suggested above, and this topic represents a fruitful area for future research.

OXIDATIVE DAMAGE

The oxidative stress theory of aging proposes that the progressive deterioration and time-associated alterations arising during aging are the cumulative result of incessant free radical generation occurring in the course of normal cellular metabolism. Dietary restriction possesses a remarkable ability to reduce oxidative damage and this protective attribute has been proposed as a possible mechanism by which DR exerts its anti-aging action. We have examined the effects of DR on age-related changes in oxidative damage to cardiac mitochondrial membranes and antioxidant defenses in cardiac muscle. In the earlier study, DR decreased the malondialdehyde production rate of cardiac mitochondrial membranes indicating that the degree of lipid peroxidation in these membranes was decreased (Figure 2). The later study confirmed the protective effect of DR. Here, 2',7'-dichlorofluorescein-diacetate (DCF) formation, an index of reactive oxygen species production and an indicator of membrane lipid peroxidation, increased from 6 to 24 months of age in the AL group (Figure 3). Even at 6 months of age, the DCF formation of the DR group was lower than the AL group and, in fact, remained lower than the 6-month-old group even up to 24 months of age (Figure 3). Measurement of the fatty acid composition indicated that membranes from control hearts exhibited a decrease in the peroxidizability index at 24 months of age, whereas in the DR group no decline in this index was observed over the same time period (Figure 4). In agreement with these results, membrane fluidity decreased with age in the AL group, while DR restriction prevented the decline (Figure 5).

ANTIOXIDANT PROTECTION

Part of these protective effects of DR may arise from dietary-induced changes in the antioxidant status of the myocardium. DR increased the activity of the following cardiac cytosolic enzymes (Figure 6): Cu/Zn...
superoxide dismutase (SOD), glutathione S-transferase (GST), and selenium-dependent glutathione peroxidase (GSH). DR exerted no effect on catalase (CAT) in this study (Figure 6).

**MYOSIN ISOZYMES**

The aging myocardium exhibits a profound shift in the myosin isoenzyme profile from the fast isoform (V1) to the slow isoform (V3). Work from our laboratory has confirmed this age-associated shift in the profile. The V1 content decreased from a value of about 60% at 6 months of age to approximately 40% at 24 months of age (Figure 7A). Surprisingly, the effect of life-long DR was actually an enhancement, rather than an inhibition, of the age-related decline in V1 isozyme content. At every age the V1 content of the DR hearts was lower than that of the AL controls. Similar effects were observed with the slower V3 isozyme (Figure 7B) where V3 increased with age in the AL group and DR increased the levels at all ages. The effect of diet depended only upon calorie input and was unaffected by changes in carbohydrate ingestion. Interestingly, older AL rats (16 months) retained the ability to respond to DR by decreasing V1 and increasing V3 isozyme contents.
Dietary restriction applied for shorter periods elicits the same results. Dillmann et al.28 and Haddad et al.4 showed that a decrease in the Ca++-activated myosin ATPase accompanied the diet-induced decrease in the V1 isozyme content. Baldwin’s group3–6 confirmed and extended these results to include decreases in \( \alpha \)-myosin heavy chain protein content and its mRNA.

**CARDIAC MECHANICS**

Transition from the fast V1 to the slower V3 isozyme profile is accompanied by a number of functional alterations, including prolongation of both the rising29–31 as well as the falling30,31 phase of the cardiac twitch. Aging itself is characterized by similar changes in the cardiac contraction cycle.31 The effects of long-term DR (9 to 12 months) on the cardiac mechanics were investigated in the isolated perfused hearts of Fischer 344 male rats.32 Contraction times (time to peak pressure) and relaxation times (time to 1/2 relaxation) were measured in control and DR hearts under conditions of high inotropic states induced by high perfusate calcium and isoproterenol and under two intraventricular volumes. The results are shown in Table 2. Regardless of the inotropic agent used (calcium or isoproterenol) or the intraventricular volume, DR hearts required a longer time to reach maximum pressure. They also took a longer time to reach the 1/2 relaxation point. Just as DR appeared to enhance the age-related change in the myosin isozyme profile (see earlier), so too with contraction and relaxation times; DR accelerated the age-related increases in cardiac twitch contraction and relaxation times.

Both developed pressure as well as diastolic pressure are affected by DR.32 At low calcium ion concentrations DR hearts developed greater pressure than AL hearts. This difference disappeared at higher perfusate calcium concentrations (Figure 8A). DR hearts also exhibited greater developed pressure than AL hearts when

<table>
<thead>
<tr>
<th>Volume</th>
<th>Isoproterenol</th>
<th>Calcium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AL</td>
<td>DR</td>
</tr>
<tr>
<td>Low</td>
<td>58.3±0.9</td>
<td>61.0±0.8*</td>
</tr>
<tr>
<td>High</td>
<td>66.6±0.9</td>
<td>72.1±0.5*</td>
</tr>
</tbody>
</table>

AL = fed ad libitum; DR = diet restricted.

*Time in msec. *Different from its respective AL group in same volume and with the same inotropic agent.
stimulated with isoproterenol (1 to 10 nM) (Figure 8B). A decrease in diastolic pressure was observed during inotropic stimulation as a result of elevated calcium in the perfusate (Figure 9A) or addition of isoproterenol (Figure 9B) and DR enhanced the decline. These results indicate that the sensitivity of the heart to inotropic agents, which is known to decrease with age, is enhanced with DR.

CARDIAC ENERGETICS

The mechanism by which DR exerts its anti-aging action is not known. One hypothesis is that it increases the efficiency of energy utilization in one or more organs. Enhanced efficiency would be beneficial from two points of view. First, it may lead to a lower metabolic rate for that organ or tissue and therefore lower the levels of injurious metabolic end-products. Second, the energy saved with greater efficiency may be utilized for greater repair and maintenance. We have examined the effects of DR on cardiac energetics to determine whether the DR heart utilizes energy more efficiently than the AL control hearts. The energy utilization by the heart can be divided into three basic components of approximately equal magnitude: energy usage for mechanical activity, for activation (contractility), and for basal metabolism.

FIGURE 8. A, Effect of DR on the maximum developed pressure over a range of calcium ion concentrations in the perfusate. Heights of the columns and bars represent means ± SEM for 10 and 7 hearts from the AL and DR groups, respectively. * represents a significant (P < 0.05) difference from the AL group. B, Effect of DR on the maximum developed pressure over a range of isoproterenol concentrations in the perfusate. Heights of the columns and bars represent means ± SEM for 10 and 7 hearts from the AL and DR groups, respectively. * represents a significant (P < 0.05) difference from the AL group. Redrawn from Reference 32.

FIGURE 9. A, Effect of DR on the end-diastolic pressure over a range of calcium ion concentrations in the perfusate. Heights of the columns and bars represent means ± SEM for 10 and 7 hearts from the AL and DR groups, respectively. * represents a significant (P < 0.05) difference from the AL group. B, Effect of DR on the end-diastolic pressure over a range of isoproterenol concentrations in the perfusate. Heights of the columns and bars represent means ± SEM for 10 and 7 hearts from the AL and DR groups, respectively. * represents a significant (P < 0.05) difference from the AL group. Redrawn from Reference 32.
The oxygen cost of tension development represents the energy consumed for mechanical activity. The greater the tension developed by the muscle, the greater the oxygen consumption. Over a wide range of developed pressures, the oxygen consumption was unaffected by DR. Whether pressure was altered by changes in external calcium (Figure 10A) or β-adrenergic stimulation (Figure 10B) no differences in oxygen consumption could be detected between AL and DR hearts. Thus, DR did not affect the efficiency of energy utilization for mechanical activity.

The oxygen cost of contractility represents the energy utilized for electrical and excitation-coupling processes, the latter arising mainly from energy required for calcium pumping into the sarcoplasmic reticulum. DR did not alter the oxygen cost of contractility when calcium was used as the inotropic agent to change contractility (Figure 11). Interestingly, the DR heart exhibited a lower efficiency than AL hearts when isoproterenol was used to change contractility. As shown in Figure 11, the oxygen consumption due to isoproterenol-induced changes in contractility was greater for the DR hearts than that of the AL hearts.

Finally, the basal metabolic rate represents the oxygen consumption under conditions of zero tension development and zero contractility. This rate can be measured directly when the external calcium is completely removed from the perfusate. Figure 12 demonstrates that DR had no effect on the basal metabolic rate of the heart when compared with that of the AL controls.

In summary, these studies provide no evidence that long-term DR enhances the energy efficiency of the heart.

**CARDIAC ADRENERGIC FUNCTION**

The decline in β-adrenergic receptor responsiveness is a hallmark of the aging process. It has been documented in most organs studied and the age-related decrease in cardiac responsiveness has been observed consistently. Both short-term and long-term DR enhance the cardiac response to β-adrenergic stimulation. The effects of DR on the β-adrenergic receptors and post-receptor activity have not been explored.

**CARDIAC SYMPATHETIC NERVES**

The aging heart, like most aging organs, exhibits profound changes in its sympathetic innervation, including a loss in the norepinephrine content. An early study by McLean et al showed that the cardiac
sympathetic axons degenerated with age and suggested that part of the decline arose from the loss of sympathetic nerves to the heart. We also have noted a decline in cardiac norepinephrine content with age and this decline is prevented by long-term DR (Figure 13). The mechanism by which DR prevents the age-related decline in norepinephrine content is not known. It may be related, however, to preservation of sympathetic axons or the lower sympathetic nervous system activity that occurs with DR (see below) may also contribute to the elevated content. Roberts’ group has examined the effects of age and DR on the handling of norepinephrine by cardiac synaptosomes. Norepinephrine release from as well as its uptake into the synaptosomes decline as the heart ages and DR was shown to inhibit the aging effects. These results agree with our findings, where DR of 4.5 months’ duration increased the norepinephrine uptake by cardiac synaptosomes (Figure 14).

**AUTONOMIC NERVOUS SYSTEM**

It is widely believed that the activity of the sympathetic nervous system increases with age (for reviews see References 37–39 and 44) and this belief has led to the idea that “old age may represent a hyperadrenergic state.” Short-term DR or fasting decreases sympathetic nervous system activity. The effects of long-term DR on this activity have not been studied.

Very few data are available on the effects of DR on the parasympathetic innervation of the aging heart. Results from our laboratory (see below) suggest that DR rats possess a higher resting parasympathetic tone than AL controls.

**HEART RATE**

Resting heart rate is not markedly affected by age in humans or in rodents. Of the 34 studies reviewed earlier over 70% reported that heart rate did not change substantially with age and the remaining 30% observed a decrease. DR has been shown to consistently lower resting heart rate. The dietary-induced decline in heart rate was noted in the earliest studies (McCay as quoted by Comfort). In our own laboratory, DR rats exhibited significantly lower heart rates than AL rats at 7, 14, and 21 months of age (Figure 15).

Heart rate is regulated by both the sympathetic and parasympathetic components of the autonomic nervous system. Removal of both components results in the intrinsic rate of the heart. Most studies report that intrinsic heart rate either decreases or at least shows a tendency to decrease with age. Blockade of the
parasympathetic nervous system with atropine results in a greater increase in heart rate in the DR group, suggesting that the DR groups possess a higher parasympathetic tone than the AL group (Figure 16). Total blockade of the autonomic nervous system with both atropine (parasympathetic) and propranolol (sympathetic) decreased heart rate to a lower level in the AL group than the DR group, indicating that DR may inhibit the age-related decline in intrinsic heart rate.

**BLOOD PRESSURE**

In humans, aging is associated with increasing blood pressure. In modern industrial societies, elevated systolic, diastolic, and mean arterial pressures are observed and are associated with an increasing prevalence of hypertension. Comparative studies have shown, however, that minimal changes in diastolic and mean pressures occur with age in rural subjects in contrast to their urban counterparts. This suggests that the age-related changes occurring in diastolic and mean pressures are more related to environmental factors, such as diet and stress, rather than to aging per se. The rise in systolic pressure is associated with an age-related decline in arterial compliance. This stiffening of the arteries appears to be a primary aging process and the consequent impedance mismatch contributes to the cardiomyopathies observed in the aging heart.

![Figure 13](image1.png)

**FIGURE 13.** Effects of age and DR on the norepinephrine content of the heart. Circles and bars represent the mean ± SEM for 10 hearts in each group. * represents a significant ($P < 0.05$) difference from the AL group at the same age. Redrawn from Reference 54.

![Figure 14](image2.png)

**FIGURE 14.** Effect of DR (4.5 months) on the norepinephrine uptake of cardiac synaptosomes. Heights of the columns and bars represent the means ± SEM for 5 hearts in each group. Redrawn from Reference 43.

![Figure 15](image3.png)

**FIGURE 15.** Effects of age and DR on the resting heart rate of Fischer 344 rats. Red and blue circles represent means for the AL and DR rats, respectively. Bars represent the SEM for 6 to 8 rats in each group. * indicates a significant ($P < 0.05$) difference between the means. Redrawn from References 48 and 49.
Dietary restriction leading to weight loss is considered a major nonpharmacologic therapy for controlling high blood pressure, especially in overweight subjects. The decline in pressure with dietary restriction appears to be independent of salt intake. In most studies weight loss accompanies DR and it is commonly believed that DR exerts its hypotensive action through its effect on body weight rather than the effect of energy restriction per se.

In laboratory animals, the data on the changes in blood pressure with age are inconclusive. In this review, most studies examined reported no changes in pressure with age. Where changes did occur, the method of blood pressure measurement appeared to qualitatively affect the outcome. For example, with the tail cuff method, pressure increases were generally reported, whereas pressure decreases with age were observed when measured by direct cannulation. In the Fischer 344 rat, Yu et al. reported an increase in tail cuff pressure between 9 and 19 months of age. In contrast, from the same rat colony we have observed a moderate, but progressive, decrease in mean arterial pressure during aging (Figure 17).

The data on the effects of DR on blood pressure in the rat are also inconclusive. Most studies report that DR elicits either no change or a decrease in blood pressure. When DR exerted a hypotensive action, the study usually utilized hypertensive rats and this observation is consistent with the observation from clinical studies that the higher the initial pressure the greater the hypotensive action of DR. Very little change in blood pressure is observed in normotensive rats subjected to DR. The longitudinal study of Yu et al. observed no effect of DR on the tail cuff pressures measured throughout life. Our own data generally confirmed these results except at 22 months where a slight decrease in mean pressure was observed in the DR group (Figure 17).

**BAROREFLEX**

The arterial baroreflex is the major mechanism responsible for buffering acute changes in blood pressure. In humans and laboratory rodents, the responsiveness of the reflex declines with age. Very little work has been done on the effects of DR on the age-related changes in baroreflex sensitivity. We have examined the sensitivity of the cardiac component of the reflex by measuring the increase in heart rate that results from hypotensive episodes induced by nitroprusside administration (Figure 18). A decrease in sensitivity occurred with age in AL control rats. At every age, the sensitivity of the DR group was higher than...
that of the AL group. Interestingly, the sensitivity of the DR group declined with age, albeit at a higher level (Figure 18).

**SUMMARY**

It is clear that DR alters many age-related changes that occur in the cardiovascular system. In some cases, DR exerts an anti-aging action in that it reverses or at least inhibits age-related changes. In other cases, DR seems to enhance the aging process in that it itself produces alterations that mimic the age-related changes. Although these paradoxical effects make it difficult to erect a unified picture of cardiovascular aging and the effect of DR, they do offer hints on future avenues of research that may prove fruitful in unraveling the effect of age on the cardiovascular system.

**ACKNOWLEDGMENTS**

Dr. Herlihy’s work was supported by NIA Grant AG01188 and NIA Grant T32AG00205. The secretarial assistance of Ms. Nancy Markham in the preparation of this manuscript is greatly appreciated.
REFERENCES


ABSTRACT:

The electrocardiogram (ECG) is the "gold standard" for the rhythm of the heart. The length of the intervals between the waveforms and the shape, frequency, and duration of the waveforms reveal a great deal about the ability of the heart to deliver blood to the tissues of the body. While the ECG is the "accepted" measure of the heart's electrical activity, it can also provide information on the size and shape of the various heart chambers. Clinicians rely on the ECG to allow them to sort out arrhythmias that may be produced by the patient's disease or may be side effects of the drugs they have prescribed to treat the disease. Serial ECG tracings recorded over the course of a patient's cardiac disease reveal changes that are helpful to the clinician in making medication adjustments as well as providing prognostic information to the client. ECG used routinely in this manner has provided a wealth of information about what ECG changes are likely to occur in dogs as they age with an abnormal and/or compromised cardiovascular system. The Purina 448 study is the first time a group of normal dogs were followed routinely with ECG tracings as they aged through all stages of their lives. ECG tracings were done at the beginning of this study and at least once each year until these animals died. The tracings of each of these animals were evaluated year to year with the changes noted over time for each animal. The tracings were also compared within their sex and feeding trial groups. This 14-year study provides ECG aging information in a controlled situation that heretofore has never been reported. The ECG changes seen in these dogs, although often not outside what is considered normal ranges for dogs, provide valuable insight on how the electrical system within the heart changes over the life span of a Labrador retriever.
Dr. Robert Hamlin received his DVM and PhD degrees from The Ohio State University (OSU). Dr. Hamlin is a Diplomate of American College of Veterinary Internal Medicine (Cardiology/Internal Medicine). Dr. Hamlin is a Stanton Youngberg Professor of Veterinary Physiology/Pharmacology and Professor of Biomedical Engineering at OSU. His clinical interests are cardiopulmonary medicine and physical examination, while his research interests include pathophysiology of heart failure and arrhythmias, comparative electrocardiography, and drug-induced ventricular arrhythmias. Dr. Hamlin's teaching responsibilities include cardiovascular physiology, pulmonary pathophysiology, electrocardiography, noninvasive diagnosis of cardiopulmonary diseases, and diagnosis and management of heart failure. He has received the Mahanna Award of the American Heart Association, Career Development Award of NIH, Robert Kirk Award from ACVIM, National and International Waltham Awards, and Distinguished Teaching Award at OSU. He has written more than 250 scientific publications and 12 textbook chapters.

ABSTRACT:
The prevalence, pathophysiology, and diagnosis of systemic arterial hypertension in dogs remain problematic. We assume that a blood pressure exceeding 165 mmHg in systole or 135 mmHg in diastole is above normal. Most animals with hypertension have abnormal renal function, but it is not known whether it is the cause or the consequence of hypertension. Hyperadrenocorticism is a common cause of hypertension. Hypertension results in renal (glomerular disease with proteinuria), ocular (retinal hemorrhage, edema and detachment), and possible cardiac consequences (ventricular hypertrophy). Dogs fed a diet restricted in calories tend to have lower blood pressure, lower pressure pulse, slower heart rates, and much lower rate-pressure products—a prime determinant of myocardial oxygen consumption. Caloric restriction also tends to decrease pulse pressure, indicating a decrease in arterial impedance. Considering extrapolations from experience with human hypertension and presuming that all dogs would respond as Labrador retrievers, caloric reduction in dogs and reduction in arterial pressure and arterial pulse pressure should translate into decreased morbidity and mortality.
ABSTRACT:
A study was designed to evaluate the effect of diet restriction on body lean, fat, and bone mass in Labrador retrievers throughout their life span. Eight-week-old pups were allotted to two treatment groups in a paired-feeding design with 24 pairs. One pair-mate was control-fed and the other pair-mate received 25% less food on a daily basis. Body lean, fat, and bone mass were collected by dual-energy x-ray absorptiometry (DEXA) starting at 6 years of age. Pair-mates received the same nutritionally complete diet through their entire life span. Control-fed (CF) dogs received an average of 1,745 kcal metabolizable energy (ME) per day, whereas the restricted-fed (RF) dogs received an average of 1,352 kcal ME per day ($P<0.01$) through 12 years. Caloric intake data after 12 years was erratic due to increased incidence of illness, especially in the CF group. Mean body weight of RF dogs was, on average, 26% lower than the CF group ($P<0.01$). Mean body condition score (range: 1 to 9, emaciated to grossly obese) taken during the 6- to 12-year period was 6.7 in the CF group as opposed to 4.6 in the RF group. Mean lean body mass remained constant from 6 through 9 years of age for CF dogs and from 6 through 11 years of age for RF dogs. From 6 through 9 years of age, mean lean body mass was significantly ($P<0.01$) greater for CF dogs than for the RF group. A progressive decrease in lean body mass was detected among CF dogs after 9 years of age, but a similar decrease was not detected among RF dogs until after 11 years of age. Mean percentage lean body mass decreased significantly ($P<0.05$) in both groups from 6 through 12 years of age; however, the RF dogs always had a significantly ($P<0.01$) greater mean percentage lean body mass. Mean absolute and percentage body fat mass increased significantly ($P<0.05$) in both groups from 6 through 12 years of age. Body fat mass, expressed as an absolute (i.e., grams of fat tissue) or as a percentage of body mass, was always significantly ($P<0.01$) higher among CF dogs than among RF dogs. Mean percentage body fat mass for the entire period from 6 through 12 years of age was significantly ($P<0.01$) higher for the CF dogs (29.9%) than for the RF dogs (16.8%). Results for bone mass were similar to results for lean body mass. Dogs in the CF group had significantly ($P<0.05$) higher bone mass than did RF dogs from 6 through 9 years of age. After 9 years of age, bone mass among CF dogs decreased significantly ($P<0.05$), whereas bone mass among RF dogs remained constant. The decrease in lean body mass late in life (after 9 years of age among CF dogs and after 11 years of age in RF dogs) might have been a consequence of deteriorating physiologic function associated with aging or disease, with delayed expression among RF dogs. The range in estimated body fat for the RF group ranged from 10 to 22% and suggested that body fat less than 22% is a reasonable goal for attaining maximum quality and quantity of life in dogs.
Dr. Joe Kemnitz received his PhD from the University of Wisconsin-Madison and joined the staff of the Wisconsin Regional Primate Research Center (WRPRC) immediately thereafter. His early work with rhesus monkeys focused on hypothalamic mechanisms for the regulation of energy balance and the influences of gonadal hormones on food intake and body composition. He has also examined these relationships in free-ranging baboons and in zoo-housed orangutans. Since 1989, he has been characterizing the changes that occur in middle age and older adulthood of rhesus monkeys, and assessing the effects of dietary restriction on aging in this species. He was elected Fellow in the Gerontological Society of America in 1993, and is an active member in the American Society for Nutritional Sciences, the American Physiological Society, the North American Association for the Study of Obesity, the Endocrine Society, and the American Diabetes Association. He is now Director of the WRPRC and Professor in the Department of Physiology and the Interdepartmental Graduate Program in Nutritional Sciences at the University of Wisconsin-Madison.

BODY COMPOSITION

Overview

Body composition is defined as the relative contributions of different components to the whole body mass. Such components include, but are not limited to, fat, muscle, organ, water, and bone. The definition of the components of body composition relies on the use of body composition models. The classic two-compartment model of body composition divides body weight into fat mass and fat-free mass. The direct measurement of fat mass is a significant challenge for most body composition techniques. Given a measurement of fat-free mass, however, fat mass can be estimated based on the two-compartment model as the difference between body weight and fat-free mass. Three methods traditionally exist for two-compartment body composition analysis:

- measurement of body density by underwater weighing,
- measurement of body cell mass by whole-body potassium counting, and
- measurement of total body water by isotope dilution.

As more measurement techniques were developed the basic two-compartment model evolved into multicompartment models of body composition. Currently, a comprehensive five-level, multicompartment system is used to systematically characterize all of the major body-composition components. According to this system the more than 30 major body composition components are organized into five levels: atomic, molecular, cellular, tissue-system, and whole body. Each component is distinct, clearly identified, and occupies only one level within the hierarchy.¹ ²

Importance

Body composition is an important variable of interest in many circumstances. By itself, body composition is a measure of energy economy reflecting the difference between energy intake and expenditure. A change in an individual component may be defined as pathology (e.g., the loss of skeletal muscle mass suggesting sarcopenia, or the loss of bone mineral indicating osteoporosis). In addition, body composition is often used as a covariate for normalizing variables of interest for statistical analysis (e.g., the use of lean body mass as a covariate in the analysis of metabolic rate).

Of major importance, body composition is independently an excellent marker of health risk and disease. For example, increased body fat, especially increased visceral adiposity, is related to cardiovascular risks such as hypertension and hyperlipidemia, and to glucose regulation problems such as hyperglycemia, hyperinsulinemia, insulin resistance, and diabetes. Therefore, the anatomic distribution of body fat is an additional important factor for predicting morbidity and mortality.
Body composition is known to change as a function of age. Progressive loss of skeletal muscle mass is a well-established characteristic of aging. This loss of muscle mass is associated with decreased strength and increased physical frailty. With age, the ratio of fat tissue mass to lean tissue mass tends to increase even with constant weight. Men undergo a progressive loss of lean body mass (primarily skeletal muscle) and a steady gain of body fat. Early in the aging process there is often a net gain of body weight and a considerable increase in whole body energy store because of the greater energy content of adipose tissue compared with muscle. Similar changes in body composition occur for women with advancing age, but the rate of change is slower than for men, and it begins later in life. These changes lead to an overall increase in percent body fat with advancing age and a decrease in lean tissue and bone mass with age.

Measurement

All in vivo measurements of body composition are by nature indirect and are therefore approximations. The only way to assess body composition directly is by dissection and chemical analysis. Although the results can be very accurate, this approach can be done only once and as a terminal procedure.

The most basic, traditional in vivo assessments of body composition are somatometric indices (e.g., body weight, height, skinfold thicknesses, body circumferences) and derived estimates (e.g., body mass index for estimating body fat). These measurements are easy to acquire, cost effective, and require little specialized equipment or training. Although such indices have been very useful, they can misrepresent the true body composition (e.g., very high body mass index in body builders reflects increased muscle, not fat mass) and can be difficult to translate to animal models.

Underwater weighing for the measurement of body volume was developed in the 1940s based on the two-compartment model of body composition. Although still used as the standard in many laboratories, this technique has several limitations. The major technical difficulty with this method is that the subject must be completely submerged under water and air in the lungs must be quantified. A second major problem with this technique is the assumption that the density of fat-free mass is constant, while in fact the density of fat-free mass is known to vary with many factors including age, gender, and ethnicity.

Unlike the tissue density, the water content of fat-free mass is roughly constant (73.2%) in healthy individuals. This provides the basis for the assessment of fat-free mass from total body water measurements. This method involves the administration of a known dose of a stable isotope of water, a mixing period, and then assessment of the tracer concentration in bodily fluids. The major limitation of this method is in the cost of the isotope and spectroscopic analysis.

Bioelectrical impedance analysis is an alternative method of body water analysis based on the electrical properties of tissues. This technique is based on the premise that when electrical current is passed through the body, the voltage drop between the two electrodes is proportional to the body’s fluid volume in that region. This method is inexpensive and easy to perform; however, its biological interpretation may be limited as it is based on many, sometimes inaccurate, assumptions.

Similar to bioelectrical impedance analysis, total body electrical conductivity is based on the electrical conductivity properties of tissues. Specifically, the subject is placed inside a large electromagnetic coil and the amount of energy absorbed is recorded. The amount of energy absorbed is related to the quantity of conducting material present in the tissue. This rarely used method suffers from the same limitations as bioelectrical impedance analysis in addition to being costly.

More recently three imaging techniques have been applied to body composition analysis: computed tomography (CT), magnetic resonance imaging (MRI), and dual-energy x-ray absorptiometry (DEXA). These
methods allow for relatively rapid, precise, and accurate multicompartment analysis of body composition. Their limitations are cost of the equipment and the need for highly trained technical staff. In addition, CT involves relatively high radiation exposure and entails risk for children, women of childbearing years, or use in longitudinal studies. Despite its limitations, DEXA has become a well-established tool for the assessment of soft tissue composition and bone mineral in humans and animals.

**THE WISCONSIN DIETARY RESTRICTION STUDY**

Dietary restriction (DR), or reduced caloric intake without malnutrition, is the only intervention that has repeatedly and strikingly increased the maximum life span and retarded the rate of aging in laboratory rodents. The ability of DR to increase life span has also been shown in fish, spiders, water fleas, and other lower animals. Study into DR dates to the early 1900s. The first studies reported reduced tumor growth under conditions of severe DR. These were followed by numerous studies documenting DR’s effectiveness in reducing cancer and increasing life span in mice and rats. An ongoing phase of research, begun in 1970, shows that rodents on DR stay younger longer. Another phase of DR research concerns the mechanisms by which DR retards disease and aging in rodents. Finally, the prospective study of DR in primates has recently begun, with one such study underway at the Wisconsin Primate Research Center at the University of Wisconsin–Madison (UW study) and another at the National Institute on Aging (NIA study). The UW Study has three major aims:

- to advance the development of the rhesus monkey as a model for aging research,
- to determine the influence of DR on the rate of aging in this primate species, and finally
- to identify and validate biomarkers of aging in the rhesus monkey.

Approximately 165 rhesus monkeys, both males and females, are being used in the two primate DR studies. To date, results from these two studies are remarkably similar even given differences in experimental design. Two major differences between these two studies are in the age of the animals at the onset of DR and the way in which the reduced diet was calculated. The NIA study began with animals in three age groups: juvenile, adult and old. The juveniles were all clearly still growing and it is likely that the adult males (3 to 5 years old at onset of DR) were still growing as well. The UW study began with all fully adult animals (8 to 14 years of age at onset of DR). Because the NIA animals were still growing, total ad libitum dietary intake was based upon National Research Council Guidelines, while for the UW study, individual baseline intakes were determined for each animal. Both studies proceeded to impose a 30% restriction of ad libitum intake on the subjects.

Specifically, the UW study began in 1989 with a group of 30 adult male rhesus macaques (Macaca mulatta; Group 1). In 1994, a group of 30 adult females (Group 2) and an additional group of 16 adult males (Group 3) were added. Within each group, half of the animals were randomly assigned to the control group (allowed ad libitum access to food for 6 to 8 hours per day) and the remainder assigned to the DR group (70% of individual ad libitum food intake).

Animals are fed a pelleted, semi-purified diet (Teklad, Madison, WI) which contains 15% lactalbumin, 10% corn oil, and approximately 65% carbohydrate in the form of sugars and corn starch. The macronutrient content of the control and restricted diets is similar, but the restricted diet is supplemented with an additional 30% of the vitamin and mineral content to assure that the groups, on average, consume a similar amount of micronutrients. Food intake is measured daily for each animal.

In addition to daily food intake measurements, animals are weighed weekly, assessed for body composition and overall health semi-annually, and studied in a six-week battery of specialized tests yearly. Body
composition is measured by somatometric assessment (body lengths, trunk and limb circumferences, skinfold thicknesses) and DEXA (DPX-L, GE/Lunar, Madison, WI). Overall health checks include, among other measurements, serum chemistries and complete blood counts, blood pressure, physical examination, and dental examination. During the six-week annual assessment period, additional measurements include an electrocardiogram and echocardiographic examination of cardiac function, radiographic examination for osteoarthritis, energy expenditure by indirect calorimetry methods, physical activity, as well as glucose tolerance and insulin sensitivity testing. In addition, serum, plasma, and urine samples are banked twice yearly for assessment of among other analytes, reproductive hormones, lipid profiles, and biochemical markers of bone metabolism and skeletal relevance.

As predicted from rodent studies, monkeys on DR show rapid alterations in body composition. Body weight is significantly lower in restricted compared with control monkeys in all three groups. The DEXA data suggest (and are confirmed by somatometrics) that the difference in body weight between control and restricted animals is primarily attributable to differences in body fat.7, 8 Restricted animals have significantly less body fat compared with controls at all time points following restriction and a difference is seen between groups in longitudinal comparisons of rate of change in body fat over time. In all animals, abdominal fat mass accounted for ~50% of the difference in total body fat mass between groups. This difference in abdominal fat mass is clear in several somatometric variables as well.7,8 In addition, in Group 1, there has been an emerging difference in fat distribution between control and restricted animals in which the restricted group has a lower percent abdominal fat compared with controls.7 In light of the health problems associated with abdominal fat, this reduction can be seen as a positive health benefit of DR. In the near future we plan to begin acquiring CT images of the abdomen and mid-thigh for more direct measurement of visceral fat mass and fat content of skeletal muscle, respectively. In Group 1 animals show an additional treatment difference in lean body mass due primarily to an increase in the control group.7,9 In contrast to Group 1, in Groups 2 and 3 lean body mass differences to this point have been very slight. In all groups, DEXA-measured bone mass is lower in DR compared with control animals. We believe this lower bone mass is expected as a direct consequence of lower body weight, i.e., less mechanical load.

**SUMMARY**

Body composition is of interest for many reasons including its important direct relationship with overall health. Many techniques have been established to assess body composition based upon both the original basic two-compartment model as well as the more complex multicompartment models. Our study of DR in rhesus macaques has shown that an approximately 30% DR can be safely maintained long-term in both male and female rhesus macaques, and that restricted animals have favorable changes in body composition and improved health compared with controls.

**REFERENCES**


Food restriction is the only nutritional intervention that has been shown effective in delaying morbidity and mortality. This has been documented in numerous species, now including dogs.1-5 One might question whether it is food restriction that is the dietary intervention, or if overfeeding is the dietary intervention. After all, obesity is a disease of domestication. Abundant availability of food and reduced activity are hallmarks of modern life and are risk factors for obesity. Rodents kept in cages and provided with food ad libitum, which is typical of “control” groups in food-restriction studies, hardly reflect rats or mice in a “natural” environment. Nevertheless, it is not unlike life for humans and pets in developed countries. While the semantics and implications of this point could be argued several ways, the present discussion begins with the acceptance that lean body condition promotes longevity whereas excess body fat is associated with enhanced morbidity and reduced longevity.

WHEN SHOULD CALORIE RESTRICTION BE PRACTICED?

According to the recent canine findings, even a mild degree of excess body fat can be a risk factor for disease and early mortality.2 The quantitative benefit of calorie restriction, e.g., enhanced life span, is greatest when started early in life,3 but calorie restriction initiated in adulthood provides significant benefits as well.3,6 Similar controlled studies initiated in adulthood are not available for dogs, but the evidence that does exist suggests a similar pattern. Dogs that were obese at one year of age had a greater risk for developing mammary cancer later in life.8,9 Puppies overfed during growth have a greater likelihood of developing hip dysplasia or other orthopedic diseases.10,11 Restricting calories, along with limited exercise, to achieve weight loss in overweight, lame dogs resulted in enhanced mobility.12 Thus, while benefits can be derived from controlling body condition throughout life, it is believed that achieving and maintaining lean body condition can be of benefit at any time.

WHEN SHOULD CALORIE RESTRICTION BE AVOIDED?

The studies that have demonstrated a health benefit to calorie restriction mostly involved select strains of rodents. The sole canine study involved a single breed of dog, the Labrador retriever, which is an obese-prone breed. While most rodent studies have shown beneficial results from calorie restriction, at least one study showed a difference based on genetic strain. Mean and maximum life spans were extended by food restriction in male B6CBAF1 hybrid mice and in genetically obese (ob/ob) B6 mice, but the exact opposite effect was observed when male B6(C57BL/6J) mice were food restricted.1 Whether or not such strain or breed differences may be observed in other species remains to be seen.
While postweaning calorie restriction generally provides health benefits, preweaning and in utero nutrient restriction appears to have the opposite effect. There is now considerable evidence that nutritional restriction during reproduction can induce health risks in the offspring that may not be evident until months to years later. Human infants and rodents that are calorie or protein restricted in utero or as neonates experience shortened life spans or have increased risk for developing diabetes mellitus or cardiovascular disease later in life. Puppies whose dams were protein restricted during gestation showed delayed evidence of compromised immune function during adolescence. In separate research with mice it was documented that calorie restriction enhanced, but protein restriction compromised, survival.

From these studies, it can be seen that calorie restriction should not extend to restriction of other nutrients, and that calorie restriction should not be practiced during reproduction or before weaning. Whether this is appropriate for all breeds of dogs remains to be seen, yet obesity appears to be a major health risk in all breeds and species evaluated to date. Thus, restriction of calories to minimize obesity seems a reasonable goal.

**HOW SHOULD CALORIE RESTRICTION BE APPLIED TO PET DOGS?**

One of the issues with applying calorie restriction outside the research laboratory is the question, "restriction from what?". The maintenance energy requirements (MER) of individual dogs vary greatly. Even among adult dogs of similar breed, age, gender, activity level, and body condition, MER can vary by 50% or more. Differences among breeds can further increase this variation. In addition, total energy needs of healthy dogs can be increased by activity, reproduction, growth, and cold environments or decreased by neutering and age. Further complicating the issue is the physiologic response to varying energy intake: basal metabolic rate, heat increment, and intestinal nutrient uptake associated with food consumption will increase or decrease in most species in response to excess or restricted energy intake.

Thus, for practical purposes, one cannot simply apply "calorie restriction" for individual dogs. Rather, one must feed individual dogs to whatever calorie intake is needed to maintain an ideal body condition. This may be accomplished by feeding initially using a calculated estimate of average MER (e.g., metabolizable energy/kg body weight = 110 · kg \(^0.75\)), and then adjusting based on the dog's response to that intake.

**WHAT CAN VETERINARIANS DO TO PROMOTE LEAN BODY CONDITION IN PETS?**

Dogs with an ideal body condition score (BCS) between 4 and 5 (using the Purina 9-point BCS system) lived 15% longer than dogs with a BCS between 6 and 7. In a recent survey involving 200 dogs and their owners, it was demonstrated that owners do not recognize their own dog as overweight. In that survey, the mean BCS determined by trained pet experts was 6.3 while the mean BCS determined by the dog owners was 5.3. About 27% of the owners underestimated BCS by 2 units: 2 units on this BCS system correlates to 20% to 30% excess body weight. In other research, it was shown that the primary way that dog owners recognized their pet as overweight was based on their veterinarian's assessment.

Thus, veterinarians should begin or continue to evaluate BCS on all patients, and to discuss with clients the importance of maintaining an ideal BCS. Veterinarians may want to provide illustrated BCS charts for their clients, or to post them within their clinic. A videotape teaching pet owners how to monitor and control BCS can be a valuable client education tool that practitioners may wish to provide.
Puppy owners should be taught how to assess BCS in their puppies, and advised to adjust food allowances to maintain a lean body condition while promoting a slow, healthy rate of weight gain. Neutering in both sexes is associated with a reduction in energy requirements. All pet owners should be advised to alter the feeding management of their pet following spay or castration.

To assure that all essential nutrients are provided despite energy control, it is important that the caloric density of the diet fed be appropriate to the energy needs of the individual pet. Those dogs with very low energy requirements should be fed products with an enhanced nutrient:calorie ratio, such as properly formulated “lite” or weight management diets. Dogs with high energy needs may benefit from performance or high calorie foods. Consideration must be given to calories provided from sources other than complete and balanced pet foods, to reduce the risk of nutrient dilution as well as calorie excess.

WHAT FURTHER RESEARCH IS NEEDED?

One of the questions that remains to be answered involves the relative roles of lean body mass (LBM) compared with fat mass. Does increasing LBM provide incremental health benefits? In the canine food restriction study, LBM decreased later in the food-restricted group coincident with delayed morbidity; and the decrease in LBM appeared to precede an increase in morbidity in both groups of dogs. It is not currently known whether the decrease in LBM is a marker of subclinical disease, a contributor to disease, or an unrelated event. Assuming a causal relationship, can disease be delayed further by nutritional enhancements to LBM, such as increased dietary protein and metabolically active amino acids? These areas require further investigation.

Do the canine, rodent, and primate findings apply to cats as well? Calorie restriction studies have not been completed in cats. However, studies have shown similar associations between excess body condition and increased morbidity in cats. Scarlett showed that obesity in middle-aged cats was associated with early mortality.26 Overweight cats also demonstrate compromised insulin sensitivity and have a fourfold increased risk for developing diabetes mellitus.25–27 Pending further research findings, it seems reasonable to feed cats to maintain a lean, healthy body condition. For cats of any age, a BCS of 5 (of 9) appears to be an appropriate target.

REFERENCES


Improved insulin sensitivity is a hallmark of a moderate calorie restriction (CR; 20% to 40% below ad libitum, AL) in many species, including mice, rats, nonhuman primates, and humans. In rat skeletal muscle, we have found that the effect of CR on glucose transport is specific to the insulin-stimulated pathway (i.e., neither basal glucose transport nor activation by in vitro hypoxia, an insulin-independent stimulus, is increased by CR). The increased insulin-stimulated glucose transport is attributable to an enhanced recruitment of the GLUT4 glucose transporter to the cell surface, without any increase in total GLUT4 abundance. In this context, we suspect that CR enhances insulin action secondary to an amplification of the insulin signaling pathway. Activation of phosphatidylinositol 3-kinase (PI3K) is essential for insulin-stimulated glucose transport under normal conditions, but we have found that brief CR (20 days) results in no significant change in insulin-stimulation of insulin receptor substrate1 (IRS-1)-, IRS-2-, or phosphotyrosine-PI3K compared with AL controls. However, by incubating muscles with PI3K inhibitor (wortmannin) we were able to completely eliminate the CR-induced increase in insulin-stimulated glucose transport. These findings led us to assess a key post-PI3K step in insulin signaling: Akt (also called protein kinase B). We found that CR did not alter abundance of the predominant isoforms expressed by skeletal muscle (Akt1 and Akt2). However, insulin stimulation of Akt phosphorylation was significantly higher in muscles from CR versus AL animals. In conclusion, these findings indicate that brief CR can enhance insulin action in skeletal muscle by a wortmannin-inhibitable and presumably PI3K-dependent mechanism which likely involves enhanced phosphorylation of Akt.
As rhesus monkeys age, a large number of them become obese and develop type 2 diabetes. We have shown that there is a step-wise decrease in insulin sensitivity (as assessed by whole-body insulin-mediated glucose disposal rates) as monkeys progress in time from young/normal to older/obese/hyperinsulinemic to old/obese/type 2 diabetic. In these monkeys, insulin sensitivity is significantly positively correlated to in vivo insulin action on skeletal muscle enzyme activity including (1) insulin receptor substrate-1 (IRS-1)–dependent phosphatidylinositol 3-kinase (PI3K) activity, (2) atypical (z/l/t) protein kinase C (PKC) activity, and (3) glycogen synthase fractional activity. Prevention of obesity by caloric restriction prevents the development of insulin resistance; calorie-restricted monkeys, although similar in age to the old type 2 diabetic monkeys, have normal insulin action to increase whole-body glucose disposal, normal IRS-1–dependent PI3K activity, and normal atypical PKC activity during a euglucemic hyperinsulinemic clamp. These findings suggest that decreased glucose disposal rate, and concomitantly decreased ability of in vivo insulin to activate IRS-1–dependent-PI3K and atypical PKC activity, are secondary to obesity. Interestingly, calorie-restricted monkeys have significantly higher basal skeletal muscle glycogen synthase activity compared with any of the ad libitum-fed groups. These findings suggest that protein kinase B (PKB) is not involved in the regulation of glycogen synthase in rhesus monkeys. In addition, calorie-restricted monkeys do not have normal insulin activation of skeletal muscle glycogen synthase during a euglycemic hyperinsulinemic clamp. Thus a defect in insulin activation of glycogen synthase is not likely to be secondary to obesity. We conclude that examining the skeletal muscle of calorie-restricted primates will provide invaluable information on insulin signaling pathways and the mechanisms by which calorie restriction improves insulin sensitivity.
Caloric restriction (CR) in laboratory mice significantly increases life span and prevents age-associated disease. GHR/GHBP-KO, "Laron" mice live significantly longer than normal (N) animals, but the mechanism that is responsible for delayed aging is unknown. We are studying the effects of CR on longevity of GHR-KO mice. Thirty percent CR was initiated at the age of approximately 2 months and caused the expected reduction in body weight (BW). In both N and KO animals food consumption expressed in terms of metabolic BW showed that CR significantly increased the efficiency of food utilization. At nine months of age plasma glucose levels were reduced by CR in both N and KO mice with a significant interaction between genotype and treatment ($P < 0.0003$). Insulin levels were also significantly reduced by CR in both N and KO animals with a significant interaction between genotype and treatment ($P < 0.0001$). As in previous studies, plasma insulin levels in KO mice were markedly reduced in comparison with levels measured in N animals ($0.128 \pm 0.013$ vs. $0.225 \pm 0.028$ ng/mL). Insulin levels in N-CR and KO ad libitum (AL) groups were indistinguishable ($0.122 \pm 0.019$ vs. $0.128 \pm 0.013$ ng/mL). Plasma corticosterone levels were significantly elevated by CR in N mice, but not affected in KO animals. The results allow for comparison of the effects of CR in GHR-KO animals which are GH-resistant, insulin-like growth factor-1 (IGF-1)-deficient, hypoinsulinemic, and long-lived with the effects of the same treatment in normal mice from the same line. In both N and KO animals, CR improved feed efficiency and reduced plasma glucose and insulin levels. However, CR failed to alter plasma corticosterone levels in KO mice. It remains to be determined whether CR will affect longevity of GHR-KO animals.

Supported by the Illinois CFAR and NIH (AG19899).
Percentages of CD8 memory cells were examined as a function of age in a group of 46 Labrador retrievers. At birth dogs were divided into age- and sex-matched pairs and, from age 8 weeks, restricted dogs received 75% of the total calories consumed by their maintenance-fed pair-mates. Commencing at 4 years of age, immunologic parameters were examined annually and periodic lymphocyte samples were cryopreserved. Lymphocyte subset analysis (B, T, CD4, CD8) from 4 to 13 years revealed significant age-related decreases in percentages of CD4 cells and B cells, with restricted dogs demonstrating lower B-cell percentages. Age-related increases in CD8 and T-cell percentages were observed overall, but the rate of increase in T-cell percentages in restricted females was not significant. While it was not initially feasible to monitor canine memory cells, subsequent availability of a suitable CD44 reagent allowed us to examine immune memory as a function of diet and age using the cryopreserved cells. For each dog pair, samples from three “freeze” dates, spanning an age range of 4 to 13 years, were evaluated on the same test date. The percentages of CD8 memory cells (as defined by CD44 bright staining) increased markedly with age; diet restriction was found to be minimally beneficial in retarding this increase.
Moderate food, energy, and/or calorie restriction delays age-related immune dysfunction and prolongs life span in multiple animal models. The amount and type of dietary fatty acids can also profoundly affect life span. Marine-derived fish oils contain (n-3) fatty acids which have potent anti-inflammatory properties. We therefore examined the influence of food restriction (FR) (40% overall reduction in intake of all of the dietary components) combined with substitution of fish oil (FO) for corn oil (CO) (5%). Autoimmune-prone female (NZB x NZW)F(1) (B/W) mice, which develop fatal autoimmune renal disease, were used. The food-restricted/fish oil diet maximally extended median life span to 645 days (vs. 494 days for the food-restricted corn oil diet). Similarly, fish oil prolonged life span in the ad libitum (AL)-fed mice to 345 days (vs. 242 for the ad libitum/corn oil diet). Increased life span was partially associated with decreased body weight, decreased renal proinflammatory cytokine (interferon-g, interleukins-10 and –12, and tumor necrosis factor-a) mRNA levels, and lower nuclear factor kappa B (NF-kB). Reductions in NF-kB were preceded by enhanced superoxide dismutase, catalase, and glutathione peroxidase activities. We report here that CO/FR and FO/FR and to a lesser extent FO/AL offset disease-associated losses in Th-1 cytokine production, CD69 expression, and NF-kB activation in splenic T lymphocytes activated ex vivo. Similarly, CO/FR and FO/FR prevented the disease-dependent rise in Th-2 cytokine production ex vivo and CD69 expression in vivo. In summary, the T-lymphocyte phenotype in the old CO/FR and FO/FR groups was identical to that in the young disease-free mice. Taken together, the data suggest that both CO/FR and FO/FR increase life span, in part, by maintaining a youthful immune phenotype in autoimmune prone mice.

Supported by NIH Grants R01 AG14541 (to G.F.) and F32 AG05826 (to C.J.).
There is a general belief that, as cats age, energy requirements decrease, which is attributed to a decrease in activity. Old age–associated body weight (BW) losses often contradict this belief, however, suggesting that it might be more prudent to assess old cats individually for the best feeding practice. Data records were analyzed to investigate energy requirements of colony cats. Daily energy requirements of cats \((n = 138)\) from 1 to 15 years of age were calculated from the calorie intake required to maintain BW (± 3%) over a 4-week period. A significant \((P < 0.001)\) decline in energy requirements with age was observed and suggests a 20% lower energy requirements for cats over 7 years of age. A plot \((n = 444)\) of BW by age (1 to 20 years) shows a significant \((P > 0.001)\) BW decline with age. The decline primarily appears to affect cats after the age of 12, with an increased incidence of underweight cats (< 2 kg) in that age group. Cats showed a slow and progressive loss of BW starting at least two years prior to their natural death, irrespective of cause of death. Average BW losses from one to two years prior to death were over 6% and losses over 10% were seen during the year prior to death when the cause of death was cancer, chronic renal failure, or hyperthyroidism. These data suggest that assessment of energy requirements over a 4-week weight maintenance period may not be a suitable method of assessing energy requirements in old cats because BW declines slowly over longer periods of time. Cats appear to undergo two old age life stages: senior, from 7 years to approximately 12 years, and geriatric, over 12 years. The energy requirements for these two life stages may be different. Further work is needed to investigate the causes of general loss of BW in geriatric cats, which contributes to the decline of their quality of life.
To gain insight into the pathways by which caloric restriction (CR) slows aging, gene expression levels were assessed for each of 2352 genes in liver of 9-month-old CR and control mice. 352 genes were found to be significantly increased or decreased by CR. The distribution of affected genes among functional classes was similar to the distribution of genes within the test set. Surprisingly, a disruption or knockout of the gene for the growth hormone receptor (GHR-KO), which also produces life extension, had a much smaller effect on gene expression, with no more than 10 genes meeting the selection criterion. There was, however, an interaction between GHR-KO mutation and the CR diet: the effects of CR on gene expression were significantly lower in GHR-KO mice than in control mice. Of the 352 genes altered significantly by CR, 29 had shown a significant and parallel alteration in expression in a previous study of liver gene expression that compared mice of the long-lived Snell dwarf stock (dw/dw) to controls. These 29 genes, altered both by CR and in dwarf mice, provide a list of biochemical features common to both models of delayed aging, and thus merit confirmation and more detailed study.
A number of studies using a bonnet/mask technique have been published where estimation of resting energy expenditure (REE) is done in dogs. Since gas measurement can be profoundly influenced by inconsistent gas flow and mixing, we hypothesized that a mask method of indirect calorimetry could produce data significantly different from a flow controlled/equilibrated open flow cage system.

Methods and Materials. Twenty-five healthy dogs of various breeds, body size, and condition (>20 kg [n = 12], <20 kg [n = 13]) had REE measured on a single day by the same operators; cage studies preceded mask studies. Dogs were fasted for 12 hours prior to calorimetry; body weights were recorded on a calibrated scale to the nearest 0.1 kg. Daily variation (n = 9) was investigated using a cooperative 20-kg dog, body condition 2.5/5, maintained on a consistent measured energy intake at a stable body weight.

Masks were custom made and fitted to each dog with shapes suited to their muzzle physique. An attempt was made to provide consistent mask-face contact during measurements. Airtight cages (except for entry and exit portals) were made either from modified air flight cages or were specially constructed of Plexiglas. Cage “dead space” was reduced with styrofoam inserts. Room temperature was controlled to 18˚C. Initial gas flow was provided at a minimum of 750 mL/min/kg0.67 and was modified to achieve CO₂ change between 0.4 and 0.8%. Animal movement/cooperation during recordings were recorded; only recordings during quiet intervals were used.

Cage gas flow was determined with calibrated mass flow controllers and confirmed with nitrogen dilution. Mask flow was maintained at the same rate as cage flow (mass flow meter) immediately after cage measurements. Water absorbent was positioned in line before mass flow meters, CO₂ absorbent before the O₂ analyzer, in series following the CO₂ analyzer. CO₂ concentrations were determined with an infrared-based analyzer and O₂ concentrations with a disposable fuel cell. Gas analyzers were calibrated with pure nitrogen and a span gas (20.0% O₂, 5.0% CO₂) with guaranteed analysis before each session. After habituation to chamber confinement or mask (15 to 30 minutes for most), expiratory gases were continuously sampled and recorded every 6 seconds, for 30 minutes. A customized software program automated chamber and baseline samplings throughout recording sessions. Total energy expenditure was calculated using VO₂ and VCO₂ and the equation of DeWeir:

$$\text{TEE (kcal/day)} = \{[3.94 \times \text{VO}_2 \text{ (mL/min)}] + [1.10 \times \text{VCO}_2 \text{ (mL/min)}]\} \times 1.44$$

Respiratory quotient (RQ) = VCO₂/VO₂
Statistical Analysis. Since most data were non-gaussian (box and whisker plots), significant differences in REE, REE/kg, REE/kg^{0.67}, and RQ were evaluated with the Wilcoxon signed rank test (two-tailed); P values < 0.05 were considered significant. Interassay differences between methods were evaluated similarly. Bland-Altman plots were used to demonstrate data variation.

Results. Descriptive statistics of REE, REE/kg, and REE/kg^{0.67} showed lowest values for cage measurements in 15 dogs. Significantly lower REE, REE/kg, and REE/kg^{0.67} occurred in dogs <20 kg. Bland-Altman plots displayed discordant values at high REE. The interassay study yielded significant differences in REE, REE/kg, REE/kg^{0.67}, and RQ values; mask values were higher.

Conclusion. This study shows that mask collections can generate data significantly different from a calibrated open flow cage system in dogs ≤20 kg. In addition, interassay variability with the mask method may importantly confuse data interpretation when REE and RQ measurements are made on different days.

BIBLIOGRAPHY


The BODY CONDITION SYSTEM was developed at the Nestlé Purina Pet Care Center and has been validated as documented in the following publications:


Laflamme, D.P. Development and Validation of a Body Condition Score System for Dogs. Canine Practice July/August 1997; 22:10-15

Kealy, et. al. Effects of Diet Restriction on Life Span and Age-Related Changes in Dogs. JAVMA 2002;
Ribs visible on shorthaired cats; no palpable fat; severe abdominal tuck; lumbar vertebrae and wing of ilia easily palpated.

Shared characteristics of BCS 1 and 3.

Ribs easily palpable with minimal fat covering; lumbar vertebrae obvious; obvious waist behind ribs; minimal abdominal fat.

Shared characteristics of BCS 3 and 5.

Well-proportioned; observe waist behind ribs; ribs palpable with slight fat covering; abdominal fat pad minimal.

Shared characteristics of BCS 5 and 7.

Ribs not easily palpated with moderate fat covering; waist poorly discernible; obvious rounding of abdomen; moderate abdominal fat pad.

Shared characteristics of BCS 7 and 9.

Ribs not palpable under heavy fat cover; heavy fat deposits over lumbar area, face and limbs; distention of abdomen with no waist; extensive abdominal fat deposits.

The BODY CONDITION SYSTEM was developed at the Nestlé Purina Pet Care Center and has been validated as documented in the following publications:

Laflamme DP. Development and Validation of a Body Condition Score System for Cats: A Clinical Tool. Feline Practice 1997; 25:13-17


Call 1-800-222-VETS (8387), weekdays, 8:00 a.m. to 4:30 p.m. CT