Effects of hydrocortisone administration on leptin and adiponectin synthesis in dogs

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OBJECTIVE
To determine effects of hydrocortisone administration on serum leptin and adiponectin concentrations, abdominal fat distribution, and mRNA expression of leptin and adiponectin in abdominal adipose tissue of dogs.

ANIMALS
12 healthy dogs.

PROCEDURES
Dogs received hydrocortisone (8.5 mg/kg; n = 6) or a placebo (6) orally every 12 hours for 90 days. Serum leptin and adiponectin concentrations were measured with a canine-specific ELISA on the day before (day 0; baseline) and during (days 1, 3, 7, 30, 60, and 90) administration. On days 0, 30, 60, and 90, abdominal fat mass was quantified with CT, and mRNA expression of leptin and adiponectin in abdominal fat was analyzed by use of a PCR assay.

RESULTS
Hydrocortisone administration resulted in an increase in visceral fat mass on days 60 and 90, compared with the mass at baseline. Visceral fat mass at the level of L3 increased during hydrocortisone administration. Serum leptin concentration began to increase on day 1 and was significantly higher than the baseline concentration on days 30 and 60. Serum adiponectin concentration on days 30, 60, and 90 was significantly lower than the baseline concentration. Leptin and adiponectin mRNA expression in abdominal fat was greater on day 30, compared with expression at baseline, but lower on days 60 and 90, compared with expression on day 30. Serum leptin concentration and visceral fat mass were correlated.

CONCLUSIONS AND CLINICAL RELEVANCE
Hydrocortisone administration affected abdominal fat distribution and serum leptin and adiponectin concentrations through dysregulation of leptin and adiponectin expression. (*Am J Vet Res* 2019;80:771–778)

Leptin and adiponectin are the most common adipokines in dogs. They are proteins produced in adipose tissue and are important factors in the pathophysiologic processes of obesity and its related conditions.[1] Leptin is encoded by the *ob* gene and is responsible for the regulation of food intake and energy expenditure.[2,3] Leptin can also regulate reproductive and immune function and modulate insulin sensitivity.[1]

Hyperadrenocorticism (commonly referred to as Cushing syndrome) describes the clinical manifestations of chronic hypercortisolemia that result from various metabolic changes associated with exogenous administration of glucocorticoids or endogenous overproduction.[4] Those metabolic changes include dyslipidemia, glucose intolerance, hypercoagulability, arterial hypertension, and obesity with an increase in visceral adiposity.[5–7] Abdominal visceral obesity is one of the most common features of humans with hyperadrenocorticism.[8] In addition, redistribution of body fat in human patients with hyperadrenocorticism leads to increases in the proportion of fat in the visceral compartment and metabolic complications.[9–11]

Serum leptin concentrations are positively correlated with total body fat, but serum adiponectin concentrations are inversely correlated with visceral adiposity in humans.[12] Thus, changes in fat mass may dysregulate the production and secretion of adipokines and contribute to the pathogenesis of several metabolic and cardiovascular complications.[13–15] Visceral obesity might also occur in dogs with hypercortisolemia, but it has not been definitively verified. Similar to results for humans[12] and rodents,[16] circulating leptin concentrations are positively correlated with body fat in dogs, but there is controversy regarding the correlation between adiponectin concentrations and body fat in dogs.[17]

In humans, glucocorticoids act directly on adipose tissue, increase leptin secretion and expression, and inhibit adiponectin secretion.[3,18] In another study[19] conducted by our research group, we reported that the serum leptin concentration in dogs with pituitary-dependent hyperadrenocorticism was significantly higher than the concentration in healthy dogs. The increase in visceral fat

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distribution in humans with hyperadrenocorticism is associated with changes in the production and expression of leptin.[11,20] However, there is a lack of information about fat redistribution and leptin mRNA expression in the visceral adipose tissue of dogs with hypercortisolemia.

Therefore, the objective of the study reported here was to examine the effects of oral administration of hydrocortisone on serum leptin and adiponectin concentrations, mRNA expression of leptin and adiponectin in visceral fat, and abdominal fat distribution in dogs. Furthermore, we intended to evaluate the correlation between the serum leptin concentration and visceral fat mass.

Materials and Methods

Animals

Twelve 1-year-old male research Beagles were used in the study. The dogs were evaluated and treated at the Laboratory Research Center of Chungbuk National University. Body weight ranged from 10.19 to 12.21 kg (median, 11.35 kg). Dogs were considered healthy on the basis of results of a physical examination, indirect measurement of systolic blood pressure, examination of fecal specimens for parasites by use of a flotation technique, heartworm antigen test, CBC, serum biochemical analysis, urinalysis, ACTH response test,[19] thyroid gland function test, and diagnostic imaging (survey radiography and abdominal ultrasonography).

Dogs were housed separately in cages with a light cycle of 12 hours of light and 12 hours of darkness. They were fed a commercial diet[a] (amount of the diet was determined on the basis of the energy requirement for maintenance calculated with the body weight) and had ad libitum access to drinking water. None of the dogs received any drugs before the onset of the study.

Dogs were handled in accordance with published guidelines.[21] The study was approved by the Institutional Animal Care and Use Committee of the Laboratory Animal Research Center of Chungbuk National University (CBNUA-713-14-01).

Study design

Dogs were randomly allocated (block randomization method) into 2 groups (6 dogs/group) by use of commercial software.[b] Dogs in the control group received an empty gelatin capsule. For dogs in the hydrocortisone group, hypercortisolemia was induced by the administration of hydrocortisone, which is the synthetic glucocorticoid most similar to cortisol.[22] The hydrocortisone dosage was chosen on the basis of studies[8,23] in which hypercortisolemia was induced in dogs. Dogs received hydrocortisone[c] (8.5 mg/kg) orally every 12 hours for 90 days, after which the dose was tapered over a 2-month period until administration was stopped. Day 0 (baseline) was the day before the start of hydrocortisone or placebo administration. Dogs were monitored daily for development of clinical signs of hypercortisolemia (eg, polyuria, polydipsia, polyphagia, and skin abnormalities).

Collection of blood samples

Blood samples (2 mL) were collected before (day 0) and during (days 1, 3, 7, 30, 60, and 90) the treatment period. Food was withheld from the dogs for 12 hours before blood collection, but water was freely available during food withholding. The investigators were extremely careful to ensure food was not visible to any dogs at the time of blood collection.

Blood was collected from a jugular vein into serum separating tubes. Serum was separated from clotted blood by centrifugation at 1,200 X *g* for 10 minutes. All samples were centrifuged within 30 minutes after blood collection. Serum was stored at –80°C until assayed as a batch analysis (maximum duration of storage, 97 days).

Assessment of abdominal fat distribution

Dogs were anesthetized, and total body fat, visceral body fat, and subcutaneous body fat in the abdomen were measured by use of a CT method. Anesthesia was induced by administration of propofol[d] (5 mg/kg, IV) and maintained with isoflurane[e] in oxygen. The CT imaging was performed before (day 0) and during (days 30, 60, and 90) administration of hydrocortisone. Dogs were positioned in dorsal recumbency, and all images were obtained by use of helical CT[f] with the following parameters: rotation time, 0.7 seconds; slice thickness, 3 mm; x-ray tube potential, 120 kV; and x-ray tube current, 130 mA. All images were acquired from the apex of the heart through the caudal aspect of the sacroiliac articulation. Thresholds of –105 to –135 HU were used to assess fat.[24]

Visceral and subcutaneous fat mass were assessed separately. Regions of interest[4] were manually drawn around the abdominal cavity on the CT image of each dog. Visceral fat and subcutaneous fat were measured at the level of each cross section by quantifying the fat mass. Subcutaneous fat mass was calculated by subtracting the visceral fat mass from the total fat mass. Total fat mass, visceral fat mass, and subcutaneous fat mass for the entire abdomen were calculated as the sum of each fat value from the sum of all slices from the heart apex to the caudal aspect of the sacroiliac articulation.

A single 3-mm-thick slice at the level of L3 was used for fat mass analysis.[24] To compare changes of the abdominal fat mass attributable to administration of hydrocortisone, several values for fat distribution were used, which included the total, visceral, and subcutaneous fat masses[25]; percentage of visceral fat mass, which was calculated as the ratio of visceral fat mass to total fat mass; and ratio of visceral fat mass to subcutaneous fat mass. All CT images were analyzed by an experienced investigator (DC) who was not aware of the treatment group of each dog.

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Collection of adipose tissue samples

Immediately after CT was completed, visceral adipose tissue samples were surgically obtained from the anesthetized dogs. All dogs received cefazolin[g] (30 mg/kg, IV) prophylactically before the procedure.

Dogs were positioned in dorsal recumbency. Hair was shaved from the incision site (caudal right quadrant of the abdomen), and the skin was aseptically prepared. Dogs were then moved to an operating room. The incision site was sprayed with 4% chlorhexidine gluconate and 70% ethanol until the skin was saturated. After a 3-minute period, dry sterile gauze was used to soak up excess antiseptic solution, and the abdomen was draped.

An incision (3 to 5 cm) was made. Samples of visceral adipose tissue were obtained aseptically. Approximately 3 cm^3 of visceral adipose tissue were procured from the abdominal region. The extracted visceral adipose tissue was excised with a surgical blade. Biopsy resulted in minimal bleeding. The muscle and skin were sutured with a simple interrupted pattern.

After sample collection was completed, dogs received saline (0.9% NaCl) solution (60 mL/kg, IV, for 24 hours), cefazolin (30 mg/kg, IV, q 12 h for 3 days), famotidine[h] (0.5 mg/kg, IV, q 12 h for 3 days), and butorphanol tartrate[i] (0.2 mg/kg, IV, q 12 h for 3 days). The incision site was carefully managed by application of povidone-iodine solution to prevent postoperative infection. Rectal temperature, pulse and respiration rates, indirect arterial blood pressure, mucous membrane color, and capillary refill time were monitored every 15 to 30 minutes after sample collection until dogs had completely recovered from anesthesia; these variables then were monitored every 8 hours for 1 week. No complications were observed in any dogs during or after biopsy, and none of the dogs were removed from the study.

Biopsy samples were immediately rinsed with saline solution.[j] An aliquot (1 mL) of guanidinium thiocyanate[k] was added to each biopsy sample. Samples were inspected to verify tissue homogeneity, snap-frozen in liquid nitrogen, and stored at –80°C until analyzed.

Assay of serum concentrations

Serum biochemical analyses were performed with an autoanalyzer.[l] All samples were assayed in duplicate. Serum leptin concentrations were analyzed by use of a canine-specific ELISA kit[m] used in accordance with the manufacturer's instructions. Sensitivity for the leptin assay was 0.78 ng/mL, intra-assay variability was 2%, and interassay variability was 6%. Serum adiponectin concentrations were analyzed by use of a commercially available ELISA kit[n] that had been validated for use with canine serum[26]; the kit was used in accordance with the manufacturer's instructions. Sensitivity for the adiponectin assay was 0.47 ng/mL, intra-assay variability was 3.9%, and interassay variability was 6.0%.

Assessment of mRNA expression

Samples of mRNA were extracted from the abdominal adipose tissue. The RNA was extracted with guanidinium thiocyanate; the concentration of total RNA was determined at 260 nm. The amount of 18S rRNA served as an indicator of the quantity of total RNA. Changes in leptin and adiponectin mRNA expression were quantitated by use of a real-time PCR assay. Total RNA (1 µg) was reverse transcribed into cDNA by use of reverse transcriptase[o] and a random primer.[p] An aliquot (1 µL) of cDNA was used for the PCR assay, which was performed with standard conditions[27,28] (denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute). Primer sequences for leptin (GenBank accession No. NM_001003070.1; forward, GCCCTATCTGTCCTGTGTTG; and reverse, AGACTGCGTGTGTGAAATGT) and adiponectin (GenBank accession No. NM_001006644.1; forward, AGAGAAAGGAGATGCAGGTCT; and reverse, CTCCAACCCCACACTGAATG) were used. Aliquots (10 µL/aliquot) of the PCR assay product were separated on 2% agarose gels and stained with ethidium bromide. Gel images were analyzed by use of a software package[q] designed to record and analyze 1-D electrophoresis gels.

Statistical analysis

Statistical analyses were performed with commercially available statistical software.[r] The Friedman test and Dunn post hoc test were used to compare changes in serum leptin and adiponectin concentrations before and during hydrocortisone administration. Differences between the hydrocortisone and control groups were analyzed by use of the Mann-Whitney *U* test. Correlations between serum leptin concentrations and body fat mass were evaluated with the Spearman correlation test. Values were considered significant at $P < 0.05$.

Results

Long-term administration of hydrocortisone resulted in the development of clinical signs consistent with hypercortisolemia, including polyuria, polydipsia, polyphagia, no regrowth of hair within 1 month after the area was shaved for surgery, and thinning of the skin with prominent subcutaneous veins in the abdomen, in all dogs of the hydrocortisone group. Abnormalities of laboratory analyses, including a stress leukogram, high alkaline phosphatase activity, and isosthenuria, were also consistent with cortisol excess in all dogs of the hydrocortisone group. Some dogs of the hydrocortisone group also developed abdominal distention (n = 5) and muscle atrophy (2). However, all dogs of both groups remained bright, alert, and afebrile during the entire study period. All dogs were in good health throughout the study, as determined on the basis of no abnormalities for physical examination findings and results of laboratory analyses.

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Changes were detected in serum leptin and adiponectin concentrations **(Figure 1)**. Serum leptin concentrations increased significantly (*P* < 0.001) from baseline and peaked on day 60. Compared with the leptin concentration on day 0, the concentration was significantly higher on days 30 (*P* = 0.016) and 60 (*P* < 0.001) for dogs of the hydrocortisone group. It also differed significantly (*P* = 0.002) between treatment groups on days 1, 3, 7, 30, 60, and 90. The serum adiponectin concentration was significantly lower on days 30 (*P* = 0.018), 60 (*P* = 0.002), and 90 (*P* < 0.001) than at baseline, and it was lowest on day 90 for the hydrocortisone group. It also differed significantly between treatment groups on days 3 (*P* = 0.004), 7 (*P* = 0.004), 30 (*P* = 0.002), 60 (*P* = 0.002), and 90 (*P* = 0.002). Additionally, the ratio of the adiponectin concentration to the leptin concentration for the hydrocortisone group was significantly lower on days 60 (*P* < 0.001) and 90 (*P* = 0.005), compared with the ratio on day 0. The ratio also differed significantly (*P* = 0.002) between treatment groups on days 1, 3, 7, 30, 60, and 90.

Leptin mRNA expression for the hydrocortisone group was significantly (*P* = 0.010) greater on day 30, compared with the expression at baseline **(Figure 2)**. However, leptin mRNA expression for the hydrocortisone group began to decrease on day 60 and was further decreased on day 90, compared with the expression on day 30. Leptin mRNA expression differed significantly (*P* = 0.002) between treatment groups on day 30. Adiponectin mRNA expression for the hydrocortisone group was significantly (*P* = 0.042) greater on day 30 than on day 0. Adiponectin mRNA expression then progressively decreased on days 60 and 90, compared with the expression on day 30. It was significantly (*P* = 0.002) greater for the hydrocortisone group than the control group on day 30.

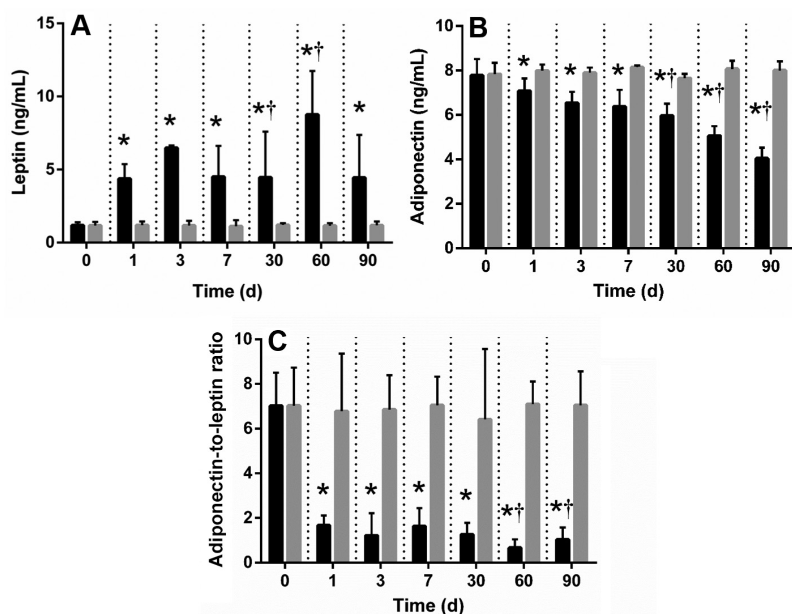

Figure 1—Median (interquartile [25th to 75th percentile] range) values for serum concentrations of leptin (A) and adiponectin (B) and the ratio of adiponectin concentration to leptin concentration (C) of dogs before (day 0) and during (days 1, 3, 7, 30, 60, and 90) hydrocortisone administration (8.5 mg/kg, PO, q 12 h; black bars; n = 6) or administration of a placebo to a control group (gray bars; 6). *Within a day, value differs significantly (*P* < 0.05) from the value for the control group. †Within the hydrocortisone group, value differs significantly (*P* < 0.05) from the value at day 0.

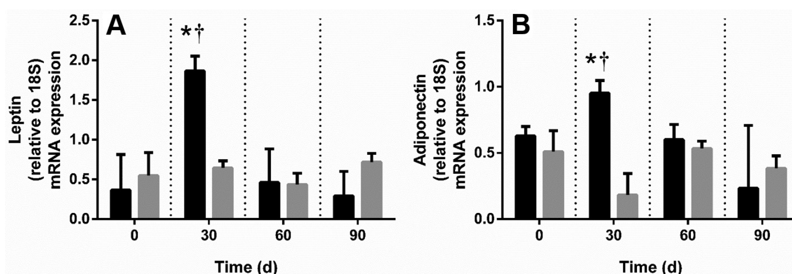

Figure 2—Median (interquartile range) values of leptin (A) and adiponectin (B) mRNA expression in dogs before and during administration of hydrocortisone (black bars; n = 6) or a placebo (gray bars; 6) for 90 days. Notice that the scale on the y-axis differs between panels. ***See*** Figure 1 for remainder of key.

Body weight was similar to that at baseline for all dogs receiving hydrocortisone. The CT images obtained at the level of L3 before and during administration of hydrocortisone were evaluated **(Figure 3)**.

Total, visceral, and subcutaneous fat mass on days 0, 30, 60, and 90 were determined **(Figure 4)**. Total fat mass of dogs in the hydrocortisone group was significantly (*P* = 0.002) greater on day 90, but not on days 30 or 60, compared with the total fat mass at baseline. Total fat mass differed significantly (*P* = 0.041) between treatment groups on day 90. Subcutaneous fat mass did not differ significantly over time within the hydrocortisone group or between treatment groups. However, visceral fat mass of dogs in the hydrocortisone group was significantly greater on days 60 (*P* = 0.022) and 90 (*P* < 0.001), compared with the visceral fat mass at baseline, and peaked on day 90. It also differed significantly (*P* = 0.002) between treatment groups on days 60 and 90.

Total, visceral, and subcutaneous fat mass, percentage of visceral fat, and visceral fat-to-subcutaneous fat ratio for the dogs before and during administration of hydrocortisone were calculated **(Table 1)**. There were significant increases in the visceral fat mass, percentage of visceral fat, and visceral fat-to-subcutaneous fat ratio during hydrocortisone administration. Total fat mass for the hydrocortisone group was sig-

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nificantly greater on day 90, compared with the values at baseline ($P = 0.044$) and on day 30 ($P = 0.022$). It was significantly ($P = 0.041$) greater for the hydrocortisone group than the control group on day 90.

No significant differences in subcutaneous fat mass were detected on days 30, 60, and 90, compared with the mass at baseline. However, visceral fat mass for the hydrocortisone group was significantly greater on days 60 ($P = 0.042$) and 90 ($P < 0.001$) than at baseline. It differed significantly ($P = 0.002$) between treatment groups on days 30, 60, and 90. The percentage of visceral fat for the hydrocortisone group was significantly higher on days 60 ($P = 0.005$) and 90 ($P = 0.002$) than at baseline. It was significantly higher for the hydrocortisone group than the control group on days 30 ($P = 0.002$), 60 ($P = 0.015$), and 90 ($P = 0.004$). In addition, the visceral fat-to-subcutaneous fat ratio for the hydrocortisone group was also significantly higher on days 60 ($P = 0.005$) and 90 ($P = 0.002$) than at baseline. It differed significantly between treatment groups on days 30 ($P = 0.002$), 60 ($P = 0.015$), and 90 ($P = 0.004$).

Correlations between visceral fat mass and serum adipokine concentrations were determined **(Figure 5)**. There was a significant positive correlation ($r = 0.710$; $P < 0.001$) between visceral fat mass and the leptin concentration. In contrast, visceral fat mass was not significantly correlated ($r = -0.397$; $P = 0.055$) with the adiponectin concentration.

Discussion

Analysis of results of the present study indicated that administration of hydrocortisone at a dosage of 8.5 mg/kg, PO, every 12 hours for 90 days increased serum leptin concentrations and decreased serum adiponectin concentrations. Additionally, mRNA expression of leptin and adiponectin in visceral fat was greater on day 30, compared with expression at baseline. Fat distribution changed with an increase in visceral fat mass. To our knowledge, the study reported here was the first in which fat distribution of dogs was quantified after hydrocortisone administration and the first in which a correlation was identified between serum leptin concentration and visceral fat mass in dogs with hypercortisolemia.

In the present study, serum leptin concentration significantly increased with hydrocortisone administration. In particular, the leptin concentration increased over time in the hydrocortisone group and was higher after the first day of hydrocortisone administration, compared with the concentration in the control group. These results strongly suggested that cortisol was involved in the regulation of leptin secretion in vivo. In a previous study[19] conducted by our research group, the serum leptin concentrations were significantly higher in dogs with hyperadrenocorticism, and there was a linear association for the leptin concentration in dogs with hyperadrenocorticism. Additionally, injection of dexamethasone in dogs increased plasma leptin concentrations in another study.[29] These findings indicate that cortisol increases leptin secretion in dogs, suggesting that cortisol may have direct effects on leptin secretion.

In rodents, glucocorticoids increase leptin expression in vivo and in vitro.[30–32] Acute administration of

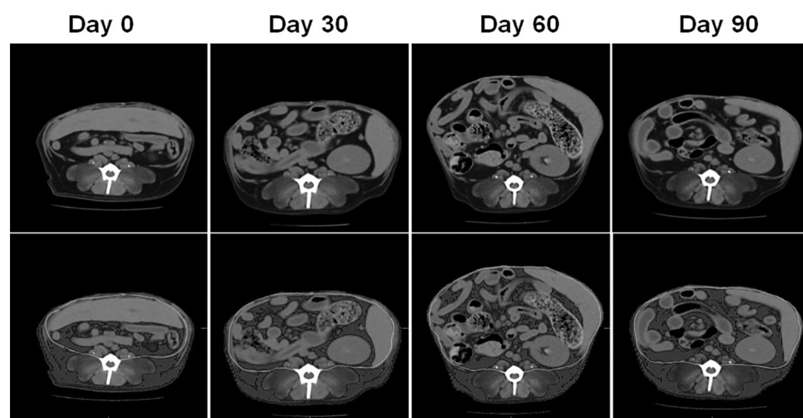

Figure 3—Representative transverse CT images of a dog obtained at the level of L3 before (day 0) and during (days 30, 60, and 90) hydrocortisone administration (top row). For the same transverse CT images, pixels with an attenuation range of –135 to –105 HU are depicted as dark gray (bottom row).

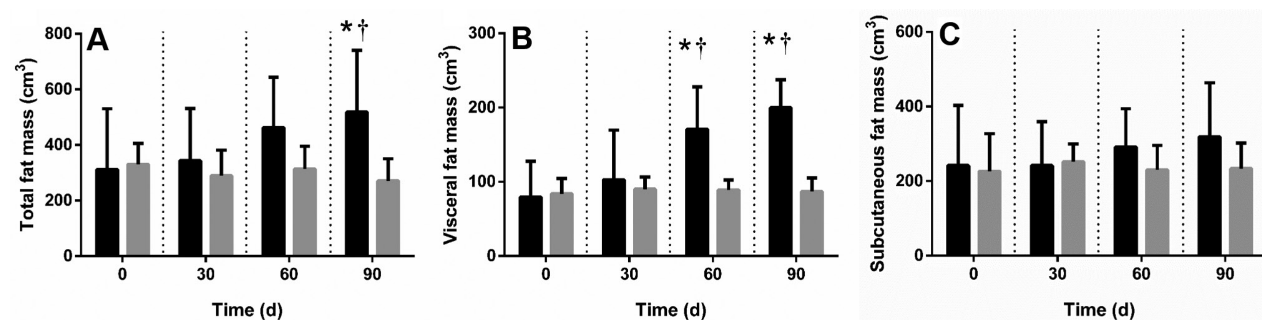

Figure 4—Median (interquartile [25th to 75th percentile] range) values of the total (A), visceral (B), and subcutaneous (C) fat mass in the abdomen of dogs before and during (days 30, 60, and 90) administration of hydrocortisone (black bars; n = 6) or a placebo (gray bars; 6). Notice that the scale on the y-axis differs among panels. ***See*** Figure 1 for remainder of key.

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Table 1—Body weight and fat distribution in the abdomen of dogs before (day 0) and during (days 30, 60, and 90) administration of hydrocortisone (8.5 mg/kg, PO, q 12 h; n = 6) or a placebo (6).

	Day 0		Day 30		Day 60		Day 90	
Variable	Hydrocortisone	Control	Hydrocortisone	Control	Hydrocortisone	Control	Hydrocortisone	Control
Body weight (kg)	11.35 (10.58–12.21)	10.67 (10.38–10.82)	10.58 (10.08–10.99)	10.53 (10.39–10.72)	11.09 (10.24–11.80)	10.61 (10.33–10.71)	11.22 (10.37–11.95)	10.38 (10.19–10.74)
Total fat mass (cm^3)	18.17 (12.09–26.04)	17.81 (15.15–21.79)	18.32 (13.52–25.91)	13.85 (12.65–21.24)	20.52 (13.87–33.93)	15.94 (10.47–18.94)	26.22*†‡ (16.43–42.55)	15.29 (12.09–20.92)
Visceral fat mass (cm^3)	5.73 (3.56–7.06)	5.83 (5.00–6.38)	9.64* (8.76–13.02)	4.73 (3.79–5.76)	11.30*† (8.89–18.31)	4.81 (3.44–6.02)	15.35*† (10.47–22.71)	4.96 (3.74–5.95)
Visceral fat (%)	30.85 (23.19–40.21)	31.59 (23.60–38.50)	53.90* (48.84–60.74)	31.37 (20.44–40.50)	57.04*† (51.16–61.97)	36.38 (18.21–45.03)	59.51*† (55.85–63.28)	27.44 (22.96–49.27)
Subcutaneous fat mass (cm^3)	13.74 (7.29–18.31)	13.02 (9.35–15.95)	8.70 (4.93–12.89)	9.81 (7.59–16.31)	9.22 (5.29–15.62)	10.14 (5.77–15.49)	10.87 (5.96–17.50)	11.30 (6.15–15.72)
V:S	0.45 (0.30–0.68)	0.47 (0.31–0.63)	1.17* (0.96–1.57)	0.47 (0.26–0.68)	1.33*† (1.05–1.66)	0.57 (0.22–0.86)	1.47*† (1.29–1.73)	0.38 (0.30–0.98)

Values reported are median (interquartile [25th to 75th percentile]) range.

*Within a day, value differs significantly ($P < 0.05$) from the value for the control group. †Within the hydrocortisone group, value differs significantly ($P < 0.05$) from the value for day 0. ‡Within the hydrocortisone group, value differs significantly ($P < 0.05$) from the value for day 30.

V:S = Ratio of visceral fat mass to subcutaneous fat mass.

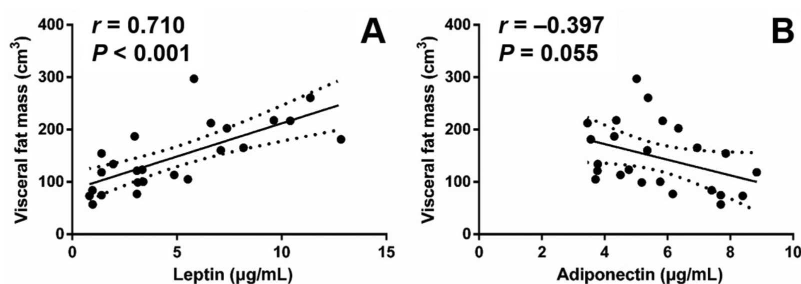

Figure 5—Correlation curves between visceral fat mass and serum leptin (A) or adiponectin (B) concentrations for 6 dogs receiving hydrocortisone for 90 days. The line of best fit (solid line) and 95% confidence interval (dotted lines) are indicated.

glucocorticoids in humans increases leptin concentrations,[33] and glucocorticoids increase leptin secretion and expression of the *ob* gene in cultured human adipose tissue.[3] These results suggest that glucocorticoids act directly on adipose tissue and increase leptin synthesis and secretion in humans. In another study[29] of dogs, induction of hypercortisolemia increased leptin mRNA expression in subcutaneous adipose tissues and increased plasma leptin concentrations, although the method for induction of hypercortisolemia differed from that of the study reported here. In the present study, leptin expression for the hydrocortisone group was greater on day 30, compared with the expression at baseline and the expression for the control group. Therefore, the parallel increase in serum leptin concentrations and leptin mRNA expression in visceral adipose tissue after administration of hydrocortisone suggested that the effects of glucocorticoids on leptin secretion were mediated by mRNA expression. However, we did not determine the leptin mRNA expression on day 1, and this was an important limitation of the study. Thus, it will be necessary to determine the effects of glucocorticoids on leptin expression in cultured canine adipose tissues.

Leptin concentrations in humans with hyperadrenocorticism reportedly are similar[34,35] or elevated,[36] compared with concentrations for body mass index–matched control subjects. In 1 study,[37] leptin concentrations were correlated with the amount of total adipose tissue and subcutaneous adipose tissue in women with hyperadrenocorticism; however, leptin concentrations were not correlated with the amount of visceral adipose tissue. In another study[19] conducted by our research group, serum leptin concentrations in dogs with hyperadrenocorticism were significantly higher than concentrations in similarly overweight dogs with physiologically normal adrenal glands. Additionally, the leptin concentrations in dogs with hyperadrenocorticism after trilostane treatment in that study[19] were higher than those in healthy dogs, which suggests that increases in the visceral fat mass lead to excessive hyperleptinemia in dogs. Thus, quantitative evaluation of the visceral fat mass would have helped to clarify whether hyperleptinemia in dogs with hyperadrenocorticism is related to visceral obesity. In the present study, a correlation was detected between serum leptin concentration and the visceral fat mass in dogs receiving hydrocortisone. These findings suggested that an increase in the abdominal visceral fat mass elevated the serum leptin concentration in dogs.

In the present study, serum adiponectin concentrations of dogs decreased significantly during administration of hydrocortisone for 90 days. However, another study[19] conducted by our research group found that serum adiponectin concentrations did not differ significantly between dogs with hyperadrenocorticism and healthy dogs. Although the effects of glucocorticoids on adiponectin expression of humans have been studied, no consensus has been reached.[38]

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It has been suggested[17,39] that adiponectin expression might not be inversely related to adiposity in obese dogs and that differences in adiponectin physiology between dogs and humans might be expected. In rats, glucocorticoids decrease serum adiponectin concentrations and adiponectin mRNA expression in white adipose tissue.[40] The authors of that study[40] proposed that the decrease in serum adiponectin concentration was attributable to inhibited adiponectin mRNA expression. Dexamethasone treatment of dogs did not cause changes in adiponectin expression of canine adipocytes.[41] In contrast to results of an in vitro study,[41] adiponectin mRNA expression in abdominal visceral fat for the dogs of the present study was significantly greater on day 30. The reason for this discrepancy is unknown, but it is possible that adiponectin mRNA expression is altered under different conditions.[17] Thus, additional studies will be necessary to clarify the effects of obesity on relationships between serum adiponectin concentrations and cortisol concentrations in dogs and the relationships of these hormones between obese and nonobese dogs.

The amount of abdominal visceral fat was greater after administration of hydrocortisone in the present study. Currently, CT is the criterion-referenced standard technique for confirmation of visceral obesity, which is characterized as increased amounts of adipose tissue surrounding the intra-abdominal organs,[42] in humans and dogs.[24,42] Hypercortisolemia is associated with visceral obesity in humans and dogs. Some studies[11,37] have revealed that fat distribution changes in humans with hyperadrenocorticism, compared with results for control subjects. However, visceral obesity in dogs with hyperadrenocorticism has not been evaluated. Total fat mass differed significantly on day 90, compared with the mass on day 0. Visceral fat mass increased sequentially, whereas subcutaneous fat mass did not differ significantly during hydrocortisone administration. Therefore, the lack of a significant difference in the subcutaneous fat mass suggested that the increase in total fat mass associated with hydrocortisone administration was caused by an increase in the visceral fat mass.

In addition, single-slice images were evaluated to quantify multicompartmental fat distribution between the subcutaneous and visceral compartments in the abdomen. Results indicated a significant increase in the proportion of visceral fat after administration of hydrocortisone, compared with the proportion before administration. Therefore, fat redistribution appeared to be associated with visceral obesity in dogs receiving hydrocortisone.

The present study had several limitations. First, the leptin concentrations could have been influenced by a stress reaction (eg, reaction to drug administration, collection of blood samples, insertion of the catheter in a jugular vein, and pain caused by biopsy). Evaluations of site-related differences in leptin expression of canine adipocytes have not been reported. Therefore, additional studies will be necessary to identify mRNA expression of leptin and adiponectin in the subcutaneous and visceral adipose tissues of dogs.

In the present study, serum leptin and adiponectin concentrations in dogs administered hydrocortisone might have been affected directly by hypercortisolemia and dysregulation of mRNA expression of leptin and adiponectin in visceral adipose tissues. Additionally, analysis of the results suggested that serum leptin concentrations might have been related to visceral obesity. Additional studies will be necessary to clarify the role of leptin in the pathogenesis of complications related to chronic hypercortisolemia.

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The authors declare that there were no conflicts of interest.

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Footnotes

a. Natural Balance, Natural Balance Pet Foods Inc, Los Angeles, Calif.
b. Microsoft Excel, version 2013, Microsoft Co, Redmond, Wash.
c. Inist Biopharma Co, Kyunggi, Republic of Korea.
d. Provive 1%, Myungmoon Pharmaceutical Co, Seoul, Repubic of Korea.
e. Terrell, Piramal Critical Care Inc, Bethlehem, Pa.
f. Hi Speed CT/e, GE Medical Systems Inc, Chicago, Ill.
g. Yuhan, Seoul, Republic of Korea.
h. Gaster injectable, Dong-A ST, Seoul, Republic of Korea.
i. Butophan, Myungmoon Pharmaceutical Co, Seoul, Republic of Korea.
j. Daihan Pharmaceutical Co, Seoul, Republic of Korea.
k. TriZol reagent, Life Technologies Co, Carlsbad, Calif.
l. Hitachi 7020, Hitachi High-Technologies Corp, Tokyo, Japan.
m. Canine leptin ELISA kit, Millipore Co, Billerica, Mass.
n. Canine adiponectin ELISA kit, Millipore Co, Billerica, Mass.
o. M-MLV reverse transcriptase, Invitrogen, Carlsbad, Calif.
p. 9-mer, Takara Bio Inc, Shiga, Japan.
q. Gel Doc EQ, Bio-Rad Laboratories Inc, Hercules, Calif.
r. GraphPad Prism, version 6, GraphPad Software Inc, La Jolla, Calif.

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