

# Secretion of sex hormones in dogs with adrenal dysfunction

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**Objective**—To evaluate adrenal sex hormone concentrations in response to ACTH stimulation in healthy dogs, dogs with adrenal tumors, and dogs with pituitary-dependent hyperadrenocorticism (PDH).

**Design**—Prospective study.

**Animals**—11 healthy control dogs, 9 dogs with adrenal-dependent hyperadrenocorticism (adenocarcinoma [ACA] or other tumor); 11 dogs with PDH, and 6 dogs with noncortisol-secreting adrenal tumors (ATs).

**Procedure**—Hyperadrenocorticism was diagnosed on the basis of clinical signs; physical examination findings; and results of ACTH stimulation test, low-dose dexamethasone suppression test, or both. Dogs with noncortisol-secreting ATs did not have hyperadrenocorticism but had ultrasonographic evidence of an AT. Concentrations of cortisol, androstenedione, estradiol, progesterone, testosterone, and 17-hydroxyprogesterone were measured before and 1 hour after IM administration of 0.25 mg of synthetic ACTH.

**Results**—All dogs with ACA, 10 dogs with PDH, and 4 dogs with ATs had 1 or more sex hormone concentrations greater than the reference range after ACTH stimulation. The absolute difference for progesterone, 17-hydroxyprogesterone, and testosterone concentrations (value obtained after ACTH administration minus value obtained before ACTH administration) was significantly greater for dogs with ACA, compared with the other 3 groups. The absolute difference for androstenedione was significantly greater for dogs with ACA, compared with dogs with AT and healthy control dogs.

**Conclusions and Clinical Relevance**—Dogs with ACA secrete increased concentrations of adrenal sex hormones, compared with dogs with PDH, noncortisol-secreting ATs, and healthy dogs. Dogs with noncortisol-secreting ATs also have increased concentrations of sex hormones. There is great interdog variability in sex hormone concentrations in dogs with ACA after stimulation with ACTH. (*J Am Vet Med Assoc* 2005;226:556–561)

**H**yperadrenocorticism (HAC) is defined as excessive production of adrenal hormones and is a com-

mon endocrine disorder in dogs with a reported prevalence in the general population of 0.1%.<sup>1,2</sup> **Pituitary-dependent HAC (PDH)** accounts for approximately 85% of cases, with the remainder caused by benign or malignant adrenocortical tumors (**adrenal-dependent HAC [ADH]**).<sup>3–6</sup> The most common hormone secreted from the adrenal gland in dogs and cats with HAC is cortisol; however, excessive secretion of mineralocorticoids and sex hormones has been reported.<sup>7–15</sup> Adrenal sex hormone concentrations are high in dogs with PDH.<sup>7,14</sup> Measurement of sex hormones may increase the sensitivity of the ACTH response test in the diagnosis of PDH. High adrenal sex hormone concentrations have also been reported in dogs with noncortisol-secreting adrenocortical carcinomas.<sup>8,9,14</sup> Adrenal sex hormone concentrations in dogs with ADH have not been evaluated systematically.

The purpose of the study reported here was to determine whether dogs with ADH have higher concentrations of sex hormones than healthy dogs, dogs with PDH, and dogs with noncortisol-secreting **adrenal tumors (ATs)** without HAC; whether dogs with adrenal **adenocarcinomas (ACAs)** secrete higher concentrations of sex hormones than dogs with adrenal adenomas; and whether noncortisol-secreting ATs secrete high concentrations of adrenal sex hormones.

## Materials and Methods

**Dogs**—Four groups of dogs (all neutered) were enrolled in the study: healthy control dogs (n = 11), dogs with ADH (9), dogs with PDH (11), and dogs with noncortisol-secreting ATs (6; **Table 1**). The healthy control dogs, owned by staff and students of the **Purdue University Veterinary Teaching Hospital (PUVTH)**, were age- and sex-matched to dogs with PDH. Dogs were determined to be healthy on the basis of results of physical examination, history, CBC, serum biochemical profile, and urinalysis. All dogs were fully vaccinated and had negative results for heartworm antigen. An ACTH stimulation test was performed, and cortisol and adrenal sex hormone concentrations (androstenedione, estradiol, progesterone, testosterone, and 17-hydroxyprogesterone) before and after ACTH administration were measured. The study was approved by the Purdue Animal Care and Use Committee and signed informed owner permission was obtained.

Dogs with adrenal disorders were enrolled in the study after referral to the PUVTH or the University of California,

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Table 1—Variables (mean  $\pm$  SD [range] or ratio) in healthy control dogs and dogs with hyperadrenocorticism (HAC) caused by adrenal adenocarcinoma (ADH with ACA), a tumor of unknown type (ADH unknown), pituitary-dependent hyperadrenocorticism (PDH), or a noncortisol-secreting adrenal tumor (AT without HAC).

Variable	ADH with ACA	ADH unknown	PDH	AT without HAC	Control
Male:female	3:4	0:2	5:6	5:1	5:6
Age (y)	12.0 $\pm$ 1.73* (10–14)	(12–14)†	9.36 $\pm$ 2.29 (7–13)	11 $\pm$ 2 (9.2–38.9)	9.1 $\pm$ 1.86 (7–12)
Weight (kg)	14.1 $\pm$ 8.75 (7.5–30)	(5.4–33.4)†	17.3 $\pm$ 10.9 (3.8–34.5)	27.4 $\pm$ 14 (9.1–45)	30.9 $\pm$ 10.9 (9.2–48.0)

\*Significantly ( $P \leq 0.05$ ) different from other groups. †Range (other data for these dogs not included in analyses).  
To convert kilograms to pounds, multiply by 2.2.

Davis. Dogs with HAC had clinical signs; physical examination findings; and CBC, serum biochemical panel, urinalysis, and ACTH stimulation test or low-dose dexamethasone suppression (LDDS) test results consistent with a diagnosis of HAC.

A diagnosis of ADH was confirmed by finding no suppression of cortisol concentrations after administration of dexamethasone (0.01 mg/kg [0.005 mg/lb], IV), endogenous ACTH concentration  $< 10$  pg/mL, and a mass identified in the area of 1 adrenal gland via abdominal ultrasonography.<sup>4,16</sup> Dogs were classified as having ACA on the basis of results of histopathologic examination of tissue removed at surgery or necropsy or by detection of invasive or metastatic neoplasia via ultrasonography or radiography.

At least 2 of 3 criteria were required for a diagnosis of PDH including  $> 50\%$  suppression from baseline cortisol concentration at 4 or 8 hours during an LDDS or high-dose dexamethasone suppression test, an endogenous ACTH concentration  $> 12$  pg/mL, or bilaterally enlarged adrenal glands of approximately equal size detected via abdominal ultrasonography.<sup>4,16</sup>

Diagnosis of a noncortisol-secreting AT was made if the history, clinical signs, ACTH stimulation test results, and LDDS test results were inconsistent with HAC. In all instances, an adrenal mass was detected via abdominal ultrasonography. Histopathologic examination of adrenal masses was performed if possible.

**Hormone testing**—The ACTH stimulation tests were performed between 8:00 and 10:00 AM with synthetic ACTH<sup>a</sup> (0.25 mg, IM). A 10-mL sample of blood was collected from the jugular vein prior to and 1 hour after administration of ACTH and allowed to clot in a glass clotting tube. All samples were centrifuged within 30 minutes of collection and stored at  $-20^{\circ}\text{C}$  prior to analysis.

Endogenous ACTH concentration was measured with a chemiluminescent assay validated for use in dogs. Samples for measurement of endogenous ACTH concentration were collected in silicon-coated EDTA tubes, frozen immediately at  $-20^{\circ}\text{C}$ , and stored  $< 8$  hours prior to assay.<sup>17</sup> Cortisol concentrations were measured with a chemiluminescent assay validated for use in dogs.<sup>18</sup>

Plasma collected from blood obtained before and after ACTH administration was shipped overnight to the Clinical Endocrinology Service, Veterinary Teaching Hospital, University of Tennessee, for sex hormone analysis. All samples were frozen and packed with dry ice for shipping. Sex hormone analysis (androstenedione, progesterone, 17-hydroxyprogesterone, estradiol, and testosterone) was performed with a radioimmunoassay validated for dogs.<sup>19</sup>

**Statistical analyses**—All data analyses were performed with standard statistical software,<sup>b</sup> and differences were considered to be significant at  $P < 0.05$ . The 4 groups of dogs enrolled in the study were compared with respect to age, weight, pre- and post-ACTH stimulation hormone concentrations, absolute differences between pre- and post-ACTH stim-

ulation hormone concentrations, and cortisol-to-hormone ratios. The comparisons between the 4 groups were performed with nonparametric<sup>20</sup> and parametric ANOVA.<sup>21</sup> Data are presented as mean  $\pm$  SD values unless otherwise noted.

## Results

**Breed, age, and weight**—No breed was overrepresented in any of the groups. The male-to-female ratio was approximately evenly distributed except for the AT group (5 males, 1 female; Table 1). Dogs with ACA were significantly ( $P = 0.005$ ) older (median age, 12.0 years) than the healthy control dogs (median age, 8.0 years). Dogs with ACA were also older than dogs with PDH (median age, 8.0 years), but this difference was not significant. Dogs with ACA (median weight, 8.9 kg [19.6 lb]) were smaller than dogs in the other 3 groups, although this difference was not significant.

**Ultrasonographic and histopathologic findings**—Of the 9 dogs with ADH, 2 had small noninvasive adrenal tumors visualized by use of abdominal ultrasonography. Histopathologic examination was not performed on tissues from either of these dogs; therefore, it was not possible to determine whether these were adrenal adenomas or ACAs. These 2 dogs were not included in the statistical analyses.

Unilateral adrenomegaly was visualized by use of ultrasonography in 7 dogs with ADH that were classified as having ACA. The size of the contralateral adrenal gland was reported in 6 of the 7 dogs; 1 dog had a small contralateral adrenal gland, and the other 4 dogs had a normal-sized contralateral adrenal gland. One dog had large bilateral adrenal tumors with obvious invasion of the vena cava. The diagnosis of ACA was made on the basis of histopathologic findings in 4 dogs; the other 3 dogs had a presumptive diagnosis based on detection via abdominal ultrasonography of large tumors invading the caudal vena cava.

All dogs with PDH had bilaterally enlarged adrenal glands of approximately equal size as judged via abdominal ultrasonography. A necropsy was performed on 1 dog with PDH, which confirmed a microscopic pituitary adenoma.

All dogs with noncortisol-secreting ATs had evidence of unilateral adrenomegaly at the time of ultrasonography. In 3 dogs, there was evidence of invasion into the vena cava. Of the 6 dogs with noncortisol-secreting ATs, histopathologic findings confirmed a pheochromocytoma in 3 dogs and a nonfunctional ACA in 1 dog. This dog had a nonfunctional ACA because there were no clinical signs of hypercorti-

solemia, urine cortisol-to-creatinine ratio was low, and results of the ACTH stimulation test were within reference range before and after surgical removal of the tumor. Two dogs had a presumptive diagnosis of pheochromocytoma based on the presence of an adrenal mass, hypertension, and no evidence of HAC.

**Cortisol and sex hormone analyses**—One dog in the control group had slightly high basal concentrations of progesterone, 17-hydroxyprogesterone, and androstenedione, although these values were not above reference range after ACTH stimulation. Five of 10 dogs with PDH had high basal concentrations of androstenedione, and another dog with PDH also had high basal concentrations of estradiol, progesterone, and 17-hydroxyprogesterone. All 7 dogs with ACA had high basal androstenedione concentrations, and 3 of 7 had high basal progesterone concentrations. One dog with ACA and 2 of 11 dogs with PDH had high basal cortisol concentrations (Table 2).

There was wide variation between and within groups for all the adrenal sex hormones after administration of ACTH (Table 3). All dogs in the ACA group, all but 1 dog in the PDH group, and all but 2 dogs in the noncortisol-secreting AT group had 1 or more adrenal sex hormone concentrations greater than the reference range. The dog classified as having a non-functional adrenal ACA had unremarkable sex hormone concentrations before and after administration of ACTH. None of the dogs in the control group had values greater than the reference range.

**Cortisol**—Six of 7 dogs with ACA, 10 of 11 dogs with PDH, and 1 of 6 dogs with noncortisol-secreting AT had post-ACTH stimulation cortisol concentrations greater than the reference range. Further testing of the dog with hypercortisolemia in the noncortisol-secreting AT group revealed suppression of cortisol concentrations with the LDDS test, and a necropsy revealed a pheochromocytoma with no evidence of PDH or ADH. Mean cortisol concentration after administration of ACTH for the ACA group was significantly higher than for the healthy control dogs and dogs with noncortisol-secreting AT. Mean cortisol concentration for dogs

with PDH was significantly greater than that of the healthy control dogs. There was no significant difference in cortisol concentrations between the ACA and PDH groups.

**Androstenedione**—Six of 7 dogs with ACA, 8 of 10 dogs with PDH, and 4 of 6 dogs with noncortisol-secreting AT had increased androstenedione concentrations after ACTH stimulation. In dogs with ACA, mean androstenedione concentrations after administration of ACTH were significantly greater than that of the healthy control dogs and dogs with noncortisol-secreting AT. Dogs with PDH had significantly greater mean androstenedione concentration than the healthy control dogs. There was no significant difference between androstenedione concentrations in dogs with ACA, compared with dogs with PDH.

**Progesterone**—All 7 dogs with ACA, 8 of 10 dogs with PDH, 1 of 6 dogs with noncortisol-secreting AT, and 1 dog with unclassified ADH had increased progesterone concentrations after ACTH stimulation. Mean progesterone concentration after administration of ACTH in dogs with ACA was significantly greater than for the other 3 groups. The increase in progesterone concentrations after ACTH stimulation for the PDH group was significantly greater than that for the healthy control dogs.

**17-Hydroxyprogesterone**—Six of 7 dogs with ACA, 9 of 11 dogs with PDH, 4 of 6 dogs with noncortisol-secreting AT, and both dogs with unclassified ADH had 17-hydroxyprogesterone concentrations outside the reference range after ACTH stimulation. Mean 17-hydroxyprogesterone concentration after administration of ACTH in dogs with ACA was significantly greater than that of the other 3 groups. There were no significant differences among the latter 3 groups.

**Testosterone**—Two of 7 dogs with ACA had testosterone concentrations outside the reference range after ACTH stimulation. Mean testosterone concentration after stimulation with ACTH in the dogs with ACA was significantly greater than the other 3 groups.

Table 2—Basal hormone concentrations (mean  $\pm$  SD [range]) in dogs with HAC caused by ACA, PDH, and noncortisol-secreting ATs and healthy control dogs before stimulation with ACTH.

Hormone	ADH with ACA	PDH	AT without HAC	Control dogs
Cortisol (ng/mL)	53.75 $\pm$ 32.75 (24–106.7)	36.7 $\pm$ 23.26 (22.7–83.5)	15.9 $\pm$ 14.4 (2.1–42.1)	12.6 $\pm$ 14.2 (2.9–52.4)
Androstenedione (ng/mL)	9.25 $\pm$ 4.7 (4.9–18.7)	9.04 $\pm$ 10.9 (3–39)	3.25 $\pm$ 1.3 (1.4–4.8)	2.6 $\pm$ 1.90 (1.4–8)
Progesterone (ng/mL)	0.40 $\pm$ 0.28 (0.09–0.87)	0.24 $\pm$ 0.12 (0.09–0.46)	0.08 $\pm$ 0.03 (0.04–0.13)	0.09 $\pm$ 0.07 (0.02–0.27)
17-OH progesterone (ng/mL)	0.34 $\pm$ 0.23 (0.1–0.69)	0.27 $\pm$ 0.21 (0.16–0.79)	0.2 $\pm$ 0.08 (0.09–0.32)	0.18 $\pm$ 0.12 (0.07–0.5)
Testosterone (ng/mL)	0.04 $\pm$ 0.02 (0.03–0.09)	0.04 $\pm$ 0.01 (0.03–0.05)	0.03 $\pm$ 0.01 (0.02–0.04)	0.03 $\pm$ 0.01 (0.02–0.05)
Estradiol (pg/mL)	49.69 $\pm$ 10.48 (34.6–65.5)	46.5 $\pm$ 12 (39.8–70.3)	37.1 $\pm$ 14.1 (12.1–55.2)	44.5 $\pm$ 3.9 (38.8–49.7)

Table 3—Hormone concentrations (mean  $\pm$  SD [range]) in dogs with HAC caused by ACA, PDH, and noncortisol-secreting ATs and healthy control dogs after stimulation with ACTH.

Hormone	ADH with ACA	PDH	AT without HAC	Control dogs
Cortisol (ng/mL)	442.9 $\pm$ 334.3 (88.2–1149.6)	277.9 $\pm$ 92.5 (91.9–423.7)	122.7 $\pm$ 111.1 (27.2–329.2)	83.5 $\pm$ 26.9 (44.1–126.3)
Androstenedione (ng/mL)	104.17 $\pm$ 47.3 (29.5–161.6)	65.3 $\pm$ 47.7 (20–183.6)	40.9 $\pm$ 24.30 (7.2–74.8)	12.1 $\pm$ 6.56 (3.6–21.3)
Progesterone (ng/mL)	19.6 $\pm$ 21.3 (2.3–54.7)	3.2 $\pm$ 1.4 (1.03–4.9)	1.3 $\pm$ 1.1 (0.2–3.4)	0.7 $\pm$ 0.4 (0.2–1.4)
17-OH progesterone (ng/mL)	16.6 $\pm$ 13.7 (2.53–38.6)	5.1 $\pm$ 3.4 (1.53–12.8)	2.4 $\pm$ 1.2 (0.7–4.3)	1.5 $\pm$ 1.2 (0.2–4.4)
Testosterone (ng/mL)	0.2 $\pm$ 0.21 (0.05–0.54)	0.05 $\pm$ 0.019 (0.03–0.1)	0.04 $\pm$ 0.015 (0.03–0.07)	0.04 $\pm$ 0.014 (0.03–0.08)
Estradiol (pg/mL)	53.0 $\pm$ 12.8 (31.3–65.0)	44.4 $\pm$ 9.7 (29.9–58.9)	36.2 $\pm$ 12.00 (14.1–47.8)	42.3 $\pm$ 5.6 (35.1–53.1)

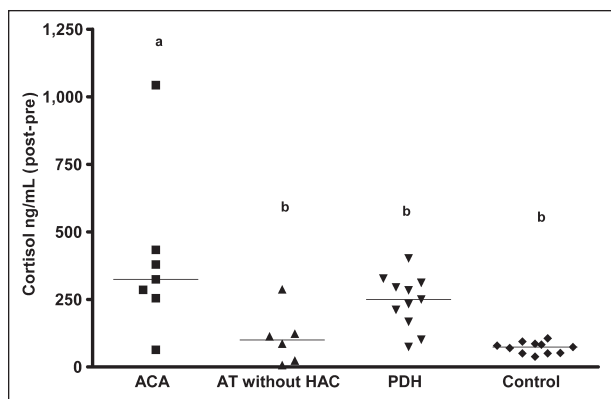


Figure 1—Dot plot of absolute differences of cortisol concentrations (concentration after ACTH stimulation minus value before ACTH stimulation [post-pre]) in 7 dogs with adrenal-dependent hyperadrenocorticism caused by adrenal adenocarcinoma (ACA), 6 dogs with noncortisol-secreting adrenal tumors (AT without hyperadrenocorticism [HAC]), 11 dogs with pituitary-dependent hyperadrenocorticism (PDH), and 11 healthy control dogs. Horizontal bars represent mean values in each group. Different lowercase letters indicate significant ( $P < 0.05$ ) differences among groups.

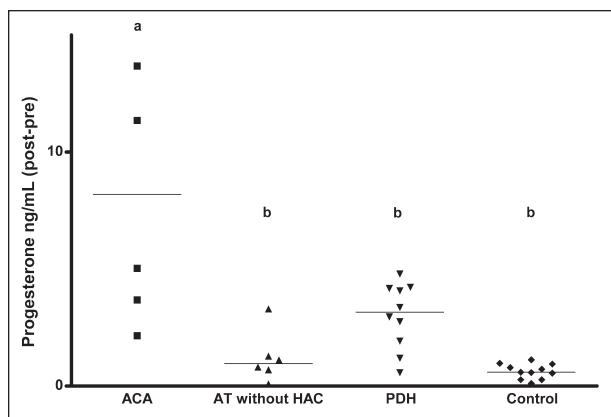


Figure 2—Dot plot of absolute differences of progesterone concentrations in dogs. See Figure 1 for remainder of key.

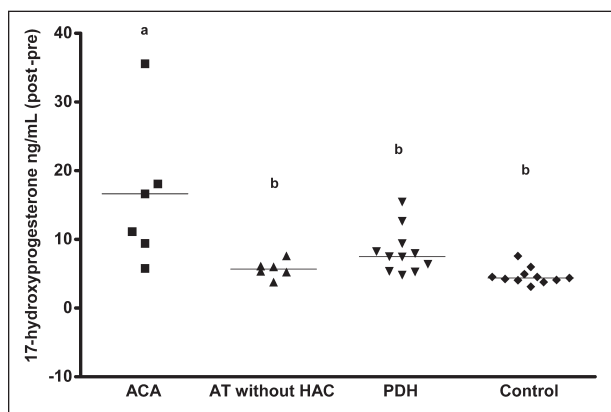


Figure 3—Dot plot of absolute differences of 17-hydroxyprogesterone concentrations in dogs. See Figure 1 for key.

**Estradiol**—Post-ACTH stimulation concentrations of estradiol were within reference range for all dogs in the study. A significant difference was not found among the groups.

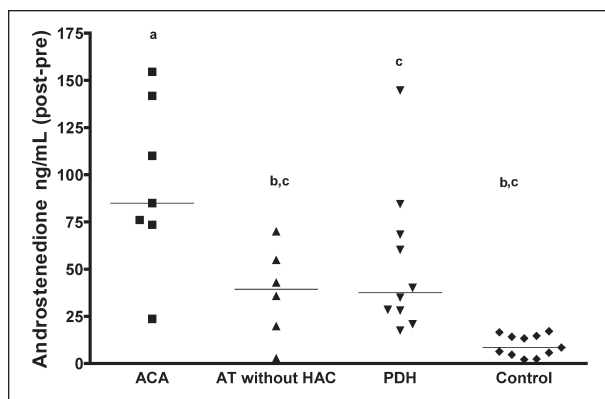


Figure 4—Dot plot of absolute differences of androstenedione concentrations in dogs. See Figure 1 for key.

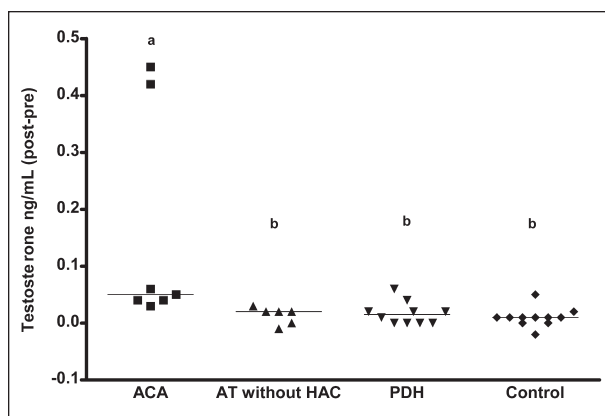


Figure 5—Dot plot of absolute differences of testosterone concentrations in dogs. See Figure 1 for key.

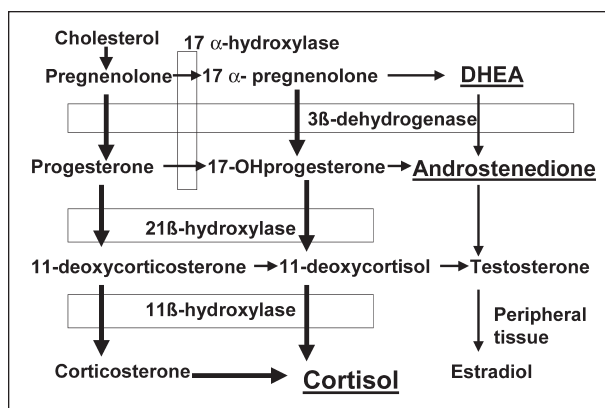


Figure 6—Schematic diagram of the steroidogenic pathway in the zona fasciculata and zona reticularis of the adrenal gland. DHEA = Dehydroepiandrosterone. The major enzymes that catalyze the reactions are listed in boxes. The major products of the adrenal gland are underlined.

**Absolute difference in hormone concentrations**—Because the basal concentrations of sex hormones among groups were all similar and concentrations after administration of ACTH varied, the absolute differences in hormone concentrations (post-ACTH administration value minus pre-ACTH administration value) were compared among the 4 groups.

For cortisol concentration, there was no significant

difference for the absolute difference between the PDH and healthy control group, although the value for the ACA group was still significantly greater than that of the AT and healthy control groups. For sex hormones, results for the absolute difference for each hormone were identical to the results for the absolute values (Figures 1–5).

Differences among groups for the cortisol-sex hormone ratio and percentage change in sex hormone concentration after ACTH administration were also compared. The results were similar to the results for the absolute values.

## Discussion

The results of this study suggest that as a group, dogs with HAC caused by adrenal ACAs secrete higher concentrations of certain adrenal sex hormones, compared with healthy control dogs, dogs with PDH, and dogs with noncortisol-secreting ATs. However, there is great variability in sex hormone concentrations among individual dogs with adrenal dysfunction. Because of the wide interdog variability and overlap among the groups, measurement of adrenal sex hormones is unlikely to be clinically useful for differentiation of individual dogs with PDH from dogs with ADH caused by an ACA. Whether the measurement of adrenal sex hormones is helpful in the differentiation of adrenal ACAs from adrenal adenomas could not be determined from this study because no dog was confirmed to have an adrenal adenoma.

Adrenal steroids are synthesized from cholesterol derived from the diet (Figure 6). Adrenal steroids contain either 19 or 21 carbon atoms. Steroids with 19 carbons (dehydroepiandrosterone sulfate [DHEAS], androstenedione, and testosterone) are termed 17-ketosteroids and have predominantly androgenic activity. Steroids with 21 carbons (17-hydroxyprogesterone, progesterone, aldosterone, and cortisol) are termed 17-hydroxycorticosteroids and have either glucocorticoid or mineralocorticoid properties.

Hyperadrenocorticism is defined as excessive production of adrenal steroids and is most commonly associated with hypercortisolemia in dogs and cats, although excess production of mineralocorticoids and sex hormones can occur. In previous reports,<sup>8-10,14,15</sup> animals with excessive concentrations of sex hormones, but cortisol concentrations within reference range, had similar clinical signs as dogs with hypercortisolemia. This suggests that sex hormones such as progesterone and 17-hydroxyprogesterone may act as glucocorticoid agonists. In several previous studies, sex hormone concentrations in dogs with adrenal dysfunction were determined. Frank et al<sup>7</sup> measured sex hormone concentrations in 11 dogs with PDH before and after stimulation with 5 µg of synthetic ACTH/kg (2.3 µg/lb) and detected an increase in 1 or more of the sex hormones in all dogs. Ristic et al<sup>14</sup> reported 11 dogs with HAC (7 with confirmed PDH, 2 with confirmed ATs) in which concentrations of 17-hydroxyprogesterone were measured before and after stimulation with 0.25 mg (IV) of synthetic ACTH. All 11 dogs had increased concentrations of 17-hydroxyprogesterone after administration of ACTH. Our study found that all dogs with ACA (7/7) and 10 of 11 dogs with PDH had an increase in 1 or more of the sex hormone concentrations after ACTH stimulation. This is in accordance with previ-

ous studies.<sup>7,14</sup> The dog with PDH that had results of an ACTH stimulation test that were within reference range also had 17-hydroxyprogesterone concentrations within reference range after stimulation with ACTH, but unfortunately, there was not enough serum available from that dog to determine progesterone and androstenedione concentrations.

Other studies have focused on dogs with atypical HAC in which cortisol concentrations are within reference range (ACTH stimulation test and LDDS test), but the dogs have clinical signs of HAC. Norman et al<sup>9</sup> measured 17-hydroxyprogesterone concentrations in 8 dogs with ATs that had cortisol concentrations within reference range after ACTH stimulation and equivocal results of an LDDS test. Two dogs with ACAs had increased concentrations of 17-hydroxyprogesterone after stimulation with ACTH. Ristic et al<sup>14</sup> also reported 13 dogs with atypical HAC (9 with PDH and 4 with ATs) and found the concentrations of 17-hydroxyprogesterone to be increased after ACTH stimulation in all dogs. Results of these studies suggest that sex hormones may be increased in dogs with PDH and ATs that have cortisol concentrations within reference range (ie, atypical HAC). In these dogs, it has been hypothesized that progestins may act as glucocorticoid agonists. Measurement of sex hormone concentrations in dogs with suspected atypical HAC may be useful to confirm the diagnosis.

The dog in our study with an ACA and results of the ACTH stimulation test within reference range had markedly increased concentrations of androstenedione, progesterone, and 17-hydroxyprogesterone after stimulation with ACTH. This dog clearly did not have suppression on the LDDS test, which is different from dogs described by Norman et al<sup>9</sup> in which interpretation of the LDDS tests was complicated because of low cortisol concentrations.

Our study focused on dogs with hypercortisolemia and ATs; however, some dogs with functional ATs and clinical signs of HAC have low cortisol concentrations but high sex hormone concentrations. The 2 dogs with adrenal ACAs reported by Syme et al<sup>8</sup> had a subnormal cortisol response to ACTH stimulation, but both dogs had increases in progesterone and 17-hydroxyprogesterone concentrations after ACTH stimulation.

The reason for the great interdog variability in dogs with adrenal ACA is not known, although it may be related to tumor size. The 2 dogs with the greatest tumor burden also had the greatest increases in progesterone, 17-hydroxyprogesterone, androstenedione, and testosterone after ACTH stimulation. This could be attributable to the tumor causing a disruption of the enzymatic metabolic pathways, which would favor sex hormone production. Deficiencies in the enzymes 21β-hydroxylase or 11β-hydroxylase have been identified in humans with adrenal ACAs.<sup>22,23</sup> Either an enzyme deficiency or disruption of the enzymatic pathway required to produce cortisol may result in accumulation of cortisol precursors and shunting into other metabolic pathways, such as androgen biosynthesis. Clinical signs in humans with these tumors are often those of virilization. Measurement of high concentrations of urinary 17-ketosteroids (a metabolite of DHEAS) confirms the diagnosis and may also be useful for differentiating adrenal

adenomas from adrenal ACAs.<sup>22,23</sup> To the authors' knowledge, there is presently no validated assay for the measurement of urinary 17-ketosteroids in dogs.

The high adrenal sex hormone concentrations in 4 dogs with ATs without HAC was an unexpected finding. Androstenedione concentrations were the most markedly increased, and the concentrations of 17-hydroxyprogesterone were just outside the reference range after stimulation with ACTH. Two of the dogs with high concentrations of sex hormones had histopathologic findings that confirmed a pheochromocytoma, whereas the other 2 dogs had a presumptive diagnosis based on clinical signs and diagnostic tests. Pheochromocytomas are tumors of the chromaffin cells of the adrenal medulla, which usually secrete catecholamines. Pheochromocytoma and adrenocortical tumors can be found together,<sup>24</sup> and it is also possible that there will be invasion of adrenocortical tissue resulting in disruption of enzymatic pathways. One human study<sup>25</sup> revealed an increase in ACTH-stimulated plasma 17-hydroxyprogesterone concentrations in patients with pheochromocytomas, which resolved after unilateral adrenalectomy. Results of that study suggested that the increase in 17-hydroxyprogesterone concentrations might be attributable to adrenocortical hyperplasia secondary to pro-opiomelanocortin-derived peptides secreted by the pheochromocytoma.

Limitations of our study included the small numbers of dogs with adrenal adenomas and the lack of histopathologic confirmation of disease in 5 dogs. We were also unable to determine whether dogs with adrenal ACAs secreted higher concentrations of adrenal sex hormones than dogs with adrenal adenomas because no dog in our study had histologic confirmation of an adrenal adenoma. It was unfortunate that at the time of the study, a validated canine assay for DHEAS was unavailable. This is the hormone most commonly measured in humans with adrenal adenocarcinomas and is the major precursor of urinary 17-ketosteroids.

Our study revealed that dogs with hypercortisolemia and ADH caused by an ACA and most dogs with hypercortisolemia and PDH had an increase in 1 or more adrenal sex hormone concentrations after ACTH stimulation. These results support quantifying the entire adrenal sex hormone panel because one particular hormone was not consistently increased after ACTH stimulation. Dogs with adrenal ACAs as a group had higher concentrations of some adrenal sex hormones after ACTH administration, but there was great variability among dogs. A larger study would be required to determine whether measurement of adrenal sex hormones could be used to differentiate adrenal ACAs from adrenal adenomas.

- Cortrosyn, Organon Inc, West Orange, NJ.
- SAS, version 8.1, SAS Institute Inc, Cary, NC.

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