Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses

M. Judith Radin¹, Leslie C. Sharkey², and Bethany J. Holycross¹
¹Department of Veterinary Biosciences, The Ohio State University, Columbus, OH, USA; and ²Department of Veterinary Clinical Sciences, University of Minnesota, St. Paul, MN, USA

Key Words
Adipokines, adiponectin, leptin, obesity, renin-angiotensin system, resistin

Correspondence
M. Judith Radin, Department of Veterinary Biosciences, The Ohio State University, 1925 Coffey Road, Columbus, OH 43210
E-mail: radin.1@osu.edu
DOI:10.1111/j.1939-165X.2009.00133.x

Abstract: In addition to its role as an energy storage depot, adipose tissue is now recognized as a complex endocrine organ. Adipose tissue releases a variety of factors, termed adipokines, that regulate energy metabolism, cardiovascular function, reproductive status, and immune function. Some of the better-studied adipokines include leptin, adiponectin, and components of the renin-angiotensin system such as angiotensinogen. The function of more recently discovered adipokines such as resistin are under intense scrutiny. Abnormal production or regulation of adipokines occurs in obese individuals and is implicated in the development of a variety of associated co-morbidities including metabolic syndrome, type 2 diabetes, atherosclerosis, heart disease, and cancer in people, although evaluation in domestic species is just beginning. Adipokines are now being examined as potential biomarkers for risk assessment for development of complications related to obesity. This article summarizes the function and regulation of some better-characterized adipokines. It also reviews the current information on the characterization of adipokines in some domestic species in which rates of obesity and obesity-related disorders are increasing, such as the dog, cat, and horse.

Introduction

Obesity and associated metabolic diseases are of increasing prevalence and importance in companion animal medicine.¹–⁵ Health risks reported to be associated with obesity include diabetes mellitus and osteoarthritis in dogs and cats. There is also evidence for obesity-associated shortened lifespan in dogs that are fed ad libitum compared with dogs fed an energy-restricted diet that results in a more optimal body condition.¹,³ In horses, obesity may predispose to laminitis, hyperlipemia, and strangulating lipoma.⁵ Adipokines, which are proteins derived from adipose tissue that have local and systemic effects, are important factors in the pathophysiology of obesity and its related conditions in people. As rates of obesity in companion animals climb, the impact of altered adipokine balance on animal health is expected to increase. The biological characterization of and the measurement tools available for the more thoroughly investigated adipokines in dogs, cats, and horses

This review article has been peer-reviewed.
will be the focus of this review. Much of the information regarding the basic biology of adipokines is derived from human and rodent data, while current information describing what has been established in dogs, cats, and horses is described in specific sections. A comprehensive understanding of adipokines is facilitated by a brief review of current concepts in adipose biology.

**Adipose as an Organ**

Adipose tissue makes up a diffuse organ comprised of adipocytes (~50% of the total cellular content), preadipocytes, multipotent mesenchymal stem cells, endothelial cells, pericytes, monocyte/macrophages, and nerve cells (Figure 1). Previous models of adipocyte biology suggested that the number of adipocytes was fixed in childhood, and that expansion and contraction of adipose mass was due solely to modulation of the balance of triglyceride storage and fatty acid mobilization. Newer research demonstrates that there is a large pool of stem cells and preadipocytes in adipose tissue in individuals of all ages that may be recruited once existing adipocytes reach a critical level of hypertrophy.6

Adipose tissue has traditionally been divided into 2 types: white adipose tissue (WAT) consisting of cells with a single large droplet of triglycerides, and brown adipose tissue, consisting of cells containing multilocular lipid droplets (Figure 2). Classical functions of WAT include insulation and mechanical support along with storage of surplus energy,7 while brown adipose tissue is associated with thermogenesis as a result of the expression of distinct genes such as uncoupling protein-1.8 Adipocytes from different anatomic locations may vary in their biology due to local influences on differentiation and gene expression, and may be considered “mini-organs.” The distribution of adipose tissue influences the pathologic sequelae characterizing obesity. Preferential deposition of fat into visceral deposits instead of subcutaneous deposits increases the risk of insulin resistance, atherosclerosis, and diabetes mellitus in people.9 With impaired deposition of lipid in adipocytes due to aging or lipodystrophy, lipids may be deposited in nonadipocytes in the liver, muscle, pancreas, and kidney, resulting in altered fat and glucose metabolism and impaired organ function due to “lipotoxicity.”10 This illustrates the importance of adipose as a specialized storage organ for excess energy. WAT is becoming regarded as an important endocrine organ that secretes a wide variety of substances including steroid hormones, growth factors and cytokines, eicosanoids, complement proteins, binding proteins, vasoactive factors, regulators of lipid metabolism, and others.7 Collectively, these factors are labeled “adipokines” and they function as part of a complex set of physiologic control systems that regulate local tissue and whole organism physiology.

**General Biology of Adipokines**

Adipokines are defined generally as biologically active substances produced in adipose tissue that act in an autocrine/paracrine or endocrine fashion.11 Some adipokines are produced exclusively or predominantly by adipose tissue; however, others may be produced in a variety of different tissues as outlined below for individual adipokines. Production of adipokines by adipose tissue may be influenced by sex hormones and nutritional status.8 While the classic adipokines such as
leptin undoubtedly have an important role in the regulation of energy balance and metabolism, the list of adipokines and their potential functions is expanding rapidly. There is evidence that adipokines may contribute to the regulation of diverse biological processes, including inflammation and immune function, hemostasis and vascular biology, hematopoiesis, and cell proliferation and angiogenesis.

**Obesity, Adipokines, and Disease**

At a basic level, obesity is the result of imbalance between energy intake and energy expenditure. Major theories regarding the underlying causes for obesity include recent changes in environmental factors such as food availability and activity levels, genetic influences, and developmental programming. Normally, highly redundant and tightly regulated physiologic systems (mostly neural and endocrine) function to maintain body weight within a narrow range with >99% precision in most individuals over a period of years. This regulation system requires a sensing mechanism for energy stores (ie, a “lipostat”) to be coordinated with eating behaviors and energy metabolism. The “thrifty gene” hypothesis predicts that this system will tend to favor mechanisms promoting weight gain, due to the survival value of that characteristic in environments with unpredictable and restricted access to food. This hypothesis is supported by the observation that overfeeding produces fewer compensatory changes in energy expenditure than underfeeding. Obesity in an individual likely is the result of complex interactions between environmental factors and genes.

A consequence of increased fat mass is dysregulation of production and secretion of adipokines that impact glycemic control, inflammation, and cardiovascular function. As a result of these diverse functions of adipose, altered adipokine production in obesity has been implicated in the pathophysiology of a diverse group of diseases in people and in rodent models, including diabetes mellitus, cardiovascular disease, and cancer. Because of the increasing significance of adipokines in health and disease, there is a need for awareness of their biology and measurement in veterinary medicine. While the list of recognized adipokines continues to grow, this review will focus on those that have been at least partially characterized in companion animals, including leptin, adiponectin, the renin-angiotensin system (RAS), and resistin. In particular, we will review these adipokines in dogs, cats, and horses, species with a documented increased risk for developing obesity and subsequent health consequences of excess weight gain.

**Selected Adipokines**

**Leptin**

**Synthesis and regulation**

Leptin is the prototypic adipokine and is the best characterized adipokine in domestic animal species. Leptin is a 167 amino acid protein that is encoded by the *ob* gene. Both the nucleotide and amino acid sequences are highly conserved, with homologies of 83–95% for nucleotide and 79–96% for amino acid sequence in vertebrate species examined thus far. Adipocytes are the main site of leptin synthesis and the main contributor to serum leptin levels. However, lower levels of expression of leptin mRNA are detectable in other tissues such as placenta, mammary gland, gastric mucosa, and liver.

Leptin is constitutively secreted by adipocytes; its transcriptional regulation depends upon energy flux within adipocytes, so circulating levels correlate closely with body mass index. As a result, the serum concentration of leptin is predominantly defined by body fat mass but will transiently increase following a meal and decrease with fasting. Transcription of the *ob* gene is controlled by a variety of metabolic and inflammatory mediators. For example, binding of glucocorticoids or peroxisome-proliferator–activated receptor-γ (PPARγ) to specific sites in the promoter region of the *ob* gene will increase expression of leptin mRNA. Transcription also is increased by insulin endotoxin and proinflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-1β, and interleukin-6 (IL-6). Serum leptin concentration increases during the luteal phase of the estrus cycle, and estradiol appears to increase leptin mRNA expression as well as protein secretion by WAT. In contrast, circulating leptin levels are inversely correlated with serum testosterone levels in males. Studies in rats suggest that testosterone increases leptin clearance but does not modify leptin synthesis and secretion.

**Leptin receptor and signaling**

Leptin is a cytokine, and its receptor (Ob-R) is closely related to gp130, a member of the IL-6 family of receptors. Multiple variable-length isoforms of Ob-R are possible due to alternative splicing of the cytoplasmic domain. While Ob-R is expressed in highest concentrations in the satiety centers of the hypothalamus, both long and shorter splice variants are widely distributed in many tissues throughout the body,
reflecting the involvement of leptin in the regulation of diverse physiologic processes. The long form of the receptor, designated Ob-Rb, mediates most of leptin's physiologic actions; however, short forms also are capable of signal transduction.

With the exception of the soluble Ob-Re isoform, which acts as a binding protein for leptin in the circulation, full length and the shorter receptor isoforms have a cytoplasmic domain that associates with the Janus kinase family of tyrosine kinases (JAK). Binding of leptin to full-length Ob-Rb results in activation of JAK and phosphorylation of signal transducers and activators of transcription (STAT). Because STAT activation depends on phosphorylation of tyrosine1138 on Ob-Rb, shorter isoforms cannot signal via STAT despite being able to phosphorylate and activate JAK. Ob-Rb also signals via activation of extracellular-signal-regulated kinases (ERK1/2), phosphatidylinositol-3 kinase via activation of insulin receptor substrate 1/2, and Akt (protein kinase B). Signal transduction through ERK1/2 and Akt may also be initiated by short isoforms without concurrent STAT activation, thus bypassing potentially important negative feedback mechanisms, as described below.

A consequence of activation of STAT signaling is induction of expression of suppressors of cytokine signaling (SOCS), especially SOCS-3. SOCS proteins are part of an important negative feedback loop that inhibits cytokine signaling by binding to phosphorylated cytokine receptors. SOCS-3 binding to Ob-Rb has the potential to dampen multiple leptin-induced signaling pathways. In addition, there is potential for crosstalk to modulate signaling initiated by other cytokines or insulin, thus modifying their actions. Increased expression and production of SOCS-3 in brain and adipocytes occurs in rodent models with obesity and has been proposed as a mechanism mediating impaired response to leptin and central leptin resistance. Because shorter leptin receptor isoforms lack the ability to activate STAT, increased SOCS-3 production is not anticipated following leptin binding to these shorter splice variants. The physiologic significance of the shorter isoforms and their importance in the development of leptin resistance and obesity-associated diseases are still largely unexplored.

**Functions of leptin**

The primary actions originally described for leptin are mediated via binding to Ob-R in the brain, resulting in suppression of appetite and increased energy expenditure (thermogenesis). Appetite suppression is mediated via binding of leptin to its full length receptor (Ob-Rb) in the hypothalamic satiety centers, with stimulation of anorexigenic/catabolic neurons (via neurotransmitters such as cocaine and amphetamine regulated transcript [CART] and α-melanocyte stimulating hormone [α-MSH]) and suppression of orexigenic/anabolic neurons (via neurotransmitters such as neuropeptide Y and agouti-related peptide). Leptin also suppresses the release of endocannabinoids, which are postsynaptic regulators of presynaptic activity of orexigenic neurons. As a result, endocannabinoid antagonists such as rimonabant are being explored as appetite suppressors for obesity treatment. Increased thermogenesis is mediated by central binding of leptin to Ob-Rb, resulting in sympathetic nerve stimulation and activation of β3-adrenergic receptors in brown fat, thus increasing fat oxidation.

In addition to the primary function of regulation of energy stores, leptin has been implicated as a growth factor by virtue of its ability to stimulate angiogenesis, suppress apoptosis, and act as a mitogen. These functions may play a role in the increased incidence of cancer observed with increasing body mass index and waist-to-hip ratio in humans. Leptin has been suggested to have a role in hypothalamic development, thereby providing a mechanistic link between altered nutritional states during fetal and neonatal development and long-term programming of adiposity. Other important physiologic functions of leptin include regulation of reproductive and immune functions as well as modulation of insulin sensitivity. Thus, leptin levels regulated by fat stores may provide a mechanism by which reproductive readiness is tied to nutritional status as well as an explanation for suppression of immune function in states of malnutrition.

**Leptin and obesity**

Failure to produce leptin (ob/ob mouse) or failure to produce a functional leptin receptor (db/db mouse and the Zucker, Koletsky, and SHHF rat models) results in massive obesity. Such genetic mutations occur rarely in humans and to our knowledge, have not been documented in dogs, cats, or horses. Obesity in species other than genetically engineered rodents is usually attributed to other factors, such as high caloric or fat intake that is disproportionate to the level of physical activity. Obesity unrelated to specific mutations in leptin or its receptor is characterized by a syndrome of at least partial leptin resistance and hyperleptinemia. Hyperleptinemia is also seen with aging and may occur secondary to weight gain during middle age, independent of or disproportionate to increases in body fat mass, and as a consequence of aging itself. With leptin
resistance, the ability of high levels of leptin to regulate energy homeostasis is blunted, abetting further weight gain and predisposing to the development of metabolic abnormalities and type 2 diabetes mellitus. The blunted response to leptin may be due in part to saturated transport systems for leptin across the blood–brain barrier or to acquired defects in downstream signaling in hypothalamic neurons. In adipose tissue, leptin gene expression increases with age. Adipocytes from aged obese rats have increased levels of SOCS-3, which has a feedback inhibitory effect on leptin signaling and further potentiates leptin resistance.

Leptin resistance with obesity appears to be selective for metabolic functions. This leaves other functions of leptin intact, contributing to the development of a variety of co-morbidities associated with obesity, including cardiac, renal, and vascular dysfunction. These are at least partially mediated through a combination of leptin-mediated sympathetic nervous system hyperactivity, impaired nitric oxide generation, and increased endothelin production. For example, selective leptin resistance has been observed in agouti mice in which the anorexic effect of leptin is blocked by overexpression of agouti protein (an inhibitor of α-MSH), but the ability of leptin to stimulate renal sympathetic nerve activity remains intact. Leptin has similar sympathoexcitatory effects in diet-induced obesity in mice. Circulating leptin concentration correlates with renal sympathetic nerve activity and urine norepinephrine spillover in men with varying degrees of obesity. In obese humans and rodents, increases in endothelin contribute to increased vascular tone, and may play a role in the development of salt sensitivity. Generally, leptin appears to be pro-inflammatory, prothrombotic, and pro-oxidant, therefore opposing the effects of adiponectin, which will be addressed in the next section.

Measurement of leptin

The reported half-life of leptin in the circulation is 25 minutes for humans, 1.5 hours for lean mice, 3 hours for obese mice, and 5–13 minutes for rats. Several factors may account for this wide variability among species, including methodological differences in the studies, protein binding, and route or rate of clearance. Leptin binds to the soluble leptin receptor (Ob-Re), oxidized z2-macroglobulin, and several other poorly characterized serum binding proteins. Leptin does not appear to bind to albumin or lipid but may aggregate with itself due to its hydrophobicity. All of these factors may affect distribution and clearance of leptin from the circulation. Human serum leptin has been shown to be stable for 2 months at 4°C and for 2 years at −20°C, and to tolerate at least 5 freeze–thaw cycles. Our experience suggests leptin stability is similar in rodents; however, to our knowledge, systematic studies have not been published for dogs, cats, or horses. Multiple variables in reference populations and methods influence measured leptin levels, as illustrated in Tables 1 and 2. Owing to the variation in values, readers are encouraged to consult the original references that are most relevant to their needs.

To date, the majority of studies have used radioimmunoassays (RIAs) to measure leptin; however, ELISAs are becoming more available. The Linco Multispecies RIA (Linco Research/Millipore, St. Charles, MO, USA) is the most frequently used commercial kit and has been used in cats and horses. One commercially available enzyme immunoassay (EIA) also has been evaluated for use in the horse. Leptin has been assessed in relatively few studies in dogs due to lack of a validated, commercially available test kit, although one research group has developed an in-house ELISA. While a few studies have used the Linco Multispecies RIA in dogs with comparable results (Table 1), the specificity of the kit for canine leptin is only 3% as reported by the manufacturer. Millipore has recently released a new ELISA kit that is specific for canine leptin, which should aid studies in this species. Serum and EDTA- or heparin-anticoagulated plasma samples yield similar results for leptin measured by RIA in humans and this appears to hold true in domestic animal species (Tables 1 and 2).

When collecting samples for leptin measurement, a variety of preanalytical factors should be considered. Circadian rhythm can account for a 30–100% difference between daily peak and trough in human leptin concentration. The fed state may result in serum leptin concentrations as much as 2-fold higher than those in the fasted state. As reviewed above, both sex and stage of estrus cycle can influence circulating leptin levels. Fewer studies are available that address the effects of drug therapy. However, corticosteroids are well documented to increase leptin gene expression and secretion and to potentiate the effect of insulin on leptin production in humans and rodent models. Circulating concentrations of leptin for dogs, cats, and horses are summarized in Tables 1 and 2 and are discussed in more detail below.

Dogs

Like humans and rodents, circulating leptin correlates with body fat mass in the dog. Circulating leptin is
Table 1. Circulating leptin concentrations in dogs and cats.

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Sex</th>
<th>Breed</th>
<th>Body condition</th>
<th>Other factors</th>
<th>Leptin (ng/mL)</th>
<th>Assay</th>
<th>Sample</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>NR(13)</td>
<td>NR</td>
<td>Beagle</td>
<td>NR</td>
<td>NR</td>
<td>3.0 ± 0.3 (1.4–5.6)</td>
<td>ELISA*</td>
<td>Serum</td>
<td>Iwase et al.83</td>
</tr>
<tr>
<td></td>
<td>3 yr M (5)</td>
<td>M (5)</td>
<td>Beagle</td>
<td>Lean</td>
<td>Fasted (16 hr)</td>
<td>2.4 ± 1.2</td>
<td>ELISA*</td>
<td>Plasma</td>
<td>Ishioka et al.84</td>
</tr>
<tr>
<td></td>
<td>5 mo–15 yr</td>
<td>M F (36)</td>
<td>Various</td>
<td>BCS 3</td>
<td>Fasted (overnight)</td>
<td>2.7 ± 0.3</td>
<td>ELISA*</td>
<td>Plasma</td>
<td>Ishioka et al.84</td>
</tr>
<tr>
<td></td>
<td>M F (8)</td>
<td>M F (11)</td>
<td>F (18)</td>
<td>BCS 3</td>
<td></td>
<td>9.7 ± 0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>M (18)</td>
<td></td>
<td></td>
<td>BCS 3</td>
<td></td>
<td>12.3 ± 1.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.4 ± 0.9 yr</td>
<td>CM (6), F (3), SF (3)</td>
<td>Beagle</td>
<td>Lean, BCS 5/9</td>
<td>Fasted (24 hr)</td>
<td>2.3 ± 0.4</td>
<td>ELISA*</td>
<td>Plasma</td>
<td>Jeusette et al.85</td>
</tr>
<tr>
<td></td>
<td>4.7 ± 0.6 yr</td>
<td>CM (6), F (3), SF (3)</td>
<td>Beagle</td>
<td>BCS 4</td>
<td>Obese, BCS 7–8/9</td>
<td>13.2 ± 1.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6 mo–17 yr</td>
<td>M (19), CM (2), F (14), SF (5)</td>
<td>Various</td>
<td>BCS 3</td>
<td>Fasted (&gt; 10 hr)</td>
<td>3.0 ± 0.4</td>
<td>ELISA*</td>
<td>Plasma</td>
<td>Ishioka et al.86</td>
</tr>
<tr>
<td></td>
<td>Median 8.8 yr</td>
<td>M (14), CM (5), F (16), SF (11)</td>
<td></td>
<td>BCS 4</td>
<td></td>
<td>8.6 ± 0.7</td>
<td></td>
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<tr>
<td></td>
<td>M (15), CM (5), F (27), SF (28)</td>
<td></td>
<td>BCS 5</td>
<td></td>
<td>12.8 ± 0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adult</td>
<td>M (20)</td>
<td>F (20)</td>
<td>Mixed breed</td>
<td>13.5–16.4 kg BW</td>
<td>Fasted (14 hr)</td>
<td>2.3 ± 0.1</td>
<td>RIA†</td>
<td>Serum</td>
<td>Yılmaz et al.87</td>
</tr>
<tr>
<td>Cat</td>
<td>1.1–3.5 yr</td>
<td>M (19)</td>
<td>NR</td>
<td>3.8–7.1 kg BW</td>
<td>Fed</td>
<td>2.9 ± 0.2 (1.6–4.9)</td>
<td>RIA†</td>
<td>Serum</td>
<td>Backus et al.88</td>
</tr>
<tr>
<td></td>
<td>Young adult</td>
<td>CM (11)</td>
<td>F (12)</td>
<td>BCS 3.17 ± 0.41</td>
<td>Fasted (12 hr)</td>
<td>6.54 ± 2.18</td>
<td>RIA†</td>
<td>EDTA+aprotinin</td>
<td>Appleton et al.89</td>
</tr>
<tr>
<td></td>
<td>SF (15)</td>
<td>CM (6)</td>
<td>F (12)</td>
<td>BCS 3.90 ± 0.32</td>
<td></td>
<td>6.31 ± 2.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SF (10)</td>
<td></td>
<td>F (12)</td>
<td>BCS 4.56 ± 0.52</td>
<td></td>
<td>31.4 ± 1.61</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>19.5 ± 0.3 mo</td>
<td>M (11)</td>
<td>Short haired</td>
<td>23.8 ± 1.0% body fat</td>
<td></td>
<td>20.4 ± 6.95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>F (12)</td>
<td>CM (10)</td>
<td></td>
<td>30.1 ± 1.7% body fat</td>
<td></td>
<td>4.2 ± 0.9</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>SF (9)</td>
<td></td>
<td></td>
<td>32.9 ± 1.7% body fat</td>
<td></td>
<td>4.7 ± 0.5</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>M (10)</td>
<td></td>
<td>35.5 ± 1.8% body fat</td>
<td></td>
<td>6.7 ± 0.5</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>SF (9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 mo–15 yr</td>
<td>M (10), F (14)</td>
<td>NR</td>
<td>4.0 ± 0.6 kg BW</td>
<td>Presurgical</td>
<td>2.08 ± 0.36</td>
<td>RIA†</td>
<td>Serum</td>
<td>Hoenig and Ferguson81</td>
</tr>
<tr>
<td></td>
<td>CM (10)</td>
<td>F (10)</td>
<td></td>
<td>4.0 ± 0.6 kg</td>
<td>16 wk post</td>
<td>3.18 ± 0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SF (10)</td>
<td></td>
<td></td>
<td>3.4 ± 0.3 kg</td>
<td>Presurgical</td>
<td>2.43 ± 0.67</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.4 ± 0.4 kg</td>
<td>16 wk post</td>
<td>2.34 ± 0.66</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 mo–15 yr</td>
<td>M (10), F (14)</td>
<td>NR</td>
<td>2.2–6.8 kg BW</td>
<td></td>
<td>4.5 ± 1.3 (0.3–29.7)</td>
<td>ELISA†</td>
<td>Plasma</td>
<td>Shibata et al.92</td>
</tr>
</tbody>
</table>

Data are mean ± SEM unless otherwise indicated.
*Noncommercially available sandwich ELISA as described in reference 83.
†Linco Multispecies RIA, Linco Research (now Millipore), St. Charles, MO, USA.
§Noncommercially available sandwich ELISA as described in reference 92.
NR, not reported; M, male; F, female; CM, castrated male; SF, spayed female; number of animals is indicated in parentheses; BW, body weight; BCS, body condition score (scale 1–5); yr, years; mo, months; hr, hours.

[Correction added after online publication on 6 April 2009: The original Table 1 contained misaligned text. The correct table, above, shows the correctly aligned text.]
increased in dogs with experimentally induced obesity as well as in pet dogs with increased body condition scores (BCS). The *ob* gene is expressed in preadipocytes and mature adipocytes in WAT from multiple sites in the dog. Unlike species such as human, rodent, and chicken in which the *ob* gene may be expressed in other tissues, expression of the *ob* gene in dogs appears to be confined to WAT; whether this finding is dependent on experimental methodology remains to be determined.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Breed</th>
<th>Body condition</th>
<th>Other factors</th>
<th>Leptin (ng/mL)</th>
<th>Assay</th>
<th>Sample</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 10 yr</td>
<td>F [11]</td>
<td>TB</td>
<td>NR</td>
<td>July</td>
<td>4.77 ± 0.45</td>
<td>Multispecies RIA*</td>
<td>Serum</td>
<td>Fitzgerald and McManus*33</td>
</tr>
<tr>
<td>8–24 yr</td>
<td>M [14], G [15]</td>
<td>QH</td>
<td>BCS 3–8</td>
<td>Mid Sept</td>
<td>8.07 ± 1.02</td>
<td>Equine RIA†</td>
<td>Serum</td>
<td>Buff et al*25</td>
</tr>
<tr>
<td>8–24 yr</td>
<td>F [42]</td>
<td></td>
<td></td>
<td>Dec–Jan</td>
<td>4.37 ± 0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 2 yr</td>
<td>M [5], G [6], F [12]</td>
<td></td>
<td></td>
<td>Jan–Mar</td>
<td>5.72 ± 0.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2–4 yr</td>
<td>M [1], G [3], F [10]</td>
<td></td>
<td></td>
<td></td>
<td>1.86 ± 0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–12 yr</td>
<td>M [4], G [3], F [10]</td>
<td></td>
<td></td>
<td></td>
<td>2.38 ± 0.32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 12 yr</td>
<td>M [4], G [3], M [10]</td>
<td></td>
<td></td>
<td></td>
<td>2.64 ± 0.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6–23 yr</td>
<td>F [46]</td>
<td>LIP</td>
<td>535 ± 10 kg BW</td>
<td>Lactating (1 wk postpartum)</td>
<td>9.5 ± 0.8</td>
<td>Ovine RIA‡</td>
<td>Plasma</td>
<td>Heidler et al*24</td>
</tr>
<tr>
<td>5–19 yr</td>
<td>F [11]</td>
<td></td>
<td>522 ± 10 kg BW</td>
<td>Nonlactating, nonpregnant</td>
<td>15.0 ± 2.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–9 yr</td>
<td>F [5]</td>
<td>TB</td>
<td>BCS 3 (exercised)</td>
<td>Fed</td>
<td>4.23 ± 0.03</td>
<td>EIÀ§</td>
<td>Serum</td>
<td>Piccione et al*95</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fasted (48 hr)</td>
<td>3.96 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fed</td>
<td>4.24 ± 0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Fasted (48 hr)</td>
<td>4.05 ± 0.04</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4–14 yr</td>
<td>F [8]</td>
<td>Pony</td>
<td>BCS 6</td>
<td>Fed</td>
<td>17.20 ± 0.41</td>
<td>Equine RIA†</td>
<td>Plasma</td>
<td>Buff et al*26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Feed-restricted (48 hr)</td>
<td>7.29 ± 0.41</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3–4 yrs</td>
<td>G [6]</td>
<td>STB</td>
<td>BCS 4.1 ± 0.1</td>
<td></td>
<td>1.66 ± 0.59 [SD]</td>
<td>Multispecies RIA*</td>
<td>Serum</td>
<td>Pratt et al*97</td>
</tr>
<tr>
<td>10–16 yr</td>
<td>F [8]</td>
<td></td>
<td>BCS 4.8 ± 0.5</td>
<td></td>
<td>2.96 ± 2.05 [SD]</td>
<td>Median 11.0 (1.0–30.5)</td>
<td>Equine RIA†</td>
<td>Plasma</td>
</tr>
<tr>
<td>7–16 yr</td>
<td>F [5]</td>
<td>Various</td>
<td>BCS 7–9 (obese)</td>
<td></td>
<td>3.47 ± 0.50</td>
<td>Multispecies Plasma RIA*</td>
<td>Kerns et al*98</td>
<td></td>
</tr>
<tr>
<td>10 ± 3 mo</td>
<td>F [23]</td>
<td>STB</td>
<td>514 ± 12 kg BW</td>
<td></td>
<td>3.47 ± 0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 ± 3 mo</td>
<td>F [10], F [2]</td>
<td></td>
<td>QH × BEL, 227 ± 11 kg BW</td>
<td></td>
<td>1.90 ± 0.34</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 ± 2 yr</td>
<td>F [12]</td>
<td>STB</td>
<td>BCS 6.7 ± 0.2 (unfit; unconditioned)</td>
<td></td>
<td>4.4 ± 2.4</td>
<td>Multispecies Plasma RIA*</td>
<td>Gordon et al*59</td>
<td></td>
</tr>
<tr>
<td>2–8 yr</td>
<td>M, F, G [34]</td>
<td></td>
<td>BCS 4.8 ± 0.1 (fit; exercised)</td>
<td></td>
<td>1.0 ± 0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SEM unless otherwise indicated.
*Linco Multispecies RIA, Linco Research, (now Millipore), St. Charles, MO, USA.
†Noncommercially available RIA as described in reference 25.
‡Noncommercially available RIA as described in reference 94.
§EIA-2395, DRG Instruments GmbH, Germany.
NR, Not reported; M, male; F, female; G, gelding; STB, Standardbred; QH, Quarter Horse; QH × BEL, Quarterhorse × Belgium cross; TB, Thoroughbred; LIP, Lipizzaner; Number of animals shown in parentheses; BW, body weight; BCS, body condition score (scale 1–9); yr, years; mo, months; hr, hours.
[Correction added after online publication on 6 April 2009: The original Table 2 contained misaligned text. The correct table, above, shows the correctly aligned text.]
The main factor influencing circulating leptin concentration in dogs appears to be fat mass. However, like other species, dogs show a transient increase in circulating leptin following a meal. This postprandial peak occurs about 5–8 hours after feeding and leptin concentration may reach 2–3 times fasting levels. Thus, postprandial levels in lean dogs are comparable to levels observed in fasted dogs with high BCS of 4 to 5/5, suggesting that serum or plasma leptin concentrations must be interpreted with knowledge of the fed/fasted state. Interestingly, this postprandial peak appears blunted in thin dogs with BCS of 2/5.

When dogs are grouped by BCS, there is no apparent effect of age on plasma leptin, although puppies (<1 year of age) tended to have lower plasma leptin concentrations. This was speculated to be due to low body fat mass and a high energy requirement for growth at a young age. In this same study, there was no apparent effect of sex or neutering in dogs grouped by BCS; however, others have suggested an influence of sex and estrus cycle.

Breed may influence leptin levels. In one study, Shetland Sheepdogs within BCS groups tended to have higher leptin concentrations than dogs of other breeds including Miniature Dachshunds, Shih Tzus, and Labrador Retrievers. However the number of animals per breed was too small to be conclusive and some common breeds found in the United States were absent from this study.

Glucocorticoid administration may affect circulating leptin concentration as well as leptin secretion in response to a meal. The effect appears to be dependent on the type and dose of glucocorticoid used. For example, dexamethasone increases serum leptin levels and potentiates the leptin peak in response to a meal, while oral prednisolone does not appear to affect leptin levels. The effect of methylprednisolone appears to be dose dependent, with low doses causing an increase and high doses suppressing leptin concentration. These findings suggest that treatment with corticosteroids or increased endogenous steroid production may affect circulating leptin concentrations and should be considered when evaluating leptin levels in clinical patients.

Cats

Feline leptin is partially bound to protein in the circulation. Like other species, circulating leptin primarily reflects body fat mass in cats, and weight loss is associated with a fall in peripheral blood leptin levels. Leptin levels are mildly increased in the fed compared with fasted state. The effect of dietary composition on leptin levels is not consistent between studies and appears to be modulated by the relative insulin resistance and body fat mass of the cats. Regardless of fat mass, insulin-resistant cats have higher circulating leptin concentrations compared with insulin sensitive cats.

Several studies have looked at the effect of neutering on body weight and circulating leptin in cats. In general, increases in leptin occur following neutering and are correlated with the amount of body fat gained postsurgery, although this finding is not consistent in all studies. There also are conflicting results regarding the effect of sex. There was no difference in serum leptin concentration between neutered male and female cats with normal fat mass or between intact male and female cats. In 1 study, neutered females gained more weight and had higher leptin levels compared with castrated males while in another study castrated males had greater fat gain and a trend towards higher leptin levels compared with neutered females. In both studies, the group that had the highest percent body fat had the highest average leptin concentration. If presurgical body weight was maintained by caloric regulation, males showed small but significant increases in leptin subsequent to castration, while females did not show changes in leptin following ovariohysterectomy. This may reflect the leptin lowering effect of testosterone, as observed in other species. Although the difference between castrated males and neutered males was statistically significant, the actual difference was small in comparison to the effect of weight gain and thus may be of minor clinical significance (Table 1). No assessment of the effects of the estrus cycle was done in any of the studies in cats.

Horses

While fat mass appears to be the primary determinant of serum leptin concentration in the horse, a number of other factors have been shown to modulate circulating levels. Fasting results in a decline in serum leptin, which may decrease by as much as 50% compared with the fed state after 48 hours of feed restriction. Leptin shows a circadian pattern in horses, with levels peaking at night and a nadir reached during daytime hours. However, this finding is not consistent in all studies, and daily fluctuations in leptin levels result from both the feeding schedule as well as composition of the feed. Leptin levels will rise 8–10 hours subsequent to a high carbohydrate (grain) meal and follow the rise in insulin levels. In contrast, peaks and troughs in both insulin and leptin concentrations are blunted in horses given frequent meals, given ad libitum hay, or on
pasture.122 Thus, husbandry practices have significant impact on hormonal fluctuations that may contribute to the development of insulin resistance and hyperleptinemia, an increasingly common problem in overweight horses. When horses were matched for BCS, those animals with consistently high serum leptin levels also were hyperglycemic and hyperinsulinemic and had exaggerated glucose and insulin responses to dexamethasone.123 These findings are similar to those in humans with type 2 diabetes mellitus or metabolic syndrome.124 However, not all overweight mares are hyperleptinemic and 1 study found that only 35% of mares with high BCS were hyperleptinemic and insulin resistant.125 This again is similar to humans in which not all obese individuals have metabolic derangements.124 To date, definitive documentation of central or selective leptin resistance has not been achieved in the horse.

The interaction of sex hormones and leptin is currently being explored in horses. Geldings and stallions have similar circulating leptin levels and several studies have noted mild but significantly higher leptin levels in males compared with females.25,120 These data contrast with those in humans in which leptin levels are higher in women than in men.33,34,126 Neither administration of testosterone to mares127 nor ovariotomy96 affected peripheral leptin concentration.

Several studies have looked at reproductive status and leptin in mares. Mares with lower BCS and lower leptin levels become seasonally anestrua while mares with higher BCS and higher serum leptin levels continue to cycle throughout the year.93,117 Blood leptin levels decrease during the first few days following parturition and remain below prepartum levels during early lactation.94,128,129 This decrease in leptin concentration occurs even if BCS remains stable and may be an adjustment to allow increased caloric intake and avoid negative energy balance during lactation. Interestingly, this decline in leptin does not seem to impact resumption of postpartum ovulation.94,130 Leptin also is present in mares’ milk, with the concentration in colostrum approximately 2- to 3-fold greater than that found in blood.128,131 It is uncertain if this high concentration reflects active secretion/concentration from the blood or local glandular production. The role of colostral leptin is still uncertain; it has been speculated to aid in gut development or thermoregulation in the foal. The concentration of leptin in milk falls within days to below that found in the mare’s blood and remains stable thereafter. The concentration of leptin in the foal’s blood rises after birth and stabilizes within a few days to levels similar to those in milk. No work has documented whether maternal leptin contributes to blood leptin levels in the foal.

### Adiponectin

**Synthesis and regulation**

Adiponectin (also named apM1, Acrp30, GBP28, and AdipoQ) has very restricted tissue expression and is thought to be produced almost exclusively by mature adipocytes. Adiponectin is a 244-amino acid protein and consists of a signal sequence, a variable domain, a collagen-like domain, and a globular C-terminal region.132 The C-terminal globular domain has sequence homology to complement factor C1q and shows structural but not sequence similarities to TNFα. Adiponectin proteins that have undergone extensive post-translational modification are secreted by the adipocyte as homotrimers. In the circulation, adiponectin may form trimers, hexamers, or high molecular weight multimers (HMW, 4–6 noncovalently bonded trimers). In addition, the globular domain may circulate independently of the rest of the molecule. Regulation of multimer formation and secretion as well as the biological significance of the various multimeric forms are still under investigation. Currently, it is thought that the HMW forms have the most biological activity and are best correlated with insulin sensitivity.

Adiponectin is one of the most highly expressed genes in WAT. Secretion of adiponectin by fat cells is stimulated by insulin, and a number of drugs and dietary constituents modulate blood levels of adiponectin. The best known pharmaceutical regulators of adiponectin expression and secretion are the thiazolidinediones (TZD), which activate PPARγ and subsequently upregulate adiponectin expression.133 Rimonabant, a selective cannabinoid-1 receptor (CB-1) antagonist, also stimulates adiponectin expression and increases adiponectinemia, suggesting a role for CB-1 in the regulation of adiponectin production.134 Various dietary supplements have been shown to affect circulating adiponectin levels in rodent models. For example, supplementation with fish oil,135 linoleic acid,136 and soy protein137 increased circulating adiponectin concentration in rats in the absence of a change in body weight.

Females have higher total circulating adiponectin levels compared with males.138,139 This sexual dimorphism is accounted for by females having a significantly greater proportion of HMW forms compared with males, but no difference in the mean concentration of trimeric or hexameric forms.139,141 Testosterone mediates some of this difference by decreasing the secretion of HMW adiponectin by adipocytes.142 Studies on the effects of exercise have produced somewhat conflicting results in humans.143 In general,
chronic exercise training results in small increases in adiponectin levels. The effect of acute exercise is more variable and appears to depend on the intensity of exercise as well as the fitness of the participants. For example, trained athletes usually show an increase in circulating adiponectin while unfit or obese individuals may show a decline in adiponectin levels following a bout of exercise.

Adiponectin receptors and signaling

The physiologic effects of adiponectin are related to tissue-specific receptor expression and differences in binding affinity for the various molecular forms of adiponectin (reviewed in Kadowaki et al132). Two specific adiponectin receptors have been cloned, AdipoR1 is primarily expressed in skeletal muscle, with affinity for the globular and trimeric forms of adiponectin, while AdipoR2 is highly expressed in the liver with affinity for the hexameric and HMW forms of adiponectin. Both receptors stimulate AMP-activated protein kinase (AMPK) and PPARα. In the liver, this results in increased β-oxidation of fats and decreased gluconeogenesis. In muscle, receptor binding results in increased β-oxidation of fats as well as increased glucose uptake. Overall, liver and muscle effects contribute to increased insulin sensitivity, lowering of blood glucose, and decreased tissue triglyceride content.

Functions of adiponectin

The best characterized effects of adiponectin include enhancement of insulin sensitivity, anti-inflammatory properties, and inhibition of the development of atherosclerosis.144 Adiponectin appears to play an important role in increasing insulin sensitivity by stimulating phosphorylation of AMPK. Activation of AMPK subsequently increases glucose uptake by promoting translocation of the glucose transporter, GLUT4, to the cell surface, increasing glycolysis by phosphorylation of phosphofructokinase and increasing fatty acid oxidation by inactivation of acetyl CoA carboxylase.

A role for adiponectin in cardiovascular function has been suggested, and treatment with adiponectin may have a palliative effect on pathologic cardiac remodeling after myocardial infarction.145 These cardiovascular effects may stem from adiponectin’s function as a vasodilator.146 Adiponectin promotes vasorelaxation through increased vascular expression of endothelial nitric oxide synthase and prostacyclin synthase.147 It also is likely that stimulation of AMPK by adiponectin increases endothelial nitric oxide generation by endothelial nitric oxide synthase, affecting vascular tone. Improved insulin sensitivity in endothelial cells may increase vasorelaxation via Akt-mediated activation of endothelial nitric oxide synthase. A recent study suggests that adiponectin relaxes isolated aortic rings and mesenteric arteries via opening of K+ channels, a mechanism that is independent of the presence of the endothelium.146

Adiponectin has anti-inflammatory and anti-atherogenic properties. These functions appear to be mediated in part by the ability of adiponectin to suppress TNF-α production by macrophages and to both suppress myelomonocytic progenitor cell growth and induce apoptosis.148 Expression of adhesion molecules by endothelial cells and subsequent adherence of monocytes is also reduced.149 This curtails movement of monocytes into subendothelial areas and inhibits the development of atherosclerotic plaques. These same effects may also provide some protection against carcinogenesis by inhibiting cancer cell growth and angiogenesis associated with tumor formation.150 Low adiponectin levels have been observed in humans with a variety of cancer types and have been speculated to contribute to the increased incidence and severity of cancer in obesity.150,151

Adiponectin and obesity

Unlike leptin, increases in fat mass result in decreased circulating adiponectin while weight loss results in increased adiponectin concentrations in humans, primates, and rodents.152,153 In humans, circulating adiponectin levels are negatively correlated with body mass index, fasting insulin concentrations, and plasma triglyceride concentration but are positively correlated with high density lipoprotein cholesterol concentrations.154 The decrease in adiponectin in obesity is more severe with visceral than subcutaneous adiposity in humans. Changes in adiponectinemia reflect not only total adiponectin production but changes in the relative proportions of different molecular weight forms. Overweight and obese individuals have a relatively lower proportion of the HMW form of adiponectin compared with other forms, and the percentage of HMW adiponectin relative to total adiponectin increases with weight loss.155 The mechanisms underlying these changes are still being explored. Increased production of pro-inflammatory cytokines such as TNF-α and IL-6 as well as reactive oxygen species within the enlarging fat mass may act as autocrine or paracrine inhibitors of adiponectin gene expression.156,157 Insulin will downregulate AdipoR1 and AdipoR2 expression. Therefore, hyperinsulinemia, often associated with obesity and metabolic syndrome,
may contribute to a state of “adiponectin resistance” through alterations in receptor availability.\textsuperscript{132}

In humans, decreased circulating adiponectin has been linked with insulin resistance, type 2 diabetes mellitus, essential hypertension, and development of progressive ventricular hypertrophy and diastolic dysfunction.\textsuperscript{132,158} Spontaneous mutations of the adiponectin gene in humans and in engineered rodent models corroborate the association of hypo-adiponectinemia with the predilection to develop metabolic syndrome, type 2 diabetes mellitus, and cardiovascular disease.\textsuperscript{132,159,160} While these syndromes are common co-morbidities associated with obesity, adiponectin is persistently lower in these conditions even when subjects are matched for body mass index and adiposity. This suggests that hypo-adiponectinemia may be an independent risk factor for the development of metabolic and cardiovascular complications.

**Measurement of adiponectin**

Adiponectin is present in high concentrations (\(\mu\)g/mL) in human plasma where it has a half-life of 14 hours.\textsuperscript{140} Studies in mice suggest that differences in half-life may depend on the molecular form, with HMW adiponectin remaining in circulation longer than smaller forms (9 vs. 4.5 hours, respectively).\textsuperscript{141} Studies in dogs and horses have used the commercially available RIA from Linco as well as an ELISA (Otsuka, Pharmaceuticals, Tokushima, Japan) to measure adiponectin (Table 3). Millipore (formerly Linco, St. Charles, MO, USA) now offers a canine-specific ELISA. These commercial assays measure total adiponectin. Recent studies in humans have suggested that measurement of various molecular weight forms may provide additional risk assessment for the development of disease such that newer, less complicated, ELISAs are being developed to assess individual molecular weight forms.\textsuperscript{139,155} The clinical importance of differentiating the various molecular weight forms have not been addressed in dogs, cats, or horses.

Adiponectin can be measured in serum or plasma. Samples anticoagulated with EDTA or heparin have comparable results. One study in humans suggested that adiponectin is stable for up to 36 hours in whole blood before separation of plasma if kept on ice.\textsuperscript{165}

<p>| Table 3. | Circulating adiponectin concentrations in dogs and horses. |</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Sex</th>
<th>Breed</th>
<th>Body condition</th>
<th>Adiponectin ((\mu)g/mL)</th>
<th>Assay</th>
<th>Sample</th>
<th>Ref. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>21.6 ± 1.2 mo</td>
<td>SF (7)</td>
<td>Beagle</td>
<td>Lean</td>
<td>94 ± 12 ng/mL</td>
<td>RIA*</td>
<td>Plasma</td>
<td>Gayet et al.\textsuperscript{104}</td>
</tr>
<tr>
<td></td>
<td>2–6 yrs</td>
<td>SF, M (5)</td>
<td>Beagle</td>
<td>After 25% weight gain</td>
<td>52 ± 6 ng/mL</td>
<td>Plasma</td>
<td>Omachi et al.\textsuperscript{161}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Various</td>
<td>1–3 yrs</td>
<td>M (9), F (4)</td>
<td>9.3–29.5 kg BW</td>
<td>1.22 (range 0.85–1.5)</td>
<td>Murine/rat ELISA†</td>
<td>Serum</td>
<td>Brunson et al.\textsuperscript{162}</td>
</tr>
<tr>
<td></td>
<td>Various</td>
<td>CM (6), SF (16)</td>
<td>Beagle</td>
<td>Before weight gain</td>
<td>37.7 ± 2.0</td>
<td>Murine/rat ELISA†</td>
<td>Plasma</td>
<td>Ishioka et al.\textsuperscript{163}</td>
</tr>
<tr>
<td></td>
<td>5 mo–16 yr</td>
<td>M, F (34)</td>
<td>Various breeds</td>
<td>After ~30% weight gain</td>
<td>28.1 ± 2.3</td>
<td>Murine/rat ELISA†</td>
<td>Plasma</td>
<td>Moore et al.\textsuperscript{164}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various (20)</td>
<td></td>
<td></td>
<td>33.4 ± 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Various (17)</td>
<td></td>
<td></td>
<td>24.0 ± 2.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>M</td>
<td></td>
<td></td>
<td>16.8 ± 3.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>F</td>
<td></td>
<td></td>
<td>33.8 ± 4.6</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32.9 ± 3.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NR</td>
<td>M, F (4)</td>
<td>Mix</td>
<td>Various breeds</td>
<td>Lean</td>
<td>23.1 ± 0.6 kg BW</td>
<td>28.1 ± 2.3</td>
<td>Murine/rat ELISA†</td>
<td>Plasma</td>
</tr>
<tr>
<td>Horse</td>
<td>11 ± 2 yr</td>
<td>F (12)</td>
<td>STB</td>
<td>Lean</td>
<td>6.7 ± 0.2 (unfit)</td>
<td>1.3 ± 0.1</td>
<td>RIA*</td>
<td>Plasma</td>
</tr>
<tr>
<td></td>
<td>2–8 yr</td>
<td>M, F, G (34)</td>
<td>Standardbred</td>
<td>4.8 ± 0.1 (fit)</td>
<td>1.8 ± 0.05</td>
<td>RIA*</td>
<td>Plasma</td>
<td>Kearns et al.\textsuperscript{98}</td>
</tr>
<tr>
<td></td>
<td>10 ± 3 yr</td>
<td>F (23)</td>
<td>STB</td>
<td>514 ± 12 kg BW</td>
<td>2.69 ± 0.17</td>
<td>RIA*</td>
<td>Plasma</td>
<td>Pratt et al.\textsuperscript{97}</td>
</tr>
<tr>
<td></td>
<td>4 ± 3 mo</td>
<td>F (10), F (2)</td>
<td>Quarter Horse, Belgian</td>
<td>227 ± 11 kg BW</td>
<td>4.34 ± 0.12</td>
<td>RIA*</td>
<td>Plasma</td>
<td>Pratt et al.\textsuperscript{97}</td>
</tr>
<tr>
<td></td>
<td>3–4 yrs</td>
<td>G (6)</td>
<td>STB</td>
<td>4.84 ± 0.66</td>
<td>2.34 ± 0.59</td>
<td>RIA*</td>
<td>Plasma</td>
<td>Pratt et al.\textsuperscript{97}</td>
</tr>
<tr>
<td></td>
<td>F (8)</td>
<td></td>
<td></td>
<td>4.49 ± 0.59</td>
<td></td>
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<td></td>
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</table>

Data are mean ± SEM unless otherwise indicated.\textsuperscript{9} Linco Research (now Millipore), St. Charles, MO, USA.\textsuperscript{9} Otsuka Pharmaceuticals, Tokushima, Japan.\textsuperscript{9} M, male; F, female; G, gelding; MC, castrated male; SF, spayed female; STB, Standardbred; QB, Quarter Horse; QB × BEL, Quarter Horse × Belgium cross; number of animals indicated in parentheses; BW, body weight; BCS, body condition score (dog scale 1–5; horse scale 1–9); yr, years; mo, months; hr, hours. [Correction added after online publication on 6 April 2009: The original Table 3 contained misaligned text. The correct table, above, shows the correctly aligned text.]
Similar evaluations have not been done in domestic animal species.

Adiposity appears to be the primary factor affecting circulating adiponectin levels. Unlike leptin, adiponectin does not have a circadian pattern in humans. There is no consensus as to whether adiponectin levels change between the fed and fasted state or in response to glucose tolerance testing. The changes are generally mild and likely influenced by the metabolic state of the test subjects and the composition of the meal.

Adiponectin in dogs, cats, and horses

In dogs, both the nucleotide and amino acid sequences of canine adiponectin are highly homologous to those of other species and canine adiponectin appears to circulate as variably sized molecular weight complexes. The adiponectin gene is expressed in a variety of WAT depots in dogs; expression is not observed in other tissues. Adipocytes are the source of adiponectin, and gene expression increases with adipocyte differentiation. Similar to primates and rodents, adiponectin is inversely related to adiposity in dogs (Table 3). Diet-induced obesity is accompanied by a decrease in adiponectin gene expression in visceral WAT and a decrease in circulating adiponectin concentration.

The feline adiponectin gene and protein show strong homology with human and canine adiponectin. Expression was demonstrated in fat pads, and gene expression was significantly greater in visceral compared with subcutaneous depots. One study demonstrated that serum adiponectin is lower in obese cats and increases following weight loss.

Circulating adiponectin in horses has been shown to be negatively correlated with fat mass, percent body fat, BCS, and leptin levels (Table 3). Unlike leptin, adiponectin levels do not appear to fluctuate in a circadian pattern, or in response to feeding or exercise. With horses, fat mass is usually estimated from rump fat thickness and the role of visceral versus subcutaneous fat mass in regulating leptin or adiponectin levels has not been studied.

Angiotensinogen and the renin-angiotensin system (RAS)

Synthesis, receptors, regulation, and function

The RAS plays an important role in normal adipocyte differentiation and metabolism. All components of the RAS are found within fat pads and WAT is a major source of angiotensinogen, second only to the liver. Renin and angiotensin-converting enzyme for conversion of angiotensinogen to angiotensin II are present within the fat pad. The physiologic actions of angiotensin II are mediated by specific angiotensin receptors (AT1 and AT2). Local production of angiotensin II in fat depots appears to play a role in normal adipocyte differentiation, size, and insulin sensitivity.

The RAS and obesity

Increased production of angiotensinogen with excess gain in WAT contributes to the development of cardiovascular and renal disease. Obesity in humans, rodent models, and dogs is associated with activation of the RAS as evidenced by increased levels of circulating angiotensinogen, plasma renin activity, angiotensin converting enzyme activity, angiotensin II, and aldosterone. Increased intraabdominal fat is associated with a more pronounced activation of the RAS compared with peripheral adiposity. For example, obesity induced by feeding a high fat diet to mice results in increased angiotensinogen expression in visceral fat but not in subcutaneous fat or other tissues. With increased WAT mass, adipose-derived angiotensinogen spills over into the circulation, resulting in increased plasma concentrations of angiotensinogen, which may then be acted upon by the kidney and other tissues to produce angiotensin II. Increases in angiotensin II promote cardiovascular and renal dysfunction by direct vasoconstrictor activity as well as by increasing renal sodium retention via aldosterone release.

As evidence of dysregulation of the RAS, salt loading failed to appropriately suppress plasma renin activity and aldosterone in obese male humans. In a rat model of obesity due to a leptin receptor defect, a high-salt diet suppressed aldosterone secretion, but aldosterone levels remained abnormally high, suggesting a change (increase) in the set point for aldosterone secretion. Furthermore, weight loss in humans resulted in decreases in circulating components of the RAS.

In addition to direct vasoactive effects, increased activity of the RAS contributes to local inflammation in fat tissue. Angiotensin II also furthers progression of the metabolic syndrome and insulin resistance by inhibiting the insulin-stimulated translocation of GLUT4 subsequent to JAK/STAT signaling and production of SOCS-3.

Cross-talk between components of the RAS and other adipokines promotes development of metabolic syndrome. For example, leptin production by adipocytes appears to be stimulated by angiotensin II. Hyperleptinemia may further hyperactivity of the RAS.
by stimulating renal sympathetic nerves and subsequent increasing renin release by the kidney. Angiotensin II may also regulate adipocyte production of adiponectin. Infusion of angiotensin II into rats resulted in decreases in plasma adiponectin and in adiponectin mRNA expression in fat pads, changes that were ameliorated by treating with an antioxidant. In humans with metabolic syndrome, both plasma adiponectin levels and insulin sensitivity increased subsequent to use of angiotensin-converting enzyme activity inhibitors or AT1 receptor blockers. The mechanism by which RAS antagonism increases adiponectin production is uncertain. Some AT1 receptor blockers appear to increase adiponectin secretion via activation of PPARγ and differentiation of smaller, more insulin-sensitive adipocytes. This effect on PPARγ appears to be drug specific, and other inhibitors of the RAS may work through alternate mechanisms to improve insulin-sensitivity. Some inhibitors of the RAS also may promote redistribution of fat from visceral (metabolically disadvantageous fat) to subcutaneous depots. The mechanism underlying this redistribution is less certain but it appears to be related to increased expression or activation of PPARγ.

While a few studies have documented activation of the RAS in diet-induced obesity in dogs, the impact of alterations of RAS on obesity-related disorders in dogs, cats, and horses is incompletely characterized at this time. The demonstrated significance of RAS in people and laboratory animals suggests this system is a good candidate for evaluation in the pathophysiology of obesity-related disorders in domestic species, especially because many of the assays required are functional and should have broad species applicability.

Resistin

Resistin is a relatively recently described adipokine and was originally demonstrated as a product of murine adipocytes. While resistin also is expressed by human adipocytes, it appears to be primarily produced by macrophages in this species. Resistin expression has been demonstrated in fat and mammary tissue from cattle, and in fat and predominantly leukocytes from pigs. Expression of resistin has not yet been documented in dogs but this may be due to technical issues. Resistin is a cysteine-rich protein that is secreted as a dimer. The amino acid homology between human and murine resistin is relatively low (~60%) but appears to be higher between human, bovine, and porcine sequences. The receptor for resistin currently is unknown.

Most studies on the effects of resistin have been performed in rodent models (recently summarized by Lazar). Resistin secretion follows a similar pattern to that observed with leptin. Circulating levels increase with increasing fat mass and following a meal. Hyper-resistinemia contributes to the development of insulin resistance and metabolic derangements compatible with type 2 diabetes mellitus. Resistin is proinflammatory and induces upregulation of vascular adhesion molecules and stimulation of proinflammatory cytokine secretion by macrophages. Increased circulating resistin in humans correlates with atherosclerosis. Resistin is an adipokine that has not been well studied in domestic species, but is a logical candidate for evaluation in obesity-related disorders once analytical issues are worked out.

Inflammatory cytokines

Fat pads are a source for a variety of inflammatory cytokines, which also may be considered as adipokines. Recent data suggest that both macrophages and white adipocytes are the source of inflammatory cytokines such as TNF-α, interferon-γ, interleukins such as IL-1, IL-6, IL-8, and IL-10, monocyte chemotactic protein-1, and complement proteins. Obesity is considered to be a cause of chronic inflammation, in which macrophages infiltrate the enlarging adipose mass and local and circulating levels of proinflammatory cytokines are increased. Some authors feel that macrophages do not initiate the inflammatory process, but rather amplify a response to hypoxia in poorly vascularized areas of the expanding adipose tissue mass.

Inflammation is associated with insulin resistance via poorly characterized mechanisms, with insulin resistance contributing to the metabolic derangements that commonly accompany obesity. Excessive circulating free fatty acids that are present with overnutrition/obesity appear to activate the innate immune response via Toll-like receptor 4 and increased NF-κB signaling, although the exact mechanism for the induction of insulin resistance is not clear. Given the newly documented anti-inflammatory, antithrombotic, and vasodilatory functions of insulin, impaired insulin responsiveness has multiple pathways by which it may modulate comorbidities observed with weight gain. There is also evidence that insulin resistance may contribute to breast cancer development, potentially via its effect on tissue availability of insulin-like growth factor-1.

Interestingly, TNF-α has effects on adipocyte tissue as well: it inhibits adipocyte differentiation, thereby...
leading to decreased lipid storage capacity; promotes lipid mobilization; and impairs brown adipose tissue function. In a nonobese individual, these effects may promote fuel availability; however, in obesity, they exacerbate hyperlipidemia and lipotoxicity in other organs.

### Summary

Adipose is an important endocrine organ. It is a developmentally dynamic tissue with the potential to produce a broad spectrum of biologically important modifiers. Adipokines secreted by adipose tissue are key regulators of energy metabolism, cardiovascular health, and immune function, and their production and function may be influenced by nutrition and reproductive status, with some species-specific considerations. Dysregulation of adipokines appears to contribute to the development of a number of complications associated with obesity, including metabolic syndrome, type 2 diabetes, cardiovascular disease, and cancer via changes in whole organism physiology or at the local tissue level. These connections have mostly been explored in humans and rodent models; similar mechanistic studies are in their early stages in dogs, cats, and horses. Evaluation of individual adipokines shows promise as means to assess pathophysiologic mechanisms and risk for development of obesity-related conditions in veterinary patients. An understanding of the biology and regulation of adipokines, and of preanalytical factors that may influence circulating adipokine levels, will be important in interpreting measurements of these endocrine factors.

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Adipokines in domestic animals


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