COMPANION ANIMALS SYMPOSIUM:
Nutrigenomics: Using gene expression and molecular biology data to understand pet obesity

M. R. C. de Godoy* and K. S. Swanson*†‡

*Department of Animal Sciences, †Division of Nutritional Sciences, and ‡Department of Veterinary Clinical Medicine, University of Illinois, Urbana, IL 61801

ABSTRACT: Approximately 55% of dogs and 53% of cats in the United States are considered overweight or obese. The domestication of dogs and cats and, more recently, their anthropomorphism, have drastically changed their environment and social behavior. A greater manifestation of chronic diseases is observed with pet obesity (e.g., insulin resistance, type-2 diabetes, musculoskeletal disorders). The advances in “omics” technology may provide new tools to investigate the complexity of obesity and its comorbidities. The field of nutrigenomics focuses specifically on the mechanisms by which nutrients and dietary bioactive molecules affect gene expression. The main objective of this review is to discuss factors involved in the etiology of pet obesity and demonstrate how the field of nutrigenomics has been used to better understand and characterize this disease. Currently, most of the genomics literature available on companion animal obesity has focused on adipose tissue, with fewer studies focused on other tissues (e.g., skeletal muscle, liver). Initial studies focused on the sequence and functionality of a few specific genes, such as leptin and adiponectin, and identified their association with obesity. Subsequent studies focused on gene expression levels across tissues and how they were impacted by BW status or if animals were intact, spayed, or neutered. Dietary interventions to induce obesity, promote BW loss, or alter dietary nutrient profile have also been investigated. Diets including prebiotics, green tea extract, or increased concentrations of protein have been shown to modify the expression of several genes related to glucose and lipid metabolism in adipose [e.g., uncoupling protein-2, carnitine palmitoyltransferase-1, PPARα, lipoprotein lipase (LPL), and glucose transporter 4] and skeletal muscle (e.g., PPARα and LPL) tissues. In general, the outcomes derived from these studies demonstrated that dogs and cats share similar adipokines and hormones to other species, and they are affected in a similar fashion during obesity. They also indicate that gene transcription modifications may preclude clinical signs, which may become a useful tool in the management and prevention of obesity.

Key words: adipose tissue, canine, feline, genomics, obesity, transcriptome


INTRODUCTION

Human and pet obesity is a health concern around the globe but is most prevalent in developed nations. In the United States, approximately two-thirds of humans and over 50% of pets are either overweight or obese (Hedley et al., 2004; Calabash, 2011; WHO, 2011). Genetics, decreased physical activity, and increased availability of highly palatable, energy-dense diets are the main contributors of obesity. Comorbidities associated with excessive weight include hypertension, diabetes or insulin resistance, cardiovascular diseases,
metabolic syndrome, musculoskeletal disorders (e.g., osteoarthritis and fractures), hyperlipidemia, and some forms of cancer (Allison et al., 1999; American Cancer Society, 2011; American Heart Association, 2011; WHO, 2011). In addition to an increased incidence of chronic disease, obese people and pets have a decreased life expectancy (Kealy et al., 2002; Peeters et al., 2003). Although a wide variety of BW maintenance and loss strategies are currently available, maintaining a healthy BW over the long term is often difficult. A better understanding of obesity and its comorbidities at a molecular and/or tissue level may prove useful.

The long coevolutionary history between humans and pets, specifically dogs and cats, was hypothesized to result in their predisposition to similar genetic-based pathophysiologies (Lindblad-Toh et al., 2005). Because the dog and the cat are good comparative models for humans, sharing about 200 genetic diseases (O’Brien, 2004), their genome sequences were completed with the support of the National Institutes of Health (NIH). The canine genome sequenced by NIH was first reported in 2004 and was based on the DNA from an adult female Boxer (Lindblad-Toh et al., 2005). The feline genome sequenced by NIH was completed in 2007 and was based on the DNA of an adult female Abyssinian cat (Pontius et al., 2007). In addition to studying genetic diseases, canine and feline genome sequence data provide an opportunity to understand the role of particular genes on metabolic pathways or tissue function, how these genes respond in or affect different physiological stages and pathophysiological conditions (e.g., aging, obesity), and their interplay with nutrients.

The term “genomics” is broadly defined as a set of high-throughput technologies for the generation, processing, and application of scientific data describing the composition and biological purpose of genomes (Afman and Muller, 2006). Table 1 defines similar terms in the “omics” field. Nutrigenomics, specifically, relates to how nutrients and bioactive food compounds affect host gene expression. It is a multidisciplinary field that studies nutrient–gene interactions, which includes changes in RNA transcription (i.e., transcriptome), protein expression (i.e., proteome), metabolite profiles (i.e., metabolome), DNA structure (i.e., epigenome), and genetic variation within species (i.e., nutrigenetics; German et al., 2003; Fenech et al., 2011).

Traditionally, nutritionists have based their hypotheses and findings on phenotypical variables. Recent advancements in molecular biology, bioinformatics, and computer science, however, have provided new tools to study and unravel complex biological systems. With the availability of genomic technology, nutritionists are now capable of gaining a more holistic and mechanistic understanding of organisms and an appreciation of how specific genes and proteins may affect physiological processes (Swanson and Schook, 2006). Understanding how nutrients affect tissue gene expression may identify strategies in treating canine and feline diseases such as obesity and its comorbidities. The main objective of this review is to discuss factors involved in the etiology of pet obesity and demonstrate how the field of nutrigenomics has been used to better understand and characterize this disease.

### THE OBESITY PANDEMIC IN PETS

The incidence of obesity in companion animals has skyrocketed and is considered the most common nutritional disorder in pets (German, 2006). In the United States, it is estimated that 55% of dogs and 53% of cats are overweight or obese (Calabash, 2011). Dogs and cats are considered overweight or obese when their BW exceeds 15 or 30%, respectively, of their ideal BW (Burkholder and Toll, 2000). A common method to assess body composition in dogs and cats is BCS. There are a few different systems (e.g., 5-point or 9-point scales), but all have a common goal of assessing BW status. Even though BCS systems are subjective measurements done by visual observation and body palpation, they have been reported to have a high correlation with body composition as determined by dual-energy X-ray absorptiometry (Mawby et al., 2004). Additional benefits of using a BCS system are that it is not invasive or painful, it requires no extra cost or expensive equipment, and it is easily performed by practitioners and owners.

The etiology of companion animal obesity shares many similarities with humans. The domestication of dogs and cats and, recently, their increased anthropomorphism have drastically changed the environment and social behavior of these animals. Pets have moved away from hunting, competing for food resources, and living in harsh environmental conditions. Most current pets live indoors, are individually housed (e.g., 62% of U.S. households have only 1 pet; APPA, 2011), and have plenty of food available. In addition, most of these animals have minimal reproductive purpose (75% of U.S. pets are neutered; ASPCA, 2011). As a consequence of these social and environmental changes, the average pet has a lower energy requirement compared with its ancestors.

A greater manifestation of chronic diseases has also been observed in veterinary clinics in recent years. The excessive adiposity observed with obesity predisposes animals to health disorders by physical and metabolic mechanisms (German et al., 2010). Obese dogs and cats are more likely to suffer from endocrine, orthopedic, cardiorespiratory, reproductive, urogenital, and neoplastic disorders (German, 2006). Similar to humans, excess fat
Nutrigenomics and pet obesity

1.8 yr longer than ad libitum fed dogs that had increased mass leads to shorter lifespan in companion animals. For example, food-restricted dogs were reported to live about 1.8 yr longer than ad libitum fed dogs that had increased body fat mass (Kealy et al., 2002).

Common therapeutic options include dietary intervention, mainly by energy restriction, and increased physical activity. Although these strategies are logical, in practice, they are not often successful. Therefore, a better understanding of the molecular changes that occur in metabolically active tissues (e.g., adipose, skeletal muscle, and hepatic tissues) with BW gain and their response to dietary intervention may identify methods that prevent BW gain or aid in BW loss as well as ameliorate some of the chronic health problems related to obesity.

**Role of Adipose Tissue in Obesity**

Adipocytes (i.e., fat cells) are dispersed throughout the body in loose connective tissues (Ross and Pawlina, 2006). In adipose tissue, however, adipocytes are the predominant cell type, representing one-third to two-thirds of total cells (Ailhaud et al., 1992). Fibroblasts, pericytes, blood cells, endothelial cells, and macrophages represent the remaining cell types (Ailhaud et al., 1992; Tilg and Moschen, 2006; Poulos et al., 2010). Adipose tissue development begins in the secondary phase of fetal growth, when mesenchymal stem cells differentiate to adipocytes by expressing PPARγ (Poissonnet et al., 1984; Ross and Pawlina, 2006). Adipocyte precursors are not exclusive to the neonatal developmental period, however, as pre-adipocyte precursors are present throughout life (Ailhaud et al., 1992). Brown adipose has the same embryonic origin as white adipose; however, its differentiation and early proliferation is under the control of norepinephrine. This catecholamine regulates the gene expression of the mitochondrial *uncoupling protein (UCP)-1*, which uncouples the oxidation of fatty acids from ATP synthesis, leading to thermogenesis (Ross and Pawlina, 2006). During the postnatal period, there is a rapid and dramatic transformation of brown to white adipose tissue, achieved by its high plasticity. This phenomenon varies among species and fat depots; for instance, the process takes only a few days in ruminants, a few weeks in dogs and cats, and a few years in humans whereas this transformation is never fully completed in rodents (Loncar et al., 1986; Holloway, 1989; Guerra et al., 1998). In dogs, there is evidence that brown adipose tissue can be reactivated by stimuli (e.g., cold temperature, drug induction) that require compensatory thermogenesis (Holloway, 1989; Toseland et al., 2001).

Adipogenesis is closely associated with angiogenesis. In growing adipose tissue, angiogenic vessels contribute to adipogenesis by several means. Blood vessels are important for the delivery of nutrients, oxygen, growth factors, and cytokines to the adipocytes. The production of various growth factors and cytokines from the endothelial cells of the vessels communicate with adipocytes in a paracrine fashion to promote their growth and expansion and remove waste products from adipose tissue (Cao, 2010). During the development of obesity, however, the rapid expansion of adipose tissue is not necessarily followed by the proliferation of blood vessels, which may result in hypoxia. This low availability of oxygen to adipocytes serves as a stimulus for the synthesis and release of inflammatory cytokines and angiogenic factors to promote vascularization (Trayhurn and Wood, 2004).

Classically, adipose tissue was considered an inert and passive type of connective tissue; its main functions were thought to be confined to the maintenance of energy homeostasis and to the cushioning and insulation of the body. Due to the limited ability of the body to store carbohydrate- or protein-based energy sources, lipids in the form of triglycerides are stored in adipocytes during adequate food supply under influence of the hormone *insulin* and the enzyme *lipoprotein lipase (LPL)*. Under fasting conditions or food deprivation, triglycerides stored in adipocytes are broken down to glycerol and NEFA by the upregulation of *glucagon* and *hormone sensitive lipase (HSL)* as well as by the action of the enzymes diglyceride and monoglyceride lipases. Additionally, anti-insulinemic hormones such as GH, cortisol, and catecholamines can also be involved in the latter process. Once released into the systemic circulation, the liver and, to some extent, the kidney, take up glycerol whereas NEFA reach the target tissues (e.g., skeletal muscle) where they serve as an energy substrate.

The recent discovery of the secretory capacity of adipose tissue has redefined its purpose and it is now considered a major endocrine gland. In 1994, the discovery of *leptin* became a hallmark in the field of endocrinology, challenging the postulated purpose of adipose tissue (Zhang et al., 1994). From that time,

### Table 1. Common “omic” terms

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genomics</td>
<td>The study of genomes, including genome mapping and sequencing.</td>
</tr>
<tr>
<td>Transcriptomics</td>
<td>The study of the gene expression (mRNA or transcripts) profile present in a particular cell, tissue, or organism.</td>
</tr>
<tr>
<td>Proteomics</td>
<td>The study of the full complement of proteins present in a particular cell, tissue, or organism.</td>
</tr>
<tr>
<td>Metabolomics</td>
<td>The study of the full complement of metabolites present in a particular cell, tissue, or organism.</td>
</tr>
<tr>
<td>Nutrigenetics</td>
<td>The study of how the genetics of an organism affect response to diet.</td>
</tr>
<tr>
<td>Nutrigenomics</td>
<td>The study of how nutrients or bioactive dietary components affect host gene expression.</td>
</tr>
</tbody>
</table>
various substances synthesized by adipose tissue have been characterized and are known as “adipokines” or “adipocytokines.” These terms refer to cell-signaling proteins released by cells residing in adipose that have a specific effect on cell differentiation, energy metabolism, tissue remodeling, immune response, or inflammation, and that may function in an endocrine, paracrine, or autocrine fashion (Wang et al., 2008). Among the hormones synthesized by adipose tissue, leptin has been the most studied. More recently, adiponectin and resistin have also received substantial attention.

Leptin is secreted predominantly from adipose tissue. It is an anorexigenic peptide that acts via its receptors present predominantly in the arcuate nucleus of the hypothalamus, which is an important region in the brain responsible for the control of food intake, glucose homeostasis, and other endocrine functions (Myers, 2010). Circulating leptin concentrations reflect adiposity. Greater leptin concentrations in the obese individual signal the brain to decrease food intake and increase energy expenditure (Sagawa et al., 2002; Shibata et al., 2003; Sousa et al., 2009). Even though hyperleptinemia is common in obesity, the development of leptin resistance blunts its anorexigenic effects and exacerbates the development of this disease (Wang et al., 2008; Zoran, 2010). Age, gender, physical activity, and caloric ingestion can also affect leptin secretion (Klok et al., 2007). Leptin is involved in thermogenesis via activation of the sympathetic nervous system or by the stimulation of thyroid hormones (Cusin et al., 2000; Nonogaki, 2000).

Adiponectin is secreted exclusively by adipocytes and circulates as low molecular weight multimers (Kadowaki and Yamauchi, 2005; Ishioka et al., 2006). Not only do circulating adiponectin concentrations vary among species, but the range of concentrations of this hormone within species is also quite wide (Ishioka et al., 2006, 2009; Brunson et al., 2007). Total blood adiponectin concentration may not be that informative, however, because it has been suggested that the ratio between the high and low molecular weight adiponectin forms is more important in determining its effects on the body (Bobbert et al., 2005). In contrast to leptin, total adiponectin concentrations are decreased in obese or insulin-resistant human subjects. A greater proportion of low molecular weight adiponectin is present in the obese (Maeda et al., 2002). In some studies, obese dogs and cats have had reduced adiponectin concentrations, indicating that it has similar metabolic functions as in humans (e.g., glucose metabolism, anti-inflammatory properties) in these animals (Ishioka et al., 2006; Zoran, 2010). Others have not observed such changes in serum adiponectin concentrations. In our laboratory, for example, rapid BW gain in dogs did not alter circulating adiponectin concentrations (Grant et al., 2011). In contrast to humans, healthy lean dogs have greater high molecular weight adiponectin concentration, and obesity does not appear to decrease its circulating concentrations (Verkest et al., 2011). Future research should be conducted to better characterize the role of adiponectin in pet obesity.

Resistin is a hormone known to be secreted from adipocytes and other body tissues in mice and humans (Steppan et al., 2001; Kusminski et al., 2005). In dogs, obesity increases serum resistin concentrations (44.4 to 88.5 pg/mL) compared with that of dogs after BW loss (32.3 to 67.1 pg/mL; Wakshlag et al., 2011). However, the expression of this hormone has not yet been reported in cats (Zoran, 2010). Similar to leptin, greater resistin concentrations are observed in overweight or obese subjects, during insulin resistance, and in type-2 diabetes mellitus patients. Resistin also increases the production of pro-inflammatory cytokines [e.g., IL-1, IL-6, and tumor necrosis factor α (TNF-α); Steppan et al., 2001; Kaser et al., 2003], which are thought to contribute to insulin resistance. Further research is warranted in this area to determine the presence of this hormone and its metabolic functions in dogs and cats.

White adipose tissue is involved in the synthesis and secretion of over 50 other adipokines engaged in multiple physiological conditions such as metabolism, immunity, and inflammation (Lago et al., 2007). Tumor necrosis factor-α, IL-1, and IL-6 have been well characterized, showing local and systemic pro-inflammatory effects (Bulló et al., 2003), and have been attributed to the development of insulin resistance (Kern et al., 2003) and as a local regulator of insulin action (Kern et al., 2001). Another relevant aspect of IL-6 is that it controls hepatic C-reactive protein (CRP) production, an important inflammatory marker associated with obesity (Bulló et al., 2007). It is likely that as more research is conducted in this area, new adipocyte secretory products will be discovered and their mechanisms and functions will be revealed. As yet, little is understood about adipokines in pets.

**Role of Skeletal Muscle and Hepatic Tissues in Obesity**

Metabolic disorders related to overweightness and obesity are not exclusive to adipose tissue. Skeletal muscle and the liver are key contributors to the maintenance of whole-body euglycemia and, therefore, are targets of metabolic and hormonal imbalances and of increased inflammatory processes associated with obesity.
The liver plays a vital role in several metabolic processes, including protein synthesis, nutrient metabolism, waste removal, and the production and assimilation of glucose. **Insulin-like growth factor-1** is a hormone synthesized in the liver, which has a similar molecular structure to insulin and has been suggested to be a biomarker of obesity. It has anabolic effects in several tissues of the body. In obese dogs but not in cats, increased serum IGF-1 concentrations were reported whereas serum IGF-1 concentrations were decreased after BW loss (Blanchard et al., 2004; Tvarijonaviciute et al., 2012a,b,d).

In healthy subjects, the liver is responsible for the disposal of approximately 30% of ingested glucose after a carbohydrate load whereas the other two-thirds reach the systemic circulation and peripheral tissues, with 40% of it being taken up by the skeletal muscle (Meyer et al., 2002). Under normal physiological conditions, insulin is the primary hormone responsible for blood glucose homeostasis during the postprandial state. After ingestion of a meal, insulin concentrations rise to promote uptake of glucose into insulin-sensitive tissues (e.g., skeletal muscle, liver) and decrease hepatic glucose production. Concomitantly, insulin stimulates LPL activity in adipose tissue, which results in increased esterification of NEFA from very low density lipoprotein (VLDL) and chylomicrons, decreasing their availability in the systemic circulation as fuel to other tissues.

Obese humans and some animals (e.g., cats) are predisposed to develop insulin resistance and type-2 diabetes. The lack of insulin sensitivity by adipose, skeletal muscle, and hepatic tissues leads to disturbances in the metabolism of lipids and carbohydrates. **Insulin** resistance in adipose tissue prevents the suppression of HSL, which precludes the esterification of NEFA in this tissue. This unresponsiveness of hepatic and peripheral tissues to insulin results in an inadequate uptake of glucose and in the mobilization of endogenous substrates, such as glycogen, protein, and fat reserves. The latter may result in increased lipid accumulation in skeletal muscle cells (e.g., triglycerides, diglycerides, and ceramides), due to increased fatty acid uptake and to decreased β-oxidation (Cahová et al., 2007). Increased lipid content in myocytes leads to defects in the insulin cascade signaling, mainly through dysfunction in the phosphorylation of the accessory proteins, insulin receptor (IRS)-1 and IRS-2, resulting in the inability of these cells to translocate the insulin-responsive glucose transporter (GLUT) 4 from the cytosol to the apical membrane, and by reducing the glycogen synthase activity levels in these cells (also regulated by IRS-1 and -2 proteins), which further exacerbates this condition. **Insulin** resistance is characterized by hyperglycemia and insulinemia and it may progress to type-2 diabetes mellitus, which over time can lead to exhaustion of pancreatic β cells and need of exogenous insulin for maintenance of physiological blood glucose concentration. Because the skeletal muscle and the liver are the main organs responsible for glucose disposal, understanding the onset of insulin resistance in these tissues is fundamental to preventing disturbances in lipid and carbohydrate metabolism, the development of type-2 diabetes mellitus, and other clinical complications associated with obesity.

**USE OF GENOMIC BIOLOGY TO UNDERSTAND PET OBESITY**

Currently, most of the genomics literature available on companion animal obesity has focused on adipose tissue whereas fewer studies have investigated the role of other body tissues (e.g., skeletal muscle, liver). Common gene expression responses to BW gain/obesity are presented in Fig. 1. In addition, gene expression profiles of dogs and cats under different physiological status have also been investigated, with researchers comparing obese vs. lean or intact vs. spayed or neutered animals. Dietary interventions to induce obesity, promote weight loss, or alter dietary nutrient profile have also been investigated, all of which have been reviewed below.

Although the advances in molecular biology (e.g., genome sequencing, microarrays, quantitative real-time PCR (RT-PCR) have opened new research frontiers, most of the techniques and the research in this field have been applied to humans or rodent models. Despite some progress, limited genomic information and molecular tools have been available to study the biological function of adipokines in the domestic dog and cat. As a consequence, several scientific questions have to be answered before a good understanding of canine and feline obesity is obtained. Those include but are not limited to whether assays developed for humans and rodents can be used to study canine or feline adipose physiology, whether hormones and peptides involved in obesity in other species have similar biological activity in dogs and cats, and what factors and mechanisms contribute or respond to canine and feline adiposity.

**Canine Studies**

In 2000, the canine *leptin* gene was cloned and described (Iwase et al., 2000). The AA sequence of canine leptin was 76 to 88% identical to other mammal and nonmammal species (e.g., cow, pig, human, mouse, rat, and chicken), with only 8 AA commonly found in other species being altered in dogs (Iwase et al., 2000). The canine leptin cDNA sequence was most similar to the pig (92% similarity) and least similar to the chicken (82% similarity). Although several tissues were analyzed for the expression of leptin in the dog, only adipose tissue had a detectible product, indicating that it is the major
production site of this hormone. Biological functions of canine leptin were reported to be similar to that of other species. For instance, recombinant canine leptin induced phosphorylation of signal transducer and activators of transcription and mitogen-activated protein kinase (Iwase et al., 2000). The use of plasma leptin as a marker for adiposity in dogs was verified by Sagawa et al. (2002). Twenty spayed female Beagles were used in that study. Dogs were subjected to different feeding and exercise regimens, with the objective of yielding distinguished BW and BCS over a period of 21 wk. Predictably, strong positive correlations were observed between body fat content and plasma leptin concentration, BW, and subcutaneous tissue thickness. Similarly, plasma leptin concentration was highly and positively correlated with BW, BCS, and subcutaneous tissue thickness. It seems that measurement of plasma leptin concentration can be a useful tool in the quantification of adiposity in obese dogs. It also may provide further benefits in assessing adiposity and obesity of dogs of different breeds and ages, in which reasonable variability in somatoscopic characteristics can exist. A few items must be considered when measuring plasma leptin concentration, however, including diurnal variation associated with feeding–fasting period, variation associated with estrous cycle, and regulation by neuroendocrine factors (Sagawa et al., 2002).

Canine adiponectin is exclusively expressed in adipose tissue, with both visceral and subcutaneous depots having been studied thus far. Like leptin, canine adiponectin has a high nucleotide sequence homology with other species including the domestic cat (91% similarity), human and Rhesus monkey (87% similarity), pig (86% similarity), and rat (80% similarity). Diet-induced obese research dogs (14 wk of a high-energy diet; 3,500 to 5,300 kJ/d) and client-owned overweight and obese dogs have reduced plasma adiponectin concentrations. The inverse relationship between adiponectin and leptin in relation to fat mass may become a useful marker in the management and prevention of obesity (Ishioka et al., 2006). Adiponectin, however, seems less sensitive to variation in BW than leptin, with obese dogs showing only a slight reduction in adiponectin when compared with overweight dogs. Further investigation is warranted to understand the metabolic function of adiponectin in canine obesity (Ishioka et al., 2006).
A few studies have used primary cell culture systems to learn more about adipose tissue. Those studies have demonstrated that canine white adipose tissue is capable of expressing and secreting a variety of cytokines. Eisele et al. (2005) showed that canine adipocytes collected from different body fat depots and differentiated in vitro expressed several adipokines involved in inflammatory responses [e.g., leptin, adiponectin, plasminogen activator inhibitor-1, IL-6, haptoglobin, metallothionein-1 and -2, and nerve growth factor (NGF)]. Additionally, canine pre-adipocytes and differentiated adipocytes from major fat depots (i.e., subcutaneous, omental, perirenal, gonadal, and falciform ligament) expressed and secreted NGF. This adipokine is involved in the development and maintenance of the sympathetic nervous system in various tissues and in inflammatory responses in adipose tissue. In adipocytes, NGF was highly responsive to TNF-α and lipopolysaccharide (LPS) treatment, indicating that canine adipocytes are very reactive to inflammatory processes (Ryan et al., 2008).

The ubiquity and lack of extensive differences in expression of leptin, adiponectin, IL-6, monocyte chemoattractant protein-1 (MCP-1), and TNF-α in several canine adipose tissues (e.g., subcutaneous, omental, perirenal, gonadal, and falciform ligament) indicates that adipose depots are more equally distributed among body compartments in the dog (Ryan et al., 2010). Similar to human cell culture, results from a canine cell culture system demonstrated that pre-adipocytes were capable of expressing IL-6, MCP-1, and TNF-α mRNA but not leptin and adiponectin mRNA, which were only expressed in differentiated adipocytes (Ryan et al., 2010). Expression and secretion of IL-6, MCP-1, and TNF-α mRNA were highly responsive to inflammatory mediators, including TNF-α and LPS. The use of anti-inflammatory agents (dexamethasone and rosiglitazone) resulted in decreased expression and secretion of MCP-1 mRNA but stimulated leptin gene expression. Although administration of dexamethasone has similar effects on leptin expression in humans and rodents (Yoshida et al., 1996; Masuzaki et al., 1997), the effects of rosiglitazone in canine adipose tissue contrasted with that of humans and rodents where leptin expression was suppressed in adipocytes (De Vos et al., 1996; Kallen and Lazar, 1996), indicating differences in the biological functions of PPARγ nuclear receptor in dogs (Ryan et al., 2010). Increased 11β-hydroxysteroid dehydrogenase type 1 mRNA is often observed in obese adipose tissue. Although its role is not fully understood, it has been related to hypoadiponectinemia and decreased insulin sensitivity in obese subjects (Alberti et al., 2007). In addition, 11β-HSD-1 is involved in the conversion of inert cortisone to cortisol (Ryan et al., 2011). The latter study investigated the 11β-HSD-1 gene expression in several canine adipose depots (e.g., subcutaneous, omental, perirenal, gonadal, and falciform) during adipocyte differentiation in vitro gene expression of 11β-HSD-1 was greatest in the omental depot and least in the perirenal depot. Differentiation of pre-adipocytes isolated from subcutaneous and gonadal adipose depots resulted in increased and decreased 11β-HSD-1 mRNA, respectively, when compared with pre-induction levels. Treatment of canine adipocytes with TNF-α and LPS in vitro resulted in increased 11β-HSD-1 mRNA in subcutaneous and gonadal adipocytes (Ryan et al., 2011).

Because adipose tissue is so actively involved in the synthesis of several signaling molecules and hormones related to energy balance, several studies have focused on understanding the metabolic changes that occur in this tissue during BW gain or BW loss. During the development of obesity, adipose tissue undergoes major anatomical and physiological modifications; however, little is known about this process. To our knowledge, our laboratory was the first to investigate the physiological and transcriptome changes during the early stages of BW gain and obesity (i.e., wk 4, 8, 12, and 24) in dogs using microarray technology (Grant et al., 2011). In that study, intact female Beagles were fed ad libitum or at maintenance for a period of 24 wk. As expected, ad libitum feeding led to increased BW (wk 0 = 8.4 ± 0.34 kg and wk 24 = 14.6 ± 0.34 kg), BCS (wk 0 = 4.4/9 and wk 24 = 8.1/9), fat mass (wk 0 = 1.4 ± 0.24 kg and wk 24 = 6.5 ± 0.24 kg), and adipocyte size (wk 0 = 114.7 ± 17.4 µm² and wk 24 = 321.0 ± 18.2 µm²). The onset of obesity also led to altered expression of 1,665 genes in adipose tissue, with major functional gene classes being related to transcription, transport, metabolism, signaling, cell cycle, differentiation and growth, and RNA processing. Moreover, several genes related to oxidative stress (e.g., GSTM4, GSTT2, GSTO1, and GPX7) were upregulated with BW gain, mainly during early BW gain (i.e., wk 4 and 8), which may reflect changes occurring during acute adipose tissue expansion. Expression of genes associated with carbohydrate and lipid metabolism were also altered with weight gain. For example, the expression of elongation of long-chain fatty acids family member 6 (ELOVL6) and zinc-2 a glycoprotein (ZAG) were drastically increased at wk 4 by approximately 62- and 64-fold, respectively, whereas acetyl-CoA carboxylase-A (ACACA) was decreased 0.30-fold. From wk 4 to 24, expression of ACACA remained constant whereas expression of ELOVL6 and ZAG fluctuated, showing smaller increases overtime. The many changes in gene expression that occurred with BW gain in that study indicates that subcutaneous adipose tissue may respond differently during early BW gain and acute development of obesity compared with chronic deleterious effects of this disease (Grant et al., 2011).
Although Grant et al. (2011) studied diet-induced obesity in research dogs, many have studied naturally occurring obese client-owned dogs. In naturally occurring obese dogs (n = 26), increased concentrations of fasting plasma TNF-α, haptoglobin, CRP, and insulin and increased insulin to glucose ratio were observed (German et al., 2009). After an average BW loss of 22%, all variables mentioned above were decreased, indicating improved insulin sensitivity and attenuation of inflammatory processes associated with obesity (German et al., 2009). Obese female dogs (4 to 12 yr of age) have also been shown to have decreased glucose sensitivity (Vargas et al., 2004). These dogs had reduced plasma membrane GLUT4 content in parametrical adipose tissue. Because increased microsomal GLUT4 was detected in this same tissue, it is thought that insulin resistance during obesity is a result of reduced translocation of intracellular GLUT4 to the cell membrane (Vargas et al., 2004). Obesity has also been shown to decrease the efficiency of plasma insulin uptake by the central nervous system, an event that seems to occur independently of the reduced insulin sensitivity in peripheral tissues. Decreased central nervous system sensitivity to insulin further exacerbates BW gain because the effect of insulin on food intake inhibition, lipid oxidation, and long-term energy balance is no longer sensed at that site (Kaiyala et al., 2000).

High-fat or high-calorie diets have been used to investigate changes in insulin-sensitive tissues during obesity. Adult Beagles fed a combination of extruded and canned diets, both high in protein and fat content (>30% CP and 20% fat), for a period of approximately 7 mo had BW increased 43% and became insulin resistant, as confirmed by the euglycemic-hyperinsulinemic clamp technique (Leray et al., 2004). In these dogs, PPARγ and UCP-1 mRNA was decreased in visceral adipose tissue, indicating their potential contribution in the development of obesity (Leray et al., 2004). Gayet et al. (2007) also examined the effects of canine obesity and insulin resistance on the expression of PPARγ and other genes implicated in glucose and lipid metabolism in the skeletal muscle and adipose tissues. In that study, a steady state of obesity was achieved after 55 wk of feeding a high-fat diet [i.e., 53% fat and 5,527 kcal/d] vs. control diet [i.e., 33% fat and 3,885 kcal/d] for a period of 16 wk led to larger adipocytes, predominantly, in visceral depots (Kabir et al., 2011). Visceral adipocytes larger than 75 μm were considered to be good predictors of whole body and hepatic insulin resistance whereas size of adipocytes in subcutaneous adipose tissue was not. In that same study, administration of rimonabant, a selective type-1 cannabinoid receptor antagonist, resulted in reduced adipocyte cell size and distribution of fat cells in both visceral and subcutaneous adipose tissue. These findings provide further support to the portal theory and indicate that visceral adipocyte hypertrophy favors the development of insulin resistance (Kabir et al., 2011).

Hyperlipidemia is another common metabolic disorder observed with obesity. Le Bloc’h et al. (2010) investigated the effects of nicotinic acid (NA) treatment on triglyceride and cholesterol metabolism, including mRNA transcription of genes implicated in the metabolism of apolipoprotein B 100 (apoB100)-containing lipoproteins, in obese-induced insulin-resistant dogs fed 1.6 times that of the NRC (2006) maintenance requirement for a period of 60 wk. Obese dogs (16.8 ± 0.7 kg BW) treated with NA during a 4-wk
Nutrigenomics and pet obesity

TDF). Restricted feeding resulted in a dramatic decrease in weight loss on the reestablishment of insulin sensitivity and was correlated with increased postprandial adipose gene expression (Leray et al., 2008). In that experiment, adult female dogs were fed a high-fat, low-energy diet (i.e., 34% CP, 10% fat, and 20% TDF). Restricted feeding resulted in a dramatic decrease in fasting insulin concentrations compared with baseline (11.1 ± 0.8 kg BW). Results from a kinetic study using a stable leucine isotope (precursor of apoB100) demonstrated that NA treatment decreased the concentration of VLDL apoB100 and LDL apoB100.

This reduction was associated with a reduced absolute production rate. Additionally, hepatic gene expression of diacylglycerol acyltransferase 2, a key enzyme in triglyceride synthesis, was decreased after NA treatment. This was the first study to demonstrate such an effect of diacylglycerol acyltransferase 2 in vivo and implied that inhibition of this enzyme by NA could result in less plasma triglyceride by reducing VLDL synthesis.

A few researchers have studied the impact of weight loss on the reestablishment of insulin sensitivity and the gene expression of genes associated with the lipogenic and lipolytic processes that may be involved. Subcutaneous and visceral adipose depot changes were studied in dogs before and after BW loss by Leray et al. (2008). In that study, obesity was induced by feeding 300 g (i.e., approximately 171.3 kcal/kg of metabolic BW, a 30% increase compared with the maintenance energy requirement) of food daily of a hyperenergetic diet (i.e., 30% CP, 20% fat, and 7.5% TDF) daily whereas BW loss was achieved by restricted feeding (i.e., 0.6 times the NRC requirement for maintenance; NRC, 2006) of a high-protein, low-energy diet (i.e., 34% CP, 10% fat, and 20% TDF). Restricted feeding resulted in a dramatic decrease in fat mass (i.e., 34.1 vs. 17.6% of BW). Insulin sensitivity, as determined by euglycemic-hyperinsulinemic clamp, was improved with BW loss and was correlated with the increased IRS-2 gene expression in subcutaneous adipose tissue. Several genes associated with lipogenesis were decreased in visceral adipose with weight loss, including SREBP-1, fatty acid synthase (FAS), adipose differentiation related peptide, fatty acid binding protein, and PEPCK. The expression of these genes was not altered in subcutaneous adipose tissue as a consequence of weight loss, confirming significant differences in lipid metabolism between these fat depots (Leray et al., 2008).

Wakshlag et al. (2011) measured changes in adipokines in a client-owned dog population (n = 25) before and after a BW-loss program. On average, it took 26 wk for dogs to lose about 23% of their initial BW and to decrease their BCS from 8 to 5 in a 9-point BCS system, with a target weekly BW loss of 2%. As expected, BW loss resulted in decreased fasting serum CRP, MCP-1, leptin, and resistin concentrations. In contrast to humans, fasting serum total and high molecular weight adiponectin concentrations were unchanged after BW loss. Altogether, this study indicates that the inflammatory processes related to excessive fat mass are suppressed after BW loss. It also implies that, in dogs, differences in adiponectin functionality and response to changes in BW status may be responsible for the reduced incidence of insulin resistance and type-2 diabetes in this species compared with humans and cats.

Alteration is in the serum proteome of 5 female Beagles before and after weight loss has also been investigated (Tvarijonaviciute et al., 2012c). After 3 mo of ad libitum feeding of a hyperenergetic diet (i.e., 31% CP and 21% fat), animals were 30% overweight with a BCS of 5 in a 5-point BCS system. Once obese, a blood sample was collected and dogs were fed a restricted amount of a hypoenergetic diet (i.e., 34% CP and 10% fat) for an additional 3 mo until their BW returned to ideal range (i.e., BCS of 3 in a 5-point BCS system). At the end of the BW-loss period, a blood sample was collected and protein mapping of canine serum was analyzed. Obesity affected 3 serum proteins: retinol-binding protein 4 (RBP4), clusterin precursor (CLU), and α-1 antitrypsin (α-AT). Expression of RBP4 and CLU were increased during obesity whereas α-AT was decreased. The authors suggested that RBP4, CLU, and α-AT can be used as biomarkers of obesity of obesity-related disorders or as measurement of therapeutic effectiveness of weight loss (Tvarijonaviciute et al., 2012c).

Diet has been a major contributor, among other environmental factors, of the obesity pandemic and chronic diseases related to BW gain. In companion animals, however, few have focused on identifying mechanisms by which diet may improve health in the obese. Respondek et al. (2008) evaluated the effects of feeding 1% short-chain fructooligosaccharides (scFOS) on insulin resistance and adipose tissue metabolism in obese dogs. In that experiment, adult female dogs were fed twice their maintenance energy requirement for a period of 14 wk to induce obesity (BW = 19.2 ± 2.3 kg, a 50% increase from baseline). Once obesity was established, food intake was adjusted to maintain that BW. During the maintenance phase, dogs were randomly assigned to a basal diet or a basal diet plus 1% scFOS in a crossover design to evaluate the beneficial effects of scFOS supplementation. Obese dogs were insulin resistant, as measured by a euglycemic-hyperinsulinemic clamp, and had increased plasma insulin (135 ± 13 pmol/L) concentration compared with baseline (69 ± 7 pmol/L). Despite the increased concentration of insulin, euglycemia was maintained (approximately 4.9 mmol/L). Subcutaneous adipose expression of HSL tended to be increased in obese dogs. Supplementation of scFOS resulted in improved glucose disposal during the euglycemic-hyperinsulinemic clamp technique, which was potentially explained by the increased postprandial adipose gene expression...
of UCP-2 and a tendency for greater postprandial carnitine palmitoyltransferase-1 mRNA.

Another study demonstrated that oral supplementation of green tea extract at 80 mg·kg BW$^{-1}·d^{-1}$ for 12 wk was sufficient to improve insulin sensitivity, also determined by euglycemic-hyperinsulinemic clamp, and to enhance lipid profile of obese dogs (13.9 to 15.1 kg BW and 5.2 to 5.8 kg of fat mass) by modifying gene expression of genes involved with the glucose and lipid metabolism (Serisier et al., 2008). Green tea supplementation did not result in BW loss or reduced fat mass but did decrease plasma triglyceride concentrations in obese dogs (1.16 and 0.58 mmol/L before and after green tea supplementation, respectively), improved insulin sensitivity index (60% increase), and increased gene expression of PPARγ, LPL, GLUT4, and adiponectin in visceral and subcutaneous adipose tissues when compared with baseline at wk 0. In skeletal muscle, expression of PPARα and LPL were increased after green tea supplementation whereas no changes in the expression of PPARα and LPL were observed in hepatic tissue. Findings reported in both studies indicate that dietary intervention influences the transcription of genes involved in glucose and lipid metabolism (Respondek et al., 2008; Serisier et al., 2008). However, more research in this area is needed to further investigate what and how nutrients and phytochemicals may improve life quality and health of companion animals.

**Feline Studies**

Domestic cats also suffer from a high incidence of obesity. Similar to humans, obese cats are highly susceptible to developing type-2 diabetes and insulin resistance. As described previously, leptin plays a crucial role in energy balance and, therefore, in obesity. In 2001, the primary structure of the feline leptin gene was determined by cloning its cDNA (Sasaki et al., 2001). It was discovered that feline leptin had high homology to that of other species (e.g., canine, porcine, bovine, human, mouse, and rat), having the greatest and least nucleotide identity with the dog (93.5% similarity) and the mouse (82.7% similarity), respectively. Feline leptin gene expression was measured in white adipose tissue but not in liver, heart, kidney, lung, pancreas, brain, or skeletal muscle tissues (Sasaki et al., 2001). The production of recombinant feline leptin and the development of an ELISA enabled the analysis of plasma leptin concentrations of 24 privately owned cats with varying ages (i.e., 7 mo to 10 yr) and BW (2 to 7 kg). Although the average plasma leptin concentration was 4.5 ng/mL, it ranged from 0.2 to 12.8 ng/mL and was positively correlated with body fat mass (Shibata et al., 2003).

Adiponectin has been highlighted as an important hormone involved in the development of insulin resistance (Kondo et al., 2002; Kadowaki et al., 2006). Therefore, it is crucial to understand the physiological role of adiponectin in the cat. Ishioka et al. (2009) determined the nucleotide and AA sequence of the feline adiponectin and adiponectin receptor (AD-R1) genes. Both genes had high nucleotide sequence similarity with other species (adiponectin gene with dog, human, mouse, rat, cow, and pig and AD-R1 gene with human, mouse, and rat), especially with the dog (91% similarity for adiponectin gene) and the human (96% similarity for AD-R1 gene). Feline adiponectin mRNA was present only in adipose tissue whereas AD-R1 mRNA was distributed in various tissues, including adipose, liver, pancreas, kidney, heart, lung, and skeletal muscle. Plasma adiponectin concentrations of lean and obese cats were also determined. Lean cats had considerably greater (18.0 ± 3.2 μg/mL) adiponectin concentrations than obese cats (7.2 ± 1.5 μg/mL). In obese cats that undergo BW loss, plasma adiponectin has been shown to increase to concentrations similar to those of lean cats (Tvarijonaviciute et al., 2012d).

Even though diabetes is a serious concern in the feline population, little is known regarding the genes involved in insulin signaling in this species. Only recently were the feline mRNA sequences of genes associated with obesity and inflammation-induced insulin resistance (e.g., adiponectin, insulin receptor, GLUT1 and GLUT4, PPAR-1 and -2, plasminogen activator inhibitor-1, and MCP-1) sequenced in healthy cats (Zini et al., 2009). In that study, feline mRNA sequences were determined using conserved regions of canine and human gene alignments. Those feline mRNA sequences were more similar to dogs (i.e., 90 to 95% similarity) than humans (i.e., 86 to 92% similarity). Except for MCP-1 that was only detected in the adipose tissue, all aforementioned genes were expressed and detected in visceral adipose, subcutaneous adipose, and skeletal muscle tissues (Zini et al., 2009).

As a strict carnivore, the cat is normally thought of as being less sensitive to insulin and, therefore, not capable of coping with large carbohydrate loads in contrast to omnivorous species, such as the dog. In addition, cats have a very low hepatic glucokinase activity due to trivial amounts of portal glucose. As a consequence, adult cats rely on gluconeogenesis to maintain euglycemia, and this metabolic process is maximized in the absorptive phase in contrast to omnivorous species (Kirk et al., 2000). These unique qualities that pertain to the feline metabolism may explain the greater incidence of type-2 diabetes in this species when compared with dogs (Mori et al., 2009b). To investigate differences in the glucose and lipid metabolism between dogs and cats, tissue samples from liver, abdominal adipose, and skeletal muscle tissues of a healthy adult cat and dog were collected and analyzed for mRNA transcription of genes involved in the insulin signaling cascade [e.g., IRS-1 and -2 and
phosphatidylinositol 3-kinase (PI3-K) and enzymes involved with glucose and lipid metabolism [e.g., malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G6PDH), and FAS]. In general, feline hepatic tissue had less expression of IRS-1 and -2, PI3-K, and MDH than dogs. Similarly, gene expression of IRS-2, PI3-K, MDH, and FAS in skeletal muscle was less in cats than in dogs. In abdominal adipose tissue, MDH, G6PDH, and FAS mRNA was less in cats than in dogs. All together, these data support the hypothesis that cats have less insulin sensitivity than dogs (Mori et al., 2009b). A similar study in that laboratory examined mRNA abundance of insulin signaling genes (i.e., IRS-1 and -2 and PI3-K), activity of enzymes related to lipid and carbohydrate metabolism in insulin-sensitive tissues (e.g., liver, skeletal muscle, and abdominal fat), and plasma metabolite concentrations in lean and overweight cats (Mori et al., 2009a). Increased BW was induced by providing a 2-fold increase of their daily energy requirement for 4 wk. Although all cats had similar fasting plasma glucose and insulin concentrations, overweight cats had less mRNA transcription of IRS-2 (28 and 48%, respectively) and PI3-K (48 and 60%, respectively) in hepatic and skeletal muscle tissues. Overeating increased BW 27% (approximately 1 kg BW increase), BCS from 3 to 4 (on a 5-point scale), and plasma triglyceride (48 ± 6 mg/dL, an approximately 118% increase) and alanine aminotransferase (131 ± 24 U/L, an approximately 92% increase) concentrations when compared with the control group (Mori et al., 2009a). Despite having decreased mRNA abundances of MDH and HSL in the liver and adipose tissues, respectively, their activities were not altered in these tissues. Similarly, Brennan et al. (2004) reported that obese cats (5.1 ± 0.7 kg BW and 31.8 ± 5.6% body fat mass) fed ad libitum for 6 mo had less protein GLUT4 expression in subcutaneous adipose (lean = 23.8 ± 11.5 counts mm² × 10³ and obese = 8.1 ± 7.6 counts mm² × 10³ in obese dogs) and skeletal muscle (lean = 26.5 ± 16.2 counts mm² × 10³ and obese = 3.9 ± 4.7 counts mm² × 10³ in obese dogs) tissue as determined by western blot analysis and greater insulin (lean = 34.8 ± 12.9 nmol/L and obese = 42.0 ± 11.2 nmol/L) area under the curve data during a glucose tolerance test yet fasting glucose concentrations were within the normal physiological range (lean = 85.1 ± 11.9 mg/dL and obese = 88.4 ± 40.9 mg/dL) for cats (Brennan et al., 2004). Overall, the data demonstrate that modifications at the transcription level preclude clinical signs observed with obesity-induced insulin resistance.

Because obesity can result in alterations in lipid metabolism, it is important to understand the role of lipases and inflammatory cytokines in lipid deposition. Lipoprotein lipase is an important enzyme in the distribution and uptake of fatty acids into different body tissues. Compared with lean controls (approximately 3.4 kg BW), obese research cats (approximately 6.4 kg BW) had less plasma LPL activity (lean = approximately 8 μmol oleic acid h⁻¹·min⁻¹ and obese = approximately 4 μmol oleic acid h⁻¹·min⁻¹, respectively), greater LPL mRNA in skeletal muscle [lean = 1.3 ± 0.7 arbitrary units (AU) and obese = 2.1 ± 0.7 AU], and less LPL mRNA in subcutaneous adipose tissue (lean = 46.9 ± 10.6 AU and obese = 19.4 ± 10.6 AU; Hoenig et al., 2006). Skeletal muscle HSL mRNA was increased 2-fold in obese vs. lean cats. In subcutaneous adipose tissue, TNF-α gene expression was greater in obese cats vs. control (lean = 1.7 ± 0.3 AU and obese = 11.2 ± 5.6 AU). Tissue specific regulation of lipases and TNF-α indicates a shift in fatty acid deposition from adipose tissue to skeletal muscle, where fatty acids can be stored or metabolized for energy (Hoenig et al., 2006).

A recent study examined the effects of overweight status on the expression of SREBP-1c and downstream lipogenic genes in abdominal subcutaneous adipose, omental adipose, and hepatic tissues. After a 6-wk period of overfeeding, overweight cats had increased BW (control = 3.6 kg and overweight = 4.7 kg), BCS (control = 2.7 and overweight = 3.9), and plasma triglyceride (control = 22.0 mg/dL and overweight = 48.3 mg/dL), NEFA (control = 0.27 mEq/L and overweight = 0.38 mEq/L), and alanine aminotransferase (control = 68.0 U/L and overweight = 131.3 U/L) concentrations compared with control. Hepatic and subcutaneous adipose tissues of overweight cats also had less SREBP-1 mRNA transcription (0.29- to 0.71-fold change, respectively) and ATP citrate lyase (ACL) mRNA transcription (0.32- to 0.90- and 0.24- to 0.48-fold change, respectively). Transcription of FAS mRNA was also decreased in subcutaneous adipose tissue (0.0- to 0.79-fold change) vs. control whereas SREBP-1 (1.0- to 4.9-fold change) and FAS (1.2- to 7.6-fold change) mRNA transcription was increased in the abdominal omental adipose tissue of overweight cats vs. control (Lee et al., 2011). Comparison of subcutaneous and omental adipose depots between lean and overweight cats showed that changes in gene expression of SREBP-1, FAS, and ACL may depend on depot. In lean cats, SREBP-1, FAS, and ACL mRNA were greater in subcutaneous vs. omental adipose tissue. In overweight cats, however, the expression of most of these genes was downregulated in the subcutaneous vs. omental adipose tissue, except for ACL. These data indicate that in an overweight state, subcutaneous adipose and hepatic tissues have a reduced capacity for lipid storage whereas omental adipose tissue continues to foster lipid deposition (Lee et al., 2011).

Gonadectomy is a major risk factor of companion animal obesity. Belsito et al. (2009) examined the effects of ovariohysterectomy and food intake on body composition, voluntary physical activity, and subcutaneous adipose gene expression in cats that were food restricted and ad
libitum fed. After a 4-wk baseline period, healthy female young adult cats (3.52 ± 0.72 kg BW) were spayed. After spaying, cats were fed to maintain BW for 12 wk. During that period, a decrease of approximately 30% in food intake was required to avoid BW gain. In addition, total voluntary physical activity was drastically decreased (52% less at 24 wk vs. baseline) from ovariohysterectomy. From wk 12 to 24, cats were fed ad libitum, resulting in severe increase (i.e., 120%) in body fat mass. Similar increases in BW and fat mass have been observed in other studies evaluating cats after ovariohysterectomy (Harper et al., 2001). Along with BW gain, relative gene expression of IL-6 was increased (wk 0 = 210 ± 18 AU and wk 24 = 463 ± 71 AU) in adipose tissue whereas gene expression of LPL (wk 0 = 968 ± 159 AU and wk 24 = 543 ± 50 AU), HSL (wk 0 = 702 ± 120 AU and wk 24 = 436 ± 49 AU), and adiponectin (wk 0 = 1,070 ± 335 AU and wk 24 = 460 ± 124 AU) were decreased at wk 24 when compared with baseline (Belsito et al., 2009). Another study from our laboratory investigated the effects of ovariohysterectomy on body composition, blood metabolite concentrations, voluntary physical activity level, and mRNA transcription in subcutaneous adipose and skeletal muscle tissues of cats fed either a high-protein [i.e., 53% CP and 10% nitrogen free extract (NFE)] or a moderate-protein (i.e., 34% CP and 34% NFE) diet (Vester et al., 2009b). Food intake and body fat were increased after gonadectomy regardless of diet, but food intake tended to be greater in cats fed the high-protein diet. In agreement with previous findings (Belsito et al., 2009), voluntary physical activity level of the cats decreased during the light (9.6 activity counts/15-s epoch) and dark (11.9 activity counts/15-s epoch) periods 24 wk after spaying compared with baseline (28.0 and 35.9 activity counts/15-s epoch, respectively; Vester et al., 2009b). In that study, mRNA transcript abundance in adipose and skeletal muscle tissues was affected by BW gain but not by dietary treatment. In adipose tissue, adiponectin, HSL, insulin receptor, toll-like receptor-4, and UCP-2 gene expression was decreased with BW gain whereas GLUT4 was increased after ovariohysterectomy and BW gain. In skeletal muscle, GLUT1, HSL, UCP-2, and insulin receptor mRNA transcription decreased with BW gain, independent of diet. These last 2 studies demonstrate that ovariohysterectomy may establish a new “set point” characterized by increased food intake and BW and accompanied by physiological changes. They also demonstrated that a high-protein diet did not control food intake or improve satiety and that ad libitum feeding should be avoided after spaying or neutering to prevent BW gain (Belsito et al., 2009; Vester et al., 2009b).

Another study in our laboratory evaluated the effects of in utero and postnatal feeding of a high-protein (i.e., 53% CP and 10% NFE) or a moderate-protein (i.e., 34% CP and 34% NFE) diet on adipose tissue gene expression, blood metabolites, and voluntary physical activity in kittens (Vester et al., 2009a). Two-month-old kittens fed the high-protein diet had increased fat mass (17% body fat) when compared with kittens fed the moderate-protein diet (12% body fat). It was suggested that the greater fat mass at 2 mo of age was related to a potential greater ingestion of milk or the smaller litter size of the high-protein fed group. However, at 8 mo of age there were no differences in fat mass and BW between the 2 groups. In addition, kittens fed the high-protein diet tended to have greater nocturnal voluntary physical activity (43.1 vs. 26.1 activity counts/epoch) but less light to dark activity ratio (0.56 vs. 0.87 light:dark activity counts/epoch) at 6 mo of age when compared with kittens fed the moderate-protein diet. In adipose tissue, mRNA abundance of HSL (205.4 vs. 134.5 AU), insulin receptor (109.7 vs. 77.6 AU), leptin (233.3 vs. 151.4 AU), and UCP-2 (132.1 vs. 102.6 AU) were increased in kittens fed the high-protein diet when compared with moderate protein diet fed kittens (Vester et al., 2009a).

Hyperlipidemia is commonly observed in obese and insulin resistant cats. However, the connections between increased lipid concentrations and insulin resistance in cats are still largely unknown. Zini et al. (2010) investigated the effects of hyperlipidemic clamp on insulin sensitivity, inflammation, and glucose metabolism-related genes in healthy adult cats. After 10 d of lipid infusion to mimic concentrations of plasma triglyceride concentrations reported in untreated diabetic cats (3 to 7 mmol/L), hepatic steatosis and systemic inflammation was depicted by increased concentrations of adiponectin (50%) and by a 2- to 3-fold increase in α1-acid glycoprotein and MCP-1. Despite an increase in inflammatory processes, insulin sensitivity in lipid-infused cats was not impaired when compared with saline-infused cats, probably due to the protective effect of increased plasma circulating concentrations of adiponectin and upregulation of transcription of GLUT4 in visceral (1.54 vs. 0.82 AU) and subcutaneous (1.48 vs. 0.49 AU) depots and PPARγ2 in the subcutaneous (2.19 vs. 0.98 AU) adipose tissues (Zini et al., 2010). Another study from that same laboratory investigated the influence of hyperlipidemia on cortisol metabolism in insulin-sensitive tissues (Sieber-Ruckstuhl et al., 2010). Similar to the aforementioned study, healthy adult cats were subjected to a hyperlipidemic clamp for 10 d followed by biopsy samples of adipose (subcutaneous and visceral depots), muscle, and hepatic tissues for determination of gene expression of 11β-HSD-1 and 11β-HSD-2 and glucocorticoid receptor by RT-PCR. Induced hyperlipidemia increased 11β-HSD-1 transcription by approximately 2-fold in subcutaneous and visceral adipose tissue whereas 11β-HSD-2 was decreased in visceral adipose (expression approximately one-third of control) and hepatic (expression approximately one-fourth
of control) tissues. Glucocorticoid receptor expression was also decreased in both fat depots (expression approximately one-sixth of control in subcutaneous adipose and expression approximately one-fifth of control in visceral adipose), which may indicate a tissue defense mechanism against the rise of cortisol concentrations in response to the upregulation of 11β-HSD-1 and downregulation of 11β-HSD-2. In contrast, serum cortisol concentrations of lipid-infused cats were similar to the control group (Sieber-Ruckstuhl et al., 2010).

Obesity is ultimately a result of an imbalance between energy intake and expenditure. Thyroid hormones, triiodothyronine (T3) and thyroxine (T4), are important in the maintenance of energy homeostasis. Previous research has demonstrated that obese cats have increased free T4 concentrations, indicating a T4 resistance in negative feedback at the hypothalamus and/or pituitary gland (Ferguson et al., 2007). The same group of researchers also investigated the effects of T3 (75 µg/d orally) administration in obese (6.9 ± 0.4 kg BW) and lean (3.9 ± 0.2 kg BW) cats (Hoening et al., 2008). Obese cats before and after T3 treatment had increased serum leptin (lean = 1.32 ± 0.21 ng/mL, pre-T3 obese = 8.77 ± 2.22 ng/mL, and post-T3 obese = 8.56 ± 0.41 ng/mL) and NEFA (lean = 0.43 ± 0.08 mEq/L and obese = 0.84 ± 0.17 mEq/L) concentrations. Obese cats had increased UCP-3 (lean = 0.52 ± 0.10 AU and obese = 1.1 ± 0.17 AU) and deiodinase-1 (lean = 0.37 ± 0.07 AU and obese = 1.27 ± 0.31 AU) mRNA transcription in skeletal muscle and UCP-2 (lean = 0.83 ± 0.15 AU and obese = 1.79 ± 0.43 AU) in adipose tissue when compared with lean cats. Administration of T3 resulted in increased thermogenesis and NEFA concentrations in obese and lean cats whereas adipose tissue expression of PPARγ was only increased in lean animals. No changes in the expression of UCP were observed after administration of T3. Overall, these data indicate that in cats, regulation of thermogenesis by T3 is independent of UCP expression and that PPAR may play a role in feline obesity (Hoening et al., 2008).

SUMMARY AND CONCLUSION

Obesity is the health problem of the century in the human and pet populations. The use of new research tools and the continued development of the genomic biology field will hopefully provide the means to further elucidate the role of the adipose tissue in obesity as well as to enlighten the crosstalk and interaction of metabolic pathways among insulin-sensitive tissues affected by obesity and its comorbidities. Gene sequencing of canine and feline leptin and adiponectin have shown that these hormones may be good markers in the management of obesity. In adipose tissue, the onset of obesity has been shown to alter the expression of thousands of genes involved in transcription, transport, metabolism, cell signaling and cycle, and oxidative stress. Dietary and environmental factors have been demonstrated to play a role in the development of obesity. Diet-induced obesity modified expression of several genes implicated in glucose and lipid metabolism in insulin sensitive tissues. In contrast, BW loss suppressed inflammatory processes related to excessive fat mass. In addition, dietary intervention using scFOS, green tea extract, or high-protein diets has shown to modify the expression of several genes related to glucose and lipid metabolism in adipose (e.g., UCP-2, carnitine palmitoyltransferase-1, PPARγ, LPL, and GLUT4) and skeletal muscle (e.g., PPARα and LPL) tissues. Overall, outcomes of recent canine and feline genomics projects have demonstrated that dogs and cats share similar adipokines and hormones to other species and, in general, are affected in a similar fashion during obesity. They also indicate that gene transcription modifications may preclude clinical signs, which may become a useful tool in the management and prevention of obesity. A better understanding of metabolic disorders that afflict companion animals will not only benefit these species but also provide further insight in the mechanisms involved in several human clinical conditions (e.g., obesity) because they share a large gene homology, live in the same environment, experience a similar lifestyle, and have comparable dietary habits.

LITERATURE CITED


