Nestlé PURINA Nutrition Forum

Focus on Felines

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Preface and Dedication

The objective of the Nestlé Purina Nutrition Forum is to promote advances in veterinary nutrition related to dogs and cats. Our goal is to further the creation of new knowledge through quality research and encourage the sharing of that knowledge by providing suitable venues and programs to facilitate effective communication with a variety of audiences. By staying true to these objectives, this annual program has come to be regarded by many as one of the best meetings on veterinary nutrition.

This year, we have taken the opportunity provided by the Nestlé Purina Nutrition Forum to recognize something else that is often regarded as one of the best: the research duo of Dr. James G. Morris and Dr. Quinton R. Rogers. While true long-term collaborations are rare in the world of science, anyone who studies feline nutrition instantly recognizes the names Morris and Rogers. This duo has spent the better part of their careers working together, exploring the unique nutritional needs of cats. Outside of the pet food industry, these two were the first to seriously pursue studies in feline metabolism and nutrition. Beginning in the early 1970s, Dr. Morris set out to build a world-class program in feline nutrition. He enlisted Dr. Rogers to join him, and the rest, as they say, is history.

Over the next 30 or so years, significant advances were made in the field of nutrition. Concurrent with this, Drs. Morris and Rogers expanded our understanding of feline nutrition. When they began studying this field, little was known about the needs of cats. For example, when they started exploring the amino acid requirements of cats, neither the essentiality of, nor the quantitative requirement for, specific amino acids had been determined. Much had been extrapolated from other species, but the unique features of feline metabolism identified by Drs. Morris and Rogers and others showed that such extrapolation was often not appropriate.

Between the two of them, Drs. Morris and Rogers have shared the results of their research through hundreds of journal articles and research abstracts. Their research helped define minimum requirements for protein and essential amino acids, vitamins, and minerals for growing and adult cats, and they contributed to both the National Research Council and the Association of American Feed Control Officials nutritional guidelines for cats and dogs. In addition, Drs. Morris and Rogers played a key role in elucidating the link between feline dilated cardiomyopathy and taurine.

Now retired, Drs. Morris and Rogers leave a lasting legacy in the knowledge they built and in the students they trained. As well stated by a former colleague, the generation to follow them has giant footsteps to fill.

On behalf of Nestlé Purina PetCare, it is with great pleasure that we dedicate the 2007 Nestlé Purina Nutrition Forum to Dr. James G. Morris and Dr. Quinton R. Rogers.

Dottie Laflamme, DVM, PhD, DACVN
Nestlé Purina PetCare Research
Animals were originally classified into groups on the basis of comparative anatomic and physical traits. Because of anatomic resemblances, sometimes animals that eat similar diets were grouped together; however, animals that eat similar diets are often in divergent groups. Therefore, zoological grouping does not reflect diet. More modern systems use cladistic classification, which arranges organisms by their order of branching in an evolutionary tree, not by their morphologic similarity. Determination of cladistic relationships has been greatly facilitated by the application of molecular techniques.

The two most common companion animals belong to the order Carnivora in the families Felidae and Canidae. Although the term carnivore (derived from the Latin carne, which means “flesh,” and vorare, which means “devour”) is used to denote eating of animal tissue, all animals belonging to Carnivora are not carnivores. Some are herbivores or omnivores, and a number of strict carnivorous animals belong to families other than Carnivora. A strict carnivorous diet is high in protein, moderately high in fat, and very low in carbohydrates. This diet also contains the essential vitamins and minerals (if the skeleton is consumed) and fatty acids to provide a complete diet. One can debate what constitutes a strict dietary carnivore. Certainly, raptors and carnivorous fish, such as salmonids, belong in this category. Although cats share many of the same characteristics of these carnivores, cats can also utilize starch. It would appear that in the evolution of cats, this facility has been maintained, whereas it either has been lost or did not exist in raptors and carnivorous fish.

Although we have worked on feline nutrition for many years, we are still fascinated by the nutritional peculiarities of cats and how, in contrast to dogs or rats, they have modified their metabolism. This brief review concentrates on some of the nutritional peculiarities of cats and the mechanisms by which these adaptations have occurred.

**FOOD INTAKE**

The early studies of nutritional requirements of animals were restrained by lack of purified diets that contained defined quantities of nutrients. Whereas rats and dogs readily ate purified diets, it was difficult to induce cats to consume them. Nevertheless, deficiencies of certain vitamins and minerals were observed and studied in cats before the development or use of satisfactory purified diets. It was not until the 1950s that purified diets that supported near-normal growth were developed.

Another major obstacle during the early studies of feline nutrition was a lack of control of respiratory and other viral diseases in colony cats. This situation was especially acute in studies of kitten postweaning growth. The development of an effective panleukopenia vaccine decreased morbidity and mortality associated with this disease, but it was not until specific pathogen-free colonies were developed that controlled studies could be undertaken.

An additional stumbling block was the often finicky feeding behavior of cats. Besides being particularly sensitive to flavor, cats find the texture of the diet very important. Various research groups were able to improve consumption of purified diets by increasing the water content of the diet using gelatin or agar or formulating the diet as a mash. It has been consistently found in various laboratories that weanling kittens more readily adapt to purified diets than adult cats. However, we have induced adult cats that were not previously fed purified diets to accept purified diets by using pelleted or gel diets and gradually mixing the purified diet with a previously acceptable commercial diet over a period of a week or more (sometimes it takes several weeks). Even then, some adult cats will not eat enough of the purified diet to maintain their original weight, whereas others may eventually become overweight. Although proteins are neither selected nor avoided, amino acids, peptides, and nucleotides show positive palatability for cats.
PROTEIN AND AMINO ACIDS

A key difference between the nutritional needs of cats and omnivores (e.g., rats, dogs) is the quantitatively higher crude protein (CP) requirement of cats for maintenance and the higher requirement for arginine, sulfur amino acids, and aromatic amino acids. Cats also show a greater tolerance for excess CP and several essential amino acids and a lesser tolerance for glutamic acid than other animals. Other key qualitative differences between cats and omnivores in relation to protein include cats' requirement for taurine and niacin, which can be synthesized by most animals from cysteine and tryptophan, respectively.

Protein Requirement

The requirement of bioavailable CP for adult cats is about 160 g/kg diet, whereas the requirement for dogs is about half as much (80 g/kg diet) and the requirement for rats is less than one-third as much (50 g/kg diet). The high CP requirement of adult cats is reflected by the quantity of protein in all commercial diets formulated for the maintenance of cats, which for several decades has generally contained at least 280 to 300 g CP/kg diet; however, the CP requirement for growing kittens, rats, and puppies is 180, 150, and 180 g/kg diet, respectively. For cats, there is a small difference between maintenance and growth requirements due to a considerably higher CP requirement for maintenance, whereas dogs and rats have a higher CP requirement for growth component. Puppies have a high CP requirement for growth but a low CP requirement for maintenance, which is consistent with the growth rates of postweaning kittens, rats, and puppies of about 1.5% to 2%, 5% to 10%, and 2% to 5% of body weight/day, respectively. Thus, the increased need for CP by growing rats and puppies is due to the higher rate of growth in these species, which also results in a higher percentage of the dietary nitrogen being used for protein synthesis than for kittens.

The reason for the high CP requirement of adult cats for maintenance appears to be the metabolic profile of the nitrogen catabolic enzymes, those moving nitrogen into the liver for the urea cycle and those involved in synthesizing urea. These enzymes do not downregulate when cats are given low-protein diets, as occurs in omnivores and herbivores; therefore, cats cannot conserve nitrogen to the same extent as these species. Although some adaptation takes place for some of the essential amino acid catabolic enzymes in cats, the changes are minor (about 0.5- to 2-fold) when compared with rats (2- to 10-fold). In contrast, although cats do not adapt to low-protein diets, cats adapt well to diets containing medium to high protein. Adaptation is achieved by increasing flow through enzyme systems, including the urea cycle via substrate regulation, via allosteric regulation, and by increasing metabolic intermediates (e.g., ornithine in the urea cycle), all without the necessity of increasing enzyme activities. This lower ability of cats to conserve nitrogen results in a higher urinary obligatory nitrogen loss in adult cats fed a protein-free diet of 360 mg × kg body weight× day-1 compared to rats or dogs at 128 and 210 mg × kg body weight× day-1, respectively. Long-term food deprivation also causes a much higher urinary nitrogen loss in cats than it does in omnivores. This same lack of downregulation of nitrogen catabolic enzymes results in the protein-efficiency ratio and net protein utilization for the same proteins being much lower for kittens (about one-half or less) than for rats, which is another measurement that shows lower efficiency of utilization of protein for kittens than for rats or dogs.

Essential Amino Acids

When we began our work on the amino acid requirement of cats, neither the essentiality nor the requirement for dietary amino acids had been determined. We began by showing which amino acids were essential for the cat. As might be expected, because all animals studied (from single-cell animals to higher animals) had been shown to require eight amino acids—leucine, isoleucine, valine, methionine, threonine, phenylalanine, lysine, and tryptophan—we found these also essential for cats. Not surprisingly, we also found histidine and arginine to be essential. Most surprising were some of the clinical signs of the deficiencies that we observed. Arginine deficiency produced the most dramatic effect: When near-adult cats were food deprived overnight and fed a single meal of 4 to 11 g of an arginine-free diet, within 2 hours all cats exhibited emesis and lethargy; and shortly thereafter they also vocalized and exhibited frothing at the mouth, ataxia, emprosthotonos, and exposed claws. One cat, which had eaten 8 g of the arginine-free diet, showed bradypnea and cyanosis and died in apnea. These clinical signs were caused by severe hyperammonemia resulting from a lack of ornithine, an essential intermediate in the urea cycle, thus shutting down urea synthesis. Normally, under these conditions, ornithine is produced from dietary arginine via liver arginase. Acute short-term deficiencies of any one essential amino acid for a week or less (except arginine) resulted in no overt clinical signs except a gradual decrease in food intake and weight...
Amino Acid Intolerances

Other interesting differences in amino acid nutrition between cats and omnivores, such as the rat and chick, are the lower tolerances for glutamic acid and the higher tolerances for most of the essential amino acids except methionine. The upper limit for dietary glutamic acid for the kitten is about 5% to 6% of the energy.43 When more than 7% of energy from glutamic acid was given to kittens, occasional emesis occurred, and kittens given only their normal requirement of thiamine (4.4 mg thiamine/kg diet) also became thiamine deficient. With higher dietary thiamine or lower glutamic acid, the kittens grew normally and exhibited no observable clinical signs. Rats and chicks tolerate more than twice these concentrations of glutamic acid. Among the essential amino acids, the lowest tolerance is for methionine, which is about 1.5% of energy.14,44 An example of higher tolerance is that of branched-chain amino acids. A leucine–isoleucine and valine antagonism could not be shown in kittens unless isoleucine was limiting, and even then it was mild and transitory.35 Kittens tolerated 10% leucine without a depression in food intake or weight gain. Also, kittens chose the high-leucine diet even when isoleucine was limiting.46

The phenylalanine plus tyrosine requirement of cats is interesting in that only about a 7 g/kg diet is required for maximal growth,47 yet even in adult animals, at least twice this amount is required to produce enough eumelanin in black hair to maximize the black color.48

All of these differences in the nutrition and metabolism of cats versus omnivores can be explained on the basis of cats being strict carnivores and having evolved to eating small prey that is medium in fat and high in protein that contains less glutamic acid than that found in cereal proteins. The low tolerance of methionine that we have found in kittens fed purified diets may seem to contradict this evolutionary explanation; however, a diet of meat containing 65% of the energy from protein and 35% of the energy from fat and carbohydrates (assuming a bioavailability of sulfur amino acids of 85%) would provide the amount of energy right at this upper limit. It is known that cats eating high-protein, low-carbohydrate diets seldom, if ever, become obese. Perhaps it is the methionine tolerance that limits food intake in these animals.

Taurine

In 1975, Hayes and coworkers49 reported that feline central retinal degeneration is caused by taurine deficiency. Another highlight involving taurine in the nutrition of cats is the recognition...
in 1987 that taurine deficiency also results in dilated cardiomyopathy.\textsuperscript{50} Between these dates, other clinical signs of taurine deficiency were described, including reproductive and developmental problems and neurologic, osmoregulatory, and immunologic defects.\textsuperscript{8,52} The puzzling part of the finding of dilated cardiomyopathy in cats was that the cats were normally eating diets containing 1,200 to 1,400 mg taurine/kg dry matter when the requirement had been determined, using purified diets, to be 400 mg/kg.\textsuperscript{53} After much research, it was found that the cause of the higher requirement was the lower digestibility of protein or Maillard reaction products when most commercial diets (especially canned diets) were fed. These diets resulted in bacterial conjugated bile acid hydrolase\textsuperscript{54} activity in the ileum sufficient to cause the hydrolysis of taurocholic acid and the further destruction of taurine, thus interfering with the enterohepatic reutilization of taurocholic acid.\textsuperscript{35-39} Thus, it was not a problem of bioavailability in the sense of absorption of dietary taurine but in the efficiency of reutilization of taurocholic acid. This was supported by the use of dietary antibiotics, which resulted in a restoration of taurine homeostasis in cats given such a diet.\textsuperscript{54} Thus, there is no single requirement of taurine for cats but instead a variable requirement between 300 and 2,000 mg/kg diet, depending on the composition and nature of the diet and its processing.

**CARBOHYDRATE UTILIZATION**

Because a diet of animal tissue contains only low concentrations of carbohydrate (primarily glycogen), a question arises whether cats have the ability to utilize plant carbohydrates. Digestion studies on cats show that starch disappears from the gut and may be more highly digested by cats than dogs, even when uncooked.\textsuperscript{60,61} About four isoenzymes occur in mammalian liver that catalyze the formation of glucose-6-phosphate from fructose. The major hexokinase in most animals is hexokinase D or type IV, often referred to as glucokinase. Glucokinase is absent in the liver of cats,\textsuperscript{62-64} which is consistent with the low glucose loads cats experience from an all-animal tissue diet. Glucokinase is also absent from cat leukocytes but is present in dog leukocytes.\textsuperscript{55} Using molecular techniques, Hiskett et al\textsuperscript{66} found that the expression pattern of glucose-sensing proteins in feline liver differed from dogs, humans, and rodents but that pancreatic expression of these proteins in cats and other species was similar.

The absence of glucokinase in the liver of cats limits cats’ ability to handle high-glucose loads but does not pose a potential problem unless cats ingest a high-carbohydrate diet, such as may come from a non–all-animal-tissue diet. Even then, the normal eating behavior of cats results in the ingestion of a number of small meals, which would tend to smooth out the glucose load. Despite the absence of glucokinase, a number of enzymes related to glucose metabolism (hexokinase, fructokinase, pyruvate kinase, glucose-6-phosphate dehydrogenase, fructose-1, 6-bisphosphatase, glucose-6-phosphatase) are higher in feline liver than in canine liver.\textsuperscript{64}

High intakes of sucrose in cats result in fructosuria and fructosuria.\textsuperscript{67} This observation indicates that although fructokinase activity in the liver of cats is higher than in dogs, there is an impediment in the metabolism of fructose beyond fructose-1-phosphate. Normally, fructose-1-phosphate is catalyzed to dihydroxyacetone and glyceraldehyde by the enzyme fructose-1-phosphate aldolase, of which there are three isozymes of aldolase: A, B, and C. Aldolase B is expressed exclusively in the liver, kidney, and intestines. Aldolases mediate two other reactions besides the cleavage of fructose-1-phosphate: the cleavage of fructose 1,6-diphosphate and condensation of the triose phosphates, glyceraldehyde phosphate, and dihydroxyacetone phosphate to form fructose 1,6-diphosphate. Reduced cleavage of fructose-1-phosphate leads to its cellular accumulation and inhibition of fructokinase, causing accumulation of free fructose in the blood, which would explain the fructosuria.

The evidence suggests that aldolase B activity is probably low in feline liver, but apparently this has not been measured. In humans, hereditary fructose intolerance is caused by a deficiency of aldolase B,\textsuperscript{68} which has been identified as being due to mutations in the aldolase B gene.\textsuperscript{69,70} More than 25 enzyme-impairing mutations of the aldolase B enzyme have been identified.\textsuperscript{71} Therefore, it appears that there is a high probability that cats have an inactive aldolase B, which impedes the metabolism of fructose.

A consequence of the poor utilization of fructose by cats is the diarrhea and diuresis that follow ingestion of either aqueous solutions of sucrose or diets containing high amounts of sucrose. Providing only sucrose-containing solutions to cats can result in death. Although sucrose improves the physical texture of purified diets for cats (compared to starch and glucose, which produce more powdery diets), for the above reasons, the amount in the diet should be restricted.

**VITAMINS**

Although differences in the protein and amino acid metabolism of cats and omnivores may be anticipated from cats’
high-protein diet, major differences also occur in the vitamin requirement of cats and other animals. For many years, the vitamin A requirement of cats was set at a high level, principally because of the initial studies of feline nutrition by Patricia Scott and coworkers. This group found that kittens given casein diets developed retinal degeneration, and she proposed that retinal degeneration was a consequence of vitamin A deficiency. Subsequent to the discovery of the role of taurine in production of central retinal degeneration, the preformed vitamin A requirement of cats was shown to be similar to other mammals; however, it is in the utilization of the vitamin A precursor carotenoids that cats are different from most other animals, including dogs. Carotenoids require cleavage to retinal, the aldehyde form of vitamin A, and it has unequivocally proven that the major, if not the sole, pathway of beta-carotene cleavage to vitamin A is by oxidative cleavage of the central ethylenic bond of beta-carotene to yield two molecules of retinal. The enzyme undertaking this cleavage is beta,beta-carotene 15,15′-dioxygenase (previously known as beta-carotene 15,15′-dioxygenase), a cytosolic enzyme located in the duodenal mucosa and, to some extent, in the liver of animals undertaking the carotene conversion. Although the enzyme has been cloned from chickens and humans, to our knowledge no studies have been done with cats to determine whether the enzyme is present or, if present, what factors prevent its activity. The eccentric cleavage of beta-carotene resulting in the formation of apocarotenoids does not appear to be significant and is present only in in vitro systems in the absence of α-tocopherol. Until further information is available, it appears that the inability of cats to utilize carotenoids as precursors of vitamin A is due to lack of or very low activity of beta,beta-carotene 15,15′-dioxygenase in the intestines and liver.

Vitamin A plays a key role in the development (as retinoic acid) and maturation of tissues. In all species of animals, including cats, excessive dietary intakes of vitamin A produce pathologic changes in fetal and adult tissues. Animals that obtain their vitamin A from carotenoids have the ability to protect against excess vitamin A by downregulation of the enzyme that converts carotene to vitamin A. This step does not occur in cats, as all the vitamin A is absorbed from tissues that contain retinol and retinyl esters, which could increase the susceptibility of cats to vitamin A toxicity; however, kittens and adult cats can tolerate intakes of vitamin A that would induce toxicity in other species. The tolerance of cats appears to stem from a combination of two factors: the cat’s ability to sequester larger quantities of vitamin A in the liver with no apparent adverse effect and the form of circulating retinoids in plasma, which is predominantly retinyl stearate rather than retinol. In general, carnivores differ from humans, rats, and pigs, which have predominantly retinol in their plasma combined with retinol-binding protein. Retinyl esters only appear in plasma when these animals have excessive intakes. The liver of cats given high vitamin A diets contains concentrations of vitamin A in excess of those recorded in animals such as polar bears (another carnivore), an animal often cited as storing such large quantities of vitamin A in the liver that it is toxic when eaten by humans and dogs.

Most animals are independent of a dietary source of vitamin D through ultraviolet (UV) activation of 7-dehydrocholesterol in the skin; however, cats and dogs are unable to synthesize adequate vitamin D even when shaved and subjected to UV radiation. Cats and dogs synthesize 7-dehydrocholesterol, which is also a precursor of both vitamin D and cholesterol, but cat and dog skin contain only low concentrations, compared with animals that can undertake vitamin D synthesis. When cats are given an inhibitor of the enzyme that converts 7-dehydrocholesterol to cholesterol (7-dehydrocholesterol-Δ(reductase), the concentration of 7-dehydrocholesterol in the skin is elevated, and when cats are exposed to UV radiation, they synthesize vitamin D and have adequate concentrations of 25-hydroxyvitamin D in the plasma. Therefore, the peculiarity of cats in regard to vitamin D synthesis is high activity of the enzyme that depletes the precursor pool for synthesis, not that the enzymes of the synthetic pathway are absent.

Most animals are able to supply their needs for nicotinic acid by the metabolism of tryptophan in excess of that required for protein synthesis. Depending on the species, the molar yield of nicotinic acid from tryptophan is variable (about 30 to 40 mol tryptophan in rats; 60 mol tryptophan in humans). The catalytic pathway of tryptophan to nicotinic acid has an intermediate: α-amino-β-carboxymuconic-ε-semialdehyde, which can either proceed to nicotinic acid synthesis or be metabolized to acetyl coenzyme A (acetyl-CoA) and CO₂ by the enzyme picolinic carboxylase. The activity of picolinic carboxylase is so high in cats that virtually none of the intermediate is available for nicotinic acid synthesis but is metabolized to acetyl-CoA and CO₂, which renders niacin a dietary requirement. Therefore, although cats have the necessary pathway for nicotinic acid synthesis, the activity of an enzyme (picolinic carboxylase) is so high that it diverts the intermediate for synthesis along an al-
ternate pathway, a situation analogous to the cat’s inability to synthesize vitamin D. It has been speculated that because animal tissue is a good source of nicotinamides, there was no evolutionary pressure to maintain synthesis, and some of the intermediates have carcinogenic potential.

No other specific peculiarities in the cat’s vitamin requirement have been identified (with the possible exception of thiamine, which was discussed in the section on amino acids). Similarly, the propensity of cats to exhibit clinical signs of vitamin E deficiency is more a reflection of the diet than a difference of requirement.

Finally, to our knowledge, the essentiality of dietary inositol has not been tested in cats. Under specific conditions of high-saturated-fat diets (e.g., coconut oil), inositol is required by female gerbils to prevent fatty infiltration of the liver and intestines.81

**FATS AND ESSENTIAL FATTY ACIDS**

Fats play a significant role in the attractiveness of food for cats, as well as being an important source of energy. Cats exhibit a distinct preference for some animal fats over other fats (e.g., chicken fat is preferred over beef tallow, which in turn is preferred over butter fat). The latter ranking may be related to olfactory and flavor components in these fats or to the reported aversion of cats to short-chain fatty acids.

Cats, like other mammals, require preformed n-3 and n-6 long-chain essential fatty acids in their diet, as they are unable to introduce double bonds (desaturate) to precursor fatty acids beyond carbon 9. These long-chain essential fatty acids, through chain elongation and desaturation, result in families of highly active metabolic eicosanoids, such as prostaglandins, prosta-cyclines, leukotrienes, and thromboxanes.

There is a general consensus that cats, like other animals, require linoleate (an n-6 fatty acid) in the diet along with n-3 fatty acids, but the exact requirement of cats for long-chain fatty acids has not been well defined. For most animals, arachidonate (an n-6 fatty acid) is not essential in the diet, as the metabolic need for arachidonate can be met through chain elongation and desaturation of linoleate. There is also a general consensus that cats have a limited capacity to synthesize arachidonate,82,83 which is attributed to low desaturase activity of cat liver.84,85 Pawlosky and colleagues86 demonstrated that cats possess low Δ6-desaturase activity but are capable of limited synthesis. Arachidonate-free diets permit similar growth rates in kittens and reproductive success in males when they achieve adulthood as toms given diets containing arachidonate.87 The essentiality of arachidonate in the diet for multiple litters in queens is somewhat equivocal. Limited numbers of litters have been reported in queens receiving linoleate and no arachidonate in the diet.88 It has been suggested that some of the problems encountered with purified diets may be due to the balance of n-3 to n-6 fatty acids. In view of the above, the addition of a source of arachidonate in the diet of breeding queens is prudent.

Among dietary ingredients, animal fat is highly palatable; however, the cat has an aversion to medium-chain triglycerides.89

**MINERALS**

Although the quantitative requirement for mineral elements in the diet vary across mammalian species (often a function of relative growth rates), there is general concurrence regarding which elements are essential. Many minerals (e.g., iron, copper, zinc) are often involved at catalytic sites of the enzymes that occur across species, whereas elements such as sodium and potassium have common roles in osmoregulation and calcium and phosphorus have common roles in the skeleton.

Dietary selection based on minerals is relatively rare. Some notable exceptions are sodium and phosphorus in ruminants and several minerals by rats. Many herbivores exhibit a preference for sodium salts or sodium salt solutions that presumably have survival value, as most plants do not require sodium for growth, and hence vegetable material is low in sodium. In contrast to herbivores, cats show no preference or aversion to sodium salts. Even when severely depleted of sodium and given a choice of diets, cats do not correctly choose a diet containing adequate sodium over a sodium-deficient diet.90 It would appear that as animal tissue always contains adequate sodium, the redundant neural pathways required for detection of sodium either did not develop or have not been maintained in cats. Although the required concentration of calcium in the diet of growing kittens is much less than that for large breeds of dogs, it is similar to that for small breeds of dogs.

**CONCLUSIONS**

This review is intended to update our earlier reviews on nutritional peculiarities of cats82,83,91 to indicate how modification of the metabolism of cats has resulted in their distinct nutrient requirement. The high protein requirement of cats for maintenance is a consequence of the cat’s limited ability to downregulate aminotransferases of general nitrogen metabolism and the urea cycle enzymes, which is similar to that observed in other nonfelid carnivores. It is suggested that this
confers an advantage to cats because they are always prepared to ingest a high-protein meal.

Four nutrients plus arginine (which is not essential in the diet of several mammals, including humans) are essential in the diet of cats. Arginine and taurine synthesis are limited by low activities of enzymes in the synthetic pathway. The other three nutrients are vitamins, two of which (vitamin D and niacin) are required in the diet even though the pathways for their synthesis are present. For these two vitamins, degradation of intermediates by high activities of enzymes for alternate pathways results in no effective synthesis. The third vitamin (vitamin A) cannot be synthesized from precursor carotenoids because of an apparent lack of the monooxygenase enzyme required to cleave the carotenoids. Besides requiring the above nutrients in the diet, cats show greater sensitivity than omnivores to a number of compounds that occur in plants but not animal tissue. Fructose is an example of a plant carbohydrate that is poorly utilized, probably due to low activity of the aldolase B enzyme. Other compounds include benzoate, sulphydryl compounds from onions, aspirin, and so forth.14

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SCIENTIFIC PROGRAM: FOCUS ON FELINES

Advances in Knowledge about Feline Metabolism

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The high dietary protein requirement of cats is perhaps the most often cited attribute for classifying cats as carnivores. Although other nutritional peculiarities of cats support a carnivore designation, such as dietary requirements for preformed vitamin A, arachidonic acid, taurine, and niacin, protein requirement seems to receive the most attention. The high protein requirement applies to all stages of life for cats, with the maintenance requirement most outstanding compared with other species. The protein requirement to maintain adult cats is about twice that needed to maintain adult dogs (160 and 80 g protein/kg of diet, respectively). An even greater difference in maintenance protein requirement is observed between adult cats and rats. The requirement for a nutrient such as protein is the minimum intake or dietary concentration of a nutrient needed for an “optimal” response. The classically targeted response in kittens is maximal growth, whereas in adults, nitrogen balance or constant body weight is targeted. As noted more than 10 years ago by Morris and Rogers, these commonly evaluated responses may not be optimal for establishing protein requirement. In the case of the adult maintenance state, nitrogen balance may be achieved when protein reserves are depleted and therefore might not be optimal for long-term health. Greater dietary concentrations or intakes of protein may support a healthier lean mass, optimize immunologic response, or generally result in freedom from degenerative disease.

Thirty years ago, Rogers et al reported findings that indicated the metabolic basis of the high protein requirement of cats. The investigators determined enzyme activities in the livers of adult cats when given diets that were high (700 g/kg) and low (175 g/kg) in protein and when food was withheld for 5 days. They evaluated enzymes known in rats to be key for regulating amino acid and nitrogen metabolism, gluconeogenesis, and lipogenesis. In comparing results between cats and rats, the investigators made several seminal observations. Especially pertinent to the high protein requirement of cats was that dietary protein concentration and food withholding have, with few exceptions, little effect on urea cycle, transaminase, and “first-limiting” amino acid catabolic enzyme activities. They also found that activities of the enzymes in cats, irrespective of diet, were maintained at levels similar to those in rats given intermediate (400 to 500 g/kg) to high (800 to 900 g/kg) levels of dietary protein. These observations contrasted sharply with wide variations in enzyme activities (as much as 13-fold) observed in rats given similar dietary treatments. Compared with cats, enzymatic variations in rats were acutely and intuitively adaptive. The variations in rats were appropriate for sparing amino acids during food deprivation and intake of little dietary protein. The investigators concluded that cats cannot sufficiently downregulate expression of amino acid and amino nitrogen catabolic enzymes to survive on diets that would marginally meet the protein requirements of omnivores and herbivores.

AMINO ACIDS AND PROTEIN NITROGEN

In a strict sense, cats and other animals do not have a dietary requirement for protein; they require amino acid nitrogen, specific amino acids, and carbon skeletons of other amino acids that dietary protein provides. This can be well appreciated from a review of many nutritional studies of cats conducted by Morris and Rogers and others in which crystalline amino acid mixtures were substituted for dietary protein. Normal growth rate and maintenance of body weight are observed in these studies (i.e., when amino nitrogen and essential amino acids are sufficiently abundant and presented in correct proportions). Specific amino acids needed in the diet of cats, so-called indispensable or essential amino acids, were identified in studies using crystalline amino acids in place of intact protein. Two years after postulating a cause for the high protein requirement for cats, Rogers and Morris demonstrated that the 10 amino acids essential for growth in rats were also essential for growth in kittens. During the following decade, these and other investigators using crystalline amino acids determined the minimal dietary concentrations of essential amino acids needed by kittens for optimal growth.

Rogers and Morris found that dietary requirements for essential amino acids in kittens were similar to or only moderately greater (14% to 67%) than those in rat pups, with the
notable exception of taurine.7 The similarity of essential amino acid requirements between cats and rats indicated to the investigators that the high dietary protein requirement of kittens relates principally to a high requirement for amino nitrogen, not essential amino acids that protein provides. This realization was consistent with the 1977 observation that expression of liver urea cycle enzymes and transaminases in cats are not reduced in response to intake of diets low in protein. It was also consistent with results of the species comparisons. Differences between cats and rats in first-limiting amino acid catabolic enzyme activities were much less than differences between the species in enzyme activities of nitrogen metabolism.

Since it was proposed, limited hepatic enzymatic adaptability as a cause for high dietary protein requirement in cats generally has been accepted; however, confirming studies were lacking. In recent years, this issue has received renewed attention.

Using indirect calorimetry, Russell et al8 evaluated protein oxidation in cats consuming high (550 g/kg) and moderate (442 g/kg) levels of dietary protein. The investigators found that protein oxidation matched protein intake and concluded that adapting to varying protein concentrations was not a problem for cats. They also concluded that there must be another explanation for the high protein requirement of cats. The inconsistency of their conclusions with those posited 25 years earlier appears to reflect different interpretations of adaptability and the basis of dietary protein requirement.

With respect to adaptability, implicit in the kind of measurements conducted by Rogers et al9 was that cats have a limited ability to up- and downregulate enzyme mass per unit of liver mass. Enzyme activities were measured at presumed maximal velocity conditions, which generally indicate enzyme mass. Although not specifically stated, other means of regulation were not discounted by Rogers et al.9 As addressed by Rogers and Morris,7 cats should be able to modulate nitrogen metabolism and amino acid catabolism by changing liver mass and allosteric regulation of rate-controlling enzyme activities. Indeed, when cats consume high-protein diets, it has been observed that their livers become enlarged.10 Additionally, because of the nature of enzyme kinetics, enzyme activity varies with the availability of substrate. Enzymes in vivo function at rates much lower than their maximum, and their activities increase with substrate concentration. This behavior of enzymes, to some extent, should account for increasing nitrogen and amino acid metabolism with increasing dietary protein concentration in cats.

Green et al10 reported findings of protein oxidation in cats that were given a wider range of dietary protein concentrations than were given by Russell et al9 (511 to 77 g/kg versus 550 to 442 g/kg). Using indirect calorimetry, they also found that protein oxidation positively varied with dietary protein content; however, when cats consumed the lowest level of dietary protein, the ratio of protein oxidation to protein intake increased to above unity. This result indicated that net catabolism of body protein occurred at the lowest level of dietary protein, findings consistent with the protein requirement of cats being greater than those of dogs and rats. Net catabolism of body protein would not be observed in rats given the lowest level of dietary protein. The results were also consistent with earlier findings showing adaptability of protein oxidation in cats; however, it is important to note that they also supported limited adaptability to tolerate low levels of dietary protein in cats relative to dogs and rats. Observations of nitrogen balance in anorectic cats12 and in cats given protein-free diets13 have indicated that cats have a diminished and slow-reacting ability to conserve body protein relative to many other species. Limited regulation of nitrogen catabolic enzymes still appears to be a tenable cause for these observations.

A fixed and moderately high amino acid and nitrogen metabolism would seem detrimental to cats because of their inefficient use of dietary protein and poor conservation of body protein; however, these attributes are probably inconsequential to an animal ingesting food that does not vary greatly in protein content, such as in small mammals, reptiles, amphibians, and insects. Morris, Rogers, and others have suggested advantages to carnivores: Though cats naturally ingest a low-carbohydrate diet, glucose is made readily available for catching prey by a high rate of gluconeogenesis from amino acid catabolism. Also, after periods of food deprivation, ammonium produced after ingestion of prey is less likely to cause toxicity with a moderately high rather than downregulated nitrogen metabolism.

**CARBOHYDRATE METABOLISM**

Rogers et al5 found that activities of key regulatory gluconeogenic enzymes in cats were greater than those in rats fed high-protein diets. As with nitrogen-metabolizing enzymes, they also found that activities of the gluconeogenic enzymes were affected little by dietary protein concentration or carbohydrate concentration because sucrose and corn starch were reciprocally substituted for soy protein to vary dietary protein concentration. These findings were consistent with amino acid use for glucose production in cats during the fed
as well as food-deprived state. As the investigators noted, the all-meat diet of carnivores would contain little carbohydrate; therefore, use of amino acids for gluconeogenesis over oxidation would favor meeting obligate tissue needs for glucose.

Subsequent studies appear to confirm the lack of adaptability and substantial glucose production in cats as a result of amino acid catabolism. Cats given a high-protein (630 g/kg), low-carbohydrate (60 g/kg) diet were found to possess liver glycogen in amounts similar to those in rats given a high-protein diet; however, unlike rats, gluconeogenesis in liver slices of cats given a high-protein diet is not reduced by food deprivation. Studies on isolated hepatocytes of cats indicate that catabolic pathways for some amino acids, such as glycine, are uniquely directed more toward gluconeogenesis than oxidation. Such studies also indicate that as dietary protein concentration is increased, oxidation, not gluconeogenesis, becomes a more likely fate of amino acids in cat liver. A mechanism for this trend was not postulated. Hence, although maximal activities of the gluconeogenic enzymes are not observed to change with dietary protein concentration, adaptation in flux of amino acids away from glucose production in the liver may occur with increasing dietary protein concentration. This trend may prevent overproduction of glucose and facilitate glycemic regulation in animals consuming high-protein diets.

Concern about a rising prevalence of diabetes mellitus in cats in recent years has prompted the suggestion that protein concentration should be increased in feline dry diets even though the protein content of such diets is greater than that needed for maintenance of body weight and nitrogen balance. The basis of this suggestion seems to be that increasing dietary protein concentration will necessarily reduce intake of dietary carbohydrate and that intake of an “unnaturally high” amount of carbohydrate is detrimental to the “relatively glucose-intolerant” cat. If reduction of glucose load is beneficial, it is reasonable to consider that increasing dietary protein in exchange for carbohydrate will increase glucose entry from the liver, whereas glucose entry from the intestines is decreased. In humans, between 50 and 80 g of glucose is estimated to be derived from 100 g of dietary protein.

Nonetheless, relative to dietary carbohydrate, dietary protein probably contributes less to glucose entry, and glucose derived from dietary protein is probably less dependent on insulin for disposal. Findings of reduced need for exogenous insulin in diabetic cats given a high-protein, low-carbohydrate diet appear to support this. Also, in normal, healthy cats, plasma glucose and insulin concentrations are lower when meals of high-protein, low-carbohydrate diets are consumed than when low-protein, high-carbohydrate meals are consumed. This effect does not seem to be mediated by improvement of glucose disposal, although increased insulin sensitivity or effectiveness of glucose is promoting its own disposal.

A benefit of increasing dietary protein may be through reduction of risk for obesity. It is clear that obesity is a contributing factor to development of diabetes in cats. Recent findings of Hoenig et al indicate that consumption of high protein results in a greater heat increment in lean cats but not obese cats. This thermic effect of protein may be beneficial for obesity prevention by increasing energy expenditure and satiety. Dietary protein appears to be beneficial in obesity management through maintaining or reducing the loss of lean body mass, where glucose is mostly disposed, and facilitating loss of body fat, which positively affects insulin sensitivity.

**LIPID METABOLISM**

Activities of a few hepatic enzymes of lipogenesis were evaluated in the 1977 report of Rogers et al. As with the other enzymes studied, diet had little to no effect on enzyme activities in this study. This finding, along with findings of undetectable to low activities of malic enzyme and citrate cleavage enzyme, indicated that cats, relative to rats, have a limited capacity for de novo synthesis of fatty acids in liver. From this, the investigators inferred that cats have a limited capacity for lipogenesis in general.

To the author’s knowledge, little has been reported on lipogenesis in cats. In apparent agreement with the suggestion of Rogers et al, Ibrahim et al found undetectable fatty acid synthesis in livers of cats using an in vivo, deuterated water-labeling method; however, these investigators determined fatty acid synthesis in cats during weight loss, when substantial synthesis would not be expected. Rogers et al acknowledged that fatty acid synthesis may occur in tissues other than livers of cats. Richard et al determined fatty acid synthesis rates in liver and adipose slices of cats given a diet low in fat (80 g/kg), adequate in protein (300 g/kg), and presumably high in carbohydrate. They found that fatty acid synthesis in the liver preparations from cats was low when either glucose or acetate was used as substrate. In this way, cats seemed similar to ruminants. A much greater (about 20-fold) rate of fatty acid synthesis occurred in adipose tissue compared with liver tissue. Cats had an intermediate rate of adipose lipogenesis among species, lower than that in dogs but greater than that...
in rats and humans.

Adipose tissue in cats does appear to be capable of substantial fatty acid synthesis under the right conditions. In cats deficient in lipoprotein lipase (LPL) activity, body tissues have impaired access to circulating fatty acids and hyperlipidemia results. In these cats, fatty acids in subcutaneous adipose triacylglycerol are enriched in palmitic acid, the most abundant product of de novo fatty acid synthesis.26 Although LPL-deficient cats are generally lean, neutering causes accumulation of body fat mass in the cats to the point that overweight to obese body conditions are observed with ad libitum food intake.29 For this degree of body fat accretion to have developed, it is believed that an extraordinary fatty acid synthesis occurred in adipose tissue.

FUTURE DIRECTIONS

The research of Morris and Rogers,30–39 subsequent to their initial studies on the high protein requirement of cats, has revealed other unique metabolic and nutritional attributes of cats and demonstrated the complexity of establishing dietary protein requirements. For some amino acids, minimal dietary concentrations needed for optimal growth in cats were found to be too low for other body functions. For these amino acids, dietary requirements were based on more sensitive biomarkers than growth, such as prevention of cataracts in the case of histidine30 and minimizing urinary excretion of orotic acid in the case of arginine.31 For some amino acids, the dietary matrix or proportion of nutrients affected requirements. Tau- rine and lysine requirements were found to vary with dietary protein quality, quantity, and processing.32,33 Also, optimizing the coat color of cats was found to vary with absolute and relative amounts of dietary phenylalanine and tyrosine.34 As in other species, the requirement for methionine was found to depend on dietary cyst(e)ine concentration,35 phenylalanine on dietary tyrosine concentration,36 and arginine on dietary protein concentration.37 Unique to cats were discoveries that optimal growth is supported by a very wide range, in ratio, of dietary essential to nonessential amino acids38 and that requirements for many essential amino acids are reduced with increasing dietary protein content.39 These and other discoveries revealed that some aspects of control of food intake, palatability, and metabolism of protein and amino acids in cats cannot be directly extrapolated from other species.

In considering areas of future research, classical methods for determining protein and amino acid requirements have been mostly applied to the growth stage of cats. With the exception of lysine and sulfur amino acids, the reported amino acid maintenance requirements of cats are not based on dose responses to optimize a biomarker of adequacy.7 Maintenance requirements would be better defined with dose-response studies. Given that obesity is the most common nutritionally induced disease of cats and is associated with diabetes mellitus and immune dysfunction, it seems worthwhile to investigate whether maintenance protein and amino acid requirements should be based on minimizing obesity and diabetes risk and optimizing immune function.

For studies aimed at minimizing obesity risk, recently neutered (orchietomized or ovarietomized) young cats that have finished growing are probably good models. Such cats are representative of a large fraction of privately owned cats that will become overweight. Before neutering, cats are typically lean even when they are continuously presented with food. After neutering, an average increase in body weight of 25% to 30% is observed experimentally with continuous presentation of food.29 Body condition scoring of cats presented to veterinary clinics show that by middle age (~7 years old), more than one-third of neutered cats will be overweight or obese.40 The effect of neutering is so potent that postneutering weight gain is also observed in feral cats.41 Such cats expectedly would experience more exercise and less food abundance than privately owned neutered cats.

An optimal protein:carbohydrate ratio that reduces postneutering weight gain may facilitate owner efforts to prevent weight gain in their cats. Relative to carbohydrate, dietary protein is reputed to have a greater satiating potency, lower energy utilization efficiency, and, as shown recently in lean cats, may induce greater thermogenesis25; however, a few factors would make determination of an optimal protein:carbohydrate ratio difficult. Variation in palatability among test diets and amino acid composition of protein sources undoubtedly would be encountered, which in turn may mask an effect of changing protein:carbohydrate ratio. Burger and Smith42 experienced a similar problem while investigating protein requirement for maintenance. Because their low-protein diet was not universally accepted, the protein requirement was based on observations of selected cats. The greater satiating potency and thermogenesis and lower energy utilization efficiency of protein over carbohydrate might vary with amino acid composition. In demonstrating that cats accommodate maximal growth over a wide range of protein and amino acid concentrations, Taylor et al.43 found that adjustments in dietary concentrations of methionine and arginine affected food...
intake. Toxicity effects of high concentrations of the amino acids were suggested by the investigators.

The protein and amino acid requirements of overweight and obese cats should perhaps also be evaluated. These cats constitute a large fraction of privately owned cats, and corrective weight loss is difficult to achieve, even with diets formulated for weight loss. The obese condition is believed to increase free-radical production. Therefore, obese relative to lean cats may have a greater requirement for sulfur amino acids (cyst(e)ine, methionine). Cyst(e)ine that is derived directly from the diet or from methionine provides reducing equivalents to affect redox status and is a rate-limiting substrate for synthesis of glutathione, a major cellular antioxidant. The redox status of cells appears to have roles in modulating signal transduction, gene expression, and apoptosis. Also, methionine, which is consumed in cysteine and glutathione synthesis, is a methylation substrate, and as such has an important role in epigenic regulation. Recently, hypomethylation of DNA and associated proteins has been implicated in the cause of cancers and type-2 diabetes, diseases for which obesity increases the risk of their development in cats.

Dietary enrichment of leucine may also be beneficial in managing overweight and obese cats. Leucine is not metabolized extensively in the liver like other branched-chain amino acids; hence, it reaches skeletal muscle in direct proportion to dietary concentrations. In skeletal muscle, leucine is a regulator of initiation of protein synthesis, a fuel source, and a principal donor of nitrogen for alanine and glutamine produced by skeletal muscle. Additionally, leucine is believed to modulate insulin signaling and glucose use in skeletal muscle. These roles of leucine may be especially important in overweight cats with insulin resistance because skeletal muscle is both a major determinant of insulin sensitivity and a consumer of glucose. Obese humans have improved glucose and insulin responses to meals when dietary protein is substituted with carbohydrate. The mechanism of this benefit is presently unknown but could plausibly involve leucine from dietary protein.

Protein and amino acid requirements of gestation and lactation are based on analyses of diets that support accepted but arbitrarily defined levels of performance, such as birth weight, litter size, and lactational period weight loss in queens and weight gains in kittens. Breakpoints indicating the minimal dietary concentrations for optimal responses have not been determined. In the context of increasing recognition of nutrient programming and interest in the health of aged cats, it seems valid to ask whether dietary protein and amino acid requirements are optimized for longevity of offspring. The protein:carbohydrate ratio in the diet appears to have an effect on the body composition of queens during lactation. Queens given dry-type diets have less lactational weight loss than queens given canned diets. The higher carbohydrate content of dry relative to canned diets is suggested to reduce lactational loss through greater insulin-mediated inhibition of fat mobilization. Changes in glycemia and abundance of endocrine factors important to regulation of body composition in dams are believed to affect eventual body composition and the propensity for development of diabetes in offspring. To the author’s knowledge, this relationship and the potential mediating role of dietary protein:carbohydrate ratio has not been studied in cats.

As surveys of clinically presented cats indicate, the lifespan of privately owned cats is increasing, as many as one-third to one-half of such cats are older than 7 years old. Protein and amino acid requirements in aging cats may be unique. With increasing age, maintenance energy requirement in cats is reported to decrease by some but not all investigators. A tendency for protein digestibility to decrease with age is also observed. Because animals eat to meet their energy requirements, protein and amino acid intake will decrease with decreasing energy requirement. The recent findings of taurine deficiency in dogs exemplify how changing energy requirements may influence amino acid requirements. Dietary sulfur amino acid concentration must be increased in some diets to prevent taurine deficiency in dogs with low-maintenance energy requirement. Hence, protein and amino acid requirements may be increased in aged cats that consume less energy than younger cats. Appreciation of this appears to be the motivation in recent years for slight increases in the protein content of commercial diets for senior cats.

In old cats, incidence of obesity decreases while, as observed by some investigators, loss of lean mass increases. The loss of lean mass is presumably from reduction in skeletal muscle, as observed in aging rodents and humans. Skeletal muscle loss is believed by some to be the result of reduced postprandial protein synthesis and increased visceral amino acid extraction. Loss of muscle mass is a concern to human health providers because of associated declines in mobility and health. Stimulation of skeletal muscle protein synthesis by dispensable (nonessential) and indispensable amino acids (in particular, leucine) has prompted investigation of the use of amino acids to reduce age-related muscle loss. Similar investigations may be warranted in cats. Study of dietary ma-
Manipulation to reduce the loss of lean mass is not without precedent in cats. Cupp reported that loss of lean mass in senior (~8 to 12 years old) and geriatric (~>12 years old) cats can be altered by nutrient compositional changes unrelated to protein changes.

Aging, as with obesity, is associated with increased oxidative stress, impaired glucose tolerance, and diabetes. For this reason, aged cats may benefit from dietary sulfur amino acid enrichment, which, as described for obesity, might compensate for possible depletion of glutathione. Indeed, low intracellular glutathione concentrations in peripheral blood mononuclear cells are observed in elderly humans. Although several factors are suggested as causal, an inadequate supply of precursor amino acids could be responsible. Unfortunately, sensitive biomarkers of sulfur amino acid status have not been identified in cats. This is perhaps a worthwhile subject of future study.

The growing practice of clinical nutrition continues to foster interest in the use of varying dietary nutrient concentrations and profiles for treatment and prevention of disease. Metabolism is variably altered, and usage efficiency is variably decreased in disease states. The research activities of Morris and Rogers have provided a valuable foundation upon which to base dietary protein and amino acid manipulations for disease treatment. For some diseases, deficiencies in branch-chain amino acids, arginine, methionine, threonine, histidine, taurine, and normally dispensable amino acids are suggested; however, the need for modification of dietary amino acid profiles is often uncertain because amino acid requirements for a disease condition generally are not known and markers of amino acid deficiency used in the healthy state may not apply to the diseased state. Also, if preexisting malnutrition is not encountered, amino acid mobilization due to metabolic response to disease may be sufficient to meet short-term tissue needs. A worthwhile first step in assessing adequacy of amino acid profile in diseased cats might be kinetic measurements of plasma amino acids with parenteral or enteral feeding. Although difficult to achieve, measurements of intracellular amino acid concentrations would be of additional value for assessing adequacy. Evaluating the effectiveness of manipulating dietary amino acid profiles to correct a detected imbalance would be a necessary second step.

In recent years, hypothesized benefits of dietary enrichment in lysine and glutamine for treatment of disease have been investigated in cats. Interpretation of such studies is often complicated by the necessary experimental design choices. The benefit of an amino acid depends on the supplementation mode, timing, and matrix of administration. For example, symptoms of feline herpesvirus infection are reduced with lysine given alone in oral capsules but not when enriched in a diet. Differences in circulating levels of lysine and nutrient antagonism are suggested to account for the difference in effectiveness.

Glutamine was evaluated in cats using gastric instillation of a purified diet that contained glutamine and protein in the form of crystalline amino acids. Glutamine administered in this way was not protective of methotrexate-induced injury of intestinal epithelium. One might ask if the outcome would have been different if intact protein, protein-bound glutamine, or parenterally administered glutamine were used. The latter condition may be important to the outcome because compared with villus cells, intestinal crypts derive nutrients more from the intestinal arterial supply than from the lumen. When glutamine is listed as an ingredient in commercial diets, protein-bound glutamine is most likely being counted because the free amino acid is labile. Tracer studies on the rate of protein digestion indicate that amino acids in protein would be absorbed more slowly than crystalline amino acids. Hence, exposure time, the degree of intestinal metabolism, and the peak circulating levels of an amino acid depend on whether it is in free or bound form. These variables should be considered in amino acid manipulations intended for clinical applications.

SOME CHALLENGES

Studies on the use of protein and amino acid manipulations for clinical applications have additional complexities. Perhaps foremost of these is that patient populations are heterogeneous. Because of this, large numbers of patients are needed to identify inadequacies and evaluate the effects of manipulations. Another complexity is measuring end points quantitatively, such as wound healing and immune response. Making measurements in a clinical setting is often difficult or, at best, inconvenient and requires an ardent commitment of investigators.

Nitrogen balance largely has been relied on to estimate feline maintenance requirements for protein and amino acids. This measurement and many newer isotopic methods are heavily influenced by whole-body protein synthesis. In addition to being substrates of protein synthesis, dietary amino acids become energy sources (e.g., glutamine, glutamate, aspartate), precursors of compounds (e.g., creatine, glutathione,
nitric oxide), and cellular regulators. Optimizing these latter functions may require greater dietary amino acid concentrations than those needed to achieve nitrogen balance. A worthwhile direction of future research may be identification and use of biomarkers of amino acid adequacy that are more sensitive measures of amino acid requirement than body protein turnover. An example of this would be to measure glutathione levels in the liver as a means to optimize dietary sulfur amino acid requirement. Using sulfur amino acids for protein synthesis has a higher priority than glutathione synthesis.73 Liver glutathione content is important because it serves as a reservoir of cysteine that may be used for synthesis of glutathione and maintenance of redox status in other tissues.

Another challenge is predicting bioavailabilities of amino acids in diets prepared for cats. Recommended dietary amino acid concentrations are greater than the minimum amino acid concentrations used in defining dietary requirement. This is because the bioavailabilities of amino acids in ingredients are typically less than 100%, and processing has a variable effect of further decreasing bioavailabilities.74 Lysine is especially susceptible. Heat treatment of good-quality protein in the presence of moisture and reducing sugar has been shown to reduce lysine bioavailability in cats by more than 40%.33 Bioassays, rather than chemical assays, presently are more reliable for accurately determining amino acid bioavailabilities. This was recently demonstrated with several feline diets.77 Unfortunately, routine use of bioassays for the evaluation of diets is cost prohibitive. A major advance in this area would be the development of simple and rapid laboratory tests that reliably indicate amino acid bioavailabilities in feline diets and ingredients used in the diets.

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SCIENTIFIC PROGRAM: FOCUS ON FELINES

Trace Mineral Requirements in Cats: Challenging How We Define “Requirements”

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Despite advances in our knowledge of companion animal nutrition, very little information is available about trace mineral requirements in cats. Historically, it has been hypothesized that such requirements are greater during growth than in other life stages; however, research determining copper and zinc requirements in queens during gestation offers an interesting opportunity to examine this hypothesis and raises a larger, overarching question of how we define nutrient requirements.

COPPER

In 1983, Doong et al1 demonstrated that copper is an indispensable trace mineral in cats. On the basis of that study and copper requirements in rats, the National Research Council (NRC) proposed a copper requirement of 5 mg of copper/kg of diet for kittens for growth2; however, while testing diets in Association of American Feed Control Officials protocols, Morris and Rogers observed clinical signs compatible with copper deficiency in kittens born to queens consuming a number of different commercial diets with copper contents exceeding this recommendation (J. G. Morris and Q. R. Rogers, personal communication, 1994). Clinical signs included neonatal death, premature birth, hypochromotricia, and collagen abnormalities. These observations were the stimuli for further investigations concerning dietary copper requirements for gestation and copper metabolism in cats.

In a study using a depletion–repletion design, queens were fed a copper-deplete purified diet (0.8 mg copper/kg diet) for 4 months and then randomly allocated to one of three dietary treatment groups receiving copper (supplied as copper sulfate) at 4, 5.8, or 10.8 mg copper/kg diet.3,4 Dietary copper concentration had a significant effect on the time to conception ($P = 0.04$). There was also a negative, linear relationship between dietary copper ($x = \text{mg copper/kg diet}$) and mean time ($y = \text{days}$) for queens to conceive ($y = 43.38 - 2.87x; R^2 = 0.97$). Based on these findings, it was concluded that the NRC recommendations at the time the study was conducted (5 mg copper/kg diet) were marginal for reproduction in cats and that at least 5.8 mg copper/kg diet is necessary.

ZINC

Zinc is an essential trace element for all animals and plays a role in the function of more than 200 known enzymes. The ubiquitous distribution of zinc among cells and the fact that it is the most abundant intracellular trace element suggest that it has a very basic biologic role. Therefore, zinc is especially important during reproduction and fetal development. While evaluating the effects of zinc concentrations on reproductive efficiency in queens consuming copper-deplete and copper-replete purified diets, I observed a significant number of cleft palates and low birth weights in kittens born to queens consuming diets with low zinc concentrations (<21 mg zinc/kg).5 The amount and form of dietary copper had no influence on these findings. At the time this experiment was conducted, the NRC recommended a minimum of 15 mg zinc/kg diet in kittens fed diets containing a low quantity of compounds known to decrease zinc bioavailability (e.g., phytate, fiber).6 Our findings challenge that recommendation.

A subsequent study was undertaken with the objective of determining zinc requirements for queens during gestation (A. J. Fascetti, unpublished data, 2001). Queens were fed purified diets containing 5, 15, 25, or 50 mg zinc (provided as zinc sulfate)/kg diet. Reproductive performance in the queens consuming the lower zinc diets was extremely poor, and virtually all kittens were born dead or died within a day of parturition; in addition, most of the kittens had congenital abnormalities, such as cleft palates, clubfeet, or curled long bones. Queens consuming diets with the greater concentrations of zinc had higher conception rates and more live births, and their offspring had fewer congenital defects. These findings suggest that the NRC-recommended minimum requirement at the time (15 mg zinc/kg diet) was not adequate for reproduction.
CURRENT RECOMMENDATIONS

The NRC provides recommendations for growth, maintenance, and gestation/lactation for many of the required nutrients. Previous guidelines generally listed only a minimum requirement for each nutrient. Nutrient recommendations now span minimum requirements or adequate intakes, recommended allowances, and, where possible, safe upper limits. The current NRC-recommended allowance for queens during gestation and lactation is 8.8 mg copper/kg of diet. This is greater than the current recommended allowances for growing kittens, which are 8.4 mg copper/kg diet following weaning and 5.0 mg copper/kg diet for maintenance. The current NRC-recommended allowances for zinc in growing kittens is 75 mg zinc/kg diet following weaning and 74 mg zinc/kg diet for maintenance. These recommended allowances are greater than that for gestation/lactation, which is 60 mg zinc/kg diet. This recommendation for queens during gestation/lactation was based on findings from two studies, one that determined the nitrogen requirements for gestation/lactation and one that used the factorial calculation method for determining the zinc requirement for lactating queens.

CONCLUSION

The examples of copper and zinc requirements in the queen during gestation serve as interesting foils for the consideration of how nutrient requirements are determined. Traditionally, it has been suggested that growth is the most demanding life stage and that by determining the requirements for growth, one may subsequently apply the findings to other life stages. Certainly, the example of the copper requirement for feline growth versus that for gestation challenges this assumption. Although the story is not as complete with respect to zinc, this mineral may be more in line with previous assumptions that the requirements are greater for growth. Currently, there is spirited debate regarding how nutrient requirements are determined and how recommendations for minimal and adequate intake and recommended allowances can be made. All can agree, however, that more information on the basic requirements for each life stage is necessary to achieve this goal.

REFERENCES

Obesity is the most prevalent form of malnutrition in veterinary medicine. Surveys suggest that 25% to 30% of cats presented to veterinary clinics are overweight or obese. Obesity is defined as a pathologic condition characterized by an accumulation of fat in excess of that required for optimal body function. The significance of obesity pertains to its role in the pathogenesis of a variety of diseases and the ability to exacerbate preexisting disease. "Is my cat fat?" is a question that cat owners ask veterinarians daily. The ability to answer this question with objective data requires the ability to accurately measure body fat. Measurement of body fat also facilitates understanding the response to weight-reduction programs.

Numerous methods exist for the assessment of body composition; however, techniques such as densitometry, total body potassium measurement, and neutron activation analysis are not readily available. This discussion will focus on clinically relevant methods, such as body weight measurement, body condition scoring, morphometric measurements, dilutional techniques, bioelectrical impedance analysis (BIA), and dual-energy x-ray absorptiometry (DEXA).

Body weight can be subdivided into two or more physiologically distinct components. The traditional two-compartment model divides body weight into fat mass (FM) and fat-free mass (FFM). This model forms the basis of the majority of our current knowledge of body composition and depends on assumptions regarding the character of FM and FFM. The composition of FFM is assumed to be relatively constant, with a density of 1.1 g/mL at 37°C, a water content of 72% to 74%, and a potassium content of 50 to 70 mmol/kg. In addition, the major constituents of FFM are presumed to be present in fixed ratios. In comparison, FM is relatively homogenous in composition, anhydrous, and potassium free, with a density of 0.900 g/mL at 37°C.

The assessment of body composition in the form of FM and FFM provides valuable information about the physical and metabolic status of the individual. FM can be considered to represent a calorie or energy storage depot, whereas FFM represents the actual health of the animal. FFM is a heterogeneous entity consisting predominantly of intracellular fluid (ICF) and extracellular fluid (ECF), minerals, glycogen, and protein. FFM contains body cell mass (BCM), which is the metabolically active part of the body responsible for determining most of the resting energy expenditure. BCM encompasses those lean tissues most likely to be affected by nutrition or disease over relatively short periods. Furthermore, FFM is generally accepted as an index of protein nutrition, and therefore changes in FFM over time are assumed to represent alterations in protein balance.

**BODY WEIGHT MEASUREMENT**

Body weight measurement is the simplest technique, and it should be included in the examination of every cat. It provides a rough measure of total-body energy stores and changes in weight-parallel energy and protein balance. In healthy cats, body weight varies little from day to day. There can be wide variations between scales, however. To avoid interscale variation, the same scale should be used each time for an individual cat. In addition, it is preferable to use a pediatric scale and to routinely calibrate the scale to maintain accuracy.

It is important to note that a measurement of body weight by itself has little meaning. For instance, knowing that a Maine coon weighs 18 lb means little because the cat could be overweight, underweight, or in ideal body condition. In addition, body weight can be falsely altered by dehydration or fluid accumulation. Therefore, body weight should not be used in isolation.

**BODY CONDITION SCORING**

Body condition scoring provides a quick and subjective assessment of an animal’s overall body condition. The two most commonly used scoring systems in small animal practice are a 5-point system, where a body condition score (BCS) of 3 is considered ideal, or a 9-point system, where a BCS of 4 to 5 is considered ideal. BCS in conjunction with body weight gives clinicians a more complete perspective on a patient’s body condition and should be recorded in the medical record at every visit. Limitations of body condition scoring include the subjectivity inherent in the scoring system and...
interobserver variation. Finally, like body weight, BCS gives an overall assessment of body condition; it cannot differentiate between body compartments and does not provide any precise quantitative information concerning alteration in FFM or lean body mass relative to FM.

**MORPHOMETRIC MEASUREMENTS**

Height and circumferential measurements of the abdomen, hip, thigh, and upper arm are commonly used to estimate percent body fat in humans. Circumferential measurements have also been developed to estimate the percent body fat in cats.6 The Feline Body Mass Index™ is determined by measuring the rib cage circumference at the level of the ninth cranial rib and the leg index measurement (LIM), which is the distance from the patella to the calcaneal tuber. The percent body fat can be calculated using a simplified equation, such as 1.5(rib cage – LIM) – 9, or determined by consulting a reference chart. Cats with more than 30% body fat are candidates for a weight-loss program. The Feline Body Mass Index is a very simple, yet objective, tool for determining a cat’s body fat content. In addition, it is particularly valuable for convincing clients that their cat is indeed overweight and in need of weight loss.

**DILUTIONAL TECHNIQUES**

**Total Body Water**

Dilutional techniques rely on the principle of \( C_1V_1 = C_2V_2 \); that is, the volume of a biologic fluid can be calculated following the administration and equilibration of a known concentration of tracer. The total body water (TBW) method relies on the assumption that fat has negligible water content and FFM has fairly constant and known water content (73%). FFM can be calculated as TBW/0.73. Because body weight = Fat + FFM, an estimation of body composition can be made.

Isotopes of hydrogen (deuterium oxide \( \text{D}_2\text{O} \) and tritium oxide \( \text{H}_2\text{O}^{18} \)), urea, alcohol, \( N \)-acetyl-4-aminopyrine, and \( \text{H}_2\text{O}^{18} \) distribute in the TBW compartment and have been employed to quantify TBW. The approach used in most laboratories is dilution of the stable isotopes \( \text{D}_2\text{O} \) or \( \text{H}_2\text{O}^{18} \). These techniques have been successfully completed in cats7 and are appropriate for noninvasive studies; however, they do require expensive analytical equipment. Deuterium and tritium undergo some exchange with nonaqueous \( \text{H}^+ \), and hence can overestimate TBW by 3% to 5%. Similarly, \( \text{H}_2\text{O}^{18} \) will exchange with labile oxygen atoms, and hence can overestimate TBW by 0% to 1%. Consideration also needs to be given to urinary and respiratory losses of isotope. TBW can be measured with a precision and accuracy of 1% to 2%. The potential concern with this technique is the assumption of hydration factor, which may change with age, sex, species, breed, or disease.8

**Extracellular Fluid**

ECF is an important physiologic component of TBW that may be altered in illness. ECF can be measured by use of compounds such as inulin, \( ^{35}\text{S}_2\text{O}_3^- \), \( ^{35}\text{SO}_4^{2-} \), SCN-, Br-, and \( ^{82}\text{Br}^- \) that distribute within the extracellular space; however, ECF markers may not distribute uniformly in the subcompartments of the ECF (plasma, interstitium, lymph, connective tissue), some markers penetrate cells to an extent that cannot be precisely determined, or the markers may bind to some degree to endogenous components. Bromide is the most useful, safe, and widely used tracer for determination of extracellular water (ECW) volume. Determination requires high-performance liquid chromatography, and correction factors can be applied to account for the Gibbs-Donnan equilibrium, serum water, and distribution in nonextracellular sites. Simultaneous measurement of ECF and TBW enables the estimation of intracellular water (ICW) volume (i.e., ICW = TBW – ECW). ICW volume most closely approximates BCM.

**BIOELECTRICAL IMPEDANCE ANALYSIS**

BIA is an electrical method of assessing body composition that has the potential of quantifying TBW, ECW, ICW, BCM, FFM, and FM. Electrical conductance is used to calculate the composition of the body by measuring the nature of the conductance of an applied electrical current in the patient. Body fluids and electrolytes are responsible for conductance, whereas cell membranes produce capacitance. Because adipose tissue is less hydrated than lean body tissues, more adipose tissue results in a smaller conducting volume or path for current and larger impedance to current passage. FFM contains virtually all the water in the body. Thus, if bioelectrical impedance is measured, a value for FFM can be determined.

Two types of BIA systems are currently available: single frequency, which applies a 50 kHz current, and multifrequency, which uses frequencies from 5 kHz to 1,000 KHz. A BIA test is performed by placing four small electrodes on the body. The electrical current is introduced into the patient from the distal electrodes; it then travels through the body and is detected by the proximal electrodes. Low frequencies (e.g., 5 kHz) pass primarily through the ECW because of high cell
membrane capacitance. In contrast, at higher frequencies the effect of cell membrane capacitance is diminished so the current flows through both the ICF and ECF environments (or TBW). The proportion of the current in the ICF and ECF is frequency dependent.

Reliable BIA requires standardization and control of these variables, such as hydration status; consumption of food and water; skin and air temperature; recent physical activity; conductance of the examination table; patient age, size, shape, and posture; and electrode positioning. However, BIA has been shown to be a safe, noninvasive, rapid, portable, and reproducible method for estimating body composition in healthy cats. Calculation of ECF–ICF takes approximately 1 minute; hence, BIA provides virtually instantaneous online information of body composition that has never before been available.

**DUAL-ENERGY X-RAY ABSORPTIOMETRY**

DEXA is a technique originally developed for precise measurement of bone mineral content; however, it is now also used to measure both body fat and nonbone lean tissue. DEXA uses photons of two different energy levels (70 and 140 kVp) to distinguish the type and amount of tissue scanned. The x-ray source is positioned underneath the table supporting the patient, with the detector housed in an arm above the patient. During a scan, the source and detector move together over the patient. The detector measures the amount of x-rays that pass through the subject. The x-rays of the two different energy levels are impeded differently by bone mineral, lipid, and lean tissue. Algorithms are used to calculate both the quantity and type of tissue in each pixel scanned. DEXA calculates bone mineral density, bone mineral content, FM, and lean body mass.

DEXA’s low coefficient of variation for measuring bone mineral content (~1%) makes it a very precise technique. DEXA is also safe and quick, requiring 10 to 30 minutes for a whole-body scan. Similar to other body composition techniques, DEXA relies on the assumption that lean body mass is uniformly hydrated at 0.73 mL water/g.

**SUMMARY**

With the advent of technology and application of clinically relevant techniques, veterinarians can offer an objective answer to the question, “Is my cat fat?” The ability to accurately measure body fat and FFM (lean body mass) is vital for understanding the causes and effects of obesity. In addition, these techniques allow critical appraisal of the effect of nutrient composition on body composition.

**REFERENCES**

Adipokines and Their Importance in Obese Cats

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Obesity is a chronic disease that is growing at an alarming rate in both humans and their feline companions. Obesity increases the risk for a number of metabolic abnormalities in humans and cats, including insulin resistance, type 2 diabetes mellitus (T2DM), hypertension, and dyslipidemia. Obesity has been regarded as a simple energy imbalance, with energy intake exceeding energy expenditure; however, this view has changed over the last 10 years based on a greater understanding of adipose tissue and its function in normal states and disease. We now know that adipose tissue reserves are carefully maintained and regulated by complex systems that integrate food intake, substrate partitioning, and energy expenditure and can be influenced by environmental conditions and individual genetics. Obesity results from dysregulation of these complex systems and is a disease that is now recognized to have significant metabolic and health implications. Alterations in the secretion of adipokines, protein signals, and factors originating in adipose tissue underlie many of the abnormalities in obesity.

White adipose tissue has been viewed as a passive storage depot for excess energy in the form of triglycerides and a site of release of fatty acids when energy is needed. This view was partially held because adipose tissue appears to be a simple tissue composed primarily of large cells filled with triglyceride (~85% of content); however, white adipose tissue is now understood to be a complex tissue composed of multiple functional cell types, including mature adipocytes, preadipocytes, fibroblasts, endothelial cells, and macrophages, and the cell composition can change in response to various conditions. Functionally, white adipose tissue is an active endocrine and paracrine organ that secretes a wide array of mediators that participate in regulation of diverse metabolic processes, including food intake, energy expenditure, lipid and carbohydrate metabolism, angiogenesis, reproduction, vascular remodeling, blood pressure, and coagulation. A major advance in our understanding of adipose tissue was made with the discovery of leptin, a secreted signal from adipose tissue to the hypothalamus that signals the size of body fat stores as part of its wide range of biologic functions. In addition to leptin, many other adipokines have been discovered and are now in the process of being characterized.

Adipokines have important physiologic effects on multiple organs, including the brain, liver, muscle, adipose (paracrine effect) bone, reproductive organs, immune cells, and blood vessels; however, most adipokines are dysregulated in response to alterations of body fat mass, one of the best examples being obesity. Adipokine responses are also influenced by the distribution of body fat increase, as excessive fat is not only stored in adipose tissue, but is abnormally deposited in muscle and liver tissue as well. The majority of adipokines studied to date are hypersecreted in obesity, a notable exception being adiponectin, which declines. Adipokine alterations cause abnormalities in insulin action, glucose and fat metabolism, immune function, coagulation, and endothelial cell function, eventually leading to a proinflammatory state characterized by insulin resistance and altered immune function. Adipokine alterations appear to provide the link between obesity-related diseases as diverse as T2DM and cancer. The list of adipokines is ever increasing and includes leptin, adiponectin, resistin, retinol-binding protein 4 (RBP4), apelin, omentin, monocyte chemoattractant protein-1 (MCP-1), transforming growth factor-β, interleukin (IL)-1β, IL-6, IL-8, IL-10, macrophage migration inhibitory factor, haptoglobin, serum amyloid-A, nerve growth factor, adipin, plasminogen activator inhibitor-1 (PAI-1), fasting-induced adipose factor, metallothionein, angiotensinogen, complement C3, fibroinectin, and vascular endothelial growth factor. This review will attempt to highlight a few of the better-understood adipokines and their role in obesity.

LEPTIN

Leptin, a 16-kDa, cytokine-like protein, is encoded by the ob gene and is secreted primarily by adipose tissue in proportion to body fat stores and immediate nutritional state. Leptin is a pleiotropic hormone with multiple actions on the brain, pancreas, liver, immune system, and adipose. The importance of leptin was first investigated in loss-of-function
Adipokines and Their Importance in Obese Cats

Rodent models of obesity, such as ob/ob and db/db mice. Leptin is one of a few adipokines that has also been investigated in cats. Several studies have demonstrated that, similar to other species, plasma leptin concentrations are correlated with body fat content and concentration increase or decrease in response to weight gain or weight loss, respectively. 24–29 Although few studies have investigated the physiologic effects of leptin in cats, it is likely that leptin behaves similarly in cats as it does in other species.

Leptin is normally a signal of energy sufficiency that results in decreased food intake and increased energy expenditure, actions that are mediated primarily by central sympathetic activation. 30–33 Centrally, leptin interacts with hypothalamic pathways involved in energy regulation and acts to inhibit release of the orexigenic peptides, neuropeptide Y and agouti gene-related peptide, and increase release of the anorexigenic peptides, pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript. In peripheral tissue, leptin action is mediated in part by increased expression and activation of AMP-activated protein kinase (AMPK). 34,35 AMPK inhibits the enzyme acetyl-coenzyme A (CoA) carboxylase, leading to reduced levels of malonyl-CoA and increased entry of fatty acids into the mitochondria, where they undergo β-oxidation. Leptin may also stimulate fatty acid oxidation by activation of peroxisome proliferator-activated receptor-α (PPARα). 36 In liver, pancreatic islet, and adipose tissue, leptin inhibits the expression of sterol response element-binding protein-1c, resulting in inhibition of lipogenesis in these tissues. 37 Through these mechanisms and others, leptin causes enhanced glucose uptake in skeletal muscle due to increased translocation of glucose transporter 4 (GLUT 4). 49 These effects, which lead to decreased triglyceride content in tissue and enhanced insulin sensitivity, and its emerging antiinflammatory role make adiponectin one of the key candidate molecules mediating negative changes in obesity.

Resistin

Resistin is a 12-kDa peptide hormone expressed primarily by adipocytes in rodents and macrophages in humans. 50 Circulating concentrations of resistin are increased in rodent models of diet-induced and genetic obesity. 51 Based on its actions in rodents to increase blood glucose and insulin levels and to impair insulin function, resistin was believed to be a major link between obesity and insulin resistance. In addition, deletion of the resistin gene in ob/ob mice leads to improved glucose tolerance and insulin sensitivity through increased AMPK activity in liver and increased insulin-mediated glucose disposal in muscle and adipose tissue; 22 however, human resistin shares only 59% identity with murine resistin and is expressed primarily in macrophages, not in adipocytes. 53 In humans, there is no convincing link between patients with T2DM and coronary artery disease, and low adiponectin concentrations have been proposed as risk markers for these diseases. Adiponectin concentrations are decreased in obesity but can be increased by weight loss or treatment with thiazoladinediones. 46 Few data are available in cats regarding adiponectin, but plasma concentrations have been shown to correlate with body fat mass and to increase in response to weight loss. 29

In mice and humans, adiponectin has been shown to function as both an insulin-sensitizing factor and an antiinflammatory agent, antagonizing many of the negative effects of tumor necrosis factor (TNF)-α. 46 Overexpression or exogenous administration of adiponectin results in glucose lowering and amelioration of insulin resistance in murine models of obesity and diabetes. Adiponectin is the most abundantly secreted adipokine and circulates at high concentrations (1000-fold higher than most polypeptide hormones). 31 It is rarely found as a monomer and circulates in plasma as trimer, hexamer (low molecular weight form), or multimeric forms of 12 to 18 subunits (high molecular weight form). 47 The various forms appear to have different roles, with the high molecular weight form having a predominant action in the liver. Similar to leptin, adiponectin activates AMPK and increases fatty acid oxidation in skeletal muscle, liver, and other tissues. 37,46 It also causes enhanced glucose uptake in skeletal muscle due to increased translocation of glucose transporter 4 (GLUT 4). 49

Adiponectin, or adipocyte complement-related protein, is a 35-kDa protein that is almost exclusively expressed in white adipose tissue. 41,42 In humans, adiponectin levels are inversely correlated with body fat content, hepatic fat content, dyslipidemia, and insulin resistance. 43–46 Levels are very low in patients with T2DM and coronary artery disease, and low adiponectin concentrations have been proposed as risk markers for these diseases. Adiponectin concentrations are decreased in obesity but can be increased by weight loss or treatment with thiazoladinediones. 46 Few data are available in cats regarding adiponectin, but plasma concentrations have been shown to correlate with body fat mass and to increase in response to weight loss. 29

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plasma resistin concentrations and body fat stores or insulin resistance. Tissue sites and the role of resistin in obese and diabetic cats are unknown, but if these factors in cats are similar to those in rodents, they could contribute to obesity-related insulin resistance.

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ADIPOKINES AND THEIR IMPORTANT IN OBSE cats


The Nestlé Purina Nutrition Forum, honoring Drs. James Morris and Quinton Rogers, provides an opportunity to reflect not only on the legacy of their work and its contributions to animal health but also on the evolution of animal nutrition and medicine over the course of their careers. This presentation describes the evolution of approaches to the study of nutrient function over the past several decades, including whole animal nutrition, metabolism, and physiology; the molecular mechanisms of nutrient metabolism and regulation; and the study of these mechanisms in the context of living cells. The parallels between the fields of nutrition, pharmacology, and toxicology in the wake of new technologies for illuminating the mechanisms of action of nutrients and drugs at the cellular level is also described.

**WHOLE ANIMAL NUTRITION, METABOLISM, AND PHYSIOLOGY**

The legacy of Rogers’ and Morris’ collaboration results from their classic studies in whole animal nutrition, combined with a deep understanding of nutritional biochemistry and metabolism, to define the unique dietary requirements of cats.1–3 It is now widely appreciated that these requirements are consistent with the evolutionary influence of a strict carnivorous diet. The studies by Rogers and Morris of cats’ essential amino acid requirements not only defined the dietary requirements but also suggested that the extreme response of cats to arginine deficiency resulted from certain biochemical defects in the synthesis or transport (or both) of urea cycle intermediates. They showed that ornithine prevents the hyperammonemia of arginine deficiency but does not provide for normal growth and that citrulline is capable of substituting for arginine in the diet.4,5 With regard to essential fatty acids, cats fed diets lacking arachidionate have extremely low levels of arachidonic acid in plasma and erythrocyte lipids6 as a result of a low level of the first enzyme in the desaturation pathway.7 Linoleate prevents or ameliorates many, but not all, of the signs of essential fatty acid deficiency in cats.8–11 In particular, linoleate appears to supply the needs of male cats for reproduction and prevention of testis degeneration, but female cats require preformed arachidionate for successful pregnancies and normal litters.8,9

The role of arachidonic acid as an essential component of membrane phospholipids and as a precursor for the eicosanoids allowed us to formulate some hypotheses regarding the consequences of arachidonate deficiency in cats. Because thromboxane A₂, a potent thrombotic agent, is derived from arachidonic acid, we hypothesized that a deficiency of dietary arachidionate would affect platelet function. Consistent with this hypothesis, we showed that arachidionate is a requirement in cats for normal platelet aggregation.11 The central role of arachidionate in cellular function in mammals has subsequently been highlighted by the discoveries of various enzymes with a preference for arachidonoyl-containing substrates. Selective enzymes include the arachidonate-selective acyl-CoA synthetase, acyl-CoA thioesterase, and phospholipase A₂ as well as a membrane-bound arachidonoyl-specific diacylglycerol kinase. The latter enzyme functions to enrich cell membranes with arachidonic acid–containing phospholipids by selectively phosphorylating arachidonoyl-diacylglycerol. The arachidonate-acid–selective acyl-CoA synthetase is a hormone-dependent, obligatory protein in the signal transduction pathway of steroidogenic hormones.16

Many of these effects occur at the level of the whole cell and result in changes in cell motility, growth, adhesion, and other properties. Cells are complex systems in which a multitude of biochemical reactions and molecular events take place concurrently and need to be finely orchestrated to preserve cell homeostasis and direct cell-specific functions. For example, some of the effects of arachidonate deficiency may be related to the essential role of arachidonate in preserving cell membrane fluidity and architecture, including the interactions of proteins within lipid rafts. It has been shown that arachidonate is important for the maintenance of numerous cellular functions, including the functions of receptors in the...
membrane and nucleus of the cell. \(^{17}\) Finally, arachidonate and other polyunsaturated fatty acids directly modulate gene transcription by binding to nuclear transcription factors, including peroxisome proliferator-activated receptors (PPARs). \(^{18}\) Because cells are controlled by the physical interactions of these proteins, cell-based methods may provide an efficient way to understand how nutrients and other small molecules affect not just their primary targets but also individual or multiple biochemical pathways within cells.

**MOLECULAR MECHANISMS OF NUTRIENT METABOLISM AND REGULATION**

The field of molecular cell biology has evolved substantially over the past 10 years, enabling such studies on a large scale. Cellular screening techniques allow for the study of the effects of exogenous molecules (e.g., nutrients, drugs, toxins) in living cells. The ability to work with live cells at the level of individual proteins, including receptors, signaling proteins, and enzymes, opens the door to understanding the links between the biochemical function or metabolism of a nutrient and its role in health and disease. Specifically, the development of new technologies based on real-time imaging of fluorescence or luminescence has enabled the direct visualization and quantification of these events in real time.

Whole-cell assay technologies vary with respect to the assay principle but largely have in common a form of luminescence or fluorescence for detection. Luminescent, fluorescent, or bioluminescent signals are easily detected and quantified with a variety of automated or high-throughput instrumentation systems, including fluorescence multi-well plate readers, fluorescence activated cell sorters, and automated cell-based imaging systems that provide spatial resolution of the signal at the subcellular level. A variety of instrumentation systems have been developed to automate these measurements, allowing the accumulation of data on thousands of living cells arrayed in microtiter plates. If combined with a suitable assay system, the effects of any compound can be measured on a large scale at the level of any cellular process or pathway, whether in the membrane, cytosol, or nucleus of the cell, of any cell type. All that is needed is a universal quantitative method of monitoring the dynamic processes that occur at the level of the proteins, which are the targets of the compound (e.g., nutrient, drug) of interest.

Dr. Stephen Michnick and colleagues\(^ {19–21}\) at the University of Montreal proposed that cell-based measurements of protein–protein interactions could be used to monitor the dynamic association and dissociation of proteins, both to monitor the activity of a biochemical pathway in living cells and to directly study the effects of chemicals on the pathways. Michnick’s laboratory developed protein fragment complementation assays (PCAs), a general strategy for monitoring the dynamics of protein–protein interactions in vivo and in real time. PCAs enable fluorescent, real-time analysis of signaling events by measuring the association, dissociation, and movements of protein–protein complexes within cells. PCAs are created by expressing mammalian genes linked in frame to fragments of rationally dissected reporter genes. The association of two proteins of interest brings together complementary reporter fragments and enables productive folding of the fragments into an active structure that generates a fluorescent or luminescent signal. The resulting signals can be spatially localized and quantified in living cells using high-content imaging instrumentation.\(^ {22,23}\) We and others have recently applied these methods to de novo drug discovery in the identification of the mechanism of action of nutrients and hormones and in the discovery of new uses for known drugs.\(^ {24,25}\) These new technologies will aid in the design of in vivo studies to advance our understanding of the relationships between the biochemical functions of nutrients and drugs and their functions in health and disease.

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NEW TECHNOLOGIES FOR PHARMACEUTICAL AND NUTRITION RESEARCH


Diseases of advanced life have been viewed as chance events that potentially end life. This understanding is reflected in cataloging approaches to describing diseases of aging and recommending preventive or therapeutic responses. In a different view, aging phenotypes often are associated with complex underlying biology at genomic, cellular, organ, and systemic levels. Prolonged investment of metabolic energy at this level of complexity is not consistent with chance events that simply disrupt “natural aging.” Rather, perhaps late-life diseases should be regarded as integral to the aging process, with response programming that is plastic and adaptable.

Within this redirected frame of reference, numerous aspects of aging have been evaluated from a database consisting of postmortem findings from nearly 700 adult cats that were maintained for life as residents of the same colony from 1979 to 2001. Cats that died or were euthanized because of renal disease lived longer than those that died from other causes. The cats that died from renal disease had higher but uniform mean renal histologic scores across ages compared with cats that died from other causes.

Among cats that died from nonrenal causes but that had histologic renal changes, mean lifespan was longer than in cats without renal changes or renal causes of death. Cats that succumbed to nonrenal causes of death also were evaluated across categories of death-causing diseases. Specific problems did not underlie the difference in mean age at death between these two subgroups, underscoring the observation that the outcomes were not consequent to the structure and function of the colony. Additionally, the inbreeding coefficients in this colony were low.

Cats that succumbed to renal failure frequently had morphologic and preterminal metabolic changes, suggesting varying but substantial retained functional capacity. Although standard clinical chemistry is insensitive, the total body of data suggests also that other factors may direct transition to renal failure. Considering histological morphology, the usual sequelae of ischemia (cell swelling, karyolysis, lysosomal rupture, massive inflammation) are not commonly observed in cat kidneys or in a similar mouse model. In addition, the low frequency of acute renal lesions among a large number of cats that died from nonrenal causes suggests that a discrete initializing event, such as ischemic episode, infection, or toxicity, may not be a prominent feature of this process.

In a feline model of renal tubular disease, fibrosis was principally peritubular, suggesting that hypoxic sequelae result mainly from local compromise of diffusion. In a murine renal model, upregulated Fas (apoptosis-mediating surface antigen; APO1, CD95) in tubular epithelial cells was shown to bind to Fas ligand of adjacent tubular cells, suggesting that tubular loss is a signaled (fratricidal) apoptosis. Thus, the histologic nature of tubule loss in feline and murine models appears to be more compatible with an adaptive mechanism that operates at the cellular level. Frequent observation of tubulointerstitial changes in younger adults documents early onset, which also is compatible with defensive adaptation because symptomatic renal failure is much less frequent during early adulthood but very common during late adult life.

A specific underlying explanation for these observations is not obvious at present. Domestic cats are seasonally polycystous and multiparous, with declining fertility often initially detectable over the 84- to 96-month age range. Thus, the onset of tubulointerstitial changes that appear before this age may have evolved as one homeostatic response to help preserve a fertile reproductive lifespan by selective elimination of dysfunctional renal tubular cells and nephrons. Such an adaptation could, for example, increase the likelihood of successful reproduction through greater metabolic stability. A hypothesis that stress-response programming of this type evolved to modulate population survival is within the present scope of the debate about aging theories. However, this hypothesis is incomplete because it does not account for a long postreproductive lifespan. Indeed, a long postreproductive lifespan can occur even in simple organisms. Additionally, Mitteldorf suggests that longevity and fecundity may have evolved independently. This idea jeopardizes the older hypothesis that longevity and reproduction represent evolutionary trade-offs.
Another point to consider is that deterioration during more advanced stages of chronic renal failure often is accompanied by progression to increasingly severe cachexia, which signals a death trajectory. In studies of aging populations across species, death generally tends to be associated with more precipitous declines of body mass that are recognizable around the time that late-life mortality increases. However, factors that influence body composition before termination may not reflect only secondary, preterminal degenerative processes. The higher percentage of cats with kidney-related death and thin body condition suggests prior transition from more obese body condition in at least some subjects. This observation aligns well with typical clinical observations of an early onset of very gradual change in body mass in patients that eventually develop renal disease.

Aside from the role of body composition in the death trajectory, serial body composition dual-energy x-ray absorptiometry data from 119 cats over years of healthy adult life resulted in heritability and principal component outcomes indicating that multiple genes are involved in phenotypic expression of healthy body composition in cats. Two heritable principal components, PC2 ($h^2 = .40$, $P < .01$) and PC6 ($h^2 = .74$, $P < .01$), explained 24.7% and 1.3% of the population variance, respectively. The first principal component, PC1, which accounted for 55% of population variance ($h^2 = .33$), was less strongly significant ($P = .038$), possibly as a result of the number of variables tested. The observation that terminal body condition has a quantitative genetic component was unexpected and indicates a need for reevaluation of the underlying role of body composition in “diseases” of aging.

Interestingly, individual components of body composition related to each other only moderately in the principal component analysis, further suggesting that phenotypic expression of individual body composition components might result from multiple underlying genetic and epigenetic mechanisms. The specific genes involved in control of body composition and the relative contributions of those genes are presently unknown; slowly progressive, preterminal loss of body mass may reflect additional plastic genetic programming. In a renal context, death occurs as the apparent outcome of a pathologic process only at the point of systemic adaptive failure associated with very advanced nephron deletion or (probably more frequently in domestic cats) by extranephron, extrarenal, or extrinsic metabolic factors. Whether these putative events could result from threshold effects is not known at present, but this seems an attractive hypothesis.

Genetic evaluation did not reveal directly heritable components of renal tubulointerstitial phenotypes in this population, indicating that the phenotypes are either totally environmental in origin or that they reflect fixed traits (or both). It is critically important to recognize that aging, although reflecting conserved programming, also remains a highly plastic process that is subject to interactions with stress-response phenotypes that may be the actual fixed traits. Therefore, measured heritabilities should be modest at best and are not expected in the case of fixed traits. The possibility of modulating interactions between fixed alleles and epigenetic influences also is compatible with a working hypothesis that ultimately may recharacterize the role of overt disease in aging, centered around an emerging understanding of the role of genetic–epigenetic interactions.

The ultimate implications of these observations may involve altering approaches to intervention and prevention, probably with species and breed or strain specificity. It must be recognized that at least some components of long-term intrinsic disease (aging) processes likely represent life-preserving adaptations. Nonspecific attempts at entire abolition of these processes or use of specific interventions that are applied indiscriminately, universally, or based on insensitive measures may deprive the individual of selected (or convergent) protective mechanisms.

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Kidney disease is defined as the presence of functional or structural abnormalities in one or both kidneys; chronic kidney disease (CKD) is defined as kidney disease that exists for at least 3 months. CKD is the most common kidney disease in cats. As with most species, CKD is primarily a disease of older cats. One recent publication estimated the prevalence of CKD among cats of all ages to be 1.12 cases per 1,000 cats. Among cats 10 years of age and older, prevalence was estimated to be 269 cases per 1,000, while the prevalence in cats 15 years of age and older was 491 per 1,000. CKD appears to be two to three times more prevalent in cats than in dogs; the reason for this is unknown. Although CKD is an irreversible and progressive condition, most affected cats survive for several months to years, and many ultimately die of conditions other than CKD.

RECOGNITION AND STAGING
Although CKD may be recognized initially via physical examination, serum biochemistries, urinalysis, or imaging studies, it is most commonly detected as reduced renal function (azotemia). Differentiating renal azotemia from prerenal azotemia is usually based on examining urine concentration concurrent with detection of azotemia. Because cats tend to have an exceptional ability to concentrate their urine, it is not surprising that cats, compared with dogs or humans, typically maintain a greater degree of urine concentrating ability as renal function declines. As a consequence, less advanced CKD may be associated with relatively concentrated urine in some cats. Absent other causes for dilute urine (e.g., hyperthyroidism, diabetes mellitus), serum creatinine values of 1.6 mg/dl or greater associated with urine specific gravity values less than 1.035 should generally be interpreted as consistent with renal azotemia. Cats with more advanced CKD typically have urine specific gravity values below 1.020. Urine specific gravity values between 1.035 and 1.040 constitute a “gray zone” in which azotemia may be renal or prerenal; however, cats may occasionally present a diagnostic dilemma because they remain persistently azotemic for months to years with urine specific gravity values greater than 1.040. These cats most likely have CKD.

To facilitate application of appropriate clinical practice guidelines for diagnosis and treatment, patients with CKD are categorized into four stages along a continuum of progressive kidney disease. (For more information on staging chronic kidney disease, visit the International Renal Interest Society website at www.iris-kidney.org.) The stage of CKD is assigned based on the level of kidney function ascertained by two or more determinations of serum creatinine concentration obtained while the patient is well hydrated:

- Stage 1: Serum creatinine <1.6 mg/dl
- Stage 2: Serum creatinine 1.6–2.8 mg/dl
- Stage 3: Serum creatinine 2.9–5.0 mg/dl
- Stage 4: Serum creatinine >5.0 mg/dl

The stage is further elucidated by proteinuria status and the presence of systemic hypertension because these factors appear to influence prognosis and are amenable to therapeutic modification. Cats with urine protein:creatinine ratios less than 0.2 are classified as nonproteinuric, ratios between 0.2 and 0.4 indicate borderline proteinuria, and ratios greater than 0.4 indicate proteinuria. Cats with systolic blood pressure less than 150 mm Hg are considered to have minimal risk of experiencing hypertensive end-organ injuries (e.g., renal, ocular, cardiac, or nervous system lesions). Cats with systolic blood pressure between 150 and 159 mm Hg, 160 and 179 mm Hg, or greater than 180 mm Hg are considered to have a low, moderate, or high risk, respectively, of experiencing hypertensive end-organ injuries.

CAUSE AND PATHOLOGY
CKD in cats may be initiated by a variety of familial, congenital, or acquired diseases. Unfortunately, the initiating cause(s) of CKD often cannot be identified at the time of diagnosis. In one study, chronic tubulointerstitial nephritis was observed in 70% of cats with CKD, whereas glomerulonephropathy occurred in 15%, lymphoma in 11%, amyloidosis in 2%, and
tubulointerstitial nephritis does not help to identify the underlying cause of kidney disease and probably represents the final common pathway for progression of many feline renal diseases. The initiating causes of diseases believed to originate in the tubulointerstitium have been especially elusive; however, one possible cause for the higher prevalence of CKD in cats has recently been proposed. Subcutaneous administration in kittens of feline herpesvirus 1, calcivirus, and panleukopenia virus vaccines grown in feline tissue culture systems has been shown to induce production of antifeline renal tissue antibodies in serum and a tubulointerstitial inflammatory response within the renal tubulointerstitium. This observation prompts the question of whether repeated vaccinations play a role in the development of CKD in cats.

Another interesting observation that appears to be unique to cats is the high frequency of nephroliths and ureteroliths in those with CKD. These uroliths are composed predominantly of calcium oxalate. The origin of these uroliths and whether they develop before, during, or after the onset of CKD are not known. Although ureteroliths have become an important cause of acute uremic crises in cats, the presence of nephroliths does not appear to adversely affect clinical outcomes in cats with CKD. Although it may be necessary to surgically remove ureteroliths associated with complete, persistent ureteral obstruction, removal of nephroliths is generally not recommended.

BIologic Behavior

A progressive decline in kidney function over months to years is typical of naturally occurring CKD. Although it is logical to assume that CKD progresses as a consequence of ongoing renal injury associated with the disease process that initiated kidney disease, the initiating cause for CKD cannot be identified at the time of diagnosis in most patients. The preponderance of clinical and experimental evidence suggests that in dogs and cats with stages 3 and 4 CKD, progressive loss of kidney function results, at least in part, from factors unrelated to the inciting disease. These factors may include intraglomerular hypertension, glomerular hypertrophy, hypertension, proteinuria, intrarenal precipitation of calcium phosphate, and tubulointerstitial disease.

Whereas progression of CKD in humans and dogs is often characterized by a linear pattern of decline in glomerular filtration rate (GFR), progression of CKD in cats more commonly appears as abrupt, usually unpredictable, increases in serum creatinine concentration. In a clinical trial performed at the University of Minnesota, renal function as measured by serum creatinine remained stable for up to 24 months in 40 of 45 cats. Five of the 45 cats developed uremic crises associated with abrupt increases in serum creatinine concentrations after having had stable renal function for 3 to 21 months. Upon retrospective evaluation of the clinical data on these cats, no clear indicators were found to be useful in predicting an impending decline in kidney function.

The seeming stability of renal function in many cats with CKD translates into relatively long survival time. Compared with dogs having similar levels of renal dysfunction, cats typically live many months or years longer. In fact, many older cats succumb to other diseases before their CKD becomes severe enough to cause significant morbidity.

MODIFYING CLINICAL OUTCOMES

Even though feline CKD tends to be generally less progressive than CKD in dogs, many cats still progress to a point where it becomes difficult or impossible to have a satisfactory quality of life. Recent studies have indicated that certain medical interventions may delay or prevent progression of CKD, thereby extending survival with a good quality of life. Factors that have been shown to influence survival times for cats with CKD include the severity of reduction in GFR (stage of CKD) and magnitude of proteinuria. There may also be an interaction between systemic hypertension and proteinuria on survival. With greater severity of intrinsic renal dysfunction or magnitude of proteinuria, shorter survival time is likely. Some other factors that may or may not influence progression of CKD directly include systemic hypertension, pyelonephritis, and presence of nephroliths.

All patients with CKD are potentially at risk for progressive kidney disease. Progression may occur as a consequence of the primary renal disease, in association with a variety of secondary factors that may promote progressive renal disease, or both. An important therapeutic goal for managing patients with CKD is to minimize or prevent progressive loss of renal function. Treatment designed to limit progression of kidney disease may involve a variety of interventions, including diet therapy, minimizing proteinuria, controlling hypertension, and modulating the renin-angiotensin-aldosterone system.

There is substantial clinical trial evidence supporting the effectiveness of dietary intervention in prolonging survival of cats with CKD; there is no credible clinical evidence to the contrary. In a nonrandomized clinical trial, cats fed a renal
diet survived significantly longer than cats that continued to consume their usual diet (633 versus 264 days). It was not possible to detail the differences between the diets used in this study, but the therapeutic renal diet had a reduced protein and phosphorus content. The renal diet was shown to be beneficial in lowering serum phosphorus and parathyroid hormone concentrations, and it was suggested that the beneficial effect of the diet may have been related to this effect. A randomized, controlled clinical trial from the University of Minnesota Veterinary Medical Center further confirmed the beneficial effects of diet therapy in prolonging survival of cats with CKD. In this study, the effect of a renal diet on survival was compared with that of a maintenance diet in 45 cats with spontaneous CKD. Renal-related mortality in 23 cats fed an adult maintenance diet was 17.4%, whereas no deaths were observed in 22 cats fed the renal diet, which was restricted in protein and phosphorus content. In a retrospective study of cats with CKD treated at 31 veterinary clinics in the Netherlands, feeding a renal diet compared with a typical feline diet was found to be associated with a significant increase in median survival time (7 months among cats consuming conventional cat foods; 16 months for cats consuming a renal diet).

A common misconception is that renal diets are simply low-protein diets. Renal diets encompass a variety of modifications beyond just a limitation of protein content, and, indeed, the principal beneficial effects of these diets may not accrue from their reduction in protein content. Thus, simply replacing a renal diet with a standard manufactured diet that is lower in protein content does not meet the guideline for feeding a renal diet. Because inappropriate diets can exacerbate clinical signs of uremia or promote progression of CKD, cats with CKD should be fed a renal diet.

Treatments designed to reduce glomerular proteinuria are recommended for managing proteinuric cats with CKD stages 1 through 4. Intervention is indicated when the urine protein:creatinine ratio exceeds 2.0 in cats with CKD stage 1 and 0.4 in cats with CKD stages 2 through 4. Proteinuria has been shown to adversely affect outcome in humans, dogs, and cats with CKD, presumably because proteinuria itself appears to injure the renal tubules, thereby promoting progression of CKD. It is well established in human patients that reducing proteinuria by suppressing the renin-angiotensin-aldosterone system ameliorates the adverse effects of proteinuria on the kidneys. While qualitatively similar, evidence in cats is less compelling. Although studies have shown that proteinuria is closely linked to progression of CKD in cats and that angiotensin-converting enzyme (ACE) inhibitors are effective in reducing proteinuria in cats with CKD, the effectiveness of ACE inhibitor therapy in altering the course of CKD in cats remains to be confirmed. Nevertheless, ACE inhibitors such as benazepril and enalapril are recommended for patients with CKD that meet the above criteria. Interestingly, treatment of systemic hypertension in cats with CKD using amlopidine besylate has been shown to be associated with a reduction in the magnitude of proteinuria. Ideally, therapy should be adjusted so that the urine protein:creatinine ratio is reduced to 0.4 or lower; however, this may be difficult or impossible in many patients and may require higher doses of ACE inhibitors or the addition of angiotensin II receptor-blocking drugs (e.g., losartan, irbesartan).

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Feline Urolithiasis: Understanding the Shift in Urolith Type

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Naturally occurring urolithiasis is affected by many known and unknown risk factors. Known risk factors that influence urolith formation include diet, urine pH, water homeostasis, breed, abnormalities of metabolism, urinary tract infection (UTI), and anatomic and functional abnormalities of the urinary tract. Each factor may play a significant or limited role in the development or prevention of different types of uroliths. Recognition and control of lithogenic risk factors is the primary goal to prevent urolith formation and minimize urolith recurrence.

The Minnesota Urolith Center has performed quantitative analysis of uroliths from cats for more than two decades. During this period, we have observed substantial shifts in urolith type (Figure 1). In 1981, struvite was the most common stone, representing 78% of urolith submissions. A decade later, struvite remained the most common stone; however, its prevalence had declined to 59%. By the end of the second decade, the ever-present struvite had been supplanted by the emergence of calcium oxalate (CaOx). In 2001, uroliths were retrieved from 6,185 cats and submitted for quantitative analysis; 55% were CaOx, and 34% were struvite. Epidemiologic shifts in feline urolith type were not confined to the United States: Increased prevalence of CaOx was also observed in Asia and Europe. Because of the short time span in which this occurred, we hypothesized that changes in husbandry and nutrition represent significant contributing factors influencing this epidemiologic shift in urolith type.

THE RISE AND DECLINE OF STRUVITE

In the early 1970s, the association between dry diets and feline lower urinary tract disease (FLUTD) became a topic of intense discussion in England, Denmark, and the United States. Also in the early 1970s and continuing for the next decade, several groups of investigators experimentally produced magnesium hydrogen phosphate and then magnesium ammonium phosphate (MAP) uroliths in clinically normal cats by adding various types of magnesium salts to their diets. The cats developed typical signs of FLUTD, including urethral obstruction, but they did not produce the struvite-matrix urethral plugs commonly encountered in cats with naturally occurring urethral obstruction. The general consensus of many investigators and clinicians was that consumption of dry diets with excessive magnesium was an important primary cause of FLUTD.

Following the development of dietary protocols to induce dissolution of naturally occurring struvite uroliths in dogs, dietary protocols to dissolve naturally occurring sterile struvite urocystoliths in cats emerged in 1983. Their effectiveness justified the emphasis on dietary factors in the prevention of sterile struvite urolithiasis.

In 1985, the results of studies on the effects of feeding diets containing alkalinizing and acidifying salts of magnesium to clinically normal cats were reported. These laboratory studies shifted the focus from dietary magnesium content to alkaline urine pH as a primary factor in the development of struvite crystalluria. Results of these studies had a profound effect on veterinarians and the pet food industry. Many adult feline maintenance diets were eventually modified to minimize struvite crystalluria. Because of dietary modifications, the prevalence of struvite uroliths and struvite urethral plugs began to decline in the mid-1980s. Unexpectedly, the decrease in prevalence of struvite-related urolithiasis was associated with a concomitant increase in the prevalence of CaOx urolithiasis even though struvite remained the primary mineral component of urethral plugs.

THE EMERGENCE OF CALCIUM OXALATE

The exact etiologic cascade of events that led to the increased prevalence of CaOx uroliths remains unknown. However, several biologic phenomena provide plausible explanations.

The Role of Diet

Results of epidemiologic studies support the hypothesis that diets designed to minimize MAP urolith formation may have
inadvertently increased the occurrence of CaOx uroliths.\textsuperscript{5,6} Whereas diet-mediated urine acidification enhances the solubility of MAP crystals in urine, dietary acids promote CaOx crystalluria by inducing hypercalciuria. This association between aciduria, acidemia, and hypercalciuria may be explained by the fact that acidemia promotes mobilization of carbonate and phosphate from bone to buffer hydrogen ions. Concomitant mobilization of bone calcium may result in hypercalciuria. In addition, metabolic acidosis in dogs, humans, and rats may result in hypocitraturia. If consumption of dietary acid precursors is associated with hypocitraturia in cats, it may increase the risk of CaOx uroliths because citrate is an inhibitor of CaOx crystal formation.

Over the past 50 years, the incidence of CaOx uroliths in humans living in the United States has increased considerably.\textsuperscript{7} Global distribution of urolithiasis in humans indicates that CaOx uroliths predominate in the United States and other industrialized, technologically advanced regions of the world.\textsuperscript{8} Although originally attributed to the sedentary lifestyle of inhabitants of such countries,\textsuperscript{8} the increased incidence of CaOx uroliths is now believed to reflect the ability of these more affluent societies to spend disposable income for the consumption of animal protein, which leads to increased urinary excretion of acidic metabolites, calcium, and oxalate.\textsuperscript{9,10} Regional environmental factors, such as water and soil quality, may also influence urolith formation. It is logical to consider that variables that contribute to the increased incidence of CaOx uroliths in humans may also influence the incidence of CaOx uroliths in cats. In other words, are strategies that incorporate the concepts of improved nutrition or overnutrition a risk factor for CaOx urolith formation?

\textbf{The Role of Age}

In a retrospective study of feline CaOx uroliths from 922 cats, only 3 were younger than 1 year of age. Ninety-seven percent of affected cats were older than 2 years. These observations are interesting because conditions promoting urinary acidity have been identified as a risk factor for CaOx urolith formation, and the urine pH of young cats is lower than that of adult cats consuming the same diet. If acidic urine is an important risk factor for CaOx, a reasonable question is why CaOx uroliths are uncommon in immature cats in which urine is normally acidic. The answer is likely related to a combination of risk factors associated with CaOx urolithiasis, including the concentrations of minerals and nonmineral crystallization inhibitors and promoters and the quantity of urine produced. There likely is no simple cause-and-effect relationship between a single risk factor and CaOx urolithiasis.

Why MAP has remained the most common mineral of urethral plugs while the prevalence of feline CaOx has dramatically increased in uroliths is unknown. However, the observation that the average age of cats with urethral plugs is lower (approximately 2 to 4 years old) than that of cats with CaOx uroliths leads to the hypothesis that age-related changes in urine promoters for urolithiasis play an important role. As an illustration, in a case-control study comparing the age of 7,895 cats with CaOx uroliths that were submitted to the Minnesota Urolith Center between 1981 and 1997 with the age of 150,482 cats admitted to veterinary colleges in North America, cats at greatest risk of developing CaOx uroliths were those between 7 and 10 years of age.\textsuperscript{11} Cats in this age group were 67 times more likely to form uroliths than cats 1 to 2 years of age. The mean age of cats with CaOx uroliths was 7.5 ± 3.3 years. In contrast, cats at highest risk of developing MAP uroliths were between 4 and 7 years of age. These comparisons are clinically important because they emphasize the need to monitor cats receiving diets that promote urine acidification because as cats get older, the risk of developing CaOx urolithiasis increases.
The Role of Oxalobacter spp

Hyperoxaluria is an important risk factor for CaOx urolith formation. Although the majority of urinary oxalate is derived from endogenous metabolic pathways, increased urinary oxalate appears to be sustained by increased dietary load and increased intestinal absorption. Studies in rats have demonstrated that components of the intestinal microflora, particularly Oxalobacter formigenes, use oxalate in the gut, thus limiting its absorption. Of particular interest is the observation that human patients with recurrent UTIs excrete higher quantities of oxalate than do stone formers without UTIs. It was hypothesized that antibiotic control of UTIs reduces intestinal Oxalobacter populations. Conceptually, this association may be important for two reasons: (1) antibiotics are commonly used in the management of idiopathic FLUTD and (2) renal tubular damage by increased urine oxalate concentrations may serve as a nidus for crystal nucleation, adherence, and growth.

THE AGE OF NEPHROURETEROLITHIASIS

The increase in occurrence of CaOx uroliths in cats has been associated with a parallel increase in occurrence of CaOx uroliths found in their kidneys and ureters. In fact, there has been a 10-fold increase in the frequency of upper tract uroliths diagnosed in cats evaluated at veterinary teaching hospitals in North America over the past 20 years.

Between 1981 and 2003, the Minnesota Urolith Center analyzed nephroureteroliths from 1,599 cats. Seventy percent of the uroliths were composed of CaOx. In contrast, only 8% were composed of MAP. While enrolling cats with renal failure into a clinical trial, we were surprised to find that 48% had radiographic evidence of nephroliths or ureteroliths. This finding emphasizes the importance of CaOx prevention and control in cats to minimize potential life-threatening renal failure.

Is kidney disease a cause or a consequence of urolith formation? Hyperoxaluria may be the common link between these two processes. One current hypothesis proposes that excessive oxalate damages kidney tubules. The damaged tubules become mineralized (Randall’s plaques, which are sites of interstitial mineralization at or near the renal papilla found in kidneys of CaOx stone formers) and serve as a nidus for CaOx precipitation (epitaxy). By increasing urine saturation, hyperoxaluria also promotes precipitation of calcium. In turn, CaOx uroliths of sufficient size can block the ureter, promoting kidney failure.

THE EPOCH OF HOPE

Since 2001, the Minnesota Urolith Center has observed a consistent decline in the yearly percentage of cats with CaOx uroliths (Figure 1). Although factors other than a change in the occurrence of CaOx may contribute to this reduction, we have taken the optimistic perspective that increased knowledge and understanding of the risk factors associated with CaOx formation have favorably altered husbandry, nutrition, and veterinary care.

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Feline hyperthyroidism was first described in 1979 and 1980 by investigators in New York and Boston, respectively. The question at that time and ever since has been, “Is hyperthyroidism a new disease in cats?” Based on epidemiologic and hospital-acquired data, the answer appears to be “yes.” During a 14-year period (1970 to 1984), an average of 1.9 cats per year were diagnosed with hyperthyroidism; however, it is now estimated that the incidence is as high as 2% of the feline population seen in tertiary care veterinary facilities. Hyperthyroidism has become the most frequently diagnosed endocrinopathy in cats, with reports originating from North America, Europe (especially the United Kingdom), New Zealand, and Australia. Hyperthyroidism in cats has become increasingly more prevalent as a result of an increase in the number of cats that survive past 10 years of age, improved diagnostics, and increased suspicion of the disease among veterinarians. Dozens of studies have been published on the origins of feline hyperthyroidism, but none provides a definitive answer to the mystery behind this disease.

**THYROID PHYSIOLOGY**

The thyroid gland is the most important endocrine gland for metabolic regulation. The synthesis of thyroid hormone is unusual because a large amount of the active hormone is stored as a colloid within the lumen or acinus, created by the circular arrangement of glandular cells. Two molecules—tyrosine and iodine—are important for thyroid hormone synthesis. Tyrosine is a part of thyroglobulin, a large molecule (molecular weight: 660,000 D) formed within the follicle cell and secreted into the lumen of the follicle. Iodine is converted to iodide in the intestinal tract and then transported to the thyroid, where the follicle cells effectively trap the iodide through an active transport process. This allows intracellular iodide concentrations to be 25 to 200 times higher than extracellular concentrations.

As iodide passes through the apical wall of the cell, it attaches to the ring structures of the tyrosine molecules, which are part of the thyroglobulin amino acid sequence. The tyrosyl ring can accommodate two iodide molecules; if one iodide molecule attaches, it is called monoiodotyrosine. The coupling of two iodinated tyrosine molecules results in the formation of the main thyroid hormones: two diiodotyrosine molecules form tetraiodothyronine (T4), and one monoiodotyrosine and one diiodotyrosine molecule form triiodothyronine (T3). Thyroperoxidase, a key enzyme in the biosynthesis of thyroid hormones, works in concert with an oxidant, hydrogen peroxide. Thyroperoxidase catalyzes the iodination of the tyrosyl residues of thyroxine-binding globulin and the formation of T4 and T3. In addition to the unusual molecular storage form of the hormone, thyroid hormones are also unique in that they are the only hormones that contain a halide (i.e., iodine).

The main form of metabolism of thyroid hormones involves the removal of iodide molecules. The two enzymes involved in T3 and reverse T3 synthesis, 5′-deiodinase and 5-deiodinase, are also involved in the catabolism of thyroid hormones. The majority of T3 formation occurs outside the thyroid gland by deiodination of T4. The enzyme involved in the removal of iodide from the outer phenolic ring of T4 is known as 5′-monodeiodinase. Another type of T3 in which an iodide molecule is removed from the inner phenolic ring of T4, a compound called reverse T3, is also formed. Reverse T3 has little of the biologic effects of thyroid hormones and is formed only by the action of extrathyroidal deiodinating enzymes, not by activity of the thyroid.

**CLINICAL ASPECTS OF HYPERTHYROIDISM**

As noted, hyperthyroidism is the most common endocrinopathy of cats. It is caused by adenomatous hyperplasia of the thyroid gland. Middle-aged to older cats are typically affected, and there is no predilection for breed or sex, although some studies suggest a male predilection and a decreased incidence in Himalayans and Siamese. Hyperthyroidism is characterized by hypermetabolism; therefore, polyphagia, weight loss, polydipsia, and polyuria are the most prominent features of the disease. Activation of the sympathetic nervous system is also seen. Hyperactivity, tachycardia, pupillary dilatation, and behavioral changes are characteristic of the disease in cats. Long-standing hyperthy-
Feline hyperthyroidism is diagnosed by measuring total T₄ (TT₄); total T₃ measurement is generally noncontributory to a diagnosis. Because the disease has become more common and recognized in its early stages, free T₄ (FT₄) concentrations have been shown to be more diagnostic of early or “occult” hyperthyroidism; however, FT₄ concentrations should be interpreted in light of TT₄ because nonthyroidal illness (e.g., chronic renal failure) can result in spurious elevations of FT₄ as well.

**NUTRITIONAL ASPECTS OF HYPERTHYROIDISM**

An inability to secrete adequate amounts of thyroid hormone often leads to the enlargement of the thyroid gland, a condition known as goiter. In many places around the world, this condition is, or has been, caused by a deficiency of iodine in the diet, a situation that has largely been corrected through the use of iodized salt. Balanced pet foods provide sufficient iodine but vary widely in iodine content.6 The effects of this variation have been theorized to be important in cats, but there are no data to support or refute the theory. Tartellin et al7 showed an acute inverse relationship between FT₄ and di-iodotyrosine but have no data to support or refute the theory. Tartellin et al7 showed an acute inverse relationship between FT₄ and di-iodotyrosine but have no data to support or refute the theory.

Goitrogens can result in hypothyroidism, and some have theorized that chronic exposure to goitrogens can lead to toxic nodular goiter, resulting in hyperthyroidism.

It has been theorized that flavonoids from soy proteins play a role in the pathogenesis of hyperthyroidism in cats. Quercetin, a flavonoid, is capable of stimulating mitogenesis in a cell-culture line from hyperthyroid cats.9 Polyphenolic soy isoflavones, such as genistein and daidzein, were identified in almost 60% of dry cat foods tested.8 Some dry foods contain isoflavone at levels consistent with those shown to interfere with thyroid function by inhibiting thyroperoxidase in rats and 5′-deiodinase activity in cats12,13; however, these cell-culture and in vitro studies are in contradiction to epidemiologic data that show hyperthyroidism to be less common in cats fed dry foods.4,5,14 Studies in rats have demonstrated in vitro effects of soy isoflavones, especially in conjunction with iodine deficiency; however, an in vivo effect on TT₄ and thyroid-stimulating hormone (TSH) has not been observed.12,15 In a prospective study of 18 clinically normal cats eating a soy diet (400 mg isoflavones/kg diet), TT₄ and FT₄ concentrations were significantly, but modestly, increased, whereas T₃ concentrations were unchanged.9 Many human studies have shown no detrimental effect of soy isoflavones on thyroid function, particularly when incorporated into a balanced diet with adequate iodine intake.16,17 Thus, the effect of soy, if any, within complete cat foods remains controversial.

Although unproven, canned cat food has been implicated as a cause of feline hyperthyroidism in multiple epidemiologic studies.4,5,14 The suspected goitrogen is bisphenol A diglycidyl ether (BADGE), a substance used in the manufacture of the liners of easy-open pop-top cans. It is suspected that this compound can leach into food and be consumed by cats. While this BADGE-based lining is generally considered safe and is used with foods for human consumption, it is suggested that cats may be more susceptible to toxic effects of this compound because they have a greatly reduced ability to detoxify it via hepatic glucuronidation. At toxic levels, bisphenol A also reduces triiodothyronine binding and causes increased TSH secretion, resulting in hyperthyroidism and goiter in rats and some humans. On the other hand, although cat studies may not be available, rodent studies show a very high safety margin.18 It also should be noted that epidemiologic studies showing associations are not the same as cause and effect. Over 90% of cats in the United States consume commercial pet foods as their primary nutritional source, and relatively few develop hyperthyroidism.

**IMMUNOLOGIC ASPECTS OF HYPERTHYROIDISM**

The literature regarding an immunologic cause of hyperthyroidism is contradictory. Initially, feline hyperthyroidism was
believed to be similar to Grave’s disease in humans. In fact, the clinical signs of hypermetabolism associated with feline hyperthyroidism are identical to Grave’s disease; however, several studies have shown that, unlike Grave’s disease, which is caused by autoantibodies to the thyroid TSH receptor, cats with hyperthyroidism have antibodies that do not stimulate the TSH receptor. In other research, antibodies from hyperthyroid cats were shown to stimulate thyroid cellular proliferation and interfere with TSH binding. More recent reviews have indicated that an autoimmune basis for hyperthyroidism is unlikely and that the disease is more similar to toxic nodular goiter than to Grave’s disease.

MOLECULAR ASPECTS OF HYPERTHYROIDISM

More recently, investigators have honed in on the molecular aspects of feline hyperthyroidism. In cats, the disease is more similar to toxic nodular goiter in humans and is characterized by autonomous growth of thyroid follicles. The pathogenesis of toxic nodular goiter is an abnormality in the signal transduction of the thyroid cell. The TSH receptor on the thyroid cells activate receptor-coupled guanosine triphosphate-binding proteins (G proteins). Uniquely, thyroid cell proliferation and hormone production are both controlled by the TSH receptor–G protein–cAMP signaling. Overexpression of stimulatory G proteins and underexpression of inhibitory G proteins have been demonstrated in some humans with toxic nodular goiter. Mutations of the TSH receptor that result in the receptor remaining activated without ligand (i.e., TSH) have also been reported in humans with toxic nodular goiter.

The same abnormalities have been investigated in hyperthyroid cats, and it appears that activation mutation of the TSH receptor may be part of the pathogenesis of hyperthyroidism in some cats. Furthermore, abnormalities of G proteins (in particular, significantly decreased G inhibitory protein expression) have been described in tissues from hyperthyroid cats.

ENVIRONMENTAL ASPECTS OF HYPERTHYROIDISM

In one study, the use of cat litter was associated with an increased risk of hyperthyroidism; however, there was no significant difference among different brands of litter, suggesting that the use of litter is simply a marker of cats that are kept indoors. Indoor cats are likely to live longer and hence have a higher risk of developing hyperthyroidism. Exposure to pesticides and herbicides has been associated with thyroid abnormalities in other species. In particular, the use of flea-control products was associated with an increased risk of developing hyperthyroidism, but no specific product or ingredient could be identified.

A recent report implicated brominated flame retardants (BFRs) as carcinogens/goitrogens possibly associated with feline hyperthyroidism. Coincidently, BFRs were introduced 30 years ago, around the same time that feline hyperthyroidism emerged. Bromide, a halide, is an intriguing agent to implicate in feline hyperthyroidism because of the unique composition of thyroid hormones that contain the halide iodide. In this abstract, serum levels of lipid-adjusted serum polybrominated diphenyl ethers (PBDE) were 10 to 400 times higher than those found in human exposure. The authors theorized that these findings of high PBDE serum levels are in accord with the most consistently identified risk factor: indoor living. The authors also propose that cats are at increased risk because of meticulous grooming behavior and increased exposure to furniture and carpet. The small size of cats is also a possible risk factor for increased serum levels of PBDEs.

CAUSES OF FELINE HYPERTHYROIDISM

It is unlikely that autoantibodies to TSH, iodine deficiency, or iodine excess causes hyperthyroidism. There are unproven theories that goitrogens, such as BADGE or isoflavones, PBDEs, and genetic or molecular changes in predisposed individual cats might contribute to hyperthyroidism. Feline hyperthyroidism, like most diseases, is probably caused by a multitude of interactive factors, including genetics, nutrition, and environment.

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Research over the past several years has substantially expanded our understanding of feline inflammatory bowel disease (FIBD) and its underlying pathomechanisms (Figure 1). Advances in basic immunologic techniques and molecular biology have provided evidence that FIBD likely results from a dysregulated immunologic response to environmental triggers, including luminal bacteria and specific antigens. In addition to these basic research findings, emerging clinical data offer insight into the histopathology, clinical immunology, and assessment of disease severity in affected cats at diagnosis and in response to therapeutic intervention. Additionally, preliminary data suggest that some staging criteria have direct application to cats with other forms of chronic enteropathy, such as food-responsive enteropathy.

**HISTOPATHOLOGIC GUIDELINES FOR DIAGNOSING FIBD**

Histology is key to confirming diagnosis and eliminating some other diseases, but standardized grading criteria have not been adopted. Several qualitative and semiquantitative histopathologic scoring systems for IBD have been proposed (Box 1). In most instances, these scoring systems are larger, case-based studies that use a spectrum of histologic criteria of mucosal inflammation with commentary on lamina propria cellularity. Endoscopically obtained gastrointestinal (GI) tract mucosal biopsy collection remains the gold standard but presents a variety of challenges for clinicians and pathologists. It is recognized that these specimens are small, prone to procurement and processing artifacts, and difficult to optimally orient for accurate morphologic characterization. Additionally, extensive interobserver variability in interpretation between pathologists can occur. Histopathologic diagnostic criteria of IBD should clearly define morphologic evidence of mucosal inflammation; however, which criteria are most relevant is presently a matter of debate.

The World Small Animal Veterinary Association Congress GI Standardization Group is working diligently to produce and validate a workable, standardized histopathologic scoring system for GI inflammation that clinicians and pathologists can apply universally. This grading protocol covers the gastric fundus and pylorus, duodenal mucosa, and colonic mucosa. For each anatomic region, a narrative and visual (photomicrographic) template that defines the normal histologic appearance of the tissue and key morphologic and inflammatory changes (by severity) has been developed. Analysis of approximately 250 endoscopic biopsies collected by gastroenterologists worldwide using standardized reporting forms is presently under way.

**MUCOSAL CYTOKINE EXPRESSION AND CORRELATION TO HISTOLOGY IN FIBD**

In contrast to canine IBD, in which a balanced ratio of T-helper 1 (Th1) to T-helper 2 (Th2) mucosal cytokine pattern emerges, data to date suggest that FIBD cytokine expression is more overtly Th1-like and broadly correlates to histopathologic inflammation. In one small study of 12 cats with IBD, endoscopic biopsies were evaluated for the presence of cellular infiltrates and morphologic changes and then correlated to levels of cytokine mRNA quantitated by real-time polymerase chain reaction. In general, morphologic changes (e.g., epithelial alterations, villus fusion, atrophy) were associated with upregulated expression of interleukin-1β (IL-1β), IL-8, IL-12, and interferon-γ (IFN-γ) as well as IL-10 in diseased cats. Furthermore, IBD grade correlated with IL-10 and IL-12, with IL-10 highest in cats with severe FIBD. Interestingly, cytokine upregulation was not correlated with the density of the mucosal cellular infiltrate.

A separate investigation evaluated cytokine mRNA expression in cats with chronic enteropathy caused by FIBD and non-FIBD GI diseases. The results of this study were analyzed on the basis of either clinical presentation or histopathologic evidence of intestinal inflammation. Clinically normal cats and cats...
with FIBD showed increased expression of immunoregulatory (IL-10, tumor growth factor-β [TGF-β]) and proinflammatory (IL-6, IL-18, tumor necrosis factor-α [TNF-α], and IL-12p40) cytokines relative to cats with other GI diseases. Histopathologic analysis showed that cats with intestinal inflammation had upregulated expression of IL-6, IL-10, IL-12p40, TNF-α, and TGF-β, compared with those with normal intestinal morphology. These accumulated observations indicate that FIBD is characterized by immune dysregulation that parallels morphologic evidence of intestinal inflammation.

**CLINICAL MEASURES OF DISEASE ACTIVITY IN FIBD**

Well-defined clinical criteria for assessment of FIBD activity have not been published, presumably reflecting the generally sparse number of studies reported and the inability of the researchers to critically assess disease activity other than by the severity of histologic lesions. Clearly, several themes emerge from these earlier evidence-based investigations: (1) GI signs of anorexia, weight loss, and vomiting predominate with gastric or small-intestinal IBD; (2) GI signs of hematochezia, mucoid feces, tenesmus, or increased frequency of defeation are commonly observed with colonic IBD; (3) biochemical changes of altered plasma protein concentrations (e.g., hyperglobulinemia, hypoalbuminemia) and increased serum concentration of hepatic enzymes (e.g., alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP]) are often observed; and (4) histologic lesions of lymphocytic-plasmacytic mucosal cellular infiltrates predominate (Box 2).

A possible first step in the development of an FIBD activity index (FIBDAI) would be collecting a wide range of variables, including prominent GI signs (generally reported by the client or clinician) and select laboratory parameters, and correlating their association to the severity of histologic lesions. One pilot study has taken this exact approach. An FIBDAI was prospectively evaluated in 27 cats with FIBD before and during therapeutic intervention. Variables included histology, GI signs, serum total protein and phosphorous concentrations, serum ALT and ALP, and endoscopic lesions. Complete response to prednisolone therapy was observed in 22 of 27 FIBD cats, and remission was achieved in the remaining 5 cats with the addition of chlorambucil. Alterations in clinical scoring indexes were observed in all FIBD cats as a consequence of medical therapy. Pretreatment FIBDAI scores (mean score: 7.8) were markedly reduced during the 14- to 21-day treatment period (mean posttreatment FIBDAI score: 0.8). These preliminary data suggest that clinical scoring of FIBD is suitable for clinical evaluation of the therapeutic effect in these patients.

**LABORATORY (SURROGATE) MARKERS OF DISEASE ACTIVITY IN FIBD**

Acute-phase proteins (APPs), such as haptoglobin (HAP), serum amyloid A (SAA), and acid glycoprotein (AGP), are plasma proteins that increase in concentration after infection, inflammation, or trauma. Serum APPs are routinely measured in human clinical laboratories to assist in assessing the activity of disease and its response to treatment. Previous studies have shown that concentrations of some APPs correlate...
MEASURES OF DISEASE ACTIVITY IN FELINE INFLAMMATORY BOWEL DISEASE

CONCLUSIONS

Measures of disease activity for FIBD and other forms of feline chronic enteropathy are presently being designed. Cats with FIBD clearly have defined markers of intestinal inflammation, such as altered proinflammatory cytokine expression profiles and morphologic features of mucosal inflammation on review of histologic specimens. Preliminary results from pilot studies suggest that clinical variables may be useful to assess the initial disease severity and response to treatment in cats with FIBD. Future studies evaluating the role of fecal and serologic markers of inflammation in cats with chronic enteropathy are warranted.

REFERENCES


Box 2. Clinical and Laboratory Parameters Perturbed in Feline Inflammatory Bowel Disease

- Clinical scores
- Increased hepatic enzymes
- Altered plasma proteins
- Histologic grading
- Endoscopic lesions
- Hypocobalaminemia

well with human and canine IBD, but few detailed reports are available for cats. In a prospective study that included 27 cats with FIBD, we investigated whether serum APPs would be altered at diagnosis and in response to therapeutic intervention compared with healthy cats. All of the cats in this study had an extensive diagnostic workup, including a dietary trial with an elimination diet to exclude adverse food reactions and GI tract endoscopic biopsy. Additionally, all cats were clinically scored with the FIBDAI for disease severity at diagnosis and after 14 to 21 days of medical therapy.

Although it was hypothesized that cats with FIBD would have increased APPs, the highest serum concentrations were observed in cats with non-IBD chronic enteropathy. Baseline APPs (HAP, AGP) during the initial examination were marginally increased in cats with IBD compared with healthy cats. SAA was not detectable in any of the feline groups. Although medical therapy resulted in a significant reduction of clinical disease severity in FIBD, this was not accompanied by reduced serum concentrations of APPs. Serum HAP showed a negative correlation to therapy, and posttreatment values were increased compared with pretreatment levels, suggesting that glucocorticoids likely induce serum concentrations of APPs. It was concluded that APPs are not suitable markers for assessment of disease activity in FIBD.
The glycemic load of high-carbohydrate diets has been proposed to be suboptimal for cats. We hypothesized that the in vitro carbohydrate digestibility of diets would predict the in vivo glycemic response or that other dietary variables would explain any difference.

The in vitro digestibility of 18 whole dry diets was determined by simulated physiologic digestion. Diets were ranked according to the rate of glucose release over time (GGE) relative to total available carbohydrates. Six diets spanning the range of GGE were selected for in vivo assessment. Six cats were each pre-fed one of the diets for 7 days followed by a 16-hour fast; they were then fed enough diet to provide 1 g/kg of available carbohydrates. Serial blood glucose was assayed until it had returned to baseline.

Despite a wide range of in vitro digestibilities and compositions, there was little difference in incremental area under the curve (AUCinc) between diets, with glucose absorption occurring over 10 to 12 hours for all diets. There was no significant association between AUCinc and in vitro digestibility, but $GGE_{60}$ was associated with the baseline fasted blood glucose ($r^2 = .35; P = .035$). The fasted blood glucose predicted the absolute AUC (AUCabs; $r^2 = .66; P < .001$), the AUCinc ($r^2 = -.73; P < .001$), and peak glucose responses ($r^2 = .59; P < .001$). AUCinc was not associated with dietary crude fiber, fat, protein, carbohydrate, or physical biscuit characteristics. Glycemic responses (AUCinc) to whole foods are prolonged and are not predicted by the digestibility of the carbohydrate component.

When the equivalent amount of available carbohydrate is fed, incremental glycemic responses to different diets are unaffected by dietary fiber, fat, protein, carbohydrate type, or physical biscuit characteristics. Whereas fasted blood glucose after a few days of feeding appears to be a good indicator of the long-term glycemic load of a diet (AUCabs), AUCinc is probably determined simply by the rate of gastric emptying.
Although spaying is known to contribute to obesity, the role of adipose tissue is poorly understood. Thus, our objectives were to examine the effects of spaying on serum metabolite concentrations and adipose tissue and skeletal muscle gene expression in cats.

Eight adult (>1 year old) domestic shorthair cats were fed a commercial dry diet throughout the study. After a 2-week baseline period (week 0), cats were spayed and fed to maintain an ideal body weight for 12 weeks. After 12 weeks, cats were fed ad libitum for an additional 12 weeks. Blood samples were collected at weeks 0, 6, 12, 18, and 24, and adipose tissue and skeletal muscle biopsies were collected at weeks 0, 12, and 24. Data were analyzed using the mixed-model method of SAS (Cary, NC).

Fasting serum glucose and triglycerides were increased \((P < .05)\) at week 24, and plasma leptin tended to be increased \((P < .10)\) at weeks 18 and 24. Adipose lipoprotein lipase (LPL) mRNA was decreased \((P < .05)\) at weeks 12 and 24. Adipose hormone-sensitive lipase (HSL) mRNA was decreased \((P < .05)\) at week 24. Adipose tumor necrosis factor-\(\alpha\) mRNA tended to be decreased \((P < .10)\) at week 12, and interleukin-6 (IL-6) mRNA was increased \((P < .05)\) at weeks 12 and 24. Adipose leptin mRNA was decreased \((P < .05)\) at week 12, and adiponectin mRNA tended to be decreased \((P < .10)\) at week 24. Changes in HSL and LPL mRNA suggest changes in adipose tissue lipid metabolism as a result of spaying and weight gain, likely contributing to increased circulating triglycerides. Decreased adiponectin and increased IL-6 mRNA may be early signals of adipose tissue dysregulation and contribute to insulin resistance.

Our results demonstrate that adipose tissue is sensitive to spaying or weight gain (or both), justifying further research in this area.
High-protein diets have been used to promote weight loss in cats, but the effect of feeding high-protein diets after spaying to maintain weight has not been determined. The objective of this study was to evaluate cats fed either a high-protein diet (52.9% crude protein [CP] on a dry matter basis [DMB]) or a moderate-protein diet (34.3% CP DMB; 3.9 and 4.2 kcal/g calculated metabolizable energy, respectively) following ovariohysterectomy.

Food intake, body weight (BW) gain, body condition score (BCS), body composition, and activity level were measured in eight cats (four cats/treatment). Cats older than 1 year underwent ovariohysterectomy on week 0 and were fed ad libitum for 24 weeks. Food intake was measured daily, and BW and BCS were measured weekly. Activity was measured for 6 consecutive days before weeks 0, 12, and 24, and body composition was determined by dual-energy x-ray absorptiometry at weeks 0, 12, and 24.

Food intake and BW were markedly changed (P < .05) over time in all cats and tended to be increased (P < .10) in cats fed a high-protein diet. BCS was greater (P < .05) in cats fed the high-protein diet but increased (P < .05) over time regardless of dietary treatment. Total activity, measured using Actical activity collars (Mini-Mitter, Bend, OR), decreased (P < .05) from week 0 to weeks 12 and 24. Body composition did not change due to diet; however, body fat percentage increased (P < .05) over time. Grams of lean tissue showed a curvilinear (P < .05) effect over the course of the study, but percentage of lean tissue tended to decrease (P < .10) over time. Bone mineral content was increased (P < .05) at week 12 in cats fed the high-protein diet. This is likely to support the increased BW because of the large increase in food intake early after spaying in the cats fed the high-protein diet, which may have been due to the high palatability of this diet.

Based on these data, feeding a diet ad libitum after spaying, regardless of protein level in the diet, may increase the incidence of obesity in cats.
The objective of this study was to compare energy requirements for weight stabilization of cats that lost weight while consuming two different dietary protein levels. Fifteen adult neutered cats were divided into two groups: a control group and a high-protein group.

Two procedures were followed: In the first part of the study, two diets were used to achieve a controlled 20% weight loss (the control group was given a 29% crude protein [CP] diet, and the high-protein group was given a 43% CP diet). Groups had similar body composition (dual-energy x-ray absorptiometry) before weight loss; after weight loss, the control group had higher fat body mass (FM; 28.8% ± 1.6%) and lower lean body mass (LM; 68.4% ± 1.6%) than the high-protein group (FM: 23.4% ± 3.2%; LM: 73.5% ± 3.1%; P < .01).

Cats were then fed a 39% CP diet to maintain body weight for 17 weeks. Energy ingestion was divided into three periods: initial (weeks 0 to 6), middle (weeks 7 to 12), and final (weeks 13 to 17). At the end of the 17 weeks, there was no difference in body composition between groups (control group: FM = 24.9% ± 2.2%; LM = 72.8% ± 2.8%; high-protein group: FM = 24.1% ± 1.9%; LM = 72.2% ± 1.1%). From weeks 7 to 12, energy requirements gradually increased and did not stabilize in either group. Energy requirements were similar during the first 6 weeks of the study (control group: 93.6 ± 2.8 kcal/kg BW0.4; high-protein group = 97.2 ± 1.9 kcal/kg BW0.4) but significantly higher in the high-protein group during weeks 7 to 12 (control group = 96.7 ± 2.2 kcal/kg BW0.4; high-protein group = 111.9 ± 1.8 kcal/kg BW0.4; P < .001) and weeks 13 to 17 (control group = 110.6 ± 2.2 kcal/kg BW0.4; high-protein group = 127.7 ± 2.0 kcal/kg BW0.4; P < .01).

Based on our results, we conclude that high protein ingestion during weight loss periods provides higher energy requirements to stabilize body weight during maintenance. Nutritional composition of the maintenance diet may contribute to the recovery of lean body mass lost during caloric restriction.
Ingesting proteins and amino acids can impact the health of cats in two ways: (1) in a nutritive sense by supplying necessary energy and amino acid requirements, and (2) by acting as bioactive molecules and influencing functions within the body, including intestinal health. Because there is little information available on the influence of diet on gut health in cats, this preliminary study was designed to generate information on changes in gut morphology in response to a low-protein diet.

Eleven adult feral cats (six males, five females) were trapped as part of normal pest-control measures in the Manawatu region of New Zealand. Before being included in the study, the cats were sedated and screened by a veterinarian for feline immunodeficiency virus or feline leukemia virus. Cats were housed in single-metabolism cages and fed either a control (32.7% protein, 21.0% fat, 42.5% carbohydrate) diet (n = 5) or a low-protein (20.9% protein, 25.5% fat, 49.9% carbohydrate), semi-synthetic diet (n = 6) for 10 weeks before being euthanized. Samples of intestine (~5 cm in length) were excised from areas 15% (duodenum), 30% (jejunum), and 60% (ileum) along the length of the tract and processed for histologic analysis. Transverse sections (5 μm) of tissue were cut, and each specimen was stained with alcian blue, hematoxylin–eosin and examined by light microscopy (original magnification, ×100). SigmaScan (Systat Software, Chicago, IL) was used to measure villous height, crypt depth, and epithelial cell thickness of 10 villi in each tissue sample.

Similar morphologic characteristics were observed in the duodenal, jejunal, and ileal segments. Animals fed the low-protein diet had consistently longer villi, deeper crypts, and a thicker epithelial cell layer than animals fed the control diet. This indicates that the animals responded to the low-protein diet by increasing the gut surface area to maximize nutrient absorption.
Feline fatty acid metabolism may be directly affected by alterations of dietary fat intake. This study investigated the effect of different types of dietary fat on plasma triacylglycerol, total cholesterol, lipoprotein-cholesterol, and nonesterified fatty acid (NEFA) concentrations.

Thirty clinically normal, sexually intact young adult female cats were randomized into three groups of 10. Each group was fed a complete, balanced, commercial, dry, extruded-type basal diet supplemented with equal amounts of fat, differing only in fatty acid composition. The diets were designated as high-oleic sunflower (HOS), menhaden fish oil (MFO), and safflower oil (SFO). The HOS diet contained high amounts of oleic acid, the MFO diet contained high amounts of long-chain omega-3 fatty acids, and the SFO diet contained linoleic acid. Diets were fed for 28 days, with blood collected on days 0, 14, and 28.

Using repeated measures analysis of variance and post hoc comparisons, the MFO diet showed a statistically significant \( P < .05 \) triacylglycerol-lowering effect despite the already low-normal triacylglycerol levels typically observed. Lipoprotein electrophoresis revealed a statistically significant lowering of the pre-beta band (i.e., very-low-density lipoprotein) triacylglycerol in the MFO diet \( (P < .05) \) consistent with plasma triacylglycerol lowering. Whether triacylglycerol lowering in normal cats is beneficial is unknown. No main time or diet effects were found on total cholesterol concentrations, and no changes were observed in mean plasma NEFA concentrations. In some cats, an additional lipid-staining region was found on the electrophoretogram, which migrated similar to plasma albumin; however, this region did not correlate with plasma NEFA concentrations.

Additional studies to investigate these effects are in progress, including studies of plasma phospholipid and red blood cell fatty acids, cholesteryl ester and lecithin acyl transferase activities, and indices of fatty acyl desaturase enzyme activities.
Diabetes mellitus (DM) in cats is characterized by insulin resistance. Overweight and older cats are at increased risk of developing DM. In humans, dietary trans-fatty acids (TFA) increase insulin resistance, especially when fed at a level greater than 1% energy (%E). Exposure to dietary TFA may increase insulin resistance and type II DM in cats. The objective of this study was to determine if dietary intake of TFA was higher in cats at increased risk for DM.

Cats were grouped as follows: group 1 (normal; n = 27)—less than 10 years of age, body condition score (BCS) of 4 to 6; group 2 (fat; n = 23)—less than 10 years of age, BCS of 7 to 9; and group 3 (senior; n = 6)—10 years of age or older, BCS of 4 to 6. Serum was collected for general analysis, and each cat’s diet was analyzed for TFA.

Group 2 cats had a significantly higher BCS (mean: 7.6), body weight (mean: 14.1 lb), and percentage of body fat (mean: 5.0%) than group 1 cats (mean: 5.3 BCS, 10.1 lb body weight, 3.9% body fat) or group 3 cats (mean: 5.0 BCS, 9.0 lb body weight, 3.6% body fat). Group 2 cats showed a significantly higher (P < .05) concentration of serum insulin (mean: 54.5 pmol/L) and had a higher serum insulin:glucose ratio (mean: 9.8) than group 1 cats (mean: 39.1 pmol/L serum insulin, 6.8 insulin:glucose ratio) or group 3 cats (mean: 28.0 pmol/L serum insulin, 5.9 insulin:glucose ratio) cats. There were no significant differences in serum glucose, serum or dietary TFA concentrations, or dietary %E derived from TFA. Insulin concentration and insulin:glucose ratio were significantly correlated to BCS, body weight, and percentage of body fat but not to serum TFA, dietary TFA, or dietary %E from TFA.

Cats at higher risk for DM did not show elevated serum TFA or dietary intake of TFA. Although there does not appear to be a direct correlation of dietary TFA to insulin concentration, TFA could still contribute to the development of DM in predisposed individuals.
Glucokinase (GK) is an important metabolic enzyme that is the “glucose sensor” in pancreatic beta cells. GK activity in beta cells correlates with blood glucose concentration and links glucose metabolism to activation of cellular pathways that promote insulin secretion. The importance of pancreatic GK expression for normal glucose tolerance and insulin secretion is well established, and GK mutations cause diabetes in mammals. GK mRNA is known to be expressed in the feline pancreas, but the molecular details of feline pancreatic GK have not been previously investigated. This study’s objectives were to determine the sequence of feline pancreatic GK cDNA, predict the amino acid sequence of the feline pancreatic GK protein, and perform a comparative analysis of feline pancreatic GK sequence and structure.

GK mRNA from the pancreas of a normal cat was analyzed with reverse transcription polymerase chain reaction using species-specific primers. The elucidated cDNA sequence was used to predict protein sequence. Protein structure was modeled using molecular modeling software. The cDNA coding region contains 1,398 bp and encodes a 465–amino acid protein (GenBank EF121813). The predicted feline pancreatic GK protein is 89% to 94% identical to other mammalian GK proteins and contains 15 unique residues, 5 of which are nonconserved substitutions. Substrate binding, protein recognition, and other important functional motifs are conserved in feline GK. The feline GK protein model has two globular domains separated by a hinge region, similar to known GK structures. Interestingly, modeling studies indicated the region around nonconserved tryptophan35 in wild-type feline pancreatic GK has structural similarities to human GK with an R36W mutation, which causes type-2 maturity-onset diabetes mellitus of the young.

In conclusion, feline pancreatic GK has all major sequence and structural motifs found in noncarnivores, but nonconserved amino acids in the feline sequence may indicate species specificity. The aggregate effect of the nonconserved residues on protein function and the significance of these variations with respect to feline glucose metabolism or development of diabetes is unknown.
Periodontopathic conditions are the most common diseases in dogs and cats. Prophylaxis is usually limited to professional dental cleaning under anesthesia, especially in cats. Special diets that reduce plaque and calculus accumulation are formulated to extend the time between dental cleanings. Epigallocatechin (EGCG) and lactoferrin are already used in humans to maintain oral health because of these substances’ antibacterial effect. The aim of this study was to investigate the effect of EGCG singly and in combination with lactoferrin in a regular, dry, nondental diet on the oral health of cats.

In two consecutive trials, a total of 18 domestic short-haired cats (age: 3.83 ± 1.85 years old; body weight: 4.2 ± 1.1 kg) were divided on the basis of plaque score, age, and gender into two equal groups of nine animals each. For 28 days, animals were fed either a control or treated diet (trial 1: 227 mg EGCG/kg cat food; trial 2: EGCG and lactoferrin each 300 mg/kg cat food). General and dental health were investigated in the study. Because the “clean tooth model” was used, all cats received a dental cleaning before the 28-day feeding period.

EGCG alone proved able to inhibit the growth of bacteria taken from the feline oral cavity in vitro and seemed to have an effect on the cats’ antioxidant status. Supplementation with EGCG in combination with lactoferrin led to slightly lower plaque and calculus indexes compared with the control group. The gingivitis index decreased significantly in the EGCG/lactoferrin group, and compared with the control group, the probing depth of the supplemented diet group was significantly lower at the end of the experimental phase of the EGCG/lactoferrin test.

Altogether, the combination of EGCG and lactoferrin could be valuable when used along with established solutions for reducing plaque and calculus (e.g., fiber-containing kibbles or edible chews).
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This study investigated whether soy isoflavones alone or a blend of soy isoflavones, conjugated linoleic acid, and l-carnitine can promote weight loss, preserve lean body mass, and reduce oxidative stress in overweight dogs. Overweight Labrador retrievers and Siberian huskies were randomized into three groups (control, isoflavones, blend diets) and fed 70% of their maintenance energy requirement (MER) during the first 3 months of weight loss. Dogs that failed to reach their ideal body fat levels after the first 3 months of weight loss were fed 55% of their MER during the second 3 months of weight loss. Dual-energy x-ray absorptiometry scans were obtained 3 and 6 months after the study was initiated.

At the end of the study, the percentage of Labrador retrievers with their body fat reduced to ideal levels was 66.7%, 75%, and 85.7% for the control, isoflavone, and blend diets groups, respectively; the percentage of Siberian huskies with their body fat reduced to ideal levels was 33.3%, 50%, and 50% for the control, isoflavone, and blend diets groups, respectively. Compared with the control diet, both the isoflavone and blend diets groups significantly reduced plasma isoprostanes, and the blend diet completely prevented loss of lean body mass and significantly increased lean body mass after the first 3 months of weight loss.

In summary, more dogs in the isoflavone and blend diets groups tended to have their body fat percentages reduced to ideal levels than did control dogs. The blend diet prevented loss of lean body mass in overweight dogs during weight loss, and soy isoflavones in the weight-loss diets reduced in vivo oxidative damage in overweight dogs.
Dietary insoluble fiber is believed to support weight loss by increasing satiety and the mass of the food consumed without adding calories. Therefore, we measured satiety and weight loss in beagles fed a vegetable-based fiber supplement.

Two diets that differ in fiber content were fed: Purina ONE® Healthy Weight Formula (Purina ONE; Nestlé Purina PetCare, St. Louis, MO) was compared with a diet consisting of Purina ONE plus a vegetable-based fiber supplement (FS); crude fiber contents were 2.6% (Purina ONE) versus 5.4% (FS) dry matter.

For the satiety studies, 12 to 14 adult female beagles with average body fat of 38.4% ± 1.9% and body weight of 13.8 ± 0.9 kg were randomly divided into two groups. Diets were fed at 8:00 AM and 3:00 PM (7-hour interval) during one trial and at 8:00 AM and 11:00 AM (3-hour interval) for 15 minutes each during a second trial. The amounts offered at each feeding were 1.2 times the maintenance energy requirement (MER) using a crossover design. Blood samples were collected 45 and 120 minutes postprandially, and food intakes were recorded.

For the weight loss study, seven obese beagles were selected (average body fat: 45.1% ± 1.6%; body weight: 15.2 ± 1.0 kg) and divided into two groups. The diets were fed once daily (approximately 60% of obese MER) for 42 days. Postprandial blood samples were collected at 0 and 60 minutes on days 1, 28, and 42. Food intakes and body weight were recorded daily and weekly, respectively.

As expected, all dogs lost similar amounts of body weight and body fat independent of diet. In the satiety trials, intake at both the 3- and 7-hour intervals was not different from the control group; however, significantly fewer total calories were consumed with the FS diet during the 3-hour interval. FS did not affect triglyceride concentrations. Thus, FS provided fewer calories with the same degree of satiety as the higher calorie intake of the control diet. FS may improve fullness at lower calorie intake during weight loss with no effect on hypertriglyceridemia.
Adipose tissue is a highly active endocrine tissue that plays a pivotal role in glucose and lipid metabolism, energy homeostasis, and disease risk. Recent experiments suggest that fermentable dietary fibers, including short-chain fructooligosaccharides (scFOS), may beneficially impact glucose homeostasis and adipocyte metabolism. The objectives of the current experiment were to compare adipose tissue mRNA abundance in lean versus obese dogs and in obese dogs fed a diet containing 1% scFOS versus a control (fructan-free) diet.

The experiment consisted of two phases, the "obesity phase" followed by a "treatment phase." Adipose samples were collected from eight (four female, four male) neutered adult beagles with a normal body condition score (5 of 9) during fasted and fed states at baseline. All dogs were then fed ad libitum to promote weight gain (to 125% optimal body weight) and then fed to maintain this obese phenotype. In the obese state, a crossover design was used to test scFOS versus control diets. For each period, dogs were randomly allotted to a diet and fed for 6 weeks. Fasting and fed adipose samples were collected at the end of each period. Real-time quantitative reverse transcriptase polymerase chain reaction was used to measure mRNA abundance of genes involved with fatty acid metabolism, glucose metabolism, or inflammation. mRNA data were analyzed using the mixed-models procedure of SAS.

Compared with a lean phenotype, obesity increased \( P < .05 \) insulin receptor substrate 2 and interleukin-6 mRNA abundance and tended to increase \( P < .10 \) leptin mRNA. In the obese state, scFOS altered the expression of hormone-sensitive lipase, lipoprotein lipase, and uncoupling protein 2.

More research is needed to identify to what extent gene transcripts or proteins involved with leptin or insulin signaling are affected by scFOS supplementation.
Astaxanthin is a natural carotenoid with potent antioxidant properties in vitro and in vivo. Previous research in rodent models has suggested that astaxanthin can diminish cell proliferation and retard tumor growth. The exact mechanisms of action have yet to be elucidated, but it is believed that astaxanthin can decrease promitogenic autocrine mediators, inhibit cell signaling pathways, and alter cell adhesion molecules. Although astaxanthin may provide an attractive alternative therapy for neoplasia, its strong antioxidant capabilities have precluded its incorporation into cancer therapy, as it has been hypothesized that using antioxidants during radiation or chemotherapy may hinder neoplastic cell death.

To test this hypothesis, we treated three osteosarcoma cell lines with and without radiation, chemotherapy (doxorubicin), and peroxidative stress (H$_2$O$_2$) to see if astaxanthin treatment significantly alters cell death. Growth curves, cell death assays (methyl thiazolyl tetrazolium), flow cytometry, and soft agar growth assays were performed.

Astaxanthin treatment had no significant effects on radiation or chemotherapeutic or peroxidative cell death; however, it did significantly slow cell proliferation to various degrees in all three cell lines examined. To further elucidate the antioxidant capabilities of astaxanthin-treated cells, we used a commercial kit to measure total antioxidant potential of cell lysates, which showed modest antioxidant potential; however, the antioxidant potential of astaxanthin-treated cells was not enhanced beyond the natural upregulation of cellular antioxidant potential during cell stress, further supporting our cell death assays.

Overall, the results suggest that astaxanthin has the ability to significantly inhibit cell proliferation and growth of colonies in soft agar but that this inhibitory capacity is different, depending on the cell line examined, with no appreciable changes in cell cycle dynamics. Surprisingly, astaxanthin did not hinder the ability of radiation treatment, peroxidation, or doxorubicin to induce cell death. This suggests that the use of astaxanthin as a synergistic antiproliferative compound may be beneficial in neoplastic diseases. Further investigation into its use across various neoplasias and its mechanisms of action is warranted, particularly because the antioxidant capabilities do not seem to interfere with traditional cancer treatment options.
Ginkgo biloba extract (EGb) contains flavonoids, which have antioxidant, antiinflammatory, and antiviral properties and induce peripheral vasodilation. It is widely reported in the scientific literature that these properties are useful for elderly humans and animals. EGb can be administered to senior cats by adding it to their food, which ensures regular and continual administration. The presence of EGb flavonoids in this study was confirmed by high-performance liquid chromatography coupled with a diode array detector and mass spectrometry.

The goal of this study was to establish EGb flavonoid absorption in cats. Four privately owned, healthy cats of different sexes, ages, and breeds were included in the study. The standardized EGb (glycosylated flavonoids: 24%; terpene lactones: 6%) was administered at 20 mg/kg with 50 g of dry food (Bayer Fito Progres Cat Senior®) followed by qualitative and quantitative evaluation of quercetin in plasma at fixed times. Blood samples were collected in heparinized tubes before EGb administration in all four cats; after 1, 3, and 5 hours in two cats; and after 5, 7, and 9 hours in the remaining two cats. Plasma quercetin concentrations were established without knowledge of study group by liquid chromatography tandem mass spectrometry.

Table 1 shows quercetin concentration in plasma (ng/ml) in all four cats before and after the administration of EGb. Flavonoids from EGb were absorbed by cats, and their main metabolite, quercetin, was present in the blood for up to 5 hours. Furthermore, none of the cats showed any adverse effects (e.g., diarrhea).

This evidence encourages the use of EGb, administered alone or added to the diet, to improve cat wellness.
Although the intestinal microbiota of the human gut has received considerable attention as of late, very little is known about life in the feline gastrointestinal tract. Even less is known about the intestinal microbiota of growing kittens. Thus, our objective was to investigate the intestinal microbiota of growing kittens fed moderate-protein (MP) or high-protein (HP) diets using molecular qualitative and quantitative techniques.

Kittens consuming an HP diet (7 males from 2 litters) or MP diet (10 males from 4 litters) were evaluated. Kittens were weaned at 8 weeks of age and consumed the same diet as their dams. Fresh fecal samples were collected at 8, 12, and 16 weeks of age and stored at –80°C. Fecal DNA was extracted using the QIAamp DNA Stool Mini-Kit (Qiagen, Valencia, CA). DNA purity and concentration were determined using an ND-1000 NanoDrop spectrophotometer. Quantitative polymerase chain reaction (PCR) was used to quantify four microbial groups (Bifidobacterium spp, Lactobacillus spp, Clostridium perfringens, Escherichia coli) previously determined to be prevalent in cats. Mixed models of SAS (Cary, NC) were used to analyze quantitative PCR data. Qualitative analysis was performed on each sample using denaturing gradient gel electrophoresis with a 29% to 48% gradient to separate amplicons. DNA bands of interest were excised from the gel, extracted using the QIAquick Gel Extraction Kit (Qiagen), and sequenced using an ABI PRISM bigDye Terminator Cycle Sequencing Ready Reaction Kit and ABI 3730XL capillary sequencer (Applied Biosystems, Foster City, CA). 16S rRNA sequences were subject to BLAST search (GenBank) for identification.

The presence of Bifidobacterium spp and Lactobacillus spp was affected by diet, with kittens fed HP diets having lower (P < .05) counts than those fed MP diets. E. coli was also lower (P < .05) in kittens fed HP diets and was affected by age. Microbial differences in growing kittens suggest that prebiotic supplementation may be beneficial when feeding HP diets because of decreased Bifidobacterium and Lactobacillus populations.
The Actical Activity Monitor (AAM; Mini-Mitter, Bend, OR) is an accelerometer-based device that continuously measures movement for extended periods. This device might permit quantification of activity level and provide insight into the energy expenditure of pet dogs. In validating the use of the AAM in pet dogs, we wanted to determine the optimal sampling interval for this population.

Fifty-five clinically normal dogs were included. After obtaining the owners’ written consent and confirmation of no planned changes in their usual schedule, dogs had AAMs placed on collars around their necks. The collars were worn continuously for 2 weeks. Between-dog and day-to-day variability in activity counts that occurred over the 2-week period were evaluated using ANOVA. Weekdays and weekends were evaluated individually. Activity counts in week 1 versus week 2 were compared using paired t-tests to assess changes in the full 7 days as well as weekday and weekend activity counts.

There was significant variability in activity counts between dogs \( (P < .001) \). As a group, there was significant day-to-day variability in activity counts \( (P < .008) \), which was driven by increased activity counts on weekends compared with weekdays \( (P < .001) \). When comparing the first and second weeks of data, full-week and weekday activity counts were relatively stable \( (P = .31 \text{ and } P = .44, \text{ respectively}) \), but weekend activity counts were less so \( (P = .07) \).

Full week-to-week comparisons of activity showed no significant differences in counts in pet dogs that maintain their normal routines. When using the AAM to follow changes in groups of pet dogs over time (e.g., before and after an intervention), comparing dogs on a full 7-day basis offers the benefit of relatively stable estimates of activity in unchanged animals while including the days with the highest potential for changes in activity to occur (i.e., weekends).
The objective of this study was to quantify the intensity of different types of exercise by measuring heart rate and blood lactate level in sled dogs to better understand their aerobic capacities.

Fourteen Alaskan huskies (seven males, seven females; age: 2.4 ± 0.4 years old; weight: 21.9 ± 0.9 kg) were involved in mild (45-minute walk on leash), moderate (2-hour trot at 8 mph), and intense (6-minute run at 22 mph) exercise. Heart rate and activity intensity were measured using Actiheart monitors (Mini-Mitter, Bend, OR) during the exercise, preexercise, and postexercise periods. Blood lactate was measured before and after exercise.

Average heart rates during mild, moderate, and intense exercise were 159 ± 5.2, 179 ± 5.3, and 190 ± 2.7 bpm, respectively, and correlated with the increase in measured activity: 246 ± 15 counts/min (cpm), 454 ± 27 cpm, and 648 ± 27 cpm.

Preexercise lactate values for mild, moderate, and intense exercise were 0.7 ± 0.1, 1.5 ± 0.2, and 1.3 ± 0.2 mmol, respectively. Postexercise lactate values were low after mild and moderate exercise (0.8 ± 0.1 and 0.7 ± 0.1 mmol) but higher after intense exercise (4.4 ± 0.7 mmol). By regression, we identified the lactate threshold as being around 2 mmol, corresponding to 74% maximum heart rate. Walking and trotting heart rates (64% ± 1.8% and 72% ± 2% maximum heart rate, respectively) were beneath the lactate threshold, indicating aerobic pathways as the main supply of energy. Onset of blood lactate accumulation (OBLA, 4 mmol) occurred at 76.5% maximum heart rate. Intense exercise (77% ± 1% maximum heart rate) was just beyond OBLA, indicating a large contribution from anaerobic metabolic pathways.

The postexercise recovery times (time to recover preexercise heart rate) were equivalent after mild and moderate exercise but much higher after intense exercise (14 ± 2 and 15 ± 1 vs. 39 ± 2 minutes, respectively), reflecting the difference observed in postexercise lactate values and the theoretical higher oxygen debt after anaerobic intense exercise versus aerobic mild to moderate exercise.

These results validate the use of Actiheart monitors in working dogs to evaluate the intensity of exercise. Together with blood lactate values, these monitors give a clear picture of the scope of these dogs’ aerobic capacity in response to different types of exercise.
The influence of some foods on the animal psychophysical equilibrium, outlining a direct connection between nutrition and animal behavior, is well known. The purpose of this study was to evaluate the effects of isoenergetic and isonitrogenous diets on animal behavior and health. The isoenergetic diet was based mainly on vegetable proteins and was rich in carbohydrates (soybean meal added), whereas the isonitrogenous diet was based mainly on animal proteins and had a lower percentage of carbohydrates (respectively, dry matter: 91.5% vs 91.4%; protein: 27.0% vs 29.9%; fat: 14.5% vs 29.4%; crude fiber: 4.2% vs 0.5%; ash: 6.1% vs 13.4%; carbohydrate: 50.0% vs 17.3%; metabolizable energy: 4,003 vs 4,213 kcal/kg).

After a careful history and clinical and laboratory examinations confirmed good health and lack of evident behavioral disorders, 20 dogs (10 males, 10 females) weighing 10 to 30 kg and aged 1 to 7 years were randomly assigned to one of the two diets. Dogs were fed the individual diets for 40 days, including 30 days for the adaptation period and 10 days of observation. During the observation period, different stressful situations were simulated (e.g., handling, sudden light, loud noises, door opening). Each dog’s behavioral reaction was evaluated by an expert behaviorist and divided into one of two categories: aggressive reactions and nonaggressive reactions.

Differences between the groups did not reach statistical significance; however, a trend toward hyperexcitability, with a consequent increase in aggressive responses to some stimulations (e.g., sudden light: \( P = .067 \); sudden opening of a door: \( P = .06 \); sight of a cat: \( P = .070 \)), was observed in dogs fed the animal protein diet. These results suggest a possible benefit (at the limit of statistical significance) of a vegetable protein-based diet with a higher level of carbohydrates for reducing aggressive or excitable behavior in dogs.
Polymeric diets in place of elemental diets are not recommended for jejunal feeding in human patients, and at least one veterinary report recommends against polymeric diets for animal patients. We tested the hypothesis that polymeric diets could be delivered directly into the jejunum without causing diarrhea.

A thorough examination of medical records in a veterinary medical teaching hospital from 1999 to 2003 yielded 55 dogs and 6 cats that received at least 1 day of a polymeric diet administered directly into the jejunum via a surgically placed jejunostomy tube. Per hospital protocol, diluted diets were given in increasing strengths until full concentration was achieved, typically on the third day of administration. The liquid diet was discontinued when nutritional support via oral intake was satisfactory. Diarrhea was noted as a function of the presenting complaint, type and duration of diet use, signalment, and survival.

Three commercially available diets were administered: CliniCare (Abbott Laboratories, Chicago, IL; \( n = 40 \)), Ensure Plus (Ross Laboratories, Columbus, OH; \( n = 16 \)), and Jevity (Ross Laboratories, Columbus, OH; \( n = 1 \)); one group received a combination of two of these diets (\( n = 4 \)). Mean duration of use was 4.5 days (range: 1 to 13 days). The case of longest duration received concurrent Ensure Plus and Jevity without developing diarrhea. Diarrhea occurred postoperatively in two dogs 1.5 and 6 days after initiation of jejunal feeding, respectively. In one of these cases, diarrhea was a presenting complaint. Forty-five animals survived, 11 died or were euthanized, and 5 had incomplete follow-up.

Results of this study indicate that polymeric diets delivered directly into the jejunum, although not formulated for that use, can be administered for postoperative nutritional support without causing diarrhea.
Hair growth in adult short-haired cats shows a strong seasonal pattern, with maximal hair growth rates in late summer and minimal rates in late winter. The timing of the hair growth cycle is such that the densest and sparsest coats are produced during the coldest and warmest periods of the year, respectively. This study aimed to investigate differences in hair growth patterns between short- and long-haired domestic cats.

Hair growth rates in 11 short-haired (8 male, 3 female) and 7 long-haired (2 male, 5 female) adult cats 1.4 to 6.8 years of age born at the Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand) were determined throughout the year using the midside patch technique. Cats from six litters containing both long- and short-haired individuals were used in this year-long study. Hair was shaved and collected at monthly intervals and weighed before the average diameter of the hair sample was measured using an Optical Fibre Diameter Analyser (BSC Electronics, Attadale, Australia).

As previously reported, the midside hair growth rate in short-haired cats showed a strong seasonal pattern. Maximum hair growth occurred in late summer (273 μg/cm²/day), and minimum hair growth occurred in late winter (29 μg/cm²/day). In contrast, the hair growth rate in long-haired cats showed a less pronounced seasonal pattern, with an average maximum growth of 290 μg/cm²/day in late summer and an average minimum growth of 100 μg/cm²/day in late winter. The average diameter of the coat samples showed that the short-haired cats had coarser coats (28.54 μm in summer; 30.15 μm in winter) than did the long-haired cats (24.13 μm in summer; 17.37 μm in winter).

This study shows that long-haired cats grow more hair during the year and show a less seasonal hair growth pattern than short-haired cats, although the timing of the hair growth cycle is similar.

REFERENCE

Studies of human monozygotic and dizygotic twins have documented quantitative genetic contributions to phenotypic expression of clinical chemistry variables. We evaluated quantitative genetic aspects of phenotypic expressions of erythrocyte, clinical chemistry, and acid–base measures in domestic cats (*Felis silvestris catus*).

The metrics used for this study are part of a large database that is maintained to support nutrition research. To establish single representation in the database for these analyses, sequential data over healthy lifetimes of individual cats were expressed as the mean overall lifetime analyses for each chosen variable. This procedure made available data from 564 cats for erythrocytic metrics, 444 to 530 cats for serum clinical chemistry, and 629 cats for venous acid–base metrics. Extreme (nonphysiologic) values were removed, and non-normal traits were log-transformed. The “polygenic” function of SOLAR (sequential oligogenic linkage analysis routines) was used to estimate heritability as the ratio of additive genetic variance to total variance. This procedure estimates additive genetic variance by relating the additive genetic relationship matrix (2x coefficient of coancestry between pairs) to the phenotypic covariance. Multiple regression techniques were used to adjust for diet within nutrition study in the database. Inbreeding in this colony was minimal.

Heritabilities for erythrocyte, clinical chemistry, and acid–base variables ranged, respectively, between 0.41 and 0.69, 0.13 and 0.78, and 0.23 and 0.59 (P < .05). Some observations merit additional comment. The high heritability of the serum alkaline phosphatase phenotype likely explains frequently observed smaller disease-related responses in cats compared with other species. However, the quantitative genetic signals that were recognized for venous acid–base metrics were quite surprising.

The physiologic implications of similar heritabilities among species for the same variable may be different, dictating caution with interspecies phenotypic comparisons. Minimally, these data indicate that differential heritability of clinical chemistry metrics should be considered in health screening.
Hyperthyroidism is a common problem in geriatric cats. As part of a larger study of aging in cats, we examined the effect of age on thyroid hormone (T₄) concentrations as well as the prevalence of thyroid pathology postmortem.

Fifty-nine cats ranging in age from 8 to 15 years old (average age: 11.6 ± 2.3 years old) at the start of the study were evaluated for up to 7 years. The cats had no evidence of hyperthyroidism based on baseline T₄ concentration or physical examination. T₄ levels along with other routine health parameters were evaluated and physical examinations performed periodically until the cats’ natural deaths (average age of death: 15.4 years). After each cat’s death, a full necropsy was performed and both thyroid glands were submitted for histopathology.

Twenty-one cats (35.6%) had evidence of thyroid hyperplasia or adenoma on histopathology. Of these, only seven cats exhibited serum T₄ levels above the reference range at some point during the preceding years (average age at diagnosis: 14.8 ± 2.3 years). Four of the seven cats were diagnosed with hyperthyroidism by physical examination, clinical signs, and consistently elevated T₄ levels. Aging had no significant effect on serum T₄ level in this study; however, a difference between cats with hyperthyroidism (as evidenced by histopathology) and those without hyperthyroidism was noted (P = .097). Among cats that developed thyroid disease, T₄ levels gradually increased over time (P = .055); nonhyperthyroid cats showed no change with age.

In conclusion, serum T₄ level tends to increase with age in cats with thyroid pathology but does not change in cats without thyroid pathology. Based on these data, a consistent increase in T₄ level over time strongly suggests developing thyroid disease, but a large number of cats with thyroid disease have T₄ levels within the reference range.
Many species show a similar decline in immune function with age; however, little research has been done in cats. The goal of this study was to extend the information available on age-related changes in immune function in domestic cats and to compare our results with those previously reported.

The study was conducted at the Centre for Feline Nutrition at Massey University in Palmerston North, New Zealand, over a 28-day period. Whole-blood samples were collected from 138 domestic short-haired cats (71 male, 65 female) aged 7 months to 13.5 years. The cats were fed a variety of commercial moist foods (approved by the Association of American Feed Control Officials), with water freely available.

Samples were analyzed for expression of cell surface markers (CD4+ cells, CD8+ cells, B cells, CD11b+ cells), phagocytic activity, and mitogen-induced lymphocyte proliferation (concanavalin A [ConA], phytohemagglutinin antigen [PHA]). Age-related trends were assessed by simple linear regression analysis. Detailed family trees were available, and hereditary effects on immune function parameters were also determined.

Results showed a significant ($P = .0001$) age-related decline ($R^2 = .25$) in the phagocytic activity of peripheral blood leukocytes. There were no age-related trends in the relative percentages of T-helper cells (CD4+), cytotoxic T cells (CD8+), or granulocytes (CD11b+). There was a decline ($P = .02$) in the relative percentage of B cells and a decrease ($P = .011$) in lymphocyte-proliferative responses to stimulation with ConA with age; however, no change in lymphocyte blastogenic responses to PHA was observed. Parentage, particularly the father, had significant effects on phagocytic activity, percentages of CD8+ cells, CD4:CD8 ratio, and lymphocyte-proliferative responses to ConA.

Unlike other studies, we did not see significant changes in the level of expression of CD4+, CD8+, or CD11b+ cells or proliferative responses to PHA, possibly due to the age range or the genetic profile of the population studied. Decreases in both percentage of B cells and proliferative response to ConA were similar to those previously reported.
In vivo methods for determining the digestibility of companion animal diets generally involve either total fecal collection or using an indigestible marker. The total fecal collection method is labor-intensive and prone to sample losses. Therefore, using indigestible markers is generally preferred, particularly in dogs. When using indigestible markers, the diet must be ground to facilitate the homogeneous mixing of the marker into the diet; however, little work has been done to investigate whether this change in dietary form has any effect on digestibility.

A preliminary study was carried out to compare the nutrient digestibility of two different forms of a dry diet (unground vs ground) in cats and dogs. Eight male cats (3 to 7 years of age) from Massey University’s Centre for Feline Nutrition were fed a dry Association of American Feed Control Officials (AAFCO)–approved diet in either ground or unground form. In a crossover design, the cats received each diet for 12 days, which included a 7-day adaptation period followed by a 5-day total fecal collection period. The diet was fed according to maintenance requirements, and water was available ad libitum. Feed intake and fecal output were measured. Feces from each cat were subsampled, freeze-dried, and analyzed for gross energy and protein.

Similarly, six male and six female dogs (3 to 8 years of age) from Massey University’s Canine Unit were fed either ground or unground AAFCO-approved dog biscuits for a total of 12 days. Sample collection, preparation, and analysis were also carried out as described in the feline study. Preliminary data showed no difference in digestibility between the ground and unground diets in cats (respectively, energy: 86.4% vs 86.7%; protein: 82.7% vs 82.8%). In dogs, however, dietary form did appear to affect digestibility (respectively, energy: 82.81% vs 84.3%; protein: 78.2% vs 80.1%).
The objective of this study was to determine the chemical composition and protein and fiber disappearances of corn protein concentrates (CPC1, CPC2) and corn fiber (CFn), novel coproducts from the ethanol industry, compared with conventional plant protein and fiber ingredients used in the pet food industry. Novel corn coproducts were produced from a pilot modified wet milling plant.

Crude protein values for CPC1 and CPC2 were 57.3% and 49.7%, respectively. Total dietary fiber was 29% for CPC1 and 23.5% for CPC2. Acid hydrolyzed fat and gross energy were similar for these ingredients. Crude protein disappearance after 6 hours of incubation in an HCl/pepsin solution was highest for soybean meal (SBM) (53.3%), followed by corn gluten meal (CGM) (49.3%), distillers dried grains with solubles (DDGS) (49.0%), CPC2 (29.3%), corn germ meal (CGeM) (25.3%), and CPC1 (24.9%). After an additional 18 hours of incubation (24 hours total) with porcine pancreatin, CGM had the highest corn protein disappearance (94.1%), followed by SBM (87.2%). CPCs had corn protein disappearances of 77.5% (CPC2) and 74.1% (CPC1).

Crude protein concentration ranged from 0% (Solka Floc [SF]; International Fiber Corporation, St. Louis, MO) to 11.0% (CF control 1 [CFc1]). Total dietary fiber was highest for SF (100%) and lowest for beet pulp (68.8%). Corn fibers had intermediate total dietary fiber values. Acid hydrolyzed fat concentrations ranged from 0.8% (SF) to 6% (CFc1). Gross energy values were very similar among corn fiber sources. Organic matter disappearance was lowest for SF in the hydrolytic (−6.5%) and fermentative stages (−2.1% and −1.6% at 8 and 16 hours, respectively) and highest for CFc1 and beet pulp in the hydrolytic stage. Beet pulp was the only fiber source with significant fermentation (17.7% after 16 hours). CFc1 and CFc2 had intermediate fermentation values (5.7% and 5.3%, respectively), but CFc1 and CFc2 were higher than CFn (3.0%). Substrate versus time interactions were significant (P < .05) for organic matter disappearance.
Understanding the impact of different processing methods in the manufacture of fiber-rich corn coproducts is a precondition of their potential use as fiber sources for dogs. This experiment examined total tract nutrient digestibility and fecal characteristics of adult dogs fed selected fiber-rich corn coproducts from the ethanol industry.

Native corn fiber (NCF), NCF with fines, hydrolyzed corn fiber (HCF), and hydrolyzed extracted corn fiber (HECF) were included as fiber sources in a commercial-type diet matrix with poultry byproduct meal and brewer’s rice as the main ingredients and chromic oxide (0.2%) included as a digestion marker. Beet pulp (BP) was used as a positive control treatment.

Diets were fed to 15 beagles in a partially balanced incomplete block design with two blocks of 12 days, including 8 days for diet adaptation and 4 days for fecal collection.

The average daily food intake, fecal production, fecal scores, and fat and crude protein digestibilities were not significantly different among treatments. Body weight and body condition score remained unaltered throughout the duration of the experiment. Apparent dry matter (DM) digestibility coefficients were high, with the NCF treatment having a small but statistically higher value compared with the remaining treatments except for the NCF with fines. Dogs fed BP, HCF, and HECF had lower DM digestibilities compared with those fed NCF but not compared with dogs fed NCF with fines. Apparent total dietary fiber digestibility was higher for NCF, BP, and HECF treatments, but BP and HECF were no different than NCF with fines and HCF treatments.

Results of this experiment suggest that incorporation of corn fibers at the 7% inclusion level, when substituted for BP in diets of healthy adult dogs, does not dramatically impact nutrient digestibility, food intake, or fecal characteristics.
Given the need to develop in vitro methods to simulate digestion of pet food, we carried out an investigation to examine the proteolytic activity of *Streptomyces griseus* protease to determine its suitability to estimate protein digestibility for dogs.

In vitro protein digestibility was measured (according to work by Coblentz and coworkers¹) on four dry concentrates for large-breed puppies using *S. griseus* protease (sigma EC 3.4.24.31). Residual crude protein was determined after 0, 24, and 48 hours of incubation.

Protein losses at time 0, without incubation, corresponded to the soluble protein fraction. The chemical composition results were similar among the pet foods (average values for crude protein: 27.5 ± 1.27; ether extract: 11.6 ± 3.99; crude fiber: 3.54 ± 0.62%). The ranking of pet foods for soluble protein (time 0) was 1 and 4 > 2 > 3 (*P* < .01). At 48 hours, pet food 3, which was characterized by the lowest soluble protein value, showed the highest digestibility (Table 1).

These results correspond with previous data obtained in vivo in a growing trial conducted on 24 German shepherd puppies² in which pet foods 1 and 4 allowed significantly (*P* < .01) higher weight gains from the age of 60 days. In each case, all registered daily weight gains (from 66 to 93 g/d and from 93 to 150 g/d in the period 1 to 60 days and 60 to 90 days, respectively) are included in the ranges indicated by Debraekeleer and coworkers³ for the periods 1 to 2 and 3 to 5 months of age in puppies with an average adult body weight of 30.5 kg. Considering the insufficient enzymatic production and development of gut microbial population in the period immediately after weaning, the availability of soluble protein, which is immediately absorbable and utilizable, could improve nutrient availability.

These preliminary results demonstrate the validity of this rapid and reliable in vitro procedure in estimating the protein digestibility of dog foods.

### References


### Table 1

<table>
<thead>
<tr>
<th>Pet Food</th>
<th>Time 0</th>
<th>24 hr</th>
<th>48 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>65.0 ± 0.05</td>
<td>28.1 ± 0.40</td>
<td>6.55 ± 0.75</td>
</tr>
<tr>
<td>2</td>
<td>51.3 ± 1.06</td>
<td>26.7 ± 0.57</td>
<td>11.4 ± 1.02</td>
</tr>
<tr>
<td>3</td>
<td>27.3 ± 0.88</td>
<td>27.9 ± 0.56</td>
<td>25.2 ± 0.59</td>
</tr>
<tr>
<td>4</td>
<td>62.7 ± 0.11</td>
<td>28.9 ± 0.84</td>
<td>8.47 ± 0.46</td>
</tr>
</tbody>
</table>

A, B, C = *P* < .01; a, b, c = *P* < .05.
Little epidemiologic data exist about pet dog feeding habits and management in Europe.1–3 Studies show that feeding patterns, environmental conditions, and pet management all affect animal health, behavior, and welfare.4,5 Between 1995 and 2005, a retrospective epidemiologic study was carried out to analyze trends in feeding habits of pet dogs in Catalonia, Spain. The specific goals this study were to determine (1) the effect of environmental factors on the dogs’ feeding habits as well as (2) the relationship between the type of food provided and the composition of the family.

A total of 1,000 dogs (Canis familiaris) were observed for the purpose of behavioral and clinical evaluation at the Clinical Behavioral Service of the Veterinary Teaching Hospital of the Autonomous University of Barcelona. Recorded information included sex, age, breed, type of food (dry, wet, mixed), mode of administration (meal fed, free choice), family composition (family size and age, presence of children or other pets in the home), environment (apartment or house, urban or rural location, presence of a garden or terraces), exercise (walk frequency and duration), and behavioral problems. To determine the relationship between dietary habits and management and behavioral problems in the dogs, data for 500 dogs were analyzed by chi-square test (SPSS 12.0). Study results indicate that the most common types of food consumed were medium- to high-quality dry foods (74%) fed twice daily. The typical owner in the study was a young couple with no children (45%) living in an apartment (>80%).

Some management and dietary characteristics were found to be related to canine behavioral problems and welfare. Free-choice dogs showed less food-related aggression toward family members than meal-fed dogs (P < .05). This could be because when food is continuously available, dogs perceive it as a less valuable resource than when it is offered only a few times a day. Moreover, it appears that there is a relationship between dog environment and separation anxiety: dogs with more available space when alone show less anxiety than dogs living in a little room (P = .001).

Dog management (i.e., exercise level, including frequency and duration of walks) and aggressive behavior toward family members could be related: dogs with a reduced exercise level (0 or 1 walk/day) were more aggressive toward family members than dogs with a higher exercise level (3 or 4 walks/day; P = .05). This result could be partially explained by the increase in serotonin turnover caused by regular exercise, as shown in humans and other species.6,7

This preliminary study suggests that diet, feeding pattern, and management may play a role in the development of behavior problems in dogs.5,8,9

REFERENCES
The SmartPill GI Monitoring System (SmartPill Corporation, Buffalo, NY) provides ambulatory testing for gastrointestinal (GI) tract pressure, temperature, and pH; gastric emptying time (GET); combined small and large intestine transit time; and total GI transit time. This device, although extensively tested in humans, had not been previously evaluated in dogs. The objective of this study was to evaluate the feasibility of measuring GI transit time and gastric pH in dogs.

Eight (four male, four female) clinically healthy Labrador retrievers were used in the study. After an 8- to 10-hour fast, the dogs were fed one-third of their daily energy requirement. The capsule was administered immediately after food consumption (time 1 [T1]). The dogs were fitted with mesh jackets with pockets to hold the data receiver and then returned to their runs. The dogs were monitored until the unit was expelled in the feces (time 2 [T2]), and total GI transit time was defined as time from T1 to T2. Gastric emptying time was measured from T1 until the onset of a sudden and sustained increase of at least 3 pH units above baseline. Small and large intestine transit time was calculated as total GI transit time minus gastric emptying time.

The capsule was successfully administered to and retrieved from the stools of all dogs. There was a difference ($P < .05$) in gastric emptying time between sexes, averaging 15.17 hours for females versus 12.58 hours for males. The average small and large intestine transit times were 18.77 hours for females and 21.84 hours for males, and the average total GI transit times were 33.45 hours for females and 34.29 hours for males. The mean gastric pH was 2.14 for females and 2.34 for males.

From the results of this study, we can conclude that the SmartPill technology is a novel, noninvasive method for assessing several aspects of GI function. This technology has the potential for clinical and research applications to study the effect of diet or nutrients on the canine GI tract.