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Nutrient-gene interactions and their role in complex diseases in dogs

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The dog was the first nonrodent mammalian animal chosen for genome sequencing by the National Institutes of Health, with the first draft of the canine genome completed in July 2004. Sequencing of the canine genome was a remarkable feat that has advanced canine research into a new realm with unlimited potential. In addition, advances in molecular biology, nanotechnology, and computer science have supplied scientists with an extensive set of experimental tools. For veterinarians, and particularly for veterinary nutritionists, these advances provide new avenues for companion animal research. Incorporating sequence data and genomic technology into research initiatives will greatly enhance our understanding of canine health and disease.

Nutrition and Complex Diseases

The information obtained by sequencing the canine genome will likely have its greatest initial impact on the study of the >400 genetic diseases that have been identified in dogs. However, although genetic diseases are a considerable problem in many dog breeds, the vast majority of diseases affecting the health and longevity of dogs are more complex in nature. Thus, for many of these diseases, although genetic factors play a role, disease expression is also affected by age; sex; and environmental factors such as diet, activity level, and pathogen exposure. Similar to the case for humans, advances in veterinary care and nutrition have resulted in a steady increase in canine lifespan over the past few decades. Unfortunately, the trends toward more sedentary lifestyles, greater incidence of malnutrition (most commonly overnutrition), and increased risks of complex diseases in humans are also seen in dogs. Thus, the prevalence of complex diseases, including many diseases similar to those seen in humans, continues to increase. Thus, studies designed to identify strategies to aid in the prevention and treatment of diseases such as obesity, hip dysplasia, diabetes, intestinal tract disorders, and cancer are of high priority. For all of these, results may be improved through the inclusion of genomic technologies.

By definition, phenotype is a result of genotype, environment, and the interaction between genotype and environment. Nutritional status is one of the primary environmental factors influencing health and disease (ie, phenotype). In fact, diet plays a role in the development of several common highly complex diseases in humans and dogs. In 2002, for instance, 7 of the top 10 causes of death for people in the United States were diseases shown to result from complex interactions, including those in which nutrition played some role. Together, heart disease (28.9% of all deaths), cancer (22.9%), stroke (6.6%), chronic lower respiratory tract disease (5.5%), diabetes mellitus (2.8%), Alzheimer’s disease (2.6%), and renal disease (1.6%) represented >70% of total deaths in the United States. Osteoarthritis, gastrointestinal tract diseases, mental and behavioral disorders, alcoholism, and drug addiction are other complex diseases that are clinically important because they often are seen in combination with other diseases and affect quality of life. In dogs, the prevalence of complex diseases, including many diseases similar to those seen in humans, continues to increase.

The genome sequencing effort, coupled with continued advances in biotechnology, has enhanced our ability to measure gene transcripts (mRNA), proteins, and metabolites. These advances have not only improved the accuracy of tests and the limits of detection but have also led to high-throughput techniques that can provide researchers with a holistic view of a cell, tissue, or an entire organism. For example, DNA microarrays enable measurement of thousands of gene transcripts simultaneously, providing researchers with a global view of gene expression. Because complicated networks of genes and biological pathways contribute to the development of complex diseases, a holistic appreciation of gene expression may enhance our understanding of disease development or help predict responses to various drug and dietary treatments. Moreover, analysis of microarray data allows identification of gene expression profiles or “disease signatures” that may be useful for diagnostic purposes or for the development of effective treatments. Gene expression

ABBREVIATIONS

| SNP | Single nucleotide polymorphism |
| IL-1 | Interleukin-1 |

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and protein profiles have already been used to molecularly describe inflammatory bowel disease,
hepatic carcinoma and liver diseases, breast cancer, prostate cancer, and ovarian cancer in humans.

Gene expression profiles may be particularly useful in the treatment of patients with complex diseases. For example, many human patients with breast cancer are classified as having the same stage of disease on the basis of histologic criteria but respond quite differently to treatment. In addition, although chemotherapy and hormone therapy reduce the risk of metastasis in breast cancer patients by approximately a third, recent studies have shown that 70% to 80% of patients survive without these treatments. Thus, a substantial portion of patients do not benefit from these treatments but risk developing adverse effects associated with them. A recent study identified genes indicative of a poor prognosis (eg, genes regulating cell cycle, invasion, metastasis, and angiogenesis) that may be useful in developing targeted treatments and predicting disease outcome. Such prognostic profiles provide a powerful tool to tailor treatment of breast cancer patients to maintain overall effectiveness while reducing costs, both in terms of adverse effects and health care expenditures. Although such techniques have been used sparingly in dogs thus far, great potential exists for their use in diagnosis and treatment.

Given the recent advances in molecular biology and bioinformatics, researchers now have the ability to study the impact of nutrition on phenotype in fine detail, identifying nutrient-responsive genes and biological pathways, important nutrient-gene interactions, and genomic markers of disease. The interplay between nutrients and genes, and its impact on health and disease, is becoming increasingly evident. Whereas genotype may affect the absorption, metabolism, and transport of nutrients in the body, nutrition affects epigenetic, genomic, and proteomic events. Thus, nutrient-gene interactions have the potential to play a substantial role in the development of complex diseases.

Nutrigenetics

The term “nutrigenetics” may be used to describe the effect of genotype on nutrient absorption, metabolism, and transport. On average, each human gene is thought to contain approximately 10 deviations in its code from the “standard” code for that gene. Although these DNA variations, known as polymorphisms, may have no direct effect on gene function, a few may have serious health implications. In particular, a considerable number of polymorphisms are thought to have mild effects on protein functionality, contributing to the variety of diet-related responses observed in a population. Because it is generally assumed that much of the quantitative genetic variation is traceable to SNPs or insertion or deletion polymorphisms, SNP maps are now being generated for several species, including dogs. Debusk et al have compared SNPs to “variations in a recipe.” Each gene is a recipe for a specific protein or group of proteins that regulate biological functions or serve as structural building blocks for tissues. Some SNPs change the recipe, resulting in a different quantity of the protein being produced or an alteration in the protein structure. Genome sequencing and SNP mapping have shifted the search for disease genes from linkage studies, which require multigenerational families, to association studies, which scan the entire genome.

Assessment of SNPs in humans has already resulted in identification of numerous allelic variants associated with an increased disease risk, many of which are applicable to companion animals. An example is the association between allelic variation in the gene for IL-1 and the incidence of inflammatory disease. In humans, IL-1 variants associated with increased levels of inflammatory mediators are also associated with increased severity of several chronic diseases, including Alzheimer’s disease and periodontal disease. This proinflammatory IL-1 genotype pattern has also been linked with increased risk of acute coronary events, increasing the risk of heart attack 4-fold in individuals homozygous for a specific IL-1 SNP. Inflammation is emerging as an important component of many chronic diseases, including obesity, cardiovascular disease, type 2 diabetes, osteoporosis, periodontal disease, rheumatoid arthritis, degenerative neurologic disorders, and inflammatory bowel disorders. Although individuals with IL-1 genotypic variants are not the only ones at risk for inflammatory disease, knowledge of the role of IL-1 genotypic variants can be used to identify individuals who have a high risk of developing these disorders and justify the search for additional genetic variants that increase the risk of disease. This area is a prime target for development of disease prevention and treatment strategies in humans as well as in companion animals.

Although the average number of polymorphisms per gene is unknown in dogs, many canine genes have already been shown to be highly polymorphic, including genes for the major histocompatibility complex, immunoglobulin A, the cytochrome P450 enzymes, and the serotonin (3-hydroxytriptamine) receptor. To date, the presence of polymorphisms has been tested in only a few canine genes involved with nutrition or metabolism. This is expected to drastically change in the future, however, as the canine genome sequence data required to perform these analyses are now available in public databases. Identifying individual SNPs or clusters of SNPs that affect nutrient and drug metabolism or immunologic status may take us one step closer to developing personalized canine diets and medicines, similar to efforts in human medicine.

Because canine SNP maps are still under development, a considerable amount of research is needed before reliable canine SNP tests are available. Nevertheless, while it may be several years before SNP tests are used in a clinical setting, their utility can be illustrated by use of the example of copper toxicosis, an autosomal recessive disease commonly diagnosed in Bedlington Terriers. Owing to a defect in copper metabolism, affected dogs slowly accumulate copper in hepatic tissue over time. Most dogs die between 3 and 7 years of age if untreated, and in the past, the most effective test for the disease was to measure hepatic copper concentration at 1 year of age via liver biopsy. Although this method may detect diseased animals, it
is problematic because hepatic copper concentration may already be dangerously high at this age and carriers of the disease are not detected. A genetic test that could detect affected and carrier dogs at birth would enable breeders and owners to adjust dietary copper content early in life and make educated breeding decisions. Although copper toxicity has been studied for > 25 years in dogs, the genetic defect in the MURR1 gene responsible for the disease was identified only a few years ago. It is now evident, however, that several other genetic variants are linked with the disease in various breeds, complicating the matter further. Nonetheless, as all genetic variations (ie, SNPs) associated with the disease are identified, a single test designed to identify all variants simultaneously may be developed.

Although designing tests to detect single diseases would be relatively simple, future SNP tests may screen for hundreds or thousands of genes that are identified as being associated with disease. In the future, veterinary clinics may present owners with the option of using such SNP tests to genetically assess young puppies and kittens during initial health checkups. Identification of affected and carrier animals may help in managing the disease and making informed breeding decisions. In addition to detecting monogenic diseases (ie, diseases caused by a single genetic defect), results may also be used to provide a “disease susceptibility score” for complex genetic and metabolic diseases. On the basis of this score (genotype), diet and management strategies may be devised to minimize the likelihood that disease will develop.

**Epigenetic Inheritance and Metabolic Programming**

Gene expression is regulated through 2 primary mechanisms. The first is labile regulation, by which numerous factors alter production of mRNA in response to a given stimulus (eg, fasting vs postprandial state). The second is epigenetic regulation, which refers to control of gene expression that is relatively stable and can be transmitted from parental cells to daughter cells via meiosis or mitosis. Epigenetic inheritance is a result of heritable changes in gene expression and regulation that are independent of changes in DNA sequence. Epigenetic inheritance is influenced by numerous factors, including diet and hormone concentrations, during early development and throughout life.

The most frequently studied and best-understood markers of epigenetic inheritance are DNA methylation patterns. Modification of histones, DNA-binding proteins that assist in DNA folding and are involved with mRNA production, is also known to contribute. In general, methylation near gene promoter regions is associated with silencing of genes (ie, transcription is blocked), whereas histone acetylation is associated with gene activation.

The best examples of epigenetics involve cancer and were identified as early as 1983. Recent studies have also linked epigenetic inheritance with lifelong metabolic status, a process termed “metabolic programming.” Research in metabolic programming continues to gain interest because of its link to chronic diseases such as obesity, diabetes, heart disease, and behavioral disorders. Evidence now exists supporting the notion that both prenatal and postnatal nutrient intake play a role in metabolic programming. The fetal origins hypothesis proposed by Barker was based on the observation that the disproportionate size of newborn children at birth resulting from an adverse intrauterine environment was highly correlated with an increased risk of adverse adult-onset health outcomes. Epidemiologic evidence has substantiated this hypothesis, demonstrating that maternal dietary or placental insufficiency “programs” susceptibility to later development of chronic diseases.

Dietary manipulations during the early postnatal period have also been shown to lead to physiologic and anatomic changes that have a lifelong impact on metabolism. Rodent studies have reported the metabolic programming of several organs (eg, pancreatic islets, hypothalamus, liver, and muscle) that were associated with an increased incidence of adult-onset diseases. In rats, for example, the lifetime growth trajectory can be programmed by reducing litter size, so that pups in small litters receive more milk than pups in large litters. In 1 study, permanent programming was accomplished after only 3 weeks of such dietary intervention. Other studies have found that malnutrition induced by either protein or caloric restriction during gestation and lactation in female rats was accompanied by adult-onset diseases in the offspring.

Evidence now exists supporting the notion that epigenetic alterations occur throughout life, not just during early development. By studying global and locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins, Fraga et al concluded that widespread “epigenetic drift” is associated with human aging. These researchers reported that while twins were epigenetically indistinguishable during early years of life, older twin pairs exhibited remarkable differences in the amount of DNA methylation and histone acetylation that was present. The pattern of DNA methylation (ie, the amount of hyper- or hypomethylation in a specific chromosomal region) was also substantially more different between elderly twins than between young twins. While this study did not identify individual genes that were affected by alterations in methylation, it does suggest that these epigenetic alterations play an important role in the aging process, justifying further study.

Some of the most profound proof of metabolic programming in humans has been obtained by studying survivors of the Dutch famine during World War II. Offspring of women who had restricted access to food during gestation were reported to have an increased incidence of obesity, glucose intolerance, and hypertension as adults. Lower birth weight was also associated with a higher risk of adult-onset diabetes in this population. The most remarkable finding from these studies was that an increased risk of disease was still detected in the third generation (ie, the grandchildren of the women who survived the famine), demonstrating that maternal diet may affect not only a women's...
offspring but also future generations. Studies\textsuperscript{38-40} of disadvantaged populations in the United States, South Africa, India, and the Caribbean have reported similar findings.

While results of these studies have suggested that prenatal undernutrition may be playing a role in today's obesity epidemic, others have focused on the effects of overnutrition during the neonatal period. Kaati et al\textsuperscript{41} studied the effects of overeating during the slow growth period, before the prepubertal peak in growth, and its influence on risk of death from cardiovascular disease and diabetes in offspring. Results from this study demonstrated that when food was not readily available during the father's or grandfather's slow growth period, offspring tended to be protected from cardiovascular disease and diabetes as a cause of death. However, the diabetes death rate was increased 4-fold if the paternal grandfather was exposed to a surfeit of food during the slow growth period.\textsuperscript{45} Research in rodents has produced similar findings. Taken together, results of human and animal research suggest that both under- and overnutrition, in utero or during the early postnatal period, may result in permanent changes to anatomy or physiology that affect lifelong metabolism.

Numerous biological mechanisms have been proposed to contribute to metabolic programming, and various nutrients and hormonal factors are believed to have a role. As stated, DNA methylation patterns are the best-understood form of epigenetic inheritance. Methylation occurs on both strands of palindromic CpG dinucleotides (the “p” denotes the intervening phosphate group in the dinucleotide) and is critical for mammalian development. Specific patterns of CpG methylation are established in early development and are propagated during DNA replication by DNA-methyltransferase-1.\textsuperscript{43} Methylation may impact transcription directly by influencing the binding of methyl-sensitive DNA-binding proteins or indirectly by influencing regional chromatin conformation.

Early nutrition is able to influence DNA methylation because 1-carbon metabolism, which ultimately provides methyl groups for all methylation reactions, is highly dependent on dietary methyl donors and cofactors.\textsuperscript{44} Information on dietary sources of folate and other donor methyl groups provides the most compelling data on the interaction between nutrients and DNA methylation because they are directly involved in DNA methylation through 1-carbon metabolism. Waterland and Jirtle\textsuperscript{45} demonstrated the impact of dietary methyl donor supply on DNA methylation in agouti mice by supplementing diets with folate, vitamin B\textsubscript{12}, choline, and betaine during gestation and lactation. The fur of agouti mice can be yellow, brown, or a calico-like mixture, depending on the degree of DNA methylation. Offspring from dams fed a diet containing a rich supply of donor methyl groups had increased CpG methylation, altering the coat color of these animals. Mice receiving supplements had offspring with mostly brown fur, whereas mice that did not receive supplements gave birth to mostly yellow pups. Although it is uncertain how these results may apply to humans or companion animals, they provide proof of concept and demonstrate the need for further study in this area.

It is well established that folate supplementation is an effective means for preventing most neural tube defects in newborn humans. However, results from 1 study\textsuperscript{46} suggest that oversupplementation of folate or other donor methyl groups may have unintended effects on the establishment of epigenetic gene-regulatory mechanisms during embryonic development. Because the agouti gene studied in that experiment controls coat color, it was relatively easy to detect changes associated with dietary treatment. However, it is unknown what other genes were affected in these mice and what impact those effects will have on long-term health.

Although folate and other donor methyl groups are known to have a direct impact on DNA methylation, many micronutrients and vitamins are known to be indispensable in DNA metabolic pathways.\textsuperscript{47} Dietary vitamin deficiencies (eg, deficiencies in vitamins B\textsubscript{12}, C, D, and E and niacin) and low mineral concentrations (eg, magnesium, iron, zinc, and selenium) have been reported to influence DNA damage or DNA methylation patterns.\textsuperscript{48} These nutrient imbalances likely affect gene expression and processes such as cell growth rates and tissue differentiation; they also likely play a role in cancer, the aging process, and various complex diseases.\textsuperscript{49} Many of the vitamin and mineral requirements for companion animals are still poorly defined.\textsuperscript{50} This lack of micronutrient requirement data, in combination with recent findings regarding epigenetics, underscores the importance of determining optimal dietary micronutrient concentrations, especially in gestating and lactating females.

Glucose is another nutrient thought to contribute to metabolic programming, as it has been shown to act as a low-grade mutagen\textsuperscript{51,52} and is suspected in the etiology of certain birth defects that develop during the first trimester in the offspring of diabetic mothers.\textsuperscript{53} Many researchers have studied the mechanism of action of glucose in rodents and have been able to metabolically program pancreatic islet function in rat pups by feeding a high-carbohydrate milk formula early in life.\textsuperscript{54} Consumption of this high-carbohydrate formula results in an immediate onset of hyperinsulinemia that persists throughout the period of dietary intervention. This hyperinsulinemic state results in increased hexokinase activity and increased gene expression of preproinsulin and related transcription factors and kinases in the pancreatic islets. Although the acute effects of feeding a high-carbohydrate diet were expected, the impact on long-term metabolism may not have been so easy to predict. In these experiments, the adaptations that occurred at a young age were permanently programmed and expressed in adulthood, leading to chronic hyperinsulinemia throughout life and adult-onset obesity. Similar to findings in humans, female rats fed the high-carbohydrate formula have been shown to spontaneously transmit the “high-carbohydrate phenotype” to their progeny.\textsuperscript{55} Kozak et al\textsuperscript{56} reported increased neuropeptide Y release from the hypothalamic paraventricular nucleus in rats fed a high-carbohydrate diet, suggesting that intraterine and perinatal programming of the central regulatory mechanisms may also be partially
Glucocorticoids and thyroid hormones have long been recognized to play crucial roles in regulating differentiation, growth, and metabolism. Nuclear receptors for lipid-soluble molecules, such as thyroid hormone, act predominantly by directly regulating transcription via DNA binding and chromatin remodeling (ie, histone acetylation modifications). Thyroxine, for example, is crucial for myelination and CNS development, with deficiency leading to severe mental and physical retardation. Although hormones play a crucial role in normal embryonic and early postnatal development, recent evidence suggests that perturbations in perinatal nutrition may metabolically program an individual for life.

Leptin is important in the development of the arcuate nucleus of the hypothalamus, a critical component of the forebrain pathways that regulates energy homeostasis. A recent review described evidence supporting the hypothesis that altered perinatal leptin concentrations have long-lasting effects on the formation and function of these pathways, which result in an increased susceptibility to obesity. The arcuate nucleus contains 2 subpopulations of neurons that play an important role in distributing leptin signals centrally. The first subpopulation coexpresses neuropeptide Y and agouti-related protein, both of which are potent orexigenic (appetite-stimulating) molecules. The other subpopulation expresses melanocortins (eg, α-melanocyte-stimulating hormone), which are anorexigenic in nature. Nutrient status during early developmental periods may alter these subpopulations, resulting in long-term effects on leptin signaling and energy homeostasis.

A paucity of information currently exists in regard to epigenetic inheritance in dogs. A preliminary study demonstrated that, as in humans, genomic hypomethylation was a feature of neoplastic cells in most dogs with lymphoma and in approximately a third of dogs with leukemia. Although the mechanisms are not understood at present, these results confirm that dysregulation of DNA methylation plays a role in malignant transformation of lymphoid cells in some dogs. Another study found that age at neutering had a significant effect on bone sarcoma risk in dogs. Dogs neutered prior to 1 year of age had a much greater risk of developing bone sarcoma than did sexually intact animals. Because DNA methylation patterns were not measured, however, it is unknown if hormonal exposure played a role in cancer development via epigenetic mechanisms.

Because so little research has been done in this area thus far, future canine research should focus not only on the role of epigenetics in cancer but also on its role in aging and chronic diseases, such as obesity and diabetes mellitus, that are common and known to be influenced by prenatal and early postnatal nutritional and hormonal status. As a means to curb human obesity, various prevention and treatment strategies are being proposed. For example, Kral has identified 3 critical periods to target obesity in young girls: the postnatal and infancy periods (ie, from birth to approx 5 years of age), when children are vulnerable to rapid growth, a precursor of childhood and adult obesity; the early school period, which is characterized by increasing nutritional independence from the mother and family; and menarche, when gonadal steroids exert strong influences on body composition related to adipose tissue growth and bone mineralization. Kral's strategy is focused on maternal education, diet, exercise, and behavior modification to avoid obesity in young females during these critical stages, when metabolic programming is particularly prominent. To combat the obesity epidemic in dogs, similar strategies may prove to be effective and deserve study. Evaluating dietary folate and donor methyl group concentrations in maternal diets may also provide valuable information in this area.

Nutritional Regulation of Gene Expression

As stated, gene expression may be regulated by labile processes controlled by transcriptional activators and repressors for which nuclear concentrations, covalent modifications, and subunit associations may fluctuate extensively. Most lipogenic genes, for example, are transcriptionally regulated, being affected by factors such as energy source (fat vs carbohydrate) and metabolic state (fasting vs fed). Nutritional status affects the amounts of fatty acid synthase, acetyl-CoA carboxylase, ATP-citrate lyase, and malic enzyme by causing large changes in the rate of transcription of the genes for these enzymes, enhancing mRNA concentration and, ultimately, enzyme protein synthesis. Because long-term changes in the activity of these enzymes are caused by changes in the amount of enzyme protein in the cell, alterations in the transcription rate affect enzyme activity. In addition to regulating transcription rate, nutrients may also alter mature mRNA concentrations through several posttranscriptional mechanisms, influencing mRNA stability, processing of pre-mRNA (ie, splicing or polyadenylation of pre-mRNA), and nucleocytoplasmic transport.

For example, the activity of the enzyme glucose-6-phosphate dehydrogenase, the rate-limiting step in the pentose phosphate pathway, is regulated by changes in the rate of mRNA processing. Finally, nutritional status may regulate some genes via translational or posttranslational mechanisms (eg, phosphorylation, acetylation, and glycosylation).

Until recently, changes in gene expression attributed to diet were thought to be mediated predominantly through hormones or the nervous system. However, recent research has demonstrated that macronutrients (eg, glucose, fatty acids, and amino acids), micronutrients (eg, iron, zinc, and vitamins), and their metabolites can regulate gene expression in a hormone-independent manner. Some of the earliest evidence demonstrating direct effects of nutrient intake on gene expression was reported by Blanchard and Cousins who found that almost 50 genes were up- or downregulated in zinc-deficient rats. Numerous bioactive food components, including vitamins, minerals, carotenoids, flavonoids, monoterpenes, and phenolic acids, are now thought to act as transcriptional factors directly affecting gene expression.

Macronutrients such as fatty acids are important in governing gene expression, having a strong direct influ-

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ence on cell differentiation, growth, and metabolism.68 Whereas many nutrients directly affect transcription rate, others act via indirect mechanisms. For example, dietary fiber may indirectly affect transcription rate through the effects of primary by-products of colonic bacterial fermentation (short-chain fatty acids), which act as secondary messengers. Xiong et al69 reported increased adipocyte leptin expression in cultured cells provided with physiologic concentrations of short-chain fatty acids. Because short-chain fatty acids produced in the colon are absorbed and enter the bloodstream, it is possible that fermentable dietary fibers indirectly aid in controlling obesity by decreasing appetite via leptin expression. Although only speculative at present, these mechanisms are currently being tested in our laboratory in vivo.

Although the posttranslational effects of nutrition have not been well-studied thus far, numerous food components likely affect phosphorylation or glycosylation rates and, thus, have an impact on posttranslational regulatory events. Consumption of bioactive compounds, such as the allyl sulfide components in garlic, is known to result in hyperphosphorylation of certain proteins, which may result in antiproliferative properties.70 Because the impact of phosphorylation status depends on the individual genes affected, further testing is required to verify that hyperphosphorylation is, in fact, beneficial. Technologic advancements continue to improve the speed and accuracy by which protein profiles are measured. Thus, the field of nutrionial proteomics (ie, the study of the effects of nutrient intake on posttranslational modifications of newly synthesized proteins) is expected to grow immensely in the coming decade.

Unlike studies of epigenetic inheritance, which may require long-term experiments involving several generations, studies of the effects of nutrition on gene transcription or translation may be very short, requiring only that measurements be made following consumption of a single meal or alter just a few weeks or months of a particular dietary regimen. Several canine experiments of this kind have been performed in the past decade. However, researchers have previously been limited to measuring a small number of genes in each study because of the lack of high-throughput techniques for analyzing canine samples and the limited amount of information available on the sequences of various canine genes. Thus, most of these studies have focused on a single gene or a small number of genes relevant to the disease or metabolic pathway studied. Canine genome mapping and sequencing initiatives during the past few years have generated the sequence data required for production of canine microarrays.

To date, only a handful of experiments in which canine microarrays were used to study nutrition and disease have been published. A few studies have used canine microarrays to differentiate healthy from diseased tissues, including studies of osteoarthritis,71 cancer,72 and dilated cardiomyopathy.73 By comparing cardiac tissue gene expression profiles in Doberman Pinschers with end-stage dilated cardiomyopathy versus healthy control dogs, Oyama and Chittur73 identified 167 genes that were differentially expressed in the affected dogs. Several functional groups, including genes associated with metabolism, gene expression, structure or motility, cell defense and stress responses, and cell signaling or communication, were identified. Although their results may not have an immediate impact on clinical treatment strategies, the authors were able to identify biological pathways associated with diseased cardiac tissue, enhancing our understanding of the disease and highlighting areas for future studies.

Other studies have focused on gene expression in response to various diets or drugs, measuring the effects of a high-fat diet and obesity-related hypertension on gene expression of cardiac tissues74,75 and the acute phase response to pharmaceuticals.76 Our laboratory is currently investigating the effects of age and diet on gene expression in the liver,77 colon,78 skeletal muscles,79 cerebral cortex,80 and adipose tissues of dogs. Given the current incidence of obesity in dogs, studies reported by Philip-Couderc et al74,75 are of particular interest. In their first experiment,74 these researchers reported numerous gene expression changes in cardiac tissues from obese versus control dogs, including changes in expression of genes associated with metabolism and cell signaling. Genes associated with cell proliferation, protein synthesis, and tissue remodeling also were differentially expressed, suggesting they play a role in the left ventricular hypertrophy that develops with feeding a high-fat diet to dogs and obesity.80 In their second experiment,75 kinetic analysis of cardiac gene expression was performed as obesity was induced with the feeding of a high-fat diet. In this experiment, changes in similar gene categories (eg, cell signaling and tissue remodeling) were identified. Using a bioinformatics tool designed to link microarray data with biological pathways, the authors found that expression of several genes in the transforming growth factor-β pathway was altered, suggesting that this pathway has a role in cardiac adaptations to obesity. These are only a few examples of the research currently underway. As researchers continue to adopt these techniques and focus on studying canine nutritional genomics and proteomics, our understanding of canine metabolism, and the impact of nutrition on health and disease, will be greatly advanced.

**Conclusions**

By combining canine sequence data, genomic technologies, and bioinformatics into research initiatives already in place, researchers in the fields of nutritional genomics and proteomics will soon change the paradigm by which veterinarians and nutritionists understand and apply companion animal biology. Recent technologic advances enable researchers to study the impact of nutrition on phenotype in fine detail, identifying nutrient-responsive genes and biological pathways, important nutrient-gene interactions, and genomic biomarkers of disease. Thus far, nutrigenomic strategies have been lacking in the field of canine research, but they must become a priority in the future. Experiments should be designed to identify SNP profiles associated with disease, study the
impact of diet on epigenetic inheritance, and identify the effects of diet on gene transcription and translation rates. These research strategies will someday lead to the development of genotype-based diets and medicines and, ultimately, to increased health and longevity of companion animals.


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