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# Thyroid hormone concentrations in racing Thoroughbreds

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**Summery**

A study was conducted to investigate variations in concentrations of thyroxine (T4), triiodothyronine (T3), free T4 (fT4), and free T3 (fT3) in Thoroughbred racehorses. Venous blood samples were collected every 6 hours from 10 horses for 3 days. Although concentrations measured at 18.00 hr tended to be highest and those at 06.00 hr lowest, there was no clear-cut evidence of Circadian rhythmicity in these values. Racing and oral supplementation of feed with l-thyroxine tended to increase concentrations of T4, T3, and fT3, in particular. There was widespread individual variation in T4 values, which was most likely due to the effect of various exogenous stimuli. Many healthy, normally performing horses had T4 concentrations that were below the normal range. It was concluded that T3 and fT3 concentrations are relatively stable and fall within the normal range for all horses, while T4 is liable to vary considerably. Assessment of a racehorse's thyroid status should not be based on determination of T4 concentrations alone.

**Keywords:** thyroid, horse, exercise, training, racing

**Konzentration der Schilddrüsenhormone bei Galopprennpferden**

Diese Studie wurde durchgeführt, um die Schwankung der Konzentration von Thyroxin (T4), Trijodthyronin (T3), freiem Thyroxin (fT4), und freiem Trijodthyronin (fT3) im Blutserum von Galopprennpferden festzustellen. Dafür wurde drei Tage lang alle 6 Stunden venöses Blut von 10 Rennpferden gewonnen und analysiert. In der Tendenz waren die Hormongehalte um 18.00 Uhr höher als um 6.00 Uhr, jedoch war der zirkadiane Rhythmus nicht sehr ausgeprägt. Wenn die Pferde ein Rennen bestritten hatten oder sie oral mit L-Thyroxin supplementiert wurden, konnten tendenziell höhere T4-, T3- und insbesondere fT3-Gehalte in ihrem Blutserum gemessen werden. Am stärksten schwankte die T4-Konzentration. Viele gesunde Pferde, die eine der Erwartung entsprechende Rennleistung zeigten, hatten gegenüber dem Normbereich verringerte T4-Gehalte. Es zeigte sich, daß die T3- und fT3-Konzentration im Serum von Galopprennpferden relativ konstant ist und innerhalb der Normbereiche für Pferde liegt. Dagegen schwankt die T4-Konzentration so stark, daß die Schilddrüsenaktivität eines Galopprennpferdes nicht nur anhand seiner T4-Konzentration im Blutserum diagnostiziert werden sollte. Zumindest sollte auch die T3-Konzentration gemessen werden.

**Schlüsselwörter:** Schilddrüse, Pferd, Belastung, Training, Rennen

## Introduction

Thyroid function can be difficult to assess in horses, especially when such assessments are attempted on the basis of determining thyroxine (T4) concentrations in a single blood sample and/or when a thyroid stimulating hormone (TSH) stimulation test is not performed (*Morris* and *Garcia* 1983). This is partly because thyroid hormone exists in a number of forms, the best recognized of which are T4, triiodothyronine (T3, the active form of the hormone), free T4 (fT4), and free T3 (fT3). Because all forms of the hormone are in equilibrium, changes in the rate of intracellular metabolism or uptake of T3, or rate of deiodination of fT4 may affect concentrations of all forms of the hormone. Factors which are known or suspected to influence this balance in horses include albumin and total protein concentrations, treatment with protein binding medications such as phenylbutazone and anabolic steroids, inappetence or feed deprivation, metabolic rate, age and gender, level of fitness, frequency and intensity of exercise, and the time of year (*Morris* and *Garcia,* 1983; *Irvine,* 1966, 1967; *McBride* et al, 1985). A Circadian rhythm in T4 concentration has also been observed, although this finding has not been consistent (*Morris* and *Garcia,* 1983; *Sojka* et al, 1993; *Duckett* et al, 1989).

Although hypothyroidism has been stated to be a cause of poor performance and myopathies in racing horses (*Waldron-Mease*, 1979), it is not clear whether this is correct. Results from several studies have shown that T4 concentrations are often low, and sometimes undetectable in horses at peak fitness (*Irvine*, 1983). Because of the many factors that may influence serum concentrations of the various forms of thyroid hormones, in particular T4, the interpretation of serum thyroid hormone concentrations is difficult. With this in mind, this study was designed to determine the extent to which equine thyroid hormone concentrations of fit racehorses: (a) differ from those which have been determined for their sedentary counterparts; and (b) exhibit a Circadian rhythm in a field situation.

## Materials and methods

Ten horses that were actively racing and training were studied. Their ages ranged from 2 to 6 years. There were 6 geldings, 1 stallion, 1 filly, and 2 colts. All horses were in regular race training and under the care of one trainer, although diets, training sche-

dules, and medication practices varied between horses. Individual horse records were not available until after the completion of our investigation.

The investigation was conducted in late June. The horses' regular routines were maintained throughout the study. Jugular venous blood samples were obtained every 6 hours at 06.00, 12.00, 18.00, and 00.00 until 12 samples had been obtained (i.e., for 66 hours) in order to evaluate daily rhythmicity of T3, T4, fT3, and fT4 concentrations.

Following collection, samples were allowed to clot. Serum was then frozen and shipped to a clinical pathology laboratory for evaluation of T3, T4, fT3, and fT4 concentrations by radioimmunoassay using commercially available kits (Ciba Corning Diagnostics, East Walpole, Massachusettes; *Messer* et al, 1995). Data for cross-reactivity of related compounds at 50% suppression of the total binding was provided for each kit by its manufacturer. All results were expressed as the mean ± SEM for each variable at each sampling time. Individual results were compared by analysis of variance of repeated measures, and when the F-statistic was significant, means were further compared using the least significant difference post-hoc test. Values were regarded as being different when $P<0.05$.

## Results

Data from the samples collected at 6 hourly intervals for 66 hours are shown in Fig. 1. Despite the appearance of the curves for T4 and fT4, there was no clear-cut statistical evidence of the existence of a significant Circadian rhythm. There were no significant differences between any T4 or fT3 concentrations. Variability in values for each sampling time was large, especially for T4 (Tab. 1). The T3 concentration at 18.00 hr on day 3 was significantly greater than that at 06.00 hr on the same day, and values at 00.00 hr on days 1, 2, and 3. The concentration at 18.00 hr on day 2 was also significantly greater than that obtained 12 hours later. fT4 concentration tended to be highest at 18.00 hr with the value at this time on day 2 being significantly greater than those recorded at 06.00 hr on days 1 and 2, and 00.00 hr on day 1. The concentrations at 06.00 on days 1 and 2 were also significantly lower than that at 18.00 hr on day 3.

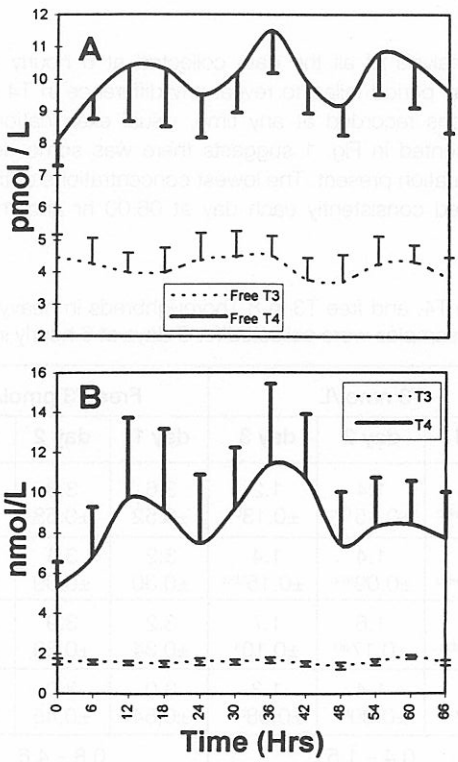


**Fig. 1:** Fluctuations in fT3 and fT4 (A) and T3 and T4 (B) concentrations in 10 Thoroughbred racehorses sampled at 6 hourly intervals for 66 hours.

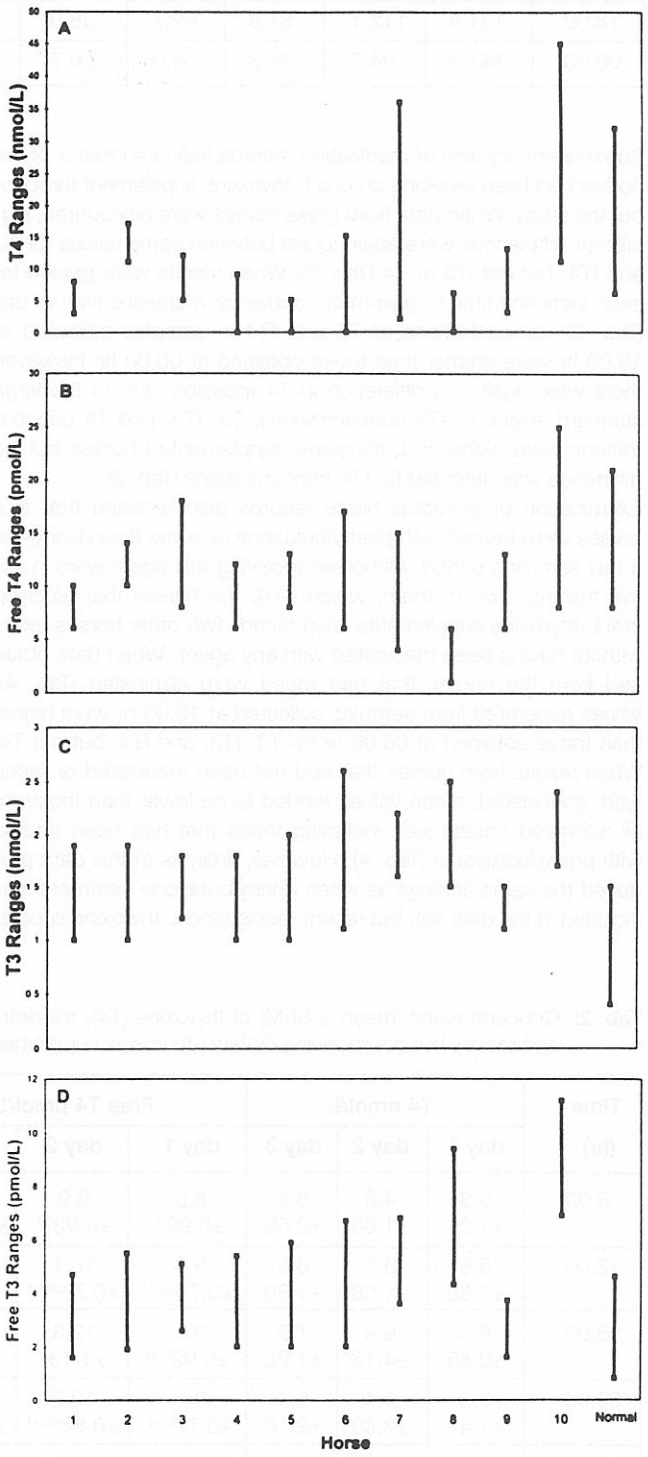


**Fig. 2:** Ranges in concentrations of T4 (A), fT4 (B), T3 (C), and fT3 (D) of individual horses sampled at 6 hourly intervals for 66 hours. The normal range of values for sedentary horses is also shown on the right side.

**Tab. 1:** Coefficients of variation (%) for plasma concentrations of thyroxine (T4), triiodothyronine (T3), free T4, and free T3 for each sampling time. Samples were obtained every 6 hours over a 66 hour period.

| Time | T4 nmol/L | | | Free T4 pmol/L | | | T3 nmol/L | | | Free T3 pmol/L | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 |
| 6.00 | 75.0 | 143.0 | 112.6 | 31.0 | 44.7 | 32.7 | 30.8 | 33.9 | 37.7 | 49.8 | 60.5 | 73.1 |
| 12.00 | 115.3 | 104.4 | 93.0 | 24.9 | 38.9 | 37.2 | 24.8 | 25.7 | 30.7 | 60.4 | 57.1 | 66.4 |
| 18.00 | 131.4 | 112.1 | 81.8 | 52.7 | 36.0 | 41.9 | 22.3 | 26.6 | 17.9 | 49.1 | 43.3 | 39.0 |
| 00.00 | 141.3 | 94.3 | 94.3 | 54.9 | 30.7 | 41.7 | 31.4 | 26.7 | 27.5 | 61.1 | 57.4 | 54.2 |

Subsequent scrutiny of medication records indicated that 2 of the horses had been receiving an oral L-thyroxine supplement throughout the study. When data from these horses were discounted, significant differences were again found between some values for T3 and fT4, but not fT3 or T4 (Tab. 2). When results were pooled for each sampling time to give mean values for a specific time of day (Tab. 3), concentrations of T3 and fT4 in samples collected at 18.00 hr were greater than those obtained at 06.00 hr. However, there was, again, no difference in T4 (possibly due to the large standard errors) or fT3 concentrations. T3, fT3, and T4 concentrations were higher in L-thyroxine supplemented horses but no difference was detected for fT4 concentrations (Tab. 3).

Examination of individual horse records also revealed that four horses were treated with phenylbutazone at some time during the 3 day sampling period. All horses receiving this agent were in active training. Two of them, which were the horses that received oral L-thyroxine supplements, also raced. Two other horses raced without having been medicated with any agent. When data obtained from the horses that had raced were eliminated (Tab. 4), values generated from samples collected at 18.00 hr were higher than those obtained at 06.00 hr for T3, fT3, and fT4, but not T4. When results from horses that had not been medicated or raced were considered, mean values tended to be lower than those for all nonraced horses (i.e., including those that had been treated with phenylbutazone) (Tab. 4). However, analysis of this data produced the same findings as when phenylbutazone treatment was included in the data set, but recent racing (and L-thyroxine supplementation) was excluded. When results from the 2 horses which were administered phenylbutazone but did not race or receive L-thyroxine were compared to those from 4 animals that were treated with nothing and did not race, T4 and fT4 concentrations were higher in the 2 medicated horses at each time period. However, the small sample sizes made the relevance of such comparisons questionable.

Pooling of all results collected over the 3 day period from nonmedicated, nonraced horses revealed that the mean concentration of T4 was considerably below that regarded as normal by the laboratory performing the analysis (Tab. 4). Overall variability tended to be lower when the effects of racing and/or L-thyroxine supplementation were discounted, but was still large, especially for T4 (Tab. 4). There were also notable differences in the range of values obtained from individual horses over the 3 day period (Fig. 2). The greatest variations were seen in two of the horses that had raced. Horse #7 had received no medication, while horse #10 had been given both phenylbutazone and oral L-thyroxine.

## Discussion

Although analysis of all the data collected at 6 hourly intervals over a 3 day period failed to reveal any difference in T4 and fT4 concentrations recorded at any time, visual examination of the results presented in Fig. 1 suggests there was some degree of diurnal fluctuation present. The lowest concentrations of these values occurred consistently each day at 06.00 hr and maximum

**Tab. 2:** Concentrations (mean ± SEM) of thyroxine (T4), triiodothyronine (T3), free T4, and free T3 in 8 Thoroughbreds in heavy training and racing, but not receiving dietary l-thyroxine supplement. Venous blood samples were collected for 3 days at 6 hourly intervals.

| Time | T4 nmol/L | | | Free T4 pmol/L | | | T3 nmol/L | | | Free T3 pmol/L | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| (hr) | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 | day 1 | day 2 | day 3 |
| 6.00 | 5.3 ±1.21 | 4.6 ±1.50 | 6.3 ±2.58 | 8.5 ±0.90$^{d}$ | 8.9 ±0.93$^{cd}$ | 9.6 ±0.94$^{abcd}$ | 1.5 ±0.14$^{abc}$ | 1.4 ±0.15$^{1bc}$ | 1.2 ±0.13$^{c}$ | 3.6 ±0.52 | 3.3 ±0.58 | 2.7 ±0.49 |
| 12.00 | 5.5 ±1.88 | 6.7 ±1.58 | 6.6 ±1.80 | 9.6 ±0.75$^{abcd}$ | 10.1 ±0.72$^{abcd}$ | 11.5 ±0.98$^{abc}$ | 1.4 ±0.11$^{abc}$ | 1.4 ±0.09$^{abc}$ | 1.4 ±0.15$^{abc}$ | 3.2 ±0.30 | 3.4 ±0.39 | 3.3 ±0.51 |
| 18.00 | 6.5 ±0.85 | 9.9 ±4.13 | 7.3 ±1.22 | 9.4 ±0.92$^{bcd}$ | 12.3 ±1.13$^{a}$ | 11.6 ±1.15$^{ab}$ | 1.4 ±0.10$^{abc}$ | 1.6 ±0.17$^{ab}$ | 1.7 ±0.10$^{a}$ | 3.2 ±0.34 | 3.9 ±0.59 | 3.8 ±0.39 |
| 00.00 | 5.8 ±1.44 | 9.4 ±3.40 | 6.9 ±2.10 | 9.1 ±0.77$^{bcd}$ | 10.2 ±0.82$^{abcd}$ | 10.4 ±1.03$^{abcd}$ | 1.4 ±0.13$^{bc}$ | 1.4 ±0.09$^{bc}$ | 1.3 ±0.08$^{bc}$ | 3.0 ±0.54 | 3.0 ±0.45 | 3.0 ±0.40 |
| lab normals* | 6 – 32 | | | 8 – 21 | | | 0.4 – 1.5 | | | 0.8 – 4.6 | | |

Values with the same superscript are not significantly different. There were no differences between any values for T4 or fT3.
*As supplied by Animal Health Diagnostic Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan.

**Tab. 3:** Mean (±SEM) concentrations of thyroxine (T4), triiodothyronine (T3), free T4, and free T3 from blood samples taken on 3 consecutive days at 06.00, 12.00, 18.00, and 00.00 hr from 8 Thoroughbred racehorses which were not receiving oral l-thyroxine (no supplement) and two which were (supplement).

| Time (hr) | T4 nmol/L | | free T4 pmol/L | | T3 nmol/L | | free T3 pmol/L | |
|---|---|---|---|---|---|---|---|---|
| | no supplement | supplement | no supplement | supplement | no supplement | supplement | no supplement | supplement |
| 6.00 | 5.4 ± 1.03 | 12.2 ± 5.05 | 9.0 ± 0.52$^{b}$ | 8.7 ± 2.32 | 1.3 ± 0.008$^{b}$ | 2.2 ± 0.10* | 3.2 ± 0.30 | 8.0 ± 0.67* |
| 12.00 | 6.3 ± 0.98 | 15.3 ± 5.07* | 10.4 ± 0.49$^{ab}$ | 9.2 ± 2.64 | 1.4 ± 0.06$^{ab}$ | 2.1 ± 0.08* | 3.3 ± 0.23 | 8.5 ± 0.97* |
| 18.00 | 7.9 ± 1.43 | 17.7 ± 6.73* | 11.1 ± 0.65$^{a}$ | 9.7 ± 3.50 | 1.6 ± 0.07$^{a}$ | 2.0 ± 0.08* | 3.6 ± 0.26 | 6.8 ± 0.62* |
| 00.00 | 7.3 ± 1.39 | 16.5 ± 6.04* | 9.92 ± 0.50$^{ab}$ | 10.0 ± 3.56 | 1.4 ± 0.06$^{b}$ | 2.1 ± 0.14* | 3.0 ± 0.26 | 7.2 ± 0.64* |

Results with the same superscript are not significantly different. No differences were detected in T4 or fT3 concentrations for the control group.
*Designates values from l-thyroxine supplemented horses that were significantly different to those from non-supplemented horses for the same sampling time.

daily concentrations occurred at 18.00 hr. Failure to demonstrate differences in values was likely due to the considerable individual variation in concentration which resulted in the sizeable standard errors and large coefficients of variation. When values obtained from horses that had been supplemented with L-thyroxine were eliminated, evidence of diurnal variation became more apparent with significant differences in some T3 and fT4 concentrations being demonstrated. This was further emphasized when possible effects of racing were discounted and results were grouped according to the time of sample collection. Under these more homogeneous conditions, concentrations of T3, fT3, and fT4 were all higher at 18.00 hr than at 06.00 hr.

As Tab. 3 indicates, there was a great difference in T4 concentrations between horses that were supplemented with L-thyroxine and those that were not. Also, provision of this agent appeared to result in much higher fT3 concentrations and higher T3 values. When the effects of racing were discounted, mean concentrations of T4 were even lower than for the entire non-L-thyroxine supplemented group (Tab. 3 and 4), although there was still considerable inter-individual variation. Inclusion of horses that had been treated with phenylbutazone but not raced, resulted in higher values of T4. This was surprising as phenylbutazone has been shown to bind with albumin, hence decreasing the amount of protein-bound T4 in the circulation (*Morris* and *Garcia,* 1983). Consequently, it was to be expected that including horses that had been treated with phenylbutazone in the population under consideration would have had the effect of further reducing T4 concentrations. Treatment with phenylbutazone had no apparent effect on T3 or fT3 concentration.

It is clear that T4 appears to be very susceptible to the effects of a variety of exogenous stimuli, and as such, likely represents a very unreliable means on which to base an assessment of a racehorse's thyroid status. T3 and fT3 concentrations appear to be much more stable than their corresponding forms of T4 and the normal laboratory range for sedentary horses appears to be applicable to their actively training and racing counterparts. Therefore, it is recommended that if a concerted effort at evaluating a horse's thyroid status is to be made, then at least T3 concentrations should be determined. The relative consistency of T3 and fT3 was underscored by the lack of experimental control associated with

**Tab. 4:** Concentrations (mean ± SEM) and overall variability (CV, %) of thyroxine (T4), triiodothyronine (T3), free T4, and free T3 from samples collected at 6 hourly intervals for 3 days in 4 Thoroughbreds in training which did not race or receive any medication or dietary thyroid supplement (control), and 6 Thoroughbreds which did not race or receive a thyroid supplement, but may have been medicated with phenylbutazone (PBZ, 2 extra horses) during the sampling period.

| Time (hr) | T4, nmol/L | | free T4, pmol/L | | T3, nmol/L | | free T3, pmol/L | |
|---|---|---|---|---|---|---|---|---|
| | control | PBZ | control | PBZ | control | PBZ | control | PBZ |
| 6.00 | 2.4 ± 0.85 | 4.8 ± 1.08 | 8.4 ± 0.60$^{b}$ | 9.2 ± 0.52$^{b}$ | 1.2 ± 0.08$^{a}$ | 1.2 ± 0.07$^{a}$ | 2.5 ± 0.24$^{a}$ | 2.7 ± 0.24$^{a}$ |
| 12.00 | 3.4 ± 1.09 | 6.1 ± 1.25 | 9.5 ± 0.58$^{ab}$ | 10.6 ± 0.62$^{ab}$ | 1.3 ± 0.05$^{a}$ | 1.3 ± 0.05$^{a}$ | 2.8 ± 0.17$^{a}$ | 3.0 ± 0.20$^{ab}$ |
| 18.00 | 5.3 ± 0.86 | 7.1 ± 0.94 | 10.4 ± 0.73$^{a}$ | 11.5 ± 0.68$^{a}$ | 1.7 ± 0.12$^{b}$ | 1.6 ± 0.09$^{b}$ | 3.9 ± 0.43$^{b}$ | 3.6 ± 0.32$^{b}$ |
| 00.00 | 4.3 ± 1.26 | 6.8 ± 1.31 | 9.3 ± 0.50$^{ab}$ | 10.4 ± 0.55$^{ab}$ | 1.3 ± 0.05$^{a}$ | 1.3 ± 0.04$^{a}$ | 2.8 ± 0.26$^{a}$ | 2.8 ± 0.19$^{a}$ |
| overall mean | 3.9 ± 0.52 | 6.2 ±0.58 | 9.4 ± 0.31 | 10.4 ± 0.31 | 1.3 ± 0.05 | 1.4 ± 0.04 | 3.0 ± 0.16 | 3.0 ± 0.13 |
| overall CV (%) | 93.8 | 78.6 | 23.0 | 25.0 | 25.0 | 22.3 | 37.1 | 35.1 |

Results with the same superscript were not significantly different. No differences were detected between T4 concentrations within either group.

this study. All the work was performed under field conditions with the intent of developing data that is relative to the racetrack environment. Even when blood samples were obtained at the same time of day, there was considerable variation between horses with respect to the time of feeding, exercising, and racing. This was further confounded by variation in medication and dietary supplementation practices.

This study demonstrated that extreme fluctuations in T4 concentrations may be observed in racehorses that are actively training and racing, and these must be considered when contemplating a diagnosis of equine hypothyroidism. Despite the low values that were observed in many of these horses (Fig. 2), there was no clinical reason to believe that any of these animals were hypothyroid. Therefore, results from thyroid hormone assays performed on a single blood sample in a poorly performing race horse may provide little indication of the true thyroid hormone status of the patient. Assessment of thyroid status on such animals would be best if done in conjunction with performance of the thyroid hormone stimulation test, although thyroid stimulating hormone can be difficult to obtain. Furthermore, there may be little justification for providing thyroid hormone supplements to fit Thoroughbred racehorses on the basis of results from a single blood test, especially if T4 is the only form of the hormone assessed, as normal resting values for this form of the hormone are comparatively low in race horses. If a TSH stimulation test cannot be performed, interpretative emphasis should be placed on T3 rather than T4 concentrations, as the former represents the active form of the hormone and also appears to be much less susceptible to variations attributable to the many exogenous influences commonly associated with horse racing in North America.

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