

Standard Article

J Vet Intern Med 2017;31:705–710**Effects of Levothyroxine Administration and Withdrawal on the Hypothalamic-Pituitary-Thyroid Axis in Euthyroid Dogs**V. Ziglioli , D.L. Panciera, G.C. Troy, W.E. Monroe, K.M. Boes, and K.R. Refsal

Background: Chronic supplementation can suppress the hypothalamic-pituitary-thyroid axis (HPTA) and make it difficult to assess thyroid function after withdrawal of levothyroxine.

Objectives: To determine whether the HPTA is suppressed after levothyroxine administration in euthyroid dogs and the time required for resolution of any suppression.

Animals: Twenty-eight healthy euthyroid dogs.

Methods: A prospective, randomized study administering levothyroxine to euthyroid dogs for 8 weeks (group 1) or 16 weeks (group 2). Serum concentrations of total thyroxine (T₄), free thyroxine (fT₄) by equilibrium dialysis, thyroid stimulating hormone; thyrotropin (TSH), and 3,5,3'-triiodothyronine (T₃) were measured every 4 weeks during supplementation and for 16 weeks after levothyroxine was discontinued.

Results: Mean serum concentrations of T₄ and fT₄ were significantly higher ($P < .0001$) and TSH was lower ($P < .0001$) in all dogs during levothyroxine administration compared to baseline. Mean serum concentrations of T₄, fT₄, and TSH in both groups, beginning 1 week after levothyroxine was discontinued, were significantly different ($P < .01$) compared to values during levothyroxine administration but not compared to baseline values ($P > .3$).

Conclusions and Clinical Importance: Assessing thyroid function tests 1 week after cessation of levothyroxine at 26 µg/kg once a day for up to 16 weeks will provide an accurate assessment of thyroid function in healthy euthyroid dogs.

Key words: Canine; Endocrinology; Hypothyroidism; Thyroid function tests.

Dogs suspected of hypothyroidism are sometimes administered levothyroxine without confirmation of the diagnosis. This can occur because measurement of serum total thyroxine (T₄) concentration, a commonly utilized thyroid function test, has limited specificity and is influenced by drugs and concurrent illnesses.^{1–5} When evaluating a diagnosis of hypothyroidism in a dog receiving levothyroxine, it is necessary to withdraw treatment before thyroid function testing. Because levothyroxine administration suppresses the hypothalamic-pituitary-thyroid axis (HPTA), thyroid function tests could be altered after cessation of therapy.⁶

Thyroid hormone replacement therapy in euthyroid patients suppresses hypothalamic and pituitary function

Abbreviations:

fT ₄	free thyroxine
HPTA	hypothalamic-pituitary-thyroid axis
T ₃	triiodothyronine
T ₄	total thyroxine
TRH	thyrotropin releasing hormone
TSH	thyroid stimulating hormone; thyrotropin

by negative feedback of thyroid hormones on thyrotropin releasing hormone (TRH) and thyroid stimulating hormone (thyrotropin; TSH).^{7–10} Chronic suppression of the HPTA will result in pituitary thyrotrope atrophy and subsequently, thyroid gland atrophy and impaired secretion of thyroid hormones.^{7,9,11}

In humans, withdrawal of therapy after prolonged treatment can result in serum thyroid hormone and TSH concentrations below their respective reference intervals, with the length and degree of suppression influenced by the type, dose, and duration of replacement therapy.^{9,10,12,13} Additionally, thyroid function tests can be affected for months after long-term thyroid hormone administration. During recovery from suppression, serum TSH concentration increases prior to thyroid hormones and can result in hormone levels similar to those found in primary hypothyroidism. Studies evaluating the effects of levothyroxine administration on the HPTA in euthyroid dogs are conflicting.^{6,14} Levothyroxine administration to healthy dogs for 5 weeks did not suppress T₄ response to TRH administration in 1 study,¹⁴ while another study documented complete suppression of TRH induced T₄ secretion after 6 weeks of treatment. More importantly, suppression of the thyroid hormone response to TSH stimulation persisted 4 weeks after withdrawal of levothyroxine treatment.⁶

The suppressive effects of thyroid hormone therapy and subsequent recovery of the HPTA present a

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diagnostic challenge when evaluating thyroid function. The influence of dosage, duration of treatment, and effects on serum TSH concentrations of levothyroxine administration has not been studied in the euthyroid dog. The effect of levothyroxine supplementation in euthyroid dogs on thyroid function tests has been investigated in only 1 study.⁶ That study contained a small number of dogs, did not measure serum TSH or free thyroxine (fT4) concentrations, administered twice daily levothyroxine rather than the currently recommended once daily treatment, and evaluated a single duration of treatment. In addition, the duration of follow-up after discontinuing treatment was insufficient to document full recovery of the HPTA. It is not known whether supplementation for more than 8 weeks will increase the time it takes for the HPTA to normalize.⁶ In this study, we evaluated the degree and duration of suppression of the HPTA that occurs after chronic levothyroxine^a administration to euthyroid dogs in order to identify when thyroid function tests accurately document euthyroidism after withdrawing supplementation. We hypothesized that levothyroxine administration would suppress the HPTA in euthyroid dogs and that the HPTA would recover within 8 weeks in all dogs, regardless of the duration of treatment.

Materials and Methods

Dogs

This study was approved by the Institutional Animal Care and Use Committee at Virginia Maryland College of Veterinary Medicine and by the Veterinary Teaching Hospital Board. This was a prospective, randomized study performed at the Virginia Maryland College of Veterinary Medicine between July 2014 and May 2015. Dogs enrolled in this study were recruited from faculty, staff, and students. Inclusion criteria included dogs 1–7 years of age with body weight >5 kg that were documented to be healthy based on results of history, physical examination, complete blood count, serum biochemistry, urinalysis, and serum concentrations of T₄ and TSH. Dogs were excluded from the study if they were a sight-hound breed, had been diagnosed previously with hypothyroidism or another chronic disease, or received medication known to affect thyroid function (glucocorticoids, phenobarbital, sulfonamides, and tricyclic antidepressants) within 2 months of enrollment in the study. However, flea and tick and heartworm prevention was allowed to be administered. Dogs with a serum T₄ concentration below the reference interval or a TSH concentration above the reference interval were excluded.

Treatment and Sampling

Twenty-eight dogs enrolled in the study were randomly assigned to 1 of 2 equal groups using number generated randomization^a. Dogs in group 1 received levothyroxine^b for 8 weeks (denoted as weeks 1–8 of the supplementation period), and those in group 2 received levothyroxine for 16 weeks (denoted as weeks 1–16 of the supplementation period). Levothyroxine (20–26 µg/kg rounded to the nearest 0.1 mg) was dispensed to the owner to be administered orally every 24 hours 30 minutes prior to a morning meal.

Prior to administration of levothyroxine, 2 blood samples for measurement of serum concentrations of T₄, fT₄, 3,5,3'-triiodothyronine (T₃), and TSH were obtained 1 week apart, with the mean concentration of each analyte serving as the pretreatment

baseline value for each dog. One week after initiating treatment, a blood sample was obtained 4–6 hours after levothyroxine administration (anticipated peak serum T₄ concentration) for measurement of serum T₄.²³ If the serum T₄ concentration was outside the target therapeutic range (40–70 nmol/L), the levothyroxine dose was increased or decreased by 25% or to the nearest tablet size. The serum T₄ concentration was re-evaluated 1 week later, and dogs not reaching the target therapeutic range were excluded from the study. Week 1 of the supplementation period was designated as the time the target therapeutic range was reached. Dogs were evaluated every 4 weeks during the supplementation period by standardized history and physical examinations, and a blood sample was obtained 4–6 hours after levothyroxine administration for measurement of serum concentrations of T₄, fT₄, T₃, and TSH. At each evaluation, prescribed levothyroxine tablets were counted to determine owner compliance. After completion of levothyroxine administration (denoted as week 1–16 of the withdrawal period), dogs were evaluated at 1 and 4 weeks and then every 4 weeks for a total of 16 weeks after cessation of treatment by standardized history and physical examination and measurement of serum T₄, fT₄, T₃, and TSH concentrations. Any dog that developed an illness or required administration of a drug other than routine antiparasite prophylaxis were reviewed by 2 of the investigators to determine whether it should be excluded from the study. At each sample collection, 8–10 mL of blood was obtained by jugular or cephalic venipuncture, allowed to clot for 30–60 minutes at room temperature and then centrifuged at 2,500 × *g* for 15 minutes. Harvested serum was stored at –70°C until analysis.

All thyroid function tests (T₄, fT₄, T₃, and TSH concentrations) used for statistical analysis were measured at Michigan State University, Diagnostic Center for Population and Animal Health.

Hormone Measurements

Serum concentrations of T₄ were measured with a commercially available radioimmunoassay kit.^c The volume of samples and reagents were used as per the manufacturer's protocol but the incubation period was extended to 2 hours in a 37°C water bath. The analytical sensitivity, estimated as the mean concentration of T₄ at 90% specific binding (10 assays), was 3.4 nmol/L (range 3.1–4.0 nmol/L). Aliquots of canine serum with T₄ concentrations of 8 and 85 nmol/L were mixed in volume combinations of 1 : 2, 1 : 1, 2 : 1, and 4 : 1 to assess parallelism. The results from assay of the mixtures showed respective % observed/expected recovery rates of 90, 96, 86, and 90%. When aliquots of a canine serum sample were mixed with an added stock of 26, 64, 129, and 193 nmol/L T₄, the respective % recovery rates were 86, 89, 99, and 101%. Assay repeatability was determined from 3 pools of canine serum with mean concentrations of T₄ of 12, 26, and 85 nmol/L. The respective intra-assay coefficients of variation (CVs) were 8.5, 9.5, and 8.1% for 10 replicates. In 10 assay runs, the respective interassay CVs for each pool were 19.6, 13, and 9.5%.

Serum concentrations of TSH were measured with a solid-phase chemiluminescent immunometric assay.^d The manufacturer reports an analytical sensitivity to 0.01 ng/mL. Two canine serum samples with TSH concentrations of 0.10 and 2.91 ng/mL were mixed in respective volume combinations of 1 : 1, 1 : 2, 3 : 1, and 5 : 1 to assess parallelism. The results from assay of the various mixtures showed % observed/expected recovery rates of 106, 105, 110, and 102%, respectively. Assay repeatability was assessed with 4 pools of canine serum with mean concentrations of 0.12, 0.43, 1.45, and 3.70 ng/mL. The respective intra-assay CVs for 10 replicates of each pool were 3.4, 2.2, 2.4, and 2.3%. Five replicates for each pool were run on 3 consecutive days and the respective interassay CVs for the daily means of each pool were 1.0, 1.0, 1.1, and 1.7%.

Free thyroxine was measured in canine serum with a commercially available radioimmunoassay originally developed by Nichols Institute Diagnostics and currently manufactured by Antech Diagnostics.^c Assay procedures were performed as per the manufacturer's protocol instructions. The manufacturer reported negligible cross-reaction with other iodothyronines (0.001–0.044%) in the assay. The sensitivity of the assay, defined as the calculated concentration of free T₄ at 2 standard deviations below total specific binding (11 assay runs), was 3.6 pmol/L. For other estimates of assay performance, 3 pools of canine serum were made with aliquots from clinical samples. In canine serum pools with mean concentrations of 7 (Low), 26 (Mid-range), or 56 (High) pmol/L, the interassay %CV were 28, 16.3, and 9.5%, respectively (n = 11 assays). The interassay repeatability for the Low pool seems disappointing but small numerical differences are magnified in the low concentrations near the sensitivity of the assay. The intra-assay % CV from the same pools were 11.0, 8.3, and 8.7%, respectively (n = 10–14 replicates). After incubation in dialysis cells, aliquots of dialysate from serum pools were mixed in different combinations for assessment of assay parallelism. When aliquots of dialysate from the "High" and "Low" pools were mixed (parts High: parts Low) in combinations of 9 : 1, 3 : 1, 3 : 2, 1 : 1, and 3 : 7, the recovery (%observed/expected) rates of measured fT₄ were 84, 84.3, 84.8, 96.2, and 86.8%, respectively, with an overall average recovery of 87.2%. When aliquots of the "Mid-range" pool were mixed at 3 : 1 rates with solutions of fT₄ with known concentrations 19, 46, and 108 pmol/L, respective recovery rates of added fT₄ were 85.0, 93.5, and 103.5%, with an overall average recovery of 94.1%.

Statistical Analysis

The minimum number of dogs in the study was determined to be 8 per group based on a power analysis, setting the level of significance at 0.05 and power at 0.8, assuming data distribution similar to that previously described.⁶

Normal probability plots showed that serum concentrations of T₄, T₃, fT₄, and log TSH as well as changes in the hormones from baseline values (an average of 2 measurements) had a Gaussian distribution. Subsequently, data were summarized as means ± standard deviation. Effect of time on each outcome within each group was assessed using mixed model ANOVA followed by Tukey's procedure for multiple comparisons. The linear model specified sample week as a fixed effect and dog identification as the random effect. Correlation among residuals was modeled by specifying the AR(1) covariance matrix. For change from baseline and for individual hormonal concentrations during treatment (weeks 0, 1, 4, and 8) the treatment groups were compared using mixed model ANOVA. The linear model specified group, time, and the interaction between group and time as fixed effects while dog identification within group constituted the random effect. Correlation among residuals was modeled by specifying the AR(1) covariance matrix. To specifically compare the groups at time point as appropriate, the sliceddiff option of proc glimmix was applied to the interaction between group and time. Results are expressed as means ± standard deviation and were considered significant at *P* < .05. All analyses were performed using SAS version 9.4.^f

Results

Of 37 dogs evaluated, nine were excluded, totaling 28 dogs that completed the trial. Three dogs were excluded initially for having a serum T₄ or TSH concentration outside the reference interval, 3 were excluded after 2 weeks on supplementation because the T₄

concentration did not reach the target therapeutic range, and 2 were excluded due to failed compliance. One dog was excluded due to an elevated serum T₄ (>100 nmol/L) and concurrent weight loss documented at week 4 of supplementation.

Twenty-eight dogs completed the study period; 14 were castrated males and 14 were spayed females. Breeds included mixed (n = 14) Labrador Retriever (n = 2), Staffordshire Terrier (n = 2), German Shorthair Pointer (n = 2), and 1 each of the following: Siberian Husky, Catahoula Leopard Dog, Golden Retriever, Bull Mastiff, Rottweiler, French Bulldog, Australian Cattle Dog, and German Shepherd. The mean ± SD age was 4.5 ± 1.9 years and weight was 25.04 ± 9.8 kg. There were no significant differences in age or weight between the 2 groups. No clinically relevant abnormalities were identified on physical examination or routine laboratory testing.

The mean initial levothyroxine dose in all dogs was 0.024 ± 0.002 mg/kg every 24 hours. There was a significant difference (*P* < .05) in the mean initial levothyroxine dose between group 1 (0.023 ± 0.002 mg/kg) and group 2 (0.025 ± 0.001 mg/kg). The levothyroxine dose was increased in 3 dogs and decreased in 2 dogs based on failure to reach the target therapeutic range during week 1. The mean final levothyroxine dose in all dogs was 0.024 ± 0.003 mg/kg and was not significantly different from the mean initial dose of levothyroxine in all dogs. There was a significant difference (*P* < .05) in mean final dose of levothyroxine between group 1 (0.023 ± 0.002 mg/kg) and group 2 (0.026 ± 0.002 mg/kg).

Compliance with levothyroxine administration was 100% in 15 (54%) dogs. Of the remaining 13 dogs, 5 in group 1 and 8 in group 2 missed an average of 2 doses over their respective treatment periods. The overall compliance for all the dogs in the study was 98.4%.

Supplementation Period

Mean serum T₄ and fT₄ concentrations (Figure 1) in both groups during the supplementation period were higher than baseline (*P* < .0001). Mean serum TSH concentrations in both groups were lower during the supplementation period compared to baseline (*P* < .0001). The mean serum T₃ concentration in both groups (data not shown) was not different between any other time periods (*P* > .7).

At week 4, T₄, fT₄, and T₃ concentrations were higher in group 1 compared to group 2 (*P* = .009, .02, and .01, respectively). There were no significant differences between groups at any other time during the supplementation period. No dog that completed the study had clinical signs or physical examination abnormalities consistent with hyperthyroidism.

Withdrawal Period

There was no difference in mean serum T₄ or fT₄ concentrations (Figure 1) in either group during the withdrawal period compared to baseline (*P* > .9). Mean serum T₄ and fT₄ concentrations in both groups were

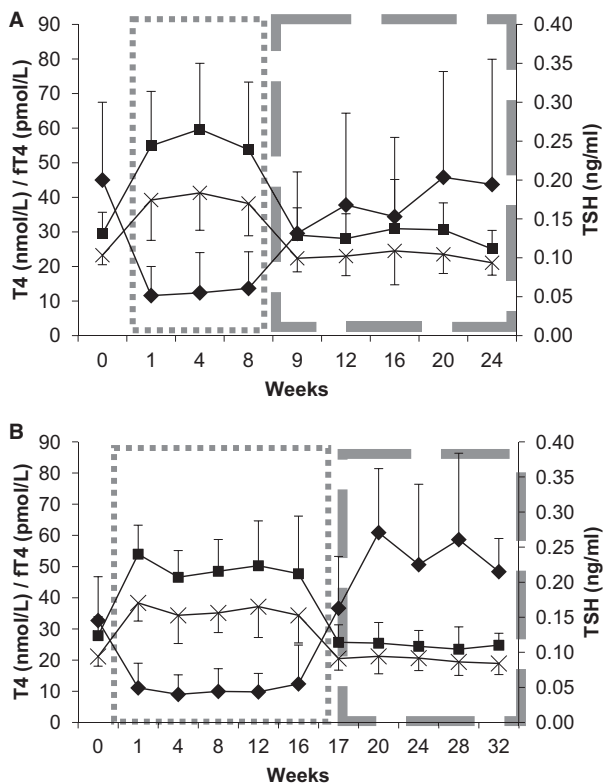


Figure 1. (A) Mean \pm standard deviation serum total thyroxine (T_4) (■), fT_4 (X), and thyroid stimulating hormone; thyrotropin (TSH) (◆) concentrations in euthyroid dogs administered once daily oral levothyroxine during the supplementation period, weeks 1–8 (outlined in “----”) and withdrawal period, weeks 9–24 (outlined in “—”) in Group 1. Week 0 represents mean baseline hormone concentrations. (B) Mean \pm standard deviation serum T_4 (■), fT_4 (X), and TSH (◆) concentrations in euthyroid dogs administered once daily oral levothyroxine during the supplementation period, weeks 1–16 (outlined in “----”) and withdrawal period, weeks 17–32 (outlined in “—”) in Group 2. Week 0 represents mean baseline hormone concentrations.

lower during the withdrawal period compared to the supplementation period ($P < .0001$). The mean serum T_3 concentrations (data not shown) in both groups were not different between any time periods ($P > .1$). Mean serum TSH concentrations in both groups were not different during the withdrawal period compared to baseline ($P > .3$). The mean serum TSH concentration in group 1 was higher at all times during the withdrawal period compared to the supplementation period, except week 1 of withdrawal ($P < .01$ and $P > .06$, respectively). The mean serum TSH concentration in group 2 was higher at all times during withdrawal period compared to the supplementation period ($P < .001$).

The serum TSH concentration was higher in group 2 than in group 1 at week 1 ($P = .05$), week 4 ($P = .04$), and week 8 ($P = .01$) of the withdrawal period. There were no significant differences between groups at any other time during the withdrawal period.

The serum T_4 concentration (10 nmol/L) was below the reference interval in 1 dog in group 2 at week 12 of

the withdrawal period, but the serum concentrations of TSH (0.17 ng/mL), fT_4 (13 pmol/L), and T_3 (1.2 nmol/L) were within their respective reference intervals. One dog in group 1 had a serum T_4 (74 nmol/L) and fT_4 (56 pmol/L) concentration that was above the reference interval at week 8 of the withdrawal period, but had a normal serum TSH concentration (0.02 ng/mL). In addition, 1 dog in group 2 had elevated serum TSH concentrations (0.59–1.27 ng/mL) throughout the withdrawal period that persisted for 14 months after completion of the study, but normal serum T_4 and fT_4 concentrations. At no point during the withdrawal period did a study subject show clinical signs of hypothyroidism or have low serum T_4 and fT_4 with an elevated serum TSH concentration.

Discussion

Results of this study demonstrate that TSH secretion is suppressed during levothyroxine administration to euthyroid dogs, but the effect does not persist after discontinuation of treatment. The anticipated suppression of serum T_4 , fT_4 , T_3 , and TSH concentrations after up to 16 weeks of treatment with levothyroxine was not present. Therefore, thyroid function can be accurately investigated as early as 1 week after cessation of levothyroxine supplementation of similar dosage and duration.

Levothyroxine administration suppresses pituitary thyrotrope function and could result in atrophy as well as suppressed secretion of TSH. As a consequence of prolonged reduction in plasma TSH, thyroid gland atrophy can occur.^{15–17} Dogs in the present study showed little evidence of residual effects of the negative feedback of exogenous levothyroxine on the HPTA. Only 1 dog had a sustained effect potentially attributable to levothyroxine treatment, with increase in serum TSH concentrations above the reference interval (0.59–1.27 ng/mL) up to 16 weeks after discontinuing supplementation, with normal serum concentrations of T_4 , fT_4 , and T_3 . However, the serum TSH remained elevated when evaluated 14 months after the conclusion of the study. The sustained TSH increase is unlikely to be related to levothyroxine treatment but may represent developing lymphocytic thyroiditis. Another dog exhibited a low T_4 at 1 time point during the withdrawal period, but the concurrent TSH concentration was normal. At no point during the withdrawal period did a study dog exhibit a low serum T_4 or fT_4 and a high TSH concentration. This emphasizes the value of measuring serum T_4 and/or fT_4 and TSH concentrations concurrently when assessing thyroid function in euthyroid dogs inappropriately supplemented with levothyroxine. However, the HPTA may not be completely recovered in 1 week despite normalization of thyroid hormone concentrations and TSH.

Similar to a previous study of levothyroxine administration to euthyroid dogs, suppression of the HPTA was documented during the supplementation period in both groups.⁶ Suppression of TSH was quite marked in group 2 dogs, with all having a decrease in the TSH

concentration by more than 38%, which is the 1-sided critical different of the assay (i.e., estimate of the analyte's individual biological variation and the assay's imprecision).¹⁸ While dogs in the present study exhibited normalization of the HPTA 1 week after discontinuation of levothyroxine, the HPTA was suppressed for at least 4 weeks after cessation of supplementation in a previous study.⁶ The assessment of HPTA function using dynamic thyroid testing (TRH and TSH stimulation tests) may account for the difference because endogenous TSH concentrations may be less sensitive than tests of thyroid reserve. In addition, levothyroxine was administered at approximately twice the daily dosage in the previous study compared with the present one.

A significantly higher mean serum TSH concentration was noted 1, 4, and 8 weeks after withdrawal of levothyroxine in dogs treated for 16 weeks compared with those supplemented for 8 weeks. This is likely the result of thyroid gland atrophy as found in another study¹⁹ caused by suppression of TSH during more prolonged levothyroxine treatment. Because serum thyroid hormone concentrations were not suppressed after ceasing treatment, it is likely the elevated serum TSH concentration stimulated secretion of T₄ and T₃ from the thyroid gland sufficient to maintain normal function. This phenomenon has previously been shown histologically by an increase in pituitary thyrotrope number and size with concurrent high activity of the thyroid gland after withdrawal of levothyroxine.¹⁹ Therefore, levothyroxine administration for longer than 16 weeks may affect thyroid function tests to a greater degree than shorter duration supplementation.

The investigators chose to administer levothyroxine once daily based on the resolution of clinical abnormalities of hypothyroidism in dogs supplemented in a similar manner.^{20,21} Although once daily dosing results in more fluctuation in serum T₄ concentrations compared to twice daily dosing, the duration of action of T₄ is longer than its plasma half-life.²²⁻²⁶ In previous studies of the HPTA, dogs were administered levothyroxine twice daily, making comparisons difficult.^{6,14}

Humans can have marked suppression of the HPTA after thyroid hormone supplementation, with more protracted treatment associated with prolonged recovery that could require many months.^{10,11} It might be inappropriate to extrapolate findings in humans to dogs because the half-life of T₄ in humans is substantially longer than in dogs, which would cause a greater degree of both thyrotrope and thyroid gland atrophy. Additionally, the magnitude of TSH suppression in hypothyroid dogs supplemented with levothyroxine is directly correlated with the T₄ serum concentration.⁸ Because the degree of HPTA suppression is dependent on the dose and frequency of levothyroxine administration, this might explain the serum TSH, T₄, and fT₄ concentrations normalizing within 1 week after cessation of once daily administration of levothyroxine in the present study. Moreover, the findings of the present study cannot be extrapolated to dogs receiving levothyroxine supplementation at a different dose, frequency of

administration, duration, type of product or with non-thyroidal illness.

Shortcomings of the present study are that dogs acted as their own controls and using client owned animals introduced intrinsic differences in environment and husbandry. However, these effects may be more clinically applicable as it closely resembles the practice setting compared to facility owned and housed dogs. Compliance can contribute a variable that might affect hormone concentrations, particularly given the relatively short half-life of levothyroxine in the dog. To circumvent this problem, tablets were counted at every recheck appointment, which has been shown to be the most practical assessment of compliance²⁷ and the overall compliance was 98.4%. Moreover, once a day dosing demonstrates a higher degree of owner compliance compared to twice or 3 times a day dosing.²⁷ Additionally, the canine TSH assay is not sufficiently sensitive to determine when TSH is below the reference interval that would indicate excessive supplementation, which makes it difficult to assess the appropriateness of treatment. We used a wide range of acceptable serum T₄ concentrations that others have considered representative of adequate treatment in this study.²⁰ In humans where accurate and precise measurement of serum TSH is possible, it is used as a more appropriate marker of tissue thyroid hormone concentrations.

In conclusion, suppression of the HPTA occurred during levothyroxine supplementation for 8 or 16 weeks, with mean serum T₄, fT₄ and TSH concentrations returning to the reference interval by 1 week after discontinuation in both groups. It appears that assessing thyroid function tests 1 week after cessation of once daily levothyroxine supplementation will likely provide an accurate assessment of thyroid function in euthyroid dogs that are otherwise healthy.

Footnotes

^a Microsoft® Excel 2011

^b Soloxine®, Virbac AH, Inc, Fort Worth, TX

^c T₄ MAb Solid Phase Component System, MP Biomedicals, Diagnostics Division, Orangeburg, NY

^d Immulite 2000 Canine TSH, Siemens Healthcare Diagnostics, Llanberis, Gwynedd, UK

^e Free T₄ – by Equilibrium dialysis, Antech Diagnostics, Irvine, CA

^f SAS version 9.4, Cary, NC

^g Rick M, Refsal KR, Nachreiner RF, et al. Effects of dose of levothyroxine on serum concentrations of thyroid hormones and TSH in hypothyroid beagle dogs. 14th ECVIM-CA. Barcelona, Spain, 2004

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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