Recent Advances in the Diagnosis of Cushing’s Syndrome in Dogs

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Hypercortisolism is a common condition in dogs and can be defined as the physical and biochemical changes that result from prolonged exposure to inappropriately high plasma concentrations of (free) cortisol, whatever its’ cause. This disorder is often called Cushing’s syndrome, after Harvey Cushing, the neurosurgeon who first described the human syndrome in 1932.

Cushing’s syndrome is sometimes iatrogenic, in most cases due to administration of glucocorticoids for the treatment of a variety of allergic, autoimmune, inflammatory, or neoplastic diseases. The development of clinical signs of glucocorticoid excess depends on the severity and duration of the exposure. The effects also vary among animals owing to interindividual differences in cortisol sensitivity. Corticosteroid administration causes prompt and sustained suppression of the hypothalamic-pituitary-adrenocortical axis. Depending on the dose and the intrinsic glucocorticoid activity of the corticosteroid, the schedule and duration of its administration, and the preparation or formulation, this suppression may exist for weeks or months after cessation of the corticosteroid administration.

This article focuses on the diagnosis of spontaneous hypercortisolism. In 80% to 85% of the spontaneous cases, hypercortisolism is adrenocorticotropic hormone (ACTH)-dependent, usually arising from hypersecretion of ACTH by a pituitary corticotroph adenoma. Ectopic ACTH-secretion syndrome is rare in dogs.1 The remaining 15% to 20% of cases of spontaneous hypercortisolism are ACTH-independent and result from autonomous hypersecretion of glucocorticoids by an adrenocortical adenoma or adenocarcinoma. In addition to an adrenocortical tumor, ACTH-independent hypercortisolism can be caused by bilateral (macro)nodular adrenocortical...

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hyperplasia because of aberrant adrenal expression of either ectopic or overactive eutopic hormone receptors.\textsuperscript{2–4}

**CLINICAL MANIFESTATIONS OF HYPERCORTISOLISM**

All endocrine tests used for the diagnosis of endogenous hypercortisolism entail measurement of cortisol in plasma or urine (or saliva). Regardless of which test is used, a high degree of clinical suspicion is mandatory to avoid false-positive test results. Positive test results in patients that have developed several clinical signs of Cushing’s syndrome over a relatively short period of time are more likely to be diagnostic than positive test results obtained in patients with more unusual presentations. Obviously, presentations that are more unusual require more confirmatory tests than a dog with a typical history and clear-cut physical and biochemical changes. Notably, several dogs with Cushing’s syndrome do not present the full-blown picture originally described in textbooks. Instead, they often have milder hypercortisolism with less pronounced symptomatology. Thus, making a diagnosis requires considerable clinical insight.

Spontaneous hypercortisolism is a disease of middle-aged and older dogs, although, very rarely, it may occur as early as 1 year of age. There is no gender predilection. It occurs in all dog breeds, with a slight predilection for small breeds such as dachshunds and miniature poodles. The incidence is much higher in dogs than in humans and cats and has been reported to be 1 to 2 cases per 1000 dogs per year.\textsuperscript{5}

Many of the clinical signs can be related to the biochemical effects of glucocorticoids, namely increased gluconeogenesis and lipogenesis at the expense of protein (Fig. 1). In dogs, the cardinal physical features are centripetal obesity and atrophy of muscles and skin (Fig. 2). Polyuria and polyphagia are also dominating features. The polyuria is known to be due to impaired osmoregulation of vasopressin release and interference of the glucocorticoid excess with the action of vasopressin in the

![Fig. 1](image.png)

*Fig. 1. Effects of cortisol excess. Increased gluconeogenesis leads to hyperglycemia, which is controlled initially by increased insulin secretion. This causes increased lipogenesis. Thus, the result of glucocorticoid excess is the catabolism of peripheral tissues such as muscle and skin to deliver the substrate for increased gluconeogenesis and lipogenesis.*
kidney. Abdominal palpation may reveal hepatomegaly. For a complete overview of the clinical signs related to hypercortisolism the reader is referred to the work of Galac and colleagues.6

Increased plasma alkaline phosphatase (AP) activity is a frequent finding in dogs with hypercortisolism. This is mainly because of the induction of an isoenzyme having greater stability at 65°C than other AP-isoenzymes and, therefore, easily measured by a routine laboratory procedure.7 In about 50% of dogs with hypercortisolism plasma thyroxine (T₄) is decreased as a consequence of altered transport, distribution, and metabolism of T₄, rather than due to hyposecretion. For a complete overview of the changes in routine laboratory data related to hypercortisolism the reader is again referred to the work of Galac and colleagues.6

Diagnostic imaging may help to complete the picture of the physical and biochemical changes associated with glucocorticoid excess. Although hepatomegaly and a distended urinary bladder may be seen, abdominal radiography is of little use in the diagnostic work-up of a dog suspected of having hypercortisolism. Thoracic radiographs may show bronchial and interstitial mineralization.8 Dystrophic calcification in the skin and subcutis may also be seen in the areas of predilection for calcinosis cutis. Ultrasonography, CT, and MRI are the imaging techniques used most frequently, especially in characterization of the source of the hormone excess.

**DIAGNOSIS OF HYPERCORTISOLISM**

The endocrine diagnosis of hypercortisolism depends on the demonstration of two principal characteristics of all forms of the condition: (1) increased production of cortisol and (2) decreased sensitivity to glucocorticoid feedback. Measurement of a single plasma cortisol concentration has little diagnostic value because the pulsatile secretion of ACTH results in variable plasma cortisol concentrations that may at times be within the reference range. There are two ways to overcome this problem: (1) to test the integrity of the feedback system, and (2) to measure urinary corticoid excretion.

In the first approach, the sensitivity of the pituitary-adrenocortical system to suppression is tested by administering a synthetic glucocorticoid in a dose that discriminates between healthy dogs and dogs with hypercortisolism. A potent glucocorticoid such as dexamethasone is used so that the dose will be too small to contribute significantly to the laboratory measurement. In this so-called dexamethasone screening test or low-dose dexamethasone suppression test (LDDST),

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Fig. 2. A 9-year-old dog with pituitary-dependent hypercortisolism. The hypercortisolism resulted in a thin hair coat and an enlarged abdomen. Furthermore, the dog had polyuria and polyphagia.
0.01 mg dexamethasone per kg body weight is administered intravenously (IV). Blood for cortisol measurement is collected before, and 4 hours and 8 hours after dexamethasone administration. The finding of a plasma cortisol concentration exceeding 40 nmol/L at 8 hours after dexamethasone administration, in dogs with physical and biochemical changes pointing to hypercortisolism, confirms hypercortisolism with a predictive value of a positive test result of 0.92 (and a predictive value of a negative test result of 0.59). The measurements at 0 hour and 4 hours are not needed for the diagnosis per se but may be useful in the differential diagnosis. If the plasma cortisol concentration at either 4 hours or 8 hours is at least 50% lower than the 0-hour value, the hypercortisolism is pituitary-dependent. The iv-LDDST can have a false-positive result because of stress, for example because of the hospital visit and blood collection.

This IV-LDDST is increasingly replaced by the measurement of urinary corticoids. Because urine is stored and mixed in the bladder for several hours, an integrated reflection of corticoid production is obtained, thereby adjusting for fluctuations in plasma concentrations. The urinary corticoids (largely cortisol) are related to the creatinine concentration in the urine, resulting in the urinary corticoid to creatinine ratio (UCCR). This test requires little time (from the veterinarian and the owner), is not invasive (no blood collection), and has a high diagnostic accuracy. In addition, the test procedure has the advantage of combining a test for basal adrenocortical function and a dynamic test for differential diagnosis (see below). To avoid the influence of stress, the urine for the UCCR determination has to be collected at home, at least 1 day after the visit of the veterinary clinic. Nonadrenal disease may also result in endogenous stress and elevated cortisol secretion and, therefore, high UCCRs in dogs that do not have a high degree of clinical suspicion should be interpreted with care. The owner collects a morning urine sample on 2 consecutive days and the UCCRs in these two samples are averaged. In our laboratory, the basal UCCR in healthy pet dogs varies from 0.3 to 8.3 × 10⁻⁶. In dogs with physical and biochemical changes pointing to hypercortisolism the predictive value of a positive test result is 0.88 and that of a negative test result is 0.98. In some dogs there is considerable day-to-day variation in the UCCR, which in mild forms of hypercortisolism occasionally leads to UCCRs just within the reference range, whereas collections on other days might have revealed one or two elevated UCCRs. The uncertainty can be resolved by measuring the UCCR in urine samples collected on 10 consecutive days.

In dogs in which results of the UCCR or the IV-LDDST have been inconclusive or negative but in which there is still suspicion of hypercortisolism, an oral LDDST may be performed. The owner collects urine at 8.00 hours (at home) for measurement of the UCCR. After collection of the urine sample, the owner administers 0.01 mg dexamethasone per kg body weight orally. The dog is walked at 12.00 hours and 14.00 hours to empty its bladder and the second urine sample is collected at 16.00 hours for measurement of UCCR. In seven healthy pet dogs, the UCCR at 16.00 hours was less than 1.0 × 10⁻⁶. In dogs with mild pituitary-dependent hypercortisolism, the UCCR following dexamethasone was greater than 1.0 × 10⁻⁶.

Another popular test to screen for hypercortisolism is the ACTH stimulation test. The main indication for the ACTH stimulation test is to test the adrenocortical reserve capacity; that is, to diagnose primary or secondary adrenocortical insufficiency. Thus, the ACTH stimulation test can be used very well to diagnose iatrogenic hypercorticism. In cases of spontaneous hypercortisolism, ACTH stimulation may result in an exaggerated adrenal response; that is, a higher plasma cortisol concentration than in healthy dogs. About 85% of dogs with pituitary-dependent hypercortisolism have exaggerated cortisol responses to ACTH, while only about 55% of dogs with...
hypercortisolism due to adrenocortical tumor have such a result. The main advantages of the ACTH stimulation test are its simplicity and the short duration of the test. However, the diagnostic accuracy for hypercortisolism of this test is less than that of the UCCR and the LDDST. Therefore, this test is no longer recommended in the diagnostic approach of dogs with hypercortisolism.

When hypercortisolism has been confirmed, it is necessary to distinguish between the different forms of the disease.

**PITUITARY-DEPENDENT HYPERCORTISOLISM**

In most cases, ACTH-dependent hypercortisolism arises from hypersecretion of ACTH by a pituitary corticotroph adenoma. The ACTH excess may originate in both the anterior lobe and the pars intermedia of the pituitary gland. In about 75% to 80% of cases, there is an adenoma in the anterior lobe. Despite decreased sensitivity to glucocorticoid feedback, the hallmark of Cushing’s syndrome, (a high dose of) dexamethasone can suppress ACTH secretion in most dogs with pituitary-dependent hypercortisolism (PDH) due to a corticotroph adenoma in the anterior lobe.

In about one-fourth to one-fifth of cases there is a corticotroph adenoma in the pars intermedia. This is of clinical interest, not only because the pars intermedia tumors tend to be larger than anterior lobe tumors, but also because of the specific hypothalamic control of hormone synthesis in the pars intermedia. The pars intermedia is under direct neural control, principally tonic dopaminergic inhibition, which suppresses the expression of glucocorticoid receptors. This explains why PDH of pars intermedia origin is resistant to suppression by dexamethasone. In other forms of spontaneous hypercortisolism, the hypersecretion of cortisol is not dependent on pituitary ACTH and is therefore also not influenced by the administration of dexamethasone.

The impaired sensitivity to glucocorticoid feedback in PDH due to an anterior lobe tumor can be demonstrated by performing a high-dose dexamethasone suppression test (HDDST). Two procedures are used, one employing plasma cortisol and the other employing the UCCR. In both, a decrease of more than 50% from baseline values confirms PDH. For the IV-HDDST, blood for measurement of plasma cortisol concentrations is collected immediately before and 3 to 4 hours after intravenous administration of 0.1 mg dexamethasone per kg body weight. When UCCRs are used, the owner has to administer three oral doses of dexamethasone (0.1 mg per kg body weight) at 8-hour intervals after collection of the second basal urine sample (see above). As mentioned earlier, the urine samples should be collected by the owner at home under conditions free of stress.

When PDH has been proven, the pituitary gland can be detected by CT or MRI. Pituitary imaging is necessary if either hypophysectomy or pituitary irradiation is to be used for treatment, but also provides information with regard to the prognosis. Dynamic contrast-enhanced CT facilitates contrast enhancement of the neurohypophysis and the adenohypophysis. Absence of the pituitary flush indicates atrophy of the neurohypophysis due to compression by a pituitary tumor. Displacement or distortion of the pituitary flush in the early phase of dynamic CT can be used to identify and localize microadenomas originating from the anterior lobe or pars intermedia in dogs.
HYPERCORTISOLISM DUE TO AN ADRENOCORTICAL TUMOR

Hypersecretion of cortisol by an adrenocortical tumor (AT) cannot be suppressed by administration of dexamethasone. As indicated by either plasma cortisol concentration or the UCCR, resistance to suppression by a high dose of dexamethasone is, with similar probability, due to AT or dexamethasone-resistant PDH.\(^{19}\) In some dogs with a cortisol-secreting AT, dexamethasone causes a paradoxical rise in both the UCCR and plasma cortisol.\(^{19}\) Hypercortisolism due to AT can be differentiated from nonsuppressible forms of PDH by measuring the plasma ACTH concentration. In addition, an AT is often readily detected by ultrasonography. Hence, it is common practice in cases of nonsuppressible hypercortisolism to measure the plasma ACTH concentration and to perform ultrasonography of the adrenal glands. If an AT is found, ACTH measurement is still useful. Plasma ACTH concentrations should be low. If not, further studies are warranted to determine if PDH is also present.\(^{23}\)

A recent study showed that intravenous administration of \(4 \text{ mg} \) desmopressin (DDAVP) did not increase plasma cortisol concentration in seven dogs with AT, whereas 75% of 46 dogs with PDH had increases in plasma cortisol concentrations of more than 10% compared with baseline concentrations.\(^{24}\) The results of this study suggest that a desmopressin stimulation test may be a useful tool in differentiating PDH from AT, but additional dogs with AT must be tested before this test can be recommended in clinical practice.

The preferred procedures for imaging of the adrenal glands are MRI and CT. Ultrasonography is less expensive, requires less time and usually no anesthesia, and so it is often used first even though it is more difficult to perform and to interpret than CT or MRI. Ultrasonography provides a good estimate of the size of the tumor and may reveal information about its expansion.\(^{25,26}\) Because it is sometimes difficult to distinguish between (macro)nodular hyperplasia and AT by ultrasonography, CT or MRI may be needed. Most ATs are unilateral solitary lesions, the two glands being affected about equally, but bilateral tumors occur in approximately 10% of cases.\(^{26–28}\)

When an AT has been confirmed, the possibility of distant metastases should be considered. During abdominal ultrasonography, the liver should be examined for metastases. If possible metastases are found, ultrasound-guided biopsy can be performed. Thoracic radiography or a CT scan of the thorax should be performed to exclude metastases in the lungs.

HYPERCORTISOLISM DUE TO ECTOPIC ACTH SECRETION

Ectopic ACTH hypersecretion has been documented in an 8-year-old German shepherd dog.\(^{1}\) The UCCRs and plasma ACTH concentrations were very high and not suppressible with dexamethasone. These findings were initially interpreted as being consistent with PDH. However, histologic examination of the tissue removed by transsphenoidal hypophysectomy did not confirm the presence of an adenoma. Within 2 weeks after hypophysectomy the clinical manifestations were exacerbated and both the UCCR and plasma ACTH concentration were further increased. CT of the abdomen revealed a tumor in the region of the pancreas. Laparotomy revealed a 5-mm nodule in the pancreas, a 3-cm metastasis in an adjacent lymph node, and metastases in the liver. Partial pancreatectomy and excision of the lymph node were performed, and a neuroendocrine tumor with metastasis in the lymph node was diagnosed by histopathology. Based on this report, ectopic ACTH secretion should be considered in cases of severe hypercortisolism in which plasma ACTH concentrations are very high and are not suppressible with high doses of dexamethasone, and in which diagnostic imaging does not reveal a pituitary tumor. In patients
with PDH, intravenous administration of 1 μg corticotropin-releasing hormone (CRH) per kg body weight results in a significant increase in plasma concentrations of ACTH and cortisol; but in patients with ectopic ACTH secretion CRH does not increase these plasma hormone concentrations. The neuroendocrine tumor causing the ectopic ACTH syndrome may be detected by a whole-body scan, but in human patients with ectopic ACTH syndrome the tumors are frequently small and often not found. Based on reports of individual cases in which ectopic ACTH secretion may have caused hypercortisolism, the condition may not be extremely rare in dogs.

HYPERCORTISOLISM DUE TO ECTOPIC OR HYPERACTIVE EUTOPIC ADRENOCORTICAL RECEPTORS

In addition to autonomous cortisol secretion by an AT, ACTH-independent hypercortisolism can also be caused by aberrant adrenal expression of either ectopic or over-expressed eutopic hormone receptors. Most of these hormone receptors belong to the superfamily of G protein-coupled receptors. In humans, various adrenocortical membrane-bound receptors functionally coupled to steroidogenesis have been reported, including glucose-dependent insulinotropic polypeptide (GIP), catecholamine, vasopressin, serotonin, and luteinizing hormone receptors.

In a recently published case report of a dog with food-dependent hypercortisolism, the ACTH-independent hypercortisolism was most likely due to aberrant adrenocortical expression of GIP receptors. The hormone GIP is secreted in the gastrointestinal tract in response to a meal and normally serves to enhance postprandial insulin secretion. In human patients with aberrant adrenocortical expression of GIP receptors, a meal not only results in augmented insulin secretion but also in increased steroidogenesis. The dog described in the case report had clinical manifestations of hypercortisolism and slightly elevated UCCRs. Basal and CRH-stimulated plasma ACTH concentrations were low, but diagnostic imaging did not reveal an adrenocortical tumor. Basal and CRH-stimulated plasma ACTH concentrations were low, but diagnostic imaging did not reveal an adrenocortical tumor. Ingestion of a meal resulted in significant increases in plasma cortisol concentration and UCCR. Consistent with the diagnostic criteria for food-dependent hypercortisolism in humans, IV administration of 3 μg octreotide per kg body weight completely prevented the meal-induced hypercortisolemia. The dog had a good clinical response to medical treatment with trilostane, administered shortly before the main meal.

Thus, a distinct increase in UCCR and plasma cortisol concentration after ingestion of a meal, low or undetectable plasma ACTH concentrations, and prevention of a meal-induced rise in plasma cortisol concentration by octreotide administration strongly suggest food-dependent hypercortisolism.

SUMMARY

The recognition of new causes of hypercortisolism, such as ectopic ACTH secretion and food-dependent hypercortisolism, and changes in technology, such as advances in imaging procedures, have reshaped the diagnostic scenario. An array of tests is available for the diagnosis of Cushing’s syndrome, but once the diagnosis of hypercortisolism is made considerable expertise is still required to determine its cause, to allow selection of the best treatment, and to avoid misdiagnosis.

REFERENCES


