Identification of Canine Markers Related to Obesity and the Effects of Weight Loss on the Markers of Interest

Ryan M. Yamka, PhD
Kim G. Friesen, PhD
Nolan Z. Frantz, PhD

*Hill's Pet Nutrition, Inc.
Topeka, KS*

KEY WORDS: biomarkers, body composition, dog, obesity, weight loss

ABSTRACT

Study 1 included 30 lean and 30 overweight beagles (15 each spayed female and 15 each neutered male) to determine the effect of obesity and gender on marker differences. Animals were weighed and given a body condition score (1 = lean, = ideal, and 5 = overweight), and a blood sample was drawn. Average body condition scores were 4.7 and 2.5 and average body weights were 17.3 and 11.2 kg for the overweight and lean groups, respectively. Serum was analyzed for chemistry screens, obesity markers, thyroid markers, and arthritis markers. The overweight group had higher levels of alkaline phosphatase ($P = 0.04$), cholesterol ($P = 0.04$), triglycerides ($P = 0.06$), total protein ($P < 0.01$), albumin ($P < 0.01$), thyroxine ($P = 0.05$), calcium ($P < 0.01$), phosphorous ($P = 0.04$), glucose ($P < 0.01$), insulin ($P < 0.01$), insulin-like growth factor-1 ($P < 0.01$), low-density lipoprotein ($P < 0.01$), leptin ($P < 0.01$), and type II cartilage synthesis ($P < 0.01$). The overweight group had lower levels of creatinine ($P = 0.01$), serum urea nitrogen ($P < 0.01$), C-reactive protein ($P < 0.01$), and chloride ($P < 0.01$), and overweight males had lower levels of testosterone ($P = 0.04$). No other gender-specific differences were observed. The markers identified in Study 1 were then utilized in a weight loss study (Study 2) to determine the effects of weight loss on the biomarkers. Twenty dogs were fed Prescription Diet® r/d® dry or canned for 90 days for weight loss. At the completion of the study, all dogs lost weight ($P < 0.01$; dry -20% and can -28%). Dogs fed the canned food had a decrease in total protein ($P < 0.01$), alkaline phosphatase ($P = 0.03$), albumin ($P < 0.01$), cholesterol ($P < 0.01$), triglycerides ($P < 0.01$), leptin ($P < 0.01$), and phosphorus ($P < 0.01$), and an increase in calcium ($P = 0.02$) and chloride ($P < 0.01$). Dogs fed the dry food had a decrease in albumin ($P < 0.01$), total protein ($P < 0.01$), cholesterol ($P < 0.01$), and leptin ($P < 0.01$), and an increase in calcium ($P < 0.01$), chloride ($P < 0.01$), and serum urea nitrogen ($P = 0.03$). These data indicate that obesity is directly related to other disease states in dogs (ie, arthritis and diabetes). Managing obesity through weight loss and calorie restriction corrects the differences observed between lean and overweight blood markers.

INTRODUCTION

Obesity is a common nutritional disorder in dogs and the incidence has been reported to range from 24% to 44%.[1] Obesity typically results when an animal consumes more calories than calories expended (decreased physical activity). As in humans, obesity in dogs is also commonly associated with increased risk for other diseases, such as diabetes,[2,3] pancreatitis,[4] hypothyroidism,[5] dyslipidemia,[6] osteoarthritis,[7,8] hypertension,[9,10] altered kidney function,[10] and respiratory distress.[1] Therefore, preventing or treating obesity may delay and or prevent many of these obesity-related diseases.

A successful weight loss program requires a reduction in caloric intake (owner compliance) and an increase in physical activity.[11] Weight loss studies in dogs have found positive effects on biomarkers associated with obesity-related diseases. Diez et al[11] found that weight loss in dogs was associated with a reduction in triglycerides, cholesterol, and thyroxine. Blanchard et al[3] reported an increase in insulin sensitivity (via tumor necrosis factor alpha and insulin-like growth factor-1) with weight loss. Understanding how canine obesity may relate to other diseases states (eg, arthritis, hypothyroidism) by measuring biomarkers would be useful diagnostics and beneficial in the prevention or early treatment of associated diseases. Therefore, the objective of these studies is to determine what biomarkers differ between lean and overweight dogs and show the effects of weight loss on these biomarkers.

MATERIALS AND METHODS

Study 1

Thirty lean and 30 overweight spayed/neutered beagles were identified for this study. The dogs were cared for in accordance with Institutional Animal Care and Use Committee protocols. Dogs were weighed, given a body condition score (BCS; 1 = lean, 3 = ideal, and 5 = overweight), and a blood sample was drawn. Dogs with a BCS of 4 or 5 were classified as overweight for purposes of this study. Dogs with a BCS <3 were classified as lean.[12] Of the dogs identified for the study, 50% of the dogs were female (15 lean and 15 overweight) and 50% were male (15 lean and 15 overweight). Serum was harvested and stored at -20ºC in 1-mL aliquots.

Study 2

Dogs and Treatments

Twenty dogs were utilized in the weight loss study. The dogs were cared for in accordance with Institutional Animal Care and Use Committee protocols. All dogs began the study with >37% body fat (of total weight) and remained on the weight loss study for 3 months. Dogs were allotted to 1 of 2 treatments (Table 1). Each food was kibbled and formulated in accordance with the Association of American Feed Control Officials[13] nutrient guide for dogs and balanced to meet adult maintenance requirements. All dogs underwent dual-energy x-ray absorptiometry (DXA; DXA-QDR-4500, Hologic, Inc., Waltham, Mass) scans and blood was pulled at 0, 1, 2, and 3 months. Serum was harvested and stored at -20ºC in 1-mL aliquots. Additionally, dogs were offered enrichment toys, received routine grooming, and had daily opportunities for socialization with other dogs and people.

Serum Analysis

Serum was analyzed for chemistry screens, obesity markers, thyroid markers, and arthritis markers. Chemistry screens were preformed at the Hill's Pet Nutrition Center (Topeka, KS). Insulin analysis was performed by Michigan State University (Lansing, MI). Thyroxine, thyroid-stimulating hormone, glucagons-like protein-1, insulin-like growth factor-1, ghrelin, leptin, angiotensin I and II, C-reactive protein, high-density lipoprotein 1 and 2, low-density lipoprotein, very-low-density lipoprotein, chylomicron, testosterone, estradiol, cortisol, osteocalcin, amino-terminal crosslink telopeptide, type II cartilage synthesis, and cartilage oligomeric matrix protein were performed by MD Biosciences, Inc. (St. Paul, MN).

Table. 1 Nutrient Composition of Foods Fed to Dogs in the Weight Loss Study.

Nutrient, 100% Dry Matter Basis	Food A*	Food B†
Crude protein, %	25.6	24.9
Crude fat, %	8.6	7.9
Crude fiber, %	21.4	21.1
Ash, %	5.6	5.1
Calcium, %	0.67	0.91
Phosphorous, %	0.54	0.64
Lysine, %	1.41	1.43
Methionine + cystine, %	0.73	0.79
Tryptophan, %	0.29	0.24
Threonine, %	0.91	0.90
Arginine, %	1.43	1.53
Isoleucine, %	0.83	1.05
Valine, %	1.23	1.26
Leucine, %	1.81	2.03
Histidine, %	0.72	0.57
Phenylalanine + tyrosine, %	1.70	1.64
Carnitine, ppm	300	300
Metabolizable energy, kcal/kg	2992	2966

*Food A = Hill's® Canine Prescription Diet® r/d® Canned. Ingredients: water, pork by-products, soybean mill run, rice, pork liver, powdered cellulose, soybean meal, chicken liver flavor, vegetable oil, iron oxide, taurine, L-carnitine, minerals (calcium carbonate, dicalcium phosphate, salt, zinc oxide, ferrous sulfate, copper sulfate, manganous oxide, calcium iodate, sodium selenite), beta-carotene, vitamins (choline chloride, vitamin D3 supplement, vitamin E supplement, ascorbic acid, thiamine mononitrate, niacin, calcium pantothenate, pyridoxine hydrochloride, riboflavin, folic acid, biotin, vitamin B12 supplement).

†Food B = Hill's® Canine Prescription Diet® r/d® Dry. Ingredients: corn meal, peanut hulls 28.2% (a source of fiber), chicken by-product meal, soybean meal, soybean mill run, chicken liver flavor, dried egg product, vegetable oil, taurine, L-carnitine, preserved with butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ethoxyquin, minerals (salt, ferrous sulfate, zinc oxide, copper sulfate, manganous oxide, calcium iodate, sodium selenite), beta-carotene, vitamins (choline chloride, vitamin A supplement, vitamin D3 supplement, vitamin E supplement, L-ascorbyl-2-polyphosphate [a source of vitamin C], niacin, thiamine mononitrate, calcium pantothenate, pyridoxine hydrochloride, riboflavin, folic acid, biotin, vitamin B12 supplement).

Statistical Analysis

Data were analyzed using General Linear Models procedure of SAS[14] to determine treatment means. The experimental unit was dog and differences were considered significant when $P < 0.05$ and trends were determined when $P < 0.10$. Data was then analyzed to predict BCS by using the Regression procedure of SAS.[14] In Study 1, regression analysis was also performed to determine which markers in addition to body weight contributed to the prediction of BCS or degree of obesity.

RESULTS

Study 1

The results of Study 1 are presented in Tables 2–4. The overweight group had higher levels of alkaline phosphatase (P = 0.04), cholesterol (P = 0.04), triglycerides (P = 0.06), total protein (P < 0.01), albumin (P < 0.01), thyroxine (P = 0.05), calcium (P < 0.01), phosphorous (P = 0.04), glucose (P < 0.01), insulin (P < 0.01), insulin-like growth factor-1 (P < 0.01), low-density lipoprotein (P < 0.01), leptin (P < 0.01), and type II cartilage synthesis (P < 0.01) than the lean group. The overweight group had lower levels of creatinine (P = 0.01), serum urea nitrogen (P < 0.01), C-reactive protein (P < 0.01), and chloride (P < 0.01), and overweight males had lower levels of testosterone (P = 0.04) than the lean group. Through stepwise regression it was determined that body weight, glucose, sodium, chloride, C-reactive protein, and thyroid-stimulating hormone were the important parameters for determining BCS (Figure 1). The resulting equation is as follows:

$$\text{BCS} = 3.62352 + (0.17443 \times \text{body weight in kg}) + (0.01621 \times \text{glucose in mg/dL}) + (0.06496 \times \text{sodium in mmol/L}) - (0.12439 \times \text{chloride in mmol/L}) - (0.05575 \times \text{C-reactive protein in ng/mL}) + (1.72392 \times \text{thyroid stimulating hormone in ng/mL})$$

Stepwise regression was again utilized to determine which parameters were necessary in determining BCS via routine assays performed by the veterinarian. Stepwise regression determined that weight, serum

Table 2. Average Measurements for Lean and Overweight Dogs in Study 1

Measurement	Average Lean (n = 30)	Average Overweight (n = 30)	Standard Error	Lean vs Overweight
Age, years	8.25	6.70	0.65	0.10
Body condition score	2.53	4.70	0.08	<0.01
Body weight, kg	11.18	17.26	0.42	<0.01
Glucose, mg/dL	84.10	93.20	2.00	<0.01
Insulin, pmol/L	67.33	126.54	14.1	<0.01
Alanine aminotransferase, U/L	53.40	55.57	9.71	NS
Alkaline phosphatase, U/L	115.28	191.30	25.17	0.04
Cholesterol, mg/dL	201.93	228.37	9.06	0.04
Triglycerides, mg/dL	91.87	242.77	56.53	0.06
Total bilirubin, mg/dL	0.40	1.36	0.48	NS
Total protein, g/dL	6.28	6.97	0.14	<0.01
Creatinine, mg/dL	0.68	0.60	0.02	0.01
Serum urea nitrogen, mg/dL	12.45	9.50	0.48	<0.01
Albumin:globulin	1.20	1.18	0.06	NS
Albumin, g/dL	3.35	3.63	0.06	<0.01
Thyroxine, µg/dL	1.71	2.02	0.11	0.05
Thyroid-stimulating hormone, ng/mL	0.19	0.21	0.02	NS
Calcium, mg/dL	9.92	10.49	0.13	<0.01
Phosphorous, mg/dL	4.19	4.65	0.15	0.04
Chloride, mmol/L	116.27	113.50	0.45	<0.01
Potassium, mmol/L	4.52	4.43	0.06	NS
Magnesium, mg/dL	2.74	2.83	0.07	NS
Sodium, mmol/L	158.00	157.47	0.51	NS
Sodium:potassium	35.17	35.73	0.46	NS
Glucagon-like protein-1, pM	7.38	13.09	2.31	0.09
Insulin-like growth factor-1, ng/mL	102.0	183.6	17.4	<0.01
Ghrelin, ng/mL	2.60	2.04	0.23	0.09
Leptin, ng/mL	0.96	5.14	0.49	<0.01
Angiotensin I, ng/mL	0.61	0.66	0.05	NS
Angiotensin II, ng/mL	0.67	1.22	0.33	NS
C-reactive protein, ng/mL	5.54	2.54	0.62	<0.01
Non-esterified fatty acids, mM	0.75	0.80	0.07	NS
High-density lipoprotein-1, % of total	15.4	11.5	1.6	0.10
High-density lipoprotein-2, % of total	68.5	67.0	2.3	NS
Low-density lipoprotein, % of total	11.8	17.4	1.4	<0.01
Very-low-density lipoprotein, % of total	3.98	3.19	0.70	NS
Chylomicrons, % of total	0.43	0.92	0.20	0.08
Testosterone, pg/mL	82.3	68.7	16.5	NS
Estradiol, pg/mL	5.65	5.22	0.21	NS
Cortisol, µg/dL	4.06	4.62	0.32	NS
Osteocalcin, ng/mL	1.76	2.03	0.35	NS
Amino-terminal crosslink telopeptide, nM BCE	22.8	23.3	1.4	NS
Type II cartilage synthesis, µg/mL	617.6	742.7	32.5	<0.01
Cartilage oligomeric matrix protein, U/L	2.13	2.26	0.09	NS

*NS = Not significant and $P > 0.10$.

Table 3. Average Measurements for Female Lean and Overweight Dogs in Study 1.

Measurement	Female Lean (n = 15)	Female Overweight (n = 15)	Standard Error	Lean vs Overweight
Age, years	8.42	6.91	0.91	NS
Body condition score	2.40	4.47	0.12	<0.01
Body weight, kg	10.76	15.29	0.60	<0.01
Glucose, mg/dL	84.46	93.07	2.82	0.04
Insulin, pmol/L	59.07	106.80	19.81	0.09
Alanine aminotransferase, U/L	54.20	53.00	13.74	NS
Alkaline phosphatase, U/L	116.07	182.27	35.28	NS
Cholesterol, mg/dL	196.13	230.00	12.81	0.07
Triglycerides, mg/dL	68.47	251.00	79.95	NS
Total bilirubin, mg/dL	0.23	1.09	0.68	NS
Total protein, g/dL	6.01	5.89	0.20	<0.01
Creatinine, mg/dL	0.68	0.57	0.03	0.01
Serum urea nitrogen, mg/dL	12.89	8.92	0.67	<0.01
Albumin:globulin	1.31	1.24	0.08	NS
Albumin, g/dL	3.35	3.69	0.08	<0.01
Thyroxine, µg/dL	1.71	2.25	0.15	0.01
Thyroid-stimulating hormone, ng/mL	0.18	0.21	0.03	NS
Calcium, mg/dL	9.85	10.53	0.19	0.01
Phosphorous, mg/dL	4.34	4.49	0.21	NS
Chloride, mmol/L	116.93	113.67	0.63	<0.01
Potassium, mmol/L	4.59	4.41	0.08	NS
Magnesium, mg/dL	2.76	2.93	0.10	NS
Sodium, mmol/L	158.40	157.47	0.72	NS
Sodium:potassium	34.67	25.80	0.65	NS
Glucagon-like protein-1, pM	8.16	15.74	3.26	NS
Insulin-like growth factor-1, ng/mL	94.7	191.2	24.5	<0.01
Ghrelin, ng/mL	2.39	2.08	0.33	NS
Leptin, ng/mL	1.00	4.16	0.69	<0.01
Angiotensin I, ng/mL	0.60	0.66	0.07	NS
Angiotensin II, ng/mL	0.62	0.84	0.46	NS
C-reactive protein, ng/mL	4.87	2.89	0.88	NS
Non-esterified fatty acids, mM	0.62	0.73	0.10	NS
High-density lipoprotein-1, % of total	12.0	12.2	2.3	NS
High-density lipoprotein-2, % of total	91.8	65.2	3.3	NS
Low-density lipoprotein, % of total	11.9	18.6	1.9	0.02
Very-low-density lipoprotein, % of total	4.03	3.05	0.99	NS
Chylomicrons, % of total	0.20	0.87	0.28	0.09
Testosterone, pg/mL	52.1	96.3	23.3	NS
Estradiol, pg/mL	5.72	5.13	0.30	NS
Cortisol, µg/dL	3.90	4.88	0.45	NS
Osteocalcin, ng/mL	1.53	2.00	0.49	NS
Amino-terminal crosslink telopeptide, nM BCE	23.5	23.9	2.0	NS
Type II cartilage synthesis, µg/mL	658.5	741.0	46.0	NS
Cartilage oligomeric matrix protein, U/L	2.11	2.25	0.13	NS

*NS = Not significant and $P > 0.10$.

Table 4. Average Measurements for Male Lean and Overweight Dogs in Study 1.

Measurement	Male Lean (n = 15)	Male Overweight (n = 15)	Standard Error	Lean vs Overweight*
Age, years	8.07	6.48	0.91	NS
Body condition score	2.67	4.93	0.12	<0.01
Body weight, kg	11.60	19.22	0.60	<0.01
Glucose, mg/dL	83.73	93.33	2.82	0.02
Insulin, pmol/L	75.60	146.29	20.14	0.02
Alanine aminotransferase, U/L	52.60	81.13	13.74	NS
Alkaline phosphatase, U/L	114.5	200.33	35.88	0.10
Cholesterol, mg/dL	207.73	226.73	12.81	NS
Triglycerides, mg/dL	115.27	234.53	79.95	NS
Total bilirubin, mg/dL	0.57	1.63	0.68	NS
Total protein, g/dL	6.54	7.05	0.20	0.07
Creatinine, mg/dL	0.68	0.63	0.03	NS
Serum urea nitrogen, mg/dL	12.01	10.09	0.67	0.05
Albumin:globulin	1.09	1.13	0.08	NS
Albumin, g/dL	3.34	3.57	0.08	0.04
Thyroxine, µg/dL	1.71	1.79	0.15	NS
Thyroid-stimulating hormone, ng/mL	0.20	0.21	0.03	NS
Calcium, mg/dL	10.00	10.46	0.19	0.09
Phosphorous, mg/dL	4.05	4.81	0.21	0.02
Chloride, mmol/L	115.60	113.33	0.63	0.01
Potassium, mmol/L	4.45	4.45	0.08	NS
Magnesium, mg/dL	2.73	2.72	0.10	NS
Sodium, mmol/L	157.60	157.47	0.72	NS
Sodium:potassium	35.67	35.67	0.65	NS
Glucagon-like protein-1, pM	6.61	10.44	3.26	NS
Insulin-like growth factor-1, ng/mL	109.3	176.1	24.5	0.06
Ghrelin, ng/mL	2.81	1.99	0.33	0.08
Leptin, ng/mL	0.91	4.16	0.69	<0.01
Angiotensin I, ng/mL	0.62	0.65	0.07	NS
Angiotensin II, ng/mL	0.71	1.61	0.46	NS
C-reactive protein, ng/mL	6.21	2.20	0.88	<0.01
Non-esterified fatty acids, mM	0.89	0.87	0.10	NS
High-density lipoprotein-1, % of total	18.7	12.2	2.3	0.02
High-density lipoprotein-2, % of total	65.1	68.8	3.3	NS
Low-density lipoprotein, % of total	11.6	16.3	1.9	0.10
Very-low-density lipoprotein, % of total	3.94	3.33	0.99	NS
Chylomicrons, % of total	0.65	0.97	0.28	NS
Testosterone, pg/mL	112.5	41.2	23.7	0.04
Estradiol, pg/mL	5.57	5.32	0.30	NS
Cortisol, µg/dL	4.22	4.36	0.45	NS
Osteocalcin, ng/mL	1.99	2.06	0.49	NS
Amino-terminal crosslink telopeptide, nM BCE	22.1	22.8	2.0	NS
Type II cartilage synthesis, µg/mL	576.7	744.4	46.0	0.01
Cartilage oligomeric matrix protein, U/L	2.15	2.27	0.13	NS

*NS = Not significant and *P* > 0.10.

Figure 1. Prediction of BCS using body weight and lean/overweight markers.

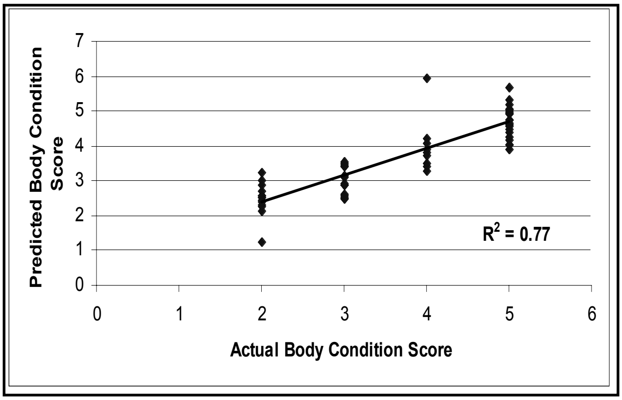
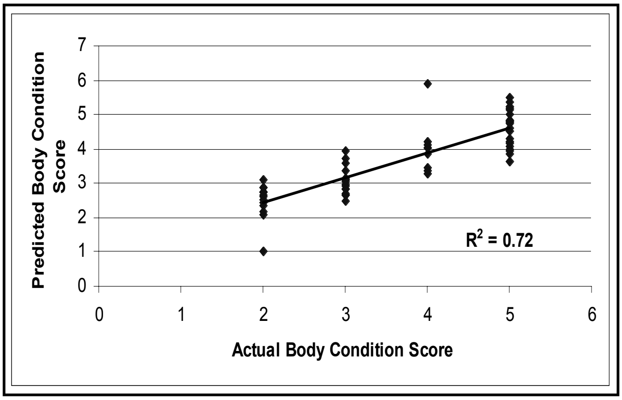


Figure 2. Prediction of BCS using body weight and chemistry screens only.



urea nitrogen, sodium, and chloride were important parameters in determining BCS (Figure 2). The resulting equation is as follows:

BCS = 3.64120 + (0.18614 × body weight in kg) - (0.05289 × serum urea nitrogen in mg/dL) + (0.08935 × sodium in mmol/L) - (0.14088 × chloride in mmol/L)

Study 2

The changes in body condition throughout are presented in Table 5. Dogs fed Food A had significant weight loss (-4924 g; $P < 0.01$), lean loss (-721 g; $P < 0.01$), and fat loss (-4167 g; $P < 0.01$) at Day 90 when compared with Day 0. Dogs fed Food B had significant weight loss (-3466 g; $P < 0.01$) and fat loss (-3363 g; $P < 0.01$) at Day 90 when compared with Day 0. No differences were observed for lean when dogs were fed Food B.

Serum chemistry screens and electrolytes are presented in Table 6. Dogs fed the Food A had a decrease in globulin ($P < 0.01$), total protein ($P < 0.01$), alkaline phosphatase ($P = 0.03$), alanine aminotransferase ($P = 0.02$), albumin ($P < 0.01$), cholesterol ($P < 0.01$), triglycerides ($P < 0.01$), phosphorus ($P < 0.01$), sodium ($P < 0.01$), and sodium:potassium ($P = 0.02$), leptin ($P < 0.01$), insulin ($P < 0.01$), and cartilage oligomeric matrix protein ($P < 0.01$), and an increase in calcium ($P = 0.02$), potassium ($P = 0.05$), and chloride ($P < 0.01$). Dogs fed Food B had a decrease in albumin ($P < 0.01$), cholesterol ($P < 0.01$), sodium ($P < 0.01$), and sodium:potassium ($P < 0.01$), and leptin ($P < 0.01$), and an increase in calcium ($P < 0.01$), chloride ($P < 0.01$), potassium ($P < 0.01$), magnesium ($P < 0.01$), and serum urea nitrogen ($P = 0.03$).

DISCUSSION

The first objective of these studies is to determine what biomarkers differ between lean and overweight dogs. By identifying differences in biological markers between lean and overweight animals, veterinarians could potentially identify early symptoms of diseases associated with obesity. These markers could be utilized by the veterinarian to manage weight loss regimens with blood analysis along with body weight reduction.

In Study 1, the overweight group had elevated levels of glucose, insulin, insulin like growth factor-1, and glucagon-like protein-1, suggesting the signs of insulin resistance. The results are not surprising because diabetes and insulin resistance are commonly associated with obesity.[15] In Study 2, dogs going through weight loss had a reduc-

Table 5. Body Composition of Dogs Fed Weight Loss Foods.

Body Parameter Measured	Food A	Food B
Weight Day 0, g	17569	17257
Weight Day 30, g	15394	15798
Weight Day 60, g	13970	14715
Weight Day 90, g	12645	13791
Weight change Day 0 to 30, g	-2304	-1459
Weight change Day 0 to 60, g	-3728	-2542
Weight change Day 0 to 90, g	-4924	-3466
Day 0 vs Day 30*	<0.01	<0.01
Day 0 vs Day 60*	<0.01	<0.01
Day 0 vs Day 90*	<0.01	<0.01
Lean Day 0, g	9678	9434
Lean Day 30, g	9093	9295
Lean Day 60, g	9059	9303
Lean Day 90, g	8961	9367
Lean change Day 0 to 30, g	-585	-139
Lean change Day 0 to 60, g	-619	-131
Lean change Day 0 to 90, g	-721	-67
Day 0 vs Day 30*	<0.01	NS
Day 0 vs Day 60*	<0.01	NS
Day 0 vs Day 90*	<0.01	NS
Fat Day 0, g	7411	7343
Fat Day 30, g	5830	6028
Fat Day 60, g	4455	4952
Fat Day 90, g	3244	3979
Fat change Day 0 to 30, g	-1705	-1314
Fat change Day 0 to 60, g	-3081	-2390
Fat change Day 0 to 90, g	-4167	-3363
Day 0 vs Day 30*	<0.01	<0.01
Day 0 vs Day 60*	<0.01	<0.01
Day 0 vs Day 90*	<0.01	<0.01

*Probability of greater F-value.

tion in glucose and insulin indicating that weight loss can correct the obesity-related glucose disorders.

The overweight dogs had elevated levels of triglycerides, cholesterol, low-density lipoprotein, and chylomicrons, and lowered levels of high-density lipoprotein-1, all of which are common signs of dyslipidemia. Studies with dogs have demonstrated that dyslipidemia is often associated with insulin resistance.[15,16] Insulin resistance plays a central role in the development of hyperlipidemia.[6] The increase in blood triglyceride concentration results from the increase in the production of triglyceride-rich lipoproteins and a decrease in their catabolism. Abnormalities in insulin action can result from an increase in lipolysis in adipocytes, resulting in increased fatty acid release and repackaging of the fatty acids back into triglycerides at the liver.[16]

In Study 2, dogs on a weight loss regimen had a reduction in cholesterol and triglycerides indicating that the signs of dyslipidemia can be corrected through food and weight loss. This is consistent with other published canine weight loss studies. Diez at al[11] fed overweight beagles either a high protein (47.5% protein and 10.9% crude fiber) or a high fiber (23.8% protein and 23.3% crude fiber) diet during their weight program. They observed decreases in both triglycerides and cholesterol when dogs were fed either of the 2 weight loss foods, indicating that these observed changes were not diet related but were directly related to weight loss. The observed decrease in triglycerides and cholesterol resulting from weight loss in the current study and Diez et al[11] is something that can be measured by the veterinarian during routine chemistry screens. Measuring cholesterol and triglycerides for obesity issues and monitoring weight loss may be a way to discuss the importance of obesity without offending the pet owner. It is important to note that in the study by Diez et al[11] and the current study, elevated triglycerides and cholesterol are both within the normal published ranges for

Table 6. Blood Chemistry Screens and Markers of Dogs Fed Weight Loss Foods for 90 Days.

	Food A				Food B			
Analyte	**Day 0**	**Day 90**	**Change**	**Day 0 vs Day 90**	**Day 0**	**Day 90**	**Change**	**Day 0 vs Day 90**
Albumin, g/dL	3.60	3.33	-0.27	<0.01	3.54	3.29	-0.25	<0.01
Serum urea nitrogen, mg/dL	12.8	13.0	0.2	NS	8.8	12.5	3.7	0.03
Creatinine, mg/dL	0.60	0.64	0.04	0.09	0.59	0.63	0.04	NS
Total protein, g/dL	6.21	5.57	-0.64	<0.01	0.65	0.60	-0.05	NS
Alkaline phosphatase, U/L	307	92	-216	0.03	175	100	-76	NS
Cholesterol, mg/dL	229	166	-64	<0.01	222	169	-53	<0.01
Glucose, mg/dL	106	99	-7	NS	101	96	-5	NS
Insulin, IU/mL	10.29	3.08	-6.86	<0.01	5.50	3.47	-2.25	NS
Triglycerides, mg/dL	191	136	-55	<0.01	147	138	-10	NS
Calcium, mg/dL	9.8	10.2	0.4	0.02	9.7	10.2	0.5	<0.01
Chloride, mg/dL	114	120	6	<0.01	115	120	5	<0.01
Phosphorus, mg/dL	4.42	3.28	-1.14	<0.01	3.43	3.18	-0.25	NS
Ghrelin, ng/mL	1.69	2.19	0.50	0.02	2.38	2.38	0.01	NS
Leptin, ng/mL	3.00	0.20	-2.80	<0.01	1.89	0.29	-1.60	<0.01
Amino-terminal crosslink telopeptide, nM BCE	19.1	20.7	1.6	NS	22.3	22.1	-0.2	NS
Type II cartilage synthesis, µg/mL	1179	1099	-80	NS	1007	1049	42	NS
Cartilage oligomeric matrix protein, U/L	1.73	1.38	-0.35	<0.01	1.72	1.53	-0.19	NS
C-reactive protein, ng/mL	2.79	2.26	-0.53	NS	2.10	1.25	-0.76	NS

the dog. Thus, looking for abnormally high triglyceride and cholesterol values would not be a good indicator for obesity and more focus should be given to elevated levels within normal ranges.

The overweight group had increased levels of arthritic markers, even though they did not show any signs of arthritis (ie, lameness). Although all arthritic markers were elevated in the overweight group, only alkaline phosphatase and type II cartilage synthesis were statistically significant. The increase in both alkaline phosphatase and type II cartilage synthesis could be an early indicator of osteoarthritis[17] in overweight dogs. Alkaline phosphatase is typically elevated when dogs have bone, bile duct, and/or liver disorders. The elevated alkaline phosphatase in this study is likely associated with bone because alanine aminotransferase did not differ between the 2 groups and albumin was higher in the overweight group thus ruling out any potential liver disorders.

Type II collagen synthesis typically increases when cartilage damage occurs.[18] The cartilage matrix consists of 2 major components, type II collagen and the proteoglycan aggrecan. Collagen fibrils provide tensile strength to maintain tissue integrity. Aggrecan is interwoven with the collagen fibrils and contributes to cartilage matrix compressive stiffness. Damage to type II collagen and loss of aggrecan are fundamental features of damage to articular cartilage in osteoarthritis. This damage has been linked to proteolytic enzymes secreted by chondrocytes and synoviocytes. The matrix metalloproteinase family (ie, MMP-13) is responsible for the primary cleavage of the triple helix of type II collagen.[18] In Study 2, dogs going through weight loss had a decrease in alkaline phosphatase indicating that managing obesity may help/prevent the onset of arthritis. Because these foods do not have added joint benefits for reducing/treating arthritis (ie, n-3 fatty acids, glucosamine, and/or chondroitin), it becomes apparent that weight loss alone lowered the alkaline phosphatase levels. This is likely the result of reducing the load exerted on the joints when animals reduce the body weight.

Leptin, ghrelin, and glucagon-like protein-1 concentrations were also measured in both studies because of their known effects on appetite suppression and stimulation. The overweight group had elevated levels of leptin along with lower levels of ghrelin. These results are in agreement with Jeusette et al[6] and Sagawa et al.[19] In both studies, leptin concentrations were directly related to body fat mass in overweight beagles. In the current study, leptin concentrations decreased with decreasing fat mass during weight loss. Jeusette et al[6] also observed a decrease in ghrelin concentrations in overweight dogs and believed them to be the result of ghrelin being down-regulated resulting from excess energy storage. In the current study, it appears that ghrelin levels were not affected by weight loss when dogs were fed the dry food; however, ghrelin levels did increase when dogs were fed the canned food for weight loss. This may be the result of increased gut fill from higher intakes of the canned product. Glucagon-like protein-1 also plays a role in the control of nutrients flowing from the stomach to the small intestine through its inhibitory effects on gastrointestinal transit and gastric emptying. This glucagon-like protein-1 mechanism is believed to exert its effect on appetite.[20,21] The overweight dogs had increased levels of glucagon-like protein-1 when compared with the lean group. These results indicate that these hormones may be trying to reduce intake in the overweight dog group; however, their effects on intake are not being elicited.

CONCLUSION

The results of these studies indicate that obesity is directly related to other disease states in dogs. The markers in Study 1 indicated that overweight dogs showed early signs of dyslipidemia, arthritis, and diabetes. Study 2 demonstrated that many of these differences can be alleviated through weight loss.

REFERENCES

1. Markwell PJ, Butterwick RF: Obesity. In: Wills JM, ed. *Waltham Book of Clinical Nutrition of the Dog and Cat*. Tarrytown, NY: Pergamon Press (U.K.); 1994:131-148.
2. Gayet C, Bailhache E, Dumon H, et al: Insulin resistance and changes in plasma concentration of TNFα, IGF1, and NEFA in dogs during weight gain and obesity. *J Anim Physiol Anim Nutr* 2004;88:157-165.
3. Blanchard G, Nguyen P, Gayet C, et al: Rapid weight loss and a high-protein low-energy diet allows the recovery of ideal body composition and insulin sensitivity in obese dogs. *J Nutr* 2004;134:2148S-2150S.
4. Hess RS, Kass PH, Shofer FS, Winkle TJ, Washabau RJ: Evaluation of risk factors for fatal acute pancreatitis in dogs. J *Am Vet Med Assoc* 1999;214:46-51.
5. Panciera DL: Conditions associated with canine hypothyroidism. *Vet Clin North Am Small Anim Pract* 2001;31:935-950.
6. Jeusette IC, Lhoest ET, Istasse LP, Diez MO: Influence of obesity on plasma lipid and lipoprotein concentrations in dogs. *Am J Vet Res* 2005;66:81-86.
7. Burkholder WJ, Taylor L, Hulse DA: Weight loss to optimal body condition increases ground reactive force in dogs with osteoarthritis. In: Proceedings Purina Nutrition Forum 2000. *Compend Contin Edu Pract Vet* 2000;23:74.
8. Impellizeri JA, Tetrick MA, Muir P: Effect of weight reduction on clinical signs of lameness in dogs with hip osteoarthritis. *J Am Vet Med Assoc* 2000;216:1089-1091.
9. Philip-Couderc P, Smih F, Pelat M, et al: Cardiac transcriptome analysis in obesity-related hypertension. *Hypertension* 2003;41:414-421.
10. Henegar JR, Bigler SA, Henegar LK, Tyagi SC, Hall JE: Functional and structural changes in the kidney in the early stages of obesity. *J Am Soc Nephrol* 2001;12:1211-1217.
11. Diez, M, Michaux C, Jeusette I, et al: Evolution of blood parameters during weight loss in experimental obese beagle dogs. *J Anim Physiol Anim Nutr* 2004;88:166-171.

12. Thatcher CD, Hand MS, Remillard RL: Small animal clinical nutrition: an interactive process. In: Hand MS, Thatcher CD, Remillard RL, et al, eds. *Small Animal Clinical Nutrition*. 4th ed. Topeka, Kansas: Mark Morris Institute; 2000:4-5.
13. Association of American Feed Control Officials (AAFCO): *Association of American Feed Control Officials: Official Publication.* Atlanta, GA: AAFCO; 2005.
14. SAS: *SAS/STAT® User's Guide (Release 6.03)*. Cary, NC: SAS Institute Inc.; 1989.
15. Bailhache E, Nguyen P, Krempf M, et al: Lipoprotein abnormalities in obese insulin-resistant dogs. *Metabolism* 2003;52:559-564.
16. Bailhache E, Ouguerram K, Gayet C, et al: An insulin-resistant hypertriglyceridaemic normotensive obese dog model: assessment of insulin resistance by the euglycaemic hyperinsulinaemic clamp in combination with the stable isotope technique. *J Anim Physiol Anim Nutr* 2003;87:86-95.
17. Yamka RM, Friesen KG, Lowry SR, Coffman L: Measurement of arthritic and bone serum metabolites in arthritic, non-arthritic, and geriatric dogs fed wellness foods. *Intern J Appl Res Vet Med* 2006;4:255-264.
18. Chu Q, Lopez M, Hayasi K, et al: Elevation of a collagenase generated type II collagen neoepitope and proteoglycan epitopes in synovial fluid following induction of joint instability in the dog. *Osteo Cart* 2002;10:662-669.
19. Sagawa MM, Nakadomo 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.
20. De Graaf C, Blom WAM, Smeets PAM, et al: Biomarkers of satiation and satiety. *Am J Clin Nutr* 2004;79:946-961.
21. Strader AD, Woods SC: Gastrointestinal hormones and food intake. *Gastroenterology* 2005;128:175-191.