

ATYPICAL CUSHING'S DISEASE: IS IT REALLY ATYPICAL?

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WHAT IS ATYPICAL CUSHING'S DISEASE?

In 2002, clinical researchers from the UK described the first cases of what is often referred to as atypical Cushing's disease (1). Dogs with atypical Cushing's disease (ACD) have a history, physical examination findings, CBC, biochemical chemistry profile and urinalysis results, and adrenal imaging consistent with hyperadrenocorticism (HAC), however, routine screening tests (i.e., the low-dose dexamethasone suppression test (LDDST) and ACTH response test are both negative. In addition, one or more sex steroids are elevated (e.g., androstenedione, progesterone, or 17-hydroxyprogesterone [17-OHP]) following administration of ACTH. The UK study evaluated 24 dogs with HAC; the cause of the HAC was not determined in 2 dogs. In the remaining 22 dogs, pituitary-dependent Cushing's disease (PDH) was diagnosed in 16 dogs and 6 dogs had an adrenocortical tumor (AT). Of the 16 dogs with PDH, 3 dogs had ACD; of the 6 dogs with an AT, 4 dogs had ACD. All the dogs with ACD had elevated post-ACTH 17-OHP concentrations. In addition, all dogs in this study treated with standard therapeutic regimens of trilostane or mitotane showed resolution of clinical signs.

ROUTINE DIAGNOSTIC TESTING FOR CLASSIC HAC: WHAT ARE THE

EXPECTATIONS? The 2012 ACVIM Consensus Statement regarding the diagnosis of the classical form of spontaneous canine HAC considers the LDDST the screening test of choice (2). The reported sensitivity and specificity of the LDDST ranges from 85 to 100% and from 44 to 73% respectively (2). The ACTH response test, because of its poor sensitivity, is considered diagnostically inferior to the LDDST as a screening test for spontaneous HAC. For dogs with AT and PDH the sensitivity is 57-63% and 80-83% respectively and the specificity ranges between 50 and 93% (2). While neither screening test is 100% accurate, most dogs with spontaneous HAC have one positive screening test. For example, in a series of 64 dogs with HAC, no dog was negative on both screening tests, but such cases clearly exist (3). **Summary impact point:** *The majority of dogs with classical Cushing's disease screened with an ACTH response or LDDST and will have at least one positive test.*

ARE NONCORTISOL STEROID HORMONES ONLY ELEVATED IN DOGS WITH ATYPICAL CUSHING'S DISEASE?

In addition to the UK study where only one noncortisol steroid hormone (NCSH) was determined, there are four relatively large published studies that have evaluated multiple NCSH (sex steroids and other cortisol precursors) and cortisol concentrations post-ACTH in dogs diagnosed with HAC (4, 5, 6, 7). The diagnosis of HAC was based on results of history, clinical signs, routine biochemistry and urinalysis findings, assessment of adrenal ultrasound imaging, a positive ACTH response test and/or LDDST, and in some cases an endogenous ACTH level. In two of these studies dogs with adrenal tumors and dogs suspected of HAC but diagnosed with another condition were also included (4, 5). Overall, when the information from all these studies is combined, 76 dogs were diagnosed with HAC and 59 of these dogs had PDH. The remaining 17 dogs had either an adrenal cortical adenoma or carcinoma or the HAC was unclassified. In addition, and most importantly, one or more noncortisol adrenal steroid hormones were elevated in all but one dog diagnosed with HAC and 42-50% of dogs suspected of having HAC but ultimately diagnosed with a nonadrenal illness. The question often arises why noncortisol adrenal steroids are commonly elevated in dogs with HAC and nonadrenal illness. The rate-limiting step in the synthesis of cortisol and the major site

of action of ACTH is the conversion of cholesterol to pregnenolone. It is therefore not surprising that *any* disease causing increased concentration of ACTH results in increased concentration of cortisol precursors as well as cortisol itself, and other NCSH. **Summary impact point:** *Noncortisol steroid hormones are commonly elevated in dogs with classic HAC and nonadrenal illness and therefore, elevated levels are not specific for ACD.*

WHY IS THE ACTH RESPONSE AND LDDST NEGATIVE IN DOGS WITH ATYPICAL CUSHING'S DISEASE?

Since dogs with classical HAC usually have one positive screening test, several questions regarding ACD are often asked. 1) *Is ACD the result of dogs with classical HAC that just happen to have a normal LDDST and ACTH response test at the testing time?* 2) *is ACD really classical Cushing's in disguise because the current screening tests lack sensitivity and reference ranges and cut-offs need to be re-evaluated?* Or 3) *is ACD a distinctly different syndrome that shares similarities to unusual adrenal disorders in people such as congenital adrenal hyperplasia or food-dependent and luteinizing hormone-dependent HAC which are caused by an aberrant expression of functional hormone receptors in adrenocortical tissue?*

Current reference ranges and cut-off levels may be misleading and need to be re-evaluated.

Because the screening tests for Cushing's disease were introduced in the 1970's and 1980's, the 2012 ACVIM consensus statement for diagnosing Cushing's disease suggests that the current reference ranges and cut-offs should be re-evaluated to avoid a misdiagnosis (2). In addition, the original clinical trials to evaluate screening tests were performed at referral centers with high disease prevalence, but today the tests are often performed in first opinion clinics with low disease prevalence. Moreover, the incidence of mild cases of HAC appears to have increased over time, possibly because of increased awareness and earlier patient presentation. Milder cases would be expected to have lower cortisol hypersecretion and an increased sensitivity of the HPA axis to dexamethasone.

Cut-offs and the LDDS: The cut-off for the 8 hour cortisol following the administration of a low dose of dexamethasone can be quite variable from one veterinary laboratory to another. For example, the cut-off for the 8 hour cortisol at Antech Diagnostics, Michigan State University, and UC Davis ranges from 1.4 ug/dl, 1 ug/dl, and 0.6 ug/dl respectively. To further add to the confusion, an "inverse pattern" in which the cortisol concentration 8 hours after dexamethasone administration is below the cut-off value, but the cortisol concentration 4 hours post-dexamethasone is above the cut-off has been described in 5 dogs with PDH (8). Because this pattern is highly suspicious for HAC, further testing should be pursued.

Cut-offs and the ACTH response test: At Antech Diagnostics and Michigan State University diagnostic laboratories a post-ACTH cortisol concentration above 20 ug/dl is consistent with HAC, while at the University of Tennessee Endocrine diagnostic laboratory the reference range for cortisol in healthy dogs is different depending on the whether the dog is a female or an intact or neutered male. For example, a post-ACTH cortisol > 10.85 ug/dl in an intact male is consistent with HAC, while a post-ACTH cortisol > 10.85 but < 17.5 ug/dl would be considered normal for an intact or spayed female and therefore not consistent with HAC. **Summary impact point:** *The cut-off values previously established for screening tests may be misleading, especially with milder cases and cases worked up at clinics with low disease prevalence. In other words, because the cut-offs are too high, ACD may be in actuality misdiagnosed cases of classic Cushing's disease.*

CONGENITAL ADRENAL HYPERPLASIA, FOOD-DEPENDENT HAC, AND LH-DEPENDENT HAC: ARE THESE DISORDERS VARIANTS OF ACD?

What is congenital adrenal hyperplasia? Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders in people characterized by impaired synthesis of cortisol, aldosterone, or both (9). The most common form of CAH is caused by mutations in CYP21A2, the gene encoding the adrenal steroid 21-hydroxylase enzyme. This enzyme converts 17-OHP to 11-deoxycortisol and progesterone to deoxycorticosterone, which are the respective precursors of cortisol and aldosterone. When the pituitary gland senses relative cortisol insufficiency it releases ACTH which stimulates the adrenal cortex to produce more cortisol. However, patients with CAH have insufficient amounts of the enzyme 21-hydroxylase, needed to convert a precursor molecule (i.e., 17-OHP) into cortisol. As a result, the pituitary gland continues to sense the need for cortisol and secretes more ACTH. This leads to an overabundance of 17-OHP, which is converted in the adrenal cortex into excess androgens (e.g., androstenedione). **Summary impact point:** *Because ACD in dogs is often associated with adrenal hyperplasia, elevated 17-OHP and androstenedione concentrations, and occasionally low to low-normal cortisol concentrations post-ACTH administration, it has been suggested that some cases of ACD may be a congenital adrenal hyperplasia-like syndrome.*

What is food-dependent Cushing's disease? This disorder is an ACTH-independent form of HAC that was first described in people in 1992 (10,11) and reported in the dog in 2008 (12). In humans, various adrenocortical membrane-bound receptors, functionally coupled to steroidogenesis, have been reported, including gastric inhibitory peptide (GIP), catecholamine, vasopressin and luteinizing hormone (LH) receptors (13). In the dog, both normal and neoplastic adrenal tissue has also been shown to express aberrant LH and GIP receptors (14). With food-dependent Cushing's syndrome, the aberrant expression of functional adrenocortical GIP receptors causes "food-induced" cortisol production. The dogs with food-dependent Cushing's disease have signs and symptoms consistent with HAC, ultrasound evidence of bilaterally enlarged adrenal glands, normal ACTH response and LDDS tests, *suppressed* endogenous ACTH levels, and an increase in the urine cortisol:creatinine ratio > 50% following a meal (12). Interestingly, once considered a rare disorder, food-dependent Cushing's disease in the dog is seen on a regular basis by the endocrinology service at the veterinary school in Utrecht, Netherlands (15).

Summary impact point: *Food-dependent Cushing's syndrome, similar to cases of adrenocortical neoplasia, is an ACTH-independent variant of ACD.*

LH-dependent hyperadrenocorticism. Several cases of hypercortisolism due to aberrant LH receptors have been reported in women; all were rare examples of ACTH-independent bilateral macrodular hyperplasia (16,17). The first documented case of LH-dependent HAC was identified in a woman with transient Cushing's syndrome during sequential pregnancies (16). Persistent Cushing's syndrome developed only after the postmenopausal sustained increase of LH secretion. Administration of the long-acting gonadotropin-releasing hormone (GnRH) agonist, leuprolide acetate, resulted in suppression of endogenous LH and normalization of cortisol production. In addition to LH-independent hypercortisolism, virilization and feminizing LH-dependent adrenal tumors have been reported in humans (18,19,20). In neutered pet ferrets LH-dependent hyperadrenocorticism also occurs (21). But unlike the syndrome in people, this disorder in ferrets is usually associated with excess secretion of NCSH (e.g., 17-OHP androstenedione, and/or estradiol) and is characterized by unilateral or bilateral neoplasia or hyperplasia of adrenocortical tissue that expresses functional LH receptors (21). Clinically, these hormonal changes lead to symmetrical alopecia, vulvar swelling in neutered females, and recurrence of sexual behavior in neutered males (22-26). In addition, although uncommon,

polyuria, polydipsia and polyphagia related to the co-occurrence of hypercortisolism and excess NCSH has been reported (27). **Summary impact point:** *Because some dogs with ACD treated with melatonin are reported to undergo resolution of clinical signs consistent with Cushing's syndrome, it is tempting to speculate that these dogs may have LH-dependent hyperadrenocorticism that responded to the LH suppressive effects of melatonin.*

TREATMENT CONSIDERATIONS FOR ATYPICAL CUSHING'S DISEASE

To date there are several medical treatment options for ACD which include melatonin and lignans, trilostane, ketoconazole, and mitotane. With food-dependent Cushing's syndrome trilostane administration two hours before a meal has proved to be an effective treatment (12). In animals, with suspected or confirmed LH-dependent hyperadrenocorticism, treatment with GnRH agonists such as leuprolide acetate, and deslorelin has been shown to be very effective (28,29). With regard to the use of trilostane therapy for ACTH-dependent and ACTH-independent variants of ACD, it is not known whether high concentrations of cortisol precursors or other noncortisol steroid hormones which may increase during therapy contribute to the clinical signs of HAC. In previous studies it was demonstrated that during treatment with trilostane concentrations of 17-OHP, a cortisol precursor, did not change (30). Treatment control was achieved by suppressing post-ACTH cortisol levels into a well defined range where cortisol concentrations were highly correlated with the results of treatment (1, 31, 32). This high correlation and the fact that other cortisol precursors that were measured did not decrease, suggests that noncortisol steroid hormones are unlikely to be associated with clinical signs of the disease. Unfortunately, the treatment for ACD, especially with trilostane, is fraught with confusion. It is a common belief that because trilostane increases 17-OHP concentrations it is not the preferred treatment, and may in fact have limited efficacy with cases of ACD. Despite these beliefs, most dogs treated with trilostane have resolution of clinical signs. However, trilostane may not be the treatment of choice in dogs, especially in Scottish Terriers, that have extremely high concentrations of both androstenedione and 17-OHP. It is theorized that these dogs as adults have a congenital adrenal hyperplasia-like syndrome whereby elevated 17-OHP acts as an androgen precursor and fuels the rise of androstenedione which may be a risk factor for hepatocellular carcinoma. In these cases, mitotane may be the preferred treatment because it will consistently lower both androstenedione and 17-OHP concentrations.

SUMMARY

The major question is the following - is atypical Cushing's disease a distinctly different disorder than classic Cushing's disease? Interestingly, only 14 cases of atypical Cushing's have been reported in the veterinary literature and the majority of cases are ATs (1,33,34,35). Published and unpublished data would indicate that it is not uncommon for ATs to be associated with excessive sex steroid production and normal screening tests. In other words, many ATs often fit the description of ACD, but this description can be considered "normal" for an adrenocortical tumor, especially an adrenal carcinoma. Of even more interest is the expression of 21-hydroxylase is normal in ATs, and, therefore, a deficiency of 21-hydroxylase is not the cause of excessive steroid production, at least in ATs (15). **Summary impact point:** *Some ATs fit the description of ACD, but this presentation is not uncommon for ATs, and therefore, would not be considered atypical.*

Primary bilateral adrenal hyperplasia caused by aberrant expression of hormone receptors in the adrenal cortex is a relatively new, albeit uncommon, form of Cushing's syndrome reported in the dog which fits the definition of ACD. The primary example is food-induced Cushing's

syndrome. These cases present with obvious signs of HAC, negative screening tests, suppressed endogenous ACTH concentrations, and symmetrically enlarged adrenal glands.

A theory for the overdiagnosis of ACTH-dependent ACD is the cut-off levels for the current endocrine screening tests are simply too high, especially in mild cases. In other words, some or possibly many cases of classic Cushing's disease are misdiagnosed as ACD. And lastly, the possibility that some cases of ACD are a form of a congenital adrenal hyperplasia-like syndrome is interesting, but unclear at this time. To prove or disprove this theory, adrenal glands from dogs with ACD, preferably not treated with mitotane or trilostane, would have to be tested for deficiencies of the various enzymes involved in the cortisol biosynthetic pathways.

And finally, and maybe most important, no matter what the underlying cause of ACD, medical therapy with trilostane or mitotane is efficacious in most cases.

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