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Nonthyroidal Illness Syndrome in Adult Horses

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Background: This study was performed to determine whether sick horses have thyroid hormone (TH) alterations similar to those observed in nonthyroidal illness syndrome in other species.

Hypothesis: Horses suffering from systemic diseases have decreased THs and inappropriately low thyroid-stimulating hormone (TSH).

Animals: Seventy-one clinically normal horses; 380 hospitalized horses.

Methods: Total thyroxine (TT4), free thyroxine by equilibrium dialysis (fT4D), total triiodothyronine (TT3), free triiodothyronine (fT3), and TSH were measured in normal and hospitalized horses. Disease severity was categorized as mild, moderate, or severe by both subjective and objective criteria.

Results: Negative correlations existed between all THs, except TSH, and objective illness severity scores. These scores also increased with each subjective disease severity category. TT3 and fT3 were decreased with mild disease. TT3 progressively decreased more with moderate and severe disease. TT4 and fT4D remained normal with mild disease, but decreased progressively with disease severity. TSH increased with mild disease, but remained normal with moderate or severe disease. Horses that died or were euthanized had lower concentrations of all THs, except TSH, when compared with those that lived. In horses that received >3 doses of NSAIDs, corticosteroids, or heparin compared to 0–3 doses, TT3 and TT4 were decreased, whereas fT4D and TSH remained normal. There were minimal TH changes in horses that were not eating.

Conclusions and Clinical Importance: Thyroid hormones decrease in horses with systemic disease. TT3 decreases first, followed by TT4 and fT4D. TSH fails to increase proportionally to the changes in THs, indicating hypothalamic–pituitary axis dysregulation. NSAIDs, corticosteroids, heparin, and fasting have less effect on THs compared with disease severity.

Key words: Equine; Euthyroid; Hypothyroidism; Thyroid hormone.

Thyroid gland dysfunction is fairly uncommon in the adult horse, but clinicians often treat horses with thyroid hormone (TH) supplementation, even when documentation of dysfunction is lacking. When THs are measured, often only total thyroxine (TT4) is measured, which can be misleading. Assays for TT4 and total triiodothyronine (TT3) have long been available for use in the horse, and normal reference ranges[1–13] have been established. However, single measurements of TT4 and TT3, without free fractions, can be difficult to interpret because TH metabolism or transport can be affected by many extrathyroidal factors.

In the horse, phenylbutazone, dexamethasone, dietary imbalances, and fasting decrease TT4 without the presence of thyroid gland disease.[1–3,14,15] Nonthyroidal illness syndrome (NTIS) also may result in altered TH concentrations, but NTIS has only been studied minimally in horses.[16] In humans, dogs, and cats suffering from systemic illnesses, NTIS is characterized first by low TT3 with or without decreases in TT4.[17–25] Thyroid-stimulating hormone (TSH) often is normal, differentiating NTIS from primary hypothyroidism.[23,25–27] In foals, decreases in all THs except rT3 were seen in NTIS; TSH was not evaluated.[28] The degree of TH suppression in all these species has been correlated with disease severity and mortality.[25,26,28–30] Characterization of NTIS in the horse is important because measurement of low TT3 or TT4 in a horse with systemic illness could easily lead to a misdiagnosis of hypothyroidism and unnecessary TH supplementation. An additional benefit of such characterization would be development of a diagnostic tool for assessment of disease severity and prediction of outcome.

The primary purpose of this study was to determine the degree to which illness alters THs and TSH in the horse. Secondary goals included the following: (1) to determine the effects of fasting and of certain drugs (NSAIDs, corticosteroids, heparin) on THs and (2) to determine if alteration in any individual TH could be used to predict severity of disease or mortality.

Abbreviations:

fT3	free triiodothyronine
fT4D	free thyroxine by equilibrium dialysis
HPA	hypothalamic–pituitary axis
IL	interleukin
NTIS	nonthyroidal illness syndrome
TBG	thyroxine-binding globulin
TH	thyroid hormone
TNF	tumor necrosis factor
TRH	thyrotropin-releasing hormone
TSH	thyroid-stimulating hormone
TT3	total triiodothyronine
TT4	total thyroxine

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Materials and Methods

Animals

Control blood samples were obtained from 71 clinically normal adult horses (30 nonpregnant mares, 38 geldings, and 3 stallions; ages 3–26 years: 42 horses ≤10 years, 26 horses 11–20 years, and 3 horses >20 years) of various breeds, living in their home environments. Normal horses received no medications for ≥4 weeks before sampling, and were determined to be systemically healthy based on history and physical examination. Blood samples were obtained randomly from 380 adult horses (143 nonpregnant mares, 223 geldings, and 14 stallions; ages 1–37 years: 208 horses ≤10 years, 137 horses 11–20 years, 34 horses 21–30 years, and 1 horse >30 years) of various breeds that were admitted to the North Carolina Veterinary Teaching Hospital for diagnosis and treatment of a primary illness or injury. Horses were included in the study if adequate information was available to place them into 1 of 3 "severity of disease" categories (mild, moderate, and severe) based on clinical judgment (BB and AH). Disease severity was categorized as "mild" if the horse had sustained an injury with little systemic involvement or required minimal hospital care and could have been treated at home with a good prognosis. The "moderate" category was assigned if disease status was such that care was required at a secondary or tertiary facility, but the horse had a reasonable prognosis for survival. The "severe" category was assigned to horses with a guarded to poor prognosis for survival, even with the level of care provided at a secondary or tertiary care facility. An "illness severity score" (developed by BB) that takes into account neutrophils, bands, platelets, fibrinogen, blood glucose concentrations, mucous membrane color, capillary refill time, heart rate, and digital pulse quality (Table 1) also was calculated for each horse, using results obtained nearest the time of blood sampling. Blood lactate concentration was not included because it was not consistently available. All owners signed consent forms for blood sampling.

Blood Samples

Blood was obtained by jugular venipuncture between 8:00 AM and 12:00 PM and allowed to clot at room temperature. Samples were centrifuged at 4°C and serum was removed and stored at −70°C until assayed. All samples were assayed for TT4, fT4D, TT3, fT3, and TSH.

Hormone Measurement

Serum concentrations of TT3, fT3, fT4D, and TSH were measured with commercially available radioimmunoassay (RIA) kits (Clinical Assays Gammacoat free T3 125I RIA Kit[a]),[b] or RIA as previously described and validated in the horse.[6,16,31,32] TT3 assay sensitivity was 0.3 nmol/L. fT3 assay sensitivity was 0.1 pmol/L. fT4D assay sensitivity was 1.8 pmol/L. TSH assay sensitivity was 0.02 ng/mL. Normal ranges for TH in adult euthyroid horses are included in Tables 2–6.

Total thyroxine was measured with a commercially available solid-phase RIA kit (Clinical Assays Gammacoat M Total T4 125I RIA Kit[a]). Specificity data from the manufacturer identified 92% cross-reactivity with D-thyroxine, 2.1% with D- and L-triiodothyronine, and <0.1% with other iodothyronines. Modifications were made to the assay protocol to enhance analytical sensitivity. The volume of sample or standard was increased from 10 to 25 μL. A "lower" standard was made by mixing equal volumes of 0- and 13-nmol/L standards. After pipetting sample or standard and 1-mL radioligand solution into antibody-coated tubes, the assay was incubated at room temperature (22°C) for 3 hours. The sensitivity of the assay, defined as the concentration of TT4 at 90% specific binding was 3 nmol/L (data from 10 assays). When L-thyroxine was added to aliquots of a pool of equine serum to create increases of 26, 52, and 78 nmol/L, 106, 104, and 95% of added TT4 was measured. A pool of equine serum with a TT4 concentration of 67 nmol/L was diluted at rates of 50 and 25% in "0" standard, with respective recovery rates of 96 and 113% when corrected for dilution. In 10 assays, intraassay coefficients of variation (CV) for pools of equine serum with low (8 nmol/L), mid-range (22 nmol/L), and high (67 nmol/L) concentrations of TT4 were 0.072, 0.042, and 0.030, respectively. In 10 assays, the interassay CV for equine serum pools with TT4 concentrations of 6, 18, and 35 nmol/L was 0.237, 0.069, and 0.073, respectively.

Statistical Analysis

Commercial software was used for statistical analyses and graph generation.[c] When >2 groups were being compared, THs and TSH were compared among groups by one-way analysis of variance (ANOVA) or by the Kruskal–Wallis ANOVA on ranks whenever the data were not normally distributed. When interactions between groups were assessed, two-way ANOVA on ranks was used. Posthoc analyses were performed by Dunn's method of multiple comparisons. Spearman rank order correlations also

Table 1. Illness severity scoring system. Values tabulated for a score of 0–39.

	0	1	2	3	4	5
Neutrophils	Normal (3,400–8,500/μL) <100 Bands	High (8,600–15,000/μL) <100 Bands	Normal–high (3,400–15,000/μL) >100 Bands	Very high (>15,000/μL) Bands ±	Low (<3,400/μL) Bands present	Low (<3,400/μL) No Bands
Fibrinogen	100–499	500–799	800–999	≥1,000		<100
Platelets	Normal (94,000–232,000/μL)			Increased (>232,000/μL)		Decreased (<94,000/μL)
Mucous membrane color	Pink	Pale	Icteric	Dark pink, bright red, injected	Dark red, muddy, toxic line	Purple, blue, ± toxic line
Capillary refill time	≤2 seconds		3 seconds		4 seconds	>4 seconds
Heart rate	<50 bpm	50–59 bpm	60–69 bpm	70–79 bpm	80–89 bpm	≥90 bpm
Digital pulse quality	Normal	Mildly increased		Bounding	Clinical foot pain	
Glucose	70–120 mg/dL	121–150 mg/dL	151–200 mg/dL	201–400 mg/dL	>400 mg/dL	<70 mg/dL

Table 2. THs in normal and hospitalized horses with mild, moderate, or severe disease.

Hormone (Normal Range)	Normal, n = 71	Mild, n = 157	Moderate, n = 157	Severe, n = 66
TT3 (0.7–2.5 nmol/L)	(0.3–2.9) 0.9 ± 0.11[a]	(0.0–2.7) 0.4 ± 0.09[b]	(0.0–5) 0.1 ± 0.10[c]	(0.0–1.5) 0.0 ± 0.07[d]
fT3 (1.7–5.2 pmol/L)	(0.1–5.9) 1.7 ± 0.31[a]	(0.1–5.9) 1.1 ± 0.16[b]	(0–9.9) 0.8 ± 0.16[c]	(0.0–1.9) 0.6 ± 0.11[c]
TT4 (6–46 nmol/L)	(6–46) 19.0 ± 2.22[a]	(0–52) 15.0 ± 1.92[a]	(0–49) 5.0 ± 1.62[b]	(0–40) 0.0 ± 1.85[c]
fT4D (7–47 pmol/L)	(7–47) 22.0 ± 2.10[a]	(1–65) 23.0 ± 1.82[a]	(0–95) 15.0 ± 1.94[b]	(0–50) 8.0 ± 2.37[c]
TSH (0.02–0.97 ng/mL)	(0.02–0.97) 0.35 ± 0.05[a]	(0.02–1.33) 0.49 ± 0.34[b]	(0.03–1.01) 0.43 ± 0.04[b,c]	(0.02–0.82) 0.37 ± 0.05[a,c]

Results expressed as (range) median ± 95% CI. Columns with different letters are significantly different from each other, $P < .05$.

THs, thyroid hormones; TT3, total triiodothyronine; fT3, free triiodothyronine; TT4, total thyroxine; fT4D, free thyroxine by equilibrium dialysis; TSH, thyroid-stimulating hormone; CI, confidence interval.

Table 3. THs in hospitalized horses grouped by illness severity score.

	Illness Severity		
Hormone (Normal Range)	0–4, n = 195	5–10, n = 121	>10, n = 53
TT3 (0.7–2.5 nmol/L)	(0–2.7) 0.3 ± 0.08[a]	(0–5) 0.1 ± 0.12[b]	(0–1.0) 0.0 ± 0.05[c]
fT3 (1.7–5.2 pmol/L)	(0–5.9) 1.0 ± 0.14[a]	(0–9.9) 0.8 ± 0.19[b]	(0.1–2.0) 0.6 ± 0.12[b]
TT4 (6–46 nmol/L)	(0–52) 12.0 ± 1.69[a]	(0–49) 3.0 ± 2.03[b]	(0–30) 0.0 ± 1.54[c]
fT4D (7–47 pmol/L)	(1–95) 20.0 ± 1.83[a]	(0–66) 15.0 ± 2.02[b]	(0–31) 7.0 ± 1.89[c]
TSH (0.02–0.97 ng/mL)	(0–1.33) 0.44 ± 0.03	(0–0.99) 0.47 ± 0.04	(0.07–0.91) 0.37 ± 0.05

Results expressed as (range) median ± 95% CI. Columns with different letters are significantly different from each other, $P < .05$.

THs, thyroid hormones; TT3, total triiodothyronine; fT3, free triiodothyronine; TT4, total thyroxine; fT4D, free thyroxine by equilibrium dialysis; TSH, thyroid-stimulating hormone; CI, confidence interval.

Table 4. THs in horses that lived compared with those that died or were euthanized.

Hormone (Normal Range)	Lived, n = 309	Died/Euthanized, n = 67
TT3 (0.7–2.5 nmol/L)	(0–5.0) 0.2 ± 0.06[a]	(0–4.4) 0.0 ± 0.16[b]
fT3 (1.7–5.2 pmol/L)	(0–5.9) 0.9 ± 0.10[a]	(0–9.9) 0.6 ± 0.32[b]
TT4 (6–46 nmol/L)	(0–52) 9.0 ± 1.32[a]	(0–49) 0.0 ± 2.57[b]
fT4D (7–47 pmol/L)	(0–95) 18.0 ± 1.41[a]	(0–51) 9.0 ± 2.40[b]
TSH (0.02–0.97 ng/mL)	(0–1.33) 0.44 ± 0.03	(0–0.91) 0.43 ± 0.06

Results expressed as (range) median ± 95% CI. Columns with different letters are significantly different from each other, $P < .0.1$.

THs, thyroid hormones; TT3, total triiodothyronine; fT3, free triiodothyronine; TT4, total thyroxine; fT4D, free thyroxine by equilibrium dialysis; TSH, thyroid-stimulating hormone; CI, confidence interval.

Table 5. THs in horses administered 0–3 or >3 doses of NSAID, corticosteroid, or heparin.

Hormone (Normal Range)	Doses of NSAID, Steroid, Heparin	
	0–3, n = 149	>3, n = 232
TT3 (0.7–2.5 nmol/L)	(0–5.0) 0.2 ± 0.11[a]	(0–4.4) 0.1 ± 0.07[b]
fT3 (1.7–5.2 pmol/L)	(0–8.5) 0.9 ± 0.18	(0–9.9) 0.8 ± 0.13
TT4 (6–46 nmol/L)	(0–49) 12.5 ± 2.09[a]	(0–52) 5.0 ± 1.39[b]
fT4D (7–47 pmol/L)	(0–95) 19.0 ± 2.23	(0–66) 15.5 ± 1.51
TSH (0.02–0.97 ng/mL)	(0–1.14) 0.45 ± 0.04	(0–1.33) 0.42 ± 0.03

Results expressed as (range) median ± 95% CI. Columns with different letters are significantly different from each other, $P < .05$.

THs, thyroid hormones; TT3, total triiodothyronine; fT3, free triiodothyronine; TT4, total thyroxine; fT4D, free thyroxine by equilibrium dialysis; TSH, thyroid-stimulating hormone; CI, confidence interval.

were performed between TH or TSH and illness severity scores. The Student's *t*-test or the Mann–Whitney rank sum test was used to compare TH and TSH between 2 groups. Significance was set at $P < .05$. Data are expressed as the median, range, and 95% CI unless otherwise noted.

Results

THs and Disease States

Objective illness severity scores increased significantly with each subjective severity of disease category (mild, moderate, and severe), showing good agreement between the 2 methods of defining disease severity (Fig 1). When disease severity was determined subjectively, TT3 and fT3 were significantly decreased in horses with mild disease compared with normal horses (Table 2). TT3 continued to decrease progressively as disease severity increased from mild to moderate to severe (Fig 2). For fT3, there was no significant difference between horses with moderate versus severe

Table 6. THs in horses eating or off feed 1 day, off feed 1–2 days, or off feed ≥3 days.

Hormone (Normal Range)	Eating (Off Feed <1 Day), n = 177	Off Feed 1–2 Days, n = 149	Off Feed 3 or More Days, n = 54
TT3 (0.7–2.5 nmol/L)	(0–2.7) 0.3 ± 0.08[a]	(0–5) 0.1 ± 0.09[b]	(0–4.4) 0.1 ± 0.18[a,b]
fT3 (1.7–5.2 pmol/L)	(0–5.9) 1.0 ± 0.14[a]	(0–3.9) 0.7 ± 0.13[b]	(0.2–9.9) 0.9 ± 0.38[a]
TT4 (6–46 nmol/L)	(0–52) 9.0 ± 1.86[a]	(0–47) 8.0 ± 1.82[a]	(0–32) 1.0 ± 2.16[b]
fT4D (7–47 pmol/L)	(0–95) 20.0 ± 2.06[a]	(0–57) 14.0 ± 1.69[b]	(0–45) 13.0 ± 3.06[b]
TSH (0.02–0.97 ng/mL)	(0–1.33) 0.45 ± 0.04	(0.03–1.04) 0.42 ± 0.04	(0.13–1.08) 0.44 ± 0.08

Results expressed as (range) median ± 95% CI. Columns with different letters are significantly different from each other, $P < .05$.

THs, thyroid hormones; TT3, total triiodothyronine; fT3, free triiodothyronine; TT4, total thyroxine; fT4D, free thyroxine by equilibrium dialysis; TSH, thyroid-stimulating hormone; CI, confidence interval.

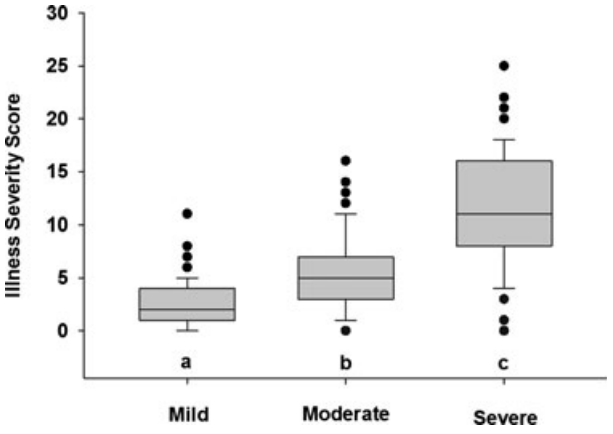

Fig 1. Objective illness severity scores in hospitalized horses subjectively grouped as having mild (n = 157), moderate (n = 157), or severe (n = 66) disease. Groups with different letters are significantly different from each other, $P < .05$.

disease (Fig 3). TT4 and fT4D remained normal in horses with mild disease, but were significantly decreased in those with moderate disease and decreased further in those with severe disease (Table 2, Figs 4, 5). TSH increased significantly in horses with mild disease compared with normal horses, but did not increase additionally with moderate disease. TSH in severe disease was not significantly different from TSH in normal horses (Table 2, Fig 6). Significant negative correlations (TT4 and fT4D $P \leq .0000002$, $r = -0.4$, TT3 and fT3 $P \leq .00000008$, $r = -0.3$) were identified between the objective illness severity scores and all THs except TSH. When objective illness severity scores were grouped as 0–4 (n = 195), 5–10 (n = 121), and >10 (n = 53), fT3 decreased significantly between 0–4 versus 5–10, but there was no additional decrease between 5–10 and >10 (Table 3, Fig 3). TT3, TT4, and fT4D decreased progressively, with significant differences among all 3 groups (Table 3, Figs 2, 4, 5). TSH was not significantly different among the 3 groups (Table 3, Fig 6).

THs and Outcome

Horses that died or were euthanized (excluding 7 horses euthanized for financial reasons) had lower concentrations of all THs, except TSH, when compared with those that lived ($P < .001$; Table 4). In sick horses that lived, TT3 and fT3 were below normal, whereas TT4, fT4D, and TSH remained normal. In horses that died or were euthanized, TT3, fT3, and TT4 were below normal, whereas fT4D and TSH remained normal.

THs and NSAIDs, Corticosteroid, and Heparin Use

Of the 148 horses that received 0–3 doses of drug (110 flunixin meglumine, 8 phenylbutazone, 11 aspirin, 2 etodolac, 1 ketoprofen, 15 corticosteroids, and 2 heparin), TT3 and fT3 were below normal; TT4, fT4D, and TSH remained normal. Of the 232 horses that received >3 doses (168 flunixin meglumine, 46 phenylbutazone, 8 aspirin, 3 etodolac, 0 ketoprofen, 7 corticosteroids, and 0 heparin), only fT4D and TSH remained normal. TT3 and TT4 were significantly decreased in horses that received >3 doses of drug compared with those that received 0–3 doses (Table 5). Because of the possibility that these drugs were given for longer periods of time in horses with greater disease severity, interactions between these 2 categories were evaluated; there were no statistically significant interactions between the 2 (TT4, $P = .822$; fT4D, $P = .451$; TT3, $P = .913$; fT3, $P = .352$; TSH, $P = .766$).

THs and Nutritional Status

When comparing horses that had been eating or were off feed <1 day, off feed for 1–2 days, or off feed for ≥3 days, TT3 and fT3 were below normal in all 3 categories, with concentrations being significantly lower after being off feed 1–2 days compared to <1 day. However, once horses had been fasted for ≥3 days, TT3 and fT3 were not significantly different compared with results in horses that were eating or off feed for <1 day (Table 6). TT4 remained normal until horses had been off feed for ≥3 days at which time it decreased significantly compared with horses off feed for a shorter period of time. fT4D remained normal for all 3 categories, but was significantly lower in horses off feed for 1–2 days and ≥3 days compared with those that were eating. TSH concentrations remained normal across all 3 categories with no significant differences among the categories. Because the duration of time off feed may be related to disease

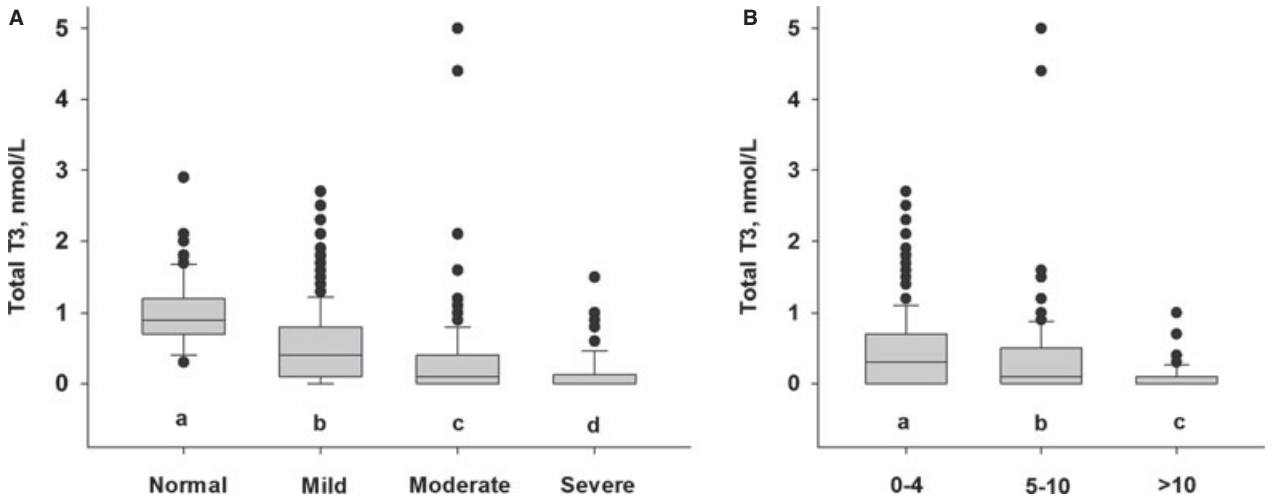

Fig 2. **(A)** Total triiodothyronine (TT3) in 71 normal horses and in hospitalized horses with mild, moderate, or severe disease. **(B)** TT3 in hospitalized horses grouped by illness severity score. Groups with different letters are significantly different from each other, $P < .05$.

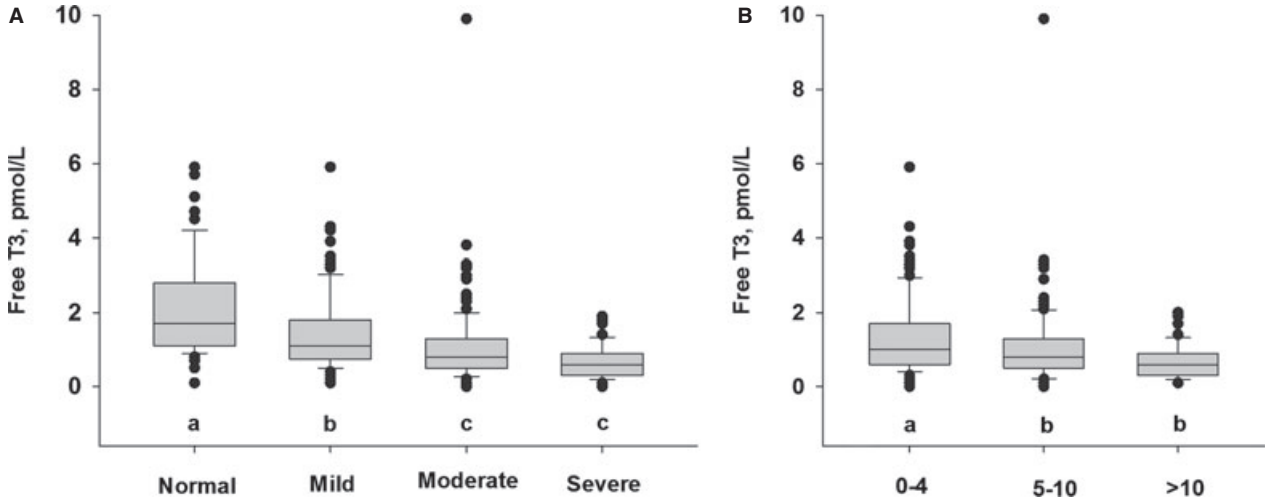

Fig 3. **(A)** Free triiodothyronine (fT3) in 71 normal horses and in hospitalized horses with mild, moderate, or severe disease. **(B)** fT3 in hospitalized horses grouped by illness severity score. Groups with different letters are significantly different from each other, $P < .05$.

severity, interactions between these 2 categories were evaluated; there were no significant interactions between the 2 (TT4, $P = .915$; fT4D, $P = .755$; TT3, $P = .862$; fT3, $P = .764$; TSH, $P = .472$).

Discussion

This study introduces an objective illness severity scoring system that appears to correlate well with subjective categorization of disease severity based on experienced clinical judgment. There was good agreement between the 2 methods in concentrations of THs and TSH during illness in the horses evaluated. Systemically ill horses in this study had decreased concentrations of all THs except TSH. Based on the subjective disease categories and objective illness severity scores, TT3 and fT3 decreased first, even in mild disease. TT4 and fT4D decreased significantly in horses with moderate disease, and continued to decrease more in horses with severe disease. Although median TT4 was below the normal reference range in horses with moderate or severe disease, fT4D remained within the normal reference range. For this reason, fT4D may be a good indicator of true thyroid gland status in ill horses. Despite decreases in T3 and T4 as disease severity increased, TSH remained normal, suggesting HPA dysregulation.

Results of this study are in good agreement with NTIS studies in other species. NTIS has been well described in children, adults, dogs, and cats suffering from systemic disease and usually is characterized by decreased TT3 and TT4. The most common finding is decreased TT3 even in mild disease, with or without decreased fT3, followed by decreased TT4 as the disease becomes more moderate to severe in nature.[17,18,30,33] Generally, fT4 decreases only in the most

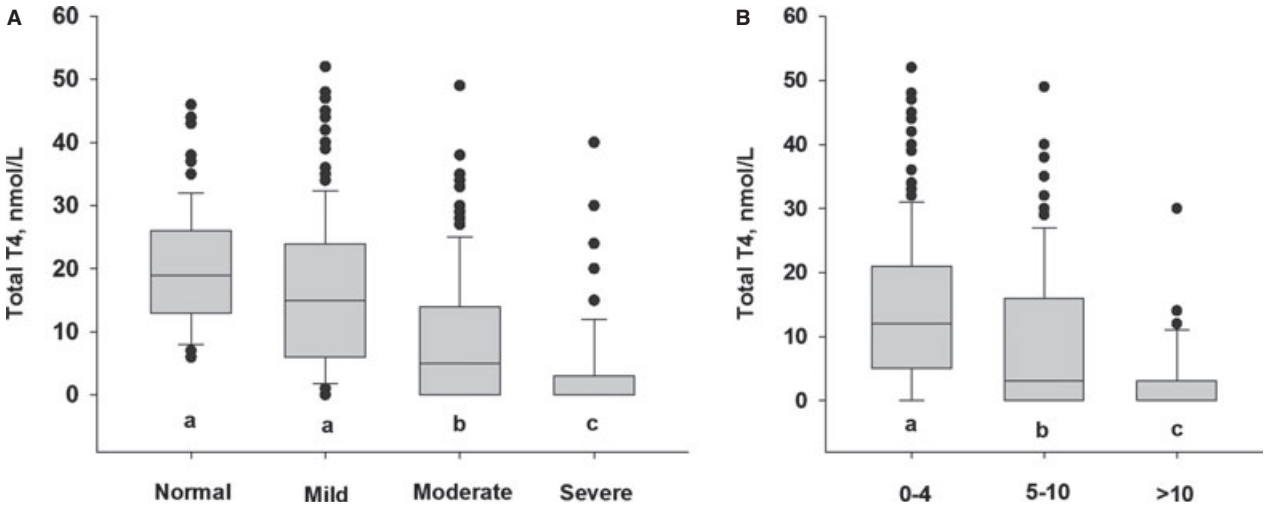

Fig 4. **(A)** Total thyroxine (TT4) in 71 normal horses and in hospitalized horses with mild, moderate, or severe disease. **(B)** TT4 in hospitalized horses grouped by illness severity score. Groups with different letters are significantly different from each other, $P < .05$.

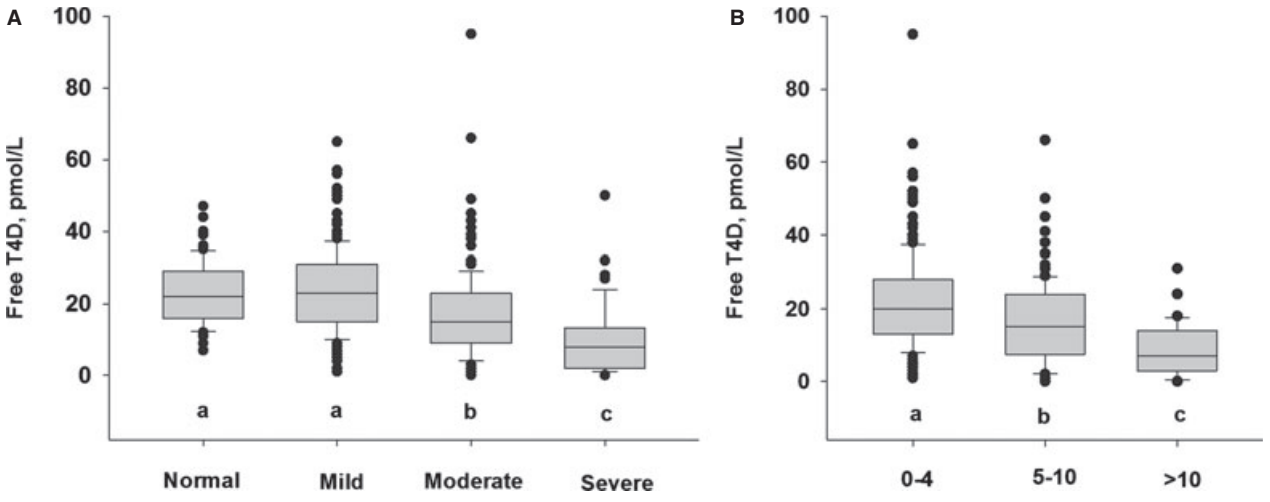

Fig 5. **(A)** Free thyroxine by equilibrium dialysis (fT4D) in 71 normal horses and in hospitalized horses with mild, moderate, or severe disease. **(B)** fT4D in hospitalized horses grouped by illness severity score. Groups with different letters are significantly different from each other, $P < .05$.

severe disease, but much variation can occur depending on analytical method. TSH typically remains inappropriately normal or even low in severe disease.[4,17,23,24,26,27,33–38] Decreases in all THs and increased rT3 recently were reported in foals with presumed NTIS.[28] Reverse T3 was not evaluated in this study, as it is often not included in routine TH panels.

Horses that died or were euthanized based on disease severity had significantly lower TT3, fT3, TT4, and fT4D compared with horses that lived; fT4D was the only TH that remained normal. TT4 remained normal in horses that lived, but decreased below normal in those that died or were euthanized, possibly making it useful in predicting mortality. In humans, low TT3 and TT4 have been associated with increased morbidity and mortality. Specifically, TT4 has been associated with decreased survival and poorer prognosis.[17,30,39,40] Because fT4D remains normal in most patients with NTIS unless illness is chronic and severe, it has been used as a sign of severity and predictor of poor outcome in humans if it also begins to decrease.[30] TSH remained inappropriately normal in horses that died or were euthanized supporting the presence of HPA dysregulation.

Many mechanisms exist by which TH decrease during illness. Decreased peripheral conversion of T4 to T3 by suppression of 5′-deiodinase is thought to be a result of systemic illness and a response to increased serum concentrations of glucocorticoids and inflammatory cytokines.[19,33–35,37] Increased concentrations of IL-1, IL-6, and TNF-α have been found to be negatively correlated with THs[19,37] and they may inhibit deiodinase, impair TH synthesis, and suppress TSH, but the mechanisms by which they do so remain

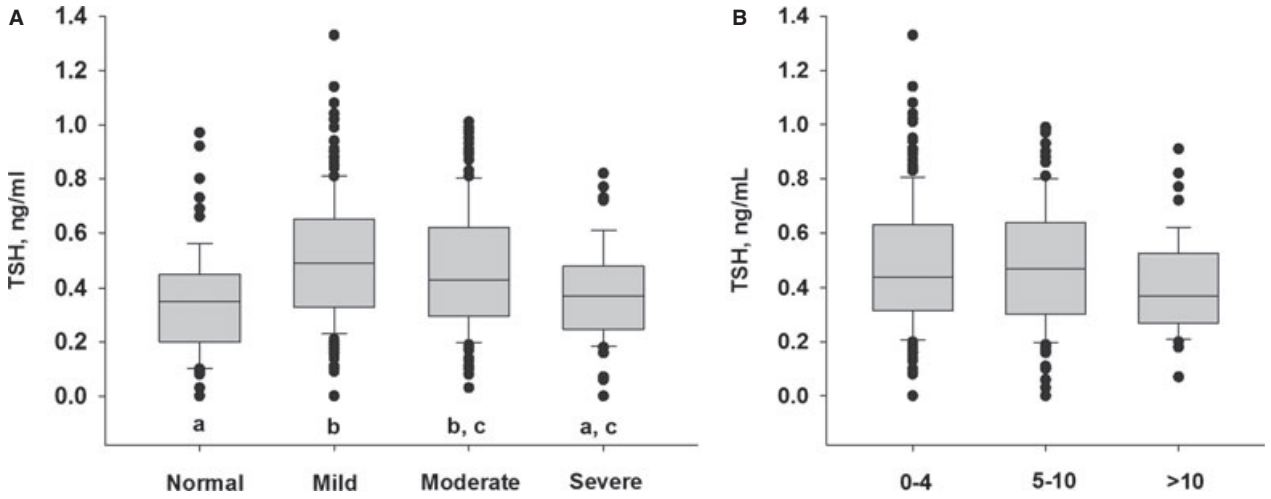

Fig 6. **(A)** Thyroid-stimulating hormone (TSH) in 71 normal horses and in hospitalized horses with mild, moderate, or severe disease. **(B)** TSH in hospitalized horses grouped by illness severity score. Groups with different letters are significantly different from each other, $P < .05$.

unknown.[19] Considering that concentrations of these cytokines are increased in severe illness, some believe that NTIS is an acute-phase response caused by activation of these cytokines.[19,34,35,37] Lipopolysaccharide, and the resulting systemic inflammatory response, has been found to decrease TH receptor expression.[35] Depletion of hepatic energy in diseased states also can lead to downregulation of receptor expression.[33,34,37]

Central downregulation of the hypothalamic–pituitary–thyroid axis plays a major role in NTIS. Inappropriately low concentrations of TSH in the face of low TH suggest decreased pituitary responsiveness to THs. TRH gene expression in hypothalamic neurons is lost owing to prolonged diminished caloric intake or cytokine-induced chronic inflammation.[34,35,39,41] Upregulation of 5′-deiodinase, converting T4 to T3 in the hypothalamus, may directly inhibit TRH neurons, thereby decreasing portal serum TRH. This excess T3 further suppresses TSH pulsatility and results in loss of nocturnal TSH surge.[19,35,37,41] Recent studies have found that coinfusion of TRH and growth hormone secretagogues in patients with chronic NTIS led to increased TSH, TT3, and TT4, further supporting the role of HPA dysregulation and a central role in low serum TH concentrations during critical illness.[19,37,39,40,42]

Altered binding of THs to serum carrier proteins is another mechanism by which THs are decreased in NTIS. Decreased availability of carrier proteins results from albumin-binding compounds that are capable of displacing THs.[34,35,37] Various drugs can affect TH concentrations in this way. Salicylates alter TH binding to carrier proteins and, in humans, result in increased fT4D.[16,17,35] Carrier proteins such as TBG, albumin, and transthyretin, all of which are negative acute-phase reactants, decrease in acute tissue injury and inflammation, enhancing the activity of low-affinity competitors of THs on binding to carrier proteins in disease.[34,35,37] In horses, phenylbutazone decreased TH by altering binding to carrier proteins, but the observed decrease in fT4D most likely was because of decreased T4 production by thyrocytes.[16,43] Glucocorticoids have been found to suppress TSH secretion, inhibit 5′-deiodinase (resulting in decreased conversion of T4 to T3 and increased conversion to rT3), and decrease TBG- and hormone-binding capacity.[16,17] In healthy horses, systemic administration of dexamethasone for 5 days resulted in increased rT3 and fT3.[5] Topical dexamethasone administration to healthy horses for 10 days decreased TT3 and TT4; no other THs were assessed.[14] Heparin also affects hormone-binding capacity to carrier proteins in humans.[37] In this study, NSAIDs, corticosteroids, and heparin did not have as significant an effect on THs as anticipated, but the majority of horses were treated with flunixin meglumine rather than phenylbutazone. Other drugs used in equine medicine such as dopamine, furosemide, phenytoin, phenobarbital, and iodine have been found to affect TH in humans,[17] but no horses in this study were receiving these drugs.

Food deprivation also can lead to changes in THs. Starvation and fasting have been reported to decrease TT3, fT3, and TT4 in humans because of decreased activity of 5′-deiodination, and also appear to contribute to HPA dysregulation.[17,18,20] Decreased concentrations of leptin result in suppression of TRH expression, decreased TSH production, and decreased THs.[17,18,20,44] Ultimately, these changes lead to increased appetite, decreased energy expenditure, and neuroendocrine function alteration to conserve energy and favor survival.[17,20] In other studies, horses fasted ≤2 days experienced decreased TT3 and fT4,[6,45] and when fasted ≤4 days, TT4 and fT4 also decreased.[6] In a different study, no changes in THs were noted for horses off feed ≤3 days.[46] In this study, horses were categorized into 3 groups: eating or off feed <1 day, off feed 1–2 days, and off feed ≥3 days. Decreases in

TT3 and fT3 occurred in all groups. TT4 was significantly decreased when horses were off feed ≥3 days. Although a significant decrease was noted in fT4D in both groups off feed for >24 hours, fT4D never decreased below normal.

In conclusion, results of this study indicate that all THs decrease with disease in the horse and HPA dysregulation occurs. Because fT4D was the only TH that remained within the normal reference range, it may be a good indicator of true thyroid function in ill horses. TT4 may be useful as a predictor of mortality. NSAIDs, corticosteroids, heparin, and fasting had far less effect on THs than did disease status, indicating that decreased THs concentrations in ill horses are not merely a result of drugs or food deprivation, but are more related to disease severity. NTIS does indeed occur in the horse, and changes in THs are similar to those noted in other species.

Footnotes

[a] DiaSorin Inc, Stillwater, MN
[b] Free T4 by equilibrium dialysis, Nichols Institute Diagnostics, San Juan Capistrano, CA
[c] Sigmastat/Sigmaplot; Systat Software, Inc., Chicago, IL

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