Canine Nutritional Model: Influence of Age, Diet, and Genetics on Health and Well-Being

Kelly S. Swanson[1,*] and Lawrence B. Schook[1,2]

[1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL,USA,* [2]*Institute for Genomic Biology, University of Illinois, Urbana, IL, USA*

Abstract: As a whole, biological research has recently shifted its focus from reductionism to holistic approaches to study complex systems, a strategy often termed "systems biology". Nutritional research has progressed similarly, incorporating phenotype, genotype, genomics, and bioinformatics into an integrative molecular nutrition research model to study the impact of nutritional status on health and disease. Given the importance of livestock and companion animals as large animal models for humans, many of these genomes including that of the dog, cow, cat, and chicken have been sequenced or are being sequenced by the National Institutes of Health (NIH). Ongoing projects to sequence the canine genome and create a SNP map, in addition to the phenotypic and genotypic similarities and unique breed structure of domestic dogs, continue to increase the experimental power of the dog model. While anatomical and physiological similarities have deemed the dog a useful model for gastrointestinal research for decades, economical and ethical concerns have recently decreased their use in this research field. This review aims to reiterate the importance of the dog model in gastrointestinal research, including the study of prebiotics and aging on intestinal health, analyzing gene expression profiles to better understand intestinal diseases such as inflammatory bowel disease, and performing whole genome association studies to identify genetic loci contributing to complex intestinal diseases.

Keywords: Canine, functional genomics, intestinal health, nutrition, systems biology.

APPLICATION OF "SYSTEMS BIOLOGY" TO STUDY HUMAN DISEASE

Healthcare programs and clinical medicine have vastly improved over the past century, increasing the estimated life expectancy from 47.3 years in 1900 (all races, both sexes) to 76.9 years in 2000 [1]. Nonetheless, the incidence of chronic diseases, due to complex genetic and environmental interactions, continues to increase rapidly. In 2002, seven of the top ten causes of death represented diseases shown to result from such interactions. Of these, heart disease (28.9%), cancer (22.9%), stroke (6.6%), chronic lower respiratory disease (5.5%), diabetes mellitus (2.8%), Alzheimer's disease (2.6%), and renal disease (1.6%) represent over 70% of total deaths in the U.S. [2]. Osteoarthritis, gastrointestinal diseases, mental or behavioral disorders, alcoholism, and drug addiction are also of clinical importance because they are often co-morbidities and affect quality of life.

The reductionist approach to biomedical research has been extremely useful in developing a basic understanding of the cells, tissues, and systems of the body. By using model organisms (e.g. cell lines, invertebrate organisms, rodents), reductionism has been successful in identifying individual components (e.g. genes, proteins, metabolites) responsible for a particular phenotype, which has led to major advances in medicine, life sciences, and agriculture. While this approach has been successful in identifying specific functions and mechanisms by which individual components perform, it has become clear that an integrative investigative approach is required to fully understand the behavior of complex biological systems [3]. Given the recent advances in biotechnology and computer science, scientists now have the ability to use a "systems biology" approach based on the simultaneous measurements of functional genomic, proteomic, and metabolomic parameters to study, dissect, and understand biological systems.

A systems biology approach not only enhances our understanding of complex systems, but identifies interconnected pathways and networks throughout the body that contribute to chronic disease states. The NIH recently devised the "NIH Roadmap", addressing the need for a holistic understanding of complex systems and diseases impacted by numerous factors such as genetics, diet, infectious agents, environment, behavior, and social structures [4]. As one of three themes emerging from this effort, "New Pathways to Discovery" addresses the technologies and approaches necessary to meet contemporary research challenges, including building blocks and pathways, molecular imaging, the development of small-molecule libraries, bioinformatics and computational biology, nanomedicine, and structural biology [4]. Given the recent sequencing of mammalian genomes, advances in molecular biology and nanotechnology, and the NIH priorities, the face of research is rapidly changing. In order for the "systems biology" approach to be successful, researchers must embrace new tools available and evolve experimental approaches.

*Address correspondence to this author at 1207 West Gregory Drive, 162 Animal Sciences Laboratory, Urbana, IL 61801, USA; Tel: 217-333-4189; Fax: 217-333-7861; E-mail: ksswanso@uiuc.edu

1573-4013/06 $50.00+.00

INTEGRATIVE MOLECULAR NUTRITION RESEARCH MODEL

Although several variables contribute to disease susceptibility, diet (nutritional status) is one of the primary environmental factors influencing the health and disease of an individual. In fact, each of the leading causes of death mentioned above are all influenced in some manner by nutritional status. Because diet is a variable that can be controlled throughout life, the nutritional sciences field has become increasingly important in the elucidation of chronic disease prevention and treatment. Given the interaction and crosstalk between organ systems, the systems biology approach is best suited to study complex research problems. To fully understand the mechanisms by which nutrition affects disease susceptibility, the research model must go beyond the study of individual cells and tissues and consider the simultaneous metabolic and genomic responses of numerous organ systems.

Nutritional research capabilities have dramatically changed over recent decades and will continue to evolve rapidly through technological advances. While phenotypic data were the primary outcome variables used by nutritionists for much of the 20th century, genotypic data (genetic background) and genomic data (gene expression, protein, and metabolite profiles) are now incorporated into the research equation. Not surprisingly, as the complexity of the research model builds, so does the complexity of interpretation. Thus, the field of bioinformatics, to manage and interpret biological information, has rapidly emerged and plays a key role in "integrative molecular nutrition" research models.

(i) Phenotype

A biomedical model can be defined as a "surrogate for a human being, or a human biologic system, that can be used to understand normal and abnormal function from gene to phenotype and to provide a basis for preventive or therapeutic intervention in human diseases" [5, 6]. Just as physical characteristics and responses were used a century ago, phenotype is still the ultimate outcome variable by which to test hypotheses. The nature of research continues to change, however, due to technological advances that improve detection limits, accuracy, and speed by which laboratory assays are performed. Methods such as high performance liquid chromatography (HPLC), mass spectroscopy, and nuclear magnetic resonance (NMR) detect limits in nanomolar (10^{-9}) and picomolar (10^{-12}) concentrations, identifying and detecting compounds unknown to nutritionists just 50 or 100 years ago. Even though other variables (e.g. genotype) have recently entered the research equation, phenotype is still the foundation from which research evolves.

Due to their size, cost, availability, short life span, and ease of being genetically manipulated (e.g. transgenesis, gene-knockouts, etc.), the use of rodents in biological research has steadily increased over the past several decades. While rodent experiments have their value in a basic research environment, anatomical and physiological shortcomings can compound experimental outcomes relevant to humans. Thus, large animal models similar phenotypically to humans are of great importance in "translational" research.

One prime example exists in the area of colon health and disease, whereby the dog and pig are much more suitable models for humans than are rodents. Although the lack of adequate sample size and difficulty accurately assessing therapeutic responses are important issues with rodent models, their greatest weaknesses are associated with the lack of anatomical and physiological similarities. First, while the primary organ of bacterial fermentation in humans is the proximal half of the colon, rats are primarily cecal fermenters [7]. The location of fermentation is largely due to the vast anatomical differences between the species (Fig. **1**) [8]. Even when consuming low fiber diets, rats have a large fully-functional cecum, whereas the ceca of humans and dogs are relatively small and with little functional importance. Because the cecum is the major fermentative organ of the rat, this disparity becomes even greater when high-fiber diets are consumed, resulting in cecal hypertrophy in rats [9]. Second, contrary to humans and dogs that normally have low concentrations of bacteria in the stomach and proximal small intestine, high bacterial concentrations exist in these compartments of rats [10]. Third, fecal concentrations of many bacterial species (e.g. lactobacilli, bifidobacteria) in rats and humans are also quite different [11-13]. Because bacteria are living organisms capable of metabolizing substances present in digesta, differences in bacterial number, type, and location in the gastrointestinal tract, affect the site and extent of absorption of many substances [14]. Differences in anatomy and intestinal microbial ecology of rats are likely the primary reason this model poorly recapitulates human responses to high fiber diets [15]. Taken together, it is evident that rodent models are not the most appropriate for the study of human gastrointestinal health and disease. Our research team and others have repeatedly used canine and porcine models to effectively study intestinal health and disease with outcomes more applicable to adult humans [16-18] and human infants [19, 20]. A more detailed discussion regarding the strengths of the dog as a model for intestinal health and disease is provided below.

(ii) Genotype

A recent component of the nutritional research paradigm is genotype. The efforts put forth by the NIH, specifically the National Human Genome Research Institute (NHGRI), have added an entirely new dimension to biological research. With what started out primarily focused on deciphering the human genetic code (Human Genome Project), NHGRI has expanded its vision to include the genomes of animal models of various evolutionary backgrounds and phenotypes. Numerous non-rodent animal models, including the dog, cow, cat, and chicken, have had their genome sequenced or are currently in the queue.

Although the progress made in the past decade in regards to genome sequencing has been astounding, the vast amount of information it provides comes without interpretation. Comparative analyses of genome sequences will be a major part of the next phase of the Human Genome Project [21]. The study of genetic variants and how they affect functioning of specific proteins and pathways will yield

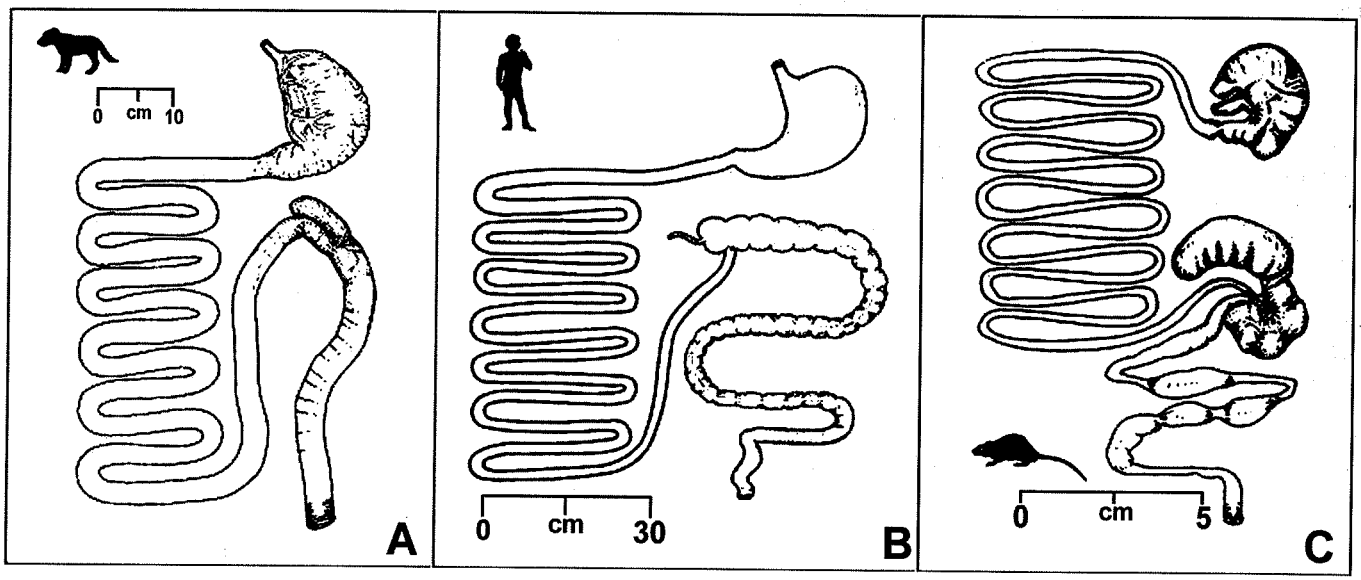

Fig. (1). Gastrointestinal tract of the: A) dog; B) human; and C) rat.

important insights about physiological processes in normal and disease states [21]. Comparative genomics promises to detail distinctive parallels in genome assemblages as a prelude to interpreting species and individual variation in a functional and evolutionary context [22]. A couple of the major goals of comparative genomics are to: 1) identify conserved and divergent DNA sequences of distant species; and 2) discriminate functional and nonfunctional DNA sequences. In addition to discriminating conserved from divergent and functional from nonfunctional DNA, comparative genomics also identifies general functional classes of DNA segments, such as coding exons, noncoding RNAs, and regulatory regions [23]. For example, regions of noncoding DNA having high similarity among species are good candidates for functional regions [24], with the possibility of being regulatory sequences [25]. While comparison of very distant species may identify functional classes of genes, comparing genomes of similar species may identify key sequence differences accounting for phenotypic differences in the organisms [23]. While the chimpanzee is probably the best comparison for humans in this regard, the dog is a promising model with several strengths (discussed below).

It is generally assumed that much of the quantitative genetic variation is traceable to single nucleotide polymorphisms (SNP) or insertion/deletion polymorphisms [26]. Given the difficulty and costs associated with searching for disease genes in the 10 million SNP that are present in the human genome, researchers have turned to first identifying haplotypes associated with disease. Haplotypes are stretches of DNA that travel as a unit, each carrying a group of SNP, when chromosomes recombine during inheritance. Because most haplotypes are between 10,000 and 20,000 bases, the cost of searching for haplotypes is drastically reduced compared to SNP detection. Haplotypes that predominate in diseased individuals may then be the focus of further studies to identify the specific SNP responsible. To start, the International HapMap Project is developing a map of DNA patterns across the genome by determining the genotypes of populations with ancestry from Africa, Asia, and Europe [27]. This project is expected to provide the tools necessary to allow the indirect association approach to be applied to any candidate gene or region suggested by family-based linkage analysis or ultimately to whole genome scans [27]. Thus, the search for disease genes has shifted from linkage studies requiring multigenerational families to association studies that scan the entire genome. The coinheritance of SNP alleles on haplotypes leads to associations between alleles known as linkage disequilibrium (LD). While whole genome association mapping is a promising strategy to understand genetic and phenotypic variation, the feasibility of such procedures depends on the level of LD present in the population. Thus, effective animal models for whole genome scans that possess extensive LD, which has already been demonstrated in several large animal models, such as the dog [28], pig [29], cattle [30], and sheep [31], currently exist. The considerable LD exhibited in these species is not surprising, given the intensive breeding programs of these domesticated livestock and companion animals.

In addition to identifying genetic loci contributing to health and disease, genome sequence data also allows researchers to create appropriate animal models through numerous gene-driven approaches (reverse genetics). In contrast to *forward genetics* in which genes responsible for a particular phenotype are identified by positional cloning (phenotype to genotype), *reverse genetics* determines the function of a gene and predicts phenotype (genotype to phenotype). Whether researchers use techniques to manipulate specific regions of a genome [e.g. gene trapping, recombineering (chromosomal engineering)] or use dsRNA to cause RNA interference (RNAi) to specific genes, genome sequence information is crucial [32]. Sequence data of large animal models will soon allow these techniques to be performed in these models, further strengthening their place in research.

(iii) Molecular Biology Toolbox

The genome sequencing effort coupled with continued advancements in biotechnology enhance our ability to measure gene transcripts (mRNA), proteins, and metabolites. These advancements have not only improved the accuracy and limits of detection, but have led to high-throughput techniques that provide researchers with a holistic view of a cell, tissue, or organism. "High-throughput" techniques, such as DNA microarrays, allow the measurement of hundreds to thousands of genes simultaneously [33, 34]. Because microarrays provide a global view of gene expression, they are often used as a starting point when studying complex disease states to identify genes or pathways involved. Genes significantly up- or down-regulated in the diseased state can then be studied in more detail in future experiments. Because microarrays are only semi-quantitative, researchers often use quantitative reverse-transcriptase polymerase chain reactions (qRT-PCR) to validate microarray data. Due to lower cost and the ability to quantify mRNA, qRT-PCR is usually used in follow-up experiments to study the key genes identified from microarray experiments. In addition to microarrays commercially available, researchers may design oligonucleotides and print their own arrays using the sequence data deposited into public databases.

While DNA microarrays have had a major positive impact on biological research, they do have limitations. The low correlation observed between protein and mRNA concentration for some genes is one such limitation with this technology. Discrepancies may be due to a number of factors, including the level at which a gene is regulated (e.g. transcription *vs*. translation) and the occurrence of post-translational modifications. This topic was recently studied in yeast by Beyer *et al.* [35], who reported a protein:mRNA ratio of 0.58 for the entire cell. In addition to identifying correlations across the whole cell, these researchers also grouped proteins based on localization and function. While protein:mRNA correlation in most spatial compartments were weak (often < 0.4), functional modules generally exhibited stronger correlations [35]. Interestingly, modules associated with "metabolism", "energy", and "protein synthesis" exhibited the strongest correlation between protein and mRNA, suggesting that they are substantially regulated at the transcription level [35]. Nonetheless, it is evident that accurate methods of protein identification and quantification are greatly needed in combination with gene expression analyses.

Although the technologies and information required for protein detection are less developed than those for detecting mRNA, a major focus is now being placed on the development of protein detection techniques. The combination of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), which separates proteins according to charge and size, and mass spectrometry, has been the most widely used method for protein analysis thus far [36]. Due to the cost and labor associated with gel-based techniques, liquid chromatography-mass spectroscopy (LC-MS) systems and protein microarrays are also being developed [37]. Because mRNA and protein concentrations are not well correlated in some instances, it is imperative that techniques measuring protein abundance continue to be developed and made available to researchers. Protein-detecting techniques will be particularly important in transferring genomics from the lab bench to bedside, a setting in which tissues have clinical relevance.

Advances in nanotechnology continue to create new tools that either compliment existing techniques or create completely new alternative approaches. Laser capture microdissection (LCM) allows the isolation and study of individual cell types from heterogeneous tissue [38]. Although cancer researchers were first to use LCM to study cancerous *vs*. "normal" cells taken from tumor biopsy samples [39], our laboratory and other nutritionists have adopted the technology to study individual intestinal cell types [40]. In combination with LCM, RNA isolation and amplification procedures enable the measurement of mRNA concentration from minute samples such as tissue biopsies. In addition to tissues such as liver, adipose, kidney, and skeletal muscle that are accessible *via* biopsy, intestinal samples may also be collected and analyzed without sacrificing animals. Thus, large animal models, including the dog, allow the design of longitudinal experiments to measure acute or long-term responses to dietary treatments and pharmaceuticals. Longitudinal experiments in which biopsy samples are collected not only reduce animal numbers and costs, but also eliminate variation within the dataset, as each animal can be used as its own control.

(iv) Bioinformatics

Computer science advances have been critical in aligning and annotating sequences of the human genome and continues to be important in other model sequencing projects. Moreover, the use of high-throughput techniques such as DNA microarrays creates enormous experimental datasets requiring computer programs for management and interpretation, since accurate interpretation is usually the rate-limiting step in microarray experiments. In addition to managing data and performing standard statistical evaluation, complex analytical programs have the ability to mine complex datasets and identify unique patterns of expressed genes associated with phenotypes. Since multiple biological systems are involved in complex disease states, the ability to identify patterns rather than individual genes or proteins is becoming increasingly important for the study of metabolic (e.g. obesity, diabetes) and immunological (e.g. inflammatory bowel disease) diseases.

As high-throughput techniques continue to be incorporated into nutritional research, the "bioinformatics" piece of the research model is becoming increasingly important. Automated learning methods, as those developed in the Automated Learning Group at the National Center for Supercomputing Applications (NCSA) at the University of Illinois, may not only aid in data management, but also enable researchers to perform predictive data mining [41]. Automated learning platforms, able to effectively generate predictive biological outcomes from large and diverse datasets, will play a vital role in the future of biomedical research. Predictive models will aid in the development of clinical screening and diagnostic tools and in devising prevention and treatment strategies for complex disease.

CANINE NUTRITIONAL MODEL

Given the time it takes for chronic diseases to develop and the expense associated with human clinical experiments, animal experimentation using appropriate biomedical models is required. The recent advent of techniques in molecular biology, genomics, transgenesis and cloning furnish investigators with a new ability to investigate vertebrates (pigs, cows, chickens, dogs) with greater precision and utilize them as model organisms. Given the availability of canine gene sequence information and canine-specific assays, the experimental power of using canine models continues to increase.

(i) Phenotype

Dogs offer several advantages as large animal models for human disease. The large body size of the dog model is more similar to humans than rodents and diminishes the problem of small sample sizes, enables longitudinal studies, allows treatment options to be tested, and is more biologically relevant to humans when evaluating therapeutics. Dogs are monitored and treated with a high standard of veterinary medicine, providing important clinical data of diseases. Although members of the Order Carnivora, the Canidae Family of which dogs belong, are omnivorous, eat a diet of similar macronutrient composition to that of humans, and have a gastrointestinal tract similar to humans (Fig. **1**). Given the advances in veterinary care, canine nutrition, and changes in lifestyle (increase in sedentary lifestyle), their life span has increased and dogs now develop many of the same complex diseases (e.g. obesity, diabetes, osteoarthritis) as humans.

(ii) Genotype

The dog is the oldest domesticated species and has served several roles for humans over time, including herding livestock, guarding homes, hunting by sight and scent, retrieving wild animals, pulling sleds, and simply providing companionship [42]. Because mating pairs were often chosen to create phenotypes best suited to serve a particular function, the modern canine breeds possess more phenotypic diversity than any other mammalian species. Although archeological records suggest that dogs were first domesticated about 14,000 years ago [43], the majority of these breeds have been developed over the past 300 years [44]. Thus, most of the 400 breeds we know today are very young and have originated from a small number of "founder" dogs. While breeding practices have generated various physical and behavioral attributes that identify specific breeds, limited genetic pools have also contributed to over 400 canine genetic diseases [45]. Interestingly, many of the canine genetic diseases are similar to that of humans. The vast phenotypic diversity, high number of genetic disorders, and high level of biological relevance to humans have made the dog an excellent model for genetic research. For more information on the role of canine genetics in human medicine, refer to Sutter and Ostrander [42] and Parker *et al.* [46].

The value of canine genetic research models is not only due to the number of genetic disorders, but the high similarity many of them have with human diseases. According to the American Kennel Club Canine Health Foundation, the top 10 diseases affecting purebred dogs include cancer, epilepsy, hip dysplasia, thyroid disease, allergies, bloat, heart disease, autoimmune diseases, blindness, and cataracts, many of which also afflict humans [42]. Disease heterogeneity, which often confounds human experiments, may be avoided in dogs because breeding practices often ensure that a small number of genes, or even a single gene, underlies a disease in a specific breed [44]. Because it is anticipated that at least 60% of canine diseases have a molecular background similar to that of humans [45], canine studies may shed light on over 250 human genetic diseases.

To date, causative gene mutations for 30 canine hereditary diseases are known, most of which share clinical and molecular features with human forms of the disease [45]. Of these diseases, genes responsible for blindness [47], renal cancer [48], hemophilia [49], narcolepsy [50], severe combined immunodeficiency (SCID) [51], muscular dystrophy [52] have been identified and applied to human medicine. Prior to having canine genome sequence data, the process of identifying disease genes was long and arduous, requiring large informative pedigrees and testing of numerous candidate genes. Now that canine genome sequence data are available on a public domain database, the search for genetic linkage to disease will become quicker and more efficient.

Demonstrating its importance as a biomedical model, the dog was the first non-rodent mammalian animal model to be sequenced. The canine initiative has provided sequence information critical to: 1) the emergence of genomic tools (e.g. microarray chips) required for functional genomics research; and 2) performing comparative genomic studies with humans and other animal models. The first draft (7X coverage) of the canine genome was completed in July of 2004, and is currently being assembled (http://www.genome.gov/12511476). To best characterize disease in dogs, it is important to have a sufficient number of markers in the genome. Therefore, in addition to the female boxer that was used for genome sequencing, NHGRI is using 9 other dog breeds, 4 wolves, and a coyote identify and map ~600,000 SNP, which will be useful in the hunt for genes contributing to disease.

As mentioned previously, the feasibility of genome association mapping is highly dependent on the level of LD in a population. Because of closed breeding pools, detailed population records, and large variation in morphology, disease susceptibility, and behavior in purebred domestic dogs, LD-based mapping approaches may be effectively used in this model to localize genes contributing to complex diseases [28]. The high quality canine genome sequence recently generated from a standard poodle (1.5X coverage) [53] and boxer (7.8X coverage; www.genome.ucsc.edu), along with the 600,000 canine SNP currently being aligned (www.genome.gov/12511476), enable effective canine genome association studies. Not only are these studies now feasible in dogs, but recent research demonstrated that LD in canines is much more extensive than humans, and thus, will require much fewer SNP for mapping traits [28]. These analyses suggest that instead of mapping hundreds of thousands of SNP required in humans [27, 54], the same

the colon due to a decrease in the ability to digest and absorb high-protein diets that has been reported in elderly people [94]. We recently observed similar responses in dogs, with geriatric (12 yr old) dogs having greater colonic ammonia, valerate, and isovalerate concentrations compared to young adults (1 yr old) [95].

The final topic to be addressed is that of age-related changes in intestinal structure and function, which is filled with controversy in the literature. Because malnutrition is prevalent in elderly populations [96], many have hypothesized that a reduced intestinal morphology (surface area) or functionality is to blame. However, while some researchers have reported age-related differences in intestinal morphology (e.g. reduced villus height, reduced surface area) [97, 98], others have reported no morphological changes due to age [99, 100]. The literature investigating age-related functional changes such as intestinal motility and nutrient transport is also contradictory.

Our laboratory recently reported age-related differences in nutrient absorption [101] and intestinal morphology [95] in young adult and senior dogs. While apparent nutrient digestibility was not different between geriatric (12 yr old) and young adult (1 yr old) dogs, apparent dry matter, organic matter, and fat digestibility was greater in the geriatric population *vs.* young growing (5 mo old) dogs [101]. Age also had a significant impact on intestinal morphology, with the geriatric population having lower duodenal villus area, jejunal villus height, and villus height:crypt depth ratio. Geriatric dogs also had greater ileal villus width and colonic crypt depth compared to young adults [95]. At the conclusion of that experimental study, intestinal tissues were collected and preserved for RNA isolation. Our laboratory is currently using microarray technology to generate gene expression profiles from intestinal tissue to identify genes associated with the changes observed in intestinal morphology, nutrient digestibility, and fermentative end-product concentrations.

(iii) Canine Model of Inflammatory Bowel Disease

Inflammatory bowel diseases (IBD), chronic inflammatory disorders of the gastrointestinal tract, are estimated to afflict 1 million Americans per year [102]. Pathogenesis of IBD is complex, being impacted by genetic, environmental, microbial, cellular, and molecular factors [103]. Dietary and microbial antigens are the most common environmental factors involved with IBD development. In fact, specific intestinal bacterial species (e.g. mycobacteria, salmonella) and bacterial end-products (e.g. ammonia) have been shown to drive IBD [104]. Thus, much of the discussion regarding the use of dogs as a model to study prebiotics, which are able to manipulate microbial populations and fermentative end-products, applies here as well.

Although IBD is commonly diagnosed and several factors are known to contribute, case-specific causes and most effective treatment strategies are ill-defined. Progress towards understanding and treating IBD remains slowed by our limited knowledge of genes involved with disease pathogenesis, coupled with the complex and heterogeneous nature of the disease [105]. It is known that an upregulation of proinflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-12, IFN-γ result in an increased number of activated leukocytes in IBD intestinal mucosa [106, 107]. However, the specific factors initiating the upregulation of these cytokines are unknown. Our lack of knowledge regarding IBD pathogenesis is evident when examining treatment strategies, which include numerous drug and (or) dietary interventions, which aim to decrease all potential environmental antigens and the body's immune response rather than efficiently targeting a specific contributing factor. These strategies are often unsuccessful and are unrealistic for the long-term. Identifying specific genes or pathways may be used to develop treatments tailored to each individual or subtype of IBD, resulting in quicker recovery time and improved long-term disease management.

Inflammatory bowel diseases consist of several subtypes or 'phenocopies' (different underlying pathophysiologic defects that share related clinical phenotype), making it difficult to identify a specific cause or treatment strategy for a given patient [105]. Two primary forms of human IBD are Crohn's disease and ulcerative colitis [108]. While Crohn's disease may occur in any region of the gastrointestinal tract, ulcerative colitis is restricted to rectal, colonic, and occasionally ileal mucosa. Several canine IBD forms exist with the two predominant types being lymphocytic-plasmacytic and eosinophilic enteritis, which are characterized by infiltration of the respective cell type into the mucosa [109]. Although histopathologic changes and area of the intestine predominantly affected by IBD are different between dogs and humans, the clinical and mechanistic similarities that exist substantiate the use of the dog model [109]. As with humans, naturally-occurring cases in dogs are known to respond similarly to antigens derived from commensal bacteria, with CD4+ T cells, TNF and IL-12 mediating the inflammatory response [109, 110]. Moreover, Sethi and Sarna [111] have demonstrated the utility of a chemically-induced colitis dog model that produces symptoms similar to those observed in humans with ulcerative colitis. While knock-out murine strains have been useful in identifying genes that may contribute to IBD pathogenesis [112, 113], large animal models such as the dog that are outbred, are biologically relevant to humans, and naturally acquire IBD, must also be used to enhance our understanding of the disease process.

Genome-wide expression analyses promise to provide unparalleled insights into the gene programs that underlie both normal physiology and disease states. Specific to IBD, array-based techniques may identify genes involved with pathogenesis and potential therapeutic targets, identify signaling pathways that perpetuate mucosal inflammation or that direct reparative programs, provide new tools for improved clinical diagnosis and characterization, and identify pharmacogenomic markers indicative of the most appropriate therapeutic intervention for each patient [105]. Gene expression and protein profiles have already been used to molecularly describe and understand IBD [114], liver diseases [115], and breast [116], prostate [117], and ovarian [118] cancers. Human IBD microarray studies have greatly expanded the list of genes differentially expressed in the mucosa of diseased patients [102, 119, 120]. Given the tools and genome sequence data now available with the canine,

similar experiments using microarray technology are possible in the dog.

(iv) Identifying Genetic Linkage to Intestinal Diseases

A few genetic diseases affecting the gastrointestinal system have been noted thus far, but with little description of their inheritance. Gluten sensitive enteropathy, present in the Irish setter breed, has been determined to be a monogenic, autosomal recessive disease that leads to vomiting, diarrhea, weight loss, and hypotrophy of intestinal microvilli [121, 122]. Because gluten sensitive enteropathy in dogs does not appear to be genetically linked to the major histocompatibility complex (MHC) class II alleles, as it is for human celiac disease, its use as a disease model is questionable [123]. Another enteric disorder identified in dogs is selective intestinal cobalamin malabsorption in giant schnauzers, leading to anemia, low blood cobalamin, and a general failure to thrive within the first few weeks of life [124]. Similar to the human form (Imerslund-Grasbeck syndrome), this disorder is autosomal recessive and is characterized by selective intestinal cobalamin malabsorption. Exocrine pancreatic insufficiency is associated with acinar cell degeneration and is present in several canine breeds. Although it may have an autosomal recessive inheritance in German shepherd dogs [125], further studies are required for validation. Finally, a genetic mutation resulting in cystinuria, a metabolic defect of amino acid transport in the kidney and intestine, has been reported in Newfoundland dogs [126]. In affected individuals, cystine can accumulate in high concentrations in the urine and form crystals leading to urinary obstruction [127]. While cystinuria has been reported in at least 60 breeds and accounts for 1% of all uroliths, the genetic polymorphism identified by Henthorn *et al.* [126] was specific to the Newfoundland breed. Thus, as in humans, cystinuria is genetically heterogeneous and further research is required to identify other genes associated with this disorder.

While only a few enteric diseases have received enough attention to identify a specific gene responsible for their existence, several other breed-specific conditions in dogs have been identified [109, 128]. While the examples provided above demonstrate the possibility of identifying monogenic enteric diseases, many gastrointestinal diseases have a complex etiology involving dietary and microbial antigens, the immune system, and genotype. Epidemiological and family studies have provided overwhelming evidence that genetics play an important role in determining the susceptibility to diseases such as IBD [129]. Thus far, rodents have provided the most information relevant to the genetics behind IBD, using chemical-induced and genetic-knockout models [130]. However, the potential now exists to perform whole-genome mapping studies, allowing researchers to go beyond autosomal recessive diseases and engage in complex diseases.

SUMMARY AND PERSPECTIVES

Given the importance of diet on health status, the nutritional sciences field has become increasingly important in the study of complex diseases that develop over years or decades. An integrative molecular nutrition research model incorporating phenotype, genotype, genomics, and bioinformatics simultaneously may be used to effectively study the impact of nutritional status on health and disease. High quality canine genome sequence data, and efforts to construct a canine SNP map, have positioned the dog as a powerful biomedical model for studying complex disease states impacted by numerous environmental and genetic factors. Ongoing and future whole genome association and nutrigenomic studies in the dog will prove to be valuable in the search for genetic loci and dietary factors contributing to complex diseases, including those impacting the gastrointestinal system.

ACKNOWLEDGEMENTS

We thank Pyxis Genomics, Inc., Chicago, IL and USDA-Hatch for providing financial support. We also thank Kristy N. Kuzmuk for her technical assistance and George C. Fahey, Jr. for the guidance he has provided with our research.

REFERENCES

[1] Arias E. United States Life Tables, 2000. National Vital Statistics Reports 2002; 51:33-34.

[2] Anderson, RN, Smith BL. Deaths: Leading causes for 2002. National Vital Statistics Reports 2005; 53: 9.

[3] Aggarwal K, Lee KH. Functional genomics and proteomics as a foundation for systems biology. Brief Genom Proteom 2003; 2: 175-184.

[4] Zerhouni E. The NIH roadmap. Science 2003; 302:63-72.

[5] Tumbleson M, Schook LB. Advances in Swine in Biomedical Research. New York: Kluwer Academic 1996.

[6] National Research Council. Biomedical Models and Resources: Current Needs and Future Opportunities. Washington, DC: National Academy Press 1998.

[7] Stevens CE, Hume ID. Microbial fermentation and synthesis of nutrients and the absorption of end products. pp. 188-228 In Comparative Physiology of the Vertebrate Digestive System, 2nd Ed. Cambridge, UK: Cambridge University Press 1995.

[8] Stevens CE. The Digestive System of Vertebrates. CD-ROM. College of Veterinary Medicine, NC State University, Raleigh, NC. 2001.

[9] Juśkiewicz J, Zduńczyk Z. Effects of cellulose, carboxy-methylcellulose and inulin fed to rats as single supplements or in combinations on their caecal parameters. Comp Biochem Physiol Part A 2004; 139: 513-519.

[10] DeSesso JM, Jacobson CF. Anatomical and physiological parameters affecting gastrointestinal absorption in humans and rats. Food Chem Toxicol 2001; 39: 209-228.

[11] Langendijk PS, Schut F, Jansen GJ, *et al.* Quantitative fluorescence *in situ* hybridization of Bifidobacterium spp. with genus-specific 16S r-RNA-targeted probes and its application in fecal samples. Appl Environ Microbiol 1995; 61: 3069-3075.

[12] Wang X, Brown IL, Khaled D, Mahoney MC, Evans AJ, Conway PL. Manipulation of colonic bacteria and volatile fatty acid production by dietary high amylose maize (amylomaize) starch granules. J Appl Microbiol 2002; 93:390-397.

[13] Sembries S, Dongowski G, Jacobasch G, Mehrländer K, Will F, Dietrich H. Effects of dietary fibre-rich colloids from apple pomace extraction juices on intestinal fermentation products and microbiota in rats. Brit J Nutr 2003; 90: 607-615.

[14] Granger DN, Barrowman JA, Kvietys PR. Clinical Gastrointestinal Physiology Philadelphia: WB Saunders 1985.

[15] Stevens CE, Argenzio RA, Roberts MC. Comparative physiology of the mammalian colon and suggestions for animal models of human disorders. Clin Gastroenterol 1986; 15: 763-785.

[16] Swanson KS, Grieshop CM, Flickinger EA, *et al.* Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities, and fecal protein catabolite concentrations in healthy adult dogs. J Nutr 2002; 132: 3721-3731.

[17] Swanson KS, Grieshop CM, Flickinger EA, *et al.* Supplemental fructooligosaccharides and mannanoligosaccharides influence

[120] Lawrance IC, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: Distinctive gene expression and novel susceptibility candidate genes. Hum Mol Genet 2001; 10: 445-456.

[121] Batt RM, Carter MW, McLean L. Morphological and biochemical studies of a naturally occurring enteropathy in the Irish setter dog: a comparison with celiac disease in man. Res Vet Sci 1984; 37: 339-346.

[122] Garden OA, Pidduck H, Lakhani KH, Walker D, Wood JL, Batt RM. Inheritance of gluten-sensitive enteropathy in Irish setters. Am J Vet Res 2000; 61: 462-468.

[123] Polvi A, Garden OA, Houlston RS, Maki M, Batt RM, Partanen J. Genetic susceptibility to gluten sensitive enteropathy in Irish setter dogs is not linked to the major histocompatibility complex. Tissue Antigens 1998; 52: 543-549.

[124] Fyfe JC, Giger U, Hall CA, *et al.* Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. Pediatr Res 1991; 29: 24-31.

[125] Weber W, Freudiger U. Heritability of chronic exocrine insufficiency of the pancreas in the German shepherd dog. Arch Tier 1977; 119: 257-263.

[126] Henthorn PS, Liu J, Gidalevich T, Fang J, Casal ML, Patterson DF, Giger U. Canine cystinuria: polymorphism in the canine SLC3A1 gene and identification of a nonsense mutation in cystinuric Newfoundland dogs. Hum Genet 2000; 107: 295-303.

[127] Segal S, Their SO. Cystinuria. Pages 3581-3601 in: Metabolic and molecular bases of inherited disease (Scriver C ed.). New York: McGraw-Hill. 1995

[128] Strombeck DR, Guiliford WG. Small Animal Gastroenterology (2nd Ed.). Davis, CA: Stonegate Publishing. 1990.

[129] Bonen DK, Cho JH. The genetics of inflammatory bowel disease. Gastroenterol 2003; 124: 521-536.

[130] Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003; 3: 521-533.

[131] Gibson GR, Beatty ER, Wang X, Cummings JH. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterol 1995; 108: 975-982.

[132] Diez M, Hornick JL, Baldwin P, Istasse L. Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrient digestibility and plasma metabolite concentrations in healthy Beagles. Am J Vet Res 1997; 58: 1238-1242.

[133] Diez M, Hornick JL, Baldwin P, Van Eenaeme C, Istasse L. The influence of sugar-beet fibre, guar gum and inulin on nutrient digestibility, water consumption and plasma metabolites in healthy Beagle dogs. Res Vet Sci 1998; 64: 91-96.

[134] Russell TJ. The effect of natural source of non-digestible oligosaccharides on the fecal microflora of the dog and effects on digestion. Friskies R & D Center/Missouri, Copyright © Friskies – Europe 1998.

[135] Tominga S, Hirayama M, Adachi T, Tokunaga T, Iino H. Effects of ingested fructooligosaccharides on stool frequency in healthy female volunteers: A placebo-controlled study. Biosci Microflora 1999; 18: 49-53.

[136] Flickinger EA, Wolf BW, Garleb KA, *et al.* Glucose-based oligosaccharides exhibit different *in vitro* fermentation patterns and affect *in vivo* apparent nutrient digestibility and microbial populations in dogs. J Nutr 2000; 130: 1267-1273.

[137] Hond ED, Geypens B, Ghoos Y. Effect of high performance chicory inulin on constipation. Nutr Res 2000; 20: 731-736.

[138] Hesta M, Roosen W, Janssens GPJ, Millet S, De Wilde R. Prebiotics affect nutrient digestibility but not faecal ammonia in dogs fed increased dietary protein levels. Brit J Nutr 2003; 90: 1007-1014.

[139] Grieshop CM, Flickinger EA, Bruce KJ, Patil AR, Czarnecki-Maulden GL, Fahey Jr GC. Gastrointestinal and immunological responses of senior dogs to chicory and mannan-oligosaccharides. Arch Anim Nutr 2004; 58: 483-493.

[140] Zentek J, Marquart B, Pietrzak T. Intestinal effects of mannanoligosaccharides, transgalactooligosaccharides, lactose and lactulose in dogs. J Nutr 2002; 132: 1682S-1684S.

[141] Twomey LN, Pluske JR, Rowe JB, Choct M, Brown W, Pethick DW. The effects of added fructooligosaccharide (Raftilose® P95) and inulinase on faecal quality and digestibility in dogs. Anim Feed Sci Technol 2003; 108: 83-93.

[142] Zentek J, Marquart B, Pietrzak T, Ballèvre O, Rochat F. Dietary effects on bifidobacteria and *Clostridium perfringens* in the canine intestinal tract. J Anim Physiol Anim Nutr 2003; 87: 397-407.

[143] Hidaka H, Eida T, Takizawa T, Tokunaga T, Tashiro Y. Effects of fructooligosaccharides on intestinal flora and human health. Bifido Microfl 1986; 5: 37-50.

[144] Hara H, Li ST, Sasaki M, *et al.* Effective dose of lactosucrose on fecal flora and fecal metabolites of humans. Bifido Microfl 1994; 13: 51-63.

[145] Bouhnik Y, Flourié B, Riottot M, *et al.* Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes on colon carcinogenesis in healthy humans. Nutr Cancer 1996; 26: 21-29.

[146] Buddington RK, Williams CH, Chen SC, Witherly SA. Dietary supplement of neosugar alters the fecal flora and decreases activities of some reductive enzymes in human subjects. Am J Clin Nutr 1996; 63: 709-716.

[147] Garleb KA, Snook JT, Marcon MJ, Wolf BW, Johnson WA. Effect of fructooligosaccharide containing enteral formulas on subjective tolerance factors, serum chemistry profiles, and faecal bifidobacteria in healthy adult male subjects. Microb Ecol Health Dis 1996; 9: 279-285.

[148] Bouhnik Y, Vahedi K, Achour L, *et al.* Short-chain fructooligosaccharide administration dose-dependently increases fecal bifidobacteria in healthy humans. J Nutr 1999; 129: 113-116.

[149] Rao VA. The prebiotic properties of oligofructose at low intake levels. Nutr Res 2001; 21: 843-848.

[150] Tuohy KM, Kolida S, Lustenberger AM, Gibson GR. The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides – a human volunteer study. Brit J Nutr 2001; 86: 341-348.

[151] Langlands SJ, Hopkins MJ, Coleman N, Cummings JH. Prebiotic carbohydrates modify mucosa associated microflora of the human large bowel. Gut 2004; 53: 1610-1616.

[152] Howard MD, Kerley MS, Sunvold GD, Reinhart GA. Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and intestinal microflora populations. Nutr Res 2000; 20: 1473-1484.

[153] Strickling JA, Harmon DL, Dawson KA, Gross KL. Evaluation of oligosaccharide addition to dog diets: Influences on nutrient digestion and microbial populations. Anim Feed Sci Technol 2000; 86: 205-219.

[154] Flickinger EA, Schreijen EMWC, Patil AR, *et al.* Nutrient digestibilities, microbial populations, and protein catabolites as affected by fructan supplementation of dog diets. J Anim Sci 2003; 81: 2008-2018.

[155] Hidaka H, Tashiro Y, Eida T. Proliferation of bifidobacteria by oligosaccharides and their useful effect on human health. Bifido Microflora 1991; 10: 65-79.

[156] Gråsten S, Liukkonen KH, Chrevatidis A, El-Nezami H, Poutanen K, Mykkänen H. Effects of wheat pentosan and inulin on the metabolic activity of fecal microbiota and on bowel function in healthy humans. Nutr Res 2003; 23: 1503-1514.

Received: June 13, 2005 Revised: August 22, 2005 Accepted: August 24, 2005