Effects of administration of glucocorticoids and feeding status on plasma leptin concentrations in dogs

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Objective—To investigate effects of short- and long-term administration of glucocorticoids, feeding status, and serum concentrations of insulin and cortisol on plasma leptin concentrations in dogs.

Animals—20 nonobese dogs.

Procedure—For experiment 1, plasma leptin concentrations and serum concentrations of insulin and cortisol were monitored for 24 hours in 4 dogs administered dexamethasone (0.1 mg/kg, IV) or saline (0.9% NaCl) solution for fed and nonfed conditions. For experiment 2, 11 dogs were administered prednisolone (1 mg/kg, PO, q 24 h for 56 days [7 dogs] and 2 mg/kg, PO, q 24 h for 28 days [4 dogs]) and 5 dogs served as control dogs. Plasma leptin and serum insulin concentrations were monitored weekly.

Results—For experiment 1, dexamethasone injection with the fed condition drastically increased plasma leptin concentrations. Furthermore, injection of saline solution with the fed condition increased plasma leptin concentrations. These increases in plasma leptin concentrations correlated with increases in serum insulin concentrations. Dexamethasone injection with the nonfed condition increased plasma leptin concentrations slightly but continuously. Injection of saline solution with the nonfed condition did not alter plasma leptin concentrations. For experiment 2, prednisolone administration at either dosage and duration did not alter plasma leptin concentrations in any dogs.

Conclusions and Clinical Relevance—Dexamethasone injection and feeding increased plasma leptin concentrations in dogs. In addition, dexamethasone administration enhanced the effect of feeding on increases in plasma leptin concentrations. Daily oral administration of prednisolone (1 or 2 mg/kg) did not affect plasma leptin concentrations in dogs. (*Am J Vet Res* 2006;67:266–270)

Glucocorticoids are commonly used in veterinary practice. Glucocorticoids have orexigenic effects[1] and can cause obesity in dogs.[2,3] In humans, glucocorticoid administration increases energy intake by 20% to 59% and also increases body weight.[4,5] It is believed that these effects relate to the ability of glucocorticoids to act on the CNS.[5] Obesity is a risk factor for the development of diabetes,[6] orthopedic disorders,[7] and cardiovascular diseases.[7] However, the pathogenesis of obesity induced by glucocorticoid treatment has not been definitively described in dogs.

Leptin was isolated as a product of the *ob* gene[8] and has important roles in energy homeostasis.[9] Leptin regulates body weight by modulation of CNS functions through the control of food intake and energy balance.[9] In humans, administration of glucocorticoids increases plasma leptin concentrations.[10-12] In addition, human patients with hyperadrenocorticism (ie, Cushing syndrome) have high blood leptin concentrations.[13,14] Analysis of these facts suggests that glucocorticoid exposure may lead to high plasma leptin concentrations. However, an anorexigenic effect of leptin has not been observed; thus, it is suggested that leptin resistance exists in these patients. Therefore, it is speculated that leptin may be implicated in obesity induced by glucocorticoid treatment.

In dogs, there are few reports about effects of glucocorticoid treatment on serum leptin concentrations. Dexamethasone administration increases serum leptin concentrations in dogs from which food is withheld.[15] In addition, feeding increases serum leptin concentrations.[16] However, effects of glucocorticoids on serum leptin concentrations in fed dogs are not known. Therefore, it is necessary to understand the pathogenesis of obesity induced by glucocorticoids for prevention or treatment of obesity. In the study reported here, 2 experiments were conducted to examine the effects of dexamethasone administration and feeding status on plasma leptin concentrations in dogs and effects of long-term (26 or 58 days) treatment with prednisolone on plasma leptin concentrations. In addition, serum insulin and cortisol concentrations were measured to investigate the relationship among these hormones.

Materials and Methods

Animals—Twenty nonobese dogs were used in the study. For experiment 1, 4 nonobese sexually intact male dogs were used. Mean ± SD body weight was 12.0 ± 0.7 kg,

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and mean age was 2.5 ± 0.58 years. For experiment 2, 16 nonobese dogs (1 sexually intact female and 15 sexually intact males) were used. Mean body weight was 12.8 ± 1.2 kg, and mean age was 3.7 ± 2.4 years. All dogs were assessed as healthy on the basis of results of physical examination, a CBC count, and serum biochemical analyses. Each dog had a body condition score of 3 (5-point scale). Dogs with a body condition score of 5/5 were considered obese. The study was performed at Gifu University and conducted in a manner consistent with the Gifu University Guidelines for Animal Experimentation (grant No. 02075).

Experiment 1—Dogs were investigated for each of 4 conditions (dexamethasone administration and fed, dexamethasone administration and not fed, saline [0.9% NaCl] solution administration and fed, and saline solution administration and not fed). Dogs were used in a Latin square design; thus, each dog underwent each treatment. There was a 1-week washout period between conditions.

Blood samples were collected at 9 AM and 10 AM. Then each dog received an IV injection of dexamethasone[a] (0.1 mg/kg) or a commensurate amount of saline solution (time 0). Blood samples were obtained at 60-minute intervals for the subsequent 24 hours. Blood samples were used for determination of plasma leptin concentrations and serum concentrations of insulin and cortisol.

For the fed condition, dogs were provided a commercially available extruded food formulated for dogs[b] at noon each day. The daily energy requirement was calculated as described by use of the following equation[17]: daily energy requirement = $552 \times BW^{0.75}$, where BW is body weight in kilograms. For the nonfed condition, dogs did not receive any food.

Experiment 2—Dogs were classified into 3 groups. There were 7 dogs in group P-1, 4 dogs in group P-2, and 5 control dogs. Dogs in groups P-1 and P-2 received daily administration of prednisolone tablets (group P-1, 1 mg/kg, PO, q 24 h for 56 days; group P-2, 2 mg/kg, PO, q 24 h for 28 days). Dosing rates were selected on the basis of the therapeutic dose (ie, these dosages are commonly used for anti-inflammatory and immunosuppressive effects in dogs).[1] The first day of prednisolone administration was designated as day 0 (ie, baseline). Dogs in the control group did not receive any medications.

All dogs were allowed ad libitum access to an extruded commercially available food formulated for dogs[b] for 30 minutes once daily; food intake was recorded after each feeding. Blood samples were collected before feeding on days 0, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56 for use in determination of plasma leptin and serum insulin concentrations. Dogs were weighed before onset of prednisolone administration and at weekly intervals thereafter.

Hormonal assays—Analysis of dilution curves revealed good linearity for each assay. Plasma leptin concentrations were determined by use of an ELISA that included an anti-canine leptin antibody.[18] Serum insulin concentrations were determined by use of an immunoradiometric assay.[c] The intra-assay and interassay coefficient of variation ranged from 2.5% to 5.9% and 2.5% to 13.6%, respectively. Serum cortisol concentrations were determined by use of a radioimmunoassay.[d] The intra-assay coefficient of variation ranged from 1.0% to 5.1%, and the interassay coefficient of variation was 3.0%. Because prednisolone cross-reacts with endogenous cortisol, serum cortisol concentrations were not determined for experiment 2.

Statistical analysis—Rates of change in plasma leptin and serum insulin concentrations were calculated and reported as the mean ± SEM. Area under the curve for serum cortisol concentration was calculated by use of the trapezoid method. Differences among groups and values before and during tests were determined by use of a 1-way ANOVA with the Fisher protected least-significant difference test and paired *t* test, respectively. Values of $P < 0.05$ were considered significant.

Results

Experiment 1—Changes in plasma leptin and serum insulin concentrations were determined (**Figure 1**). For the dexamethasone and fed condition, plasma leptin concentrations began to increase 2 hours after feeding and reached a maximum concentration (1,440% of the pretreatment [baseline] concentration) 8 hours after feeding. Plasma leptin concentrations began to decrease 9 hours after feeding and maintained a concentration similar to that for the dexamethasone and nonfed condition from 17 hours after feeding until the end of the experiment. Plasma leptin concentrations were significantly higher between 2 and 20 hours after feeding, compared with concentrations for the

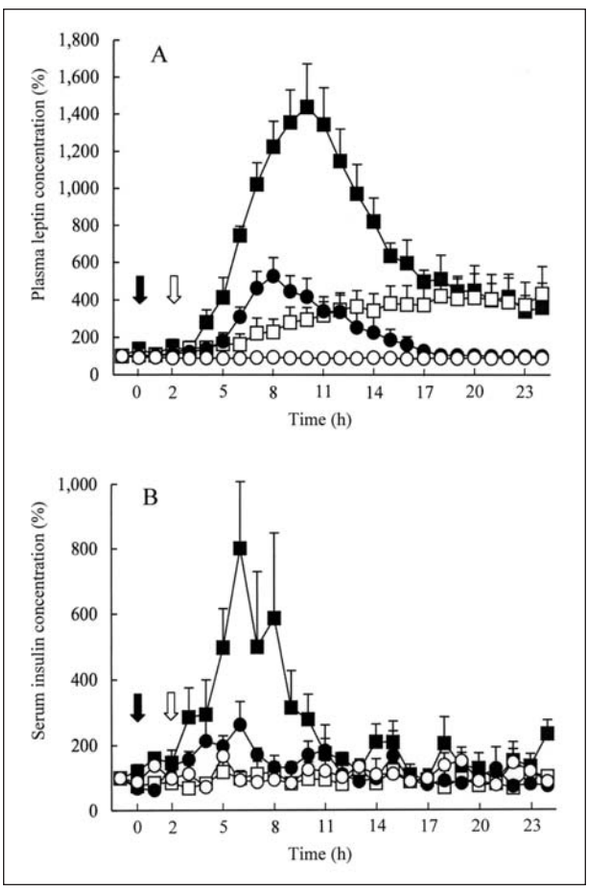

Figure 1—Mean ± SEM plasma leptin concentrations (A) and serum insulin concentrations (B) in 4 nonobese dogs for each of 4 conditions (dexamethasone injection and fed condition [black squares], dexamethasone injection and nonfed condition [white squares], saline [0.9% NaCl] solution injection and fed condition [black circles], and saline solution injection and nonfed condition [white circles]). Initial blood samples were obtained at 9 AM and 10 AM, and dexamethasone (0.1 mg/kg) or an equivalent volume of saline solution was administered IV (time 0 [ie, baseline]; black arrow). For the fed condition, dogs were provided a commercially available food formulated for dogs (white arrow). Concentrations reported represent the percentage of the baseline value.

saline solution and fed condition, and between 2 and 13 hours after feeding, compared with concentrations for the dexamethasone and nonfed condition.

For the dexamethasone and nonfed condition, plasma leptin concentrations increased mildly but continuously (435% of the pretreatment [baseline] concentration). Concentrations were significantly higher between 10 and 22 hours after treatment, compared with the concentration before treatment. Plasma leptin concentrations were significantly higher from 13 hours until the end of the experiment, compared with concentrations for the saline solution and nonfed condition.

For the saline solution and fed condition, plasma leptin concentrations began to increase 2 hours after feeding and reached a maximum concentration (528% of the pretreatment [baseline] concentration) 6 hours after feeding. Plasma leptin concentrations between 4 and 7 hours after feeding were significantly higher, compared with the pretreatment concentrations. Plasma leptin concentrations between 4 and 9 hours after feeding were significantly higher, compared with leptin concentrations for the saline solution and nonfed condition. Plasma leptin concentrations for the saline solution and nonfed condition did not change during the experiment.

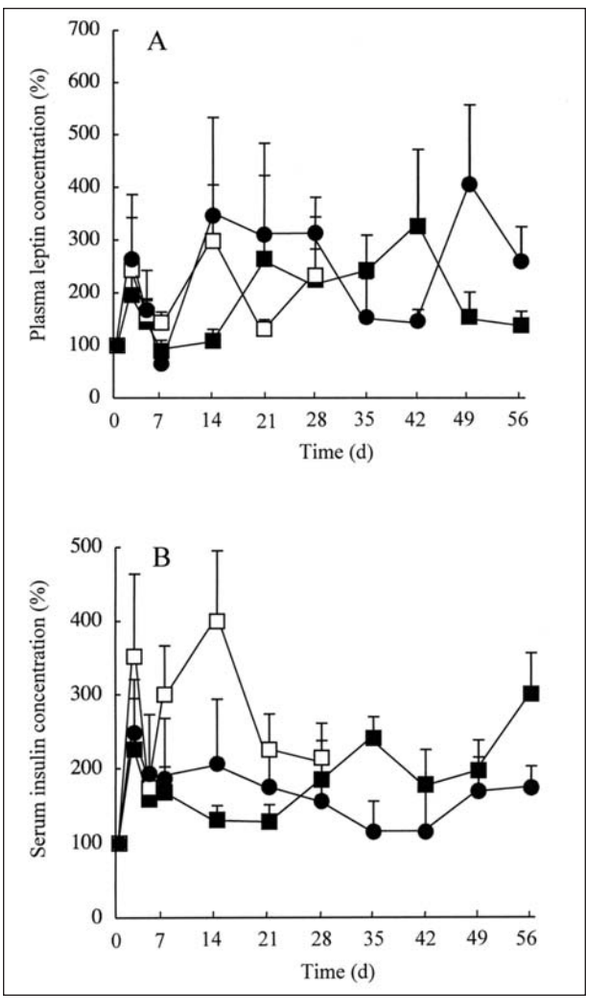

Figure 2—Mean ± SEM plasma leptin concentrations (A) and serum insulin concentrations (B) in 3 groups of nonobese dogs (7 dogs in group P-1 [black squares], 4 dogs in group P-2 [white squares], and 5 control dogs [black circles]). Dogs in groups P-1 and P-2 received daily oral administrations of prednisolone (1 mg/kg for 56 days and 2 mg/kg for 28 days, respectively). Day of the first prednisolone administration was designated as day 0 (ie, baseline). Concentrations reported represent the percentage of the baseline value.

For the dexamethasone and fed condition, serum insulin concentrations were significantly increased between 3 and 6 hours after feeding, compared with concentrations for the saline solution and fed condition. For the saline solution and fed condition, serum insulin concentrations increased between 2 and 4 hours after feeding. Serum insulin concentrations did not change for the nonfed conditions, regardless of dexamethasone administration.

Serum cortisol concentrations decreased to less than the minimum detectable concentration by 2 hours after dexamethasone injection, regardless of feeding status (data not shown). Area under the curve for serum cortisol concentrations for the saline solution and nonfed condition (mean ± SD, 830.0 ± 172.2 nmol/L/24 h) did not differ significantly from that for the saline solution and fed condition (mean, 757.3 ± 100.8 nmol/L/25 h).

Experiment 2—Changes in plasma leptin and serum insulin concentrations for the P-1, P-2, and control groups were determined (**Figure 2**). After day 3, plasma leptin concentrations were slightly higher than baseline for all groups, but we did not detect a significant difference for plasma leptin concentrations among the study groups. Dogs of group P-2 had slightly higher serum insulin concentrations, and a significant difference from control dogs was observed at day 14. Food intake did not differ among groups (mean ± SD, 366.6 ± 124.6 J/kg, 412.1 ± 100.3 J/kg, and 397.1 ± 102.0 J/kg for group P-1, group P-2, and the control group, respectively). Body weight did not change significantly for any dog throughout the experiment, regardless of prednisolone administration.

Discussion

To our knowledge, the study reported here is the first to reveal that dexamethasone administration and feeding cooperated to increase plasma leptin concentrations in dogs. The results are consistent with those for studies[10,19] in humans. In the study reported here, dexamethasone administration enhanced the postprandial increase in insulin concentrations. Insulin administration increases serum leptin concentrations in humans.[20] In addition, the combination of insulin and dexamethasone in humans increases the release of leptin from subcutaneous adipocytes to a greater extent than does insulin or dexamethasone alone.[21,22] Also, in an in vivo study[20] in humans, insulin and dexamethasone administration induced an increase in serum leptin concentrations. The increase is similar to the one observed after dexamethasone and feeding.[20] Analysis of these data suggests that insulin has an important role in the increase in blood leptin concentration after a meal. For the dogs in the dexamethasone and fed condition in our study, insulin and dexamethasone

may play an important role in the increase in plasma leptin concentrations. Dexamethasone may indirectly increase plasma leptin concentrations through the action of insulin.

Dexamethasone injection caused a mild but continuous increase in plasma leptin concentrations for dogs in the dexamethasone and nonfed condition. This result is similar to that reported in another study[15] in dogs in which the serum leptin concentration increased gradually (248% of pretreatment concentration) until 24 hours after dexamethasone injection (approx 0.14 mg of dexamethasone/kg). However, dexamethasone injection (2 mg/person) of humans in a nonfed condition did not increase serum leptin concentrations, although the injection prevented a nocturnal decrease in serum leptin concentrations. The difference between dogs and humans may relate to differences in the dose of dexamethasone.

For the saline solution and fed condition, plasma leptin concentrations began to increase 2 hours after feeding and reached maximum concentrations 6 hours after feeding. This result was consistent with that for another study[16] in dogs, whereas in a study[10] in humans, serum leptin concentrations began to increase 4 to 6 hours after a meal and reached maximum concentrations 10 to 13 hours after a meal. There may be species differences in the gastrointestinal transit time; thus, postprandial increases in blood glucose and insulin concentrations in dogs were achieved more rapidly than those in humans.[23] This may be 1 reason that the increase in plasma leptin concentrations after feeding is more rapid in dogs than in humans.

Withholding of food reduces circulating leptin concentrations in humans[10,24] and dogs.[15,16] However, in the study reported here, plasma leptin concentrations did not change throughout the experiment in dogs for the saline solution and nonfed condition. The period of food withholding before the experiment in the study reported here (ie, 24 hours) was longer than that in other studies[10,15,16,24] (ie, food withheld overnight). Plasma leptin concentrations in dogs for the saline solution and nonfed condition possibly had already been suppressed substantially; thus, decreases in plasma leptin concentrations may not have been detected.

The effects of endogenous cortisol release on leptin secretion are disputed. However, physiologic concentrations of glucocorticoid reportedly do not affect leptin secretion in humans[25,26] and dogs.[16] The study reported here revealed that feeding and withholding of food did not affect serum cortisol concentrations and feeding significantly increased plasma leptin concentrations in dogs. In agreement with those aforementioned studies,[16,25,26] analysis of results of the study reported here suggested that there was little participation in acute increases in plasma leptin concentrations attributable to endogenous cortisol concentrations in dogs.

Experiment 2 revealed that plasma leptin concentrations were slightly higher than baseline values in all groups after day 3, although body weight of all dogs did not change. In a study[27] in humans, short-term overfeeding caused an increase in blood leptin concentrations. During experiment 2, dogs consumed a larger amount of food than is typical; therefore, a positive energy balance may have induced higher plasma leptin concentrations.

In the study reported here, oral administration of prednisolone (1 mg/kg for 56 days or 2 mg/kg for 28 days) had no significant effect on plasma leptin concentrations and food intake. Administration of prednisolone for 5 weeks caused an increase in plasma leptin concentrations in children with acute lymphoblastic leukemia.[28] In addition, blood leptin concentrations were higher in humans with Cushing syndrome.[13,14] Analysis of results of these studies suggests that excessive cortisol concentrations may relate to higher blood leptin concentrations. Whether higher blood leptin concentrations in humans with Cushing syndrome are directly related to excessive amounts of cortisol or to hyperinsulinemia is not clear,[29] but it is assumed that indirect effects through the actions of insulin are most important.[30] Although insulin concentrations in dogs in the P-2 group were slightly higher than concentrations in the other groups, there was no significant difference in plasma leptin concentrations between group P-2 and the control group. Additional studies with a higher dose or longer duration of administration of prednisolone are needed to investigate whether plasma leptin concentrations respond to glucocorticoid administration in dogs.

The study reported here documented cooperative effects between dexamethasone and feeding on plasma leptin concentrations and effects of daily oral administration of predonisolone to dogs. Dexamethasone administration (0.1 mg/kg, IV) and feeding cooperated to markedly increase plasma leptin concentrations in dogs. Daily oral administration of prednisolone administration (1 mg/kg for 56 days or 2 mg/kg for 28 days) did not affect plasma leptin concentrations in dogs.

a. Dexamethasone injection A, Zenyaku Kogyo, Fukushima, Japan.
b. Hill's Science Diet Canine Maintenance, Hill's Colgate, Tokyo, Japan.
c. Insulin riabeads, Abbott Japan, Tokyo, Japan.
d. Cortisol kit TFB, TFB, Tokyo, Japan.

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