

## Frequently Asked Canine and Feline Adrenal and Thyroid Questions at the Atlantic Veterinary College Endocrinology Laboratory

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### **1. Which is the better screening test for diagnosis of canine hyperadrenocorticism - the ACTH stimulation test or the low dose dexamethasone suppression test? What about a urine cortisol / urine creatinine ratio?**

Choosing screening tests to diagnose canine hyperadrenocorticism can be challenging. All have the potential for false positive results from stress caused by non-adrenal illness<sup>1,2</sup>, so it is important to only embark on testing if the clinical suspicion for hyperadrenocorticism is high. Here are some of the advantages and disadvantages of commonly used tests:

#### *The ACTH Stimulation Test*

The ACTH stimulation test will identify ~50-60 % of dogs with adrenal cortical tumors and ~85 % of dogs with pituitary dependent hyperadrenocorticism (PDH)<sup>3,4</sup>. Therefore, it is a good screening test if positive, but a negative result does not rule out hyperadrenocorticism. Of the screening tests for hyperadrenocorticism in dogs, the ACTH stimulation test had the highest specificity and thus the fewest false-positive results in one study<sup>5</sup>. That being said, an exaggerated response to ACTH stimulation is frequently seen in stressed dogs<sup>1</sup>. The degree of exaggeration is usually mild to moderate, but can be marked and in the range easily compatible with hyperadrenocorticism, making sound clinical judgement critical.

The ACTH stimulation test is safe, relatively inexpensive (if the 5 ug/kg dose is used) and provides baseline data prior to medical therapy. It is the only test useful in the diagnosis of iatrogenic hyperadrenocorticism. An ACTH stimulation test is therefore particularly useful if you are dealing with a new client or are unsure of what medications the animal has been given. As well, it is the recommended test for evaluation of therapeutic response to mitotane<sup>6</sup>, ketoconazole<sup>7</sup> and trilostane<sup>6</sup>.

Breed and individual variability to ACTH stimulation have been observed. At the Atlantic Veterinary College (AVC), a post-ACTH cortisol concentration between 231-571 nmol/L is normal in dogs, and a concentration above 700 nmol/L tends to support hyperadrenocorticism, but severe stress can also result in values this high. Concentrations between 571-700 nmol/L can be compatible with either stress or hyperadrenocorticism, so are more difficult to interpret. These are not “hard and fast” rules and results, as always, need interpretation in view of all other patient information.

#### *Low Dose Dexamethasone Suppression Test*

The low dose dexamethasone suppression test (LDDST) is much more sensitive than the ACTH stimulation test in showing abnormal results in dogs with hyperadrenocorticism<sup>4</sup>. It is diagnostic in close to 100 % of

dogs with adrenal tumors and over 97 % of dogs with PDH<sup>8</sup>. It is a longer test (takes 8 hours as opposed to 1-2 hours for the ACTH stimulation test) and doesn't provide any baseline information for monitoring therapy. Also, stress interferes with this test<sup>1</sup>, usually to a more severe degree than for the ACTH stimulation test<sup>9</sup>. The LDDST thus has a considerably lower specificity than the ACTH stimulation test<sup>4</sup>. In fact, some authors strongly recommend that a diagnosis of hyperadrenocorticism should never be based on a LDDST only, especially in a dog who could be stressed with non-adrenal disease<sup>4</sup>. However, if there is strong clinical support for hyperadrenocorticism, no other illnesses are obvious and only 1 test can be chosen due to client finances, this test is often preferred over the ACTH stimulation test. The LDDST cannot be used to diagnose iatrogenic hyperadrenocorticism. Occasionally, it can differentiate PDH from adrenal neoplasia, acting as a discriminatory test without the need to proceed to a high dose dexamethasone suppression test.

#### *Combination testing: looking at both the ACTH Stimulation Test and the LDDST*

Older references note a combination of a high-dose dexamethasone suppression test and an ACTH stimulation test on the same day. This combination protocol was also called the "V" test<sup>10</sup>. This protocol was developed in the hope of both identifying hyperadrenocorticism and then discriminating between PDH and adrenal neoplasia with a one-day test. This test has been found to be unreliable as a discriminatory test<sup>4,11</sup> and is less reliable as a screening test than a LDDST performed alone, so doing this test is strongly discouraged.

If performing both an ACTH stimulation test and a LDDST, they therefore must be done on separate days. It is ideal to perform the ACTH stimulation test on the first day, followed by the LDDST on the second day. If the LDDST is done first, at least 2-3 days should elapse before performing an ACTH stimulation test, so that the dexamethasone is cleared from the body and the adrenal suppressive effects have had a chance to diminish. In the case of a high dose (0.1 mg/kg) dexamethasone suppression test, at least 3-4 days should elapse before proceeding with ACTH stimulation testing<sup>12</sup>.

#### *Urine cortisol / urine creatinine ratio*

Urine cortisol / urine creatinine ratios have been evaluated as a screening test for hyperadrenocorticism in dogs. Dogs with this condition do excrete more cortisol in urine than normal. The creatinine component of the ratio allows for correction of urine concentration. While increased cortisol / creatinine ratios are consistently seen in dogs with hyperadrenocorticism<sup>13</sup>, many other illnesses or conditions associated with stress also increase this ratio, often to the same degree<sup>14,15</sup>. Therefore, this test has high sensitivity but low specificity. Of the screening tests, the urine cortisol / creatinine ratio is considered most frequently abnormal in stressed dogs without hyperadrenocorticism, in some cases as high as in 76 % of such patients<sup>2</sup>. While it can be useful for ruling out hyperadrenocorticism if the ratio is normal<sup>4</sup>, a high urine cortisol / urine creatinine ratio never allows diagnosis of hyperadrenocorticism.

#### *Extra information and final thoughts:*

Diagnosis of hyperadrenocorticism is often not an easy task, even when both an ACTH stimulation test and LDDST are done. Some dogs with this condition show abnormal results with one, the other, neither or both tests! If an animal has clinical signs suggestive of hyperadrenocorticism but negative results on both a

LDDST and an ACTH stimulation test, it is ideal to evaluate the animal for other conditions. If supportive signs continue, repeating the testing in 1-2 months is warranted.

Another confusing situation occurs when both a LDDST and an ACTH stimulation test are performed but yield opposite results (normal on one test and abnormal on the other test). Clinical judgement then becomes critical. Based on the animal's history and clinical signs, a decision must be made whether to retest in 1-2 months or to use other modalities such as ultrasonography to aid in diagnosis.

As any illness causing stress can alter results, testing for hyperadrenocorticism is usually inappropriate if the patient has other significant illnesses, as the chance of false positives are high<sup>16</sup>. It is best to treat the patient for the obvious condition and delay pituitary-adrenal axis testing. If it is unclear clinically if the patient has another illness versus possible hyperadrenocorticism, testing can be embarked upon but extreme caution should be used when interpreting the results. A challenging situation like this is that of a poorly controlled diabetic patient who may or may not have underlying hyperadrenocorticism. As it is likely that the stress of the diabetes mellitus will affect results of pituitary-adrenal axis testing, testing for hyperadrenocorticism is discouraged unless significant insulin resistance is encountered or the patient has specific signs of hyperadrenocorticism<sup>13</sup>.

*A reminder of how to interpret the results of a LDDST:*

In normal dogs, the cortisol concentration drops to below ~35 nmol/L at 8 hours following dexamethasone administration in the LDDST. Dogs with either PDH or adrenal neoplasia typically have a non-suppressed cortisol concentration (appreciably above 35 nmol/L) at 8 hours. Once it has been determined that there is support for hyperadrenocorticism by seeing non-suppression at 8 hours following dexamethasone, the cortisol concentration at 4 hours is evaluated. This is only interpreted if the 8 hour result is abnormal (non-suppressed).

If the 4 hour value shows a drop of 50 % or more from the baseline value (even if the drop is not below 35 nmol/L), the patient is demonstrating an initial suppression followed by escape from suppression. Here is an example of this situation: if the baseline cortisol concentration was 200 nmol/L, a 50 % drop in cortisol concentration at 4 hours following dexamethasone would be a value of 100 nmol/L or lower, such as 80 nmol/L. When this pattern is seen, PDH can be supported and there is no need to proceed to a high dose dexamethasone suppression test<sup>17</sup>. This is how the LDDST can sometimes act as a discriminatory as well as a screening test. In utilizing the LDDST as a discriminatory test, other findings supportive of PDH include a 4 hour post-dexamethasone cortisol concentration below 30 nmol/L<sup>4</sup> and/or an 8 hour post-dexamethasone cortisol concentration less than 50 % of baseline (but still non-suppressed at greater than 35 nmol/L)<sup>17</sup>.

If there is no suppression seen at the 4 hour time period to below 50 % of baseline, PDH cannot be supported, nor can an adrenal tumor be diagnosed! In the above example of a baseline cortisol concentration of 200 nmol/L, a less than 50 % drop in cortisol at 4 hours post-dexamethasone would be 101 or higher, such as 150 nmol/L. In this case, the high dose dexamethasone suppression test is a logical next step to try discriminating between these two forms of hyperadrenocorticism.

**Short answer:** *It is ideal to do both an ACTH stimulation test and a LDDST on separate days in this order. The ACTH stimulation test has the highest specificity, is the only test that can diagnose iatrogenic hyperadrenocorticism and provides baseline data for medical therapy. The LDDST is more sensitive but*

*much less specific (is more prone to false positives) and is a longer test, but it can occasionally allow differentiation of PDH vs adrenal neoplasia. Only use the UC/UC to rule out hyperadrenocorticism. All these tests have potential false positive results from stress - avoid testing in the first place if the dog has another illness unless there is strong concurrent clinical support for hyperadrenocorticism.*

**2. *What is an acceptable result to see on an ACTH stimulation test in a dog previously diagnosed with hyperadrenocorticism who is on therapy using mitotane or ketoconazole?***

Performing an ACTH stimulation test is recommended as baseline data prior to medical treatment for hyperadrenocorticism. Assessing whether treatment has been successful depends on monitoring clinical improvement and performing one or more post-treatment ACTH stimulation tests.

Mitotane (op'DDD, Lysodren) is still most commonly used for medical therapy of PDH. It is often recommended to perform an ACTH stimulation test one week into induction therapy, one month after maintenance therapy begins, and every 3-6 months following, depending on individual response<sup>18</sup>.

Medical opinion varies as to the desired result of a post-treatment ACTH stimulation test, with some desiring a normal result and most striving for a subnormal result; clinical assessment of patient well-being also factors in this assessment. As treated dogs with normal range ACTH stimulation test results continue to show some clinical signs of hyperadrenocorticism, it is generally preferred that treated dogs have a subnormal ACTH stimulation test result<sup>19</sup>. At the AVC, we recently did a thorough literature review and sought opinions from our internal medicine clinicians. We finalized our recommendations as: < 140 nmol/L for baseline cortisol and 30-200 nmol/L for the post-ACTH cortisol concentration; this is more conservative (accepts higher cortisol values) than sometimes quoted in the literature<sup>20</sup>, which likely reflects the cautious nature of most of our AVC internists.

When ketoconazole is utilized, ACTH stimulation testing is also recommended. The ideal ranges for serum cortisol concentrations (pre- and post-ACTH) are the same as for mitotane<sup>20</sup>. As selegiline hydrochloride (L-deprenyl) is not currently recommended for the treatment of hyperadrenocorticism in dogs<sup>21</sup>, it will not be discussed here. Monitoring trilostane treatment is handled as a separate question later in this handout.

***Short answer:*** *It is desirable to see a somewhat subnormal response to ACTH stimulation, but not so low that iatrogenic hypoadrenocorticism is a concern. Clinical status is also important. If you want to follow the AVC general rules for mitotane or ketoconazole, aim for a pre-ACTH cortisol concentration < 140 nmol/L and a post-ACTH cortisol concentration of 30-200 nmol/L.*

**3. *The ACTH stimulation test results from one of my canine patients went down instead of up from baseline to the post-ACTH time frame. What happened?***

We see this situation more than we expect at the AVC Diagnostic Services Laboratory - perhaps once a month! There are several possible scenarios that can result in this findings.

The most obvious one is that the tubes could have either been mislabeled (the “pre” was actually the “post” and vice versa) at the submitting clinic or the laboratory technologist at the referral laboratory mixed them up. To address this last possibility, most laboratories will run the samples through again at no charge. If mislabeling is possible but not certain, the only solution is to repeat the administration of ACTH and collect new samples.

Another possible scenario which can result in these types of numbers are if the dog has had prednisone administered in the last ~36 hours. Prednisone (converted to prednisolone in the body) has a strong interference (49 %!) with the cortisol assay and any in serum will be read as “cortisol”<sup>22</sup>. Blood values will therefore reflect both what the adrenal is capable of for cortisol secretion as well as any circulating prednisolone. Declining blood levels of prednisolone in the face of ACTH stimulation can show up as declining “cortisol” concentrations over the time of the test. Any functional adrenal tissue should still be increasing cortisol secretion over this period, but if the prednisolone concentration dominates, the overall “cortisol” value can still decline.

Another potential scenario is if the ACTH solution is completely non-functional (out of date or stored or manufactured improperly). In this case, the body receives no stimulation for cortisol secretion, but the two sampling times of the test may “catch” a naturally declining value, as cortisol values do fluctuate over the day. Finally, we in Canada will remember dealing with defective ACTH gel several years ago. As we routinely saw declining levels of cortisol using this, we even wondered if the gel contained a corticosteroid that stimulated a drop in body production of cortisol (!)....or a substance that interfered with the assay properly identifying the cortisol which was present.

**Short answer:** Consider tube mislabeling, laboratory error, prednisone administration or outdated or altered ACTH solution. Call the laboratory to ask for a recheck on tubes, check the ACTH solution and query re: prednisone administration by owners. If all is unclear, test again with new ACTH solution and label tubes carefully.

**4. I have a dog with potential hypoadrenocorticism seen on emergency last night. He has been treated with prednisone 12 hours ago. Can I still do an ACTH stimulation test?**

The short answer to this is a qualified “yes”! Remember that corticosteroid drugs can affect test results in two ways. First, any type of corticosteroid drug causes suppression of the pituitary-adrenal axis, usually resulting in low to normal baseline cortisol concentrations with a subdued response to ACTH stimulation<sup>16</sup>. Suppression of the pituitary-adrenal axis can occur following ophthalmic<sup>23</sup>, otic<sup>24</sup> and topical<sup>25</sup> corticosteroid medication. The situation is even more complicated if the patient has been treated with prednisone or prednisolone. As stated above, these drugs have strong interference with the cortisol assay and will be read as “cortisol”<sup>22</sup>, so blood values will reflect both adrenal cortisol secretion as well as any circulating prednisolone. Depending on the time of administration, the overall “cortisol” concentration could be seen to be increasing or decreasing. Approximately 2-3 days are required for prednisone to be cleared from the body but pituitary-adrenal axis suppression may last much longer. If you have to choose an emergency corticosteroid, dexamethasone is a better drug in terms of the lack of test interference from cross-reaction with the cortisol assay (but it will still affect the function of the pituitary-adrenal axis).

Administration of any corticosteroid will suppress the adrenal gland secretion of cortisol, but I have never seen it in a short term scenario like this cause absolute suppression. In other words, we would expect a somewhat subnormal ACTH stimulation test result, but it would be very unlikely to take the pre and post-ACTH cortisol concentrations down to the very low levels (often < 10 nmol/L) typically seen in patients with hypoadrenocorticism. Although prednisone administration makes things more complex, I have not personally seen this situation get to the point of overshadowing a hypoadrenocorticoid animal with results that look like a normal dog.

**Short answer:** *A qualified yes. While always ideal to hold off on all corticosteroid treatment prior to testing, choose dexamethasone over prednisone if possible for emergency treatment of potential hypoadrenocorticism. Even if prednisone has been administered, go ahead and test but let the clinical pathologist know about the drug administration. In most cases, we can usually “walk our way” through the data to either diagnose hypoadrenocorticism or rule it out.*

**5. When should I measure free T4 in a dog? Is it better than running a total T4 concentration? Where does TSH fit into all of this?**

Measurement of total T4 remains a useful screening test for hypothyroidism in dogs<sup>26,27</sup>. It is low in ~95 % of cases of canine hypothyroidism<sup>27,28</sup>, making it a very sensitive indicator for this disease. Early hypothyroidism can result in a T4 concentration in the low normal reference interval<sup>27</sup>, accounting for some of the false negative tests. As well, the presence of antibodies against T4 can cause test interference with some assays and result in a falsely increased T4 concentration in some hypothyroid dogs<sup>27</sup>. In this case, the erroneous T4 value reported by the test can be within or even above the normal canine reference interval.

A major difficulty when evaluating T4 concentrations is that they are frequently low in dogs without hypothyroidism<sup>27,28,29</sup>. This can occasionally be due to normal fluctuations, but is most often due to the effects of many non-thyroidal illnesses<sup>28,30</sup> as well as drugs. Any situation of non-thyroidal illness can cause a decrease in baseline T4 concentration; this has also been termed the "euthyroid sick syndrome". Baseline serum T4 concentrations can be low, even to undetectable concentrations, in many acute or chronic systemic illnesses. The decrease in thyroid hormones is a protective mechanism by the body when faced with a situation of negative energy balance. The severity of illness correlates with the severity of the drop in T4 concentrations. Many drugs have been reported or suspected to cause decreases in T4 concentrations, including corticosteroids<sup>31</sup>, NSAIDs<sup>32</sup>, anticonvulsants<sup>33,34</sup> and aspirin<sup>35</sup>. Proposed mechanisms vary between drugs but include direct inhibition of thyroid hormone synthesis and alterations in protein binding. Excellent reviews of this topic has been published<sup>36,37</sup>.

There are some age and breed-related information to be aware of when evaluating baseline T4 concentrations. Young puppies have high baseline T4 concentrations and healthy euthyroid older dogs may have T4 concentrations at the lower limits of the reference range. Euthyroid Greyhounds and Scottish Deerhounds have lower basal T4 concentrations than other breeds<sup>38,39</sup>. Physiologic state can certainly affect thyroid hormone levels, with intense exercise lowering T4 concentrations<sup>40,41</sup>.

Overall, measurement of a baseline T4 concentration allows hypothyroidism to be ruled out in most cases if the value is well within the reference interval. For this reason, evaluation of baseline T4 concentrations continues as a very cost-effective screening test for possible hypothyroidism. However, as discussed above,

we realize that a low baseline T4 concentration does not mean that hypothyroidism can be diagnosed! Each time a low T4 concentration is observed, three possibilities are considered:

1. A non-thyroidal illness may be causing lowering the T4 concentration.
2. Administration of various drugs may be lowering the T4 concentration.
3. Hypothyroidism may truly be present.

In a dog with classical clinical and laboratory signs of hypothyroidism and no non-thyroidal illnesses identified, a low T4 concentration may be all that is required for tentative diagnosis and trial therapy. If clinical signs or laboratory data are not strongly supportive of hypothyroidism, if the patient has been treated with medications which can alter T4 or if a non-thyroidal illness is suspected to be present, further testing is warranted. This evaluation may consist of measurement of TSH or free T4 (fT4) concentrations or both.

Thyrotropin (TSH) concentrations are increased in ~75 % of dogs with hypothyroidism. If a low T4 and a substantively increased TSH concentration is seen, the easy diagnosis of primary hypothyroidism can be made and there is no need to proceed to a fT4 determination. It is indeed annoying that TSH concentrations are in the normal reference interval in ~25 % of hypothyroid dogs! This means that a dog with a low T4 and normal TSH concentrations still could have hypothyroidism, non-thyroidal illness or drug effects. Because some drugs and non-thyroidal illnesses can increase TSH slightly, a mildly increased TSH concentration along with a low T4 concentration can be problematic to interpret - see question 5 for information on this.

In theory, measuring only the biologically active free T4 (fT4) should provide more information about thyroid function compared to evaluating total T4 concentrations. Free T4 concentrations should be low in hypothyroid dogs and hopefully less affected by factors seen in non-thyroidal illness and drug therapy that affect protein binding<sup>26</sup>. Indeed, fT4 concentrations are reliably low in hypothyroid dogs, but debate continues as to how much effect drugs and non-thyroidal illness can have. In some studies, concentrations of fT4 remain in the normal range of most euthyroid dogs with non-thyroidal illnesses in which the total T4 is low<sup>42,43</sup>. However, other work has shown that fT4 decreases in dogs with moderate to severe non-thyroidal illness<sup>44</sup>. For example, ~25 % of euthyroid dogs with hyperadrenocorticism have a fT4 concentration lower than normal<sup>45</sup>. Even physiologic state can affect fT4 concentration. Sled dogs competing in a long distance race have lower fT4 concentrations than most dogs, even when not training<sup>40,41</sup>. Overall, there is conflicting data available as to whether fT4 more often stays within range in non-thyroidal illness or drops below it. Breed differences appear to exist in baseline fT4 concentrations, with euthyroid Greyhounds having lower concentrations than other breeds<sup>39</sup>. More studies are being published evaluating drug effects on fT4 concentrations, but there is much to learn.

By itself, fT4 is not a good replacement for measuring T4, given the higher cost, lower availability (not all laboratories offer it) and the same tendency to be low in non-thyroidal illnesses. Some investigators have found fT4 measurement to have higher specificity but lower sensitivity than total T4<sup>46</sup>. In general, measuring total T4 is still a good first diagnostic step. However, proceeding to the measurement of fT4, along with TSH, can be helpful in more challenging cases.

**Short answer:** Free T4 is not a good replacement for total T4, but can help in some cases in which the total T4 and TSH results are equivocal. Free T4 can also decrease with drugs or non-thyroidal illnesses, but not usually to the same degree as total T4, so a very low value supports hypothyroidism. The most cost effective route is to measure T4 first, then TSH, then fT4.

**6. I have a dog with a low T4 concentration but the TSH concentration is only slightly increased - can I still diagnose hypothyroidism?**

Unfortunately, the answer is no. Non-thyroidal illnesses, or the recovery from them, can increase TSH to a mild degree, as can some drugs. At the AVC, our upper limit for TSH is 0.5 ng/ml, and I never feel comfortable diagnosing primary hypothyroidism in a dog with a low T4 concentration unless the TSH is over 1 ng/ml - I consider the 0.5 - 1.0 ng/ml a "grey zone". This is not based on any scientific data; more just a sense from seeing many cases. When faced with this scenario, evaluating a fT4 may help. While concentrations will also drop with non-thyroidal illness, the degree is milder than for total T4, so a very low fT4 concentration supports hypothyroidism. Attempting to rule out other illnesses is also important here. If the patient can wait, holding off and rechecking the TSH and T4 in 1-3 months may allow a more confident diagnosis. If not, then proceeding to a therapeutic trial is acceptable if no non-thyroidal illness is obvious and the fT4 is supportive of hypothyroidism.

**Short answer:** No as non-thyroidal illnesses can also mildly increase TSH concentrations. A free T4 may help here, but if no other illnesses are identified and the clinical information looks classic for hypothyroidism, a therapeutic trial can be considered.

**7. I have a new canine patient who has been on thyroid replacement hormone for years, but I am not really sure he is truly hypothyroid. How long does he have to be off medication before I can test him?**

Veterinarians are occasionally presented with canine patients already on thyroid replacement therapy for which the previous diagnosis of hypothyroidism is suspect. In this situation, gradual withdrawal of thyroid medication over several weeks is recommended. Following complete withdrawal, it is necessary to wait at least 8 weeks for the hypothalamic-pituitary-thyroid axis to normalize so that testing (T4, TSH, fT4) can be performed<sup>35</sup>. This axis may require even more time to normalize in some dogs.

The half-life is 9-15 hours<sup>47</sup> for T4 in dogs. Therefore, administered T4 should be cleared from the body by 3-4 days following administration. One can therefore be confident that all T4 measured after that time is patient derived. However, as stated above, administered thyroid hormone medication negatively affects the pituitary-thyroid axis for a prolonged period of time. Measurement of endogenous thyroid hormones with only 3-4 days withdrawal time has no real validity as a reflection of functional thyroid capacity.

**Short answer:** After gradual withdrawal of medication, a minimum of 8 weeks is needed before testing. If you can wait even longer, that is even better!

**8. *I have a cat whose T4 is near the upper limit of the reference interval but not above it. I am still suspicious for hyperthyroidism in this patient. How can I test further?***

It is well recognized that cats in the early stages of hyperthyroidism have widely fluctuating T4 concentrations which can dip into the upper reference interval at times. If there is clinical suspicion for hyperthyroidism in a cat with a normal serum T4 concentration (also termed occult hyperthyroidism), there are several options available to further assist in diagnosis<sup>48</sup>.

If the patient is not overtly ill, the easiest option is to submit one or more samples for T4 determination. This can be done right away or a few weeks later, depending on how urgently the cat might need to be diagnosed. This is the simplest and least expensive option and is recommended as the best next step. As cats with hyperthyroidism do not have predictable diurnal T4 rhythms, one cannot predict with any accuracy the best time of day to routinely sample cats with suspected hyperthyroidism<sup>49</sup>. Therefore, sampling can be done at any time and if the results are still equivocal, yet another sampling for T4 might be needed.

Evaluation of fT4 concentrations may assist in the diagnosis of early hyperthyroidism or situations in which hyperthyroidism is complicated by a T4 suppressive situation of a current non-thyroidal illness. Free T4 concentrations do increase reliably in cats with hyperthyroidism and this test is more sensitive for diagnosis of this condition than total T4 concentrations<sup>50</sup>. Unfortunately from a diagnostic point of view, fT4 concentrations also increase in some cats (6.3 % in one study)<sup>50</sup> with non-thyroidal illnesses. Therefore false positive results do occur, and diagnosis should never solely be based on a fT4 concentration, but should have clinical support.

One less available option to assist in the diagnosis of more challenging cases of hyperthyroidism is the performance of radionuclide imaging utilizing technetium 99m pertechnetate<sup>51</sup>. This is a safe procedure which not only aids in the diagnosis of hyperthyroidism but is able to reveal ectopic sites of thyroid neoplasia or hyperplasia which may not be palpable. This option is available upon referral to some veterinary college teaching hospitals.

Administration of thyrotropin releasing hormone (TRH) can assist in the diagnosis of challenging cases of feline hyperthyroidism, but obtaining this at a reasonable cost is difficult. In healthy cats and euthyroid cats with non-thyroidal illnesses, administration of TRH should stimulate the pituitary to release TSH which will, in turn, stimulate the thyroid gland to release T4. In cats with hyperthyroidism, the prolonged excessive thyroid hormones in circulation have a negative feedback effect on the pituitary, so that stimulation with TRH does not result in much TSH released, with a resultant dampened increase in T4<sup>52</sup>. While the TRH stimulation test is reliable in differentiating hyperthyroid cats from healthy or euthyroid cats with non-thyroidal illness, it does not perform well in the situation of trying to differentiate hyperthyroid cats with concurrent severe non-thyroidal illness from those with severe non-thyroidal illness alone<sup>53</sup>. Another disadvantage is that TRH administration causes transient unpleasant cholinergic effects in cats, such as salivation, tachypnea, vomiting and defecation.

***Short answer:*** Repeating the T4 one or more times is easiest. Free T4 can also be cautiously assessed. Less available or more problematic tests include radionuclide scanning and TRH stimulation testing.

**9. *I am convinced one of my canine patients has hypothyroidism. He has a low T4 but his TSH is not increased and his fT4 is equivocal. Is a therapeutic trial a bad thing to do?***

No! Therapeutic trials still have their place in the diagnosis of hypothyroidism and are a valid step when diagnostic tests do not provide a clear diagnosis in a patient suspected to be hypothyroid. However, a rigorous evaluation attempting to rule out non-thyroidal illnesses and treatment with any medications must first be performed. As the subnormal thyroid hormone concentrations in non-thyroidal illnesses are a protective mechanism by the body, pharmacologic over-riding of this defence in a case of non-thyroidal illness could be detrimental to the patient.

Approximately one month after thyroxine replacement therapy is begun, assessment of baseline T4 concentrations at 4-8 hours post-pill (and ideally pre-pill as well) will ensure that adequate hormone replacement is occurring. Therefore, if lack of clinical improvement occurs, one can be certain that hypothyroidism was not present as opposed to a situation of inadequate dosing. For a therapeutic trial to absolutely confirm a diagnosis of hypothyroidism, therapy can be temporarily discontinued after 3-4 months to observe for recurrence of clinical signs. This is rarely done.

***Short answer:*** No, a therapeutic trial is a valid step if a diligent search has not revealed non-thyroidal illnesses, clinical signs are strongly supportive of hypothyroidism and laboratory tests are equivocal. Therapeutic monitoring is important.

**10. *I have a dog who might be hypothyroid, but he is on long term phenobarbital for epilepsy (or prednisone for atopy). I know this drug can drop T4. I can't take him off the medication but how can I rule hypothyroidism in or out? If I think he has hypothyroidism, how will I ever monitor treatment?***

This is a difficult question! Many drugs have also been either reported or suspected to cause changes in thyroid hormones concentrations, including corticosteroids<sup>31</sup>, acetylsalicylic acid<sup>35</sup>, NSAIDs<sup>32</sup> phenobarbital<sup>33,34</sup> and clomipramide<sup>54</sup>. Many other drugs might also affect thyroid hormone levels but have not yet been evaluated. While sweeping generalizations cannot be made, decreases in T4 and fT4 and sometimes increases in TSH are the most common findings in treated patients, making differentiation from hypothyroidism difficult. When discontinuation of these medications is not possible, thyroid tests should be scrutinized very carefully and not over-interpreted in these patients.

How to start? It is still worthwhile looking at the T4 concentration. As corticosteroids might be expected to decrease the T4 in a dose-dependent fashion (shown for prednisone<sup>55, 56</sup>), a very marked decrease in T4 in an animal on a low drug dosage could still be supportive of hypothyroidism. Phenobarbital treatment does decrease T4 in some dogs, but it doesn't appear dose-related<sup>34</sup>. Evaluation of TSH is still helpful; while mild increases can be seen in these two drug treatment scenarios<sup>34,45</sup>, a very high TSH concentration (perhaps over 1 ng/ml) would be beyond that expected from drug treatment and therefore supportive of hypothyroidism. Free T4 is also potentially helpful, as it may not decrease to the same degree as T4 with drug treatment; this probably varies with the drug. Therefore, a very low fT4 concentration would still be supportive of hypothyroidism in the face of corticosteroid or phenobarbital therapy. Assessment of other laboratory data and clinical signs is even more important here than usual. Does the dog have dermatologic

or weight gain signs, a very high cholesterol concentration or a mild normocytic normochromic non-regenerative anemia?

If a diagnosis of hypothyroidism is tentatively reached and thyroid replacement medication is started, T4 concentrations may not be as reliable for monitoring as usual, as concentrations would reflect a combination of administered drug and any effect by the phenobarbital or corticosteroids. Monitoring fT4 may be more useful. Although published firm therapeutic ranges are not easily found, the fT4 concentration should be in the middle to upper normal range 6 hours after dosing<sup>57</sup>.

Measurement of TSH concentrations is also utilized in the monitoring of therapy for primary hypothyroidism. In general, serum TSH concentrations should decrease by at least one-third before any effect of exogenous thyroxine supplementation can be said to have influenced the serum TSH level<sup>58</sup>. While it is likely that TSH can be collected at any time of the day to monitor therapy<sup>57</sup>, some authors have recommended that it be measured on the same serum sample submitted for T4 evaluation at 4-8 hours post-pill<sup>59</sup>. One flaw in using TSH concentrations alone for monitoring therapy is that overdosage cannot be identified, in that the reference interval for TSH often starts at zero (0-0.5 ng/ml at AVC), not allowing low values to trigger identification of overdosage<sup>60</sup>. Because therapeutic monitoring of thyroid medications is less direct here than usual, evaluation of whether clinical or laboratory abnormalities resolve is even more important than usual. The above answer does not include the possibility of using human recombinant TSH to assess the functional ability of the thyroid gland; I am not sure of availability.

**Short answer:** *Hard question! It is still worthwhile to look at T4, but fT4 and TSH concentrations might be less affected by drugs, so a very low free T4 or a very high TSH concentration still supports hypothyroidism. Looking for supportive clinical or laboratory data is very important in assessing the whole picture. If hypothyroidism is tentatively diagnosed, therapeutic monitoring is most successfully done by a combination of fT4 and TSH concentrations, and importantly, clinical signs.*

**11. I have a cat on methimazole therapy. What time of day relative to dosing should I take samples for T4 testing?**

Blood sampling can take place any time of the day as long as the normal dosing routine is maintained<sup>61,62</sup>. Embarking on methimazole therapy usually entails dosage adjustments and periodic evaluation of T4 concentrations over the first few months of therapy. T4 concentrations require 2-4 weeks to stabilize following the start of therapy (the transdermal route takes longer to stabilize than the oral route<sup>63</sup>) or any dosage adjustment. This should be remembered when choosing times to evaluate whether the therapy is effective.

**Short answer:** *If on a routine dosage regime, any time of day is fine!*

**12. My canine patient is on prednisone for atopy and I wonder if he is developing iatrogenic Cushing's disease. How do I test for that?**

Corticosteroid therapy in dogs causes rapid suppression of the pituitary-adrenal axis and adrenal cortical atrophy. Diagnosis of iatrogenic hyperadrenocorticism involves a history of corticosteroid administration,

clinicopathologic signs of the disease and no or minimal response to ACTH administration. The patient therefore has clinical features of hyperadrenocorticism, but results of ACTH stimulation testing are more typical of hypoadrenocorticism. As prednisone causes marked interference with the cortisol assay, it is ideal to switch to dexamethasone for a period of at least 3 days before doing the ACTH stimulation test. To truly assess what the adrenal glands are truly capable of in this scenario, a gradual decrease and elimination of the corticosteroid drug needs to occur if possible. In one study, the mean time to show clinical improvement after corticosteroid withdrawal was six weeks, with a mean time of 12 weeks to demonstrate complete remission<sup>16</sup>. This time frame (~3 months) is likely what is required before true testing of patient adrenal capacity using an ACTH stimulation test can occur, with recognition that individual variability can occur.

**Short answer:** *Only the ACTH stimulation test lets you test for iatrogenic hyperadrenocorticism. If this is present, the ACTH stimulation test is subnormal, sometimes even as dramatic as that seen in spontaneous hypoadrenocorticism. If the patient is on prednisone, try switching to dexamethasone for at least 3 days before testing to avoid cortisol assay cross-reactivity and make the results less confusing. If corticosteroid treatment can be discontinued, ~3 months is generally needed for the adrenal gland to return to normal function.*

### 13. ***Is there ever any value to measuring T3 or free T3 in dogs?***

The measurement of T3 is not routinely helpful in canine medicine. Although dogs with prolonged hypothyroidism have a drop in both T3 and T4, the failing thyroid gland will maintain T3 production for a time but the T4 will decrease, making the latter the helpful hormone to measure.

The only possible assistance in the diagnosis of hypothyroidism that total T3 measurement can provide is in the situation of antibody production to T3. Auto-antibodies against T3 appear to be more common than auto-antibodies to T4. This situation can cause assay interference, resulting in an artifactually increased T3 concentration, often above the reference range. This would allow suspicion for hypothyroidism to be strengthened in a patient who already has supportive clinical or laboratory signs. The basal T4 concentration in such a patient may be low, normal or high, depending on whether auto-antibodies to T4 are also present.

Each clinician must decide on the cost/benefit ratio of routinely evaluating baseline T3 in suspect hypothyroid dogs. Given that auto-antibody interference is not common and T3 measurement is a poor test overall for diagnosis of hypothyroidism, it is difficult to rationalize the cost of routine testing. I do not recommend it!

Although included on some thyroid panels offered, I could find no rational use for the measurement of fT3 concentrations. While fT3 concentrations may be low in prolonged situations of hypothyroidism, it is likely that they remain in the normal range in earlier stages, as is seen for total T3. At present, there is no advantage to the measurement of fT3 over fT4.

**Short answer:** *Total T3 is not helpful to routinely measure but can occasionally be helpful if auto-antibodies to T4 interfere with accurate T4 measurement. I have not found any good reason for measuring fT3.*

**14. *I've heard that I can just measure a baseline cortisol concentration rather than an ACTH stimulation test to diagnose hypoadrenocorticism in dogs. Is that true?***

From a study recently published looking at 13 dogs with hypoadrenocorticism<sup>64</sup>, this seems to be the case, but further work looking at bigger numbers of dogs is needed. This study found that it was very rare for a dog with a baseline cortisol concentration above 2 ug/dl (~60 nmol/L) to be hypoadrenocorticoïd. These dogs were not receiving corticosteroids, mitotane or ketoconazole. If the value was < 2 ug/dl, no conclusions could be made and an ACTH stimulation test was needed.

**Short answer:** *A baseline cortisol value higher than 60 nmol/L likely rules out hypoadrenocorticism but this is based on only 1 study - we are waiting for further work. If it is lower than 60 nmol/L, an ACTH stimulation test is definitely needed.*

**15. *What about in-house SNAP testing for T4 - is it reliable?***

Clinical pathologists often get asked about the reliability of in-house testing for various assays. This can be impossible to answer, as there is little independent research done on most marketed tests. There is good news for the SNAP total T4 test marketed by IDEXX, however, as it was rigorously evaluated in a recent independent study<sup>65</sup>. Compared to the gold standard of radioimmunoassay as well as to two human T4 tests, it performed very well and gave consistent results.

**Short answer:** *One independent study found that the IDEXX Snap T4 test performed well.*

**16. *What is the best assay for free T4? My diagnostic laboratory is now offering a 2-stage free T4 test - what is that?***

The gold standard method of measuring fT4 is via equilibrium dialysis, in which the fT4 diffuses across a semi-permeable membrane prior to measurement. Given the tedious nature of equilibrium dialysis, this technique is mainly used in research laboratories. A number of technically easier radioimmunoassays and chemiluminescence assays have been marketed for the measurement of fT4. However, when compared to the gold standard of equilibrium dialysis, they consistently underestimate the amount of fT4 present<sup>66,67</sup> and are not usually recommended.

The fT4 test that is recommended is the modified equilibrium dialysis assay. It has good correlation with traditional equilibrium dialysis<sup>67</sup> and has been the optimal one offered by veterinary diagnostic laboratories. However, reagents have been sporadically difficult to obtain, leading many laboratories to instead offer a Diasorin 2-step (also called D2S) test instead. The 2-step designation is from the protocol of antibody incubation followed by addition of a radioligand. To my knowledge, this test has been approved for use by the OFA (Orthopedic Foundation for Animals) in its thyroid certification program. The main data evaluated was provided by Cornell University and anecdotally, this 2 step test seems to perform very well and correlate nicely to the fT4 values obtained by equilibrium dialysis. Numbers on the D2S test may run a little lower, so it is important that good reference intervals are used. We are still waiting for good independent studies to be published on this 2 step test, but so far things look encouraging.

Final note: As fT4 assay results can vary between methods used at different laboratories<sup>68</sup>, it is very important to maintain consistency in a clinical setting and submit all samples from a patient to the same laboratory over time. Free T4 concentrations can increase with storage in glass tubes, so shipment in plastic vials is optimal<sup>69</sup>.

**Short answer:** *This test was offered by laboratories when obtaining reagents for free T4 by modified equilibrium dialysis was impossible at times. There are no published studies yet, but internal assessment found this to be a good test. We need more information, but in the meantime, if a modified equilibrium dialysis test cannot be done, this is the next best choice. Remember to submit samples for fT4 in plastic vials.*

**17. I am interested in trying trilostane for one of my canine patients with hyperadrenocorticism. What laboratory testing should I do to monitor response in my patient? Is it different from a situation using mitotane?**

More practitioners in the Atlantic provinces are using trilostane (Vetoryl) to treat canine hyperadrenocorticism. The drug has been available for many years in Europe. Trilostane is a competitive inhibitor of the 3 $\beta$ -hydroxysteroid dehydrogenase-isomerase enzyme system<sup>70</sup>. Following oral administration, it actively interferes with a steroid metabolic pathway and blocks the synthesis of end products, including cortisol and aldosterone<sup>70</sup>. It is the goal of therapy to block cortisol synthesis more completely while relatively sparing aldosterone synthesis. While generally considered fairly safe with fewer side effects than mitotane in many dogs, there have been reports of illness and even death due to adrenal necrosis and resultant hypoadrenocorticism, especially with higher doses given once daily. Because of this and because the action of trilostane may be less than 24 hours in some dogs, some authors are now recommending lower dose twice-daily treatment<sup>70, 71</sup> instead of the standard once daily dose.

As with other treatment modalities for hyperadrenocorticism, monitoring dogs on trilostane involves performing ACTH stimulation tests. Typically, an ACTH stimulation test should be performed 7-10 days after commencing therapy, then again at 4 weeks, 12 weeks, and every 3 months thereafter. One difference in performing this test in a dog on trilostane compared to other drugs is that the test should be performed at the time of peak activity. We currently recommend that the ACTH stimulation test be done 2-6 hours post-dosing. This was based on literature references recommending anywhere from 2-3 hours<sup>72</sup>, 2-6 hours<sup>8</sup> or 4-6 hours post-dosing<sup>73</sup>.

Optimal control of hyperadrenocorticism using trilostane is still being evaluated in the veterinary medicine. Therefore, protocols for drug administration and desired results on an ACTH stimulation test while on therapy differ. The exact goal for post-ACTH cortisol concentrations are as not clearly defined as for other drugs and thus clinical response becomes even more important<sup>6</sup>. Past recommendations varied widely, from <250 nmol/L<sup>74</sup>, 25-75 ideal but 75-125 nmol/L acceptable<sup>75</sup> and 40-120 nmol/L<sup>76</sup>. These values appear lower than typically seen for ideal results in dogs on ketoconazole or mitotane. Since some dogs are clinically stable on these low cortisol values, cortisol precursors with biologic activity may be present which are not measured by the cortisol assay<sup>8</sup>. However, since these recommendations were published, there has been increased awareness of possible adverse sequelae using trilostane, including adrenal necrosis. At the Atlantic Veterinary College, we thoroughly researched this several months ago to establish our current recommendations, which may change in the future as more information becomes available. At present, we recommend that a pre-ACTH cortisol concentration of <140 nmol/L and a post-ACTH serum cortisol

concentration of 30-200 nmol/L are desirable if clinical signs are well controlled, but a value as high as 250 nmol/L may still not warrant an increase in dosage. You will note that these are very similar to what is recommended for monitoring dogs on mitotane or ketoconazole.

**Short answer:** *Recommendations for trilostane dosing and optimal cortisol values seen following ACTH stimulation testing are still being developed. The ACTH stimulation test should be done within 2-6 hours after dosing. We recommend that the pre-ACTH cortisol values be <140 nmol/L in a treated dog. More importantly, we recommend that post-ACTH cortisol concentrations be between ~30-200 nmol/L, but a value as high as 250 nmol/L may still be acceptable if clinical signs are well controlled. These recommendations may change in the future as more information becomes available.*

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