The Effects of Anesthesia and Surgery on Thyroid Function Tests in Dogs

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ABSTRACT

Background: Many non-thyroidal factors affect thyroid function tests. Anesthesia and surgery have been documented to affect thyroid function tests in humans but have not been extensively studied in dogs.

Hypothesis: Anesthesia alone and anesthesia combined with surgery will affect thyroid function tests in dogs.

Animals: 15 euthyroid mongrel dogs.

Methods: Dogs were assigned to one of three groups: control, general anesthesia, and general anesthesia plus abdominal exploratory surgery. Blood samples were collected from each dog immediately prior to pre-medication, 20 minutes after pre-medication, 55 minutes after anesthesia induction, once daily for an additional 6 days, and 14 days post-procedures. Sampling was performed at identical times in the control group. Thyroxine ($T_4$), free $T_4$ (fT$_4$) by equilibrium dialysis, triiodothyronine ($T_3$), reverse $T_3$ (rT$_3$) and thyroid-stimulating hormone (TSH) concentrations were measured in all samples.

Results: Results of all thyroid function tests were not significantly different between control and anesthesia groups. Serum $T_3$ for the surgery group decreased significantly from baseline compared to the control and anesthesia groups at multiple times. Serum $T_4$ and rT$_3$ for the surgery group increased significantly from baseline compared to the control and anesthesia groups at multiple times. Serum fT$_4$ for the surgery group
increased significantly from baseline compared to the control and anesthesia groups at 48 hours only.

Conclusions and Clinical Importance: Surgery has a significant effect on thyroid function tests, while the anesthetic protocol used in this study does not. Because serum T₄ and fT₄ concentrations increased rather than decreased, evaluating these hormones following surgery is unlikely to lead to a misdiagnosis of hypothyroidism in euthyroid dogs.
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INTRODUCTION

Hypothyroidism is thought to be the most common endocrine disorder in dogs. Clinical signs of hypothyroidism can mimic those of other disease processes, and many non-thyroidal factors, such as drug administration and concurrent illness can affect thyroid function tests. Thus, an accurate diagnosis of hypothyroidism can be difficult in certain circumstances. Numerous drugs have been shown to have effects on thyroid function tests that could result in a misdiagnosis of hypothyroidism.\textsuperscript{1,2} Anesthesia and surgery have been documented to affect thyroid function tests in humans, resulting in a variety of abnormalities in thyroid function tests. There have not been any peer reviewed data published in the veterinary literature regarding the effects of anesthesia and surgery on thyroid function in dogs, although a decrease in serum concentrations of thyroxine (tetraiodothyronine, T\textsubscript{4}), triiodothyronine (3,5,3’-triiodothyronine, T\textsubscript{3}) and reverse triiodothyronine (3,3’5’-triiodothyronine, rT\textsubscript{3}) has been reported in dogs undergoing general anesthesia and surgery.\textsuperscript{2} However, the details of the experimental design and the data were not given. This change was reportedly present for at least 30 hours following the procedures; however it is possible that the effects lasted longer. Additionally, only T\textsubscript{4}, T\textsubscript{3}, and rT\textsubscript{3} were measured, while thyroid-stimulating hormone (TSH) and free thyroxine (fT\textsubscript{4}) are routinely measured in canine patients suspected of being hypothyroid.

In humans, T\textsubscript{3} and free T\textsubscript{3} (fT\textsubscript{3}) consistently decrease, and reverse T3 typically increases\textsuperscript{3-8} with anesthesia and surgery. The decrease in T\textsubscript{3} may be greater with surgery than with anesthesia alone,\textsuperscript{9} or greater with more extensive surgical trauma.\textsuperscript{10} There are conflicting reports in humans regarding the changes in T\textsubscript{4}, free T\textsubscript{4}, and TSH. Reasons for this variability in reported results may include the anesthetic agent used, and the type of
surgery performed. Studies have shown no change in T₄,³,¹⁰-¹² a decrease in T₄¹³,¹⁴ or an increase in T₄ as well as free T₄⁴,⁸,¹⁵ At least two studies of humans showed no change in TSH,³,⁴ while another reported a decrease.⁷ The variability of changes seen in thyroid function tests is likely multifactorial. Pre-anesthetic medications, anesthesia protocol, surgical procedure, and concurrent illnesses of the patient may all contribute to these changes. Possible explanations for the changes that have been reported in T₃, fT₃ and rT₃ associated with anesthesia and surgery include a reduction in 5’-deiodination of T₄ to T₃ in favor of the alternate pathway of T₄ to reverse T₃, decreased metabolism of rT₃, or alterations in thyroid hormone binding to plasma proteins or transport across cell membranes.¹¹-¹³ Proposed mechanisms for the increased T₄ and fT₄ concentrations include intercompartmental shifting of thyroid stores and changes in binding capacity of plasma proteins,⁵,⁸ while the decrease in T₄ could result from impaired binding to plasma proteins, increased metabolism of T₄,⁷ increased transport into cells, or decreased thyroid hormone secretion.¹⁶ The decreased TSH may be due to enhanced clearance of TSH or suppressed secretion.⁵,¹³

The objective of this study was to evaluate the effects of anesthesia alone and anesthesia combined with abdominal exploratory surgery on thyroid function in dogs.
CHAPTER I: LITERATURE REVIEW

A. Thyroid Hormone Synthesis and Secretion

The thyroid gland produces two hormones, thyroxine (tetraiodothyronine, T\textsubscript{4}) and triiodothyronine (3,5,3'-triiodothyronine, T\textsubscript{3}). Thyroxine and T\textsubscript{3} are both iodothyronines formed from the coupling of iodotyrosines in the colloid of thyroid follicles. Thyroid hormones are the only physiologically significant iodine-containing compounds in vertebrates.\textsuperscript{17}

Thyroid hormone production occurs within the thyroid follicles, which consist of a single layer of polarized epithelial cells that form a spherical structure surrounding a lumen. A specialized system has developed to allow for accumulation of iodine within the thyroid gland. Most dietary iodine is reduced to iodide in the small intestine prior to absorption. Following absorption, the majority of iodide is removed from the plasma by the thyroid gland and kidneys, with smaller amounts being removed by the salivary glands and stomach. The thyroid gland extracts iodide from plasma and concentrates it within the thyroid follicular lumen in a two-step process. The sodium/iodide symporter protein on the basolateral membrane of thyroid follicular cells mediates transport of iodide into the thyroid follicular cell via Na\textsuperscript{+}-dependent active transport. Once inside the thyroid follicular cell, iodide is passively transported across the apical membrane into the follicular lumen.\textsuperscript{18}

Once iodide is concentrated in the lumen, it is oxidized and bound to thyroglobulin (Tg), a large glycoprotein that serves as the matrix for synthesis and storage of thyroid hormones. Iodide is oxidized by thyroperoxidase (TPO) in the
presence of hydrogen peroxide and is transferred to the tyrosyl groups of Tg to form monoiodotyrosine (MIT) and diiodotyrosine (DIT). A coupling reaction between MIT and DIT catalyzed by TPO in the presence of hydrogen peroxide forms T\(_3\), while coupling between two DIT forms T\(_4\). Both thyroid hormones are thereby stored within the follicular lumen bound to Tg.\(^{17,18}\)

In order to be secreted by the thyroid gland, T\(_4\) and T\(_3\) must be released from their peptide linkage within Tg. Thyroglobulin is taken up into the thyroid follicular cell from the lumen by vesicle-mediated endocytosis, which is regulated by thyroid-stimulating hormone (thyrotropin, TSH). These vesicles ultimately fuse with lysosomes where proteolytic cleavage releases T\(_4\) and T\(_3\) from their peptide linkages. At that point, T\(_4\) and T\(_3\) rapidly leave the cell and enter the circulation through an unknown mechanism. Within the thyroid gland, about 10% of the T\(_4\) is deiodinated to T\(_3\) by the enzyme type I iodothyronine 5’-deiodinase; this conversion is enhanced by TSH. Monoiodotyrosine and DIT are also deiodinated within the thyroid gland by iodothyrosine deiodinase, thereby allowing the return of iodide to the intrathyroidal iodide pool.\(^{17,18}\)

Iodine availability and TSH are the most important factors controlling thyroid hormone synthesis, with TSH influencing most every step of synthesis and release of thyroid hormones. Iodine deficiency leads to decreased thyroid hormone production and subsequent increased TSH secretion, whereas iodine excess inhibits thyroid hormone synthesis by blocking Tg iodination. Thyroid-stimulating hormone stimulates expression of the sodium/iodide symporter protein, TPO and Tg, increases conversion of T\(_4\) to T\(_3\) and promotes internalization of Tg by thyroid follicular cells.\(^{17,18}\)
B. Hypothalamic-Pituitary-Thyroid Axis

Thyroid hormone production is regulated by the hypothalamic-pituitary-thyroid axis, with TSH produced by thyrotrophs in the anterior pituitary gland as the predominate regulator of thyroid gland activity. Thyrotropin-releasing hormone (TRH) is synthesized by neurons in the hypothalamus, transported along their axons to the median eminence of the hypothalamus, and is then released from specialized nerve terminals into the hypophyseal portal blood. It is then taken to the anterior pituitary gland, where it stimulates secretion of TSH by thyrotrophs in a dose-dependent fashion. Thyroid-stimulating hormone in turn stimulates thyroid function and growth, mainly by stimulation of the adenyl cyclase cascade resulting in an increase of intracellular cyclic AMP levels.\textsuperscript{17}

The major factors that regulate the synthesis and secretion of TSH ultimately play a large role in regulation of thyroid function. These factors include TRH and the thyroid hormones T\textsubscript{3} and T\textsubscript{4}.\textsuperscript{17,19} Triiodothyronine binds to the thyrotroph nuclear T\textsubscript{3} receptor, while T\textsubscript{4} acts mainly via its conversion to T\textsubscript{3} within the pituitary gland or within the hypothalamus; there is also a direct effect of T\textsubscript{4} on TSH gene expression. Both thyroid hormones have a direct inhibitory effect on TSH synthesis and release, as well as having indirect effects on the synthesis of TRH.\textsuperscript{19}

Numerous other substances also play a role in both TSH synthesis and secretion and TRH secretion, such as hormones, cytokines, and neuropeptides. Somatostatin, the major physiologic inhibitor of growth hormone secretion, has inhibitory effects on TSH secretion, as does dopamine, the major physiologic inhibitor of prolactin. In contrast, dopamine has a stimulatory effect on TRH secretion at the hypothalamic level. However,
it concurrently stimulates somatostatin release. Cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) can inhibit basal TSH secretion. Both IL-1_ and interleukin-6 (IL-6) acutely inhibit TSH release from the thyrotrophs, while IL-1_ (but not IL-6) also decreases hypothalamic TRH mRNA and gene expression.\textsuperscript{17,19}

C. Thyroid Hormone Metabolism

Serum thyroid hormone concentrations reflect the net result of thyroid hormone binding to carrier proteins, transfer between vascular and extravascular sites, and rates of production, clearance and degradation. More than 99 per cent of thyroid hormones in serum are bound to carrier proteins such as thyroxine-binding globulin (TBG), transthyretin, albumin, and apolipoproteins.\textsuperscript{17} In humans, 70 per cent of the serum T\textsubscript{4} is transported by TBG, 10 to 15 per cent is transported by transthyretin, 15 to 20 per cent is transported by albumin, and about 4 per cent is transported by apolipoproteins.\textsuperscript{17} In contrast, in dogs 60 per cent of the serum T\textsubscript{4} is bound to TBG, 17 per cent is bound by transthyretin, 12 per cent is bound by albumin, and 11 per cent is bound by apolipoproteins.\textsuperscript{20} Although TBG has the lowest serum concentration of the thyroid-hormone binding proteins, it has an extremely high affinity for T\textsubscript{4}, with 10-20 times less affinity for T\textsubscript{3}. Both transthyretin and albumin also have a lower affinity for T\textsubscript{3} than T\textsubscript{4}.\textsuperscript{17} Thyroxine-binding globulin has the highest affinity yet lowest binding capacity for T\textsubscript{4} in both dogs and humans, although TBG binding capacity in dogs is 25 per cent that of humans.\textsuperscript{21} Differences in the structure of TBG in dogs compared to humans may account for the differences in hormone binding.\textsuperscript{22} Albumin and transthyretin have higher binding capacities yet lower affinity for T\textsubscript{4} in both humans and dogs.\textsuperscript{20} Overall, dogs have 4.3
times lower T₄ binding and 4.8 times lower T₃ binding to serum carrier proteins than humans.²⁰ This accounts for the lower normal plasma T₄ concentrations of dogs compared with humans.

Thyroxine is the main secretory product of the thyroid gland.²³ Thyroidal secretion of T₃ contributes approximately 24 per cent to daily T₃ production in dogs,²⁴ and the majority of T₃ is produced from monodeiodination of T₄ in peripheral tissues.²³ Monodeiodination of T₄ to T₃ is catalyzed by specific enzymes, selenodeiodinases. Selenodeiodinases are membrane proteins that contain the rare amino acid selenocysteine in their active state.²⁵ Three of these deiodinase enzymes have been identified, type 1 (D1), type 2 (D2) and type 3 (D3). These enzymes can either activate or deactivate thyroid hormone, depending on whether they act on the phenolic ring (outer ring) or on the tyrosyl ring (inner ring) of the thyroid hormone molecules.²⁵,²⁶ Outer ring deiodination of T₄ produces the active T₃, while inner ring deiodination produces the inactive 3,3’5’-triiodothyronine (reverse T₃, rT₃). Triiodothyronine and rT₃ are further deiodinated to diiodothyronines. In addition to deiodination, T₄ may also be metabolized by sulfation and glucuronidation.²⁷

Type 1 deiodinase was the first deiodinase to be recognized, and is found in the liver, kidney, thyroid and pituitary gland in humans. It is the only deiodinase to possess both outer and inner ring deiodinating activity. Although D1 does catalyze the production of T₃ from T₄, its major physiologic role seems to be clearance of rT₃, sulfated rT₃ and sulfated T₃.²⁵,²⁶ Sulfation of T₃ facilitates rapid inactivation by inner ring deiodination by D1.²⁵
Type 2 deiodinase is found in the central nervous system, pituitary gland, brown adipose tissue (rats), placenta and skeletal muscle.\textsuperscript{25} Until the last several years, it was thought that the majority of extrathyroidal T\textsubscript{3} was produced by deionidation of T\textsubscript{4} by D1. However, more recent studies have demonstrated that D2 is responsible for the majority of this T\textsubscript{3} production.\textsuperscript{28} The principle reason why D2 produces more T\textsubscript{3} than D1 is because D1 catalyzes the reaction of T\textsubscript{4} to T\textsubscript{3} much more rapidly than D1 does. Type 3 deiodinase is the major inactivator of T\textsubscript{3} and T\textsubscript{4}, and possesses almost exclusively inner ring deiodination activity. Inner ring deiodination of T\textsubscript{4} via D3 produces rT\textsubscript{3} and inner ring deiodination of T\textsubscript{3} via D3 produces 3,3'-diiodothyronine, both of which are metabolically inactive. Type 3 deiodinase is found in the central nervous system, skin, placenta, pregnant uterus and fetal liver.\textsuperscript{25}

Although T\textsubscript{3} is the metabolically active form of thyroid hormone,\textsuperscript{29} the major secretory product of the thyroid gland is T\textsubscript{4}. Interestingly, the human thyroid gland preferentially secretes T\textsubscript{3} compared to T\textsubscript{4}.\textsuperscript{17,30} This has also been found to be the case in dogs, and several studies have demonstrated that thyroid secretion contains relatively more T\textsubscript{3} and rT\textsubscript{3} than would be expected from the iodothyronine content of thyroglobulin. This appears to be due to local deiodination of T\textsubscript{4}.\textsuperscript{24,30-32}

Triiodothyronine is the metabolically active thyroid hormone, and exerts its biological action by binding to receptors in target cells. This bound T\textsubscript{3} is derived from both plasma and local production from T\textsubscript{4}.\textsuperscript{29}

Depending on tissue characteristics, thyroid hormones leave the vascular space in either the free or protein-bound form. Fractional rates of transfer between plasma and extravascular sites are affected by hormone binding, tissue-specific characteristics of
vascular permeability, the transfer process between the interstitial fluid and tissues, and lymphatic flow. After intravenous injection of radiolabeled T\textsubscript{4} or T\textsubscript{3} in dogs, these hormones pass rapidly into the interstitial fluid. The majority of T\textsubscript{4} remains in the extracellular space for about 4 hours, while T\textsubscript{3} enters cells, with at least 20 per cent entering the liver. Within 15 hours, 40 per cent of injected T\textsubscript{4} and 60 per cent of injected T\textsubscript{3} is intracellular.

Studies in humans and sheep have shown that fractional transfer between serum and extravascular tissues are rapid for liver and kidney, but slow for skin, fat, muscle and brain. In the rapidly equilibrating tissues, thyroid hormones enter the extravascular space bound to binding proteins, but in slowly equilibrating tissues, they enter the extravascular space in the free form. Free hormone actively enters into cells where it binds to cell membranes, cytosol-binding proteins, nuclear receptors, and is metabolized. Because the role of binding proteins is different in slow and rapidly equilibrating tissues, alterations in binding proteins may affect transfer from the slow and rapid pools differently.

Thyroxine and T\textsubscript{3} are concentrated in the liver and secreted in bile in their normal states and as their sulfate and glucuronide conjugates. In dogs injected with radiolabeled T\textsubscript{4}, radioactivity appeared in the bile approximately 10 minutes after intravenous injection, reached a maximum level at 1 hour, and maintained those levels for at least 6 hours. Bile radioactivity was a mixture of \textsuperscript{131}I-labeled T\textsubscript{4}, T\textsubscript{3} and metabolites, including sulfates and glucuronides. The biliary clearance of radiolabeled T\textsubscript{4} is greater than that in humans.
In the same study, approximately 40 per cent of the radiolabeled T$_4$ was accounted for in the feces by 48 hours, and 41 per cent was accounted for in the urine, with urinary activity being mainly iodide. By 7 days, an average total of 52 per cent of the injected dose was excreted in the feces and 46 per cent was excreted in the urine.$^{35}$ Less than 15 per cent of conjugated bile metabolites are reabsorbed from the gut in dogs, evidenced by the fact that when radioactive bile from 1 dog was placed in the duodenum of another dog, 82 per cent of the bile activity was recovered in the feces and only 8 per cent was recovered in the urine.$^{35}$

**D. Hypothyroidism**

1. **Overview**

Hypothyroidism is the most common endocrine disorder in dogs. It can be divided into primary, secondary, and tertiary hypothyroidism. The most common form of the disease is primary hypothyroidism, due either to lymphocytic thyroiditis or idiopathic thyroid atrophy.$^{36}$ Secondary hypothyroidism results from decreased pituitary secretion of TSH, and has been rarely documented in dogs, accounting for less than 5% of all cases of hypothyroidism in dogs.$^{36}$ Tertiary hypothyroidism is caused by a deficiency of TRH, and has not been reported in dogs.$^{23}$

Hypothyroidism causes a wide variety of clinical signs that can mimic those of other disease processes. Lethargy, weakness, weight gain, and dermatologic abnormalities such as alopecia, seborrhea, pyoderma and hyperpigmentation are commonly seen in hypothyroid dogs. Additional clinical manifestations of the disease include neurological abnormalities such as vestibular disease, facial nerve paralysis and
polyneuropathies; cardiovascular abnormalities such as bradycardia; and abnormal reproductive function in females.\textsuperscript{23}

2. Diagnosis

Diagnosis of hypothyroidism is made using a combination of clinical signs and thyroid function tests. Routine laboratory tests may reveal mild non-regenerative anemia, hypercholesterolemia and hypertriglyceridemia, and less commonly elevated liver enzymes and elevated creatine kinase, none of which are specific to hypothyroidism.\textsuperscript{23}

Serum T$_4$ concentration is low in the majority of hypothyroid dogs.\textsuperscript{37} Sensitivity of this test ranges from 89 to 100 per cent.\textsuperscript{38-40} However, T$_4$ is often decreased in dogs with normal thyroid function, due to normal fluctuations, nonthyroidal illness (NTI), or drug administration, resulting in a specificity of 75-81 per cent in dogs with clinical signs of hypothyroidism.\textsuperscript{1,37,41-43} Additionally, T$_4$ autoantibodies can falsely increase serum T$_4$ concentrations.\textsuperscript{37}

Serum T$_3$ concentrations are low in the late stages of hypothyroidism, but are normal in many hypothyroid dogs.\textsuperscript{37,38,44} In one study, only 10 per cent of hypothyroid dogs had low serum T$_3$ concentrations,\textsuperscript{38} and in another study, no difference in serum T$_3$ concentrations were found between normal dogs and hypothyroid dogs.\textsuperscript{44} For this reason, serum T$_3$ concentration is not recommended for the diagnosis of hypothyroidism. As with T$_4$, T$_3$ may be decreased by NTI\textsuperscript{43} and drug administration.\textsuperscript{1,42}

Free T$_4$ is the fraction of T$_4$ that is not bound to plasma proteins. Serum fT$_4$ provides a better way to measure thyroid function, as serum fT$_4$ concentrations are less affected by non-thyroidal factors than T$_4$ and T$_3$.\textsuperscript{38} However, decreased serum fT$_4$ concentrations can still occur secondary to NTI and drug administration.\textsuperscript{1,41-43} Serum fT$_4$
is measured by standard equilibrium dialysis (SED), radioimmunoassay (RIA) or modified equilibrium dialysis (MED). Standard equilibrium dialysis is considered the gold standard, however is technically demanding, expensive, and time-consuming. Radioimmunoassays use a $T_4$ analog that binds less to serum binding proteins, and provide an estimate of $fT_4$, while the MED technique uses a dialysis step that is followed by a sensitive RIA to determine $fT_4$. Measurement of serum $fT_4$ concentration by equilibrium dialysis methods is more accurate than measurement of serum $T_4$ concentrations for assessing thyroid function in dogs. The RIA technique has been shown to produce consistently lower $fT_4$ results than SED or MED. Sensitivity of $fT_4$ by the MED technique in hypothyroidism ranges from 80 to 98 per cent, while specificity ranges from 93 to 93.5 per cent.

Measurement of serum TSH concentration is the most accurate way of diagnosing hypothyroidism in humans. Serum TSH is expected to be high in humans with primary hypothyroidism. In dogs, however, serum TSH concentrations have been reported to be normal in 18 to 38 per cent of hypothyroid dogs, and 0 to 14 per cent of euthyroid dogs have been reported to have an increased serum TSH concentration. Additionally, serum TSH concentration may be increased in dogs with NTI. Sensitivity of this test ranges from 63 to 87 per cent, while specificity ranges from 82 to 93 percent.

The TSH response test has been commonly used to diagnose primary hypothyroidism in dogs. This test measures the secretory reserve of the thyroid gland. In euthyroid dogs, serum $T_4$ concentration should increase in response to administration of supraphysiologic doses of exogenous TSH, while dogs with primary hypothyroidism will have no or minimal increase in serum $T_4$ concentrations.
Traditionally, bovine TSH has been used for the test, but pharmaceutical grade 
bovine TSH is no longer available. Recently, it has been demonstrated than human 
recombinant TSH may be used instead.\textsuperscript{50} Although the TSH response test is considered 
the most accurate noninvasive thyroid function test, it may be normal in some cases of 
early hypothyroidism, or may be abnormal in dogs with severe non-thyroidal illness or 
secondary to drug administration.\textsuperscript{23} In fact, a recent study documented only a minor 
increase in serum T\textsubscript{4} concentration in response to TSH administration in dogs with NTI 
that were euthyroid based on thyroid biopsy and scintigraphy. These dogs would have 
been misdiagnosed as hypothyroid based on earlier cutoff values for post-stimulation T\textsubscript{4} 
concentrations.\textsuperscript{51}

The TRH response test measures the responsiveness of the pituitary gland to TRH 
as well as the response of the thyroid gland itself to secretion of TSH. Either serum T\textsubscript{4} or 
serum TSH concentrations may be measured after administration of exogenous TRH. 
Dogs with primary hypothyroidism will have no change in serum T\textsubscript{4} concentrations in 
response to administration of TRH but should have an increase in serum TSH 
concentrations. Dogs with secondary hypothyroidism should have a low or low normal 
serum TSH concentration following administration of TRH, while dogs with tertiary 
hypothyroidism should show an increase in serum TSH and T\textsubscript{4} concentrations following 
administration of TRH.\textsuperscript{23,49} However, because the reference range for canine TSH 
extends below the sensitivity of the assay, the assay cannot be used to distinguish 
between normal and low TSH concentrations.\textsuperscript{47} It may be difficult to interpret the results 
of the TRH response test because the increase in T\textsubscript{4} concentration is smaller than with 
TSH administration.\textsuperscript{52} Evaluation of percentage change in serum TSH concentration in
response to TRH administration can differentiate hypothyroid dogs from normal dogs; percentage change in serum TSH concentration in response to TRH administration for hypothyroid dogs is significantly less than the percentage change for healthy dogs.\textsuperscript{53} However, the overall accuracy of this test is only slightly higher than that for measurement of baseline TSH and fT\textsubscript{4} or T\textsubscript{4} concentrations.\textsuperscript{53} The change in serum TSH concentration This test has not been shown to be useful in differentiating dogs with NTI from dogs with hypothyroidism.\textsuperscript{51}

Expected results of thyroid function tests in dogs with primary hypothyroidism are decreased serum T\textsubscript{4} and fT\textsubscript{4} concentrations, normal or increased TSH concentrations, and lack of response to TSH and TRH stimulation tests. Dogs with normal thyroid function who have a NTI or are receiving medications affecting the results of thyroid function tests may also have a similar pattern.\textsuperscript{41,43}

More recently, ultrasonography has been evaluated for its ability to diagnose hypothyroidism.\textsuperscript{54,55} These studies showed that thyroid volume and dimensions are lower in hypothyroid dogs than in dogs with NTI, therefore ultrasonography may be helpful as an ancillary test to differentiate between hypothyroid dogs and dogs with NTI.

Histological examination of thyroid tissue is the most definitive test to identify thyroid disease.\textsuperscript{23,51} One study evaluating thyroid biopsies and serum thyroid hormone concentrations in healthy versus severely sick dogs showed no difference in volume percentages of colloid and follicular epithelium in the thyroid parenchyma of healthy dogs and sick dogs with decreased serum T\textsubscript{4} concentrations.\textsuperscript{41} However, thyroid biopsy is an invasive test that requires general anesthesia and surgical expertise, and may be costly for owners.
Scintigraphic imaging of the thyroid glands using radioactive pertechnetate is another way to evaluate function of the thyroid gland. A recent study showed no overlap in thyroid uptake of radioactive pertechnetate between dogs with primary hypothyroidism and dogs with nonthyroidal illness. However, the availability of nuclear scintigraphy is limited.

E. Non-Thyroidal Effects on Thyroid Function Tests

1. Non-thyroidal illness syndrome- human

For several decades, it has been shown that NTI causes alterations in thyroid function tests. Alterations in thyroid function tests due to NTI is often referred to as “euthyroid sick syndrome,” however a more appropriate term may be non-thyroidal illness syndrome (NTIS), which does not presume that patients with NTI are necessarily euthyroid.

Decreased serum T3 and increased serum rT3 concentrations are the most common abnormality seen in human patients with NTI, with serum T3 concentrations decreasing more markedly with more severe illness. Serum fT3 concentrations typically decline less than do T3 concentrations. Decreased serum T3 concentrations are also seen within 24-48 hours of fasting, therefore malnutrition during NTI may contribute to the decrease in serum T3 concentrations. Low serum T3 concentrations have been shown to be inversely correlated to the severity of illness. Serum concentrations of rT3 and the ratio of T3 to rT3 have been documented to be prognostic for survival in a study evaluating critically ill patients in an intensive care unit with nonsurvivors having a higher serum rT3
and lower T3/rT3 than survivors. Serum T3 levels were higher on the fifth day of hospitalization in patients who survived than in patients who did not.58

With more prolonged and more critical illness, a decrease in serum T4 and TSH concentrations will also occur.56,58 Serum fT4 concentrations are often below normal concentrations in patients with severe illness, but may be normal or above normal. The variations in serum fT4 concentrations may be in part due to the type of assay used.56,59 As with serum T3 concentrations, serum concentrations of T4 and fT4 may provide prognostic information, with lower concentrations offering a worse prognosis.56,58,60 A reduction of fT4 and fT3 together has been associated with increased mortality.56 Decreased serum TSH concentrations have also been correlated with a worse prognosis,56,58 but may increase above normal as recovery begins.56,61

The mechanisms responsible for NTIS are likely multifactorial and are not fully understood. One important mechanism causing the decrease in serum T3 and increased serum rT3 concentrations is impaired activity of the selenodeiodinases, D1 and D2.25,62 In humans with NTI, D1 activity was shown to be down-regulated in the liver, and D3 activity, was induced in skeletal muscle, where it is not present in healthy humans. Type 2 deiodinase activity was not present in the skeletal muscle of critically ill patients,63 as it is in euthyroid patients.25,28 Inhibition of D1 would lead to decreased outer ring deiodination of T4 and rT3, causing decreased conversion of T4 to T3, and decreased metabolism of rT3. Inhibition of D2 would also cause decreased conversion of T4 to T3.

Alterations in thyroid hormone binding to carrier proteins likely play a role in the changes in thyroid hormones that occur in NTI.33 A marked reduction of T4, T3 and rT3 binding has been documented in critically ill patients, and may be due to decreased serum
affinity or decreased serum concentrations of thyroid-binding proteins. Decreased serum concentrations of TBG correlated with increasing disease severity in a study of children with meningococcal sepsis. The serum fT₄ concentration in that study was normal in the majority of patients, however decreased protein binding will raise serum fT₄ transiently, leading to decreased secretion of TSH via negative feedback, allowing serum concentrations to return to normal. This does not however, explain the decrease in serum fT₄ that can be seen in NTIS, strengthening the argument that multiple mechanisms are involved in NTIS.

Alterations in transfer and distribution of thyroid hormones may also contribute to the changes in thyroid function tests noted with NTIS. Inhibition of plasma membrane transport of T₄ into cells could lead to a decrease in the production of T₃ from T₄. For example, patients with liver disease have been shown to have a decreased fractional transfer rate from the extracellular to intracellular space.

Non-thyroidal illness may also lead to disturbances in the hypothalamic-pituitary-thyroid axis. Failure of serum TSH concentrations to increase despite decreased serum T₃ and T₄ concentrations suggests decreased sensitivity to negative feedback of thyroid hormones on TSH secretion. Continuous infusion of TRH to critically ill patients has been shown to markedly increase serum T₄ and T₃ concentrations, suggesting that TRH synthesis or secretion is impaired in patients with NTI. In fact, TRH messenger RNA was decreased in the paraventricular nucleus of patients with decreased serum T₃ concentrations who died from severe illness. It appears that central hypothyroidism is therefore responsible for some of the changes with NTI.
It is important to understand the changes that occur in thyroid function tests with NTI in order to provide the most appropriate therapy for patients. At the very least, NTI provides a diagnostic challenge, as patients may have clinical signs and laboratory findings suggestive of hypothyroidism from any number of illnesses, and it is up to the clinician to determine whether or not the patient is hypothyroid or suffering only from NTI. Despite extensive research on NTIS in humans, there is still no consensus as to whether patients with NTI are euthyroid, or in fact in a hypothyroid state that requires thyroid hormone supplementation.\textsuperscript{56,66}

2. Non-thyroidal illness syndrome- dogs

In comparison to humans with NTI who often have decreased serum T\textsubscript{3} concentrations alone, dogs with NTI are more likely to have decreased serum T\textsubscript{4} concentrations with or without decreased serum T\textsubscript{3} concentrations. Several studies have compared the results of thyroid function tests in hypothyroid dogs, healthy dogs, and dogs with NTI. Serum T\textsubscript{4} is decreased in approximately 30 to 59 per cent of dogs with NTI, with more severe disease producing a more dramatic decrease in serum T\textsubscript{4} concentrations.\textsuperscript{41,43} Interestingly, 59 per cent of dogs that were euthanized or died from their illness had a T\textsubscript{4} below the reference range.\textsuperscript{41} Serum fT\textsubscript{4} concentration may be decreased in 21.5 to 32 per cent of dogs with NTI, with more severe disease producing a more dramatic decrease.\textsuperscript{41,43} The study demonstrating decreased serum fT\textsubscript{4} concentration in 32 per cent of dogs with NTI evaluated dogs that had been euthanized or died from their illness.\textsuperscript{41} While the dogs in these 2 studies were determined to be healthy, hypothyroid or having nonthyroidal illness from the beginning of the study, another study evaluated dogs that were initially suspected to be hypothyroid due to clinical signs, but
were then classified as euthyroid based on the results of a TSH stimulation test of a TSH stimulation test.\textsuperscript{38} This study documented decreased serum T$_4$ and fT$_4$ concentrations in 18 and 7 per cent of dogs with NTI, respectively. Decreased serum T$_3$ concentrations are less common, occurring in only 16 per cent of dogs with NTI in one study.\textsuperscript{43} Another study reported a higher percentage of decreased T$_4$ and T$_3$ in critically ill dogs in an intensive care unit.\textsuperscript{67} In this study, 61 per cent and 56 per cent of dogs had low serum T$_4$ and T$_3$ concentrations, respectively. There was no significant difference in serum T$_4$ concentrations between dogs that survived and dogs that died, however serum T$_3$ concentration was significantly lower in dogs that died than in dogs that survived. Serum TSH concentrations may be increased in 7.6 to 12 per cent of dogs with NTI.\textsuperscript{39,41,43}

Fasting may have an effect on thyroid function tests in dogs, but this effect may not be as dramatic as that in humans. A mild decrease in serum T$_4$ concentration and mild transient increase in serum rT$_3$ concentration was reported during 4 days of fasting in dogs. A more dramatic and prolonged decrease in serum T$_3$ was reported in that same study.\textsuperscript{68} In another study of dogs fasted for 2 weeks, serum T$_3$ concentrations decreased significantly, but serum T$_4$ and rT$_3$ concentrations did not change.\textsuperscript{69}

A number of studies have evaluated the effects of specific illnesses on thyroid function tests in dogs. Diabetic ketoacidosis can decrease serum T$_4$ and T$_3$ concentrations, while renal and hepatic disease can decrease serum T$_4$ concentrations and increase serum rT$_3$ concentrations.\textsuperscript{2}

Cardiac disease may also produce alterations in thyroid function tests. Dogs with clinical signs secondary to cardiomyopathy have been documented to have decreased serum fT$_4$ concentrations with normal serum TSH and T$_4$ concentrations,\textsuperscript{70} however fT$_4$
in this study was not measured by equilibrium dialysis. Dogs with induced congestive heart failure but not dogs with spontaneous congestive heart failure have been shown to have decreased serum T₄ and increased serum rT₃ concentrations.⁷¹

Dogs with spontaneous hyperadrenocorticism have demonstrated significantly decreased serum T₄, T₃, rT₃ and fT₄, as well as a decreased (but present) response of T₄ to administration of TSH.⁷²,⁷³ In humans, glucocorticoids inhibit secretion of TSH, decrease serum TBG concentrations, and impair peripheral 5'-deiodination of T₄.⁷⁴ This results in decreased serum T₄, fT₄, T₃ and TSH concentrations and increased serum rT₃ concentrations.

Inflammatory cytokines released during NTI may play a role in the alterations seen in thyroid function tests in humans and dogs with NTI. Tumor necrosis factor α (TNF-α), interleukin-I (IL-1) and interleukin-6 (IL-6) administered to humans and rats have been documented to have an effect on thyroid function tests, producing a decrease in serum T₃, with varied effects on serum rT₃, T₄ and fT₄ concentrations.¹⁷,⁷⁵-⁷⁸ Increased serum IL-6 concentrations in humans have been demonstrated to correlate with decreased serum T₃ concentrations,⁷⁹ however one study failed to demonstrate a correlation between time of initial decrease in serum T₃ concentrations and increased serum IL-6 concentrations in patients undergoing abdominal surgery.⁸⁰

Several studies in dogs have documented changes in thyroid function tests after administration of endotoxins and interleukins.⁸¹-⁸³ Administration of endotoxin causes a release of cytokines such as IL-6 and TNF-α⁸³ and administration of interleukin-2 (IL-2) induces release of other cytokines such as TNF-α, interleukin-1 (IL-1), and interferon-γ.⁸¹ Continuous infusion of human recombinant IL-2 to dogs causes decreased serum T₄ and
Endotoxin administration to dogs has been documented to decrease serum T₃ concentrations and increase serum rT₃ and fT₄ concentrations.⁸²,⁸³ One of these studies documented a concurrent decrease in serum T₄ concentration,⁸³ while the other study did not. This difference may be due to differences in the protocol of endotoxin administration. In the study that reported no change in serum T₄, 5 µg/kg endotoxin was administered 24 hours before blood sampling on 2 separate occasions,⁸² while in the study that reported a decrease in serum T₄ concentration, 1 µg/kg endotoxin was administered every 12 hours for a total of 8 doses.⁸³

It is interesting to note that although there are similarities between alterations in thyroid function tests in dogs and humans with NTI, the alterations are not identical. Serum T₃ concentrations are less likely to be decreased in dogs with NTI than in humans with NTI. This may indicate that inhibition of selenodeiodinases is not as important a mechanism in NTIS in dogs as in humans. Dogs have been shown to have lower thyroid hormone binding to plasma proteins compared to humans. It is possible that this difference in thyroid hormone binding also contributes to the differences in the effects of NTI on thyroid function tests in dogs compared to humans.

3. Miscellaneous non-thyroidal factors

Various other non-thyroidal factors in addition to illness have effects on thyroid function tests. Although there does not appear to be a circadian rhythm to secretion of thyroid hormones by dogs, there is an episodic variation in serum T₄ and T₃ concentrations.⁸⁴,⁸⁵

Age, body size, breed and lifestyle may affect thyroid function tests in dogs. Serum T₃ concentration has been reported to be lower in medium-sized dogs than in
small and large-breed dogs, while serum T\textsubscript{4} has been reported to be greater in small-breed dogs than in medium or large-breed dogs. Serum T\textsubscript{3} and T\textsubscript{4} have been shown to fluctuate within different age groups, with nursing pups having higher concentrations of both hormones than older dogs.\textsuperscript{86}

Breed of dog may affect the values of certain thyroid function tests. Results of thyroid function tests in Greyhounds have also been found to differ from those in other breeds. In one study, serum T\textsubscript{4} and fT\textsubscript{4} concentrations were significantly lower in racing Greyhounds than in non-Greyhounds, while TSH concentrations were not significantly different between racing Greyhounds and non-Greyhounds. Additionally, racing Greyhounds had a significantly lower serum T\textsubscript{4} than non-Greyhounds after administration of TSH and TRH. The reference range for T\textsubscript{4} and fT\textsubscript{4} was calculated for the 98 Greyhounds in that study, and the lower limit was found to be lower than that previously established for all dog breeds. Possible explanations for the decreased T\textsubscript{4} and fT\textsubscript{4} seen in these Greyhounds include lower plasma protein levels, reduced affinity of binding proteins for hormones increased efficiency of conversion of T\textsubscript{4} to T\textsubscript{3}, and a more sensitive feedback mechanism leading to decreased T\textsubscript{4} and fT\textsubscript{4} concentrations.\textsuperscript{87}

Another study compared serum thyroid hormone concentrations and TSH concentrations in a small group of Greyhounds before and 5 minutes after a race, and then after 3 months of not racing. When corrected for hemoconcentration (based on serum albumin levels) T\textsubscript{4} concentrations increased significantly after racing. Thyroxine concentrations also significantly increased after 3 months of not racing. Pre-racing T\textsubscript{4} and fT\textsubscript{4} were lower in Greyhounds than in clinically normal dogs of other breeds.\textsuperscript{88}
Other sighthounds also have thyroid hormone concentrations below the reference ranges for other dogs. Whippets have been shown to have a significantly lower T₄ than other dog breeds. However, there were no significant differences in serum concentrations of fT₄, TSH or thyroglobulin autoantibodies between Whippets and other dogs. Additionally, there does not appear to be a difference between racing and non-racing Whippets.⁸⁹ In Scottish Deerhounds, the T₄ and fT₄ concentrations have both been reported to be significantly lower than those of other breeds.⁴⁹

Several studies have examined thyroid hormone and TSH concentrations in Alaskan sled dogs while training (preparing for a race), after competing in a long-distance race, and in an untrained (sedentary) state, and have demonstrated alterations in these parameters both after competing in a race and in the trained versus untrained state.⁹⁰-⁹² Plasma T₄, T₃, fT₄ and TSH concentrations have been shown to decrease in training sled dogs after competing in a long-distance race.⁹¹,⁹² One study demonstrated plasma T₄ concentrations less than the lower reference limit in 7 of 31 sled dogs immediately prior to a race and in 18 of 39 dogs after the race,⁹² while another study demonstrated plasma T₄ concentrations less than the lower reference limit in 32 of 122 dogs before a race and in 80 of 84 dogs after the race.⁹¹ In the second study, plasma fT₄ concentrations were below the reference range in 20 of 120 dogs before racing and in 37 of 84 dogs after racing, and plasma TSH concentration was above the reference range in 7 of 122 dogs before the race, but was normal in all dogs after the race.

There are also differences in thyroid hormone and TSH concentrations in the trained (preparing for a race) versus untrained (sedentary) state. Serum T₄ and fT₄ concentrations have been shown to be decreased significantly during peak training as
compared to the non-training period, and serum TSH concentrations were significantly increased in the peak training as compared to the non-training period.\textsuperscript{90} That study did not detect a difference in serum concentrations of T\textsubscript{3}, fT\textsubscript{3} or thyroglobulin autoantibodies between the training and non-training period.

There are several possible explanations for the changes in thyroid hormone and TSH concentrations seen in sled dogs. There may be decreased binding of thyroid hormones to plasma transport proteins, either from displacement of thyroid hormones from binding proteins by exercise-induced increases of free fatty acids, or by a decrease in plasma transport proteins. Decreased plasma total protein as well as albumin have been reported in sled dogs when training.\textsuperscript{92} Decreased binding of thyroid hormones to plasma proteins would be expected to increase fT\textsubscript{4} while decreasing T\textsubscript{4}, which was not seen indicating that other mechanisms must also play a role in the changes in thyroid hormone concentrations noted in sled dogs when training heavily. Nutrition, intensive exercise, and cold temperatures may all play a role in the alterations seen in thyroid hormone concentrations in sled dogs.\textsuperscript{90,91}

4. Drugs

An extensive number of drugs are known to alter the results of thyroid function tests in humans. The effects on thyroid function tests of numerous drugs have also been investigated in dogs, although the list is not as expansive as it is in humans.

a. Sulfonamides

Sulfonamides have been shown in several studies to interfere with thyroid function in dogs. These antibiotics decreased thyroid hormone synthesis by reversible
inhibition of TPO, which is necessary for iodination and coupling reactions. Decreased thyroid hormone synthesis in turn reduces negative feedback, which increases TSH secretion. Although sulfonamides only have mild effects on thyroid function in humans, they markedly decrease thyroid function in rats.¹⁴²

A number of prospective studies have evaluated the effects of sulfonamides on thyroid function tests in dogs, and have shown different effects depending on sulfonamide administered, and dose and duration of treatment. No significant effect on serum T₃, T₄ and fT₄ concentrations or response of serum T₄ concentration to TSH was seen in a controlled study evaluating the effects of the administration of trimethoprim-sulfadiazine (15 mg/kg PO q 12 hours) to normal dogs for 28 days.⁹³

However, several other studies have shown substantial suppression of thyroid function tests in dogs administered sulfamethoxazole. In one study, 6 of 7 normal dogs administered trimethoprim-sulfamethoxazole (26.5 to 31.3 mg/kg PO q 12 hours) had serum T₄ concentrations that were less than the lower reference limits within 3 weeks of beginning treatment, and had serum TSH concentrations that were greater than the upper reference limit within 4 weeks of treatment. Serum T₄ was significantly lower than pretreatment values and serum TSH was significantly higher than pretreatment values in these 6 dogs. Serum T₄ concentration was not significantly different than pretreatment concentrations 1 week after discontinuing treatment, and serum TSH concentration was not significantly different than pretreatment concentrations by the fifth week after discontinuing treatment in five out of six dogs.⁹⁴

Another study examining the effects of administration of trimethoprim-sulfamethoxazole (30 mg/kg PO q 12 hours) for 6 weeks to 21 dogs with pyoderma, also
demonstrated a significantly lower serum T4 concentration after treatment, with 12 of 21 dogs having serum T4 concentrations less than the lower reference limit. Serum T3 levels were not significantly different after 6 weeks of treatment. Serum TSH concentrations were not measured in that study, although the response of serum T4 to TSH administration was decreased in 3 dogs after 6 weeks of treatment, and in 1 dog took 12 weeks to return to normal once treatment was stopped.95

More recently, a lower dose of trimethoprim-sulfamethoxazole (14.1-16 mg/kg PO q 12 hours) administered to 6 normal dogs for 3 weeks has also been shown to decrease serum T4 and fT4 concentrations compared to pretreatment concentrations, and increase serum TSH concentrations. Serum T4 concentrations were less than the lower reference limit in 3 dogs, and only slightly greater than the lower reference limit in 2 dogs. Serum fT4 concentrations were less than the lower reference limit in 4 dogs after 3 weeks of treatment. Serum TSH concentrations were greater than the upper reference limit in 4 dogs after 3 weeks of treatment. Serum T4 and fT4 concentrations were within the normal reference range for all dogs 1 week after stopping treatment, and serum TSH concentrations were within the normal reference range in all dogs 5 weeks after treatment ended.96

Treatment with sulfonamides has been reported to cause clinical hypothyroidism in addition to simply altering the results of thyroid function tests.97,98 Two dogs receiving trimethoprim-sulfadiazine at a dosage of approximately 25 mg/kg PO BID q 12 hours for 30 to 126 days developed clinical signs of hypothyroidism.97,98 Both dogs had decreased serum concentrations of T4, and fT4, increased TSH, and decreased response of T4 to TSH. Thyroid scintigraphy performed in one dog98 showed normal uptake of
pertechnetate by the thyroid gland, consistent with sulfonamide-induced hypothyroidism, as sulfonamides do not inhibit iodide uptake.\textsuperscript{95}

In summary, sulfonamides have been shown to decrease serum T\textsubscript{4} and fT\textsubscript{4} concentrations and to increase serum TSH concentrations indicative of primary hypothyroidism. Dose, duration, and the type of sulfonamide administered may all contribute to development of hypothyroidism, and higher doses of sulfonamides given for an extended period of time may cause clinical signs of hypothyroidism.

\textbf{b. Non-steroidal anti-inflammatory drugs}

Many non-steroidal anti-inflammatory drugs (NSAIDs) have been demonstrated to affect thyroid function tests in humans by displacing thyroid hormones from their plasma protein binding sites.\textsuperscript{1,42} Inhibition of 5' - deiodination and impaired binding to the plasma or nuclear membrane or cytosol may also contribute to alterations seen in thyroid function tests with NSAID administration.\textsuperscript{42} Short-term salicylate administration results in a transient increase in free hormone concentration and a decrease in TSH concentration. With long-term administration, a new steady state is reached, reflecting an increased T\textsubscript{4} turnover rate. Decreased T\textsubscript{4} concentration with normal or decreased fT\textsubscript{4} concentration and normal TSH concentrations have been reported.\textsuperscript{42} Decreased fT\textsubscript{4} concentrations imply that other mechanisms likely contribute to the changes in fT\textsubscript{4}, as impaired binding alone should increase serum fT\textsubscript{4} concentrations. Other NSAIDs such as ketoprofen and naproxben have caused decreased T\textsubscript{3} concentration with no effect on T\textsubscript{4} or TSH concentrations.\textsuperscript{1,42} However, TBG binding capacity in dogs is 25 per cent that of humans\textsuperscript{21} and thyroid hormones in dogs have less affinity for their transport proteins than
Because of these differences, alterations seen in canine thyroid function tests secondary to NSAID administration may not be the same as those in humans. Several prospective controlled studies have evaluated the effects of various NSAIDs on thyroid function tests in dogs. Salicylates have been shown to decrease serum $T_4$, $T_3$ as well as $fT_4$. Eight normal dogs administered aspirin (24 mg/kg PO q 8 hours) for 28 days showed a significant decrease from pretreatment values in serum $T_4$, $fT_4$ and $T_3$ concentrations compared to placebo. Serum $T_4$ and $fT_4$ concentrations were less than the lower reference limit in 5 dogs and serum $T_3$ was less than the lower reference limit in 1 dog at the end of the treatment period. There were no significant changes in TSH or in the free fraction of $T_4$ ($fT_4/ T_4$). Serum thyroid hormone concentrations returned to pretreatment values 14 days after treatment was discontinued. Similarly, 18 normal dogs administered a lower dose of aspirin (25 mg/kg PO q 12 hours) for 7 days showed a significant decrease in serum $T_4$ concentration within 24 hours of starting aspirin administration, and in serum $T_3$ concentration after 1 week of aspirin administration. There were no significant changes in serum $fT_4$ concentration or serum TSH concentration with aspirin administration, however the free fraction of $T_4$ was significantly higher dogs administered aspirin than in controls. It is interesting that in one of the studies, the $fT_4$ concentration decreased, while in the other study, it did not change. These differences may be due to aspirin dose, duration of treatment, or both. The decrease in $fT_4$ seen in the 28 day study implies that mechanisms other than displacement of thyroid hormones from their binding sites play a role in the alterations seen in thyroid function tests with aspirin administration.
Carprofen is one of the most commonly used NSAIDs in dogs. Two prospective studies with contrasting results have evaluated the effects of carprofen on thyroid function tests in dogs.\textsuperscript{101,102} Twenty-one normal dogs administered carprofen (2.2-3.3 mg/kg PO q 12 hours) for 5 weeks showed a significant decrease in serum $T_4$ and TSH concentrations, with no significant effect on serum $fT_4$ concentration. Four of the dogs had serum $T_4$ concentrations less than the lower reference limit, and 3 of the dogs had undetectable serum TSH concentrations.\textsuperscript{102} In the other study 14 dogs with osteoarthritis administered carprofen (1.7-2.3 mg/kg PO q 12 hours) for 60 days did not show significant changes in serum thyroid hormone or TSH concentrations from pretreatment values.\textsuperscript{101} The differences between the studies may be due to the slight difference in dosing. The dogs in the second study did have osteoarthritis, but moderate to severe osteoarthritis has been shown to have no effect on serum $T_4$ concentrations.\textsuperscript{103} Dogs with osteoarthritis did have mild but statistically significantly higher serum $fT_4$ and TSH concentrations than dogs without osteoarthritis.\textsuperscript{103}

Several other NSAIDs have been shown to have no effects on thyroid function tests in dogs.\textsuperscript{99-101,104} Ketoprofen (1 mg/kg PO q 24 hours) administered to 18 normal dogs for 7 days\textsuperscript{99}, meloxicam (0.2 mg/kg PO once then 0.1 mg/kg PO q 24 hours) administered to 14 dogs with osteoarthritis for 60 days\textsuperscript{101}, etodolac (3.7 mg/kg PO q 24 hours) administered to 19 normal dogs for 28 days,\textsuperscript{104} and deracoxib\textsuperscript{100} (1.6 mg/kg PO q 24 hours) to 8 dogs for 28 days did not cause any alterations in serum concentrations of $T_4$.\


c. Glucocorticoids

Studies performed in rats, humans and dogs have shown that exogenous and endogenous glucocorticoids inhibit the hypothalamic-pituitary-thyroid axis and affect the peripheral metabolism of thyroid hormones. In humans, glucocorticoids inhibit secretion of TSH, decrease serum TBG concentrations, and impair peripheral 5’-deiodination of T₄. This results in decreased serum T₄, fT₄, T₃ and TSH concentrations and increased serum rT₃ concentrations.

In dogs, the effects of glucocorticoids on thyroid function tests vary depending on the dose used. Prednisone (0.55 mg/kg PO q 12 hours) administered for 35 days caused a significant decrease in serum T₃ concentrations after 14 and 28 days, but did not cause significant changes in serum T₄, fT₄, fT₃ or rT₃. However, the same dose and duration of prednisone treatment in levothyroxine supplemented thyroidectomized dogs has demonstrated not only a decreased T₃, but also a decreased fT₃ and free fraction of T₄. This was hypothesized to be secondary to changes in peripheral thyroid hormone metabolism such as decreased conversion of T₄ to T₃ and increased binding of T₄ to serum binding proteins.

Immunosuppressive doses of prednisone do not have the same effect on thyroid function tests as antiinflammatory doses. Immunosuppressive doses of prednisolone (1.1 mg/kg PO q 12 hours) administered for 3 weeks caused a significant decrease in serum T₄, T₃ and fT₄ concentrations and the response of T₄ and T₃ to TSH and TRH administration. The effects on serum T₄, T₃ and fT₄ concentrations were seen within 1 day after starting treatment, and this effect was more pronounced after 3 weeks of treatment. Similarly, immunosuppressive doses of prednisone (1.1-2 mg/kg PO q 12 hours) administered for 3 weeks also caused a significant decrease in serum T₄ and fT₄.
No significant change in serum TSH concentrations were seen in the study with prednisone, but were not measured in the study with prednisolone. Failure of serum TSH concentration to increase when serum $T_4$, $T_3$ and $fT_4$ concentrations were decreased may indicate suppression of pituitary secretion of TSH.

Excess endogenous glucocorticoids (hyperadrenocorticism) produce results similar to immunosuppressive doses of exogenous glucocorticoids. Dogs with spontaneous hyperadrenocorticism have demonstrated significantly decreased serum $T_4$, $T_3$, $rT_3$ and $fT_4$, as well as a decreased (but present) response of $T_4$ to administration of TSH.\textsuperscript{72,73}

d. Anticonvulsants

Potassium bromide does not appear to have a significant effect on thyroid function tests in dogs,\textsuperscript{109,110} however phenobarbital does. Studies in rats and in humans have shown that phenobarbital enhances hepatic metabolism of $T_4$ due to hepatic microsomal enzyme induction, thereby increasing peripheral elimination of $T_4$ via increased deiodination of thyroid hormones in the liver. Increased biliary and fecal excretion also contributes to decreased serum concentrations of thyroid hormones, and phenobarbital may also have effects on the hypothalamic-pituitary thyroid axis.\textsuperscript{1,42}

Short-term administration of phenobarbital (1.8- 3 mg/kg PO q 12 hours for 1 week, then 2.7-4.5 mg/kg PO q 12 hours for 2 weeks) does not have a significant effect on thyroid function tests in dogs.\textsuperscript{108} Long-term administration of phenobarbital to dogs, on the other hand, has caused decreased serum $T_4$ and $fT_4$ concentrations. These effects have been seen in both epileptic\textsuperscript{110-112} and normal\textsuperscript{113,114} dogs administered phenobarbital, and are not necessarily related to dose, serum phenobarbital level, or degree of seizure
control. Decreased T₄ and fT₄ may be seen within 3-5 weeks of beginning phenobarbital treatment. Although in one study no change in serum TSH concentration was seen in dogs administered phenobarbital, several studies have documented an increase in serum TSH concentrations. Serum TSH concentration increases only after long-term (greater than 6 months) phenobarbital administration.

The findings of decreased T₄ and fT₄ with increased TSH suggest that the hypothalamic-pituitary-thyroid axis in dogs treated with phenobarbital remains intact. The expected response to decreased thyroid hormones is an increase in TSH through loss of negative feedback, as has been noted in dogs administered phenobarbital chronically. Therefore, as in rats and humans, decreases in T₄ and fT₄ are likely due to enhanced hepatic metabolism of these hormones. The fact that the TSH concentrations do not increase until dogs have been receiving phenobarbital for 6 months or more may indicate that in the initial months of administration, increased deiodination of T₄ to T₃ maintains normal concentrations of T₃ within the pituitary gland, thereby maintaining normal TSH concentrations.

Interestingly, a recent study demonstrated decreased serum T₄ concentrations in dogs with idiopathic epilepsy that had not been treated with phenobarbital or other anticonvulsant medications. There was a significant correlation between seizure frequency, and dogs with longer seizure intervals had higher serum T₄ concentrations. It is possible that idiopathic epilepsy itself contributes to the alterations in thyroid function tests previously reported in dogs on phenobarbital, however these alterations have been documented in dogs without seizures, and no alterations in thyroid function tests were reported in the studies evaluating potassium bromide.
e. Tricyclic Antidepressants

Tricyclic antidepressants have been shown to impair thyroid hormone synthesis and TSH secretion in humans and rats via alteration of thyroid follicular cell iodide uptake, TPO inhibition, and interference with the hypothalamic-pituitary-thyroid axis.\textsuperscript{1,116} One study has evaluated the effects of the tricyclic antidepressant clomipramine (commonly used for separation anxiety in dogs) on thyroid function tests in dogs.\textsuperscript{116} Clomipramine (3 mg/kg PO q 12 hours) given to 14 normal dogs for 112 days caused a significant decrease in serum T\textsubscript{4}, fT\textsubscript{4}, and rT\textsubscript{3} concentrations. The study did not evaluate how long these effects are present following cessation of clomipramine.

5. Anesthesia and Surgery- humans

Anesthesia and surgery may have effects on thyroid hormones at various levels, including effects on the hypothalamic-pituitary-thyroid axis, effects on thyroid hormone synthesis and secretion, and effects on peripheral metabolism. Pre-anesthetic medications, anesthesia protocol, surgical procedure, and concurrent illnesses of the patient may all contribute to alterations in thyroid function tests.

In humans, there are numerous studies evaluating the effects of anesthesia and surgery on thyroid function tests. Unfortunately, the majority of these studies were not controlled and did not always have a sufficient number of patients. Not all of the studies provided adequate information regarding preoperative patient status, type and duration of surgery, anesthetic and analgesic protocol, and postoperative recovery to fully interpret the results.

Anesthesia and surgery consistently cause decreases in serum T\textsubscript{3} and fT\textsubscript{3} concentrations. In studies where the type of surgery performed and the anesthetic
protocol was documented, these changes seem to occur independently of surgical procedure performed, or anesthetic protocol used. Numerous studies have evaluated thyroid function tests in patients undergoing cholecystectomy, and all of these studies documented a decrease in serum T₃ concentrations, no matter which premedication, induction agents and general anesthesia protocol was used. Serum fT₃ was not measured in all of these studies, however it was decreased in the cholecystectomy studies when it was measured. Decreases in serum T₃ concentrations have also been seen in patients undergoing resection of gastric cancer, hysterectomy, and other miscellaneous intraabdominal procedures. Again, serum fT₃ concentrations also decreased in the studies in which they were measured. Extraabdominal procedures including orthopedic surgery, mastectomy and open heart surgery with cardiopulmonary bypass also cause a decrease in serum T₃ concentrations. Serum fT₃ concentration was also decreased in the orthopedic study, but was not measured in the other studies. The decreases in serum T₃ and fT₃ concentration were more dramatic in patients undergoing abdominal surgery than in patients undergoing extraabdominal surgery in one study and in patients having laparotomy for acute peritonitis rather than as an elective procedure (only T₃ was measured). This indicates that the extent of surgical trauma may contribute to the degree of decrease in T₃ and fT₃. Patients with complications such as respiratory compromise and low cardiac output after cardiovascular surgery had a lower T₃ than those that had no complications. Patients undergoing cardiopulmonary bypass are typically on a number of medications prior to surgery, and it is possible that the combination of these medications, anesthesia, bypass and the surgery itself have an effect on thyroid function tests.
The decreases in serum T₃ or fT₃ concentrations can be seen at the time of or shortly following anesthesia induction,⁷,¹²,¹²⁰ or at or shortly following skin incision.⁶,¹¹⁸ Numerous studies did not begin measuring thyroid hormone concentrations until the postoperative period, therefore the actual time when the concentrations of serum T₃ and fT₃ decreased is not known. Serum T₃ concentrations often reach a nadir at 24 hours postoperatively,⁶,¹¹-¹⁴,¹¹⁷ with serum fT₃ concentrations typically paralleling serum T₃ concentrations, although in some instances, the nadir in the serum fT₃ concentration may occur 2 or more days postoperatively.⁵,¹³ Serum T₃ and fT₃ may be decreased from baseline values for several days postoperatively,⁶,¹⁰,¹⁴,¹⁶,¹¹⁹ with one study demonstrating a reduction that continued as long as 9 days postoperatively.⁶

The timeline of the decreases seen in serum T₃ and serum fT₃ concentrations indicate that anesthesia induction, skin incision and surgery itself, and the postoperative period may all play a role in causing these changes. The fact that in many studies the lowest serum T₃ concentration was seen 24 hours postoperatively indicate that there may be a contribution of perioperative fasting to decreased serum T₃ concentration in postoperative human patients, as fasting has been shown to decrease this thyroid hormone in people within 24-48 hours of the initiation of fasting.⁵⁷ The changes seen in fasting may be less dramatic than those seen with anesthesia and surgery.¹²

In contrast to the changes that occur in serum T₃ and fT₃ concentrations, anesthesia and surgery typically cause an increase in serum rT₃ concentrations. In most studies, this increase is independent of surgical procedure performed or anesthetic protocol used, and has been documented in patients undergoing cholecystectomy,⁴,¹¹,¹²,¹⁴,¹⁶,¹¹⁷ gastric neoplasia resection,⁴ other various abdominal
surgerys, orthopedic surgery, cardiovascular surgery, and mastectomy. In two of the studies, the increase in serum rT$_3$ concentration was only seen in the patients that were anesthetized with halothane or enflurane, while in other studies the increase occurred with other anesthetic agents including isoflurane and fentanyl and nitrous oxide as well. Unfortunately, a number of the studies did not report what anesthetic protocol was used, making it difficult to determine the effect of various anesthetic agents on serum rT$_3$ concentration. The most significant increase in serum rT$_3$ concentrations is often correlated with the most significant decrease in serum T$_3$ concentration. However, serum rT$_3$ concentrations returned to baseline levels more rapidly.

There are several mechanisms that could cause the decrease in serum T$_3$ and serum fT$_3$ and increase in serum rT$_3$ concentrations reported in most studies. One proposed mechanism is impaired or altered deiodinase activity. Inhibition of D1 would result in decreased outer ring deiodination of T$_4$ and rT$_3$, leading to decreased production of T$_3$ from T$_4$, and decreased metabolism of rT$_3$. Inhibition of D2 would also lead to decreased production of T$_3$ from T$_4$. Alternately, there could be a shift in deiodinase activity to favor the alternate pathway to increase rT$_3$ production from T$_4$ via D3. This could explain the increase in serum rT$_3$ concentration as well as the decrease in serum T$_3$ concentration, as D3 also catalyzes the conversion of T$_3$ to 3,3'-diiodothyronine, therefore an increase in D3 activity would be expected to decrease serum T$_3$ concentrations. Altered binding to plasma proteins could play a role in some instances of the decreased serum T$_3$ concentrations seen with anesthesia and surgery, but this would be expected to cause an increase in serum fT$_3$ concentrations, rather than the decrease seen in most studies; however, not all studies evaluated serum fT$_3$
concentrations. In addition, serum fT₃ concentrations in humans can vary greatly depending on the type of assay used, as indirect methods give low values more often than direct dialysis methods.¹⁷ Unfortunately, dialysis methods were only used in 2 of the studies that measured fT₃,⁶,¹⁴ while radioimmunoassays were used in the others. It is possible that some of the decreases in fT₃ could be related to the assay method, rather than to the effects of anesthesia and surgery.

There are conflicting reports regarding the changes that occur in serum T₄ and fT₄ concentrations with anesthesia and surgery. This may be due to differences in pre-medications, anesthetic protocols and surgical procedures. Because fT₄ was not measured in many studies, and anesthetic protocols were not always given, it is difficult to make correlations between the anesthetic used and the changes seen in serum T₄ and fT₄ concentrations. A number of studies have shown no change in serum T₄ concentrations with cholecystectomy³,¹¹,¹² and various other intra- and extraabdominal surgeries.¹²,¹²²,¹²³ However, those studies did not evaluate serum fT₄ concentrations. Inhalant anesthetics recorded in those studies included isoflurane with nitrous oxide and methoxyflurane.³,¹²²

A postoperative increase in both serum T₄ and fT₄ concentrations together has been seen following cholecystectomy,¹⁶ and miscellaneous surgeries⁵ with unlisted anesthetics as well as with halothane and enflurane. In another study evaluating patients undergoing cholecystectomy or gastric neoplasia resection there was an increase in both serum T₄ and fT₄ concentrations only in groups receiving nitrous oxide and isoflurane or enflurane, but not in groups receiving nitrous oxide and epidural bupivicaine, pentazocine, ketamine or halothane.⁴ Another study in patients having surgery for
intervertebral disk disease showed an increase in serum $T_4$ and free $T_4$ concurrently in patients receiving enflurane, but not in patients receiving a variety of injectable anesthetics, isoflurane, or halothane. However, halothane caused an increase in serum $T_4$ concentration after 30 minutes of administration in various surgeries, while methoxyflurane caused no change in $T_4$ in the same study; the increase in $T_4$ continued in the recovery room once the patients had regained consciousness, but was not evaluated after that time. Patients undergoing hysterectomy who received general anesthesia with nitrous oxide and enflurane demonstrated an increase in serum $T_4$ concentrations, while patients who received epidural anesthesia with bupivicaine actually showed a decrease in serum $T_4$ concentration. Serum $fT_4$ concentrations were not measured in the study with methoxyflurane and halothane or in the hysterectomy study.

A decrease in serum $T_4$ concentrations with anesthesia has also been reported. A decrease in serum $T_4$ concentrations was seen in patients undergoing cholecystectomy, mastectomy, and various elective procedures. Halothane and nitrous oxide and nitrous oxide alone were reported anesthetic agents in 2 of these studies, however in the other studies, the anesthetic protocol was not stated. A decrease in serum $T_4$ and $fT_4$ both has been reported in patients undergoing cardiovascular surgery with cardiopulmonary bypass. In one study, isoflurane was the primary anesthetic, but in the other study, the anesthetic protocol was not listed. The decrease in serum $T_4$ concentration was more dramatic in those patients that had complications such as respiratory compromise and low cardiac output after surgery than in those with no complications.
There are several mechanisms that could lead to an increase in serum T₄ and/or fT₄ concentration in patients undergoing anesthesia and surgery. Based on the studies discussed above, the anesthetic agent may play more of a role in causing an increase in these thyroid hormones than surgery itself. There may be a release of these hormones from hepatic or other extrathyroidal stores,⁴,⁸ or inhibition of T₄ deiodination⁸ that causes the increase in serum T₄ and fT₄ concentrations. Decreased serum T₄ concentrations may be the result of altered binding to plasma proteins.¹¹⁷ Decreased serum concentrations of TBG have been reported in patients having surgery.⁵ If the decrease in serum T₄ concentrations were due solely to altered binding to plasma proteins, a concurrent increase in serum fT₄ concentrations would be expected, which was documented in one study.⁶ Unfortunately, not all of the studies that evaluated serum T₄ concentrations also evaluated serum fT₄ concentrations. At least one study demonstrated a decrease in serum T₄ concentration with no change in serum fT₄ concentration, indicating that other mechanisms besides altered binding to plasma proteins must be involved in the decrease in serum T₄ concentration. An increase in T₄ metabolism,⁷ increased transport of T₄ into cells, or decreased thyroid hormone secretion could all lead to a decrease in serum T₄ concentrations.¹⁶

As with serum T₄ and fT₄ concentrations, there have been conflicting reports as to the changes seen in serum TSH concentrations with anesthesia and surgery. No change has been seen in serum TSH concentrations in patients undergoing cholecystectomy³,⁴,¹²,¹⁶ and miscellaneous surgeries¹²¹ in some studies, while other studies have shown an increase in serum TSH concentrations with various elective surgeries,¹⁰,¹²⁰ and surgery for intervertebral disk disease.⁸ In patients undergoing surgery
for a prolapsed intervertebral disk, the increase in serum TSH concentration was seen postoperatively, and was more marked in patients receiving opiate anesthetic techniques than in those receiving inhalant anesthetics or ketamine and midazolam. In a number of studies in which no change in serum TSH concentration was seen, general anesthesia was maintained with inhalant anesthetics such as isoflurane, enflurane, halothane and methoxyflurane,\textsuperscript{3,4,122} although in one of the studies, fentanyl was part of the pre-medication.\textsuperscript{3} It is possible that any effect that one dose of fentanyl may have had on serum TSH concentration was not documented due to time of blood sampling in relation to time of fentanyl administration in that study. A number of studies demonstrated a decrease in serum TSH concentration in patients undergoing orthopedic procedures,\textsuperscript{13} cardiovascular surgery,\textsuperscript{7,121} uncomplicated abdominal surgery,\textsuperscript{6} and miscellaneous elective surgeries.\textsuperscript{5,123} In some of these studies, inhalant anesthetics including halothane, ethrane, isoflurane and nitrous oxide were used,\textsuperscript{5,6,123} and in one study patients were pre-medicated with morphine.\textsuperscript{6} Interestingly, although administration of morphine was reported in only one of the studies, this opiate has been demonstrated to decrease plasma or serum TSH concentrations in rats.\textsuperscript{124-128}

Mechanisms that have been proposed to explain a decrease in serum TSH concentration include enhanced TSH clearance, and direct inhibition by fT\textsubscript{4} feedback.\textsuperscript{13,123} Increased serum cortisol concentrations during surgery have been suggested as a mechanism for TSH suppression.\textsuperscript{5,7,123}

Several studies reported an increase in serum TSH concentrations with anesthesia and surgery, but these increased concentrations do not necessarily correlate temporally with the decrease in thyroid hormones.\textsuperscript{10} Although morphine administration has been
shown to decrease TSH concentrations in rats, opiates may have the opposite effects in humans, and their administration could account for the increase in serum TSH concentrations.\(^{120}\)

In summary, anesthesia and surgery typically decrease serum T\(_3\) and fT\(_3\) concentrations, increase serum rT\(_3\) concentrations and have variable results on serum T\(_4\), fT\(_4\) and TSH concentrations. Interestingly, quite a few of the studies evaluated thyroid function tests in patients undergoing cholecystecomy, but the results of these studies were varied. Based on the conflicting reports of changes in serum T\(_4\) and fT\(_4\) and TSH concentrations, it is likely that anesthetic protocol, type of surgical procedure, and patient status all contribute to the alterations in these tests, and may each act through different mechanisms to produce the changes seen.

6. **Anesthesia and surgery- Dogs**

Based on the differences in changes in thyroid function tests between humans and dogs with NTI (specifically the tendency to have decreased serum T\(_4\) but normal serum T\(_3\) concentrations in dogs), and because the changes in human thyroid function tests due to anesthesia and surgery are similar to those due to NTI, it seems likely that the changes seen in dogs undergoing anesthesia and surgery will be different than those seen in humans. As mentioned above, these differences may be due to differences in deiodinase activity in dogs compared humans, or due to the decreased protein binding of thyroid hormones in dogs.

While a review article reported a decrease in serum concentrations of T\(_4\), T\(_3\) and rT\(_3\) in dogs undergoing general anesthesia and surgery,\(^2\) the details of the experimental
design and the data were not presented. This change was reportedly present for at least 30 hours following the procedures; however it is possible that the effects lasted longer than this.

7. Importance

Because hypothyroidism is the most common endocrine disorder in dogs, it is commonly tested for. As described above, numerous non-thyroidal factors affect the results of thyroid function tests. If a dog is tested for hypothyroidism while receiving medication that is known to affect thyroid function tests, or if the dog has a medical condition in addition to suspected hypothyroidism, the results of the thyroid function tests must be interpreted in light of the non-thyroidal factors present. Alternately, the dog must be retested when the non-thyroidal factors have been eliminated as much as possible.

Veterinarians may be suspicious of hypothyroidism in dogs that have recently been anesthetized and/or had surgery. However, without information from published studies as to the effects of anesthesia on thyroid function tests in dogs, it may be difficult to appropriately interpret the results of thyroid function tests in these dogs, and to know when samples can be analyzed without the effects of these procedures interfering. Therefore, it is essential to gain an understanding of the effects of anesthesia and surgery on these thyroid function tests.
CHAPTER II: THE EFFECTS OF ANESTHESIA AND SURGERY ON THYROID FUNCTION TESTS IN DOGS

A. Introduction

Numerous drugs and systemic illnesses affect thyroid function tests in dogs, often resulting in alterations similar to those found in hypothyroid dogs. Anesthesia and surgery have been documented to affect thyroid function tests in humans. The only report evaluating thyroid function tests in dogs undergoing anesthesia and surgery did not provide details of the study design or results. A decrease in serum concentrations of total thyroxine (T₄), total triiodothyronine (T₃) and reverse triiodothyronine (rT₃) was reported in dogs undergoing general anesthesia and surgery.²

Anesthesia and surgery consistently cause decreases in serum T₃ and free T₃ (fT₃) concentrations and increases in rT₃ concentrations in humans.⁴,⁵,¹⁰-¹²,¹⁴,¹⁶,¹¹⁷,¹¹⁹ These changes may be greater with more extensive surgical trauma ¹⁰,¹² and with post-operative complications.¹²¹ Results of studies evaluating the effects of anesthesia and surgery on other tests of thyroid function are variable. Differences in pre-anesthetic medications, anesthesia protocol, surgical procedure, and concurrent illnesses of the patients may all contribute to the inconsistency of these changes.

The objective of this study was to evaluate the effects of anesthesia alone and anesthesia combined with abdominal exploratory surgery on thyroid function tests in dogs. We evaluated the hypothesis that anesthesia alone and anesthesia combined with surgery would have an effect on thyroid function in dogs. A secondary hypothesis was
that the effects of anesthesia and surgery would be greater than the effects of anesthesia alone.

B. Materials and Methods

1. Dogs

Fifteen adult, mongrel dogs (9 males and 6 females) were studied. Dogs were determined to be healthy on the basis of no significant abnormalities on physical examination, complete blood count, serum chemistries and electrolytes (glucose, blood urea nitrogen, creatinine, phosphorous, total protein, albumin, calcium, globulin, alanine aminotransferase, alkaline phosphatase, total bilirubin, cholesterol, potassium, sodium, chloride, total carbon dioxide and anion gap), as well as a negative heartworm antigen test. Dogs were determined to be euthyroid based on results of serum T4, T3, fT4 by equilibrium dialysis (fT4d), and canine TSH (cTSH). Mean body weight +/- standard deviation was 12.69 +/- 1.7 kg. Dogs were housed in indoor runs with a 12-hour light-dark cycle and were allowed to acclimate to their environment for 2 weeks prior to study. This study was approved by the Animal Care and Use Committee, Virginia Polytechnic Institute and State University.

2. Procedures

Five dogs were randomly assigned to each of three groups: control, general anesthesia, and general anesthesia plus abdominal exploratory surgery. Each group consisted of 3 males and 2 females. Dogs in the anesthesia and surgery groups were pre-medicated with acepromazine (0.05 mg/kg IM) and morphine (0.5 mg/kg IM), and anesthesia was induced with propofol (4 mg/kg IV titrated to effect). Dogs were endotracheally intubated and maintained with isoflurane (0.8-1.25%) in oxygen using a
semi-closed circle breathing circuit. Mechanical ventilation was instituted to maintain eucapnia (end-tidal carbon dioxide ranging between 35-45 mmHg). Dogs were anesthetized for a total of 100 minutes. Dogs in the surgery group also had an exploratory laparotomy performed that lasted 60 minutes from initial skin incision to skin closure. Surgery was performed via a ventral midline celiotomy, with the skin incision extending just caudal to the xyphoid to midway between the umbilicus and pubis. The abdomen was explored in a systematic method from cranial to caudal. The linea alba was closed with 2-0 polydioxanone\(^c\) in a simple interrupted pattern, subcutaneous tissues were closed with 3-0 polyglactin 910\(^d\) in simple continuous pattern, and the skin was closed using skin staples. All surgeries were performed by the same individual (MAW). Anesthesia monitoring consisted of recording oxygen flow rate, isoflurane percentage, heart rate, respiration rate, arterial oxygen saturation, invasive arterial blood pressure, end tidal carbon dioxide, temperature, end-tidal oxygen concentration and end-tidal isoflurane concentration every five minutes. Isoflurane administration was discontinued after the last skin staple was placed for dogs in the surgery group, and at 100 minutes for dogs in the anesthesia group. The dogs were then allowed to breathe 100 per cent oxygen for five minutes. Dogs were extubated when they swallowed or were able to hold their head up for five or more seconds. One dose of morphine (0.5 mg/kg SC) was administered to each dog in the surgery and anesthesia groups at the end of anesthesia. Skin staples were removed on the fourteenth post-operative day.

3. **Blood Sampling**

Eight milliliters of blood was collected via jugular venipuncture at the following intervals: immediately prior to pre-medication, twenty minutes after pre-medication, 15
minutes after skin incision (55 minutes after anesthesia induction), at the end of surgery/ anesthesia, 4, 8, 12, 34 and 36 hours post-anesthesia induction, once daily for 6 more days (48, 72, 96, 120, 144 and 168 hours), and 14 days (336 hours) post-procedures. The dogs in the control group did not receive any treatment, but had blood samples drawn on the same time schedule as the other dogs.

The blood was allowed to clot for thirty minutes, then was centrifuged for 20 minutes at 1200 x g. Serum was then divided into aliquots and frozen at -70°C until assayed.

4. Assays

Thyroxine, \( T_4 \), \( T_3 \), \( rT_3 \), \( fT_4 \) by equilibrium dialysis\(^{h}\) and cTSH\(^{h}\) were measured in all serum samples by previously validated radioimmunoassays at the Virginia-Maryland Regional College of Veterinary Medicine (\( T_4 \), \( T_3 \), \( rT_3 \)) or at the Endocrinology Section, Diagnostic Center for Population and Animal Health, Michigan State University College of Veterinary Medicine (\( fT_4 \), cTSH). Serum \( rT_3 \) concentration was not measured at times 120, 168 and 336 hours due to assay availability.

Statistical analysis

Mean difference from baseline to each sample time was compared among the 3 groups using mixed effects repeated measures analysis of variance (ANOVA) with a Bonferroni correction for multiple comparisons. Because the residual plots for \( T_4 \) and \( fT_4 \) revealed heteroscedasticity, these values were log transformed to stabilize the variance. The data were then transformed to the geometric means with 95% confidence limits. Means of the anesthesia monitoring parameters between the anesthesia and surgery groups were compared using the T-test, and mean values at baseline among the 3 groups
were compared using the GLM procedure. Statistical analyses were performed using a proprietary statistical software program.¹

C. Results

There was no significant difference between baseline values of thyroid function tests among all 3 groups. Mean serum T₃, T₄, fT₄, rT₃ and TSH concentrations are reported in tables 1 through 5. Difference from baseline for all thyroid function tests at all sampling times was not significantly different between control and anesthesia groups (Figures 1-5), or between the control and surgery groups for serum cTSH concentration (Figure 6). Serum T₃ concentration for the surgery group was decreased significantly from baseline compared to the control group at 8, 12 and 72 hours, and compared to the anesthesia group at 8, 24, 120, 144 and 168 hours (Figures 7 and 8). Serum cTSH concentration for the surgery group decreased significantly from baseline compared to the anesthesia group at 96 hours (Figure 9). Serum T₄, fT₄, and rT₃ concentrations for the surgery group were increased significantly from baseline compared to the control group at times 24, 36, and 96 hours (T₄), 168 hours (fT₄), and 8 and 24 hours (rT₃) (Figures 10, 12 and 14). Serum T₄, fT₄, and rT₃ concentrations for the surgery group were increased significantly from baseline compared to the anesthesia group at times 24 hours (T₄), 48 hours (fT₄), and 8, 12, 24, and 36 hours (rT₃) (Figures 11, 13 and 15).

The mean body temperature in the surgery group (96.21 +/- 0.96 degrees Fahrenheit) was significantly lower (P= 0.0015) than the mean body temperature in the anesthesia group (99.25 +/- 1.07 degrees Fahrenheit). The mean isoflurane percentage (1.23 +/- 0.13 percent) and end-tidal CO₂ (39.49 +/- 1.23 mmHg) in the surgery group
were significantly higher (P= 0.0172 and P= .0229, respectively) than the mean isoflurane percentage (0.99 +/- 0.12 percent) and end-tidal CO\textsubscript{2} (36.91 +/- 1.65 mmHg) in the anesthesia group. There were no significant differences in oxygen flow rate, heart rate, respiration rate, oxygen saturation or mean arterial blood pressure between the surgery and anesthesia groups.

D. Discussion

Based on the results of our study, surgery decreases serum T\textsubscript{3} concentrations, increases T\textsubscript{4}, fT\textsubscript{4} and rT\textsubscript{3} concentrations, and has minimal effects on serum cTSH concentrations. These results differ from those of another report\textsuperscript{2} where dogs subjected to anesthesia with halothane with and without abdominal exploratory surgery had a decrease in serum T\textsubscript{4}, T\textsubscript{3}, and rT\textsubscript{3} concentrations for at least 30 hours after surgery. However, the details of the experimental design were not provided, and serum fT\textsubscript{4} and TSH concentrations were not measured.

The decrease in serum T\textsubscript{3} concentration with a concurrent increase in serum rT\textsubscript{3} found in this study are similar to that in humans undergoing a wide variety of surgical procedures.\textsuperscript{4,5,10-14,16,117,119} These changes have been reported to be greater with more extensive surgical trauma\textsuperscript{10,12} or with more post-operative complications.\textsuperscript{121} The type of anesthetic agent used may also contribute to these changes, as one study demonstrated an increase in rT\textsubscript{3} in patients receiving halothane or enflurane anesthesia, but not with other anesthetic agents.\textsuperscript{4}

Several mechanisms may account for the decrease in serum T\textsubscript{3} and increase in serum rT\textsubscript{3} concentrations in our study. Decreased serum T\textsubscript{3} concentration and increased
serum rT₃ concentration are common findings in humans with NTI. Impaired activity of the selenodeiodinases has been documented in patients with NTI. A similar mechanism probably plays a role in the changes in T₃ and rT₃ noted in humans undergoing anesthesia and surgery, as well as the dogs in the current report. There may be inhibition of outer ring deiodination of T₄ and rT₃, resulting in decreased conversion of T₄ to T₃ and rT₃ to T₂. Alternatively, there could be a shift in deiodinase activity to favor the alternate pathway to increase rT₃ generation from T₄ and reduce deiodination to T₃.

Another mechanism for the decrease in serum T₃ and increase in serum rT₃ concentrations is decreased plasma membrane transport of T₄ and rT₃ that would cause a decrease in serum T₃ and increase in rT₃. Because metabolism of T₄ and rT₃ occurs intracellularly, if T₄ entry into cells decreased, T₃ production from T₄ would also decrease. Similarly, rT₃ entry into cells decreased, rT₃ concentrations in the serum would increase.

While fasting consistently decreases serum T₃ and increases serum rT₃ concentration within 24-48 hours in humans, and has also been shown to decrease serum T₃ concentrations in dogs, fasting is unlikely to have contributed to the changes in the current study. Dogs in the control and anesthesia groups, where no significant changes in thyroid function tests were found, were also fasted for a total of 24 hours, identical to the dogs in the surgery group.

The increase in serum T₄ and fT₄ concentrations in dogs that underwent surgery in our study has also been reported in some studies of human surgical patients. Impaired cellular metabolism or transport across cellular membranes of T₄ could
subsequently lead to an increase in serum concentrations of this hormone, as was demonstrated in our study. This would be consistent with the decrease in serum T_3 and increase in serum rT_3 concentrations also noted in the dogs in this study. Release of T_4 and fT_4 from hepatic or other extrathyroidal stores has also been proposed to cause the increase in serum T_4 and fT_4 concentrations.\textsuperscript{4,8}

In humans, there have been reports suggesting that increased serum T_4 and fT_4 concentrations are due to the inhalant anesthetic administered, although no single anesthetic agent has been consistently implicated.\textsuperscript{4,8,118} In our study, anesthetic agent used is unlikely to have caused the increase in serum T_4 and fT_4 concentration, as no change was seen in these hormones in the dogs that underwent general anesthesia only. However, the dogs in the anesthesia group received a significantly lower isoflurane percentage than the dogs in the surgery group, so it is possible that this difference could have contributed to the difference in serum T_4 and fT_4 concentrations.

Other studies in humans have documented a decrease in serum T_4 concentration with both anesthesia and surgery.\textsuperscript{7,12,14,117,119,121} The authors are aware of only 2 studies in which fT_4 was measured that documented a decrease in T_4, with one finding a decrease in fT_4 and the other no change in fT_4.\textsuperscript{7,14} A number of studies also documented no change in serum T_4 concentrations in human surgical patients, and the only one that measured fT_4 concurrently found that it increased.\textsuperscript{3,10,11,123} It is possible that other anesthetic protocols or surgical procedures would result in changes in serum T_4 and fT_4 concentrations different from the findings of the present study.

The only significant change in serum cTSH concentration in the present study in the present study was noted in the surgery group compared to the anesthesia group at 96
hours. The lack of changes in the surgery group compared with control dogs and the absence of a consistent change make the importance of the decreased serum cTSH concentration questionable. However, the decrease in serum cTSH concentration would be the expected physiologic response to the increased serum T_4 and fT_4 concentration noted in the surgery group.

The anesthetic protocol used in this study did not have a significant effect on thyroid function tests, indicating that the anesthetic drugs used may not be responsible for the altered thyroid function tests noted in our study. The dogs in the surgery group not only received a higher dose of isoflurane, but also had a significantly higher end-tidal carbon dioxide concentration and a significantly lower mean body temperature, which may have had an effect on the results of thyroid function tests. However, although there was a statistically significant difference between these parameters, the actual differences were quite small, making it more likely that the surgical trauma itself caused the changes in thyroid function tests.

Inflammatory cytokines released during surgery may play a role in the alterations in thyroid hormones seen in this study. Several studies in dogs have documented changes in thyroid function tests after administration of endotoxins and interleukins. Administration of endotoxin causes a release of cytokines such as interleukin-6 and tumor necrosis factor-α (TNF-α) and administration of interleukin-2 induces release of other cytokines such as TNF-α, interleukin-1 (IL-1), and interferon-γ. Continuous infusion of human recombinant interleukin-2 (IL-2) in dogs causes decreased serum T_4 and T_3 concentrations. Endotoxin administration to dogs has been documented to decrease serum T_3 concentrations and increase serum rT_3 and fT_4 concentrations. One
of these studies documented a concurrent decrease in serum T₄ concentration\textsuperscript{83} while the other study did not\textsuperscript{82}. TNF-α, IL-1 and interleukin-6 administered to humans and rats have been documented to have an effect on thyroid function tests, producing a decrease in serum T₃, with varied effects on serum rT₃, T₄ and fT₄ concentrations\textsuperscript{17,75-78}. However, the timeline of decreased serum T₃ concentrations do not necessarily correlate with the timeline of increased serum IL-6 concentrations in human surgical patients, indicating that there are likely other factors contributing to the alterations in thyroid function tests seen in surgical patients.

The diagnosis of hypothyroidism in dogs is based on a combination of clinical signs and results of thyroid function tests, most commonly a decrease in serum T₄ and fT₄ and an increase in serum TSH concentrations. Our results demonstrate that the changes induced by the anesthetic regimen and surgical technique utilized in this study are unlikely to result in a misdiagnosis of hypothyroidism. The decrease in serum T₃ concentration noted in dogs undergoing surgery provides additional evidence of the shortcomings of serum T₃ measurements for the diagnosis of hypothyroidism in the dog\textsuperscript{129}. 
CONCLUSION

In conclusion, surgery had an effect on thyroid function tests, specifically causing a decrease in serum T₃ and an increase in serum T₄, fT₄, and rT₃ concentrations, while the anesthesia protocol used in this study did not. Because serum T₄ and fT₄ concentrations increased rather than decreased, evaluating these hormones in the peri- and post-operative period is unlikely to lead to a misdiagnosis of hypothyroidism in euthyroid dogs.

As with many veterinary studies, the number of dogs in this study was fairly small, with only 5 dogs in each group. Future studies would ideally include a larger number of dogs.

In this study, dogs were premedicated with acepromazine and morphine, induced with propofol, and maintained with isoflurane. While this is a common anesthetic protocol used in small animal practice, it is by no means the only protocol used. Therefore, an important next step in investigating the effects of anesthesia and surgery on thyroid function in dogs will be to evaluate the effects of different anesthetic protocols.

This study evaluated the effects on thyroid function tests of abdominal exploratory surgery alone. Although all of the abdominal organs were evaluated and lightly manipulated, once the initial abdominal incision was made, no significant tissue trauma occurred, except for the incision. It is possible that a more invasive or prolonged surgery would affect thyroid function tests more dramatically or in different ways than the laparotomies performed in this study. Therefore, to more thoroughly evaluate the effect of surgery on thyroid function tests, future studies should involve a variety of surgeries with differing degrees of invasiveness.
The dogs in this study were clinically healthy, which is typically not the case in clinical patients undergoing general anesthesia and surgery. Aside from being anesthetized for an elective ovariohysterectomy or prophylactic gastropexy, the majority of dogs undergoing laparatomy are clinically ill. In these cases, the effects of the concurrent illness on thyroid function tests may be more significant than the effects of anesthesia and surgery. Also, as several mechanisms likely play a role in the alterations in thyroid function tests caused by NTI, anesthesia and surgery, combining these non-thyroidal factors may produce different combinations of alterations than either one would produce alone.

Similarly, the dogs in this study were determined to be euthyroid. Therefore, any conclusions made about the effects of anesthesia and surgery on thyroid function tests can only be applied to euthyroid dogs. The results of thyroid function tests in dogs with hypothyroidism may be altered in different ways than those of euthyroid dogs. Therefore, another possibility for future research is to evaluate thyroid function tests in hypothyroid dogs undergoing anesthesia and surgery.
FOOTNOTES

a Rapinovet, Schering-Plough Animal Health Corp., Union, NJ
b Isoflo, Abbott Laboratories, North Chicago, IL
c PDS II, Ethicon, Inc., Sommerville, NJ
d Vicryl, Ethicon, Inc., Sommerville, NJ
e Coat-A-Count Canine T4, Diagnostic Products Corp., Los Angeles, CA
f Coat-A-Count Canine T3, Diagnostic Products Corp., Los Angeles, CA
g Reverse T3, Adaltis, Casalecchio di Reno, Italy
h Endocrinology section, Diagnostic Center for Population and Animal Health, Michigan State University College of Veterinary Medicine.
i SAS 9.1.3, SAS Institute, Cary, NC
LITERATURE CITED


32. Laurberg P. The relative contribution of thyroxine and triiodothyronine to the hormone secretion from the perfused canine thyroid during various degrees of stimulation. Endocrinology 1977;100:656-662.


58. Peeters RP, Wouters PJ, van Toor H, et al. Serum 3,3',5'-triiodothyronine (rT3) and 3,5,3'-triiodothyronine/rT3 are prognostic markers in critically ill patients and are associated with postmortem tissue deiodinase activities. J Clin Endocrinol Metab 2005;90:4559-4565.


APPENDIX I: FIGURES

Figure 1. Difference of mean $T_3$ (nmol/L) from baseline for the control (C) and anesthesia (A) groups.
Figure 2. Difference of geometric mean of T₄ (nmol/L) from baseline for the control (C) and anesthesia (A) groups.
Figure 3. Difference of geometric mean of $fT_4$ (pmol/L) from baseline for the control (C) and anesthesia (A) groups.
Figure 4. Difference of mean rT₃ (ng/mL) from baseline for the control (C) and anesthesia (A) groups.
Figure 5. Difference of mean TSH (mU/L) from baseline for the control (C) and anesthesia (A) groups.
Figure 6. Difference of mean TSH (mU/L) from baseline for surgery (S) and control (C) groups.
Figure 7. Difference of mean T₃ (nmol/L) from baseline for the surgery (S) and control (C) groups. Times at which differences were statistically different (P < 0.05) are represented by black circles.
Figure 8. Difference of mean T₃ (nmol/L) from baseline for the surgery (S) and anesthesia (A) groups. Times at which differences were statistically different (P < 0.05) are represented by black circles.
Figure 9. Difference of mean TSH (mU/L) from baseline for the surgery (S) and anesthesia (A) groups. Times at which differences were statistically different (P < 0.05) are represented by black circles.
Figure 10. Difference of geometric mean of T₄ (nmol/L) from baseline for the surgery (S) and control (C) groups. Times at which differences were statistically different (P < 0.05) are represented by black circles.
Figure 11. Difference of geometric mean of $T_4$ (nmol/L) from baseline for the surgery (S) and anesthesia (A) groups. Times at which differences were statistically different ($P < 0.05$) are represented by black circles.
Figure 12. Difference of geometric mean of $fT_4$ (pmol/L) from baseline for the surgery (S) and control (C) groups. Times at which differences were statistically different ($P < 0.05$) are represented by black circles.
Figure 13. Difference of geometric mean of fT₄ (pmol/L) from baseline for the surgery (S) and anesthesia (A) groups. Times at which differences were statistically different (P < 0.05) are represented by black circles.
Figure 14. Difference of mean $rT_3$ (ng/mL) from baseline for the surgery (S) and control groups. Times at which differences were statistically different ($P < 0.05$) are represented by black circles.
Figure 15. Difference of mean rT$_3$ (ng/mL) from baseline for the surgery (S) and anesthesia (A) groups. Times at which differences were statistically different (P < 0.05) are represented by black boxes.
Table 1. Mean T₃ (nmol/L) +/- standard deviation for control, anesthesia and surgery groups at all time points.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control Group</th>
<th>Anesthesia Group</th>
<th>Surgery Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.64090 +/- 0.509073</td>
<td>1.6409 +/- 0.539953</td>
<td>1.55450 +/- 0.225478</td>
</tr>
<tr>
<td>0.33</td>
<td>1.57786 +/- 0.444909</td>
<td>1.62275 +/- 0.541019</td>
<td>1.35328 +/- 0.297200</td>
</tr>
<tr>
<td>1</td>
<td>1.53918 +/- 0.209127</td>
<td>1.22032 +/- 0.440946</td>
<td>1.03941 +/- 0.119359</td>
</tr>
<tr>
<td>2</td>
<td>1.44832 +/- 0.253842</td>
<td>1.29436 +/- 0.255061</td>
<td>1.01210 +/- 0.121112</td>
</tr>
<tr>
<td>4</td>
<td>1.62024 +/- 0.112070</td>
<td>1.53587 +/- 0.601519</td>
<td>1.45560 +/- 0.415171</td>
</tr>
<tr>
<td>8</td>
<td>1.43388 +/- 0.218174</td>
<td>1.25041 +/- 0.371864</td>
<td>0.67786 +/- 0.265369</td>
</tr>
<tr>
<td>12</td>
<td>1.45146 +/- 0.234071</td>
<td>1.14841 +/- 0.249007</td>
<td>0.81968 +/- 0.471788</td>
</tr>
<tr>
<td>24</td>
<td>1.50628 +/- 0.103412</td>
<td>1.84078 +/- 0.387052</td>
<td>1.08157 +/- 0.176286</td>
</tr>
<tr>
<td>36</td>
<td>1.62154 +/- 0.140408</td>
<td>1.56916 +/- 0.215710</td>
<td>1.17138 +/- 0.166375</td>
</tr>
<tr>
<td>48</td>
<td>1.72846 +/- 0.208048</td>
<td>1.47720 +/- 0.351423</td>
<td>1.31937 +/- 0.509931</td>
</tr>
<tr>
<td>72</td>
<td>1.85764 +/- 0.334926</td>
<td>1.65652 +/- 0.391023</td>
<td>1.17112 +/- 0.335695</td>
</tr>
<tr>
<td>96</td>
<td>1.77682 +/- 0.407563</td>
<td>1.95696 +/- 0.546552</td>
<td>1.45029 +/- 0.486119</td>
</tr>
<tr>
<td>120</td>
<td>1.86088 +/- 0.324650</td>
<td>1.93858 +/- 0.503742</td>
<td>1.37836 +/- 0.37924</td>
</tr>
<tr>
<td>144</td>
<td>1.80350 +/- 0.449679</td>
<td>1.90830 +/- 0.303101</td>
<td>1.32556 +/- 0.404337</td>
</tr>
<tr>
<td>168</td>
<td>1.63734 +/- 0.318428</td>
<td>2.04486 +/- 0.438544</td>
<td>1.28773 +/- 0.294208</td>
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<tr>
<td>336</td>
<td>1.75604 +/- 0.302331</td>
<td>1.82860 +/- 0.442772</td>
<td>1.67294 +/- 0.466688</td>
</tr>
</tbody>
</table>
Table 2. Mean $T_4$ (nmol/L) +/- standard deviation for the control, anesthesia and surgery groups at all time points.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control Group</th>
<th>Anesthesia Group</th>
<th>Surgery Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>22.9914 +/- 6.925184</td>
<td>20.8386 +/- 4.702275</td>
<td>13.2119 +/- 5.917239</td>
</tr>
<tr>
<td>0.33</td>
<td>27.2920 +/- 11.67448</td>
<td>20.4192 +/- 8.673445</td>
<td>13.5644 +/- 5.464534</td>
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<tr>
<td>1</td>
<td>24.8128 +/- 7.860556</td>
<td>21.4552 +/- 10.36276</td>
<td>15.7024 +/- 6.980071</td>
</tr>
<tr>
<td>2</td>
<td>28.8292 +/- 15.91220</td>
<td>17.5851 +/- 7.196816</td>
<td>12.4508 +/- 7.145857</td>
</tr>
<tr>
<td>8</td>
<td>23.3939 +/- 11.24357</td>
<td>13.4003 +/- 8.012376</td>
<td>12.3174 +/- 5.895476</td>
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<td>12</td>
<td>26.1000 +/- 17.98822</td>
<td>15.2281 +/- 8.069324</td>
<td>16.7552 +/- 13.00216</td>
</tr>
<tr>
<td>24</td>
<td>19.9422 +/- 7.950154</td>
<td>20.9210 +/- 7.484664</td>
<td>25.9370 +/- 4.573063</td>
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<td>25.0968 +/- 10.68770</td>
<td>19.3324 +/- 6.894594</td>
<td>18.7432 +/- 5.424794</td>
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<td>22.5444 +/- 8.60833</td>
<td>27.9188 +/- 7.271030</td>
<td>24.0598 +/- 7.711737</td>
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<td>25.1544 +/- 14.79465</td>
<td>23.6612 +/- 7.367517</td>
<td>21.2088 +/- 8.408973</td>
</tr>
<tr>
<td>144</td>
<td>25.8938 +/- 10.81163</td>
<td>23.5548 +/- 6.020150</td>
<td>19.1957 +/- 8.827492</td>
</tr>
<tr>
<td>336</td>
<td>24.8090 +/- 8.553884</td>
<td>22.1650 +/- 7.512665</td>
<td>21.79674 +/- 8.78672</td>
</tr>
</tbody>
</table>
Table 3. Mean fT$_4$ (pmol/L) +/- standard deviation for the control, anesthesia and surgery groups at all time points.

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Control Group</th>
<th>Anesthesia Group</th>
<th>Surgery Group</th>
</tr>
</thead>
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<td>0</td>
<td>20.4 +/- 14.36315</td>
<td>15.4 +/- 9.762172</td>
<td>13.2 +/- 6.610598</td>
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<td>0.33</td>
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<td>16.8 +/- 8.757854</td>
<td>15.8 +/- 10.25671</td>
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<tr>
<td>2</td>
<td>24.4 +/- 10.99091</td>
<td>15.6 +/- 9.864076</td>
<td>15.8 +/- 10.03494</td>
</tr>
<tr>
<td>4</td>
<td>24.4 +/- 8.648699</td>
<td>12.8 +/- 7.726578</td>
<td>12.6 +/- 7.092249</td>
</tr>
<tr>
<td>8</td>
<td>22.2 +/- 4.266146</td>
<td>10.6 +/- 6.107373</td>
<td>10.8 +/- 4.658326</td>
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<td>11.8 +/- 8.671793</td>
<td>13.2 +/- 7.155418</td>
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<td>16.2 +/- 6.83374</td>
<td>19.2 +/- 11.69188</td>
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<td>36</td>
<td>21.0 +/- 11.11306</td>
<td>16.2 +/- 8.438009</td>
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<td>22.2 +/- 13.02690</td>
<td>13.6 +/- 6.066300</td>
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<td>15.0 +/- 7.842194</td>
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<td>23.0 +/- 7.810250</td>
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<td>120</td>
<td>21.8 +/- 14.94279</td>
<td>16.8 +/- 7.328028</td>
<td>20.8 +/- 4.324350</td>
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<tr>
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<td>26.6 +/- 17.79888</td>
<td>18.2 +/- 10.52141</td>
<td>22.6 +/- 7.162402</td>
</tr>
<tr>
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<td>19.2 +/- 8.871302</td>
<td>25.8 +/- 11.45426</td>
</tr>
<tr>
<td>336</td>
<td>24.0 +/- 9.823441</td>
<td>19.6 +/- 9.502631</td>
<td>18.8 +/- 8.043631</td>
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</tbody>
</table>
Table 4. Mean rT$_3$ (ng/mL) +/- standard deviation for the control, anesthesia and surgery groups at all time points.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Anesthesia</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.92714 +/- 0.27193</td>
<td>0.75174 +/- 0.391099</td>
<td>0.77437 +/- 0.230393</td>
</tr>
<tr>
<td>0.33</td>
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<tr>
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<td>0.765544 +/- 0.13104</td>
</tr>
<tr>
<td>2</td>
<td>0.94242 +/- 0.153254</td>
<td>0.65408 +/- 0.277394</td>
<td>0.75797 +/- 0.264414</td>
</tr>
<tr>
<td>4</td>
<td>1.07679 +/- 0.178388</td>
<td>0.69899 +/- 0.343011</td>
<td>0.81251 +/- 0.184868</td>
</tr>
<tr>
<td>8</td>
<td>0.91597 +/- 0.130444</td>
<td>0.64432 +/- 0.385258</td>
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</tr>
<tr>
<td>12</td>
<td>0.918082 +/- 0.10141</td>
<td>0.60638 +/- 0.199126</td>
<td>0.97593 +/- 0.073444</td>
</tr>
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<td>24</td>
<td>0.92678 +/- 0.245806</td>
<td>0.88603 +/- 0.331268</td>
<td>1.32932 +/- 0.162528</td>
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<tr>
<td>36</td>
<td>0.82806 +/- 0.177689</td>
<td>0.56112 +/- 0.082183</td>
<td>0.93622 +/- 0.106523</td>
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<tr>
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<td>0.92177 +/- 0.176792</td>
<td>0.64622 +/- 0.256924</td>
<td>0.93343 +/- 0.228886</td>
</tr>
<tr>
<td>72</td>
<td>0.95793 +/- 0.23778</td>
<td>0.68165 +/- 0.188905</td>
<td>0.87665 +/- 0.145048</td>
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<tr>
<td>96</td>
<td>0.89801 +/- 0.295477</td>
<td>0.8807 +/- 0.104093</td>
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<td>0.68922 +/- 0.073046</td>
<td>0.72196 +/- 0.118707</td>
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</table>
Table 5. Mean TSH (mU/L) +/- standard deviation for control, anesthesia and surgery groups at all time points.

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Anesthesia</th>
<th>Surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.2 +/- 4.438468</td>
<td>7.2 +/- 4.207137</td>
<td>8.8 +/- 2.167948</td>
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<tr>
<td>0.33</td>
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<td>6.6 +/- 4.97996</td>
</tr>
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<td>1</td>
<td>7.4 +/- 4.722288</td>
<td>4.6 +/- 2.880972</td>
<td>6.4 +/- 1.81659</td>
</tr>
<tr>
<td>2</td>
<td>7.4 +/- 2.302173</td>
<td>5.6 +/- 4.037326</td>
<td>5.2 +/- 3.898718</td>
</tr>
<tr>
<td>4</td>
<td>9.0 +/- 6.041523</td>
<td>6.2 +/- 5.01996</td>
<td>4.6 +/- 3.209361</td>
</tr>
<tr>
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<td>4.6 +/- 2.701851</td>
<td>4.6 +/- 2.966479</td>
</tr>
<tr>
<td>12</td>
<td>9.2 +/- 5.403702</td>
<td>6.2 +/- 4.658326</td>
<td>6.6 +/- 5.412947</td>
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<td>9.0 +/- 6.284903</td>
</tr>
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<td>168</td>
<td>7.2 +/- 4.207137</td>
<td>9.0 +/- 14.76482</td>
<td>7.6 +/- 0.894427</td>
</tr>
<tr>
<td>336</td>
<td>9.2 +/- 6.457554</td>
<td>5.0 +/- 4.84768</td>
<td>9.0 +/- 1.581139</td>
</tr>
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</table>
Table 6. Mean +/- standard deviation of anesthesia monitoring parameters in the surgery and anesthesia groups.

<table>
<thead>
<tr>
<th>Monitoring Parameter</th>
<th>Anesthesia Group</th>
<th>Surgery Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen flow rate (liters/ minute)</td>
<td>0.9848 +/- 0.1042</td>
<td>1.0962 +/- 0.1876</td>
</tr>
<tr>
<td>Isoflurane percentage</td>
<td>0.9895 +/- 0.1162</td>
<td>1.2251 +/- 0.1319</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>91.848 +/- 12.071</td>
<td>98.842 +/- 9.3732</td>
</tr>
<tr>
<td>Respiratory rate (breaths per minute)</td>
<td>7.4476 +/- 0.8687</td>
<td>9.0644 +/- 1.3525</td>
</tr>
<tr>
<td>Body temperature (degrees Fahrenheit)</td>
<td>99.251 +/- 1.0711</td>
<td>96.209 +/- 0.9586</td>
</tr>
<tr>
<td>Oxygen saturation (per cent)</td>
<td>98.295 +/- 0.9006</td>
<td>98.720 +/- 0.6654</td>
</tr>
<tr>
<td>End-tidal CO(_2) (mmHg)</td>
<td>36.905 +/- 1.654</td>
<td>39.491 +/- 1.2276</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td>64.052 +/- 3.2641</td>
<td>63.662 +/- 4.1182</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>102.300 +/- 14.707</td>
<td>86.402 +/- 7.8250</td>
</tr>
</tbody>
</table>
VITA

Melinda Wood was born in Springfield, MA. She grew up in Braintree, MA and graduated from Bates College in Lewiston, ME with a Bachelor of Arts in French in 1996. She received her Doctor of Veterinary Medicine from The Ohio State University College of Veterinary Medicine in June, 2003. Melinda completed a rotating small animal internship at Carolina Veterinary Specialists in Charlotte, NC. She was then accepted into a program of residency in small animal internal medicine at the Virginia-Maryland Regional College of Veterinary Medicine in 2004, pursuing the degree of Master of Science in Biomedical and Veterinary Sciences. Melinda defended her thesis on July 2, 2007.