

Effects of racing and training on serum thyroid hormone concentrations in racing Greyhounds

Richard C. Hill, VetMB, PhD; Leslie E. Fox, DVM, MS; Daniel D. Lewis, DVM; Karin M. Beale, DVM; Raymond F. Nachreiner, DVM, PhD; Karen C. Scott, PhD; Deborah A. Sundstrom, BS; Galin L. Jones, MStat, PhD; Richard F. Butterwick, PhD

Objectives—To determine the effects of racing and training on serum thyroxine (T_4), triiodothyronine (T_3), and thyroid stimulating hormone (TSH) concentrations in Greyhounds.

Animals—9 adult racing Greyhounds.

Procedure—Serum thyroid hormone concentrations were measured before and 5 minutes after a race in dogs trained to race 500m twice weekly for 6 months. Resting concentrations were measured again when these dogs had been neutered and had not raced for 3 months. Postrace concentrations were adjusted relative to albumin concentration to allow for effects of hemoconcentration. Thyroid hormone concentrations were then compared with those of clinically normal dogs of non-Greyhound breeds.

Results—When adjusted for hemoconcentration, total T_4 concentrations increased significantly after racing and TSH concentrations decreased; however, there was no evidence of a change in free T_4 or total or free T_3 concentrations. Resting total T_4 concentrations increased significantly when dogs had been neutered and were not in training. There was no evidence that training and neutering affected resting TSH, total or free T_3 , or free T_4 concentrations. Resting concentrations of T_3 , TSH, and autoantibodies against T_4 , T_3 , and thyroglobulin were similar to those found in other breeds; however, resting free and total T_4 concentrations were lower than those found in other breeds.

Conclusions and Clinical Relevance—Except for total T_4 , thyroid hormone concentrations in Greyhounds are affected little by sprint racing and training. Greyhounds with low resting total and free T_4 concentrations may not be hypothyroid. (*Am J Vet Res* 2001;62:1969–1972)

(TSH) regulate and are regulated by metabolic rate. Exercise alters metabolic rate. The effect of exercise and training on serum concentrations of thyroid hormones has, therefore, been investigated in many species including humans, rodents, dogs, and horses. However, results from these studies have varied with the intensity and duration of exercise,^{1,2} the amount of training,^{3,4} the adequacy of energy intake,^{4,5} and the time when blood was sampled.^{6,7} Results from studies in which the effect of training on thyroid hormones was examined were also varied; some revealed that thyroid hormone concentrations change with training,³ whereas others have not.^{8,9}

In sled dogs that underwent severe endurance exercise during an Iditerod race, there was no change in total or free T_4 and total or free T_3 concentrations before or after the race.¹⁰ In 1 study, resting total T_4 concentrations were slightly (13%) lower in Beagles undergoing long-distance aerobic exercise on a treadmill, compared with sedentary dogs, but there was no change in free T_4 or total T_3 concentrations.¹¹ To date, however, the authors are unaware of any studies of the effect of racing on thyroid hormones in Greyhounds. Resting serum T_4 concentrations appear to be lower in Greyhounds than in other breeds of dogs,^{12,a} and it is possible that thyroid hormone concentrations may be affected differently after a short sprint race than after endurance exercise. Therefore, the purpose of the study reported here was to measure the effect of racing and training on thyroid hormone and TSH concentrations in racing Greyhounds.

Materials and Methods

Dogs—Nine Greyhounds (5 females, 4 males) 2.6 to 4.6 years of age and weighing 26 to 35 kg were used for this study. These dogs had been trained to chase a lure on a race-track and were donated by Greyhound breeding kennels. All dogs were considered to be in good health on the basis of results of physical examination, CBC, serum biochemical analysis, and urinalysis. Dogs were cared for as previously described¹³ and according to the principles outlined in the NIH guide for the care of laboratory animals¹⁴; the study protocol was approved by the Institutional Animal Care and Use Committee at the University of Florida.

All dogs were housed in 1.4 × 1.9-m cages in a room with a 12-hour light:dark cycle, a constant temperature of 24 C, and 13 to 18 air changes/h. All dogs were exercised for 15 minutes twice daily in a 30 × 30-m grass paddock and raced in pairs up to twice a week at this facility for 6 months prior to this experiment. Each race was 500 m (five-sixteenths of a mile) in length and was performed on a 400-m (quarter mile) oval soft sand-clay track with 10° banking on the corners. Dogs chased a mechanical lure maintained 10 to 20 m in front of the lead dog. Dogs were randomly assigned to race and starting position. Dogs were fed an extruded dry com-

The thyroid hormones thyroxine (T_4), triiodothyronine (T_3), and thyroid stimulating hormone

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From the Department of Small Animal Clinical Sciences and the Center for Veterinary Sports Medicine, College of Veterinary Medicine (Hill, Fox, Lewis, Scott, Sundstrom), and the Department of Statistics (Jones), University of Florida, Gainesville, FL 32610-0126; the Gulf Coast Veterinary Specialists, Houston, TX 77027 (Beale); the Department of Large Animal Clinical Sciences and the Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, MI 48824 (Nachreiner); and the Waltham Centre for Pet Nutrition, Waltham-on-the-Wolds, Leicestershire, UK (Butterwick). Dr. Fox's present address is the Department of Clinical Sciences, College of Veterinary Medicine, Iowa State University, Ames, IA 50011.

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mercially available diet each morning after exercise. No exogenous thyroid hormone supplementation was given to any dog at any time during the training or detraining period. Testosterone^b (1 mg/kg of body weight, IM) was administered to female dogs every 2 weeks to prevent estrus.

After the dogs had been training at the racetrack for 6 months, blood was drawn before racing (from the left jugular vein) while dogs were in their kennels, and blood was drawn again 5 minutes after racing (from the right jugular vein) while dogs were at the racetrack. Dogs were then neutered and did not race for 3 months; blood was again drawn while dogs were in their kennels prior to the morning exercise period. Each blood sample was collected in evacuated tubes coated with silica for clot activation and containing a gel for clot separation.^c Serum was centrifuged at 500 × g for 15 minutes at 4 C and frozen at -70 C until analysis. An automatic analyzer was used to measure serum albumin concentration.^d Thyroid hormone assays (total T₄, total T₃, free T₃, free T₄, TSH, T₄ autoantibodies, T₃ autoantibodies, and thyroglobulin autoantibodies) were performed at the Animal Health Diagnostic Laboratory at Michigan State University, using assays previously validated for dogs¹⁵⁻²¹; no validation of the free T₃ method has been published.

Statistical analyses—Postrace serum hormone concentrations were adjusted for hemoconcentration by use of the following equation: $C_{adj} = C_{obs} \times (P_1/P_2)$, where C_{adj} is the adjusted postrace concentration, C_{obs} is the observed postrace concentration, P_1 is the albumin concentration before racing, and P_2 is the albumin concentration after racing. Normal probability plots of the differences were evaluated,

Table 1—Mean (± SD) serum thyroid hormone concentrations in trained (ie, had raced for 6 months) and detrained (ie, had not raced in 3 months) Greyhounds (n = 9)

Variable	Trained dogs		Detrained dogs
	Before racing*	After racing* Observed (Adjusted†)	Before exerciset
T ₄ (nmol/L)	2.7 ± 1.8	6.5§ ± 3.2 (5.1§ ± 2.5)	7.2§ ± 2.5
T ₃ (nmol/L)	1.2 ± 0.2	1.6§ ± 0.3 (1.3 ± 0.3)	1.3 ± 0.3
Free T ₄ (pmol/L)	2.3 ± 1.4	3.5 ± 3.0 (2.8 ± 2.4)	6.0 ± 5.7
Free T ₃ (pmol/L)	4.1 ± 0.7	5.4§ ± 1.5 (4.3 ± 1.3)	4.8 ± 1.8
TSH (mU/L)	18.9 ± 3.4	20.3 ± 5.3 (15.9 ± 3.5)	15.8 ± 5.0
Albumin (g/L)	38 ± 2	48§ ± 2 (N/A)	N/A

*Blood was collected before and after a race. †Dogs had not raced for 3 months but were exercised for twice daily; blood was collected immediately preceding exercise. ‡Values obtained after racing were adjusted for hemoconcentration, using change in albumin concentration as an estimate of amount of hemoconcentration. §Significantly ($P < 0.01$) different from those of trained dogs before racing.

T₄ = Thyroxine. T₃ = Triiodothyronine. TSH = Thyroid stimulating hormone. NA = Not applicable.

Table 2—Ranges of serum thyroid hormone concentrations and autoantibodies in trained (ie, had raced for 6 months) and detrained (ie, had not raced in 3 months) Greyhounds, compared with untrained dogs of other breeds

Variable	Trained Greyhounds	Detrained Greyhounds	Untrained dogs of other breeds
T ₄ (nmol/L)*	1–6	4–12	15–50
T ₃ (nmol/L)*	0.8–1.5	0.9–1.8	1.0–2.5
Free T ₄ (pmol/L)	1–5	1–18	9–40
Free T ₃ (pmol/L)	3.2–5.0	2.8–8.5	2.8–6.5
TSH (mU/L)	14–26	11–27	0–30
T ₄ autoantibodies (%)	8–16	NA	0–20
T ₃ autoantibodies (%)	5–11	NA	0–10
Thyroglobulin autoantibodies (%)	26–63	NA	0–200

*Values in nmol/L can be converted to mg/dl by dividing by 12.87.
See Table 1 for key.

and the Shapiro-Wilk test was performed to assess whether data were normally distributed. Paired *t*-tests were used to compare serum hormone concentrations before and after racing in trained dogs and then prerace concentrations in trained dogs with resting concentrations in detrained dogs. Adjusted and unadjusted postrace serum hormone concentrations were compared with prerace concentrations separately. All analyses were performed by use of computer software.^c Values of $P \leq 0.01$ were considered significant to reduce the experiment-wise error rate. Additionally, thyroid hormone concentrations of these Greyhounds were compared with those of 91 clinically normal adult dogs of non-sight hound breeds.²⁰ Results are reported as mean ± SD.

Results

Serum total T₄, total T₃, free T₃, and albumin concentrations increased significantly in Greyhounds after racing (Table 1). After racing, when adjusted for hemoconcentration, serum total T₄ concentrations increased significantly ($P = 0.001$) by 90%, TSH concentrations decreased but not significantly ($P = 0.02$), and there was no evidence of a change in free T₄, free T₃, and total T₃ concentrations. After dogs had not been racing for 3 months, resting serum total T₄ concentrations increased significantly ($P = 0.003$) by 170%, but there was no evidence of an increase in free T₄ ($P = 0.12$). There was no evidence of an effect of racing or training on the ratio of free T₃ and free T₄ concentrations or on the ratio of TSH and free T₄ concentrations.

When compared with values obtained from clinically normal dogs of other breeds used in another study,²⁰ prerace concentrations of total T₄ and free T₄ were lower in the Greyhounds, but concentrations of total T₃, free T₃, and TSH were similar (Table 2). Concentrations of autoantibodies against T₄ (10 ± 3%), T₃ (8 ± 1%), and thyroglobulin (50 ± 12%) in Greyhounds were similar to those in other breeds.

Discussion

In our study, serum thyroid concentrations increased in Greyhounds after a short sprint race, but this increase was proportional to the increase in albumin concentration. Adjusted total T₄ concentrations increased after exercise; however, most T₄ is protein-bound, and it is the free or unbound thyroid hormones that enter cells, affect metabolism, and regulate pituitary feedback. Adjusted free T₃, T₄, and TSH concentrations and the ratio of free T₄ to TSH concentrations remained unchanged after exercise, which suggests

that the pituitary-thyroid axis was largely unaffected by this severe anaerobic exercise. Protein binding of T_4 appeared to increase with exercise (total T_4 concentrations increased, whereas free T_4 concentrations remained unchanged); however, it is possible that protein binding was affected by the 2 C increase in body temperature that occurs in Greyhounds during a 500-m race. In human serum, the dialysable fraction of thyroid hormone (free T_4) has been shown to increase by 20% when temperature increases 2 C.^{22,23}

Most human studies of trained individuals undertaking severe anaerobic exercise have also revealed there are modest (10 to 30%) increases in total and free T_3 or T_4 concentrations after exercise, which can largely be explained by exercise-induced hemoconcentration.^{6,7,24,25} However, De Nayer et al²⁶ found that free T_4 decreased slightly after severe exercise, and Galbo et al¹ reported that serum TSH concentrations increased to 107% above resting values after maximal work. In our study, the short postrace delay (5 minutes) before blood was collected may not have allowed sufficient time for a TSH response to develop, because the TSH response may be delayed as much as 4 days.⁶

We found that training had little effect on thyroid hormone concentrations. Studies of the effect of training on thyroid hormone concentrations in humans have also indicated there is either a modest (10 to 20%) decrease in resting total T_4 , free T_3 , and total T_3 with training⁴ or no change in resting free or total T_4 or T_3 .^{8,9,27,28} Thyroid hormones regulate and are regulated by metabolic rate. The energy required for exercise, however, is proportional to distance traveled, rather than speed, and each 500-m sprint requires little energy (approx 75 kcal/race).^{29,30} The maintenance energy requirement of Greyhounds that undergo this type of training is little more than that of other breeds undergoing moderate exercise (155 vs 132 kcal/kg^{-0.75}/d⁻¹).^{13,31} This may explain why thyroid hormone concentrations were largely unaffected by training. In sled dogs running in the Iditerod, where metabolic demands can be 5 times that of Greyhounds (because sled dogs run over huge distances at low ambient temperatures), thyroid concentrations also appeared to be little affected by exercise.¹⁰ Similarly, in Beagles, long-distance aerobic exercise on a treadmill had little effect on thyroid hormone concentrations.¹¹ It seems, therefore, that the effect of exercise (aerobic or anaerobic) on thyroid hormone concentrations is quite small.

This study had some limitations. Albumin concentration was used to estimate the exercise-induced change in plasma volume, but albumin as well as water may leave the vascular compartment during exercise, thereby compromising the estimate of plasma volume change. However, similar results were obtained when the correction was made using the change in PCV as an estimate of plasma volume change.³² The statistical power of the present study was also insufficient to detect small differences in thyroid hormone concentrations. Whether such small differences would be physiologically important, however, remains doubtful.

Circulating testosterone concentrations would have decreased in all dogs when they stopped racing because the males were neutered, and females were no longer administered testosterone after they were spayed.

Testosterone decreases thyroid-binding protein and can decrease total T_4 while having little effect on free T_4 .³³ Therefore, it is possible that withdrawal of testosterone was responsible for the change in total T_4 concentration when dogs stopped training. Nevertheless, the results are still of practical importance, because neutering is standard practice when dogs are retired. Some unrelated time-related factors may also have affected the results, because comparisons were not made with unexercised controls.

Serum total and free T_4 concentrations of trained and untrained Greyhounds in the present study were lower than the reference range reported for other breeds. Although healthy, the Greyhounds in our study might have been classified as hypothyroid. Unfortunately, baseline serum total T_4 concentrations are often used as the only screening test to differentiate between euthyroid and hypothyroid dogs.³⁴⁻³⁶ Greyhounds are often given T_4 orally on the assumption that they are hypothyroid because of a low single total T_4 concentration determination. The diagnosis of hypothyroidism in dogs should be made only if additional tests of thyroid function are abnormal and there are aberrant clinical findings that improve with thyroid hormone replacement therapy.³⁷⁻³⁹ Measurement of free T_4 concentration by use of equilibrium dialysis has proved an excellent test of thyroid function in humans but has been disappointing as a test of thyroid function in dogs.⁴⁰ However, when evaluated simultaneously with TSH determinations, the distinction between euthyroid and hypothyroid dogs becomes more accurate.^{18,37,41,42} The Greyhounds in the present study were clinically normal, and their serum TSH concentrations and autoantibody concentrations were within reference ranges for control dogs (non-Greyhounds). Furthermore, the measured increase in body temperature and severity of lactic acidosis after exercise was not blunted, as would be expected if the dogs had been hypothyroid.⁴³ If TSH concentrations, metabolic responses to exercise, and clinical signs are considered together, these Greyhounds should be classified as euthyroid despite low total and free T_4 concentrations.

The ranges of serum total T_4 concentrations for trained and detrained Greyhounds in the present study (1 to 6 and 4 to 12 nmol/L, respectively) were lower than those reported by Gaughan et al⁹ for either trained and detrained greyhounds (8 to 21 and 7 to 17, respectively) and by Beale et al¹² for breeding and pet dogs (11 to 27 nmol/L). However, the mean total T_4 concentration in detrained dogs in our study was slightly higher than that reported by Pamerter and Boyden⁴⁴ in Greyhounds of unspecified status (7.2 vs 5.5 nmol/L). Nevertheless, total T_4 concentrations were lower overall in Greyhounds than in other breeds. This suggests that low total and free T_4 concentrations in Greyhounds reflect interbreed variation. One possible explanation for the low T_4 concentrations in trained Greyhounds is that racing represents a stress. Greyhounds appear to become very excited about racing, and total T_4 concentrations can decrease to below normal in euthyroid dogs subjected to stress.^{45,46} This may explain why total T_4 concentrations increased slightly when dogs stopped racing, but concentrations remained below the reference range of non-Greyhound breeds even when these apparently euthyroid dogs were not raced.

Therefore, free and total T₄ concentrations should not be used alone to assess thyroid function in Greyhounds.

^aGaughan KR, Bruyette DS, Jordan FR, Comparison of thyroid function in nongreyhound pet dogs and racing greyhounds (abstr). *J Vet Intern Med* 1996;10:186.

^bTestosterone propionate (100 mg/ml), Steris, Phoenix, Ariz.

^cVacutainer tubes, Beckton Dickinson, Rutherford, NJ.

^dExpress chemistry analyzers M550, Ciba Corning, South Norwood, Mass.

^eSAS/STAT Version 6.04, SAS Institute Inc, Cary, NC.

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