

## Evaluation of Serum Thyroid-Stimulating Hormone Concentration as a Diagnostic Test for Hyperthyroidism in Cats

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**Background:** In humans, measurement of serum thyroid-stimulating hormone (TSH) concentration is commonly used as a first-line discriminatory test of thyroid function. Recent reports indicate that canine TSH (cTSH) assays can be used to measure feline TSH and results can help diagnose or exclude hyperthyroidism.

**Objectives:** To investigate the usefulness of cTSH measurements as a diagnostic test for cats with hyperthyroidism.

**Animals:** Nine hundred and seventeen cats with untreated hyperthyroidism, 32 euthyroid cats suspected of having hyperthyroidism, and 131 clinically normal cats.

**Methods:** Prospective study. Cats referred to the Animal Endocrine Clinic for suspected hyperthyroidism were evaluated with serum T<sub>4</sub>, T<sub>3</sub>, free T<sub>4</sub> (fT<sub>4</sub>), and TSH concentrations. Thyroid scintigraphy was used as the gold standard to confirm or exclude hyperthyroidism.

**Results:** Median serum TSH concentration in the hyperthyroid cats (<0.03 ng/mL) was significantly ( $P < .001$ ) lower than concentrations in clinically normal cats (0.05 ng/mL) or euthyroid cats with suspected thyroid disease (0.06 ng/mL). Only 18 (2.0%) hyperthyroid cats had measurable TSH concentrations ( $\geq 0.03$  ng/mL), whereas 114 (69.9%) of the 163 euthyroid cats had detectable concentrations. Combining serum TSH with T<sub>4</sub> or fT<sub>4</sub> concentrations lowered the test sensitivity of TSH from 98.0 to 97.0%, but markedly increased overall test specificity (from 69.9 to 98.8%).

**Conclusions and Clinical Importance:** Serum TSH concentrations are suppressed in 98% of hyperthyroid cats, but concentrations are measurable in a few cats with mild-to-moderate hyperthyroidism. Measurement of serum TSH represents a highly sensitive but poorly specific test for diagnosis of hyperthyroidism and is best measured in combination with T<sub>4</sub> and fT<sub>4</sub>.

**Key words:** Feline; Hyperthyroid; Scintigraphy; Thyroid gland; Thyroid-stimulating hormone; Thyroxine.

The finding of a high serum total thyroxine (T<sub>4</sub>) concentration allows for a straightforward diagnosis of hyperthyroidism in most older cats presented with classical clinical features of the disease (eg, weight loss despite a good appetite, palpable goiter).<sup>1,2</sup> Cats with suspected hyperthyroidism routinely are screened by measuring T<sub>4</sub> because it is inexpensive and specific. However, up to 10% of all hyperthyroid cats, and over 30% of cats with early or mild hyperthyroidism, have T<sub>4</sub> concentrations that remain within the reference interval.<sup>2,3</sup> Concurrent nonthyroidal illness also can suppress high serum T<sub>4</sub> concentrations to within the reference interval.<sup>2,4–6</sup>

Serum free T<sub>4</sub> (fT<sub>4</sub>) concentrations are high in approximately 95% of cats with occult hyperthyroidism and can aid in the diagnosis.<sup>2,3,6</sup> However, the specificity of fT<sub>4</sub> is relatively poor, with up to 20% of sick (and some clinically normal) euthyroid cats having increased serum fT<sub>4</sub> concentrations.<sup>2,5,7</sup> Tests of the

### Abbreviations:

cTSH	canine TSH
fT <sub>4</sub>	free T <sub>4</sub>
HPT	hypothalamic-pituitary-thyroid
IQR	interquartile range
T/B	thyroid-to-background
T/S	thyroid-to-salivary
T <sub>3</sub>	triiodothyronine
T <sub>4</sub>	thyroxine
TSH	thyroid stimulating hormone

hypothalamic-pituitary-thyroid (HPT) axis (eg, T<sub>3</sub> suppression test) can help in diagnosis of occult hyperthyroidism but suffer from problems with owner compliance and equivocal test results.<sup>3,6,8</sup> Consequently, quantitative thyroid scintigraphy, with calculation of thyroid-to-salivary and thyroid-to-background ratios, is considered the most accurate test for occult hyperthyroidism in cats.<sup>9</sup> Unfortunately, few veterinarians have access to scintigraphic equipment, and simpler ways of diagnosing hyperthyroidism are needed in cats with occult disease.

In human patients, measurement of serum thyrotropin (thyroid stimulating hormone, TSH) concentration is performed routinely as the first-line test of thyroid function and is the single best screening test for overt and subclinical hyperthyroidism.<sup>10,11</sup> Physiologically, even slight increases in circulating T<sub>4</sub> and T<sub>3</sub> will suppress pituitary TSH secretion through the negative feedback loop of the HPT axis, leading to low or undetectable serum TSH concentrations.<sup>12,13</sup> Recent development of ultrasensitive human TSH assays that can clearly distinguish truly low from low-normal concentrations has facilitated diagnosis of patients with mild and occult (subclinical) hyperthyroidism.<sup>10,11</sup>

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Submitted January 4, 2015; Revised June 18, 2015; Accepted June 23, 2015.

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DOI: 10.1111/jvim.13585

Although feline TSH has been cloned,<sup>14</sup> a commercial feline-specific TSH assay is not available. However, commercial assays for measuring canine TSH (cTSH) exist, and some of these are reported to detect feline TSH, potentially assisting in the diagnosis of feline hyperthyroidism.<sup>5,15–18</sup> The cTSH assays cannot accurately distinguish between low-normal and subnormal concentrations in cats; however, all hyperthyroid cats reported to date have had undetectable TSH concentrations with these assays, whereas most normal and sick euthyroid cats have had measurable TSH concentrations.<sup>5,15</sup> Indeed, some investigators have suggested that a measurable TSH concentration effectively rules out hyperthyroidism in equivocal cases, especially in sick euthyroid cats.<sup>16,17</sup>

We sought to investigate the usefulness of serum TSH concentrations in diagnosing hyperthyroidism in a large population of cats with hyperthyroidism of variable severity and in differentiating these cats from healthy cats and euthyroid cats suspected of having hyperthyroidism. To this end, we compared the test sensitivity and specificity of serum TSH concentrations, alone and in combination with that of other commonly used tests of thyroid function (ie, T<sub>4</sub>, T<sub>3</sub>, and fT<sub>4</sub>).

## Materials and Methods

### Study Design and Selection of Animals

This prospective study was conducted from June 2012 to April 2015, and included all cats referred to the Animal Endocrine Clinic for evaluation of suspected hyperthyroidism before radioiodine treatment. This evaluation included a history, physical examination, routine laboratory testing (CBC and serum biochemistry profile), and complete serum thyroid panel (T<sub>4</sub>, T<sub>3</sub>, fT<sub>4</sub>, and TSH concentrations). All cats underwent quantitative thyroid scintigraphy, which was used as the reference standard to classify cats as either euthyroid or hyperthyroid (Data S1). All cats then were assigned to 1 of 3 groups, as follows:

**Hyperthyroid Cats (n = 917).** In these cats, the tentative diagnosis of hyperthyroidism had been established by the referring veterinarian on the basis of consistent clinical signs (eg, weight loss despite a good appetite), palpable thyroid nodule(s) on physical examination, and high serum T<sub>4</sub> or fT<sub>4</sub> concentrations (or both). Of these cats, 494 (53.9%) had never received methimazole, whereas 423 (46.1%) had been treated with methimazole for periods of 1 week to 7 years. In all methimazole-treated cats, the drug was discontinued  $\geq 1$  week before evaluation. All of these cats had scintigraphic evidence of hyperthyroidism, with unilateral, bilateral, or multifocal nodular enlargement (ie, “hot” thyroid nodules) and lack of radionuclide uptake into normal thyroid tissue.<sup>9</sup> Additionally, all cats had high thyroid-to-salivary gland (T/S) ratios, high thyroid-to-background (T/B) ratios, or both.<sup>9</sup>

**Cats with Suspected Hyperthyroidism (n = 32).** These cats were referred for evaluation of hyperthyroidism because of suggestive clinical signs (eg, weight loss despite good appetite, chronic diarrhea), physical examination findings (eg, palpable mass in cervical region), or abnormal thyroid function tests (eg, high fT<sub>4</sub> concentration). However, these cats were considered euthyroid based on the absence of any “hot” scintigraphic thyroid nodules as well as T/S and T/B ratios that both were within the respective reference intervals.<sup>9</sup>

**Clinically Normal, Euthyroid Cats (n = 131).** These cats were recruited as controls to establish reference intervals for serum thy-

roid hormone and TSH concentrations. To be included in this control group, cats had to be  $\geq 7$  years of age and considered healthy based on history, physical examination (ie, none had palpable thyroid nodules), CBC, serum biochemistry profile, and urinalysis. Thyroid scintigraphy was normal in all cats, with findings similar to those of the euthyroid cats with suspected hyperthyroidism.

### Assays for Thyroid Hormone and Thyrotropin (TSH) Concentrations

Blood samples were collected from all cats to determine baseline serum total T<sub>4</sub>, T<sub>3</sub>, fT<sub>4</sub>, and TSH concentrations, immediately before the injection of pertechnetate for thyroid scintigraphy. All blood samples were centrifuged within 1 hour after collection; serum was separated and stored at 4°C until assayed by a commercial laboratory the next day.<sup>a</sup>

Serum total T<sub>4</sub> concentration was measured by a homogenous enzyme immunoassay (EIA)<sup>b</sup> on an automated biochemistry analyzer.<sup>c</sup> Serum total T<sub>3</sub> concentration was measured by chemiluminescent enzyme immunoassay (CEIA).<sup>d</sup> Serum fT<sub>4</sub> concentration was measured by the use of a validated,<sup>2</sup> commercially available kit<sup>e</sup> that separates protein-bound and fT<sub>4</sub> by equilibrium dialysis and measures fT<sub>4</sub> concentration in the dialysate by radioimmunoassay. Feline TSH was measured by CEIA<sup>f</sup> using a validated cTSH assay.<sup>5,15,17</sup> All of the CEIA assays were run on a commercial immunoassay system.<sup>g</sup> We also validated the T<sub>4</sub>, T<sub>3</sub>, fT<sub>4</sub>, and cTSH assays for use on cats at our clinic (described in Data S2).

### Data and Statistical Analyses

Data were assessed for normality by the D’Agostino–Pearson test and by visual inspection of graphical plots.<sup>19</sup> Data were not normally distributed; therefore, all analyses used nonparametric tests.<sup>20</sup> Results are reported as median (IQR, 25<sup>th</sup>–75<sup>th</sup> percentile) and are represented graphically as box-and-whisker plots. For all analyses, statistical significance was defined as  $P \leq .05$ .

Reference intervals for hormone concentrations were established by the nonparametric method of percentile estimates with confidence intervals to determine the central 95th percentile interval (ie, 2.5 through 97.5th percentile range) for results from clinically normal cats.<sup>21</sup> Reference intervals thus determined were: T<sub>4</sub> = 0.9–3.8  $\mu\text{g/dL}$ ; T<sub>3</sub> = 35–120  $\text{ng/dL}$ ; fT<sub>4</sub> = 10–51  $\text{pmol/L}$ ; and cTSH = <0.03–0.3  $\text{ng/mL}$ .

All statistical analyses were performed using proprietary statistical software.<sup>h,i</sup> Undetectable serum TSH concentrations were defined as <0.03  $\text{ng/mL}$  and detectable TSH concentrations as  $\geq 0.03$   $\text{ng/mL}$ ; all undetectable serum TSH concentrations were assigned an arbitrary concentration of 0.02  $\text{ng/mL}$  for data analysis, as previously described.<sup>17</sup>

The 917 hyperthyroid cats were further categorized into quintiles of disease severity based on total T<sub>4</sub> concentration (ie, mild, mild-to-moderate, moderate, moderate-to-severe, and severe disease).<sup>22</sup> This was done to more closely examine serum TSH and thyroid hormone concentrations in the cats with milder degrees of hyperthyroidism, because these cats represent a greater diagnostic challenge.

To examine for potential continued effects of prior methimazole treatment on thyroid function and serum hormone concentrations, hyperthyroid cats also were categorized as naïve (no methimazole treatment) and methimazole-treated.

The Kruskal–Wallis test, followed by the Dunn multiple comparisons test, was used to determine whether serum hormone concentrations differed among cats with hyperthyroidism, euthyroid cats with suspected thyroid disease, and normal cats. The Mann–Whitney test was used to determine if hormone concentrations dif-

ferred between naïve and methimazole-treated cats. The Chi-square test was used to determine differences in prevalence of measurable serum TSH concentrations between naïve and methimazole-treated cats, as well as to determine differences in prevalence of measurable serum TSH concentrations in the hyperthyroid cats divided into quintiles of disease severity. Chi-square testing also was used to determine if differences in proportions of disease severity groups existed between naïve and methimazole-treated cats.

Sensitivity and specificity were calculated for each hormone ( $T_4$ ,  $T_3$ ,  $fT_4$ , and  $cTSH$ ) and for selected combinations of test results ( $T_4$  plus TSH,  $fT_4$  plus TSH,  $T_4$  or  $fT_4$  plus TSH). The McNemar's test<sup>23</sup> was used to determine whether differences existed between the sensitivity of TSH to that of  $T_4$ ,  $T_3$ , or  $fT_4$  concentrations as diagnostic tests for hyperthyroidism in cats.

## Results

### Animals

**Hyperthyroid Cats ( $n = 917$ ).** The cats ranged in age from 6 to 19 years (median, 12.0 years; IQR, 11 to 14 years). Breeds included domestic longhair and shorthair (835 cats), Siamese (23), Maine Coon (19), Russian Blue (9), Ragdoll (5), Tonkinese (5), Persian (4), Burmese (3), Abyssinian (2), American Shorthair (2), Bengal (2), Birman (2), Norwegian Forest Cat (2), and 4 miscellaneous breeds (1 cat each). Of these, 475 (51.8%) were female and 442 were male; all had been neutered.

When subdivided into quintiles based on disease severity (determined by the serum  $T_4$  concentration), 184 cats had mild hyperthyroid disease ( $T_4 \leq 5.2$   $\mu\text{g/dL}$ ), 183 had mild-to-moderate disease ( $T_4: 5.2\text{--}7.1$   $\mu\text{g/dL}$ ), 183 had moderate disease ( $T_4: 7.2\text{--}10$   $\mu\text{g/dL}$ ), 183 had moderate-to-severe disease ( $T_4: 10.1\text{--}14.9$   $\mu\text{g/dL}$ ), and 184 had severe disease ( $T_4 \geq 15$   $\mu\text{g/dL}$ ).

Of the 917 cats, 494 (53.9%) were naïve to methimazole treatment, and 423 (46.1%) cats had been treated with methimazole for periods of 1 week to 7 years (median, 60 days; IQR, 30–300 days). In all of the methimazole-treated cats, the drug was discontinued  $\geq 1$  week (median, 10 days; IQR, 10–10 days, range, 7–180 days) before evaluation.

**Euthyroid Cats with Suspected Hyperthyroidism ( $n = 32$ ).** The euthyroid cats with suspected hyperthyroidism ranged in age from 5 to 19 years (median, 12.0 years; IQR, 9.3–13 years). Breeds included domestic longhair and shorthair (23 cats), Maine Coon (2 cats) and Abyssinian, Balinese, Chartreux, Persian, Ragdoll, Russian Blue, and Scottish Fold (1 cat each). Of these, 16 (50%) were female and 16 were male; all had been neutered.

These cats were referred for 2 distinct reasons. The first subgroup of 23 cats was referred because of high serum concentrations of  $T_4$  (2 cats),  $fT_4$  (20 cats), or both (1 cat), which had either been detected during routine health monitoring ( $n = 11$ ) or during diagnostic evaluation of primary gastrointestinal disease (eg, chronic diarrhea;  $n = 12$ ). In 9/11 cats referred for a high  $T_4$  or  $fT_4$  concentration on routine screening, normal serum  $T_4$ ,  $T_3$ , and  $fT_4$  concentrations were found on repeat testing; 1 cat had persistently high serum  $T_4$  (4.0  $\mu\text{g/dL}$ ) and  $fT_4$  (61 pmol/L) concentrations and 1

had a persistently high serum  $T_4$  (4.1  $\mu\text{g/dL}$ ) concentration alone, but neither had a palpable thyroid nodule and both had unremarkable thyroid scintigraphy. Of the 12 cats with gastrointestinal disease, 7 had normal serum  $T_4$ ,  $T_3$ , and  $fT_4$  concentrations on repeat testing, whereas 4 had persistently high  $fT_4$  concentrations (55–74 pmol/L) and 2 had slightly high  $T_4$  concentrations (both at 3.9  $\mu\text{g/dL}$ ). None of the 12 cats with gastrointestinal disease had a palpable thyroid nodule or abnormal thyroid scintigraphy. The final diagnosis in this group of 23 euthyroid cats included the following: clinically normal ( $n = 11$ ), inflammatory bowel disease ( $n = 8$ ), gastrointestinal lymphosarcoma ( $n = 2$ ), and pancreatitis ( $n = 2$ ).

The second subgroup ( $n = 9$ ) was referred for evaluation of a palpable cervical mass, associated with normal serum concentrations of both  $T_4$  and  $fT_4$ . In 5/9 cats, the palpable nodule was very small and scintigraphy identified 2 normal thyroid lobes (ie, normal size, shape, and intensity of radionuclide uptake). In 4/9 cats, the cervical mass was very large but did not display radionuclide uptake on scintigraphy. The final diagnosis in these 4 cats included thyroglossal cyst, lymphoma, and nonfunctional thyroid carcinoma ( $n = 2$ ) respectively.

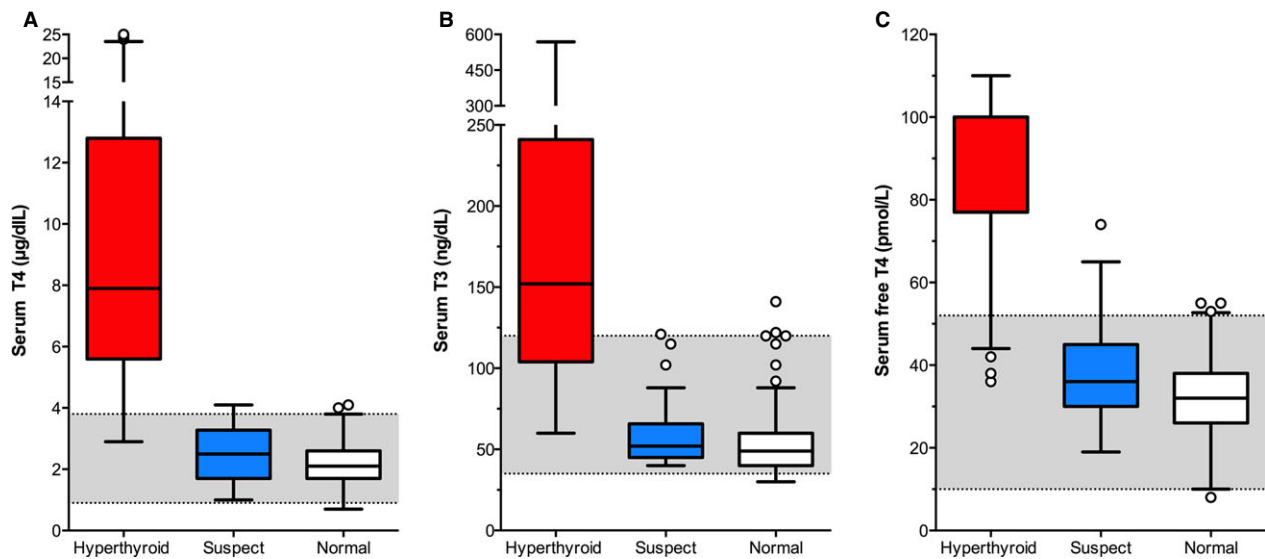
**Clinically Normal, Euthyroid Cats ( $n = 131$ ).** These cats ranged in age from 7 to 18 years (median, 9.0 years; IQR, 8–13 years). Breeds included domestic longhair and shorthair (118 cats), American Shorthair (2 cats), Siamese (2 cats), Abyssinian, Bengal, Burmese, Chartreux, Maine Coon, Ragdoll, Russian Blue, Scottish Fold, and Tonkinese (1 cat each). Of these cats, 68 (51.9%) were female and 63 were male; all had been neutered.

### Serum Thyroid Hormone Concentrations

**Serum  $T_4$  Concentrations.** The serum  $T_4$  concentrations in hyperthyroid cats were significantly ( $P < .001$ ) higher than in clinically normal or euthyroid suspect cats (Fig 1A; Table 1). Cats with even mild hyperthyroidism had significantly higher serum  $T_4$  concentrations than clinically normal or euthyroid suspect cats ( $P < .001$ ). Serum  $T_4$  concentrations did not differ between clinically normal and suspect euthyroid cats ( $P = .063$ ).

Eight hundred and seventy-three (95.2%) hyperthyroid cats had high serum  $T_4$  concentrations, and 44 (4.8%) had serum  $T_4$  concentrations within the reference interval (Fig 1A; Table 2). These 44 cats accounted for 23.9% of the cats in the mild hyperthyroid group. Of the 32 euthyroid cats in which hyperthyroidism initially was suspected, 4 (12.5%) had serum  $T_4$  concentrations that exceeded the reference interval (3.9, 3.9, 4.0, and 4.1  $\mu\text{g/dL}$ ), whereas 12 had serum  $T_4$  concentrations in the upper half of the reference interval (ie, between 2.5 and 3.8  $\mu\text{g/dL}$ ; Fig 1A; Table 2).

The serum  $T_4$  concentrations in the 423 methimazole-treated cats were significantly ( $P < .001$ ) higher than the serum  $T_4$  concentrations in the 494 naïve hyperthyroid cats (Table 3). However, when cats were compared within



**Fig 1.** Box plots of serum thyroid hormone concentrations in 917 cats with untreated hyperthyroidism, 32 euthyroid cats suspected of having hyperthyroidism but without the disease, and 131 clinically normal cats. (A) serum T<sub>4</sub>; (B) serum T<sub>3</sub>; and (C) serum free T<sub>4</sub> by dialysis. Boxes represent the interquartile range from the 25th to 75th percentile. The horizontal bar in each box represents the median value. The T-bars represent the main body of data, which in most instances is equal to the range. Outlying data points are represented by open circles. The shaded area indicates the reference interval.

**Table 1.** Median (IQR) serum concentrations of T<sub>4</sub>, T<sub>3</sub>, fT<sub>4</sub>, and TSH in 917 cats with untreated hyperthyroidism (divided into 5 quintiles of disease severity based on T<sub>4</sub> concentrations), 32 euthyroid cats suspected of having hyperthyroidism but without the disease, and 131 clinically normal cats.

Cat Group	Serum T <sub>4</sub> (µg/dL)	Serum T <sub>3</sub> (ng/dL)	Serum fT <sub>4</sub> (pmol/L)	Serum TSH (ng/mL)
Hyperthyroid (917)	7.9 (5.6–12.8)	152 (104–241)	100 (77–100)	0.02 (0.02–0.02)
Mild (184)	4.4 (3.9–4.8)	91 (70–115)	67 (56–76)	0.02 (0.02–0.02)
Mild-to-moderate (183)	6.1 (5.6–6.5)	113 (95–135)	85 (70–100)	0.02 (0.02–0.02)
Moderate (183)	7.9 (7.5–9.1)	154 (121–187)	100 (86–100)	0.02 (0.02–0.02)
Moderate-to-severe (183)	11.6 (10.7–12.8)	213 (175–259)	100 (100–100)	0.02 (0.02–0.02)
Severe (184)	20.5 (17.9–23.0)	373 (299–492)	100 (100–100)	0.02 (0.02–0.02)
Euthyroid (suspect) (32)	2.5 (1.7–3.3) <sup>a</sup>	52 (45–66) <sup>a</sup>	36 (30–45) <sup>a</sup>	0.06 (0.04–0.09) <sup>a</sup>
Clinically normal (131)	2.1 (1.7–2.6) <sup>a</sup>	49 (40–60) <sup>a</sup>	32 (26–38) <sup>a</sup>	0.04 (0.02–0.07) <sup>a,b</sup>

<sup>a</sup>Median concentration statistically different ( $P < .01$ ) from all hyperthyroid groups.

<sup>b</sup>Median concentration statistically different ( $P < .05$ ) from euthyroid suspect group.

Reference intervals: T<sub>4</sub> = 0.9–3.8 µg/dL; T<sub>3</sub> = 35–120 ng/dL; fT<sub>4</sub> = 10–51 pmol/L; and cTSH = <0.03–0.3 ng/mL.

quintiles of disease severity, only naïve cats in the most severe group had lower concentrations than similarly diseased methimazole-treated cats (Table 3; Data S3).

**Serum T<sub>3</sub> Concentrations.** The serum T<sub>3</sub> concentrations in hyperthyroid cats were significantly ( $P < .001$ ) higher than in clinically normal and euthyroid suspect cats (Fig 1B; Table 1). When the hyperthyroid cats were divided into quintiles of disease severity, the serum T<sub>3</sub> concentrations in each group were significantly higher than the concentrations in the clinically normal or euthyroid suspect cats. Serum T<sub>3</sub> concentrations did not differ between clinically normal and suspect euthyroid cats ( $P = .20$ ).

Five hundred and ninety-nine (65.3%) hyperthyroid cats had high serum T<sub>3</sub> concentrations, and 318 (34.7%) had serum T<sub>3</sub> concentrations within the reference interval (Fig 1B; Table 2). Of the cats with mild hyperthyroidism, 146 (79.3%) had serum T<sub>3</sub> concentrations within the reference interval ( $\leq 120$  ng/dL),

whereas 113 (61.7%) of the cats with mild-to-moderate disease and 45 (24.6%) of those with moderate disease had concentrations within the reference interval (Table 2). Only one of the euthyroid suspect cats had a borderline serum T<sub>3</sub> concentration (121 ng/dL), and 3 had T<sub>3</sub> concentrations in the upper half of the reference interval (ie, between 80 and 120 ng/dL; Fig 1B).

The serum T<sub>3</sub> concentrations in the methimazole-treated cats were significantly ( $P < .001$ ) higher than the serum T<sub>3</sub> concentrations in the naïve hyperthyroid cats (Table 3; Data S3).

**Serum fT<sub>4</sub> Concentrations.** The serum fT<sub>4</sub> concentrations in hyperthyroid cats were higher than the concentrations in clinically normal and euthyroid suspect cats ( $P < .001$ ; Fig 1C; Table 1). When the hyperthyroid cats were divided into quintiles of disease severity, the serum fT<sub>4</sub> concentrations in each group were higher than in the clinically normal or euthyroid suspect cats.

**Table 2.** Calculation of diagnostic test sensitivity and specificity for serum concentrations of T<sub>4</sub>, T<sub>3</sub>, free T<sub>4</sub> (fT<sub>4</sub>), and TSH in 917 cats with untreated hyperthyroidism (divided into 5 quintiles of disease severity based on T<sub>4</sub> concentrations), 32 euthyroid cats suspected of having hyperthyroidism but without the disease, and 131 clinically normal cats.

Hormone	Sensitivity (95% CI)					Specificity (95% CI)			
	Hyperthyroid (917)	Mild (184)	Mild-to-Moderate (183)	Moderate (183)	Moderate-to-Severe (183)	Severe (184)	Hyperthyroid Suspect (32)	Clinically Normal (131)	All Euthyroid Cats (163)
Serum T <sub>4</sub>	95.2% (93.4–96.5%)	76.1% (69.3–82.1)	100% (97.9–100)	100% (97.9–100)	100% (97.9–100)	100% (97.9–100)	87.5% (71.0–96.4)	98.5% (94.6–99.8)	96.3% (92.2–98.6)
Serum T <sub>3</sub>	65.3% (62.1–68.4%)	20.7% (15.1–27.2)	38.3% (31.2–45.7)	75.40% (69.1–81.5)	92.4% (87.5–95.8)	100% (98.0–100)	96.9% (83.7–99.5)	98.5% (94.6–99.8)	98.2% (94.7–99.6%)
Serum fT <sub>4</sub>	96.8% (95.5–97.9%)	85.3% (79.4–90.1)	98.9% (96.1–99.8)	100% (98.0–100)	100% (98.0–100)	100% (98.0–100)	84.4% (67.2–94.6)	97.7% (93.4–99.5)	95.1% (90.6–97.9)
Serum TSH	98.0% (96.9–98.8)	94.6% (90.2–97.4)	97.8% (94.5–99.4)	98.4% (95.3–99.6)	99.5% (97.0–99.9)	100% (98.0–100)	84.4% (67.2–94.6)	66.4% (57.6–74.4)	69.9% (62.3–76.9)
T <sub>4</sub> + fT <sub>4</sub>	93.2% (91.4–94.8)	67.9% (30.7–74.6)	98.9% (96.1–99.8)	99.5% (98.0–100)	100% (98.0–100)	100% (98.0–100)	93.8% (79.2–99.1)	99.2% (95.8 + 99.9)	98.2% (94.7–99.6)
T <sub>4</sub> + TSH	93.4% (91.5–94.9)	71.2% (64.1–77.6)	97.8% (94.5–99.4)	98.4% (95.3–99.6)	99.5% (97.0–99.9)	100% (98.0–100)	96.9% (83.7–99.5)	100% (97.2–100)	99.4% (96.6–99.9)
T <sub>3</sub> + TSH	65.1% (61.9–68.2)	20.7% (15.1–27.2)	38.3% (31.2–45.7)	74.9% (67.9–81.0)	91.8% (86.8–95.3)	100% (98.0–100)	96.9% (83.7–99.5)	99.2% (95.8–99.9)	98.8% (95.6–99.8)
fT <sub>4</sub> + TSH	95.4% (93.9–96.7)	82.6% (76.3–87.8)	96.7% (93.0–98.8)	98.4% (95.3–99.6)	99.5% (97.0–99.9)	100% (98.0–100)	100% (89.0–100)	99.2% (95.8–99.9)	99.4% (96.6–99.8)
(T <sub>4</sub> or fT <sub>4</sub> ) + TSH	97.0% (95.6–98.0)	89.1% (83.7–93.2)	97.8% (94.5–99.4)	98.4% (95.3–99.6)	99.5% (97.0–99.9)	100% (98.0–100)	96.9% (83.7–99.5)	99.2% (95.8–99.9)	98.8% (95.6–99.8)

95% CI = 95% confidence intervals.

Serum fT<sub>4</sub> concentrations did not differ between clinically normal and suspect euthyroid cats ( $P = .10$ ; Fig 1C; Table 1).

Eight hundred and eighty-eight (96.8%) hyperthyroid cats had high serum fT<sub>4</sub> concentrations, whereas 29 (3.2%) had serum fT<sub>4</sub> concentrations within the reference interval (Fig 1C; Table 2). Of the 184 cats with mild hyperthyroidism, 27 (14.7%) had serum fT<sub>4</sub> concentrations within the reference interval ( $\leq 51$  pmol/L). Of the 32 euthyroid suspect cats, 5 (15.6%) had high serum fT<sub>4</sub> concentrations (Fig 1C).

The serum fT<sub>4</sub> concentrations in the 423 methimazole-treated cats were higher than in the 494 naïve hyperthyroid cats ( $P < .001$ ; Table 3; Data S3).

### Serum TSH Concentrations

The serum TSH concentrations in hyperthyroid cats were lower than in clinically normal and euthyroid suspect cats ( $P < .001$ ; Fig 2A; Table 1). When the hyperthyroid cats were divided into quintiles of disease severity, the serum TSH concentrations in each group were significantly lower than in clinically normal or euthyroid suspect cats. The serum TSH concentrations in the euthyroid suspect cats were higher than in clinically normal cats ( $P < .05$ ; Fig 2A; Table 1; Data S4).

Only 18 (2.0%) of the hyperthyroid cats had measurable serum TSH concentrations, whereas 899 (98.0%) had serum TSH concentrations below the limit of detection. In contrast, serum TSH concentrations were detectable in 87 (66.4%) clinically normal cats and in 27 (84.4%) euthyroid suspect cats (Fig 2A; Table 2). The prevalence of detectable serum TSH concentrations in the euthyroid suspect cats was higher than in clinically normal cats ( $P < .05$ ; Data S4).

Of the 18 hyperthyroid cats with detectable serum TSH concentrations, 10 had mild disease, 4 had mild-to-moderate disease, 3 had moderate disease, and 1 had moderate-to-severe hyperthyroidism. None of the cats with severe thyroid disease had measurable serum TSH concentrations (Fig 2B). A higher proportion of cats with mild disease (5.4%) had detectable serum TSH concentrations than cats with mild-to-moderate (2.2%), moderate (1.6%), and moderate-to-severe disease (0.5%;  $P < .05$ ). Only 2 (11.1%) of these 18 cats had high serum T<sub>3</sub> concentrations; all of the cats with mild and mild-to moderate hyperthyroidism had serum T<sub>3</sub> concentrations that were within the reference interval. Of the 18 cats with detectable serum TSH concentrations, only 1 had a serum T<sub>4</sub> concentration within the reference interval, and 4 had serum fT<sub>4</sub> concentrations that were within the reference interval; all 5 of these cats were in the mild hyperthyroid group.

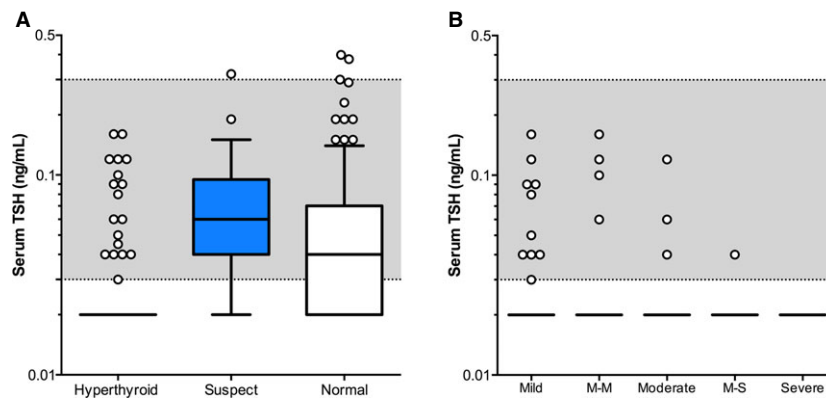
The serum TSH concentrations in the methimazole-treated cats did not differ from those in naïve hyperthyroid cats (Table 3). Of the 18 cats with measurable serum TSH concentrations, 9 had been treated with methimazole, but the drug had been discontinued 7–80 days before testing (median, 45 days; IQR, 14–60 days). Of the 899 hyperthyroid cats with undetectable serum TSH concentrations, 415 (46.2%) had

**Table 3.** Median (IQR) serum concentrations of T<sub>4</sub>, T<sub>3</sub>, fT<sub>4</sub>, and TSH in 917 cats with hyperthyroidism separated into 2 groups based on history of prior treatment with methimazole.

Cat Group	Serum T <sub>4</sub> (µg/dL)	Serum T <sub>3</sub> (ng/dL)	Serum fT <sub>4</sub> (pmol/L)	Serum TSH (ng/mL)
No methimazole (494)	7.3 (5.2–11.2)	137 (100–215)	99 (73–100)	0.02 (0.02)
Mild (121)	4.4 (3.9–4.8)	94 (69–117)	67 (56–76)	0.02 (0.02)
Mild-moderate (117)	6.1 (5.6–6.4)	114 (97–135)	85 (68–100)	0.02 (0.02)
Moderate (94)	7.9 (7.4–9.0)	152 (120–189)	100 (86–100)	0.02 (0.02)
Moderate-severe (104)	11.6 (10.9–12.8)	219 (182–269)	100 (100–100)	0.02 (0.02)
Severe (58)	19.8 (17.2–21.0)	340 (272–419)	100 (100–100)	0.02 (0.02)
Methimazole (423)	9.7 (6.4–16.6) <sup>a</sup>	172 (113–310) <sup>a</sup>	100 (84–100) <sup>a</sup>	0.02 (0.02)
Mild (63)	4.5 (3.7–4.8)	87 (75–112)	67 (59–76)	0.02 (0.02)
Mild-moderate (66)	6.2 (5.6–6.7)	112 (88–135)	86 (72–100)	0.02 (0.02)
Moderate (89)	8.0 (7.6–9.0)	155 (126–178)	100 (87–100)	0.02 (0.02)
Moderate-severe (79)	11.5 (10.6–12.7)	203 (156–259)	100 (100–100)	0.02 (0.02)
Severe (126)	20.8 (18.2–23.0) <sup>b</sup>	406 (309–513) <sup>b</sup>	100 (100–100)	0.02 (0.02)

<sup>a</sup>Median concentration of entire treated group significantly higher ( $P < .001$ ) than concentration in entire untreated group.

<sup>b</sup>Median concentration of treated group significantly higher ( $P < .05$ ) than concentration of same disease severity category in untreated group.



**Fig 2.** Box plots of serum thyroid-stimulating hormone (TSH) concentrations. (A) TSH concentrations in 917 cats with untreated hyperthyroidism, 32 euthyroid cats suspected of having hyperthyroidism but without the disease, and 131 clinically normal cats. (B) Serum TSH concentrations in the 917 cats with untreated hyperthyroidism, divided into 5 quintiles of mild, mild-to-moderate (M-M), moderate, moderate-to-severe (M-S), and severe disease on the basis of the total T<sub>4</sub> concentration. For all hyperthyroid groups, the horizontal bar represents the median value (0.02 ng/mL), as well as the interquartile range (0.02–0.02 ng/mL). The shaded area spans from the lower limit of detection (0.03 ng/dL) to the upper limit of the reference interval (0.3 ng/mL). All of the open circles represent outlying data points.

been treated with methimazole, which had been discontinued 7–180 days before evaluation. There was no obvious relationship between the finding of a measurable serum TSH concentration, methimazole treatment, or the time from discontinuing the drug and measuring the serum TSH concentration.

Overall, the sensitivity of the serum TSH concentration (positive test result = TSH concentration <0.03 ng/mL) as a diagnostic test for hyperthyroidism was higher than test sensitivity for T<sub>4</sub> or T<sub>3</sub> ( $P < .001$ ; Table 2), but not different from that of fT<sub>4</sub> ( $P = .39$ ; Table 2). Using combined data from the 163 euthyroid cats, the specificity of the serum TSH concentration was lower than the specificity of the T<sub>4</sub>, T<sub>3</sub>, or fT<sub>4</sub> concentrations (Table 2;  $P < .001$ ). Compared to serum TSH concentration alone, combining the results of a high T<sub>4</sub> plus undetectable TSH concentrations, a high fT<sub>4</sub> plus undetectable TSH concentrations, or a positive result of either a high T<sub>4</sub> or fT<sub>4</sub> plus undetectable TSH

concentrations minimally affected the test sensitivity but markedly improved the overall test specificity (Table 2).

## Discussion

Our results indicate that measurement of serum TSH concentration is a very sensitive, but nonspecific, diagnostic test for feline hyperthyroidism, with approximately 98% of hyperthyroid cats having serum TSH concentrations that are suppressed below the limit of quantification (<0.03 ng/mL). However, approximately 30% of older euthyroid cats also had undetectable serum TSH concentrations. Unfortunately, the current commercial canine TSH assay (a first-generation assay) used in this study cannot accurately measure concentrations low enough to clearly distinguish between the low-normal serum TSH concentrations found in some euthyroid cats from the truly low or totally suppressed

concentrations found in most hyperthyroid cats.<sup>5,15–17</sup> Our results, however, demonstrate that testing in parallel by combining serum TSH concentration with either  $T_4$  or  $fT_4$  concentrations improves the ability to correctly differentiate hyperthyroid cats with occult or mild disease from euthyroid cats suspected of having thyroid disease, especially when serum concentrations of  $T_4$ ,  $T_3$ , or  $fT_4$  are within the upper limits of their reference interval or are only marginally increased. Namely, finding undetectable serum TSH concentration with either a high  $T_4$  or high  $fT_4$  concentration markedly improves the overall diagnostic accuracy in this subset of hyperthyroid cats. Conversely, finding a measurable serum TSH concentration, even with high-normal  $T_4$  or  $fT_4$  concentrations, makes hyperthyroidism extremely unlikely. Thus, clinicians should consider combinatorial testing in cats with equivocal disease.

Encouragingly, the diagnostic test sensitivity and specificity for serum concentrations of  $T_4$ ,  $T_3$ , and  $fT_4$  found in this study were almost identical to results we reported in hyperthyroid and sick euthyroid cats more than a decade ago.<sup>2</sup> The total  $T_4$  test, with its relatively high sensitivity and specificity remains the initial screening test of choice for hyperthyroidism in cats.<sup>1–3</sup> Serum  $T_3$  measurements are less useful for diagnosis because of the fact that one-third of hyperthyroid cats maintain normal serum  $T_3$  concentrations, despite having clearly high total and  $fT_4$  concentrations.<sup>1–3</sup> The  $fT_4$  (equilibrium dialysis) test has very high sensitivity but low specificity (approximately 15% false-positive cats in this study). The clinical issues associated with the low test specificity of the  $fT_4$  test are highlighted in our subgroup of 21 euthyroid cats referred for radioiodine treatment because of a high serum  $fT_4$  concentration alone. None of these cats had a palpable thyroid nodule and all had normal results on thyroid scintigraphy, thus confirming that they were not hyperthyroid.

Over half of our hyperthyroid cats with detectable serum TSH concentrations ( $\geq 0.03$  ng/mL) had mild hyperthyroidism, suggesting that the hyperthyroidism was not severe or chronic enough to completely suppress pituitary TSH secretion. Accordingly, detectable serum TSH concentrations also have been reported rarely in mildly hyperthyroid human patients.<sup>11</sup> Nine of our 18 cats with measurable serum TSH concentrations had been treated with methimazole, which could have normalized TSH concentrations by the drug's action to lower high circulating  $T_4$  and  $T_3$  concentrations, thereby removing the negative-feedback inhibition on pituitary TSH secretion.<sup>13,24</sup> However, methimazole had been discontinued for at least a week before TSH testing (median time, 45 days), and all 9 of these cats had clearly high serum  $T_4$  and  $fT_4$  concentrations at the time of our evaluation. Such increases in thyroid hormone concentrations should rapidly lower serum TSH concentrations by negative feedback suppression of the HPT axis. Again, it could be argued that the minimum withdrawal time of 1 week off methimazole might have been inadequate for TSH suppression to redevelop (see Data S3 for more discussion). However, high serum thyroid hormone concentrations will suppress TSH

fairly quickly in cats, based on the standard protocols used for the 3-day  $T_3$  suppression test.<sup>25</sup> In addition, the fact that over 98% of our hyperthyroid cats had undetectable serum TSH concentrations, approximately half of which also had been treated with methimazole, suggests that prior methimazole treatment may not necessarily account for the detectable serum TSH concentrations observed in a minority of treated cats. Other factors, such as laboratory error or assay variability, also must be considered. However, the measurable serum TSH concentrations in our 18 hyperthyroid cats were verified by repeat analysis in all cases, generally with a new serum sample assayed on a different day. Again, the most likely explanation may simply be that a few hyperthyroid cats with milder, less chronic forms of the disease may not develop complete serum TSH suppression at the time of diagnosis.

Many veterinarians mistakenly believe that the finding of a high  $fT_4$  concentration alone, especially when the total  $T_4$  concentration is within the upper half of the reference interval, confirms a diagnosis of hyperthyroidism in cats. Our finding that almost 20% of these cats actually were euthyroid makes it clear that serum  $fT_4$  concentration should never be used alone to confirm a diagnosis of hyperthyroidism. Overreliance on  $fT_4$  testing will lead to misdiagnosis and inappropriate treatment or referral of many euthyroid cats. However, when we examined serum TSH concentrations in our 5 euthyroid and 33 hyperthyroid cats that had normal serum  $T_4$  with high serum  $fT_4$  concentrations, measurable serum concentrations of TSH were found in all 5 euthyroid cats, whereas none of our 33 hyperthyroid cats had a measurable serum TSH concentration. Therefore, measuring both serum  $fT_4$  and TSH concentrations in equivocal cats with normal  $T_4$  concentrations can help differentiate cats with mild hyperthyroidism from suspect euthyroid cats.

Despite the limitations of the serum cTSH test, we believe that measurement of serum TSH concentration has diagnostic value in cats with early or mild hyperthyroidism, especially when measured in conjunction with serum total and  $fT_4$  concentrations (Table 2). The test also should be helpful in cats with suspected hyperthyroidism that also are suffering from a nonthyroidal illness, a situation known to produce a relatively high prevalence of false-positive test results for the  $fT_4$  measurements. Again, the finding of a serum total  $T_4$  concentration within the upper third of the reference interval combined with high serum  $fT_4$  and suppressed TSH concentrations is consistent with a diagnosis of early hyperthyroidism. Conversely, the finding of high-normal serum  $T_4$  with a measurable TSH concentration makes hyperthyroidism very unlikely. In this latter case, the clinician should reevaluate the diagnosis, withhold treatment, and continue to monitor unless other evidence for hyperthyroidism is found (eg, compatible clinical signs, palpable thyroid nodule, positive thyroid scan results).

Clearly, a better TSH assay is needed to help in the diagnosis of cats with mild hyperthyroidism, specifically one that can accurately measure low enough concentrations to reliably distinguish a low-normal concentration

in euthyroid cats from a truly low or completely suppressed concentration in hyperthyroid cats. Based on experience with human patients, development of such a highly sensitive, feline-specific TSH assay likely would eliminate many of the problems associated with use of the cTSH assay in cats and should greatly improve the diagnostic utility of this test.

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## Footnotes

- <sup>a</sup> Antech Diagnostics, Lake Success, NY  
<sup>b</sup> DRI<sup>®</sup> Thyroxine (T<sub>4</sub>) assay, Microgenics Corporation, Fremont, CA  
<sup>c</sup> AU5400 Clinical Chemistry System, Beckman Coulter, Brea, CA  
<sup>d</sup> Immulite Total T<sub>3</sub>, Siemens Healthcare Diagnostics, Tarrytown, NY  
<sup>e</sup> Free T<sub>4</sub> – by Equilibrium Dialysis, Antech Diagnostics. Irvine, CA 92614  
<sup>f</sup> Immulite Canine TSH, Siemens Healthcare Diagnostics Products, Tarrytown, NY  
<sup>g</sup> Immulite 2000, Siemens Medical Solutions. Flanders, NJ  
<sup>h</sup> GraphPad Prism, version 6.0; GraphPad Software, La Jolla, CA  
<sup>i</sup> MedCalc, version 14.12.0, MedCalc Software, bvbnv, Ostend, Belgium
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## Acknowledgments

We thank Carol Castellano for technical assistance, as well as editing and critical reading of the manuscript.

*Conflict of Interest Declaration:* Dr Rhett Nichols serves as an internal medicine consultant for Antech Diagnostics.

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

## References

1. Broussard JD, Peterson ME, Fox PR. Changes in clinical and laboratory findings in cats with hyperthyroidism from 1983 to 1993. *J Am Vet Med Assoc* 1995;206:302–305.
2. Peterson ME, Melian C, Nichols R. Measurement of serum concentrations of free thyroxine, total thyroxine, and total triiodothyronine in cats with hyperthyroidism and cats with nonthyroidal disease. *J Am Vet Med Assoc* 2001;218:529–536.
3. Peterson ME. More than just T<sub>4</sub>: Diagnostic testing for hyperthyroidism in cats. *J Feline Med Surg* 2013;15:765–777.
4. McLoughlin MA, DiBartola SP, Birchard SJ, et al. Influence of systemic nonthyroidal illness on serum concentrations of thyroxine in hyperthyroid cats. *J Am Anim Hosp Assoc* 1993;29:227–234.
5. Wakeling J, Moore K, Elliott J, et al. Diagnosis of hyperthyroidism in cats with mild chronic kidney disease. *J Small Anim Pract* 2008;49:287–294.
6. Peterson ME. Diagnostic of occult hyperthyroidism. In: August JR, ed. *Consultations in Feline Internal Medicine*, 4th ed. Philadelphia, PA: WB Saunders; 2001:145–150.
7. Mooney CT, Little CJ, Macrae AW. Effect of illness not associated with the thyroid gland on serum total and free thyroxine concentrations in cats. *J Am Vet Med Assoc* 1996;208:2004–2008.
8. Tomsa K, Glaus TM, Kael GM, et al. Thyrotropin-releasing hormone stimulation test to assess thyroid function in severely sick cats. *J Vet Intern Med* 2001;15:89–93.
9. Peterson ME, Broome MR. Thyroid scintigraphy findings in 2,096 cats with hyperthyroidism. *Vet Radiol Ultrasound* 2015;56:84–95.
10. Bahn RS, Burch HB, Cooper DS, et al. Hyperthyroidism and other causes of thyrotoxicosis: Management guidelines of the American Thyroid Association and American Association of Clinical Endocrinologists. *Thyroid* 2011;21:593–646.
11. Ross DS, Ardisson LJ, Meskell MJ. Measurement of thyrotropin in clinical and subclinical hyperthyroidism using a new chemiluminescent assay. *J Clin Endocrinol Metab* 1989;69:684–688.
12. Larsen PR. Thyroid-pituitary interaction: Feedback regulation of thyrotropin secretion by thyroid hormones. *N Engl J Med* 1982;306:23–32.
13. Yeo PP, Lewis M, Davies TF, et al. The regulation of thyrotrophin secretion by exogenous thyroxine and triiodothyronine. *Clin Chim Acta* 1979;99:203–210.
14. Rayalam S, Eizenstat LD, Davis RR, et al. Expression and purification of feline thyrotropin (fTSH): Immunological detection and bioactivity of heterodimeric and yoked glycoproteins. *Domest Anim Endocrinol* 2006;30:185–202.
15. Wakeling J, Smith K, Scase T, et al. Subclinical hyperthyroidism in cats: A spontaneous model of subclinical toxic nodular goiter in humans? *Thyroid* 2007;17:1201–1209.
16. Wakeling J. Use of thyroid stimulating hormone (TSH) in cats. *Can Vet J* 2010;51:33–34.
17. Wakeling J, Elliott J, Syme H. Evaluation of predictors for the diagnosis of hyperthyroidism in cats. *J Vet Intern Med* 2011;25:1057–1065.
18. Puille M, Bauer R. Determination of TSH and free thyroid hormones in the diagnosis of feline hyperthyroidism. *Tierarztl Prax Ausg K Kleintiere Heimtiere* 2000;28:289–294.
19. D’Agostino RB. Tests for normal distribution. In: D’Agostino RB, Stephens MA, eds. *Goodness-of-Fit Techniques*. New York: Macel Dekker; 1986:367–420.
20. Conover WJ. *Practical Nonparametric Statistics*, 3rd ed. New York: Wiley; 1999.
21. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. *Clin Chem* 1971;17:275–284.
22. Altman DG, Bland JM. Quartiles, quintiles, centiles, and other quantiles. *BMJ* 1994;309:996.
23. McNemar Q. Note on the sampling error of the difference between correlated proportions or percentages. *Psychometrika* 1947;12:153–157.
24. Williams TL, Elliott J, Syme HM. Association of iatrogenic hypothyroidism with azotemia and reduced survival time in cats treated for hyperthyroidism. *J Vet Intern Med* 2010;24:1086–1092.
25. Peterson ME, Graves TK, Gamble DA. Triiodothyronine (T<sub>3</sub>) suppression test. An aid in the diagnosis of mild hyperthyroidism in cats. *J Vet Intern Med* 1990;4:233–238.

## Supporting Information

Additional Supporting Information may be found online in Supporting Information:

**Data S1.** Thyroid scintigraphy as the reference standard for diagnosis of hyperthyroidism in cats.

**Data S2.** Hormone validation information.

**Data S3.** Effect of prior methimazole administration of serum thyroid hormone concentrations in hyperthyroid cats.

**Data S4.** Effect of nonthyroidal disease on the TSH concentrations in the euthyroid cats referred for suspected hyperthyroidism.