Thyroid function testing in Greyhounds

Kathy R. Gaughan, DVM and David S. Bruyette, DVM

Objective—To evaluate thyroid function in healthy Greyhounds, compared with healthy non-Greyhound pet dogs, and to establish appropriate reference range values for Greyhounds.

Animals—98 clinically normal Greyhounds and 19 clinically normal non-Greyhounds.

Procedures—Greyhounds were in 2 groups as follows: those receiving testosterone for estrus suppression (T-group Greyhounds) and those not receiving estrus suppressive medication (NT-group Greyhounds). Serum thyroxine (T4) and free thyroxine (fT4) concentrations were determined before and after administration of thyroid-stimulating hormone (TSH) and thyroid-releasing hormone (TRH). Basal serum canine thyroid stimulating hormone (cTSH) concentrations were determined on available stored sera.

Results—Basal serum T4 and fT4 concentrations were significantly lower in Greyhounds than in non-Greyhounds. Serum T4 concentrations after TSH and TRH administration were significantly lower in Greyhounds than in non-Greyhounds. Serum fT4 concentrations after TSH and TRH administration were significantly lower in NT-group than T-group Greyhounds and non-Greyhounds. Mean cTSH concentrations were not different between Greyhounds and non-Greyhounds.

Conclusions and Clinical Relevance—Previously established canine reference range values for basal serum T4 and fT4 may not be appropriate for use in Greyhounds. Greyhound-specific reference range values for basal serum T4 and fT4 concentrations should be applied when evaluating thyroid function in Greyhounds. Basal cTSH concentrations in Greyhounds are similar to non-Greyhound pet dogs. (*Am J Vet Res* 2001;62:1130–1133)

Primary hypothyroidism is a common endocrine disorder in dogs.[1-4] Racing Greyhounds with poor race performance, infertility, or alopecia are often suspected of hypothyroidism, yet the clinical signs of thyroid hormone deficiency can be variable and are rarely pathognomonic. Diagnosis of hypothyroidism is usually based on clinical signs, physical examination findings, laboratory data, and results of thyroid function tests.[5-8] Interpretation of thyroid function tests can be frustrating, as no single definitive test to diagnose hypothyroidism in dogs is available.[9-10] Furthermore, nonthyroidal illness and some medications may interfere with thyroid hormone metabolism, making interpretation of thyroid function tests even more difficult.[6-7,10-13] The availability of the **canine thyroid-stimulating hormone (cTSH)** assay has improved diagnostic capabilities to diagnose hypothyroidism when used in conjunction with total **thyroxine (T4)** and **free thyroxine (fT4)** assays.[7,9,14,15]

Racing Greyhounds are often treated with medications known to interfere with the measurement of basal thyroid hormone concentrations.[10,13] Additionally, Greyhounds may have lower basal thyroid hormone concentration than do mixed-breed dogs.[8,16,17] The purpose of the study reported here was to evaluate thyroid function in healthy Greyhounds, compare the results with those of healthy non-Greyhound pet dogs, and establish Greyhound-specific reference range values for T4 and fT4.

Received Dec 13, 1999.
Accepted Aug 17, 2000.
From the Veterinary Medical Teaching Hospital, Kansas State University, Manhattan, KS 66506-5701 (Gaughan, Bruyette). Dr. Bruyett's present address is the West Los Angeles Animal Hospital, 1818 S Sepulveda Blvd, Los Angeles, CA 90025 (Bruyette).
Supported by the Kansas Racing Commission.
Abstract presented at ACVIM, San Antonio, Tex, May 1996.

Materials and Methods

Dogs—Our study included 98 healthy Greyhounds and 19 healthy non-Greyhound pet dogs. Greyhounds were in 2 groups as follows: those **receiving testosterone (T group)** for estrus suppression (n = 37) and those **not receiving testosterone (NT group**; 61). Mean age of T-group Greyhounds was 1.4 years (SD ± 0.3; range, 1 to 2 years), and all were actively racing. Females included in the NT group had not received estrus suppressive medication for at least 1 year prior to evaluation and were not actively racing (33 brood bitches and 2 retired dogs). Males included in the NT group were either actively racing (n = 10), at stud (8), or retired.[8] Mean age of Greyhounds in the NT group was 4.9 years (SD ± 2.7; range, 1 to 11 years). Non-Greyhound pet dogs served as a comparison population that was considered to represent current reference range values for thyroid function testing (n = 19). Breeds represented included 8 mixed-breed dogs, 2 Golden Retrievers, and 1 each of Labrador Retriever, English Setter, Boston Terrier, Boxer, Cocker Spaniel, German Shorthair Pointer, Chihuahua, Dalmatian, and Miniature Dachshund. Mean age of non-Greyhounds was 5.2 years (SD ± 2.9; range, 1 to 14 years).

All dogs included in the investigation were considered healthy on the basis of physical examination, CBC, and serum biochemical analysis. Exclusion criteria included the administration of thyroid supplementation, glucocorticoid, and anabolic steroid agents (with the exception of testosterone in T-group Greyhounds) within 3 months of evaluation.

Thyroid function tests and assays—Thyroid-stimulating hormone (TSH)[a] stimulation testing was performed on each dog (n = 117). Additionally, all non-Greyhounds and 56 Greyhounds were also subjected to **thyroid-releasing hormone (TRH)**[b] stimulation testing. For each dog undergoing TSH and TRH stimulation, the tests were performed on separate days not less than 3 days and not more than 60 days apart. Serum T4[c] and fT4[d] concentrations were determined with commercially available assays before and after TSH and TRH stimulation. A commercially available cTSH assay[e] was used to determine basal cTSH concentrations on available stored serum from 87 Greyhounds and 18 non-Greyhounds. All assays were performed at the Kansas State University Gastrointestinal & Endocrine Function Laboratory. All samples were processed in duplicate, and duplicate control samples were included with each test batch for each assay. Test results were considered acceptable if the control samples were within expected values and the difference between duplicate samples was less than 10%. The intra- and interassay coefficients of variation for the T4 assay were 5.6 and 7.6%, respectively. The sensitivity of the assay was 2 nmol/L. Sensitivity of the fT4 by equilibrium dialysis assay was 0.1 ng/dl, and the intra- and interassay coefficients of variation were 4.7 and 6.9%, respectively. Sensitivity of the cTSH assay was 0.02 ng/ml, and the intra- and interassay coefficients of variation were 6.5 and 8.7%, respectively. Canine reference range values for basal T4, fT4, and cTSH cited in our study were established by the Kansas State University Gastrointestinal & Endocrine Function Laboratory. Reference range values were obtained from 30 healthy (on the basis of physical examination findings and CBC, serum biochemical analysis, urinalysis, and heartworm testing results) non-Greyhounds between the ages of 2 and 11 years of age. None of dogs were on any medications, other than heartworm preventive and flea products, for the preceding 3 months.

Sample collection—Blood samples were collected from each dog immediately before and 6 hours after IV administration of 0.1 U of TSH/kg of body weight.[8,17,18] For dogs undergoing TRH stimulation, blood samples were collected from each dog immediately before and 6 hours after IV administration of 200 µg of TRH/dog.[19] Serum was harvested from blood samples within 2 hours of collection and frozen at –70 C until analysis, at which time serum T4 and fT4 concentrations were determined. Basal cTSH concentrations were determined on available serum samples after 6 to 18 months of storage at –70 C.

Statistical analysis—Mean serum T4 and fT4 concentrations for each group were compared, using a 1-way ANOVA. When significant differences ($P \leq 0.05$) were found, post hoc comparisons were made with the Student Neuman Keuls multiple comparison test and repeated measures analysis. Each dog is represented by a single data point for basal T4, fT4, and cTSH.

Basal T4 and fT4 data from all Greyhounds were used in the establishment of Greyhound-specific reference range values. Greyhound reference range values for basal T4 and fT4 were established, using 1.96 SD above and below the mean.

Results

Mean basal serum T4 and fT4 concentrations were significantly lower in Greyhound groups (T and NT group) than in non-Greyhounds (**Table 1**). After TSH administration, Greyhounds had significantly lower mean serum T4 concentrations than non-Greyhounds; however, within the Greyhound groups, mean serum T4 concentrations were significantly higher in T-group than in NT-group Greyhounds. Mean serum fT4 concentrations 6 hours after TSH administration were significantly higher in non-Greyhounds and T-group Greyhounds than in NT-group Greyhounds.

After TRH administration, mean serum T4 concentrations were significantly lower in Greyhound groups than in non-Greyhounds. However, within Greyhound groups, mean serum T4 concentrations after TRH administration were significantly higher in T-group Greyhounds than in NT-group Greyhounds. Mean serum fT4 concentrations 6 hours after TRH administration were significantly higher in non-Greyhounds and T-group Greyhounds than in NT-group Greyhounds.

Mean serum cTSH concentrations were not significantly different between Greyhounds and non-Greyhounds (Table 1). Only 2 Greyhounds (NT group) had cTSH concentrations above the reference range values (1.3 and 0.74 ng/ml, respectively).

Greyhounds in the T group were significantly younger than Greyhounds in the NT group or non-Greyhounds (Table 1). Mean age was not different between NT-group Greyhounds and non-Greyhounds. Mean age of all Greyhounds (98) was 3.6 years (SD ± 2.7, range 1 to 11).

Basal T4, fT4, and cTSH concentrations in NT-group females (n = 35) were 16.2 nmol/L (SD ± 6.7, range 6.0 to 37.0), 14.2 pmol/L (SD ± 6.7, range 1.3 to 32.2), and 0.2 ng/ml (SD ± 0.25, range 0.03 to 0.45), respectively. Basal T4, fT4, and cTSH was not different between T-group (Table 1) and NT-group females.

Mean basal T4 for all Greyhounds (98) was 13.9 nmol/L (SD ± 6.0, range 2.1 to 37.0). Mean basal fT4 for all Greyhounds was 11.3 pmol/L (SD ± 6.2, range 1.3 to 32.2). Mean basal cTSH for all Greyhounds was 0.21 ng/ml (SD ± 0.18, range 0.03 to 1.3). Greyhound-specific reference range values for basal serum T4 and fT4 were calculated to be 2.1 to 25.7 nmol/L and 0 to 23.5 pmol/L, respectively.

Table 1 Mean values ± SD (range) of serum values for thyroid function testing in healthy Greyhounds and healthy non-Greyhound pet dogs

Variables	Groups: T group (0 males, 37 females)	NT group (26 males, 35 females)	PD group (11 males, 8 females)	Reference range values*
Age (y)	1.4 ± 0.3† (1–2)	4.9 ± 2.7 (1–11)	5.2 ± 2.9 (1 –14)	NA
Basal T4 (nmol/L)	14 ± 5.6 (3–26)	13.9 ± 6.3 (2.1–37)	25.7 ± 6.9‡ (16–40)	10–45.5
Basal fT4 (pmol/L)	11.6 ± 5.8 (1.3–30.9)	11.6 ± 6.5 (1.3 –32.2)	19.3 ± 5.4‡ (11.6–30.9)	3.9–39.9
T4 after TSH (nmol/L)	50.1 ± 12.2 (31–78)	36.4 ± 10§ (13–60)	68.9 ± 6.2‡ (44–108)	NA
fT4 after TSH (pmol/L)	50.2 ± 12.3 (14.2 –72.1)	37.3 ± 13§ (12.9–78.5)	55.3 ± 12.2 (36–83.7)	NA
T4 after TRH (nmol/L)‖	32.3 ± 8.9 (21–50)	20.3 ± 10.5§ (8.2–60)	41.3 ± 9.3‡ (27–58)	NA
fT4 after TRH (pmol/L)‖	30.9 ± 8.6 (18–52.8)	16.7 ± 7.2§ (3.9–29.6)	29.6 ± 10.5 (14.2–50.2)	NA
Basal cTSH (ng/ml) ¶	0.22 ± 0.12 (0.03–0.45)	0.2 ± 0.21 (0.03–1.3)	0.32 ± 0.17 (0.08–0.59)	0–0.64

*As established by the Kansas State University-Gastrointestinal Endocrine Function Laboratory. †Significantly ($P < 0.05$) different, compared with NT and PD groups. ‡Significantly ($P < 0.05$) different, compared with T and NT groups. §Significantly ($P < 0.05$) different, compared with T and PD groups. ‖No. of dogs represented: T group = 20; NT group = 36. ¶No. of dogs represented: T group = 33; NT group = 54; PD group = 18.

T group = Greyhounds receiving testosterone. NT group = Greyhounds not receiving testosterone. PD group = Non-Greyhound pet dogs. T4 = Thyroxine. fT4 = Free thyroxine. TSH = Thyroid-stimulating hormone. TRH = Thyroid-releasing hormone. cTSH = Canine thyroid-stimulating hormone. NA = Not applicable.

Discussion

Results of our study support previous reports[13,16,17] that Greyhounds have lower basal thyroid hormone concentrations than non-Greyhound pet dogs. Because T4 is highly protein bound, the measurement of T4 in Greyhounds may be affected by low plasma protein concentrations that have been reported for athletic canine breeds.[20] Differences in plasma protein concentrations were not compared among groups in our study. It is also possible that Greyhounds may be more efficient in converting T4 to T3 or have a more sensitive feedback mechanism resulting in decreased basal T4 and fT4 concentrations.

The effect of testosterone on the measurement of thyroid hormones was not directly evaluated in our study. Mean basal serum T4, fT4, and cTSH concentrations were not different between T-group and NT-group Greyhounds; however, NT-group Greyhounds included male Greyhounds, which makes direct comparison between these groups difficult. Basal T4, fT4, and cTSH were not different between T-group and NT-group females. This may indicate that the administration of exogenous testosterone does not affect the measurement of basal serum T4, fT4, and cTSH concentrations.

The comparatively higher after-TSH and -TRH serum T4 concentration in non-Greyhounds was likely the result of higher basal serum T4 concentrations. It is unclear why T-group Greyhounds had higher after-TSH and -TRH serum T4 concentrations than NT-group Greyhounds; however, a possibility is that testosterone administration may enhance thyroid response to TSH. Additionally, basal T4 concentrations[21] and thyroid responsiveness to exogenous TSH[22] in dogs may decline with advancing age, which may have contributed to the difference in T4 concentration after TSH and TRH stimulation between T-group and NT-group Greyhounds.

The effect of exercise on measurement of thyroid hormones was not directly evaluated in our study; however, relative racing activity was not consistent between groups. All Greyhounds in the T group were in race training, whereas only 16% (10 of 61) in the NT group and no non-Greyhound pet dogs were in race training. Despite the difference in racing activity, basal T4 and fT4 concentrations were not different between Greyhound groups, and basal cTSH was similar among all groups. Basal T4 and fT4 were different between Greyhounds and non-Greyhounds, suggesting that a breed variation may exist with regard to measurement of basal T4 and fT4. Further investigation is required to fully evaluate the effect of racing activity on thyroid function in Greyhounds.

It has been reported that serum T4 and fT4 concentrations in dogs are greater during diestrus and pregnancy than in any other phase of the reproductive cycle.[23] Additionally, no differences in thyroid hormone concentrations have been observed between sexually intact males or females in anestrus, proestrus, or during lactation.[23] The stage of estrus cycle was not determined in our study with exception of T-group Greyhounds (anestrus).

A validated canine TSH assay was not available when the blood samples were obtained for our study. The cTSH assays in our study were performed, using available serum after 6 to 18 months of storage at –70 C. Although the stability of cTSH in frozen serum has not been determined beyond 2 months of storage, the glycoprotein pituitary hormones are considered stable when managed with this storage method.[f] Mean cTSH concentrations were not different between Greyhounds and non-Greyhounds in our study. Additionally, 98% (103/105) of dogs had cTSH concentrations within the reference range established for the laboratory used in our study. Two NT-group Greyhounds had high cTSH concentrations, which agrees with results of previous studies in which a small percentage of euthyroid dogs were determined to have high cTSH concentrations.[14-15, 24]

The lower limit of the Greyhound basal T4 and fT4 reference range values established in our study extend well below the lower limits of the non-Greyhound T4 and fT4 reference range values previously established (Table 1). The lower limit for Greyhound basal fT4 starts at 0 pmol/L, which may increase the difficulty in distinguishing between euthyroid and hypothyroid Greyhounds. This also emphasizes the importance of evaluating T4 and cTSH when investigating a diagnosis of hypothyroidism in Greyhounds.

Results of our study indicate that previously established canine reference range values for basal T4 and fT4 may not be appropriate for Greyhounds. Greyhound-specific reference range values for T4 and fT4 should be used when evaluating thyroid function in Greyhounds. Basal cTSH concentrations in Greyhounds are similar to non-Greyhound pet dogs.

---

[a]Thytropar, Rhone-Poulenc Rorer, Collegeville, Pa.
[b]Thyrel, Ferring Laboratories, Inc, Suffern, NY.
[c]Canine Coat-A-Count T4, Diagnostic Products Corp, Los Angeles, Calif.
[d]fT4 by Equilibrium dialysis, Nichols Institute, San Juan Capistrano, Calif.
[e]IMMULITE canine TSH, Diagnostic Products Corp, Los Angeles, Calif.
[f]Refsal K, Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, Lansing, Mich: Personal communication, 1999.

References

1. Nesbitt GO, Izzo J, Peterson L, et al. Canine hypothyroidism: a retrospective study of 108 cases. *J Am Vet Med Assoc* 1980;177:1117–1122.
2. Beale KM, Helm LJ, Keisling K. Comparison of two doses of aqueous bovine thyrotropin for thyroid function testing in dogs. *J Am Vet Med Assoc* 1990;197:865–867.
3. Campbell KL, Small E. Identifying and managing the cutaneous manifestation of various endocrine diseases. *Vet Med* 1991;86:118–135.
4. Beale KM, Bloomberg MS, Van Gilder J, et al. Correlation of racing and reproductive performance in greyhounds with response to thyroid function testing. *J Am Anim Hosp Assoc* 1992;28:263–269.
5. Nelson R. Use of baseline thyroid hormone concentrations for diagnosing canine hypothyroidism. *Canine Pract* 1997;22:39–40.
6. Nelson RW, Ihle SL, Feldman EC, et al. Serum free thyroxine concentration in healthy dogs, dogs with hypothyroidism, and euthyroid dogs with concurrent illness. *J Am Vet Med Assoc* 1991;198:1401–1407.
7. Peterson ME, Melian C, Nichols R. Measurement of serum total thyroxine, triiodothyronine, free thyroxine, and thyrotropin

concentrations for diagnosis of hypothyroidism in dogs. *J Am Vet Med Assoc* 1997;211:1396–1402.

8. Panciera DL. Canine hypothyroidism. Part II. Thyroid function tests and treatment. *Compend Contin Educ Pract Vet* 1990;12:843–856.

9. Williams DA, Scott-Moncrieff JC, Bruner J, et al. Validation of an immunoassay for canine thyroid stimulating hormone and changes in serum concentration following induction of hypothyroidism in dogs. *J Am Vet Med Assoc* 1996;209:1730–1732.

10. Panciera DL. Canine hypothyroidism. Part I. Clinical findings and control of thyroid hormone secretion and metabolism. *Compend Contin Educ Pract Vet* 1990;12:689–701.

11. Nachreiner RF, Refsal KR. The Michigan State University thyroid function profile. *Canine Pract* 1997;22:45–46.

12. Lorenz MD, Stiff ME. Serum thyroxine content before and after thyrotropin stimulation in dogs with suspected hypothyroidism. *J Am Vet Med Assoc* 1980;177:78–81.

13. Ferguson DC. Euthyroid sick syndrome. *Canine Pract* 1997; 22:49–51.

14. Scott-Moncrieff JC. Serum canine thyrotropin concentrations in experimental and spontaneous canine hypothyroidism. *Canine Pract* 1997;22:41–42.

15. Ramsey I, Herrtage M. Distinguishing normal, sick and hypothyroid dogs using total thyroxine and thyrotropin concentrations. *Canine Pract* 1997;22:43–44.

16. Rosychuk RAW, Freshman JL, Olson PN, et al. Serum concentrations of thyroxine and 3,5,3'-triiodothyronine in dogs before and after administration of freshly reconstituted or previously frozen thyrotropin-releasing hormone. *Am J Vet Res* 1988;49:1722–1725.

17. Panciera DL. Thyroid-function testing: is the future here? *Vet Med* 1997;92:50–57.

18. Kintzer P. TSH and TRH stimulation testing. *Canine Pract* 1997;22:47–48.

19. Beale KM. Current diagnostic techniques for evaluation thyroid function in the dog. *Vet Clin North Am Small Anim Pract* 1990;20:1429–1439.

20. Sullivan PS, Evans HL, McDonald TP. Platelet concentration and hemoglobin function in greyhounds. *J Am Vet Med Assoc* 1994; 205:838–841.

21. Palazzolo DL, Quadri SK. Plasma thyroxine and cortisol under basal conditions and during cold stress in the aging dog. *Proc Soc Exp Biol Med* 1987;185:305–311.

22. Ferguson DC. The effect of nonthyroidal factors on thyroid function test in dogs. *Compend Contin Educ Pract Vet* 1988;10: 1365–1377.

23. Reimers TJ, Mummery LK, McCann JP, et al. Effects of reproductive state on concentrations of thyroxine, 3,5,3'-triiodothyronine and cortisol in serum of dogs. *Biol Reprod* 1984;31:148–154.

24. Dixon RM, Graham PA, Mooney CT. Serum thyrotropin concentrations: a new diagnostic test for canine hypothyroidism. *Vet Rec* 1996;138:594–595.