Comparison of Adipokine Concentrations and Markers of Inflammation in Obese Versus Lean Dogs

Laura A. Eirmann, DVM[1,2]

Lisa M. Freeman, DVM, PhD, DACVN[3]

Dorothy P. Laflamme, DVM, PhD, DACVN[2]

Kathryn E. Michel, DVM, MS, DACVN[4]

Ebenezer Satyaraj, PhD[2]

[1]*Oradell Animal Hospital, Paramus, NJ 07652*
[2]*Nestle Purina PetCare, St. Louis, MO 63164*
[3]*Cummings School of Veterinary Medicine, Tufts University,North Grafton, MA 01536*
[4]*School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104*

Research funded by Nestle Purina's Veterinary Resident Research Grants Program

Acknowledgements
The authors thank Sue Kolkka, Kristen Cafarella, Chrissy Flora, Mary Jo Plump, Donna Stebbins, Robyn Pelker, Rachel Anderson, and Dawn Meola for technical assistance.

KEY WORDS: canine, obesity, nutrition, and inflammation

ABSTRACT

The purpose of this study was to compare the concentrations of key adipokines and markers of inflammation associated with obesity in a population of healthy lean versus obese pet dogs. Dogs were determined to be in good health based on medical history, physical examination, complete blood count, serum biochemistry profile, and urinalysis. Adult dogs between 2 and 7 years of age were assigned to either the lean group [body condition score (BCS) = 4-5/9; n=39] or the obese group [BCS= 7-9/9; n=37]. Fasting circulating concentrations of C-reactive protein (CRP), adiponectin, resistin, insulin, leptin, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF were measured. Groups were compared using Chi-Square and Mann-Whitney U tests. No significant differences between groups were detected in age (p=0.16) or gender (p=0.34). Median CRP concentration was significantly higher in the obese group [median 0.94 ug/ml (0.47-6.22 ug/ml)], versus the lean group [median 0.72 ug/ml (0.45-6.04 ug/ml); p=0.03]. The lean group had higher median adiponectin concentration [median 21.84 ug/ml (3.08-79.14 ug/ml)] compared to the obese group [median 15.27 ug/ml (1.97-42.76 ug/ml); p=0.004]. Statistical analysis for IL-6 and TNF was not performed because >50% of samples either failed due to methodological issues or were below the level of detection. Differences between groups in insulin, glucose, insulin:glucose ratio, and leptin concentrations were not detected in this study. Additional studies in dogs are warranted to

further evaluate mechanisms and clinical significance of adipokines and inflammatory mediators associated with obesity.

ABBREVIATIONS

CRP	C-reactive protein
BCS	Body condition score
IL-6	Interleukin-6
TNF	Tumor necrosis factor-α

INTRODUCTION

Obesity is a common canine nutritional problem in developed countries with 22% to 40% of dogs seen by veterinarians diagnosed as overweight or obese.[1-2] Canine obesity is associated with several adverse health conditions including insulin resistance, hip dysplasia, osteoarthritis, pancreatitis, and decreased longevity.[3-5] The precise mechanisms by which adiposity is associated with increased risk of morbidity and reduced longevity are unknown. Previously, it was believed that adipose tissue was a relatively inert tissue primarily responsible for the storage of excessive energy in the form of triglycerides. However, beginning in 1994 with the discovery of leptin,[6] a regulator of energy homeostasis, it has become clear that adipose tissue is a metabolically active organ. Additional bioactive peptides secreted from adipose tissue, termed adipokines, have been identified and postulated to have paracrine and endocrine effects on metabolic homeostasis, inflammation, and immunity. Many of these inflammatory cytokines are expressed by white adipose tissue of humans and dogs.[7-8] In a state of sustained overnutrition, these adipokines appear to have a role in the development of metabolic syndrome, insulin resistance, hyperlipidemia, and atherosclerotic cardiovascular disease in humans.[9-12] Leptin, resistin, and many pro-inflammatory adipokines, rise with increasing fat mass, while adiponectin, which has anti-inflammatory and insulin-sensitizing properties, decreases.[13]

The role of adipokines in canine obesity is an area of current investigative interest. Genes encoding for adiponectin, leptin, interleukin-6 (IL-6), and tumor necrosis factor-α (TNF are expressed in adipose tissue or in isolated adipocytes from dogs.[8] Gene expression of adiponectin in canine visceral adipose tissue was lower, while leptin and TNF expression were higher after obesity and insulin-resistance were experimentally induced in previously lean dogs.[14] A few studies have focused on circulating adipokine concentrations in experimentally induced obesity in dogs. Plasma leptin concentrations have been reported to correlate with body fat mass in dogs.[15-16] During the induction of obesity in laboratory dogs, plasma leptin[14] and TNF[17] increase and adiponectin[14, 18] decreases; leptin decreases after weight loss.[19] The clinical significance of these differences is unclear, but it is possible that the changes in adipokine expression associated with obesity play a role in insulin sensitivity. In laboratory conditions, dogs fed to a lean body condition have improved glucose tolerance compared to overweight dogs,[3] and insulin resistance has been demonstrated in dogs fed to an obese state.[14,17] A recent clinical study assessed adiponectin and leptin, as well as a measure of insulin sensitivity in a population of obese pet dogs before and after weight loss.[20] Although the results of this study supported an improvement in insulin resistance after weight loss, no changes in leptin and adiponectin were found and the obese dogs were not compared to a group of healthy lean dogs.

Another important issue with excess adipose tissue in addition to the excess production of adipokines that affect insulin resistance is that adipocytes also secrete inflammatory mediators such TNF, IL-6, and others that can contribute to systemic inflammation. Chronic low-grade systemic inflammation can contribute to a number of significant health problems in humans, such as coronary artery disease, insulin resistance, and metabolic syndrome in humans.[10-12, 21-23] This chronic inflammation may play a role in the connection between obesity and increased morbidity and mortality. C-reactive protein (CRP) is an acute phase protein synthesized primarily by the liver in

response to inflammatory cytokines and is used in people and other species as a marker of inflammation. In humans, increased CRP is associated with an increased risk for cardiovascular and all-cause mortality.[24-25] In obese humans, even mild increases in CRP concentrations are considered to reflect chronic low-grade inflammation.[26] A limited number of studies measuring CRP in obese dogs have been published, and these have yielded mixed results.[20, 27-28] Two studies reported lower CRP in overweight dogs[27-28] in contrast to the findings in humans and the recently reported canine study.[20] One of these studies found no change in CRP following weight loss,[27] while a more recent study reported decreased CRP after weight loss in obese dogs.[20] Further studies with clearly defined populations are needed to determine if these conflicting results are due to difference in study methodological issues or other factors.

Currently, there are no reported studies comparing multiple adipokines and inflammatory markers in a population of healthy client-owned lean versus overweight dogs. Therefore, the objective of this study was to compare serum concentrations of adipokines (leptin, adiponectin, and resistin), inflammatory cytokines (IL-6 and TNF), markers of inflammation (CRP), as well as insulin and glucose in a population of healthy lean and obese pet dogs. The study hypothesis was that obese dogs would have increased concentrations of leptin, resistin, CRP, TNF, and IL-1; altered glucose metabolism; and decreased adiponectin.

MATERIALS AND METHODS

Dogs and Procedures:

Healthy dogs between 1to7 years of age owned by staff or clients of the Oradell Animal Hospital were recruited by in-clinic advertisement for participation in the study. The project was compliant with the animal care and use guidelines established by the funding agency. All owners signed an informed consent form before enrolling their dogs in the study. Dogs were deemed healthy based on normal history, physical examination findings, complete blood count, serum biochemistry, and urinalysis including microalbuminuria. Pregnant dogs or dogs with evidence of chronic inflammation (eg, infection, significant dental disease, pancreatitis, cancer, inflammatory bowel disease, and osteoarthritis) were excluded. Dogs also were excluded if they received vaccinations, medication, or supplements in the past month with the exception of heartworm or flea and tick preventative.

Owners were asked to fast their dogs for 12 hours prior to presentation for blood sampling. One investigator weighed and examined each dog to screen for potential underlying inflammatory conditions. The same investigator determined a body condition score (BCS) and assigned the dog to either the lean group (BCS 4-5/9) or the obese group (BCS 7-9/9) utilizing a 9 point BCS system.[29] Dogs with a BCS of 6/9 were excluded so that two distinct populations would be compared. Dogs deemed healthy based on history and physical examination had 6 ml of blood collected by venipuncture using minimal restraint. Urine was collected by free catch. One ml of blood was placed in an EDTA tube for a complete blood count and the remaining blood was allowed to clot at room temperature for 30 minutes then centrifuged at 2500 rpm for 15 minutes. Two 0.5 ml aliquots of serum were frozen at -4^{o}C, transferred to a -80^{o}C freezer within 4 hours of collection, and stored at this temperature until analysis of adipokines, insulin, cytokines, and CRP. The remaining serum was submitted for serum biochemical analysis and urine was submitted for a urinalysis.

Sample Analysis

All serum samples were visually inspected for hemolysis and lipemia and were analyzed in a single run in duplicate for each assay. Concentrations of CRP were analyzed utilizing a commercially available enzyme-linked immunosorbent assay for canine CRP according to manufacturer's instructions (Tri-Delta PhaseTM Canine CRP assay, Tridelta Diagnostics Inc., Morris Plains, NJ). This assay had an intra- and inter-assay vari-

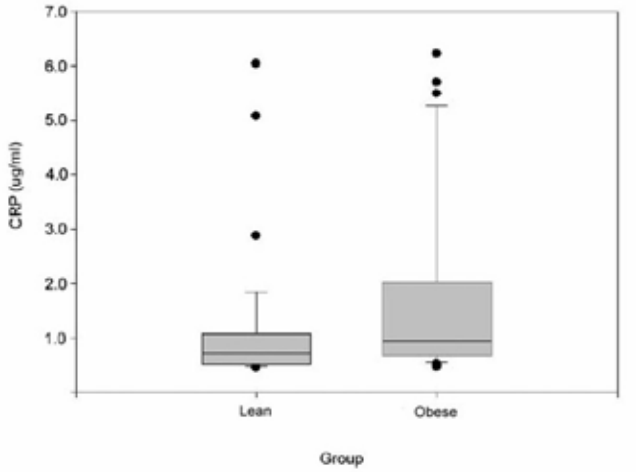
Table 1*- Signalment, body condition, and results from laboratory screening tests for lean (n=39) and obese (n=37) dogs [median (range)].*

Variable	Lean	Obese	P value	Reference Range
Age (yrs)	2.9 (0.9-6.2)	3.4 (0.9-7.3)	0.16	
Sex			0.34	
Male	18 (14 castrated)	20 (17 castrated)		
Female	21 (21 spayed)	17 (15 spayed)		
Body weight (kg)	8.0 (2.5-34.5)	34.7 (3.8-92.1)	.001	
Body condition score (1-9)	5 (4-5)	8 (7-9)	<.001	
RBC ($x10^3$/ul)	7.4 (6.5-9.7)	7.6 (6.0-9.4)	.27	6.1-8.8
WBC ($x10^3$/ul)	9.2 (4.3-14.8)	10.9 (6.0-17.4)	.001	4.7-15.1
Albumin (g/dl)	3.7 (3.2-4.2)	3.7 (3.2-4.3)	.43	2.6-4.3
Globulin (g/dl)	2.3 (2.1-3.9)	2.6 (2.1-3.9)	.001	2.0-5.0
BUN (mg/dl)	19.2 (11.4-33.6)	15.9 (10.5-29.5)	.06	7-27
Creatinine (mg/dl)	0.9 (0.4-1.5)	1.0 (0.4-1.4)	.86	0.6-1.4
Phosphorus (mg/dl)	4.0 (2.1-6.3)	3.8 (2.1-5.8)	.33	2.1-6.7
Calcium (mg/dl)	10.7 (9.0-11.5)	10.8 (9.9-11.9)	.26	8.5-12.1
Sodium (mmol/L)	151 (145-157)	152 (146-156)	.34	141-156
Chloride (mmol/L)	114 (110-119)	114 (105-118)	.05	105-115
Potassium (mmol/L)	4.6 (3.9-5.1)	4.7 (4.1-6.4)	.05	4.0-5.6
ALP (U/L)	30.5 (12.3-120.2)	48.4 (17.3-172.7)	.01	10-150
ALT (U/L)	44.3 (10.8-117.3)	37.1 (21.6-93.2)	.06	5-60
AST (U/L)	31.9 (20.1-50.7)	33.0 (16.1-65.3)	.84	5-55
Bilirubin (mg/dl)	0.1 (0.1-0.3)	0.1 (0.1-0.2)	.74	0.1-0.3
Triglycerides	59.4 (30.7-205.4)	87.0 (45.5-407.8)	<.01	26-103
Cholesterol (mg/dl)	221.2 (148.7-529.9)	242.9 (153.1-355.6)	.08	112-328
Urine Specific Gravity	1.044 (1.011-1.054)	1.035 (1.008-1.065)	.13	1.015-1.055
Urine pH	6.5 (5.0-8.5)	6.0 (5.0-8.5)	.30	5.5-7.0
Microalbuminuria (mg/dl)	10.7 (9.0-11.5)	0.15 (0.0-0.2)	.13	<0.25

Key*:*
RBC *Red blood cell count*
WBC *White blood cell count*
BUN *Blood urea nitrogen*
ALP *Alkaline phosphatase*
ALT *Alanine aminotransferase*
AST *Aspartate aminotransferase*

ability of 6.5% and 7.8%, respectively, and a minimum limit of detection of 3.75 ng/ml. Leptin and insulin concentrations were measured following manufacturer's protocol using a Luminex-based assay (MilliplexTM MAP canine endocrine hormone kit, Millipore, St. Charles, MO) . The intra- and inter-assay coefficient of variation for leptin were 4.6% and 17.6% (minimum level detection= 0.148 ng/ml) and 4.7% and 13.4% for insulin (minimum level of detection=1.8 uU/ml). Adiponectin and resistin were analyzed using a Luminex-based adipokine assay (MilliplexTM MAP canine adipokine kit, Millipore, St. Charles, MO). The intra- and inter-assay coeffients of variation were 7.9% and 9.3% for adiponectin (minimum limit of detection=22.1 pg/ml) and 9.2 and 16.6% for resistin (minimum limit of detection=1.5 pg/ml). Serum IL-6 and TNFwere measured using a Luminex-based canine cytokine assay as per manufacturer's instructions (Mil-

Figure 1 *Distribution of serum C-reactive protein (CRP) concentrations in lean (n=39) and obese (n=37) dogs. Obese dogs had significantly higher CRP concentrations than lean dogs (P=0.03). The boxes represent the central 50% of values for each group. The center horizontal line in each box represents the median value. The whiskers show the range of observed values that fall within ±1.5 x inter-quartile range. Circles beyond the ends of the whiskers are outliers.*



liplexTM MAP canine cytokine/chemokine kit, Millipore, St. Charles, MO). The intra- and inter-assay coeffients of variation were 3.9% and 15.8% for IL-6 (minimum limit of detection=12.1 pg/ml) and 13.2 and 20.0% for TNF (minimum limit of detection=0.4 pg/ml). Insulin:glucose ratios, determined as I:G= [Insulin (uU/ml) x 100/Fasting glucose (mg/dl)], were calculated as an estimate of insulin sensitivity.[20]

Statistical Analysis

Sample size calculations using data from similar studies in humans showed that 38 dogs per group would provide 80% power with alpha=0.05 to reject the null hypothesis for CRP, IL-6, and TNF concentrations.[31-32] Data were examined graphically and using the Kolmogorov-Smirnov test. Most data were not normally distributed and common mathematical transformations (eg, log, square) were not able to produce a normal distribution, so data are presented as median (range) and were compared using a Mann-Whitney test. Categorical data, such as gender, were compared between the lean and obese groups using the Chi-Square test. Correlations between continuous variables were assessed using the Spearman correlation test. All analyses were performed utilizing a commercially available statistical software program (Systat 11.0, SPSS, Chicago, IL). Probability values <0.05 were considered statistically significant.

RESULTS

Ninety-one dogs presented for evaluation. Fifteen dogs were excluded from the study for failing to meet the inclusion criteria for the following reasons: BCS 6/9 (n=4), pyoderma (n=1), significant dental disease (n=5), alanine aminotransferase greater than two times the upper reference range (n=4), hyperglycemia above laboratory reference range of 125 mg/dl (n=1). The remaining 75 dogs consisting of 39 lean dogs [BCS 4 (n=12), BCS 4.5 (n=6), BSC 5 (n=21)] and 37 obese dogs [BCS 7 (n=12), BCS 7.5 (n=4), BSC 8 (n=10), BCS 9 (n=11)] were included in the study. Age (p=0.16) and gender (p=0.34) were not different between the lean and obese groups (Table 1). A variety of breeds were represented, but the most common breeds in the lean group included shih tzu (n=6), Chihuahua (n=4), Labrador retriever (n=4), German shepherd (n=3), and poodle (n=3). The most common breeds in the obese group were Chihuahua (n=4), Labrador retriever (n=3), beagle (n=3), and German shepherd (n=3). There was a significant difference in BCS between the lean group [median=5 (4-5)] and the obese group [median=8 (7-9); p<0.001]. Body weight was significantly lower in the lean group compared to the overweight group (p=0.001). Although within the laboratory reference range, median white blood cells, serum globulins, alkaline phosphatase, and triglycerides were higher in obese dogs compared to lean dogs (Table 1).

Table 2- *Results for adipokines, insulin and glucose, and C-reactive protein concentrations in lean (n=39) and obese (n=37) dogs [median (range)].*

Variable	Lean	Obese	P value
Adiponectin (ug/ml)	21.84 (3.08-79.14)	15.27 (1.97-42.76)	.004
Leptin (ng/ml)	0.63 (0.44-9.94)	0.66 (0.43-35.79)	.28
Resistin (ng/ml)	12.4 (2.4-57.6)	14.1 (3.2-36.1)	.94
Insulin (uU/ml)	19.1 (8.3-66.6)	22.5 (9.6-91.8)	.08
Glucose (mg/dl)	100.8 (74-117)	101.8 (72-121)	.09
Insulin:glucose ratio	8.0 (3.1-28.8)	8.1 (3.7-114.8)	.16
C-reactive protein (ug/ml)	0.72 (0.45-6.04)	0.94 (0.47-6.22)	.03

There were no significant differences in median insulin (p=0.08) nor glucose (p=0.09) concentrations between the lean and obese groups (Table 2). Insulin:glucose ratios (p=0.16) also were not different between the two groups. Adiponectin was significantly higher in lean compared to obese dogs (p=0.004), but neither leptin (p=0.28) nor resistin (p=0.94) concentrations were significantly different between groups. The I:G ratio was significantly correlated with leptin (r=0.77, p<0.001) and adiponectin (r=-0.35, p=0.004), but not with BCS (r=0.16, p=0.20) or resistin (r=0.03, p=0.87). Obese dogs had a higher median CRP concentration compared to the lean dogs (p=0.03; Figure 1). There were no significant correlations between CRP and BCS (p=0.19) nor CRP and age (p=0.63). TNF concentrations were below the level of assay detection for 61% of the lean group and 56% of the obese group. IL-6 concentrations were below the level of assay detection for 79% of the lean group and 81% of the obese group. Since greater than 50% of samples were below the minimal detection limit of the assay, a Mann-Whitney test could not be performed to compare the groups.

DISCUSSION

This study documented an increase in median CRP concentrations in obese dogs compared to lean dogs. This is consistent with studies reporting higher serum CRP concentrations in obese compared lean humans, which have supported the emerging view that obesity is associated with a state of chronic, low-grade inflammation.[30-37] Studies have shown a direct association between CRP concentrations and adiposity and propose that inflammatory cytokines such as TNF and IL-6 from adipose tissue stimulate hepatic CRP synthesis, making this a commonly used marker of inflammation in obesity studies.[23] While CRP has consistently been shown to be higher in human obesity, concentrations are generally low. For example, one study showed that obese people had higher CRP concentrations (0.75 +/- 1.04 ug/ml) compared to lean people (0.41 +/- 0.75 ug/ml) even though all CRP values fell within the normal reference range.[36] In order to detect these subtle differences in CRP that have been found in human obesity studies, the current study compared two groups that were screened for underlying diseases and matched with the exception of BCS. Despite overlap in CRP values between the two populations, these data suggest that obesity in the dog is associated with higher CRP concentrations compared with lean dogs. This is consistent with similar studies in humans, cats and a recently reported canine study in which CRP concentrations decreased after weight loss.[20, 30-38] However, these results differ from two previously published studies in dogs.[27-28] The reasons for the difference are not entirely clear, but there were differences in the study populations and methodologies in these studies. In addition, the relationship of CRP to adiposity may be more complex. In humans, fat distribution may be an important factor since increased visceral abdominal fat better correlates with CRP concentrations.[39] Future studies could elucidate CRP values in a larger number of dogs with different degrees of adiposity and also evaluate the effect of fat distribution.

Elevated CRP is not a specific marker of inflammation and evaluation of the inflam-

matory cytokines, such as TNF and IL-6 would be valuable additional information for the study of obesity and inflammation. IL-6 is thought to stimulate CRP production and to play a key role in both the inflammatory processes associated with obesity and insulin resistance.[23] However, in the current study a large percentage of TNF and IL-6 samples failed due to methodological issues such as a high coefficient of variation between duplicate samples or were below the level of detection so these data were not used for concern with reliability. This finding is similar to a previous study utilizing the same assay in which a substantial number of results were below level of detection for IL-6.[20] It is possible that these cytokines circulate at very low levels in canine obesity and that their effects are primarily autocrine or paracrine. However, it is also possible that the assay used was not sufficiently sensitive. Interestingly, results from both the current study and a recent canine weight loss study showed a higher median white blood cell count in the obese population (albeit within the normal reference range) that, while nonspecific, may help to support a state of low-grade inflammation.[20] Further studies with more specific measures of inflammation are needed, however, to more fully assess this issue.

Results from the current study showed a lower median adiponectin concentration in the obese group compared to the lean group, similar to previously reported canine studies.[14,18] Since adiponectin has insulin-sensitizing and anti-inflammatory effects, future studies to assess the clinical relevance of this finding are warranted. This study was not, however, able to detect a difference in median serum leptin values between the obese and lean dogs in contrast to several other studies.[14-16, 19, 40] The reason for this difference is unclear but may be related to methodological differences since previous reported studies used a different species-specific antibody which is not commercially available. While the current study's design followed the manufacturer's instructions for collection and storage of samples, leptin concentrations were low for most samples. One other published study utilizing the same assay suggested that this assay may only detect free circulating leptin which may have limited the ability to detect differences between groups.[20]

A difference in resistin levels between the two groups could not be detected in this study. Most of the investigation of this adipokine has been performed in rodent models.[41] Further canine studies are needed to determine if the inability to detect a difference between groups was due to insufficient sample size, methodological issues with the assay, or species differences between dogs and rodents.

Few studies have examined insulin sensitivity in pet dogs with naturally-occurring obesity rather than inducing an overfeeding period in a controlled laboratory setting.[20,42] Although not a primary objective of this study, there is reason to assess insulin sensitivity since the adipokines leptin, resistin, and adiponectin are all proposed to modulate insulin sensitivity. This study was unable to detect neither a difference in insulin concentrations between the two groups nor a difference in the insulin:glucose ratio. Findings from previous canine studies are conflicting: One study reported normal insulin concentrations in obese dogs before and after weight loss,[43] while another showed a significant reduction in the insulin:glucose ratio before and after weight loss.[20] However, even in the study that showed differences, the association between body fat mass and insulin sensitivity was weak, and the authors suggested that additional factors may be involved in a diverse, outbred population of dogs.[20] This is supported by the significant correlation in the current study between the insulin:glucose ratio and leptin and adiponectin, but not with BCS.

In the current study, we attempted to carefully screen all dogs with a thorough history and physical examination and by evaluating a complete blood count, serum biochemistry profile, and urinalysis with microproteinuria to better assess dogs for

evidence of mild subclinical inflammation that could confound the results. However, there were several findings from the CBC and biochemistry profile results consistent with previously reported studies, including a higher median total white cell count,[20] alkaline phosphatase,[20, 27] and triglycerides[20,27-28, 43-45] in the obese dogs. Although these differences are unlikely to be of clinical significance, one might postulate that higher median triglycerides and a trend toward higher cholesterol in the obese dogs are supportive of dysregulation of lipid metabolism associated with obesity. Although dogs were fasted for 12 hours prior to sample collection, dietary composition was not controlled. Therefore, the difference in median triglyceride concentrations also could be secondary to higher dietary fat intake in the obese group.

There are a number of limitations to the current study which should be noted. The study population consisted of client-owned animals living in a home environment. Although a homogeneous group of dogs in a controlled environment might minimize subject variability, client-owned dogs were chosen since this group should better reflect the population seen by veterinarians in clinical practice. It is possible that increased subject diversity could influence adipokine levels suggesting that additional variables independent of body fat mass could play a role. It is interesting to note the wide range in certain parameters such as CRP in the obese groups. One might postulate that variables independent of percentage of fat mass predispose certain obese individuals to the adverse health conditions. Obesity was determined based on BCS, which is a semi-quantitative assessment of adiposity, rather than a direct measure of body fat. One investigator performed all assignments of BCS to reduce inter-observer variation. In humans, different ethnic populations have differences in visceral fat distribution which is not accounted for by body mass index.[31] Future studies comparing adipokines among dogs with increased visceral versus subcutaneous fat might yield interesting findings. Last, dietary composition and intake were not controlled in this study. There is evidence that macronutrient intake may influence inflammatory signaling pathways[11, 46] and so future studies evaluating the effect of nutrient composition on markers of systemic inflammation in dogs may be warranted.

In conclusion, this study demonstrated higher median CRP and lower adiponectin in a population of otherwise healthy client-owned obese versus lean dogs. The relationship between adipokines, inflammation, and insulin resistance is complex and requires further studies to determine the underlying mechanisms and clinical significance of these findings.

REFERENCES

1. Lund E, Armstrong P, Kirk C, Kolar L, Klausner J: Health status and population characteristics of dogs and cats examined at private practices in the United States. *J Am Vet Med Assoc* 1999; 214:1336-1341.
2. German A: The growing problem of obesity in dogs and cats. *J Nutr* 2006; 136:1940S-1946S.
3. Larson B, Lawler D, Spitznagel E, Kealy R: Improved glucose tolerance with lifetime diet restriction favorably affects disease and survival in dogs. *J Nutr* 2003; 133:2887-2892.
4. Kealy R, Lawler D, Ballam J, Mantz S, Biery D, Greeley E, Lust G, Segre M, Smith G, Stowe H: Effects of diet restriction on life span and age-related changes in dogs. *J Am Vet Med Assoc* 2002; 220:1315-1320.
5. Hess R, Kass P, Shoefer F, Van Winkle T, Washabau R: Evaluation of risk factors for fatal acute pancreatitis in dogs. *J Am Vet Med Assoc* 1999; 214:46-51.
6. Zhang Y, Proenca R, Maffei M, Barone L, Leopold L, Friedman J: Positional cloning of the mouse obese gene and its human homolog. *Nature* 1994; 372:425-432.
7. Hotamisligil G, Shargill N, Spiegelman B: Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* 1993; 259:87-91.
8. Eisele I, Wood I, German A, Hunter L, Trayhurn P: Adipokine gene expression in dog adipose tissues and dog white adipocytes differentiated in primary culture. *Horm Metab Res* 2005; 37:474-481.
9. Trayhurn P, Bing C, Wood I: Adipose tissue and adipokines-energy regulation from the human perspective. J Nutr 2006; 136:1935S-1939S.
10. Fantuzzi G: Adipose in the regulation of inflammation. *Immunol Endocr Metab Agents Med Chem* 2007; 7:129-136.
11. Shoelson S, Herrero L, Naaz A: Obesity, inflammation, and insulin resistance. *Gastroenterology* 2007; 132:2169-2180.

12. Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R: Metabolic syndrome: A comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005; 111:1448-1454.

13. AritaY, Kirhara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Simomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraquchi M, Ohmoto Y, Funhashi T, Matsuzawa Y: Paradoxical decrease in an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; 257:79-83.

14. Gayet C, Leray V, Masayuki S, Siliart B, Nguyen P: Effect of obesity-associated insulin resistance on mRNA expression on peroxisome-activated receptor-y target genes in dogs. *Br J Nutr* 2007; 98:497-503.

15. Sagawa M, Nakadoma F, Honjoh T, Ishioka K, Saito M: Correlation between plasma leptin concentration and body fat content in dogs. *Am J Vet Res* 2002; 63:7-10.

16. Ishioka K, Hosoya K, Kitagawa H, Shibata H, Hohjoh T, Kimura K, Saito M: Plasma leptin concentrations in dogs: effect of body condition score, age, gender, and breeds. *Res Vet Sci* 2007; 82:11-15.

17. Gayet C, Bailhache E, Dumon H, Martin B, Nguyen P: Insulin resistance and changes in plasma concentrations of TNF*α*, IGF 1, and NEFA in dogs during weight gain and obesity. *J Anim Physiol Anim Nutr(Berl)* 2004; 88:157-165.

18. Ishioka K, Omachi A, Sagawa M, Shibata H, Honjoh T, Kimura K, Saito, M: Canine adiponectin: cDNA structure, mRNA expression in adipose tissue and reduced plasma levels in obesity. *Res Vet Sci* 2006; 80: 127-132.

19. Jeusette I, Detilleux J, Shibata H, Saito M, Honjoh T, Delobel A, Istasse L, Diez M: Effects of chronic obesity and weight loss on plasma gherlin and leptin concentrations in dogs. *Res Vet Sci* 2005; 79:169-175.

20. German A, Hervera M, Hunter L, Holden S, Morris P, Biourge V, Trayhurn P: Improvement in insulin resistance and reduction in plasma adipokines after weight loss in obese dogs. *Domest Anim Endocrinol* 2009; 37:214-226.

21. Kershaw E, Flier J: Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab* 2004; 89: 2548-2556.

22. Wellen K, Hotamisligil G: Inflammation, stress, and diabetes. *J Clin Invest* 2005; 115:1111-1119.

23. Yudkin J, Kamari M, Humphries S, Mohamed-Ali V: Inflammation, obesity, stress and coronary heart disease: Is interleukin-6 the link? *Atherosclerosis* 2000; 148:209-241.

24. Rutter M, Meigs J, Sullivan L, D'Agostino R, Wilson P: C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham offspring study. Circulation 2004; 110:380-385.

25. Laaksonen D, Niakanen L, Nyyssonen K, Punnonen K, Tuomainen T, Salonen J: C-reactive protein in the prediction of cardiovascular and overall mortality in middle aged men: a population-based cohort study. *Eur Heart J* 2005; 26:1783-1789.

26. Heilbronn L, Clifton P: C-reactive protein and coronary artery disease: influence of obesity, caloric restriction and weight loss. *J Nutr Biochem* 2002; 13:316-321.

27. Yamka R, Friesen K, Frantz N: Identification of canine markers related to obesity and effects of weight loss on markers of interest. *Intern J Appl Res Vet Med* 2006; 4:282-292.

28. Veiga A, Price C, de Oliveira S, dos Santos A, Campos R, Barbosa P, Gonzalez F: Association of canine obesity with reduced serum level of C-reactive protein. *J Vet Diagn Invest* 2008; 20:224-228.

29. Laflamme D: Development and validation of a body condition score system for dogs. *Canine Pract* 1997; 20:10-15.

30. Visser M, Bouter L, McQuillan G, Wener M, Harris T: Elevated C-reactive protein levels in overweight and obese adults. JAMA 1999; 282:2131-2135.

31. Reinehr T, Stoffel-Wagner B, Roth C, Andler W: High-sensitive C-reactive protein, tumor necrosis factor-*α*, and cardiovascular risk factors before and after weight loss in obese children. *Metabt Clin Exp* 2005; 54:1155-1161.

32. Park H, Parak J, Yu R: Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-*α* and IL-6. *Diabetes Res Clin Pract* 2005; 69:29-35.

33. Kopp H, Kopp C, Festa A, Krzyzanowska K, Kriwanek S, Minar E, Roka R, Schernthaner G: Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. *Arterioscler Throm Vasc Biol* 2003; 23:1042-1047.

34. Yudkin J, Stehouwer C, Emesis J, Coppack S: C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: A potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999; 19:972-978.

35. Shin M, Kim O, Koh S, Chae J, Kim J, Jang Y, Lee J: Modest weight loss does not increase plasma adiponectin levels: effects of weight loss on C-reactive protein and DNA damage. *Nutr Res* 2006; 26:391-396.

36. Gomez-Ambrosia J, Salvador J, Silva C, Pastor C, Rotellar F, Gil M, Cienfuegos J, Fruhbeck G: Increased cardiovascular risk markers in obesity are associated with adiposity: role of leptin. *Thromb Haemost* 2006; 95:991-996.

37. Bozdemir A, Barutcuoglu B, Dereili D, Kabaroglu C, Habif S, Bayindir O: C-reactive protein and neopterin levels in healthy non-obese adults. *Clin Chem & Lab Med* 2006; 44:317-321.

38. Tanner A, Martin J, Saker K: Oxidative stress and inflammatory state induced by obesity in the healthy feline. *J Anim Physiol Anim Nutr* (Berl) 2007; 91:163-166.

39. Lemieux I, Pascot A, Prud'homme N, Almeras N, Bogaty P, Nadeau A, Bergeron J, Despres J: Elevated C-reactive protein; another component of the atherothrombotic profile of abdominal obesity.

Arterioscler Thromb Vasc Biol 2001; 21:961-967.

40. Ishioka K, Soliman M, Sagawa M: Experimental and clinical studies on plasma leptin in obese dogs. *J Vet Med Sci* 2002; 64:349-353.
41. Radin M, Sharkey L, Holycross B: Adipokines: a review of biological and analytical principles and an update in dogs, cats, and horses. *Vet Clin Pathol* 2009; 38:136-156.
42. Mattheeuws D, Rottiers R, Baeyens D, Vermeulen A: Glucose tolerance and insulin response in obese dogs. *J Am Anim Hosp Assoc* 1984; 20:287-293.
43. Diez M, Michaux C, Jeusette I, Baldwin R, Istasse L, Biourge V: Evolution of blood parameters during weight loss in experimental obese beagle dogs. *J Anim Physiol Anim Nutr* 2004; 88:166-171.
44. Bailhache E, Nguyen P, Krempf M, Siliart B, Magot T, Ouquerram K: Lipoprotein abnormalities in obese insulin-resistant dogs. *Metabolism* 2003; 52:559-64.
45. Pena C, Suarez L, Bautista I, Montoya J, Juste M: Relationship between analytic values and canine obesity. *J Anim Physiol Anim Nutr* 2008; 92:324-325.
46. Mohanty P, Ghanim H, Hamouda W, Aljada A, Garg R, Danadona P: Both lipid and protein intakes stimulate increased generation of reactive oxygen species by polymorphonuclear leukocytes and mononuclear cells. *Am J Clin Nutr* 2004; 80:51-57.