Evaluation of the effects of clomipramine on the canine hypothalamic-pituitary-thyroid axis

by

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EVALUATION OF THE EFFECTS OF CLOMIPRAMINE ON
THE CANINE HYPOTHALAMIC-PITUITARY-THYROID AXIS

by

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(ABSTRACT)

Tricyclic antidepressants have been shown to alter thyroid function in man and laboratory animals, but have not been evaluated in the dog. The effect of administration of clomipramine on canine thyroid function was studied in a prospective protocol in which 14 mature, healthy dogs were administered clomipramine (3 mg/kg PO q12h) for 112 days. Thyroid-stimulating hormone (TSH), total thyroxine (T_4), total 3,5,3' triiodothyronine (T_3), free thyroxine (fT_4), and 3,3',5' triiodothyronine (reverse T_3; rT_3) concentrations were measured on selected days. Thyrotropin-releasing hormone (TRH) response tests were performed concurrently. Repeated measures analysis of variance was applied to test for effects of day of treatment; when significance ($p < 0.05$) was noted, it was further investigated using orthogonal polynomial trends.

Significant decreases were found in serum T_4 (26 ± 1.2 to 17 ± 0.5 nmol/L, $p < 0.001$), fT_4, (29 ± 2.4 to 19 ± 1.3 pmol/L, $p < 0.0002$), and rT_3 (1.2 ± 0.1 to 0.83 ± 0.08 nmol/L, $p < 0.0001$) concentrations. The effect of time on serum T_3 concentration was also significant ($p < 0.0001$), but no consistent trend could be identified. No significant effect of time was noted in either pre- or post-TRH TSH concentrations.

The results of this study indicate that significant and substantial decreases in T_4 (35%), fT_4 (38%), and rT_3 can occur during clomipramine administration. Long-term administration of clomipramine may result in a misdiagnosis of hypothyroidism if a dog is tested while taking this medication and, since decreased serum fT_4 occurs, hypothyroidism may result.

DEDICATION

To my parents, Peter & Gerda, for all their love and support. I would not be where I am today without them.

To my brother, Pate, who I strive to be more like everyday.

To Ro, for making me laugh, smile, and love.

To Dr. Panciera, for all his help.

And to my research dogs, especially Madison…may this research in some way benefit your brothers and sisters in the future.

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CHAPTER 1

INTRODUCTION

A variety of drugs have been reported to alter thyroid function tests in dogs, including glucocorticoids, potentiated sulfonamides, and anticonvulsants.[1-6] Tricyclic antidepressants (TCAs) have been shown to impair thyroid hormone synthesis and thyrotropin secretion in man and rats, but have yet to be evaluated in the dog.[7-10]

Drugs alter thyroid function in various species by modification of the synthesis, secretion, transport, or metabolism of thyroid hormones. Some medications may directly inhibit the hypothalamic-pituitary-thyroid axis, but these drug-induced anti-thyroid effects vary among species. Unfortunately, the anti-thyroid consequences of many drugs have not been tested in dogs, and the unique physiologic variations between species make it impossible to extrapolate current data.

Decreased TSH secretion is one method by which some medications, including dopamine, cyproheptadine, somatostatin, and glucocorticoids, decrease thyroid function. Another influence of medications is on thyroid hormone synthesis and secretion. Medications such as amiodarone and iodine have lead to increased serum thyroid hormone concentrations, while others, including lithium, amiodarone, iodine, calcium channel blockers, thionamides, glucocorticoids and sulfonamides decrease serum thyroid hormone concentrations. A change in peripheral metabolism of thyroid hormones also occurs with some drugs, namely phenobarbital. Finally, altered binding of thyroid hormones to their serum transport proteins is affected by a plethora of medications, with glucocorticoids, ipodate, non-steroidal anti-inflammatory drugs (NSAIDs), furosemide, androgens and heparin.

Clomipramine, a tricyclic antidepressant approved for the treatment of separation anxiety in dogs, exerts potent serotonin[11,12] and norepinephrine (NE)[13-15] reuptake-inhibiting effects. Subsequently, this mechanism of action may interfere with the hypothalamic-pituitary-thyroid (HPT) axis via the serotonergic and noradrenergic systems, and administration could lead to a decrease in thyroid stimulating hormone (TSH), thyroxine (T_4) and 3,5,3' triiodothyronine (T_3) concentrations. In addition, TCAs inhibit thyroid hormone biosynthesis by altering thyroid follicular cell iodine uptake[16-18] and inhibiting thyroid peroxidase.[17,18]

If clomipramine decreases plasma canine thyroid hormones as effectively as in human and rat models, iatrogenic hypothyroidism may result. This may be of particular concern since hypothyroidism is a contributing factor to a variety of psychological illnesses in people, and the therapeutic benefit of TCAs on affective disorders has been positively modulated through thyroid supplementation in both human beings[19,20] and rats.[9] Although the relationship between hypothyroidism and behavioral disorders in dogs is tenuous, aggression has been reported.[21,22] Administration of clomipramine, if it induces hypothyroidism, may exacerbate behavioral signs or reduce the potential positive behavioral response to the drug.

As many drugs are known to affect thyroid function at all levels of the HPT axis, a basic knowledge of thyroid hormone synthesis and the HPT is essential for understanding drug effects.

LITERATURE REVIEW

Thyroid Physiology in the Dog

Thyroid hormone synthesis begins with absorption of dietary iodide, which then is bound to plasma proteins and transported to the thyroid gland (Figure 1). The concentration of iodide in canine plasma is ~12.5 times higher in dogs than in man, while the rate of uptake by the thyroid is roughly five-fold higher.[23] Iodide is concentrated in the thyroid via an energy- and oxygen-dependent "iodide pump" located on the non-follicular border of the thyroid cell. Once inside the cell, organification of iodide occurs as thyroid peroxidase (TPO) oxidizes it to form iodine, utilizing H_2O_2 as the oxidant. This enzyme then binds the iodine to tyrosine residues on the thyroglobulin molecule, which is a glycoprotein formed in the follicle cell and stored in the colloid. Depending on the amount of iodine available, the binding of iodine to thyroglobulin results in the production of mono- and di-iodotyrosine, which ultimately undergo a coupling reaction catalyzed by TPO to form T_4 or T_3 on thyroglobulin. Secretion occurs when colloid, containing thyroglobulin, is taken from the follicular lumen by thyroid follicle epithelial cell endocytosis. It undergoes proteolysis in a phagolysosome to free T_4 and T_3, which are then secreted when the phagolysosome fuses with the cell membrane. The ratio of T_4 to T_3 secreted by the canine thyroid gland is 4:1,[24] but this ratio decreases when the thyroid gland undergoes stimulation by TSH. Total secretion of T_4 is approximately twice that of man, while T_3 production in the dog is four-fold greater,[23] and it is believed that this difference is mainly due to the dog's more rapid disposal via metabolism and fecal excretion of thyroid hormones.

Synthesis and secretion of thyroid hormones is controlled by a complex negative feedback system that originates with the production of thyrotropin-releasing hormone (TRH) in the hypothalamus. This compound is secreted into the hypophyseal portal system from the paraventricular nucleus of the hypothalamus,[25] and has a direct stimulatory effect on secretion of thyroid-stimulating hormone (TSH, thyrotropin) from the anterior pituitary (Figure 2). It has been shown that somatostatin inhibits TRH secretion in human beings and primates,[25-27] while NE, histamine, dopamine, and serotonin all promote its release.[27,28] In rats, stimulation of the serotonergic system resulted in decreased TRH production.[29] This regulatory system has not been characterized in the dog.

Thyroid-stimulating hormone, which is produced by thyrotropes in the anterior pituitary, stimulates T_4 and T_3 synthesis and secretion by the thyroid gland. TSH not only upregulates the action of the iodide pumps, but stimulates endocytosis of thyroglobulin and secretion of T_4 and T_3. Thyrotropes are under direct negative feedback regulation by circulating thyroid hormone concentrations modulated by TRH.[25] In cases of primary hypothyroidism (lymphocytic thyroiditis, idiopathic follicular atrophy), TSH concentrations increase in an attempt to stimulate a damaged thyroid gland, while with secondary hypothyroidism, the adenohypophysis fails to secrete TSH, resulting in abnormally low concentrations. Additionally, glucocorticoids, growth hormone (GH), somatostatin, dopamine, and androgens have also been shown to decrease TSH secretion.[25,27] Control of TSH secretion has not been thoroughly evaluated in the dog.

Once released into the plasma, T_4 and T_3 are immediately bound by a number of plasma proteins, including thyroxine-binding globulin (TBG), thyroxine-binding pre-albumin (TBPA), albumin, and high- and low-density lipoproteins.[30] Approximately 99.9% of thyroxine is bound to plasma proteins, with TBG having the highest affinity, binding 60% of thyroxine in the dog.[30] TBPA has a lower affinity, binding 17%, while albumin, with the lowest affinity but highest serum

concentration of the binding proteins, binds 12% of canine thyroxine.[30] The lipoproteins only bind ~11% of thyroxine.[30] Only the unbound, or free portion of T_4, leaves the circulation to exert the cellular effects of thyroid hormones. Although T_4 is the main secretory product of the thyroid gland, T_3 is three to four times more effective in binding and activating α and β thyroid hormone receptors in the cell nucleus. Like thyroxine, T_3 is bound by serum proteins, mainly albumin.[31,32] It is, however, bound to a lesser degree than T_4. The protein binding of thyroid hormones is clinically important, as many medications and disease states can alter serum protein concentrations, leading to aberrancies in thyroid hormone concentrations.

Thyroid hormones undergo metabolism by progressive removal of iodine (deiodination), conjugation to sulfate and glucoronide, and ether bond cleavage.[33] The peripheral tissues, including liver and kidneys, deiodinate T_4 to form the active T_3 (5'-deiodination) or the metabolically inactive reverse T_3 (5-deiodination). The deiodination ability of the canine liver and kidneys are relatively equivalent,[34] with peripheral tissues (i.e. muscle, skin, adipose tissue) containing much lower deiodinase concentrations. These peripheral tissues, however, may be important metabolic pathways for thyroxine due to their significant mass.[33] Therefore, it is believed that deiodination decreases serum T_4 concentrations, while consequentially raising serum T_3 concentrations. The deiodinases are divided into multiple categories, with Type I deiodinase found in the highest concentration in the liver, kidneys, and thyroid, Type II deiodinase mainly present in the central nervous system, pituitary gland, placenta and brown adipose tissue, and Type III deiodinase only found in the CNS, skin and placenta.[18,25] Furthermore, the mechanisms of deiodination can be due to either degradation of the compound's phenolic ring (i.e. 5'-deiodination) or tyrosyl ring (5-deiodination).

In addition to deiodination, thyroid hormones undergo glucuronidation (in liver and kidneys) and sulfation (via hepatic phenosulfotransferases) to be excreted by the biliary system or in urine.[25,35] It is thought that these non-deiodinase pathways account for 55% of T_4 and 30% of T_3 metabolism in the dog.[32]

It is important to note that the function of deiodination in peripheral tissues is to release T_3 into the plasma for the benefit of multiple organ systems, while CNS deiodination only produces T_3 for a very limited distribution within specific portions of the brain and other neural tissue. In addition to the various deiodinases and their distribution, the process of deiodination varies between the CNS and peripheral tissues. For example, while kidney Type I deiodinase catalyses both phenolic (5') and tyrosyl (5) ring deiodination of T_4, T_3, and rT_3, CNS contains two unique isoenzymes to catalyse the production and metabolism of T_3. In the CNS, Type II deiodinase catalyses 5'-deiodination of T_4 to T_3 and rT_3 to 3,3'-T_2, while Type III deiodinase catalyses tyrosyl ring deiodination of T_4 to rT_3 and T_3 to 3,3'-T_2, thereby inactivating T_3.[36] The physiological significance of Type I deiodinase in the CNS is unknown, as the concentrations of this enzyme in the human CNS are below detectable limits.[37] Although the Type II and III deiodinase isoenzymes have been located in rat astrocytes,[38,39] this system has not been characterized in the dog.

Thyroid–Drug Interactions

Given the intricacy of the HPT axis, drug-induced alteration of thyroid hormone concentrations can be perplexing. A multitude of drugs have been reported to alter thyroid function in both rats and people by modification of the synthesis, secretion, transport, or metabolism of thyroid hormones. Furthermore, some medications may inhibit the hypothalamic-pituitary-thyroid axis. These drug-induced anti-thyroid effects vary among species, but medications frequently used in dogs that are known to alter thyroid function include phenobarbital, glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs) and the sulfonamides. A summary of the various medications affecting thyroid function in both man and dog, classified by their mechanism of action, is depicted in Table 1. Unfortunately, the anti-thyroid consequences of many of these drugs have not been tested in dogs. The differences between rodent, human, and canine thyroid physiology make it impossible to extrapolate from one species to another.

Decreased TRH or TSH Secretion

Drugs that may lead to decreased TSH secretion include dopamine, cyproheptadine, somatostatin, and glucocorticoids. The decrease in TSH release is often not a direct suppression by the drug, but a secondary response to alteration of hypothalamic or thyroid function by these medications. Unfortunately, no data exists regarding the effects of dopamine, cyproheptadine, or somatostatin in canine models.

In various studies performed on both people and rats, it has been shown that endogenous or exogenous glucocorticoids directly inhibit the hypothalamic-pituitary-thyroid axis, as TSH secretion is impaired by glucocorticoid administration. As with many drug-induced alterations, their effects are dependent upon multiple variables, including dosage, duration of treatment, route of administration and the preparation used. In dogs, various studies[1,6,40-44] have examined thyroid hormone concentrations after administration of glucocorticoids, but most have only assessed the medication's affect on T_4 and T_3 concentrations without determining the mechanism of action. Prednisone administration does not appear to have substantial effects on serum TSH concentration in either euthyroid or hypothyroid dogs.[1,45] However, euthyroid dogs treated with prednisone had decreases in T_4 and fT_4 that should have led to an increase in serum TSH concentration because of reduced negative feedback of these hormones on pituitary thyrotrophs. Failure of TSH to increase may indicate some impairment of pituitary function.

Another medication that will decrease TSH secretion is levothyroxine. Administration of levothyroxine to a euthyroid dog will cause decreased TSH secretion, and therefore decrease endogenous thyroid hormone secretion. Prolonged treatment will result in atrophy of pituitary thyrotropes and the thyroid gland. Research has revealed that after 8 weeks of administration of a replacement dose of levothyroxine, thyroid function is suppressed for at least 4 weeks.[46]

Amiodarone is a benzofuranic derivative that structurally resembles T_4, and contains 37% iodine by weight.[47] It is a class III antiarrhythmic drug that has been used in dogs. In people, amiodarone may alter thyroid function tests and may produce either hyperthyroidism or hypothyroidism. These outcomes may be due to reduced TSH synthesis and secretion by the pituitary, which may be secondary to variations in serum thyroid hormone concentrations, or may be attributable to a direct effect.[47] Hypothyroidism may occur secondary to exacerbation of pre-existing thyroiditis or because of the thyroid gland's inability to escape from the suppressive effects of iodine excess on thyroid hormone synthesis and secretion (Wolff-Chaikoff effect). The

thyrotoxicosis is thought to be due to a direct cytotoxic effect of amiodarone or the result of iodine-induced overproduction of thyroid hormones.[47] In addition, the drug has also been shown to inhibit 5'-deiodinase activity and thyroxine entry into tissue, compete with binding of T_3 to thyroid hormone receptors, and decrease thyroid cell responsiveness to TSH,[47] all of which may eventually impact TRH secretion via the feedback pathways.

Extensive documentation exists to show that administration of tricyclic antidepressants in people and rats alters thyroid function, in part by directly interfering with the hypothalamic-pituitary-thyroid axis (via manipulation of the noradrenergic and serotinergic systems that interact with the hypothalamus, directly influencing TRH release).[18] No data on the effect of this class of medications on the canine HPT axis is currently available.

Altered Thyroid Hormone Synthesis or Secretion

One of the most important effects of medications on thyroid function tests is on thyroid hormone synthesis or secretion, with medications such as amiodarone and iodine increasing hormone concentrations while lithium, amiodarone, iodine, calcium channel blockers, thionamides, glucocorticoids and sulfonamides decrease them.

One of the principle actions of glucocorticoid administration in people is inhibition of TSH secretion, resulting in decreased concentrations of T_4, and T_3.[48] Decreased concentrations of serum thyroid hormones have been demonstrated in several canine studies. Treatment of dogs with prednisolone (1.1 mg/kg PO q12h) resulted in a decrease in T_4 by 33% and T_3 by 40% the first day after initiating treatment.[6] This decrease was sustained or became greater throughout the three-week treatment period in this study. In a study of the same duration using a higher dose of prednisone (1.2-2.0 mg/kg PO q12h), serum T_4 concentration was similar to the pretreatment level two days after starting treatment, but was significantly decreased to <50% of control dogs at weeks 1 and 3 of treatment.[1] Serum fT_4 concentration measured by equilibrium dialysis was significantly decreased at week 3 of prednisone treatment, but serum T_4 and fT_4 returned to baseline concentration within 1 week of stopping prednisone.[1] Dogs given a single dose of dexamethasone (0.5-0.7 mg/kg) had a significant decrease in serum T_3 concentration 24 hours after treatment, which resolved 24 hours later. Serum T_4 was not altered by short-term dexamethasone treatment, while serum rT_3 concentration was markedly increased for more than 48 hours.[40] When administered intramuscularly at 2.2 mg/kg once daily, prednisone significantly decreased serum T_3 concentration 24 hours after the first dose; subsequent measurements were variable, but decreased T_3 was present 9 and 11 days after treatment was initiated. Serum T_4 concentration was consistently decreased to 50% or less of the pretreatment value for the duration of the 11 day treatment period.[41] A study using an identical dose and route of prednisone administration for 21 days duration showed a marked decrease in serum T_4 and T_3 in addition to suppressed T_4 response to TRH and T_3 response to TSH.[42] These latter findings could indicate a direct (decreased thyroid secretion) or indirect (decreased TSH secretion) effect, although it has been documented in human studies that the TSH-suppressive effects of glucocorticoids are transient.[48]

Although a transient decrease in serum TSH concentrations is reported in people receiving glucocorticoid therapy, it is likely that long-term glucocorticoid treatment may significantly affect thyroid hormone binding and metabolism. This theory is supported by a study from Moore and colleagues, who used a dose of prednisone of 0.55 mg/kg PO q12h for 35 days, and showed a decrease in serum T_3 concentration after 2 and 4 weeks, but failed to demonstrate any significant alteration in T_4, fT_4, free T_3, or rT_3 concentrations.[43] Serum T_3 returned to normal within 2 weeks

of discontinuing prednisone treatment, suggesting that conversion rates of T_4 to T_3 were altered by significant protein binding or variations in thyroid hormone metabolism. Using an identical dose of prednisone, Kaptien and others evaluated the effects of prednisone on T_4 and T_3 kinetics in thyroidectomized levothyroxine-replaced dogs. Prednisone treatment lowered the free fraction of T_4 by 30%, T_3 by 40%, and fT_3 by 49%; there was no change in total T_4 or fT_4 concentrations, or free fraction of T_3.[44] It is likely that the changes noted were due to several peripheral effects of chronic prednisone use, including increased T_4 binding to serum carrier proteins, decreased fractional transfer rates of thyroxine from the extravascular space into serum, redistribution of thyroid hormones from the serum to the muscle and skin, and decreased conversion of T_4 to T_3, resulting in lower serum T_3 concentration. These findings do not necessarily correlate with changes induced by higher doses of glucocorticoids in dogs with an intact HPT axis.

Although the exact mechanism of sulfonamides on thyroid function is poorly understood, it is surmised that they act by inhibiting thyroid peroxidase.[3,49] Because thyroid peroxidase mediates iodide organification and the coupling reaction to form T_4 and T_3 on thyroglobulin, the result of its inhibition is decreased serum thyroid hormone secretion. Pituitary thyrotropes respond to the decrease in thyroid hormones via reduced negative feedback inhibition, which results in increased pituitary secretion of TSH.

Studies in people show only mild decreases of thyroid function during treatment with sulfonamides, in contrast to the severe thyroid dysfunction induced in rats. Several prospective studies have evaluated the effects of sulfonamide administration on canine thyroid function. In a controlled study, trimethoprim-sulfadiazine (15 mg/kg PO q12h) administered to normal animals over four weeks had no significant effect on T_4, T_3 and fT_4 serum concentrations or the serum T_4 response to TSH administration.[50] In contrast, trimethoprim-sulfamethoxazole (30 mg/kg PO q12h) administered to a group of dogs with pyoderma for 6 weeks resulted in significantly decreased T_4 and T_3 concentrations, and subsequently increased TSH concentrations.[3,51,52] These studies seem to indicate the effects of sulfonamides on thyroid function are dose and/or time-dependent. Sulfonamides not only alter thyroid function test results, but may ultimately lead to clinical hypothyroidism in some patients.[49,52,53] Thyroid function tests in these dogs are indistinguishable from those patients with spontaneous primary hypothyroidism.

Amiodarone affects thyroid function by reducing TSH synthesis and secretion in the pituitary.[47] Thyrotoxicosis may occur, and is thought to be due to a direct cytotoxic effect of amiodarone or the result of iodine-induced overproduction of thyroid hormones.[47] In one study, amiodarone administration (22-36 mg/kg/day for 4 weeks) to normal dogs resulted in an increase in serum T_4 concentration by over 50% without an effect on serum T_3.[54] It is important to note that serum amiodarone concentrations in these dogs were 2.5-3 times higher than recommended for the desired clinical effects. These findings are consistent with decreased activity of 5'-deiodinase, concurrent with increased thyroxine secretion. Contrary to these findings, an *in vitro* study evaluating the effects of amiodarone on canine thyroid gland secretion demonstrated inhibition of TSH-induced thyroid hormone secretion.[55] The effects of therapeutic doses of amiodarone on thyroid function in the dog remain to be determined.

In addition to their effects on TRH release, tricyclic antidepressants in people and rats alter thyroid function through binding of iodine (rendering it unavailable), inhibiting TPO (decreasing thyroid hormone production), and stimulating deiodinase activity (increasing T_4 degradation).[18] As previously mentioned, no data currently exists on this medication's effects on the HPT axis in the dog, and this study attempts to clarify the influence of clomipramine on the thyroid hormones.

Potassium bromide is used extensively in the treatment of canine epilepsy, either alone or as an adjunct to phenobarbital. This medication may affect thyroid function because bromide is a halide similar to iodide. Indeed, bromide administration leads to goiter in the rat.[56] Thyroid function tests were not altered in a small study of epileptic dogs given KBr.[4] These findings were confirmed in a recent study of healthy dogs receiving KBr over six months (100 mg/kg PO q12h loading dose for 3 days, followed by 30 mg/kg PO q24h), in which thyroid function (T_4, TSH, fT_4 concentrations) remained unchanged.[57] Therefore, it appears that administration of KBr does not significantly affect thyroid function in dogs when used at appropriate dosages.

Altered Thyroid Hormone Binding

In addition to their previously noted effects, glucocorticoids also decrease the binding of T_4 to TBG, increase binding to TBPA, and may alter thyroid hormone transfer from plasma into various tissues in human beings.[58] Altered hormone binding is represented by an increase in the free fraction of T_4 (i.e. $[fT_4] / [T_4] \times 100$); if this value is increasing, it suggests shifting of thyroxine from the total pool to the free pool by reduced binding. Altered thyroid hormone binding has been shown in dogs as well. A study of nine dogs receiving prednisone (1.2-2.0 mg/kg PO q12h) for 3 weeks revealed an increase in the free fraction of T_4 up to the cessation of treatment.[1] These data were further supported by an examination of 42 dogs with hyperadrenocorticism, in which a high free fraction of T_4 confirmed diminished serum binding in 38% of the animals.[59]

In a study of thyroidectomized levothyroxine-supplemented dogs, prednisone treatment lowered the free fraction of T_4 by 30%, T_3 by 40%, and fT_3 by 49%; there was no significant change in total T_4 or fT_4 concentrations, or free fraction of T_3.[44] Contrary to the belief that glucocorticoids decrease binding of T_4 to TBG, the data from this study suggest that the changes noted were not due to decreased serum hormone binding, but rather increased T_4 binding to serum carrier proteins, decreased fractional transfer rates of thyroxine from the extravascular space into serum, redistribution of thyroid hormones from the serum to muscle and skin, and decreased conversion of T_4 to T_3, resulting in lower serum T_3 concentration. These findings may be unique to the dog, and do not necessarily correlate with changes induced by higher doses of glucocorticoids in dogs with an intact HPT axis.[1,45]

The oral cholecystographic contrast agent ipodate has also been shown in people to cause displacement of thyroxine from binding sites in hepatocytes and carrier proteins, and the excess iodine release from the medication may block thyroid gland secretion of thyroxine.[60]

Non-steroidal anti-inflammatory drugs result in altered thyroid function tests in rats and people, mainly via altered binding to plasma transport proteins. As circulating thyroid hormones are highly protein bound, various NSAIDs can displace thyroid hormones from their serum protein-binding sites, resulting in a transient increase in serum fT_4 concentration. The elevated fT_4 level serves as negative feedback to the hypothalamus and pituitary gland, ultimately resulting in decreased serum total T_4 concentrations. Deiodination of thyroid hormones may also be decreased slightly by NSAIDs.

In people, salicylates inhibit the binding of thyroxine to TBG, resulting in a decrease in T_4 and a transient increase in fT_4.[61] With sustained use, these agents cause a 20-40% decrease in T_4 concentrations, but do not alter fT_4.[62,63] Other human studies of various NSAIDs, including oxaprozin, ketoprofen and etodolac, given at therapeutic doses for >3 weeks, demonstrated no

change in T_4 concentrations, but ketoprofen and nabumetone caused a decreased in serum T_3 concentration. A similar effect has been demonstrated with diclofenac and naproxen as well.[63,64] Data on NSAIDs is limited in dogs, but carprofen, given at 2.2-3.3 mg/kg PO q12h for five weeks, resulted in a small but statistically significant decrease in T_4 (20.8 to 17.0 nmol/L) and TSH (0.16 to 0.12 ng/ml) concentration.[65] This decrease in TSH may be in response to the transient increase in fT_4 shown in human studies, but this was not documented in this study. An *in vitro* study demonstrated that phenylbutazone had no significant effect on T_4 binding to canine serum proteins, but flunixin decreased thyroxine binding.[66] The overall data in dogs seems to suggest that because the primary effect of NSAIDs is on plasma transport, serum fT_4 and TSH concentrations would be unlikely to substantially change during long-term treatment.

Administration of high doses of furosemide in people induces a decrease in serum T_4 concentration and an increase in serum fT_4, consistent with impaired plasma protein binding of thyroxine.[67-69] An *in vitro* study of canine serum demonstrated that furosemide markedly impairs binding of thyroxine to plasma proteins.[66] This would result in an increase in fT_4 and a decrease in T_4. An *in vitro* study of the effects of phenobarbital and diphenylhydantoin on thyroxine binding in canine plasma showed that phenobarbital did not alter while diphenylhydantoin decreased binding of T_4 to plasma transport proteins.[66]

Androgens consistently decrease serum concentrations of T_4 and T_3 in people by decreasing the binding capacity or circulating concentrations of TBG,[70] while effects on fT_4 and TSH are variable, depending on the specific anabolic steroid and the dose administered. Thyroid function testing in horses does not appear to be affected by administration of stanozolol.[71] In the only study in dogs available, the anabolic steroid stanozolol (2 mg PO q12h) was administered to six healthy dogs for three weeks, and no effect on serum T_4, T_3, or fT_4 concentrations were noted.[72]

Human studies have demonstrated a transient increase in fT_4 concentration following the administration of heparin,[73] and *in vitro* studies have reported this is due to inhibition of T_4 binding to plasma proteins secondary to free fatty acids generated as a consequence of heparin activation of lipoprotein lipase.[46,74,75] Although the effects of heparin on thyroid function have not been studied in dogs, a study of the *in vitro* influence of heparin on T_4 binding to canine plasma proteins demonstrated an increase in the binding of T_4. If this interaction was to occur *in vivo*, it is conceivable that this increased binding would lead to increased total T_4 and reduced fT_4 concentrations.[66]

Altered Thyroid Hormone Metabolism

Studies in people and rats have demonstrated that phenobarbital alters thyroid function tests by enhancing hepatic thyroxine metabolism secondary to hepatic microsomal enzyme induction.[35] This increases peripheral elimination of T_4 via increased hepatic deiodination of thyroid hormones that results in decreased concentrations of circulating thyroxine. Enhanced biliary and fecal excretion of thyroid hormones may also contribute to decreases in serum thyroid hormone concentrations.[76-78] This may be particularly important in dogs since about 50% of T_4 and 30% of T_3 are excreted in the feces.[32]

Chronic administration of phenobarbital to dogs consistently decreases serum concentrations of T_4 and fT_4 and elevates TSH.[2,4,5,79,80] Serum T_4 and fT_4 concentrations may be decreased below the normal range and serum TSH above the reference range in some dogs treated with phenobarbital.[2,4,5,79] The decreases in T_4 and fT_4 may occur as early as 3-5 weeks after

initiating treatment.[5,79] Serum TSH concentration increased only after protracted treatment,[5,79] suggesting that this effect was secondary to decreased T_4 concentrations.

Oral cholecystographic contrast agents, ipodate and ipanoic acid, have been shown to affect the thyroid in numerous ways, including potent inhibition of 5'-deiodinases, resulting in a substantial decrease in serum T_3 concentrations in human and feline hyperthyroid patients.[81,82] Inhibition of deiodination has been demonstrated in isolated canine thyroid glands, and an increase in T_4 and a decrease in T_3 were noted following administration of a single dose of 6 g to normal dogs, further supporting this notion.[40,83] In people, ipodate also causes a displacement of thyroxine from binding sites in hepatocytes and carrier proteins, and the excess iodine release from the medication may block thyroid gland secretion of thyroxine.[60]

Altered thyroid hormone metabolism in people has been seen with the use of the β-adrenergic blocker propranolol. Small decreases in serum T_3 are present because of decreased activity of 5'-deiodinase.[78] In the only reported study, healthy beagles given 20 mg propranolol PO q8h for two weeks, followed by 40 mg PO q8h for an additional two weeks, thyroid hormone concentrations and TSH stimulation test results were not altered.[84]

Another action of glucocorticoids is inhibition of 5'-deiodinase, resulting in decreased conversion of T_4 to T_3, and decreased rT_3 metabolism in people.[85] The 5'-deiodinase inhibition would theoretically lead to increased T_4 concentrations, yet many studies have actually demonstrated decreased serum T_4 concentrations.[1,6,41,42] The exact reason for this contradictory finding is unknown, but this discrepancy may be due to an interspecies difference between canine and human thyroid glands, or as previously noted, steroids affect thyroid hormones by multiple mechanisms, such as increased T_4 binding to serum carrier proteins or altered redistribution of thyroid hormones from the serum to muscle and skin. The inhibition of 5'-deiodinase should also lead to increased rT_3 concentrations, and this was demonstrated in dogs administered a single dose of dexamethasone (0.5-0.7 mg/kg) that had markedly increased serum rT_3 concentration for >48 hours.[40]

The canine HPT axis is affected by many different drugs, with each medication possibly acting in multiple ways to impact thyroid hormones. The list of drugs known to affect thyroid function in people is much more extensive than those evaluated in dogs. As with any medication, dosages and length of treatment are essential considerations. Finally, it is important to realize that the various mechanisms through which these drugs alter canine thyroid function have not been fully elucidated, and the effects of many other commonly used medications on canine thyroid function remain to be evaluated.

Effects of clomipramine on the hypothalamic-pituitary-thyroid axis

Review of Animal Studies

Many drugs affect the HPT axis, leading to decreased TRH/TSH secretion and/or altered thyroid hormone synthesis, binding, or metabolism, and it is thought that TCAs such as clomipramine may interact on multiple levels. A paucity of literature exists on the effect of clomipramine treatment on the HPT axis in animals. However, decreased serum concentrations of thyroid hormones are a common occurrence in people treated with TCAs.

Theoretically, alteration of the HPT axis may occur through the medication's modulation of neurotransmitters (NE, dopamine, and serotonin), leading to changes in release of TRH and TSH. This may hold true in rats, in which NE stimulates the release of TRH.[29] In addition, the thyroid gland is adrenergically innervated, supporting the fact that NE would have an impact on secretion.[18] While the stimulation of noradrenergic receptors stimulates TRH release in the rat, dopaminergic[29,86] and serotinergic[29] stimulation inhibits TRH secretion. Serotonin secretion stimulates TRH release in monkeys,[29] and this is thought to be true in people as well.[28] As such, the effects of catecholamines on the thyroid secretory hormones appears to be species-dependent, and to date no studies have been done in dogs.

In addition, as tyrosine is a precursor molecule for both catecholamines (i.e. dopamine, NE, and epinephrine) and thyroid hormones (iodotyrosine, iodothyronine, T_4 and T_3), modulation of this amino acid via upregulation of one of the pathways could theoretically decrease production of the other. As NE and serotonin reuptake is inhibited by TCAs, it is likely that their degradation is increased, and tyrosine turnover may be enhanced, leading to decreased thyroidal hormone production. To the author's knowledge, this phenomenon has not been investigated.

In a study of desmethylimipramine (20 mg/kg SQ q24h for 7 days) on rat brain β-adrenergic and serotinergic receptors, it was found that the medication down-regulated both sets of receptors, while administration of T_4 or T_3 increased their numbers.[87] As serotonin release leads to decreased TRH secretion in the rat, down-regulation of these receptors by TCAs theoretically may lead to increased TRH secretion. Conversely, as NE stimulates TRH secretion, down-regulation of these receptors in rat brain may reduce TRH secretion, and it is unknown which system predominates.

In rats given desipramine (5 mg/kg IP q12h) for fourteen days, no alterations in T_4 or T_3 concentrations were noted, despite a decreased TSH concentration.[88] *In vitro* TRH stimulation of rat thyrotropes exposed to the same treatment resulted in no significant change in TSH secretion, suggesting that the decreased serum TSH concentration observed *in vivo* was unlikely to have been due to direct or indirect effects of desipramine on pituitary thyrotrope TRH receptors.[88] The actual cause of decreased *in vivo* TSH concentration is unknown, although altered thyroid hormone binding to plasma proteins could have played a role, as could the abbreviated length of the study and decreased TRH stimulation.

In a study of cultured rat hypothalamic neurons treated with different concentrations of imipramine and desipramine for 7 days, it was found that imipramine and desipramine significantly decreased TRH concentrations in a dose-dependent manner.[89] The effect of these antidepressants on the dexamethasone induction of TRH release was also investigated, and imipramine and desipramine reduced the TRH response to glucocorticoid stimulation as well.[89] This study suggests that TCAs may have a direct effect on TRH synthesis, leading to decreased TRH secretion in the rat hypothalamus.

Another study reporting the effects of antidepressants on TSH release used TRH-response tests in a rat model of ether stress (designed to suppress TSH).[90] Contrary to previous reports of TCA-induced TSH suppression, TSH concentration was not suppressed in those subjects that were treated with clomipramine, suggesting that upregulation of the serotinergic system via TCA administration may be involved in maintaining pituitary output of TSH. In fact, these biogenic amines (serotonin and NE) may not only increase TSH secretion, but have been shown to activate thyroid follicular cells directly, acting as intrathyroidal transmitters.[91] This aminergic stimulation,

while having no direct bearing on TSH concentrations, may maintain thyroid hormone release, therefore maintaining feedback regulation of TSH concentrations. In another study regarding the role of monoamine neurotransmitters on TRH, rats were given desipramine, and hypothalamic TRH and serum TSH, T_4 and T_3 were measured.[29] The data indicated that augmented serotonergic or dopaminergic activity may inhibit TRH release.

Overall, it appears that TCAs, both directly (on synthesis) and indirectly (by their manipulation of dopaminergic and serotinergic systems) may inhibit TRH secretion, as well as down-regulate both β-adrenergic and serotinergic receptors, leading to further inhibition of TRH secretion. TCAs have also been shown to decrease serum TSH concentrations in some studies, but this is an inconsistent finding, and may be affected by alterations in thyroid hormone concentrations (due to plasma binding) and their effect on feedback mechanisms or decreased TRH stimulation. Unfortunately, many of these findings are in rat models, and other species may respond differently.

Another mechanism though to be involved in TCA modulation of thyroid hormone concentrations is their effect on intracellular hormone metabolism, especially thyroxine deiodination. In a study using desipramine (30 mg/kg q24h for 14 days), an increase in Type II and III deiodinase activity was noted in rat brain,[92,93] but no changes were noted in pituitary or liver deiodinase activity.[93] As metabolism of thyroid hormones in the CNS is unlikely to contribute to the decreased serum T_4 and T_3 concentrations often seen with TCA therapy, it is plausible that the drug's effect on neurotransmitter concentrations may be more important. Indeed, increased concentrations of NE and epinephrine have been shown to increase deiodination of thyroxine.[94] As TCA's mode of action is to increase NE concentration by preventing reuptake, it is theoretically possible that this could lead to decreased T_4 concentration via increased deiodination. This could subsequently lead to a decreased serum T_3 concentration if similar metabolic pathways for T_3 are activated, or if the decreases in serum T_4 eventually lead to a smaller substrate pool from which to form T_3.

It has been suggested that TCAs may inhibit thyroid peroxidase, thereby causing decreased thyroid hormone production. The proposed mechanism is covalent bonding of the medication to the heme portion of the enzyme, rendering it inactive.[18] Although this has been demonstrated *in vitro*,[17] specific *in vivo* studies demonstrating this effect are lacking.

Finally, TCAs have the ability to complex iodine, rendering it unavailable for thyroid hormone production.[18] When altered by clomipramine, a portion of the thyroid's iodide store is complexed by the electrodonating capacity of the drug molecule and rendered unavailable, while another part is deactivated as a triiodide ion (I_3^-).[18] The ability of clomipramine to donate electrons and impair iodine availability in the thyroid has been shown to be forty times the minimum amount required to produce hypothyroidism, while those of desipramine and imipramine are thirty and fifty times, respectively.[17]

Various behavioral studies on rats have revealed that TCA therapy decreased serum T_3 concentrations in a dose dependent manner, but did not alter TSH concentrations.[9,10] This is in concordance with other studies, one of which revealed decreased thyroid hormones in the serum of rabbits treated with imipramine,[95] and in studies of people diagnosed with clinical depression.[96] These findings support the hypothesis that altered metabolism of thyroid hormones occurs with TCA administration.

In a recent study of the effects of imipramine and desipramine on rats, it was found that after a four-week treatment cycle, serum T_4 decreased by 13% in the imipramine treated group and 20% in the desipramine group.[97] Furthermore, desipramine decreased serum T_3 by 14%, but no difference in serum T_3 concentrations was found with imipramine.[97] Both medications were shown to have accumulated in the thyroid, as thyroid:serum ratios for imipramine were 12:1 and 14:1 for desipramine.[97] It was surmised that these drugs reduce thyroid hormone secretion by complexing iodine, preventing its use for thyroid hormone production.

In the most comprehensive study of the effects of TCAs on the rat HPT axis, imipramine (10 mg/kg IP q24h for 14 days) decreased serum T_4 and T_3 concentrations in treated subjects, similar to that observed in imipramine-treated thyroidectomized rats given thyroxine-supplementation.[98] This comparison suggests that TCA therapy does not decrease thyroxine secretion, but enhances its metabolism. A corresponding decrease in free T_4 and T_3 concentrations was not evident; in fact, the free fractions of both hormones actually increased in response to imipramine treatment.[98] The study also investigated the influence of imipramine on thyroid hormone binding proteins by adding the medication to normal serum samples and evaluating hormone concentrations; no significant effect on the free fractions was noted, suggesting protein binding was not affected by the medication *in vivo*.[98] Cellular uptake or metabolism of thyroid hormones was similarly unaffected, as concentrations of thyroxine and triiodothyronine in the brain, heart and liver were unchanged from pretreatment values. Serum TSH concentrations were not significantly affected in the treated group, consistent with the lack of change noted in the serum free fractions of T_4 and T_3.[98] *In vitro* TRH stimulation testing on rat thyrotropes revealed no differences between imipramine-treated and control groups, nor was inhibition of secretion induced by bathing with T_4 and T_3. Imipramine decreased *in vitro* TSH secretion only at supra-therapeutic concentrations, and no change was noted on *in vivo* TSH concentrations, suggesting altered hypothalamic control of thyrotropes is not a mechanism for TCA-induced thyroid suppression. Therefore, while the TCA imipramine caused decreased thyroid hormone concentrations, no evidence for altered tissue concentrations or thyrotrope function was found, and it is surmised that enhanced peripheral clearance may be an important contributor to the decreased hormone concentrations. While no evidence of altered thyroid hormone binding to serum proteins was noted, interspecies differences prevent extrapolation of the data. The data presented by this research contrasts previously discussed studies, and may be impacted by the type of medication used (imipramine) or relatively short length of treatment.

In summary, it is evident that TCAs do have an effect on the HPT axis, with direct and indirect influences on TRH secretion, decreased thyroid hormone production (thyroid peroxidase and iodide concentrations), and increased metabolism (thyroxine deiodination). Various studies have indicated dose-dependent decreases in serum T_4 and T_3 concentrations, without the expected increase in serum TSH concentrations. These alterations (decreased serum T_4 and T_3 without an increase in TSH concentrations) are unclear, and may reflect altered TRH secretion or thyroid hormone binding to plasma proteins. Studies *in vivo* in rats suggest that TCA therapy does not decrease thyroxine secretion, but enhances its metabolism, and no direct effect on plasma binding proteins or thyroxine metabolism have been documented. As many of the findings of the aforementioned studies are incongruous, an important caveat must include marked species differences in response to the medication, various compounds, dosages, and durations of study used, and not all TCAs had similar effects in all studies.

Review of Human Studies

In human studies, the debate over the past 30 years has centered on whether TCAs lead to decreased thyroid function, or if decreased thyroid hormones and subclinical hypothyroidism are a cause of the depressive state and reduced response to treatment. As advances in technology for assessing thyroid function have improved, the current belief is that hypothyroidism is a relative rather than an absolute state. This is supported in the human literature, which suggests that an absolute lowering of thyroid hormone concentrations represent only the end stage of declining thyroid gland function. In the endocrinological literature, a precedent exists for using the term "subclinical hypothyroidism" to refer to biochemically measurable degrees of relative thyroid failure. However, in the psychiatric field thyroid insufficiency is considered a spectrum rather than an absolute state, and the endocrinologic categorization makes no determinations about when thyroid insufficiency becomes clinically apparent.

A study of thyroid function in 148 general medical patients found a history of treatment for depression in 50% of those with a TSH concentration >3.0 IU compared with 18% of those with TSH concentration <3.0 IU.[99] A review of the literature confirms that decreases in serum concentrations of T_4, fT_4 and reverse T_3 have been demonstrated during treatment with various antidepressants,[96,100-109] yet some of these studies are plagued by poor methodology (i.e. small sample size, open design, varied dosages). Interestingly, in seven of the above-mentioned studies the reductions in serum concentrations of T_4 (and fT_4 and rT_3, when measured) were significantly correlated to the degree of clinical response (i.e. the more the serum concentrations of the hormones declined, the better the patient's response).[102-108] These findings are in contrast to the belief that subclinical hypothyroidism is a major contributor to refractory depression, and there is considerable controversy in the psychiatric field on this subject. Obviously this discussion is complicated by the fact that depression is a multi-factorial disease process, as well as the poor understanding of neurochemistry and the mechanism of action of many drugs.

Little is known about the interaction of T_4 and its efficacy with antidepressant treatments. It is possible that the decrease in serum T_4 concentrations of both people and laboratory animals undergoing TCA therapy may reflect an increase in both T_4 degradation and T_3 production in the CNS and in peripheral tissues in which thyroid hormones are metabolized. However, a decline in serum T_4 concentration caused by a decrease in thyroidal T_4 production or enhanced elimination may be an overly simplistic explanation, as serum concentrations of both T_3 and TSH generally remain unaffected by antidepressant treatment in people. An increase in metabolism of T_4 in tissues such as the liver or kidney also seems unlikely, as experiments indicate rat 5'-deiodinase activity is unchanged in these tissues after antidepressant treatments.[92,110] Alternatively, enhanced tissue uptake of T_4 with subsequent metabolism to T_3 would be more compatible with the decrease in serum T_4 concentration and unvarying serum concentration of T_3.

In summary, subclinical hypothyroidism is associated with depressive states in people, and decreased serum concentrations of T_4, fT_4 and reverse T_3 have been demonstrated during treatment with various antidepressants.[96,100-109] Increased T_4 degradation and T_3 production in the CNS and in peripheral tissues may explain these changes, however both T_3 and TSH generally remain unchanged. Enhanced tissue uptake of T_4 with subsequent metabolism to T_3 would also be consistent with the decreased serum T_4 and unchanged serum T_3 concentrations.

Clomipramine

Pharmacology

Clomipramine is a tertiary tricyclic antidepressant medication (Figure 3) that inhibits neuronal reuptake of serotonin and norepinephrine, and its metabolite, desmethylclomipramine, is more selective for inhibiting norepinephrine reuptake.[111]

Pharmacokinetically, in rat models 80% of the radiolabeled drug was excreted in the feces, and the remaining 20% in urine.[112] There was evidence of enterohepatic circulation in the dog, but distribution data was limited.[112] In dogs given a single oral (4 mg/kg) or intravenous (2 mg/kg) dose, the drug's plasma concentration was found to decline in three distinct phases, and the half life ($t_{½}$) for the terminal elimination phase was 6.4 hours for clomipramine, and 3.8 hours for desmethylclomipramine, the primary metabolite. The volumes of distribution were relatively large, with 3.8 L/kg for clomipramine and 3.6 L/kg for the sum of clomipramine and desmethylclomipramine. Maximum concentration (15 µg/L) of desmethylclomipramine occurred 0.42-1.08 h post dosing, while relative oral bioavailability, determined from the plasma data for the sum of clomipramine and desmethylclomipramine, was 22-26%.

Clomipramine was extensively bound to plasma proteins (>97%), and a serum steady state was achieved after three days. The ratio of plasma clomipramine to desmethylclomipramine was ~3:1, and while no information on organ and tissue distribution was available for dogs, all data extrapolated from mice, rats, and rabbits indicated there is a markedly high affinity to all body tissues because of the medication's lipophilic nature. Biotransformation occurs via hepatic demethylation, aromatic hydroxylation, and glucoronide conjugation.

Canine Studies

In the initial multi-centered, double-blinded, placebo-controlled clinical trial, the goals were to compare the efficacy and adverse effect profile of two doses (1-2 mg/kg and 0.5-<1.0 mg/kg PO q12h) of clomipramine hydrochloride versus a placebo, in combination with behavioral therapy, for the treatment of separation anxiety in dogs.[113] Patients were treated for 84 days with evaluations occurring on days 0, 28, 56 and 84. The number of animals in each treatment group that showed improvement in the signs of separation anxiety was considered the primary efficacy endpoint, and overall, the higher dose was more effective.

The second clinical trial investigated not only the efficacy and safety of clomipramine in combination with behavioral modification, but compared the efficacy of once to twice daily dosing.[114] This study involved 181 dogs exhibiting one or more of the criterion of separation anxiety (salivation, destruction, urination, defecation), and patients received either placebo, or 2-4 mg/kg PO q24h, or 1-2 mg/kg PO q12h. All dogs received behavior modification consisting of desensitization and counter-conditioning. Patients received the medication for 56 days, and significant differences between the control and clomipramine treatment groups were noted during the first (29 vs. 47% improved), third (47 vs. 63%), fourth (56 vs. 75%) and sixth (44 vs. 68%) weeks of treatment. The study concluded that administration of clomipramine (2-4 mg/kg), at either dose regimen, was effective in controlling the clinical signs associated with separation anxiety, when used in conjunction with behavior modification. Although recovery was seen in both treated and control groups, the extent and rate of improvement was greater in the clomipramine-treated subjects when compared to behavior modification alone.

In the only other study evaluating the role of two doses of clomipramine in conjunction with behavior modification in canine separation anxiety, dramatically different results were noted.[115] Forty-nine dogs were assigned to three treatment groups (placebo, 0.5–1 mg/kg and 1–2 mg/kg PO q12h) and evaluated for eight weeks of treatment. Results indicated that "the typical signs of separation-related behaviour problems were not significantly affected by treatment with clomipramine, but behavioural therapy on it own was highly effective in reducing behavioral problems."[115]

A randomized, double-blinded placebo-controlled clinical trial attempted to evaluate the role of serotonergic modulation on canine aggressive behavior, specifically dominance aggression.[116] Twenty-eight dogs identified with dominance aggression were evaluated (via questioning of owners and standardized scoring) for a two-week baseline period. The dogs were then separated into two groups (15 treatment, 13 control) for six weeks of clomipramine (1.5 mg/kg PO q12h) or placebo administration with no concurrent behavior modification; results indicated no reduction of aggressiveness in either treatment group.

Toxicity Studies

Various toxicity studies have been undertaken to evaluate the potential cumulative toxicity and dose-response relationships of clomipramine. In a six-month oral toxicity in beagle dogs, the drug was administered as high as five times the recommended daily dose to 48 subjects.[117] Each dog was randomly assigned to one of four treatment groups (placebo, 4, 12 and 20 mg/kg/d), and were dosed daily for six months. The most prevalent side effect was vomiting, seen in all groups including controls. Body weight was unaffected by the treatment, and food and water consumption remained consistent in all treatment groups. Physical, ophthalmic, and electrocardiograph examinations, as well as hematologic and serum biochemistry profiles, revealed no clinically significant abnormalities, nor were there any changes in macro- or microscopic observations at necropsy.

In another study, thirty-two Pembrokeshire Corgis were randomly assigned to four groups (placebo, 12.5, 50 and 100 mg/kg/d) and were given the medication for twelve months.[118] Five dogs from the high dose group died between weeks 8 and 21, and all suffered significant weight loss, as well as convulsions, lethargy and pupil dilation. Vomiting was seen in all groups but increased in incidence in a dose-dependent manner.

In a study of clomipramine's arrhythmogenic effect, eight beagles were given either placebo or clomipramine (20 mg/kg PO q24h).[119] Dogs were treated for a seven-day period, at which time an electrocardiogram, heart rate, intra-ocular pressure (IOP), body weight, hematologic and clinical chemistry parameters were measured. Clomipramine induced reproducible bradycardia, as well as sinoatrial and atrioventricular block; ventricular extrasystoles were observed sporadically in 3 of 8 dogs. No drug-related effects were noted on body weight, blood parameters, or IOP.

PURPOSE

The purpose of the study reported here was to evaluate the effect of long-term clomipramine administration on the hypothalamic-pituitary-thyroid axis in normal dogs.

CHAPTER 2

A variety of drugs have been reported to alter thyroid function tests in dogs, including glucocorticoids, potentiated sulfonamides, and anticonvulsants.[1-6] Many more drugs have been reported to affect thyroid function in man and other species. Among these, tricyclic antidepressants (TCAs) have been shown to impair thyroid hormone synthesis and thyrotropin secretion in man and rats.[7-10]

Clomipramine is a tricyclic antidepressant that has been used to treat various human psychiatric disorders for over 30 years, and was approved in 1998 for the treatment of separation anxiety in dogs. Clomipramine exerts a potent and relatively selective serotonin reuptake-inhibiting effect by blocking the monoamine reuptake transporter of serotonergic (5-hydroxytryptophane, 5-HT) neurons.[11,12] Furthermore, the primary metabolite, desmethylclomipramine, is an inhibitor of norepinephrine (NE) reuptake.[13-15]

It has been extensively documented that TCAs inhibit thyroid hormone biosynthesis by altering thyroid follicular cell iodine uptake[16-18] and inhibiting thyroid peroxidase.[17,18] In addition, TCAs may interfere with the hypothalamic-pituitary-thyroid (HPT) axis via the serotonergic and noradrenergic systems, and therefore could lead to a decrease in thyroid stimulating hormone (TSH), thyroxine (T_4) and 3,5,3' triiodothyronine (T_3) concentrations.

To date, no studies on the effect of clomipramine on thyroid function have been performed in the dog. If dogs respond to clomipramine in a manner similar to other species, evaluation of thyroid function tests may result in a misdiagnosis of hypothyroidism. An additional concern is that if clomipramine decreases plasma thyroid hormones, iatrogenic hypothyroidism may result. This may be of particular concern since hypothyroidism is a contributing factor to a variety of psychological illnesses in people, and the therapeutic benefit of TCAs on affective disorders is enhanced by thyroid supplementation in both human beings[19,20] and rats.[9] Although the relationship between hypothyroidism and behavioral disorders in dogs is tenuous, aggression has been reported.[21,22] If administration of clomipramine induces hypothyroidism, it may exacerbate behavioral signs or reduce the potential positive behavioral response to the drug.

MATERIAL & METHODS

Fifteen random source dogs (11 males and 4 neutered females), 16-32 kg, were determined to be healthy based on physical examination, complete blood counts (Table 2), serum chemistry profile, heartworm (*Dirofilaria immitis*) antigen test, and fecal parasite examination (Table 3). Five dogs were infected with intestinal parasites (three with *Toxocara* spp., two with *Ancylostoma* spp.) and were treated appropriately prior to initiation of the study. Two of the fifteen dogs were removed from the study due to concurrent disease processes that would have affected thyroid hormone concentrations (one with antebrachial cellulitis of undetermined origin during the acclimation period, which was replaced by another dog, and one with hepatopathy, euthanized at the termination of the study after developing vomiting, anorexia and weight loss at week 13), leaving 14 subjects for data analysis. A serum chemistry profile was repeated at the end of the study to assess any systemic affect of the medication (Table 4). All subjects were housed in indoor runs with a 12-hour light-dark cycles at 22°C and conditioned for 3 weeks prior to study. Dogs

were fed a maintenance dry food[a] once per day. This protocol was approved by the Virginia Tech University animal care committee, and all animals were cared for according to NIH Guide for the Care and Use of Laboratory Animals.

Euthyroid status was confirmed by finding a normal serum T_4 response to TSH stimulation on day –7. The TSH stimulation test[120] consisted of measurement of serum T_4 before and 6 hours after administration of bovine TSH[b] at 0.1 IU/kg IV. A post-TSH serum T_4 concentration >35 nmol/L was present in all dogs. Clomipramine[c] (3 mg/kg PO q12h) was administered days 0 through 112. All dogs served as their own controls. A thyrotropin-releasing hormone (TRH) stimulation test[121] was performed on days 0, 7, 28, 42, 56, and 112 by collecting blood samples for measurement of serum TSH before and 30 minutes after IV administration of 0.2 mg/dog TRH.[d] All testing was performed between 7:00 – 8:00 a.m. Following collection of 10 ml blood by jugular venipuncture, serum was harvested within 60 minutes after centrifuging at 2000×G for 20 minutes. All samples were stored at –70°C until thawed for hormone analysis. Dogs were monitored daily for side effects of treatment.

Serum TSH was measured by an immunoradiometric assay,[e] and serum concentrations of T_4, T_3, and rT_3 were measured by radioimmunoassays.[f,g,h] Serum fT_4 was measured using a commercially available kit that utilized an equilibrium dialysis method.[i] All assays were previously validated for use in dogs.[50,84,122]

Intra-assay coefficients of variation were determined by serial measurement of 10 samples of pooled canine serum containing low, medium, and high hormone concentrations. Inter-assay coefficients of variation were determined by measuring hormone concentrations of pooled serum containing low, medium, and high hormone concentrations on 4 different dates. Intra-assay coefficients of variation for T_4 were 7.5%, 6.3%, and 7.3% in low (10.3 nmol/L), medium (24 nmol/L), and high (53 nmol/L) pools, respectively. Intra-assay coefficients of variation for T_3 were 5.2%, 4.4%, and 4.9% in low (0.62 nmol/L), medium (1.62 nmol/L), and high (2.33 nmol/L) pools, respectively. Intra-assay coefficients of variation for rT_3 were 7.9%, 6.1%, and 8.8% in low (0.57 nmol/L), medium (1.43 nmol/L), and high (2.51 nmol/L) pools, respectively. Intra-assay coefficients of variation for fT_4 were 7.6%, 9.2%, and 7.1% in low (10 pmol/L), medium (27 pmol/L), and high (79 pmol/L) pools, respectively. Intra-assay coefficients of variation for TSH were 6.2% and 5.6% in low (0.19 ng/ml) and high (1.14 ng/ml) pools, respectively. Interassay coefficients of variation for low, medium, and high pools for T_4 were 8.2%, 6.8%, and 6.9%, respectively; for T_3 were 6.6%, 5.4%, and 5.4%, respectively; for rT_3 were 6.8%, 7.2%, and 7.7%, respectively; for fT_4 were 6.3%, 7.4%, and 8.2%, respectively; and for TSH were 5.8 and 7.1% in low and high pools, respectively.

[a] Hill's® Science Diet Maintenance (Dry), Hill's Corporation, Topeka, KS
[b] bovine TSH, Sigma Chemical Corporation, St. Louis, MO
[c] Clomicalm®, Novartis Animal Health, Greensboro, NC
[d] pGLU-HIS-PRO Amide (Synthetic TRH), Sigma Chemical Corporation, St. Louis, MO
[e] Coat-A-Count® Canine TSH IRMA, Diagnostic Products Corporation, Los Angeles, CA
[f] Coat-A-Count® Canine T_4, Diagnostic Products Corporation, Los Angeles, CA
[g] Coat-A-Count® Canine T_3, Diagnostic Products Corporation, Los Angeles, CA
[h] Reverse T_3 Radioimmunoassay, Biochem Immunosystems Italia S.P.A., Bologna, Italy
[i] Free T_4 by Equilibrium Dialysis, Nichols Diagnostics, San Luis Obispo, CA

STATISTICAL ANALYSIS

Statistical analysis was performed using commercially available software.[j] Repeated measures analysis of variance was applied to test for effects of day of treatment. Covariation among repeated measurements on the same animal was modeled using a spatial power covariance structure. Significant effects of time on treatment were further investigated using orthogonal polynomial contrasts, and a p-value < 0.05 was considered significant.

RESULTS

Only mild adverse effects of the medication, such as occasional hyporexia and intermittent vomiting, were noted during the study, and all subjects' body weight remained stable. Overt serious side effects, such as tachyarrhythmias, marked lethargy, or clinical manifestations of hypothyroidism, were not noted during the treatment period.

There was a significant ($p < 0.001$) effect of treatment time on basal T_4 concentration (Figure 4). Serum T_4 concentration decreased over the treatment course, declining from a pre-treatment mean ± SE of 26 ± 1.2 nmol/L, and reaching a trough of 17 ± 0.5 nmol/L at day 112.

The effect of time on fT_4 concentration was significant ($p < 0.0002$), with a persistent decrease over the treatment course (Figure 5) from the pre-treatment mean of 29 ± 2.4 pmol/L. The lowest mean serum fT_4 concentration (18 ± 1.7 pmol/L) was present on day 56, and it remained decreased at 19 ± 1.3 pmol/L on day 112.

There was a significant ($p < 0.0001$) decrease of serum rT_3 concentration over the study period (Figure 6), from a pre-treatment mean of 1.21 ± 0.13 nmol/L to 0.83 ± 0.08 nmol/L on day 112.

The effect of time on serum T_3 concentration was significant ($p < 0.0001$) as well, but the deviation in T_3 from baseline was variable, and cyclic in nature (Figure 7). No consistent trend in T_3 concentrations could be identified, nor was it possible to fit the data to either a linear or polynomial trend. Significant increases in serum T_3 were noted at days 42 and 112. The T_3 concentration increased from a baseline concentration of 1.40 ± 0.21 nmol/L to 1.83 ± 0.30 nmol/L on day 112 of treatment, with the lowest concentration occurring on day 56 (1.18 ± 0.09 nmol/L).

Although an apparent increase occurred in basal concentrations of TSH (from 0.19 ± 0.02 ng/ml on day 0 to 0.27 ± 0.04 ng/ml on day 112), no statistically significant effect of time was noted in either basal or post-TRH TSH concentrations (Figure 8).

[j] SAS, version 6.12, SAS Institute, Cary, NC

DISCUSSION

Decreases in T_4, fT_4, and rT_3 seen in this study are consistent with the suppressive effects of TCAs on thyroid function found in other species. Although the effect TCAs have on the thyroid gland and its function varies between species, several mechanisms probably account for the clomipramine-induced depression of thyroid function. The drug binds iodine, thereby preventing T_4 synthesis. Clomipramine's capacity to donate electrons and impair iodine availability in the thyroid gland has been shown to be forty times the minimum amount required to produce hypothyroidism, and has a potency approximately 20% that of methimazole.[17] TCAs also inhibit T_4 production via their effect on thyroid peroxidase (TPO),[17,18] and *in vitro* studies have demonstrated that clomipramine irreversibly inhibits TPO by binding iodide molecules to the heme-portion of the enzyme.[17,123] The concentration of clomipramine required for 50% inhibition of horseradish peroxidase (as a substitute for TPO) was approximately 14 times the plasma clomipramine concentration obtained in dogs treated with clomipramine at 2 mg/kg twice daily.[17,124] Plasma concentrations are misleading, however, because thyroidal concentrations of imipramine and desipramine (pharmacologically similar TCAs) were 19 and 6 times, respectively, that of serum in rats administered these agents for 4 weeks.[97,124] Therefore, the thyroid gland concentration of clomipramine in dogs in the current study may have been sufficient to inhibit TPO and result in a decrease in T_4 and fT_4.

In addition to their effects on thyroid hormone synthesis, TCAs influence T_4 metabolism. In a study of rats treated with desipramine, a TCA similar to clomipramine, an increase in 5'-deiodinase activity (responsible for deiodination of T_4) was noted in the brain. In that study, decreased serum T_4 concentrations and serum T_3 concentrations were also found.[92] Although no effect was seen in peripheral deiodination by the liver or kidneys,[92] the effect of TCAs on canine deiodinases is unknown. Evaluation of the mean T_4:T_3 (Figure 9) and rT_3:T_3 (Figure 10) ratios revealed a decrease over the course of the study, suggesting increased metabolism via 5'-deiodinase. These results are only speculative, as this data could also indicate alterations in plasma protein binding for these thyroid hormones. Since serum concentrations of thyroid hormones are more dependent on deiodination in peripheral tissues such as the liver than the CNS, the stimulatory effect of TCA on 5'-deiodinase in the CNS may be of little consequence on serum thyroid hormone concentrations. Inhibition of 5'-deiodinase in tissues outside the CNS could account for the decrease in rT_3 and periodic elevations of serum T_3 concentrations, as seen in this study. Alternatively, the decrease in rT_3 found in the present study could be the result of decreased T_4 secretion, and therefore decreased substrate for conversion to rT_3.

Considerable variation in T_3 concentrations occurred over the length of the study, and may be due to both thyroidal and extra-thyroidal conversion of T_4 to T_3 via 5'-deiodinase enzymes.[23] Analysis of rT_3:T_3 ratio (Figure 10) indicates a decrease during the treatment period, suggesting decreased binding of T_3 to plasma proteins, or increased metabolism via 5'-deiodinase (increased production from T_4). Conversely, mean rT_3:T_4 ratios (Figure 11) revealed a slight increase over the 112-day treatment period, suggesting either increased 5-deiodinase activity (increased production of rT_3) or decreased 5'-deiodinase activity (decreased metabolism of rT_3). However, changes in deiodination seem an unlikely explanation for the increased serum T_3. Enhanced thyroid secretion of T_3 in proportion to T_4 appears to be dependent on TSH,[34] which was not increased in the present study. It is possible that alterations in alternative pathways of T_3 metabolism, such as sulfation, are decreased by clomipramine. However, there is no evidence to support this, and the authors are unable to explain this result.

While the 35% decrease in T_4 and 38% decrease in fT_4 seen in this study should cause a rise in TSH concentration, no significant change in basal serum TSH concentration or TSH response to TRH administration was noted. This poor response could reflect actual suppression of TSH by clomipramine, or poor sensitivity/specificity of the assay method. The smaller increase in serum TSH in response to TRH in the present study, as compared to other studies,[121,125] may have been affected by the source of TRH used, as chemical, not pharmaceutical grade TRH was administered. However, a disturbance in the hypothalamic-pituitary axis should still be detected due to the marked decrease in T_4 and fT_4 and their influence on negative feedback regulation of the pituitary. Analysis of the TSH:T_4 and TSH:fT_4 ratios both demonstrate an increase over time, suggesting that the average decrease in T_4 concentration was leading to increased TSH secretion while dogs were given clomipramine (Figures 12 and 13). It is important to note, though, that comparisons to other animals with similar reductions in T_4 and fT_4 would have to be made to ensure that these decreases are not due to the decrease in concentrations of T_4 or fT_4 alone. Furthermore, variations in secretion of TRH, and subsequently TSH, may also be due to clomipramine's inhibition of neurotransmitter reuptake at the synaptic cleft.[126,127] Studies in different species have demonstrated variable effects of serotonin on the HPT axis.[18] In rats, serotonin inhibited TRH secretion, while in monkeys it stimulated TSH secretion.[29] People administered clomipramine had a decrease in serum TSH concentrations.[96,128,129] Neurochemical regulation of the HPT axis has yet to be characterized in the dog, and any effect of TCAs such as clomipramine on TRH and TSH secretion via monoaminergic control could not be determined from these data, as that was beyond the scope of this study.

The significant decreases in serum concentrations of T_4, fT_4, and rT_3 during clomipramine administration were rapid in onset and consistent throughout the duration of treatment. The lack of a placebo-treated control group in the study detracts from the results because variables other than the effect of clomipramine treatment could have influenced hormone concentrations over time. While this study did not contain a placebo-controlled group, it is unlikely the data would have varied substantially in the treatment group, as all subjects demonstrated a decrease in T_4 and fT_4 serum concentrations. This variation would be highly unlikely if left to chance or outside influence, as different subjects would have responded differently to various factors. In addition, all dogs were allowed to acclimate for 3 weeks prior to initiation of the study, and TSH and TRH stimulation tests done on day –7 and 0, respectively, confirmed euthyroid status. All subjects were housed in strictly controlled conditions, with 12-hour light cycles, temperature, and nutritional requirements closely monitored, further decreasing the likelihood of external variability on thyroid status.

Thyroid hormone concentrations are known to fluctuate in individual dogs.[130] However, multiple studies over periods of 8 weeks or more using similar husbandry conditions have documented little variation in the mean serum T_4 and T_3 concentrations, although fT_4 might be more variable.[46,50,131] Similar study designs have been used in multiple studies that have evaluated the effects of drugs on thyroid function, particularly when the study duration is prolonged.[5,6,79] The prolonged duration of treatment in the present study was necessary, as TCA's effect on the monoaminergic systems (serotonin, norepinephrine, dopamine) via reuptake inhibition of neurotransmitters at the synaptic cleft[126,127] may work by modifying receptor site sensitivity rather than by direct action at the receptor site, thereby taking weeks to modulate their effect.[132-135] This can be supported by clinical evidence in people where beneficial effects attributable to treatment require four to six weeks.[136,137] Furthermore, negative feedback of the monoaminergic system with resultant upregulation at the level of the synapse is a prolonged process, taking weeks to reach steady state even though high plasma levels are achieved.[132,137] Power analysis during design of

the study revealed that a minimum of 12 dogs was required to ensure a type 2 error of 90% or less. In order to conserve resources because of the length of the study, all dogs were treated with clomipramine.

The clinical importance of the decrease in T_4 and fT_4 with prolonged administration of clomipramine lies in the interference with a diagnosis of hypothyroidism, the potential for hypothyroidism to be induced, and potential effects on the therapeutic response to clomipramine. The reduction of serum T_4 and fT_4 concentrations from baseline values by 35% and 38% respectively, could easily lead to a misdiagnosis of spontaneous hypothyroidism, and thyroid function should therefore be evaluated cautiously in dogs receiving clomipramine.

While the mean concentrations of T_4 and fT_4 during clomipramine treatment were in the normal range for most laboratories, our results suggest that clinical hypothyroidism could develop in some circumstances. Dogs with subclinical thyroid dysfunction, those receiving other drugs that suppress thyroid function, and those with non-thyroidal illness could be at risk for developing hypothyroidism during clomipramine administration. Although clinical evidence of hypothyroidism was not found in the present study, subclinical hypothyroidism may have existed. In addition, treatment of longer duration could result in more pronounced effects on thyroid function, and the results of this study need to be confirmed in a larger, placebo-controlled population or clinical trial.

As behavioral changes have been reported in hypothyroid dogs, administration of a TCA such as clomipramine that has the potential to cause hypothyroidism may exacerbate behavioral signs or reduce the positive behavioral response to that drug. These phenomena have been investigated in people, as a decrease in thyroid function has been reported to be associated with depression, and the supplementation of thyroxine or T_3 as an adjunct to refractory depression has been shown to be beneficial.[7] Although this has not been demonstrated in the dog, and the authors do not recommend that thyroxine supplementation be administered in refractory cases, it may be prudent to monitor thyroid function tests in addition to complete physical exam, CBC and serum chemistry profile, prior to and during clomipramine administration to ensure hypothyroidism is not misdiagnosed, or does not subsequently occur.

CHAPTER 3

CONCLUSION

Overall, the objectives of this project were met, documenting the effects of clomipramine on canine thyroid function. Clomipramine administration was accompanied by a 35% decrease in T_4 and 38% decrease in fT_4 concentrations. Several mechanisms probably account for the clomipramine-induced depression of thyroid function; binding of iodide and inhibition of TPO may both prevent T_4 synthesis, and TCAs also influence T_4 metabolism. Although no significant change in basal serum TSH concentration or TSH response to TRH administration was noted, this could have been due to actual suppression of TSH by clomipramine, or assay insensitivity. Subsequent *in vivo* studies using medical grade TRH would be prudent, as would *in vitro* studies of canine thyrotrope response to clomipramine. The variation noted in T_3 concentrations may be due to both thyroidal and extra-thyroidal metabolism, but both mechanisms are unlikely as there was no concurrent increase in TSH concentration. Alterations in alternative pathways of T_3 metabolism, such as sulfation, may play a role, and future studies of these pathways are indicated.

Systematic evaluation of the canine HPT axis may elucidate the various mechanisms by which clomipramine reduces serum thyroid hormone concentrations. In addition to *in vitro* studies of canine thyroid glands perfused with clomipramine, *in vivo* studies comparing treated groups to thyroidectomized controls may reveal clomipramine's effect on secretion and metabolism. Other studies should investigate the influence of clomipramine on thyroid hormone binding proteins by adding the medication to normal canine serum samples and subsequently evaluating hormone concentrations. Tissue metabolism should be evaluated via measurement of thyroxine, triiodothyronine and deiodinase concentrations in various peripheral tissues, including specific locations in the canine brain.

LITERATURE CITED

1. Daminet S, Paradis M, Refsal KR, et al. Short-term influence of prednisone and phenobarbital on thyroid function in euthyroid dogs. *Can Vet J* 1999;40:411-5.
2. Gaskill CL, Burton SA, Gelens HC, et al. Effects of phenobarbital treatment on serum thyroxine and thyroid- stimulating hormone concentrations in epileptic dogs. *J Am Vet Med Assoc* 1999;215:489-96.
3. Hall IA, Campbell KL, Chambers MD, et al. Effect of trimethoprim/sulfamethoxazole on thyroid function in dogs with pyoderma. *J Am Vet Med Assoc* 1993;202:1959-62.
4. Kantrowitz LB, Peterson ME, Trepanier LA, et al. Serum total thyroxine, total triiodothyronine, free thyroxine, and thyrotropin concentrations in epileptic dogs treated with anticonvulsants. *J Am Vet Med Assoc* 1999;214:1804-8.
5. Muller PB, Wolfsheimer KJ, Taboada J, et al. Effects of long-term phenobarbital treatment on the thyroid and adrenal axis and adrenal function tests in dogs. *J Vet Intern Med* 2000;14:157-64.
6. Torres SM, McKeever PJ, Johnston SD. Effect of oral administration of prednisolone on thyroid function in dogs. *Am J Vet Res* 1991;52:416-21.
7. Earle BV. Thyroid hormone and tricyclic antidepressants in resistant depressions. *Am J Psychiatry* 1970;126:1667-9.
8. Kusalic M, Engelsmann F, Bradwejn J. Thyroid functioning during treatment for depression. *J Psychiatry Neurosci* 1993;18:260-3.
9. Martin P, Massol J, Belon JP, et al. Thyroid function and reversal by antidepressant drugs of depressive- like behavior (escape deficits) in rats. *Neuropsychobiology* 1987;18:21-6.
10. Massol J, Martin P, Chatelain F, et al. Tricyclic antidepressants, thyroid function, and their relationship with the behavioral responses in rats. *Biol Psychiatry* 1990;28:967-78.
11. Dumas ML, Pons F, Cadilhac J. Histochemical data on the reuptake blockade of monoamines by chlomipramine (author's transl)]. *Ann Histochim* 1975;20:283-90.
12. Peters MDd, Davis SK, Austin LS. Clomipramine: an antiobsessional tricyclic antidepressant. *Clin Pharm* 1990;9:165-78.
13. Hewson CJ, Conlon PD, Luescher UA, et al. The pharmacokinetics of clomipramine and desmethylclomipramine in dogs: parameter estimates following a single oral dose and 28 consecutive daily oral doses of clomipramine. *J Vet Pharmacol Ther* 1998;21:214-22.
14. Martensson B, Wagner A, Beck O, et al. Effects of clomipramine treatment on cerebrospinal fluid monoamine metabolites and platelet 3H-imipramine binding and serotonin uptake and concentration in major depressive disorder. *Acta Psychiatr Scand* 1991;83:125-33.
15. Noguchi T, Shimoda K, Takahashi S. Clinical significance of plasma levels of clomipramine, its hydroxylated and desmethylated metabolites: prediction of clinical outcome in mood disorders using discriminant analysis of therapeutic drug monitoring data. *J Affect Disord* 1993;29:267-79.
16. Buxeraud J, Absil AC, Raby C. Secondary antithyroid action of drugs in relation to structure. *J Pharm Sci* 1984;73:1687-90.
17. Rousseau A, Comby F, Buxeraud J, et al. Spectroscopic analysis of charge transfer complex formation and peroxidase inhibition with tricyclic antidepressant drugs: potential anti-thyroid action. *Biol Pharm Bull* 1996;19:726-8.
18. Sauvage MF, Marquet P, Rousseau A, et al. Relationship between psychotropic drugs and thyroid function: a review. *Toxicol Appl Pharmacol* 1998;149:127-35.
19. Sokolov ST, Levitt AJ, Joffe RT. Thyroid hormone levels before unsuccessful antidepressant therapy are associated with later response to T3 augmentation. *Psychiatry Res* 1997;69:203-6.
20. Wilson IC, Prange AJ, Jr., McClane TK, et al. Thyroid-hormone enhancement of imipramine in nonretarded depressions. *N Engl J Med* 1970;282:1063-7.
21. Reinhard D. Aggressive behavior associated with hypothyroidism. *Canine Practice* 1978;5:69.
22. Dodman NH, Mertens PA, Aronson LP. Animal behavior case of the month. Dogs were evaluated because of aggression. *J Am Vet Med Assoc* 1995;207:1168-71.
23. Belshaw BE, Barandes M, Becker DV, et al. A model of iodine kinetics in the dog. *Endocrinology* 1974;95:1078-93.

24. Laurberg P. Iodothyronine release from the perfused canine thyroid. *Acta Endocrinol Suppl* 1980;236:1-50.
25. Panciera DL. Canine Hypothyroidism Part 1: Clinical Findings and Control of Thyroid Hormone Secretion and Metabolism. *The Compendium* 1990;12:689-701.
26. Hirooka Y, Hollander CS, Suzuki S, et al. Somatostatin inhibits release of thyrotropin releasing factor from organ cultures of rat hypothalamus. *Proc Natl Acad Sci U S A* 1978;75:4509-13.
27. Morley JE. Neuroendocrine control of thyrotropin secretion. *Endocr Rev* 1981;2:396-436.
28. Jackson IM. Thyrotropin-releasing hormone. *N Engl J Med* 1982;306:145-55.
29. Morley JE, Brammer GL, Sharp B, et al. Neurotransmitter control of hypothalamic-pituitary-thyroid function in rats. *Eur J Pharmacol* 1981;70:263-71.
30. Larsson M, Pettersson T. Purification and partial characterization of thyroid hormone binding proteins in canine serum. *Domest Anim Endocrinol* 1987;4:215-29.
31. Davis PJ, Handwerger BS. Thyroid hormone binding in dog plasma. *Endocrinology* 1973;93:1445-8.
32. Furth ED, Becker DV, Nunez EA, et al. Thyroxine metabolism in the dog. *Endocrinology* 1968;82:976-82.
33. Engler D, Burger AG. The deiodination of the iodothyronines and of their derivatives in man. *Endocr Rev* 1984;5:151-84.
34. Wu SY. Thyrotropin-mediated induction of thyroidal iodothyronine monodeiodinases in the dog. *Endocrinology* 1983;112:417-24.
35. Curran PG, DeGroot LJ. The effect of hepatic enzyme-inducing drugs on thyroid hormones and the thyroid gland. *Endocr Rev* 1991;12:135-50.
36. Leonard JL, Koehrle J. Intracellular pathways of iodothyronine metabolism In: L. E. Braverman and R. D. Utiger, eds. *Werner & Ingbar's the thyroid: a fundamental and clinical text*. 8th ed. Philadelphia: Lippincott Williams & Wilkins, 2000;1081.
37. Campos-Barros A, Hoell T, Musa A, et al. Phenolic and tyrosyl ring iodothyronine deiodination and thyroid hormone concentrations in the human central nervous system. *J Clin Endocrinol Metab* 1996;81:2179-85.
38. Guadano-Ferraz A, Obregon MJ, St Germain DL, et al. The type 2 iodothyronine deiodinase is expressed primarily in glial cells in the neonatal rat brain. *Proc Natl Acad Sci U S A* 1997;94:10391-6.
39. Esfandiari A, Courtin F, Lennon AM, et al. Induction of type III deiodinase activity in astroglial cells by thyroid hormones. *Endocrinology* 1992;131:1682-8.
40. Laurberg P, Boye N. Propylthiouracil, ipodate, dexamethasone and periods of fasting induce different variations in serum rT3 in dogs. *Metabolism* 1984;33:323-5.
41. Woltz HH, Thompson FN, Kemppainen RJ, et al. Effect of prednisone on thyroid gland morphology and plasma thyroxine and triiodothyronine concentrations in the dog. *Am J Vet Res* 1983;44:2000-3.
42. Kemppainen RJ, Thompson FN, Lorenz MD, et al. Effects of prednisone on thyroid and gonadal endocrine function in dogs. *J Endocrinol* 1983;96:293-302.
43. Moore GE, Ferguson DC, Hoenig M. Effects of oral administration of anti-inflammatory doses of prednisone on thyroid hormone response to thyrotropin-releasing hormone and thyrotropin in clinically normal dogs. *Am J Vet Res* 1993;54:130-5.
44. Kaptein EM, Moore GE, Ferguson DC, et al. Effects of prednisone on thyroxine and 3,5,3'-triiodothyronine metabolism in normal dogs. *Endocrinology* 1992;130:1669-79.
45. Graham PA, Refsal KR, Nachreiner RF. Oral prednisone did not suppress serum thyrotropin in radiothyroidectomized beagles. Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine 1998;732.
46. Panciera DL, MacEwen EG, Atkins CE, et al. Thyroid function tests in euthyroid dogs treated with L-thyroxine. *Am J Vet Res* 1990;51:22-6.
47. Martino E, Bartalena L, Bogazzi F, et al. The effects of amiodarone on the thyroid. *Endocr Rev* 2001;22:240-54.
48. Surks MI, Sievert R. Drugs and thyroid function. *N Engl J Med* 1995;333:1688-94.
49. Torres SM, McKeever PJ, Johnston SD. Hypothyroidism in a dog associated with trimethoprim-sulphadiazine therapy. *Vet Dermatol* 1996;7:105-8.

50. Panciera DL, Post K. Effect of oral administration of sulfadiazine and trimethoprim in combination on thyroid function in dogs. *Can J Vet Res* 1992;56:349-52.
51. Campbell KL, Chambers MD, Davis CA. Effects of trimethoprim/sulfamethoxazole on thyroid physiology in dogs. Congress of the American College of Veterinary Dermatology 1995;55-56.
52. Campbell KL, Nachreiner RF, Schaeffer DJ, et al. Effects of trimethoprim/sulfamethoxazole on endogenous thyroid stimulating hormone concentration in dogs. World Congress of Veterinary Dermatology 1996;29.
53. Gookin JL, Trepanier LA, Bunch SE. Clinical hypothyroidism associated with trimethoprim-sulfadiazine administration in a dog. *J Am Vet Med Assoc* 1999;214:1028-31, 1021.
54. Liu PM, Zhang XM, Wu W, et al. Comparison of cardiac electrophysiologic effects of experimental hypothyroidism and chronic oral amiodarone administration in dogs. *Chin Med J (Engl)* 1994;107:375-9.
55. Laurberg P. Amiodarone inhibits T4 and T3 secretion but does not affect T4 deiodination to T3 in perfused dog thyroid lobes. *Thyroidology* 1988:1-4.
56. Velicky J, Titlbach M, Lojda Z, et al. Long-term action of potassium bromide on the rat thyroid gland. *Acta Histochem* 1998;100:11-23.
57. Paull LC, Scott-Moncrieff JC, DeNicola DB, et al. Effect of potassium bromide (KBr) at anticonvulsant dosages on thyroid function and morphology in dogs. Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine 2000;753.
58. Werner SC, Platman SR. Remission of hyperthyroidism (Graves' disease) and altered pattern of serum-thyroxine binding induced by prednisone. *Lancet* 1965;2:751-5.
59. Ferguson DC, Peterson ME. Serum free and total iodothyronine concentrations in dogs with hyperadrenocorticism. *Am J Vet Res* 1992;53:1636-40.
60. Braga M, Cooper DS. Clinical review 129: Oral cholecystographic agents and the thyroid. *J Clin Endocrinol Metab* 2001;86:1853-60.
61. Larsen PR. Salicylate-induced increases in free triiodothyronine in human serum. Evidence of inhibition of triiodothyronine binding to thyroxine-binding globulin and thyroxine-binding prealbumin. *J Clin Invest* 1972;51:1125-34.
62. McConnell RJ. Changes in thyroid function tests during short-term salsalate use. *Metabolism* 1999;48:501-3.
63. Bishnoi A, Carlson HE, Gruber BL, et al. Effects of commonly prescribed nonsteroidal anti-inflammatory drugs on thyroid hormone measurements. *Am J Med* 1994;96:235-8.
64. Carlson HE, Kaell AT, Schulman PE, et al. Effects of several nonsteroidal antiinflammatory drugs on thyroid function tests. *J Rheumatol* 1999;26:1855-6.
65. Ferguson DC, Moore GE, Hoenig M. Carprofen lowers total T4 and TSH, but not free T4 concentrations in dogs. Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine 1999;709.
66. Ferguson DC. Influence of common drugs on the free thyroxine fraction in canine serum. Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine 1989.
67. Stockigt JR, Lim CF, Barlow JW, et al. Interaction of furosemide with serum thyroxine-binding sites: in vivo and in vitro studies and comparison with other inhibitors. *J Clin Endocrinol Metab* 1985;60:1025-31.
68. Stockigt JR, Topliss DJ. Assessment of thyroid function during high-dosage furosemide therapy. *Arch Intern Med* 1989;149:973.
69. Newnham HH, Hamblin PS, Long F, et al. Effect of oral frusemide on diagnostic indices of thyroid function. *Clin Endocrinol (Oxf)* 1987;26:423-31.
70. Enbring NH, Engstrom WW. Effects of estrogen and testosterone on circulating thyroid hormone. *J Clin Endocrinol Metab* 1959;19:783-796.
71. Morris DD, Garcia MC. Effects of phenylbutazone and anabolic steroids on adrenal and thyroid gland function tests in healthy horses. *Am J Vet Res* 1985;46:359-64.
72. Carr AP, Panciera DL. Hemostatic and endocrine effects of stanazolol on healthy dogs. Annual Veterinary Medical Forum of the American College of Veterinary Internal Medicine 1997.
73. Mendel CM, Frost PH, Kunitake ST, et al. Mechanism of the heparin-induced increase in the concentration of free thyroxine in plasma. *J Clin Endocrinol Metab* 1987;65:1259-64.

74. Mendel CM, Frost PH, Cavalieri RR. Effect of free fatty acids on the concentration of free thyroxine in human serum: the role of albumin. *J Clin Endocrinol Metab* 1986;63:1394-9.
75. Hollander CS, Scott RL, Burgess JA, et al. Free fatty acids: a possible regulator of free thyroid hormone levels in man. *J Clin Endocrinol Metab* 1967;27:1219-23.
76. Goldstein JA, Taurog A. Enhanced biliary excretion of thyroxine glucuronide in rats pretreated with benzpyrene. *Biochem Pharmacol* 1968;17:1049-65.
77. Oppenheimer JH, Bernstein G, Surks MI. Increased thyroxine turnover and thyroidal function after stimulation of hepatocellular binding of thyroxine by phenobarbital. *J Clin Invest* 1968;47:1399-406.
78. Cavalieri RR, Pitt-Rivers R. The effects of drugs on the distribution and metabolism of thyroid hormones. *Pharmacol Rev* 1981;33:55-80.
79. Gaskill CL, Burton SA, Gelens HC, et al. Changes in serum thyroxine and thyroid-stimulating hormone concentrations in epileptic dogs receiving phenobarbital for one year. *J Vet Pharmacol Ther* 2000;23:243-9.
80. Gieger TL, Hosgood G, Taboada J, et al. Thyroid function and serum hepatic enzyme activity in dogs after phenobarbital administration. *J Vet Intern Med* 2000;14:277-81.
81. Murray LA, Peterson ME. Ipodate treatment of hyperthyroidism in cats. *J Am Vet Med Assoc* 1997;211:63-7.
82. Beddows SA, Page SR, Taylor AH, et al. Cytotoxic effects of amiodarone and desethylamiodarone on human thyrocytes. *Biochem Pharmacol* 1989;38:4397-403.
83. Laurberg P. The effect of some iodine-containing radiocontrast agents on iodothyronine secretion from the perfused canine thyroid. *Endocrinology* 1982;111:1904-8.
84. Center SA, Mitchell J, Nachreiner RF, et al. Effects of propranolol on thyroid function in the dog. *Am J Vet Res* 1984;45:109-11.
85. Chopra IJ, Williams DE, Orgiazzi J, et al. Opposite effects of dexamethasone on serum concentrations of 3,3',5'- triiodothyronine (reverse T3) and 3,3'5-triiodothyronine (T3). *J Clin Endocrinol Metab* 1975;41:911-20.
86. Tuomisto J, Ranta T, Mannisto P, et al. Neurotransmitter control of thyrotropin secretion in the rat. *Eur J Pharmacol* 1975;30:221-9.
87. Mason GA, Bondy SC, Nemeroff CB, et al. The effects of thyroid state on beta-adrenergic and serotonergic receptors in rat brain. *Psychoneuroendocrinology* 1987;12:261-70.
88. Atterwill CK, Catto LC, Heal DJ, et al. The effects of desipramine (DMI) and electroconvulsive shock (ECS) on the function of the hypothalamo-pituitary-thyroid axis in the rat. *Psychoneuroendocrinology* 1989;14:339-46.
89. Jackson IM, Luo LG. Antidepressants inhibit the glucocorticoid stimulation of thyrotropin releasing hormone expression in cultured hypothalamic neurons. *J Investig Med* 1998;46:470-4.
90. Kadono Y, Kaneda H, Maeda K. Effects of antidepressants on thyroid stimulating hormone release in rats under ether stress. *Psychiatry Clin Neurosci* 1995;49:231-6.
91. Gershon MD, Nunez EA. Uptake of biogenic amines by thyroid glands. *Mol Cell Endocrinol* 1976;5:169-80.
92. Campos-Barros A, Meinhold H, Stula M, et al. The influence of desipramine on thyroid hormone metabolism in rat brain. *J Pharmacol Exp Ther* 1994;268:1143-52.
93. Campos-Barros A, Meinhold H, Kohler R, et al. The effects of desipramine on thyroid hormone concentrations in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* 1995;351:469-74.
94. Melander A, Westgren U, Ericson LE, et al. Influence of the sympathetic nervous system on the secretion and metabolism of thyroid hormone. *Endocrinology* 1977;101:1228-37.
95. Fischetti B. Pharmacological influences on thyroid activity. *Arch Ital Sch Pharmacol* 1962;12:33-109.
96. Schlienger JL, Kapfer MT, Singer L, et al. The action of clomipramine on thyroid function. *Horm Metab Res* 1980;12:481-2.
97. Rousseau A, Marquet P, Lagorce JF, et al. Thyroid accumulation and adverse effects of imipramine and desipramine in rats after long-term administration. *Pharmacology* 1998;57:242-8.
98. Kennedy JA, Jarrett DB, Wellby ML. Influence of imipramine on the hypothalamic/pituitary/thyroid axis of the rat. *Metabolism* 1997;46:1429-34.

99. Haggerty JJ, Marquardt MM, McAllister N. Depressive symptomatology in medical patients with antithyroid antibodies. *Biol Psychiatry* 1991;29:153A.
100. Linnoila M, Gold P, Potter WZ, et al. Tricyclic antidepressants do not alter thyroid hormone levels in patients suffering from a major affective disorder. *Psychiatry Res* 1981;4:357-60.
101. Nordgren L, von Scheele C. Nortriptyline and pituitary-thyroid function in affective disorder. *Pharmacopsychiatria* 1981;14:61-5.
102. Baumgartner A, Graf KJ, Kurten I, et al. The hypothalamic-pituitary-thyroid axis in psychiatric patients and healthy subjects: Parts 1-4. *Psychiatry Res* 1988;24:271-332.
103. Joffe RT, Singer W. A comparison of triiodothyronine and thyroxine in the potentiation of tricyclic antidepressants. *Psychiatry Res* 1990;32:241-51.
104. Hoflich G, Kasper S, Danos P, et al. Thyroid hormones, body temperature, and antidepressant treatment. *Biol Psychiatry* 1992;31:859-62.
105. Shelton RC, Winn S, Ekhatore N, et al. The effects of antidepressants on the thyroid axis in depression. *Biol Psychiatry* 1993;33:120-6.
106. Brady KT, Lydiard RB, Kellner CH, et al. A comparison of the effects of imipramine and fluvoxamine on the thyroid axis. *Biol Psychiatry* 1994;36:778-9.
107. Duval F, Mokrani MC, Crocq MA, et al. Effect of antidepressant medication on morning and evening thyroid function tests during a major depressive episode. *Arch Gen Psychiatry* 1996;53:833-40.
108. Rao ML, Ruhrmann S, Retey B, et al. Low plasma thyroid indices of depressed patients are attenuated by antidepressant drugs and influence treatment outcome. *Pharmacopsychiatry* 1996;29:180-6.
109. Balon R, Pohl R, Yeragani VK, et al. The changes of thyroid hormone during pharmacological treatment of panic disorder patients. *Prog Neuropsychopharmacol Biol Psychiatry* 1991;15:595-600.
110. Baumgartner A, Dubeyko M, Campos-Barros A, et al. Subchronic administration of fluoxetine to rats affects triiodothyronine production and deiodination in regions of the cortex and in the limbic forebrain. *Brain Res* 1994;635:68-74.
111. King JN, Maurer MP, Hotz RP, et al. Pharmacokinetics of clomipramine in dogs following single-dose intravenous and oral administration. *Am J Vet Res* 2000;61:74-9.
112. Novartis Pharma A. Committee for Veterinary Medicinal Products - European Public Assessment Report: Clomicalm®. London: European Agency for the Evaluation of Medicinal Products - Veterinary Medicines Evaluation Unit, 1998.
113. Novartis Animal Health US I. Freedom of Information Summary (NADA 141-120). Greensboro, NC: Novartis Animal Health US, Inc., 1998.
114. Simpson B. Treatment of separation-related anxiety in dogs with clomipramine: results from a multicentre, blinded, placebo controlled clinical study. First Int Conf Vet Behav Med 1997;143-154.
115. Podberscek AL, Hsu Y, Serpell JA. Evaluation of clomipramine as an adjunct to behavioural therapy in the treatment of separation-related problems in dogs. *Vet Rec* 1999;145:365-9.
116. White MM, Neilson JC, Hart BL, et al. Effects of clomipramine hydrochloride on dominance-related aggression in dogs. *J Am Vet Med Assoc* 1999;215:1288-91.
117. Goldenthal EI. Six-Month Oral Toxicity Study in Beagle Dogs with Clomipramine Hydrochloride Tablets. Mattawan, MI: MPI Research, 1998.
118. Noel P, Mawdesley-Thomas LE, Cozens D, et al. Twelve-Month Oral Toxicity Study in Pembrokeshire Corgis with Clomipramine Hydrochloride Tablets. Huntingdon, England: Huntingdon Research Centre, 1998.
119. Richard S, Champeroux P, Martel E. Evaluation of an Arrhythmogenic Effect in Eight Conscious Beagle Dogs During a 7-Day Period of Treatment by the Oral Route. Baugy, France: Centre De Recherches Biologiques, 1998.
120. Ferguson DC. Update on diagnosis of canine hypothyroidism. *Vet Clin North Am Small Anim Pract* 1994;24:515-39.
121. Scott-Moncrieff JC, Nelson RW. Change in serum thyroid-stimulating hormone concentration in response to administration of thyrotropin-releasing hormone to healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *J Am Vet Med Assoc* 1998;213:1435-8.

122. Scott-Moncrieff JC, Nelson RW, Bruner JM, et al. Comparison of serum concentrations of thyroid-stimulating hormone in healthy dogs, hypothyroid dogs, and euthyroid dogs with concurrent disease. *J Am Vet Med Assoc* 1998;212:387-91.
123. Lagorce JF, Thomes JC, Catanzano G, et al. Formation of molecular iodine during oxidation of iodide by the peroxidase/H2O2 system. Implications for antithyroid therapy. *Biochem Pharmacol* 1991;42 Suppl:S89-92.
124. King JN, Maurer MP, Altmann BO, et al. Pharmacokinetics of clomipramine in dogs following single-dose and repeated-dose oral administration. *Am J Vet Res* 2000;61:80-5.
125. Meij BP, Mol JA, Rijnberk A. Thyroid-stimulating hormone responses after single administration of thyrotropin-releasing hormone and combined administration of four hypothalamic releasing hormones in beagle dogs. *Domest Anim Endocrinol* 1996;13:465-8.
126. Langer G, Resch F, Aschauer H, et al. TSH-response patterns to TRH stimulation may indicate therapeutic mechanisms of antidepressant and neuroleptic drugs. *Neuropsychobiology* 1984;11:211-8.
127. Rang HP. *Pharmacology*. 4th ed. Edinburgh ; New York: Churchill Livingstone, 1999.
128. Brady KT, Anton RF. The thyroid axis and desipramine treatment in depression. *Biol Psychiatry* 1989;25:703-9.
129. Prange AJ, Jr. Psychotropic drugs and the thyroid axis: a review of interactions. *Adv Biochem Psychopharmacol* 1985;40:103-10.
130. Miller AB, Nelson RW, Scott-Moncrieff JC, et al. Serial thyroid hormone concentrations in healthy euthyroid dogs, dogs with hypothyroidism, and euthyroid dogs with atopic dermatitis. *Br Vet J* 1992;148:451-8.
131. Panciera DL, Johnson GS. Plasma von Willebrand factor antigen concentration in dogs with hypothyroidism [see comments]. *J Am Vet Med Assoc* 1994;205:1550-3.
132. de Montigny C, Agajanian GK. Tricyclic Antidepressants: long term treatment increases responsivity of rat forebrain neurons to serotonin. *Science* 1978;202:1303-1306.
133. Blier P, de Montigny C, Chaput Y. Modifications of the serotonin system by antidepressant treatments: implications for the therapeutic response in major depression. *J Clin Psychopharmacol* 1987;7:24S-35S.
134. Dodman NH, Shuster L. *Psychopharmacology of animal behavior disorders*. Malden, Mass.: Blackwell Science, 1998.
135. W.B. Saunders Company. *Veterinary clinics of North America - Small Animal Practice*. Philadelphia, PA: W.B. Saunders Co., 1997.
136. McTavish D, Benfield P. Clomipramine. An overview of its pharmacological properties and a review of its therapeutic use in obsessive compulsive disorder and panic disorder. *Drugs* 1990;39:136-53.
137. Mårtensson E, Axelsson R, Nyberg G. Pharmacokinetic properties of the antidepressant drugs amitriptyline, clomipramine, and imipramine: A clinical study. *Curr Ther Res* 1984;36:228.

APPENDIX

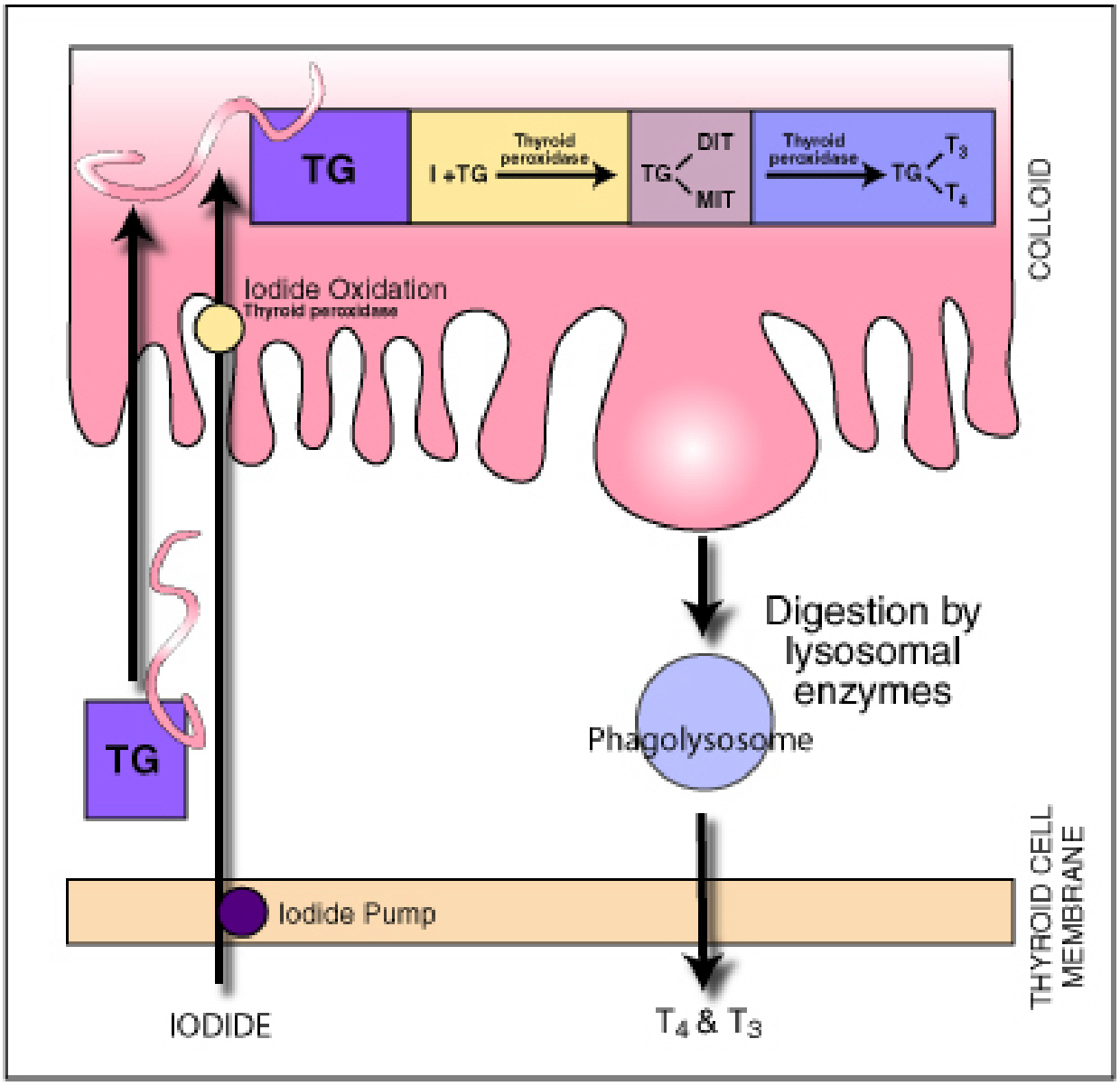

Figure 1: Thyroid Hormone Synthesis – Iodide is taken up from plasma by the iodide pump on the basal cell membrane of the thyroid follicle cell. Iodide traverses the cell and is oxidized to iodine via thyroid peroxidase at the apical cell membrane. Iodine is bound to thyroglobulin (TG) via thyroid peroxidase to form mono-iodotyrosine (MIT) and di-iodotyrosine (DIT). These compounds are coupled by thyroid peroxidase to form thyroxine (DIT + DIT = T_4) and triiodothyronine (MIT + DIT = T_3) and stored in the colloid. When secretion is stimulated, endocytosis occurs and a droplet of colloid enters the cell where it fuses with a lysosome to form a phagolysosome. Thyroglobulin then is digested and T_4 and T_3 are released and are secreted via exocytosis.

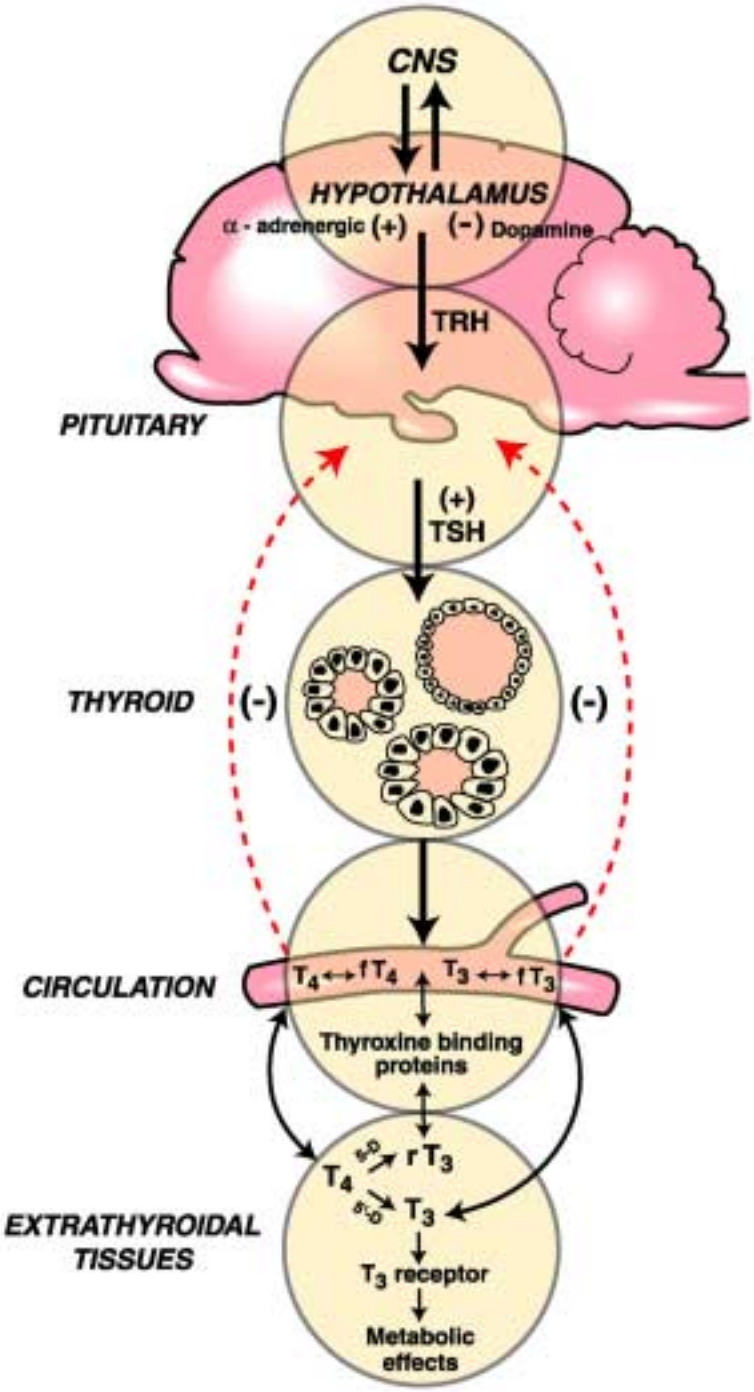

Figure 2: Hypothalamic-Pituitary-Thyroid Axis – Thyrotropin-releasing hormone (TRH) is secreted from the hypothalamus and stimulates secretion of thyroid-stimulating hormone (TSH) from the anterior pituitary. TSH stimulates the formation and release of T_4 from thyrotropes. TSH secretion is controlled by direct negative feedback of circulating thyroid hormones via TRH modulation. Once in the plasma, thyroid hormones are bound by plasma proteins, while only the unbound portion leaves the circulation to exert their effects and undergo metabolism (via 5- and 5'-deiodinase).

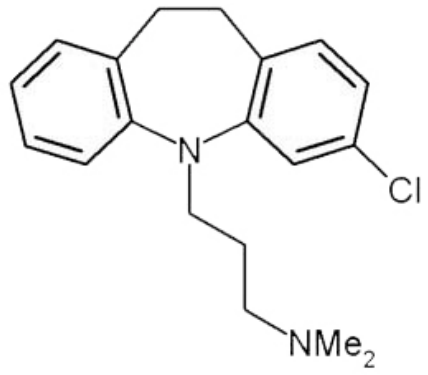

Figure 3: Clomipramine (Chemical Structure)

Table 1: A partial list of medications (classified according to mechanism of action) that affect thyroid hormones in people and dogs (in *italics*).

Mechanism of Action	Medication
Decreased TSH secretion	dopamine, *glucocorticoids*, cyproheptadine, octreotide, *sulfonamides*, *thyroxine supplementation*, *tricyclic antidepressants*
Altered thyroid hormone synthesis or secretion	Increased secretion: *amiodarone*, *iodide*
	Decreased secretion: lithium, *amiodarone*, *iodide*, calcium channel blockers, thioamides, aminoglutethimide, *tricyclic antidepressants*, *radiocontrast agents (e.g. ipodate)*
Decreased T_4 absorption (patients on supplementation)	aluminum hydroxide, ferrous sulfate, sucralfate
Altered serum transport via thyroxine-binding globulin (TBG)	Increased TBG concentration: estrogens, tamoxifen, mitotane
	Decreased TBG concentration: *androgens*, *glucocorticoids*, L-asparaginase
Displacement from plasma binding proteins	*furosemide*, *NSAIDs (e.g. salicylates)*, phenylbutazone, *heparin*, *radiocontrast agents (e.g. ipodate)*,
Increased hepatic metabolism	*phenobarbital*, rifampin, phenytoin, carbamazepine, mitotane
Decreased 5'-deiodinase ($T_4 \rightarrow T_3$) activity	propylthiouracil, *amiodarone*, *propranolol*, *glucocorticoids*, *propanolol*, *radiocontrast agents (e.g. ipodate)*, *tricyclic antidepressants*

Table 2: Complete blood count and body weight on Day –7 for each dog; abnormalities indicated by yellow, and red indicates animal removed from the study.

DOG #	096	164	181	192	194	205	217	241	242	248	251	253	254	910	961	Avg	Normal
Weight (kg)	25.0	19.1	23.7	25.7	22.0	23.3	19.5	16.7	16.0	31.5	32.0	19.8	28.8	25.0	22.3	**24**	**NA**
RBC ($\times 10^6$)	7.20	6.53	7.41	7.26	6.65	8.85	6.34	7.16	7.46	7.59	6.85	7.04	7.02	7.96	7.70	**7**	**5.5 - 8.6**
HgB (gm/dl)	17.1	15.1	18.0	16.5	16.7	18.3	15.1	17.5	18.0	16.3	17.3	18.0	17.4	17.2	17.9	**17**	**13.0 - 20.1**
HCT (%)	47.2	43.2	50.7	46.8	46.4	52.3	43.1	48.7	50.1	46.6	47.4	49.0	48.0	49.7	51.6	**48**	**37.3 - 62.0**
MCV	65.6	66.2	68.4	64.5	69.8	59.1	68.0	68.0	67.2	61.4	69.2	69.6	68.4	62.4	67.0	**66**	**58.4 - 83.0**
MCH	23.8	23.1	24.3	22.7	25.1	20.7	23.8	24.4	24.1	21.5	25.3	25.6	24.8	21.6	23.2	**24**	**22.2 - 26.2**
MCHC	36.2	35.0	35.5	35.3	36.0	35.0	35.0	35.9	35.9	35.0	36.5	36.7	36.3	34.6	34.7	**36**	**31.6 - 36.5**
nRBC	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	**0**	**/100 WBC**
Reticulocytes	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	
WBC ($\times 10^3$)	13.6	7.4	16.2	11.6	8.6	8.6	10.7	13.1	7.921	12.0	24.3	10.3	13.0	7.70	6.20	**11**	**5.4 - 16.6**
Segs	6.664	3.922	9.234	7.076	5.246	4.730	5.136	0	3.802	6.720	16.524	5.562	7.150	3.388	3.038	**6**	**3.24 - 10.7**
Bands	0	0	0	0	0	0	0	2.751	0	0	0	0	0	0	0	**0**	**0-0.25**
Lymphs	4.080	2.664	5.346	3.480	2.408	2.666	2.889	0.655	3.406	4.200	3.645	3.193	4.030	3.388	1.860	**3**	**0.75 - 5.65**
Monos	0.408	0.666	0	0.464	0.172	0.344	0.535	0.655	0.396	0.840	1.944	0.618	0.650	0.616	0.372	**1**	**0-1.11**
Eos	2.448	0.148	1.620	0.580	0.774	0.860	2.140	0	0.317	0.240	2.187	0.927	1.170	0.308	0.930	**1**	**0.36 - 2.37**
Baso	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**0**	**0 - 0.19**
Platelets	314	414	271	246	188	355	229	230	191	268	205	341	231	250	241	**262**	**179 - 473**

Table 3: Serum biochemistry panels, fecal examination, and heartworm antigen tests on Day –7 for each dog; abnormalities indicated by yellow, and red indicates animal removed from the study.

DOG #	096	164	181	192	194	205	217	241	242	248	251	253	254	910	961	Avg	Ref Range
Glucose	98	110	104	98	94	90	102	93	91	99	98	103	98	80	87	**97**	**89-135**
BUN	13	1	18	17	24	10	14	17	13	9	8	11	10	14	7	**12**	**8-27**
Creatinine	1.1	0.9	1.2	1.2	1.4	0.9	1.2	0.9	1.1	1.1	0.9	1.0	0.9	0.9	0.9	**1**	**0.6-1.4**
Phosphorus	3.5	3.0	4.0	4.7	4.9	4.0	5.8	3.9	4.0	3.4	3.3	3.7	3.4	3.5	2.8	**4**	**2.6-6.0**
Calcium	10.9	9.8	10.8	11.5	11.1	10.9	10.9	11.2	11.1	10.7	11.2	11.1	10.8	10.3	9.8	**11**	**9.5-11.6**
Total Protein	7.2	6.0	7.0	6.5	6.3	6.7	6.8	6.5	6.6	6.5	8.8	6.5	7.3	6.5	7.9	**7**	**5.4-7.2**
Albumin	3.2	3.2	3.2	3.5	3.4	3.4	3.6	3.6	3.3	3.6	3.2	3.4	3.1	3.5	3.2	**3**	**2.7-3.8**
Globulin	4.0	2.8	3.8	3.0	2.9	3.3	3.2	2.9	3.3	2.9	5.6	3.1	4.2	3.0	4.7	**4**	**2.2-4.0**
ALT	43	34	64	21	31	46	21	37	36	37	36	135	39	114	27	**48**	**13-88**
Alk Phos	59	45	78	46	123	27	80	26	49	39	63	63	96	20	24	**55**	**14-105**
Total bilirubin	0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.2	0.0	0.2	0.2	0.2	0.2	0.2	0.2	**0**	**0-0.3**
Cholesterol	301	199	225	296	236	309	336	209	225	238	183	265	160	118	107	**228**	**122-360**
Sodium	144	145	144	146	147	145	149	146	145	147	143	145	144	146	144	145	144-150
Potassium	3.8	3.9	4.2	4.5	3.8	4.3	4.1	4.0	4.2	4.0	3.8	4.0	4.0	4.0	4.1	**4**	**3.4-4.6**
Chloride	110	115	113	112	112	111	109	112	110	113	108	112	111	115	111	**112**	**108-118**
$TC0_2$	19	19	16	20	21	21	25	20	18	17	19	20	20	21	21	**20**	**16-33**
Anion Gap	18.8	14.9	19.2	18.5	17.8	17.3	19.1	18.0	21.2	21.0	19.8	17.0	17.0	14.0	16.1	**18**	**7-20**
Fecal	neg	Toxo-cara	Ancyl-ostoma	neg	Toxo-cara	Ancyl-ostoma	Toxo-cara	neg	neg	neg	neg	neg	neg	neg	neg		**negative**
HW Antigen	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg		**negative**

Table 4: Serum biochemistry panels and body weight on Day 112 for each dog; abnormalities indicated by yellow, and red indicates animal removed from the study.

DOG #	096	164	181	192	194	205	217	241	242	248	251	253	254	910	961	Avg	Ref Range
Weight (kg)	28.2	18.0	21.4	30.8	24.5	26.0	23.8	18.9	16.6	29.6		19.8	32.6	23.7	23.6	**24.11**	**NA**
Glucose	100	104	84	94	96	96	115	94	95	97		90	92	100	86	**95.93**	**89-135**
BUN	8	10	19	10	12	8	11	14	15	11		13	9	8	9	**11.21**	**8-27**
Creatinine	1.2	1.1	1.4	1.1	1.1	1.0	1.2	1.1	1.3	1.1		1.0	1.0	1.0	1.0	**1.11**	**0.6-1.4**
Phosphorus	3.2	1.9	2.9	4.0	3.7	3.5	4.4	3.8	3.8	3.8		3.5	3.6	3.2	3.8	**3.51**	**2.6-6.0**
Calcium	10.9	9.4	10.8	11.2	10.9	10.6	10.7	10.9	11.3	10.6		11.0	11.3	10.3	10.2	**10.72**	**9.5-11.6**
Total Protein	7.4	6.2	7.7	7.2	7.0	7.0	6.6	7.0	7.5	6.6		7.0	7.4	7.5	7.2	**7.09**	**5.4-7.2**
Albumin	3.1	3.2	3.5	3.4	3.2	3.2	3.4	3.6	3.4	3.2		3.4	3.4	3.0	3.0	**3.29**	**2.7-3.8**
Globulin	4.3	3.0	4.2	3.8	3.8	3.8	3.2	3.4	4.1	3.4		3.6	4.0	4.5	4.2	**3.81**	**2.2-4.0**
ALT	55	36	91	30	36	171	20	34	81	50		43	52	96	33	**120.8**	**13-88**
Alk Phos	60	47	47	29	113	59	64	28	42	38		68	109	45	22	**84.14**	**14-105**
Total bilirubin	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.2		0.3	0.1	0.1	0.0	**0.20**	**0-0.3**
Cholesterol	263	175	218	259	188	289	267	209	208	193		320	184	194	124	**220.79**	**122-360**
Sodium	146	146	145	145	146	146	147	147	148	149		147	147	148	146	**146.64**	**144-150**
Potassium	3.9	3.8	4.3	4.2	4.0	4.1	4.2	4.3	5.2	4.4		4.3	4.3	4.1	4.2	**4.24**	**3.4-4.6**
Chloride	111	114	110	111	111	111	112	112	112	116		110	112	112	112	**111.86**	**108-118**
$TC0_2$	23	21	22	23	22	22	23	21	22	20		23	20	23	19	**21.71**	**16-33**
Anion Gap	15.9	14.8	17.3	15.2	17.0	17.1	16.2	18.3	19.2	17.4		18.3	19.3	17.1	19.2	**17.31**	**7-20**

Removed from study (antebrachial cellulitis)

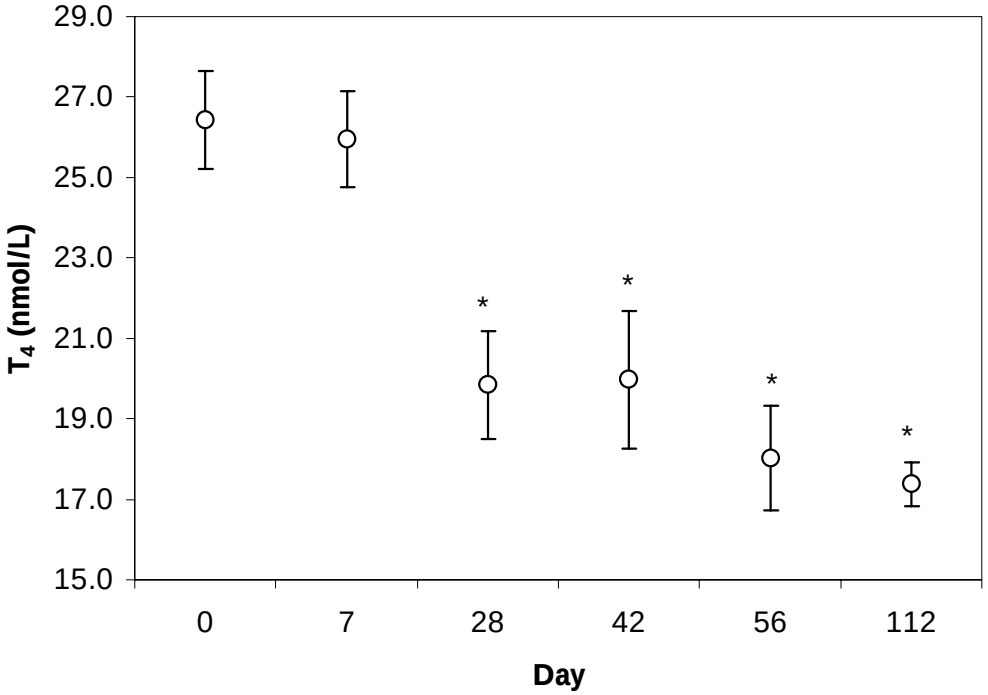

Figure 4: Mean ± SE serum T_4 concentration (nmol/L) in 14 dogs treated with clomipramine for 112 days; * denotes values significantly different from day 0.

Table 5: Mean and individual pre- and post-TRH stimulation serum T_4 concentrations (nmol/L) in 14 dogs treated with clomipramine for 112 days.

Dog #	D –7		D 0		D 7		D 28		D 42		D 56		D 112	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
096	37.83	67.15	25.00	25.26	28.68	30.87	23.41	21.18	20.80	20.53	18.59	20.27	15.57	14.33
164	24.21	51.76	24.34	27.42	32.52	25.50	22.09	20.17	19.43	21.20	21.15	17.72	16.85	17.21
181	20.60	42.99	27.06	31.18	21.19	27.79	15.86	12.90	11.00	9.43	11.28	14.08	16.48	20.31
192	18.23	55.77	20.06	21.05	17.29	18.96	12.48	15.38	12.42	16.53	15.83	14.55	15.84	13.98
194	24.79	66.55	23.07	23.28	23.63	22.11	15.16	19.90	21.42	26.54	16.05	16.46	18.02	19.55
205	21.69	58.14	22.67	26.38	25.83	20.72	16.40	17.03	12.77	18.24	10.96	15.33	14.40	15.21
217	31.19	85.09	31.19	35.07	34.40	27.27	24.46	25.28	23.16	27.59	26.03	23.82	21.55	25.04
241	33.55	84.85	28.37	26.12	27.51	32.13	22.84	25.70	16.92	22.62	21.19	22.84	17.88	17.25
242	26.09	69.20	31.92	30.18	24.50	34.17	28.71	30.53	34.62	34.18	13.38	15.96	18.38	21.14
248	27.69	55.74	28.77	31.25	26.50	26.24	18.72	16.71	21.46	21.16	20.32	21.47	16.72	22.69
253	25.11	39.63	34.61	21.05	26.68	31.71	23.56	26.91	22.04	21.26	18.70	18.24	20.12	18.29
254	22.49	33.52	21.66	19.08	24.66	29.09	18.31	18.76	19.14	20.24	14.49	17.08	18.10	18.66
910	27.55	59.77	27.93	26.61	24.46	27.55	20.56	21.04	19.83	22.42	19.38	20.04	17.52	19.16
961	31.36	72.51	23.45	16.15	25.52	21.40	15.23	13.57	24.69	24.57	24.78	23.53	15.90	18.60
Average	**26.60**	**60.19**	**26.43**	**25.72**	**25.96**	**26.82**	**19.84**	**20.36**	**19.98**	**21.89**	**18.01**	**18.67**	**17.38**	**18.67**

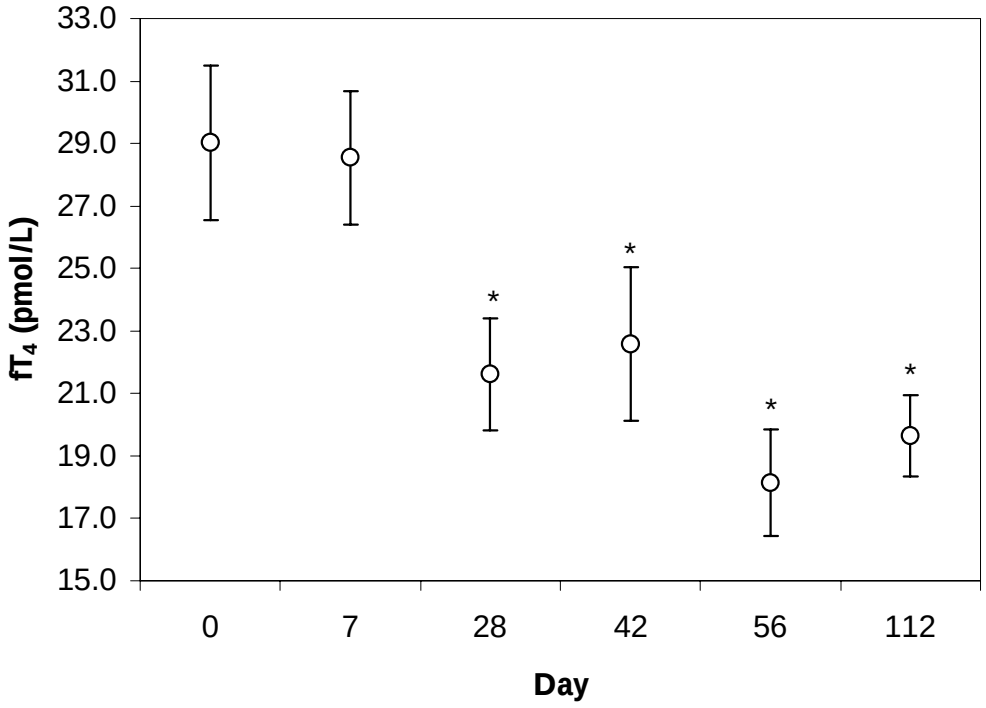

Figure 5: Mean ± SE serum fT_4 concentration (pmol/L) in 14 dogs treated with clomipramine for 112 days; * denotes values significantly different from day 0.

Table 6: Individual and mean serum fT_4 concentration (pmol/L) in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	27.40	33.65	28.88	24.70	30.35	17.71
164	33.59	48.92	28.27	22.38	22.45	26.96
181	46.62	33.10	18.82	13.43	14.56	23.11
192	22.74	19.72	14.74	12.83	13.35	16.07
194	28.35	26.92	18.76	28.55	14.01	22.63
205	20.29	21.03	17.12	15.91	15.44	13.88
217	34.91	30.53	27.71	22.50	24.08	25.62
241	27.81	30.66	24.04	21.54	25.76	17.66
242	33.08	27.01	32.94	46.64	12.38	18.06
248	37.01	28.16	15.79	21.77	19.52	20.67
253	32.51	22.83	21.82	20.79	13.14	16.32
254	14.76	22.19	13.06	15.12	10.64	12.10
910	31.22	30.57	24.27	23.42	19.33	21.74
961	16.31	24.51	17.10	26.78	18.87	22.61
Mean	**29.04**	**28.56**	**21.67**	**22.60**	**18.13**	**19.65**

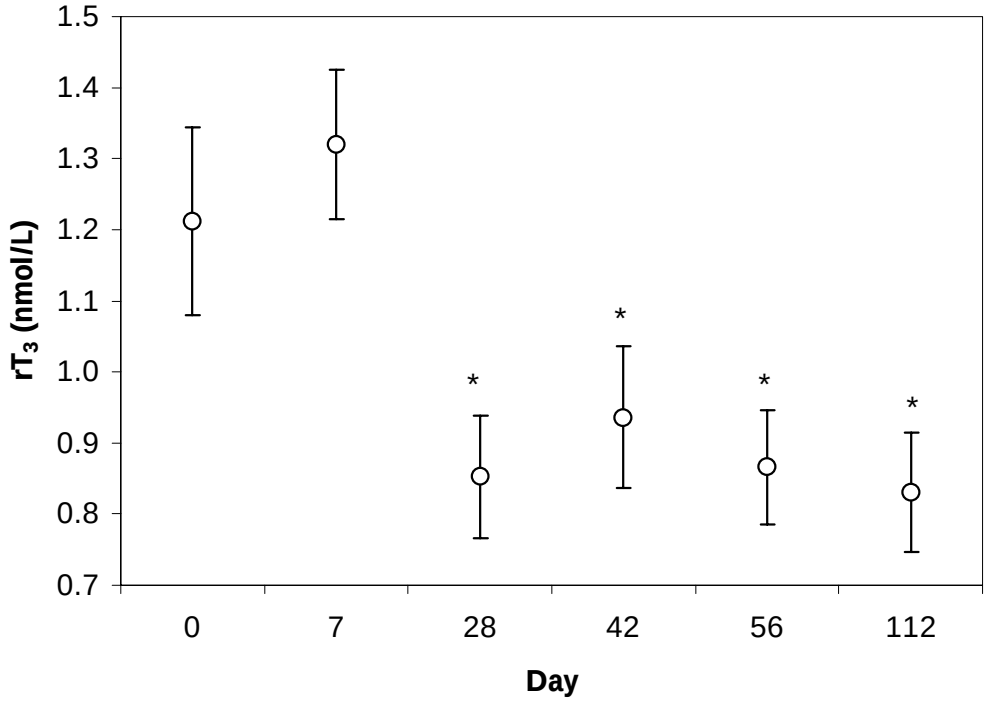

Figure 6: Mean ± SE serum rT_3 concentration (nmol/L) in 14 dogs treated with clomipramine for 112 days; * denotes values significantly different from day 0.

Table 7: Individual and mean serum rT_3 concentration (nmol/L) in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	1.07	1.24	1.07	1.08	0.94	0.85
164	1.81	1.75	1.18	1.28	1.08	1.31
181	1.61	1.44	0.72	1.00	1.31	0.98
192	1.08	1.13	0.59	0.62	0.67	0.81
194	1.53	1.94	1.25	1.50	0.97	1.12
205	0.51	0.96	0.77	0.70	0.76	0.31
217	1.53	1.39	1.18	1.17	1.13	1.19
241	1.27	1.53	0.73	0.81	1.23	0.89
242	1.82	1.60	1.24	1.47	0.84	0.84
248	1.58	1.64	0.86	1.03	0.91	0.97
253	0.75	0.96	0.74	0.48	0.43	0.53
254	0.61	0.63	0.41	0.49	0.47	0.62
910	1.25	1.39	0.79	1.05	0.98	0.88
961	0.60	0.95	0.34	0.53	0.52	0.38
Average	**1.21**	**1.32**	**0.85**	**0.94**	**0.87**	**0.83**

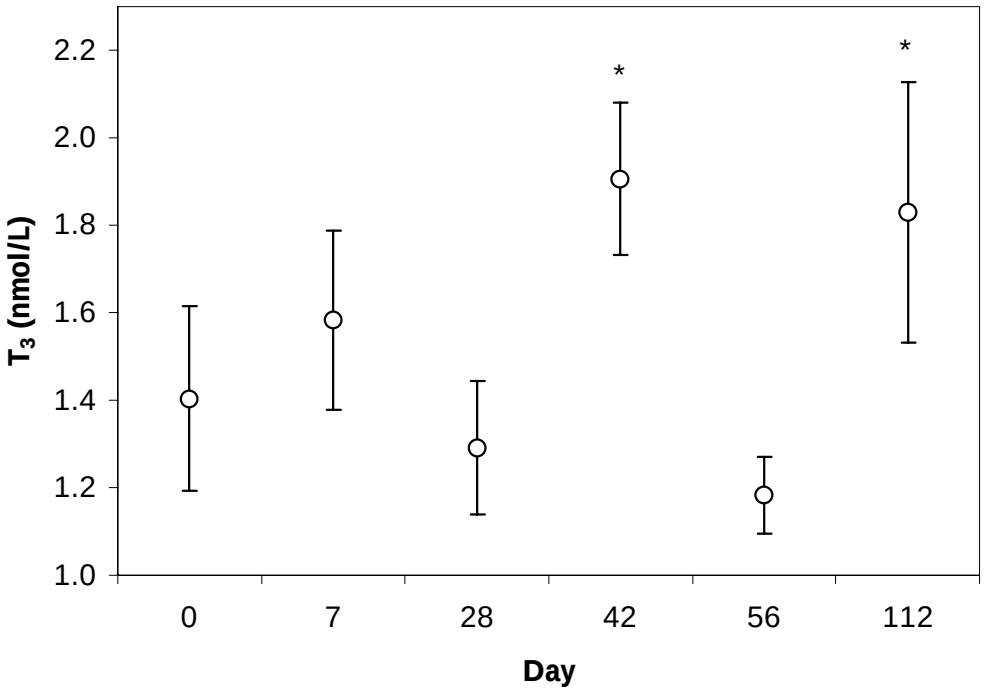

Figure 7: Mean ± SE serum T_3 concentration (nmol/L) in 14 dogs treated with clomipramine for 112 days; * denotes values significantly different from day 0.

Table 8: Individual and mean serum T_3 concentration (nmol/L) in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	0.89	0.83	1.17	1.94	0.99	1.27
164	0.49	0.57	0.90	0.93	1.28	0.67
181	1.73	1.95	1.46	1.90	1.22	1.60
192	1.67	1.98	2.04	2.05	1.57	2.68
194	1.06	1.93	1.87	3.09	1.24	1.71
205	1.43	1.24	0.85	1.07	1.21	1.26
217	1.58	1.13	1.43	2.00	1.26	1.50
241	2.96	2.13	1.14	2.08	1.55	2.05
242	2.05	7.84	1.74	2.57	1.60	2.80
248	0.75	2.00	1.16	1.98	0.79	1.20
253	2.36	3.22	2.11	1.45	0.92	4.63
254	0.93	0.90	0.42	2.30	0.51	1.60
910	1.29	1.92	1.33	2.16	1.11	2.02
961	0.34	0.86	0.49	1.21	1.23	0.63
Average	**1.40**	**2.04**	**1.29**	**1.91**	**1.18**	**1.83**

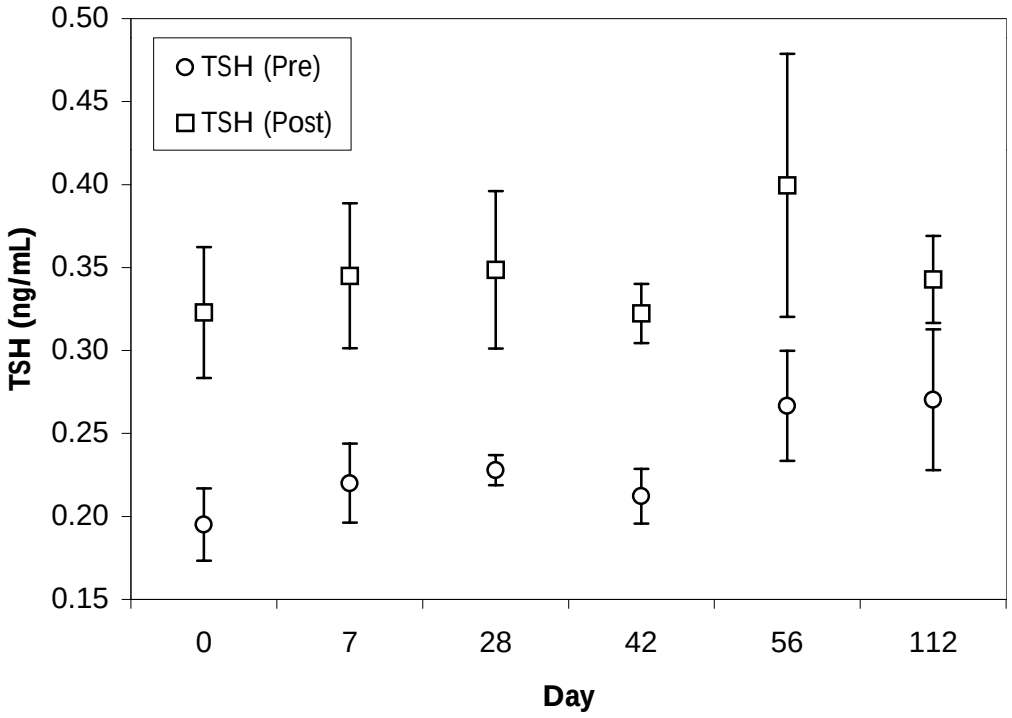

Figure 8: Mean ± SE pre- and post-TRH stimulation serum TSH concentrations (ng/mL) in 14 dogs treated with clomipramine for 112 days; no significance differences were noted.

Table 9: Mean and individual pre- and post-TRH stimulation serum TSH concentrations (ng/mL) in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0		D 7		D 28		D 42		D 56		D 112	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
096	0.10	0.15	0.10	0.18	0.15	0.29	0.14	0.22	0.14	0.20	0.14	0.25
164	0.13	0.18	0.08	0.18	0.16	0.20	0.10	0.22	0.06	0.20	0.39	0.19
181	0.14	0.22	0.16	0.39	0.16	0.22	0.15	0.25	0.18	0.35	0.17	0.25
192	0.09	0.19	0.14	0.21	0.11	0.12	0.09	0.16	0.10	0.15	0.12	0.19
194	0.10	0.19	0.10	0.18	0.13	0.20	0.14	0.20	0.17	0.18	0.13	0.19
205	0.06	0.10	0.08	0.16	0.13	0.15	0.10	0.14	0.11	0.14	0.10	0.15
217	0.10	0.16	0.15	0.18	0.13	0.21	0.09	0.20	0.15	0.20	0.11	0.22
241	0.16	0.15	0.16	0.14	0.13	0.17	0.14	0.18	0.18	0.13	0.14	0.17
242	0.14	0.18	0.13	0.17	0.17	0.17	0.16	0.17	0.16	0.16	0.13	0.37
248	0.08	0.19	0.14	0.15	0.13	0.16	0.14	0.22	0.17	0.17	0.09	0.17
253	0.14	0.23	0.21	0.19	0.16	0.16	0.16	0.27	0.22	0.24	0.19	0.22
254	0.27	0.47	0.15	0.31	0.16	0.37	0.23	0.28	0.25	0.47	0.18	0.31
910	0.15	0.22	0.16	0.23	0.14	0.29	0.19	0.26	0.19	0.33	0.27	0.23
961	0.13	0.32	0.29	0.47	0.19	0.53	0.17	0.22	0.38	0.79	0.39	0.22
Average	**0.13**	**0.21**	**0.14**	**0.22**	**0.15**	**0.23**	**0.14**	**0.21**	**0.17**	**0.26**	**0.18**	**0.22**

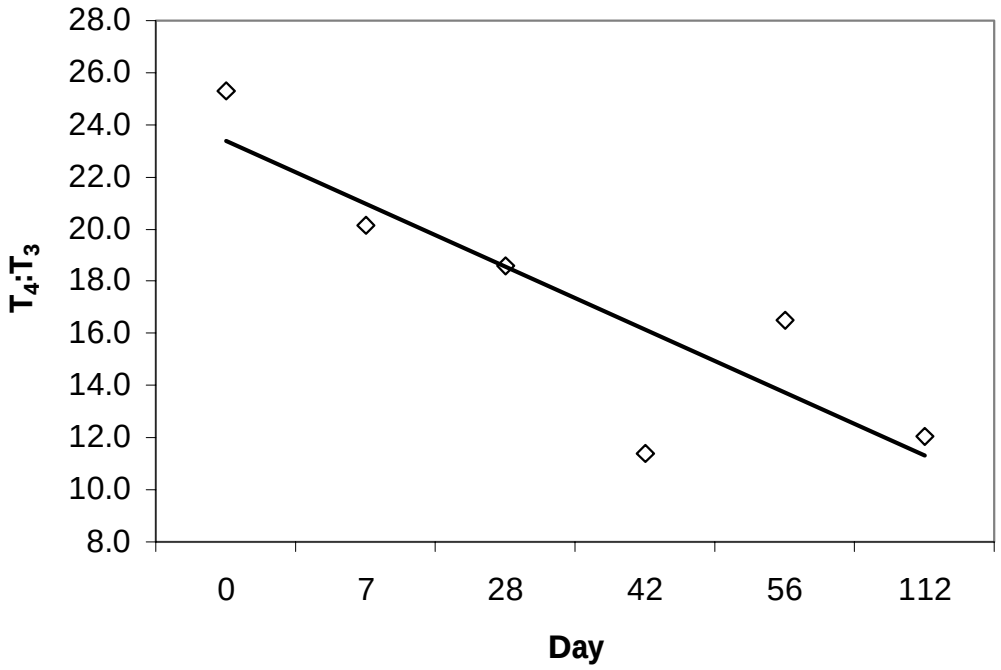

Figure 9: Mean T_4:T_3 ratios in 14 dogs treated with clomipramine for 112 days.

Table 10: Mean and individual T_4:T_3 ratios in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	28.23	34.77	20.07	10.70	18.82	12.27
164	49.24	56.99	24.46	20.98	16.48	25.30
181	15.66	10.87	10.84	5.79	9.25	10.31
192	11.98	8.71	6.12	6.06	10.11	5.91
194	21.71	12.23	8.11	6.92	12.98	10.54
205	15.81	20.78	19.22	11.99	9.03	11.41
217	19.80	30.42	17.06	11.56	20.63	14.32
241	9.57	12.94	19.97	8.15	13.64	8.74
242	15.61	3.13	16.51	13.47	8.35	6.56
248	38.40	13.27	16.19	10.81	25.73	13.94
253	14.67	8.29	11.15	15.20	20.25	4.35
254	23.32	27.47	43.89	8.32	28.27	11.33
910	21.65	12.74	15.46	9.18	17.46	8.67
961	68.48	29.65	31.38	20.42	20.15	25.16
Average	**25.29**	**20.16**	**18.60**	**11.40**	**16.51**	**12.06**

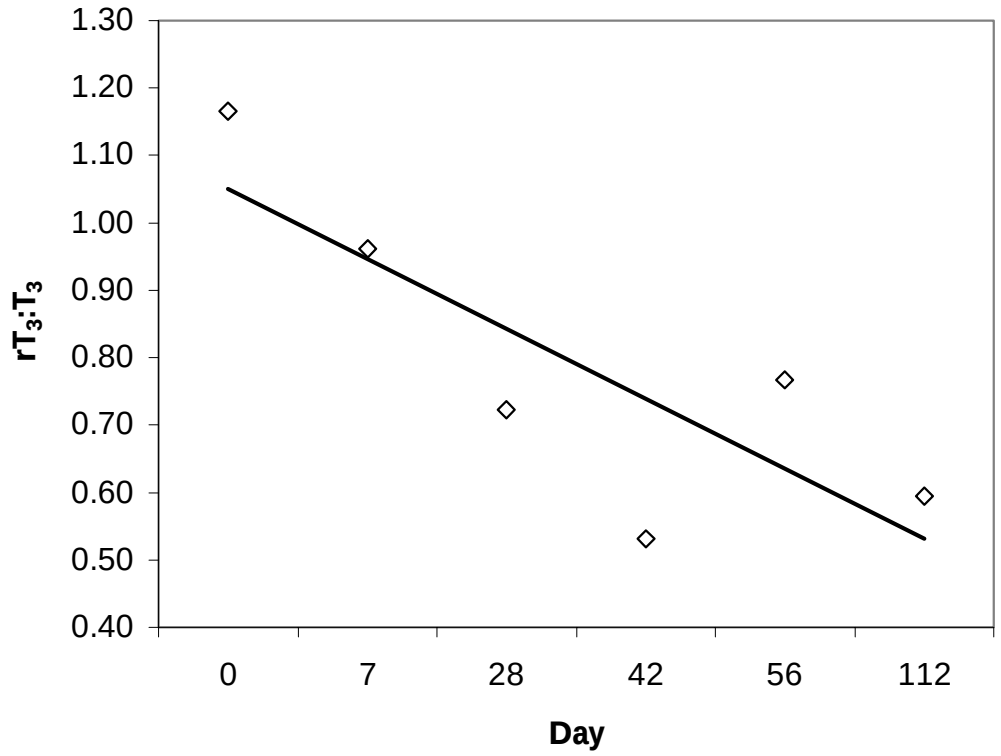

Figure 10: Mean rT_3:T_3 ratios in 14 dogs treated with clomipramine for 112 days.

Table 11: Mean and individual rT_3:T_3 ratios in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	1.21	1.51	0.92	0.56	0.95	0.67
164	3.66	3.08	1.30	1.39	0.84	1.97
181	0.93	0.74	0.49	0.53	1.08	0.61
192	0.64	0.57	0.29	0.30	0.43	0.30
194	1.44	1.00	0.67	0.48	0.78	0.65
205	0.36	0.77	0.90	0.66	0.62	0.25
217	0.97	1.23	0.82	0.58	0.90	0.79
241	0.43	0.72	0.64	0.39	0.79	0.43
242	0.89	0.20	0.72	0.57	0.52	0.30
248	2.10	0.82	0.74	0.52	1.15	0.81
253	0.32	0.30	0.35	0.33	0.46	0.11
254	0.65	0.70	0.99	0.21	0.91	0.38
910	0.97	0.72	0.59	0.49	0.88	0.44
961	1.74	1.11	0.69	0.44	0.43	0.61
Average	**1.17**	**0.96**	**0.72**	**0.53**	**0.77**	**0.60**

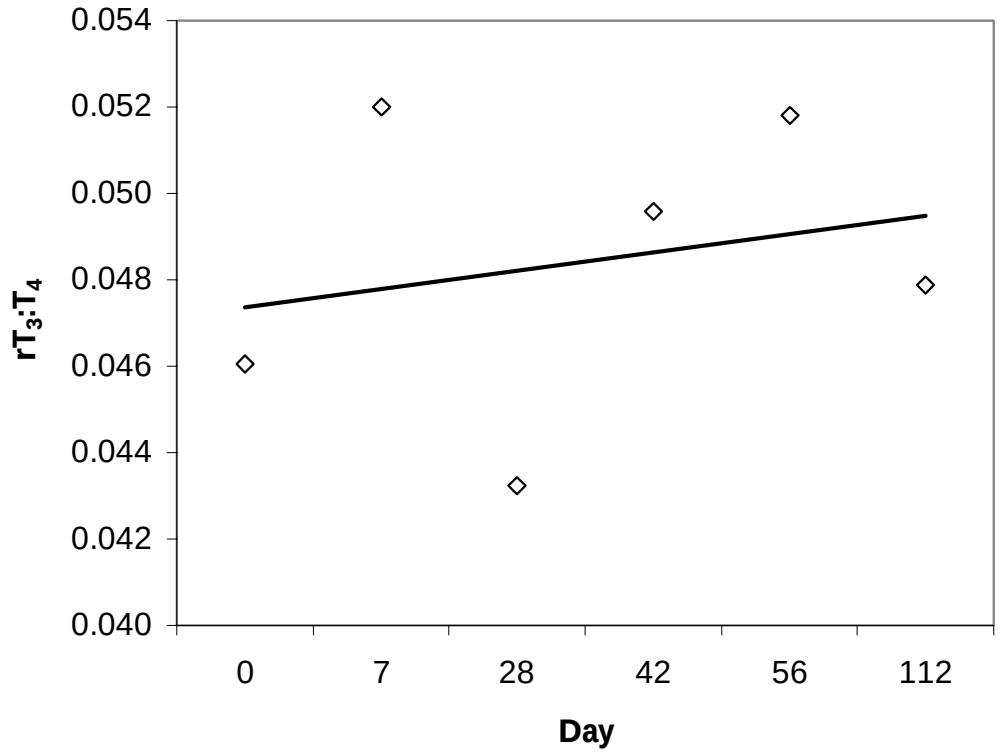

Figure 11: Mean rT_3:T_4 ratios in 14 dogs treated with clomipramine for 112 days.

Table 12: Mean and individual rT_3:T_4 ratios in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	0.043	0.043	0.046	0.052	0.051	0.055
164	0.074	0.054	0.053	0.066	0.051	0.078
181	0.059	0.068	0.045	0.091	0.116	0.060
192	0.054	0.065	0.047	0.050	0.043	0.051
194	0.066	0.082	0.083	0.070	0.060	0.062
205	0.023	0.037	0.047	0.055	0.069	0.022
217	0.049	0.040	0.048	0.050	0.044	0.055
241	0.045	0.056	0.032	0.048	0.058	0.050
242	0.057	0.065	0.043	0.043	0.063	0.045
248	0.055	0.062	0.046	0.048	0.045	0.058
253	0.022	0.036	0.032	0.022	0.023	0.026
254	0.028	0.025	0.022	0.026	0.032	0.034
910	0.045	0.057	0.038	0.053	0.051	0.050
961	0.025	0.037	0.022	0.022	0.021	0.024
Average	**0.046**	**0.052**	**0.043**	**0.050**	**0.052**	**0.048**

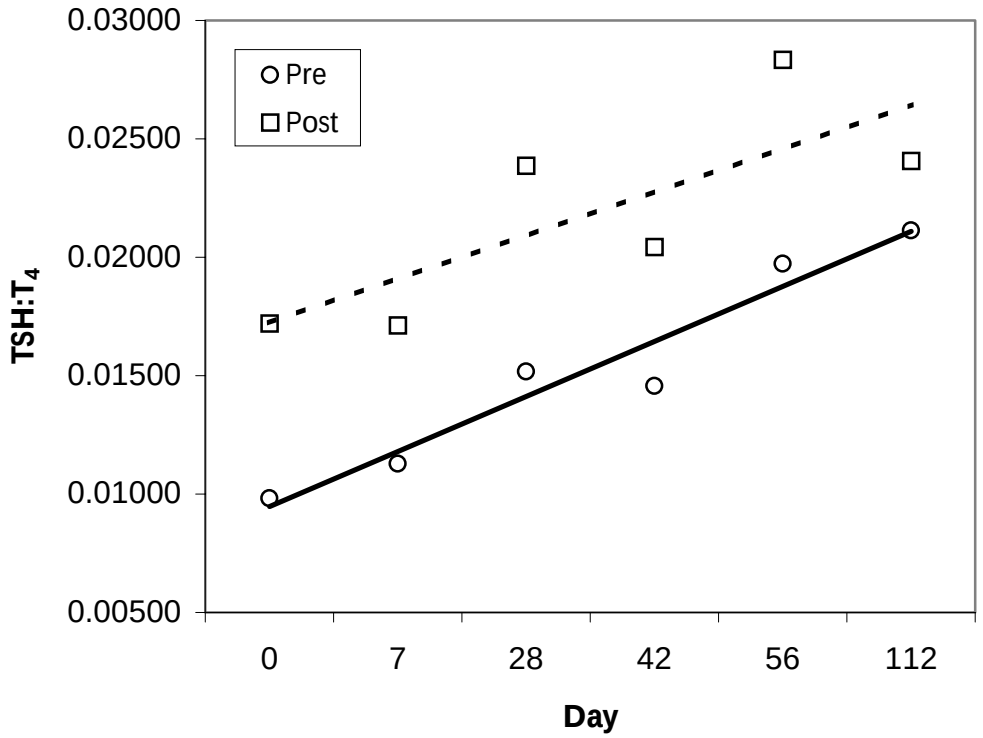

Figure 12: Mean pre- and post-TRH stimulation serum TSH:T_4 ratios in 14 dogs treated with clomipramine for 112 days.

Table 13: Mean and individual pre- and post-TRH stimulation serum TSH:T_4 ratios in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0		D 7		D 28		D 42		D 56		D 112	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
096	0.0042	0.0060	0.0035	0.0059	0.0064	0.0135	0.0065	0.0106	0.0078	0.0098	0.0090	0.0173
164	0.0055	0.0067	0.0023	0.0071	0.0075	0.0098	0.0051	0.0102	0.0029	0.0113	0.0232	0.0108
181	0.0051	0.0069	0.0074	0.0141	0.0101	0.0168	0.0132	0.0267	0.0156	0.0245	0.0102	0.0125
192	0.0043	0.0090	0.0078	0.0113	0.0089	0.0081	0.0069	0.0097	0.0066	0.0100	0.0076	0.0136
194	0.0043	0.0080	0.0044	0.0080	0.0087	0.0099	0.0066	0.0074	0.0103	0.0111	0.0070	0.0098
205	0.0027	0.0037	0.0030	0.0076	0.0078	0.0091	0.0076	0.0077	0.0097	0.0091	0.0072	0.0102
217	0.0032	0.0047	0.0044	0.0065	0.0055	0.0085	0.0040	0.0071	0.0057	0.0084	0.0052	0.0088
241	0.0056	0.0058	0.0058	0.0043	0.0058	0.0066	0.0081	0.0079	0.0083	0.0059	0.0076	0.0097
242	0.0045	0.0059	0.0052	0.0049	0.0058	0.0055	0.0045	0.0050	0.0121	0.0099	0.0073	0.0176
248	0.0028	0.0060	0.0051	0.0059	0.0072	0.0093	0.0067	0.0102	0.0082	0.0081	0.0055	0.0073
253	0.0042	0.0111	0.0077	0.0061	0.0069	0.0061	0.0073	0.0126	0.0116	0.0129	0.0094	0.0121
254	0.0123	0.0244	0.0059	0.0107	0.0088	0.0196	0.0118	0.0139	0.0170	0.0273	0.0101	0.0164
910	0.0054	0.0083	0.0065	0.0083	0.0068	0.0138	0.0096	0.0116	0.0098	0.0165	0.0154	0.0120
961	0.0056	0.0198	0.0112	0.0219	0.0123	0.0389	0.0069	0.0091	0.0153	0.0337	0.0244	0.0118
Average	**0.0098**	**0.0172**	**0.0113**	**0.0171**	**0.0152**	**0.0239**	**0.0146**	**0.0204**	**0.0197**	**0.0283**	**0.0211**	**0.0241**

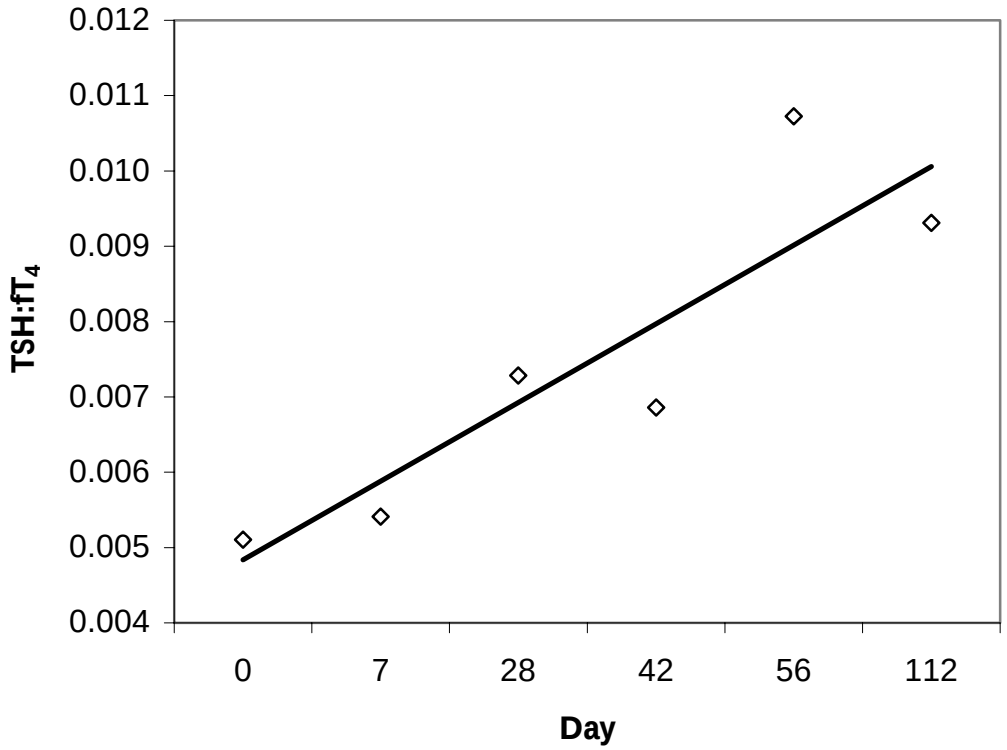

Figure 13: Mean TSH:fT_4 ratios in 14 dogs treated with clomipramine for 112 days.

Table 14: Mean and individual TSH:fT_4 ratios in 14 dogs treated with clomipramine for 112 days.

Dog #	D 0	D 7	D 28	D 42	D 56	D 112
096	0.00380	0.00300	0.00518	0.00549	0.00477	0.00789
164	0.00400	0.00154	0.00583	0.00447	0.00272	0.01452
181	0.00296	0.00471	0.00854	0.01081	0.01205	0.00730
192	0.00382	0.00685	0.00752	0.00670	0.00784	0.00750
194	0.00349	0.00385	0.00703	0.00495	0.01182	0.00557
205	0.00304	0.00366	0.00744	0.00608	0.00690	0.00744
217	0.00282	0.00499	0.00482	0.00408	0.00614	0.00434
241	0.00566	0.00516	0.00552	0.00637	0.00680	0.00769
242	0.00438	0.00471	0.00504	0.00337	0.01305	0.00745
248	0.00215	0.00483	0.00853	0.00659	0.00856	0.00443
253	0.00444	0.00899	0.00743	0.00772	0.01655	0.01156
254	0.01799	0.00657	0.01238	0.01497	0.02308	0.01505
910	0.00480	0.00523	0.00577	0.00811	0.00983	0.01242
961	0.00811	0.01164	0.01094	0.00635	0.02004	0.01719
Average	**0.00511**	**0.00541**	**0.00728**	**0.00686**	**0.01072**	**0.00931**

CURRICULUM VITAE

PERSONAL

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EDUCATION

VA-MD Regional College of Veterinary Medicine	
▪ Residency – Small Animal Internal Medicine	1999 – 02
▪ Masters of Science – Veterinary Medical Sciences	1999 – 02
Coral Springs Animal Hospital	1998 – 99
▪ Internship	
Oklahoma State University College of Veterinary Medicine	1994 – 98
▪ Doctorate of Veterinary Medicine (magna cum laude)	
Oklahoma State University College of Agricultural Sciences	1990 – 94
▪ Bachelor of Science (magna cum laude)	
▪ Entomology and Biology (Double Major)	

PROFESSIONAL MEMBERSHIPS & LICENSES

Comparative Gastroenterology Society	2001 – 02
American Veterinary Medical Association (AVMA)	1998 – 01
American Animal Hospital Association (AAHA)	1998 – 99
American Veterinary Society of Animal Behavior (AVSAB)	1998 – 00
Oklahoma Veterinary Medical Association (OVMA)	1998 – 99
Broward County Veterinary Medical Association (BCVMA)	1998 – 99
Licensed in Florida	1998 – 02

PROFESSIONAL EMPLOYMENT

Resident – VA-MD Regional College of Veterinary Medicine	1999 – 02
Internship – Coral Springs Animal Hospital (Coral Springs, FL)	1998 – 00
Relief Veterinarian – Sunrise Animal Hospital (Sunrise, FL)	1998 – 00
Student Representative – Morris Animal Foundation	1996 – 98
Senior Manager – OSU Veterinary Educational Supply, Inc.	1996 – 97
Student Representative & Intern – Novartis Animal Health, US	1995 – 97
Student Representative – The Iams Company	1994 – 97
Teaching Assistant – Oklahoma State University	1994 – 95

PROFESSIONAL TEACHING

Instructor – Veterinary Ethology: Feline Behavior (VM 8144)	2001
Instructor – Small Animal Gastrointestinal Endoscopy (CE)	2001 – 02
Instructor – Critical Care Techniques Laboratory	2000 – 01
Instructor – Clinical Techniques (VM 8354)	1999 – 00
Instructor – Small Animal Techniques (VM 8694)	2000
Gastrointestinal Endoscopy Course (VM 8964)	2000

STUDENT ACTIVITIES

Resident Departmental Representative – VA-MD Regional CVM	2000 – 02
1996 SCAVMA National Symposium Organizational Committee	1996 – 97
President – Oklahoma State University Veterinary Class of 1998	1994 – 96

PRESENTATIONS

Canine and Feline Behavior Problems — 2002
- Animal Welfare Organization (Blacksburg, VA)

Alimentary Tube Nutrition — 2002
- Virginia Veterinary Medical Association (Roanoke, VA)

Gastroenterology Special Interest Group: Case Presentation — 2001
- 19th Annual ACVIM Forum (Denver, CO)

Effects of Tricyclic Antidepressants on Canine Thyroid Hormones — 1999
Effect of Clomipramine on the Canine HPT Axis: Initial Findings — 2000
Fructosamine and Glycosylated Hemoglobin — 2001
- Resident Seminars (Blacksburg, VA)

Feline Elimination Disorders — 1999
Canine Anxiety-Based Disorders — 1999
- 1999 SCAVMA National Symposium (Blacksburg, VA)

Feather Plucking in an African Grey Parrot (*Psittacus erithacus*) — 1999
- 137th AVMA Conference (New Orleans, LA)

Feline House-soiling: Diagnosis and Treatment Options — 1998
- Student Chapter of the AVMA (University of Florida)

Behavioral Medicine and Its Role in Your Practice — 1997
- Student Chapter of the AVMA (Oklahoma State University)

HONORS & AWARDS

VA-MD Regional College of Veterinary Medicine
- EVD / BASF Nutrition Case Report Competition (3rd Place) — 2001
- Phi Zeta (ΦZ) — 2000
- Research & Graduate Studies Scholarship — 2000

Oklahoma State University
- Phi Zeta (ΦZ) — 1998
- Helen Parker Memorial Scholarship (Clinical Aptitude) — 1998
- Town & Country Kennel Club Scholarship — 1998
- North American Veterinary Conference Student Scholar — 1998
- George & Dorothy Faisy Award for Academic Excellence — 1997
- Dean's List of Distinguished Students — 1996 – 98
- Bil-Jac Pet Foods Scholastic Award — 1996
- President's / Dean's Honor Roll — 3 / 5 sems
- Phi Kappa Phi (ΦΚΦ) — 1992 – 94

PUBLICATIONS

Journals (Refereed)

Long-term monitoring of the diabetic dog: the glucose curve, fructosamine, and glycosylated hemoglobin Gulikers KP, Monroe WE. *Veterinary Medicine* Submitted	2002
Evaluation of the effects of clomipramine on canine thyroid hormones Gulikers KP, Panciera, DL. *Journal of the American Veterinary Medical Association* Submitted	2002
Canine Zinc Toxicity: Case Report and Literature Review Albano N, Gulikers KP. *Journal of the American Veterinary Medical Association* In preparation	2002
Recurrent Spontaneous Lung Lobe Torsion in a Pug Spranklin DB, Gulikers KP, Lanz OI, Monroe WE. *Journal of the American Animal Hospital Association* Submitted	2002
Influence of various medications on canine thyroid function Gulikers KP, Panciera, DL. *Compendium on Continuing Education for the Practicing Veterinarian* In press	2001

Electronic

Iams "Food for Thought" – Case Study 6 Exocrine Pancreatic Insufficiency (EPI) in a German Shepherd Dog Gulikers KP www.iams.com/vetstu/index.asp	2002

GRANTS

Evaluation of the long-term administration of the tricyclic anti-depressant clomipramine on the canine hypothalamic-pituitary-thyroid axis – Virginia Veterinary Medical Foundation ($7498)	2000