Effects of oral administration of levothyroxine sodium on serum concentrations of thyroid gland hormones and responses to injections of thyrotropin-releasing hormone in healthy adult mares

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Objective—To determine the effects of levothyroxine sodium (L-T_4) on serum concentrations of thyroid gland hormones and responses to injections of thyrotropin-releasing hormone (TRH) in euthyroid horses.

Animals—12 healthy adult mares.

Procedure—8 horses received an incrementally increasing dosage of L-T_4 (24, 48, 72, or 96 mg of L-T_4/d) for weeks 1 to 8. Each dose was provided for 2 weeks. Four additional horses remained untreated. Serum concentrations of total triiodothyronine (tT_3), total thyroxine (tT_4), free T_3 (fT_3), free T_4 (fT_4), and thyroid-stimulating hormone (TSH) were measured in samples obtained at weeks 0, 2, 4, 6, and 8; 1.2 mg of TRH was then administered IV, and serum concentrations of thyroid gland hormones were measured 2 and 4 hours after injection. Serum reverse T_3 (rT_3) concentration was also measured in the samples collected at weeks 0 and 8.

Results—Treated horses lost a significant amount of weight (median, 19 kg). Significant treatment-by-time effects were detected for serum tT_3, tT_4, fT_3, fT_4, and TSH concentrations, and serum tT_4 concentrations were positively correlated (r, 0.95) with time (and therefore dosage) in treated horses. Mean ± SD serum rT_3 concentration significantly increased in treated horses (3.06 ± 0.51 nmol/L for week 8 vs 0.74 ± 0.22 nmol/L for week 0). Serum tT_3, tT_4, fT_3, and TSH concentrations in response to TRH injections differed significantly between treated and untreated horses.

Conclusions and Clinical Relevance—Administration of levothyroxine sodium increased serum tT_4 concentrations and blunted responses to TRH injection in healthy euthyroid horses. (Am J Vet Res 2005;66:1025–1031)

S ynthetic thyroid hormone (**levothyroxine sodium [L-T_4]**) is commonly prescribed as treatment for adult horses with conditions attributed to idiopathic hypothyroidism, including myositis, laminitis, obesity, redistribution of adipose tissue, lethargy, poor performance, agalactia, and infertility.[1] Despite the popularity for the use of L-T_4 in horses, little

information is available to guide clinicians in selection of dosage or monitoring requirements. Only 1 report[2] addresses L-T_4 pharmacokinetics in horses, and those results were based on measurements obtained after oral administration of a single dose (10 mg) of L-T_4. Furthermore, serum **total triiodothyronine (tT_3)** concentrations peaked 1 hour after administration and then returned to baseline by 2 hours after administration, whereas **total thyroxine (tT_4)** concentrations peaked 2 hours after administration and were still increased 24 hours after administration in that study.[2]

Levothyroxine has also been administered orally (2.5 µg/kg) at 12-hour intervals in combination with **triiodothyronine (T_3)** to thyroidectomized horses with the objective of returning serum concentrations of thyroid gland hormones to concentrations measured before thryoidectomy.[3] Serum tT_3 concentrations increased in treated horses and approached baseline concentrations, but tT_4 concentrations remained undetectable even after 56 days of treatment.

When treating humans with primary hypothyroidism, optimal dosing of L-T_4 is achieved when the prescribed dose returns serum concentrations of **thyroid-stimulating hormone (TSH)** to the reference range.[4] Increased serum TSH concentrations have been detected in horses with experimentally induced primary hypothyroidism,[5,6] but to our knowledge, the effects of L-T_4 on serum TSH concentrations in horses have not been examined.

Function of the thyroid gland can be assessed in horses by IV administration of **thyrotropin-releasing hormone (TRH)**.[7,8] Serum concentrations of thyroid gland hormones increase within 6 hours after injection of TRH when thyroid gland function is normal.[7,8] Horses that have recently been treated with L-T_4 may require TRH testing, but to our knowledge, the effects of L-T_4 on responses to TRH injection have not been determined.

The purposes of the study reported here were to investigate the effects of L-T_4 administration on serum

concentrations of thyroid gland hormones and evaluate the impact of L-T_4 on responses to injections of TRH. We hypothesized that administration of L-T_4 would significantly alter serum hormone concentrations and the results of TRH testing.

Materials and Methods

Animals—Twelve healthy mixed-breed and Quarter Horse-type mares were selected for use in the study. Mares alone were used to reduce variability associated with differences attributable to sex. Immature and older horses were not included. Horses ranged from 5 to 13 years of age (median, 8 years) and weighed from 426 to 525 kg (median, 478 kg). Horses were housed in indoor stalls (2.75 × 3.5 m); each stall had an attached outdoor drylot (3.75 × 10 m). Each mare was fed 2 flakes of mixed-grass hay (approx 4.5 kg) twice daily, and 0.5 kg of grain was fed once daily in the morning. Horses were fed in the morning between 7 AM and 8 AM and in the afternoon between 4 PM and 5 PM. **Digestible energy (DE)** content of feeds was measured independently by personnel at a forage-testing laboratory.[a] Water was available for ad libitum intake. The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental design—Horses were allowed to acclimate to their environment for 2 weeks. Horses were then randomly assigned to 2 groups. Eight horses were administered increasing doses of L-T_4 for 8 weeks (first week of administration was week 1). The L-T_4[b] (24, 48, 72, or 96 mg of L-T_4/d; each dose was administered for 2 weeks) was mixed with 30 mL of water and 0.5 kg of grain (morning feeding). Four control horses received 30 mL of water without L-T_4 in their grain for 8 weeks. Testing by IV administration of TRH was performed at the beginning of the study (before administration of L-T_4; week 0) and at 2-week intervals thereafter. Horses were observed daily, and complete physical examinations were performed weekly. **Heart rate (HR)**, **respiratory rate (RR)**, and rectal body temperature were recorded. Body weight of each horse was measured before and at the end of the 8-week study period.

Sample collection—Blood samples were collected at 2-week intervals throughout the study (ie, weeks 0, 2, 4, 6, and 8). Samples were collected between 8 AM and 9 AM (ie, approx 24 hours after L-T_4 was administered on the preceding day). Blood samples were allowed to clot at 22°C for 1 hour; samples were then subjected to low-speed centrifugation, and serum was harvested for use in hormone measurements. Serum was frozen at –20°C until analyzed.

Measurement of thyroid gland hormones—Serum concentrations of T_3 and **thyroxine (T_4)** were measured by use of radioimmunoassays[c,d] validated for use with equine sera.[9,10] Samples obtained from the horses and standards provided by the manufacturer were concurrently analyzed in duplicate. Results of duplicate analyses were examined, and an intra-assay **coefficient of variability (CV)** < 5% was required for acceptance of results. Interassay variability was assessed by use of duplicate control serum samples[e] that contained a known concentration of canine T_3 and T_4. Values for these control samples were compared among assays, and variability < 10% was required for acceptance of results. **Reverse T_3 (rT_3)** was measured by use of a commercially available radioimmunoassay[f] that has been validated for use with horse serum.[11]

Measurement of free T_3—Free T_3 (fT_3) concentrations were measured in serum by use of a commercially available solid-phase radioimmunoassay,[g] which was based on competition of endogenous fT_3 with a ^{125}I-labeled T_3 derivative. Antibody-coated tubes, radioligand, and standards were provided in the kit. According to materials provided with the kit, the assay achieved 100% antibody cross-reactivity with L-T_3 and < 0.2% cross-reactivity with all other iodothyronines tested. The volumes of samples, standards, and radioligand were used in accordance with the manufacturer's protocol.[g] We slightly modified the assay procedure to extend the duration of incubation from 90 minutes to 3 hours in a water bath at 37°C. This modification was intended to ensure equilibration of maximal binding for assays that consisted of a standard curve and 53 samples. Sensitivity of the assay, defined as the concentration of fT_3 at 90% specific binding, was 1.2 pmol/L (mean of 10 assays). Analogue-based radioimmunoassays for fT_3 provide multiple binding interactions between the endogenous hormone, T_4 derivative, assay antibody, and endogenous binding proteins. Thus, assessment of dilutional parallelism and recovery is not possible. For pooled equine serum with concentrations of 1.7 and 5.6 pmol/L (10 replicates), the intra-assay CV was 0.099 and 0.034, respectively. Interassay CV for pooled equine serum with concentrations of fT_3 of 1.4, 4.3, and 7.7 pmol/L was 0.140, 0.095, and 0.082, respectively.

Measurement of fT_4 by use of equilibrium dialysis—Assays of fT_4 concentrations in equine sera were performed by use of a commercially available kit.[h] The procedures for dialysis of serum and radioimmunoassay of T_4 in dialysate were performed in accordance with the manufacturer's protocol.[h] According to materials provided with the kit, there was < 0.044% cross-reactivity of other iodothyronines for the assay. Sensitivity of the assay, defined as the concentration of fT_4 at 90% specific binding, was 1.8 pmol/L (mean of 10 assays). Estimates of dilutional parallelism and recovery were made in dialysates of equine serum. When a pool of dialysate with a concentration of fT_4 of 25 pmol/L was serially diluted with dialysis buffer at dilutions of 1:2, 1:4, and 1:8, the assay measured 88%, 96%, and 96% of expected amounts of fT_4, respectively. When aliquots of T_4 equivalent to 4, 11, 31, and 68 pmol/L were added to the same pool of equine dialysate, the assay measured 104%, 137%, 109%, and 105% of the respective added T_4 concentration. Estimates of repeatability were determined by use of pooled equine serum with fT_4 concentrations of 13 and 24 pmol/L. For 10 replicates of each pool, the intra-assay CV was 0.075 and 0.078, respectively. The interassay CV was 0.166 and 0.074, respectively, for each serum pool (10 assays).

Measurement of equine TSH—Equine TSH concentrations were measured by use of a double-antibody radioimmunoassay, which was based on the use of anti-TSH serum[i] and highly purified equine TSH.[j] The techniques and validation of this assay have been published elsewhere.[12]

TRH response test—At approximately 9 AM on days selected for testing, blood samples used to obtain baseline values were obtained and 1.2 mg of TRH[k] was injected IV. Blood samples were collected 2 and 4 hours after TRH injection. On those days, the L-T_4 was not administered to treated horses until after the 4-hour blood samples had been collected.

Statistical analysis—Initial body weight and changes in body weight during the 8-week period were compared between groups by use of the Wilcoxon rank sum test after normality was assessed by use of the Shapiro-Wilk method. Values for HR, RR, and body temperature were compared among time points within each treatment group by use of

the Wilcoxon signed rank test. Serum hormone concentrations in samples obtained at baseline after TRH injection were examined by use of a repeated-measures ANOVA in a statistical program.[1] Treatment, time, and treatment-by-time interaction terms were examined. When significance was established, multiple comparisons were made by comparing differences of least squares means for weeks 2, 4, 6, and 8 with values for week 0 by use of the Bonferroni test. Relationships between serum concentrations of thyroid gland hormones and dose of $L-T_4$ were evaluated within each group by use of Pearson correlation coefficients. Mean rT_3 concentrations were compared between weeks 0 and 8 in each group independently by use of paired t tests. Thyrotropin-releasing hormone (1.2 mg, IV) was administered at 9 AM, and serum concentrations of thyroid gland hormones were measured 2 and 4 hours after injection. Mean values were reported for all variables except body weight. Significance was defined as values of $P < 0.05$.

Results

Body weight at the beginning of the study did not differ significantly between untreated (range, 431 to 489 kg; median, 470 kg) and treated (range, 426 to 525 kg; median, 485 kg) horses. Median body weight significantly decreased by 19 kg (range, loss of 32 kg to gain of 5 kg) during the 8-week period in treated horses but remained unchanged in untreated horses
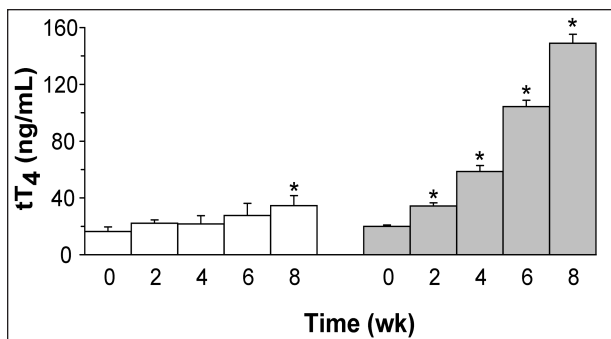
(range, loss of 4 kg to gain of 20 kg; median, 0 kg). Grain and hay fed to the horses contained 3,210 and 1,720 kcal of DE/kg, respectively, on an as-fed basis. Therefore, each horse received approximately 17.1 Mcal of DE/d, which met calculated DE requirements for maintenance (14.2 to 17.2 Mcal/d) for horses weighing 426 to 525 kg.[13] Treated horses were easily agitated during the 2 weeks that they were administered 96 mg of $L-T_4$/d, but variables measured during physical examinations did not differ significantly throughout the study.

Serum tT_4, fT_4, and TSH concentrations were significantly altered over time in treated horses, compared with corresponding concentrations for the untreated horses (**Figures 1 to 3**). A significant posi-
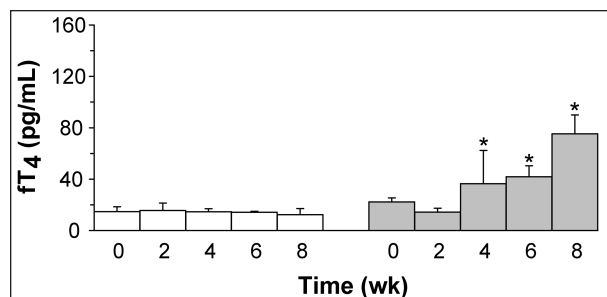

Figure 2—Mean ± SEM serum concentrations of free thyroxine (fT_4) for 4 untreated horses (white bars) and 8 horses treated with $L-T_4$ for 8 weeks in accordance with an incrementally increasing dosing regimen (gray bars). *See* Figure 1 for remainder of key.

Figure 1—Mean ± SEM serum concentrations of total thyroxine (tT_4) for 4 untreated horses (white bars) and 8 horses treated with levothyroxine sodium ($L-T_4$) for 8 weeks in accordance with an incrementally increasing dosing regimen of 24, 48, 72, and 96 mg of $L-T_4$/d, with each dose administered for 2 weeks (gray bars). Blood samples were collected approximately 24 hours after administration of $L-T_4$. Week 0 was before administration of $L-T_4$ was initiated. *Within a treatment group, value differs significantly ($P < 0.05$) from the value for week 0.
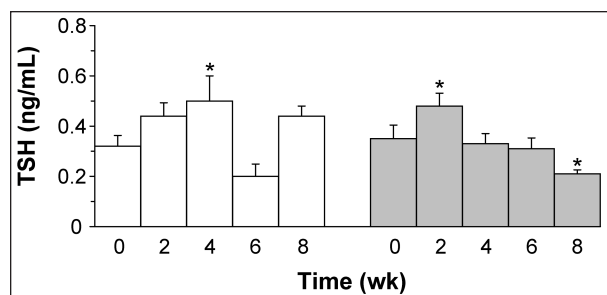

Figure 3—Mean ± SEM serum concentrations of thyroid-stimulating hormone (TSH) for 4 untreated horses (white bars) and 8 horses treated with $L-T_4$ for 8 weeks in accordance with an incrementally increasing dosing regimen (gray bars). *See* Figure 1 for remainder of key.

Table 1—Mean ± SD baseline serum concentrations of total triiodothyronine (tT_3) and free triiodothyronine (fT_3) for 4 untreated horses and 8 horses treated with levothyroxine sodium (L -T_4) for 8 weeks in accordance with an incrementally increasing dosing regimen of 24, 48, 72, and 96 mg of L -T_4/d, with each dose administered for 2 weeks.

| Variable | Group | Time (wk) | | | | |
		0	2	4	6	8
tT_3 (ng/mL)	Untreated	0.49 ± 0.05	0.43 ± 0.06	0.44 ± 0.05	0.35 ± 0.03	0.53 ± 0.05
	Treated	0.71 ± 0.05	0.39 ± 0.05*	0.53 ± 0.05*	0.54 ± 0.02*	0.88 ± 0.05*
fT_3 (pg/mL)	Untreated	0.5 ± 0.2	0.9 ± 0.1	1.0 ± 0.2*	0.6 ± 0.1	1.2 ± 0.2*
	Treated	1.0 ± 0.3	0.9 ± 1.0	1.3 ± 0.3*	1.1 ± 0.3	2.5 ± 0.6*

Blood samples were collected approximately 24 hours after administration of $L-T_4$. Week 0 was before administration of $L-T_4$ was initiated.
*Within a row, value differs significantly ($P < 0.05$) from the value for week 0.

Table 2—Mean ± SD fold increase* for various thyroid gland hormones in response to 1.2 mg of thyrotropin-releasing hormone (TRH) IV for 4 untreated horses and 8 horses treated with L-T_4 for 8 weeks in accordance with an incrementally increasing dosing regimen.

Variable†	Group	Time (wk)				
		0	2	4	6	8
tT_3 at 2 hours (ng/mL)	Untreated	2.2 ± 0.3	2.0 ± 0.1	2.6 ± 0.4	2.7 ± 0.5	1.5 ± 0.3
	Treated	1.9 ± 0.2	2.7 ± 0.5‡	1.3 ± 0.2	1.1 ± 0.0‡	1.0 ± 0.1‡
tT_4 at 4 hours (ng/mL)	Untreated	1.6 ± 0.2	1.4 ± 0.1	2.1 ± 0.5‡	1.6 ± 0.3	1.3 ± 0.1
	Treated	1.6 ± 0.1	1.2 ± 0.1‡	1.1 ± 0.1‡	1.0 ± 0.0‡	1.0 ± 0.0‡
fT_3 at 2 hours (pg/mL)	Untreated	2.9 ± 1.1	1.1 ± 0.3‡	2.2 ± 0.6	3.0 ± 1.8	0.7 ± 0.6‡
	Treated	1.8 ± 1.0	1.6 ± 1.5	0.3 ± 0.5‡	0.4 ± 0.3‡	–0.1 ± 0.2‡
fT_4 at 4 hours (ng/mL)	Untreated	1.4 ± 0.2	1.5 ± 0.4	0.9 ± 0.6	1.1 ± 0.7	0.8 ± 0.5
	Treated	1.4 ± 0.5	1.1 ± 0.6	0.2 ± 0.3	0.3 ± 0.2	–0.1 ± 0.1
TSH at 2 hours (ng/mL)	Untreated	3.6 ± 3.0	2.1 ± 1.4	3.7 ± 6.4	6.8 ± 8.7	0.3 ± 0.5
	Treated	3.0 ± 4.3	0.1 ± 0.7‡	0.9 ± 0.7	–0.4 ± 0.3‡	0.8 ± 0.5

*The fold increase was calculated by dividing the concentration after TRH injection by the preinjection concentration. †Represents the specific thyroid gland hormone and the time after TRH injection at which the sample was obtained. ‡Within a row, value differs significantly ($P < 0.05$) from the value for week 0.
tT_4 = Total thyroxine. fT_4 = Free thyroxine. TSH = Thyroid-stimulating hormone.
See Table 1 for remainder of key.

tive correlation (r, 0.95) was detected between serum tT_4 concentrations and time (and therefore dosage) in treated horses. A significant but weaker correlation (r, 0.76) with dosage was also detected for fT_4 concentrations in horses that received L-T_4. Concentrations of tT_3 and fT_3 in serum obtained from horses approximately 24 hours after L-T_4 administration were significantly altered over time in treated horses (**Table 1**); however, responses did not correlate well with dosage (r, 0.33 and r, 0.68 for tT_3 and fT_3, respectively). Serum TSH concentrations varied widely throughout the treatment period, but by week 8, they were significantly lower than the mean value for week 0 (r, –0.44). Mean ± SD serum rT_3 concentrations were significantly higher at week 8 (3.06 ± 0.51 nmol/L), compared with values for week 0 (0.74 ± 0.22 nmol/L) in treated horses, but rT_3 concentrations did not differ significantly between weeks 8 and 0 (0.64 ± 0.18 and 0.74 ± 0.16 nmol/L, respectively) in untreated horses. Concentrations of tT_3, tT_4, fT_3, and TSH in response to TRH injection decreased significantly over time in treated horses, but the fT_4 concentration after TRH injection was not significantly affected (**Table 2**).

Discussion

Horses treated with L-T_4 had a significant reduction in body weight during the 8-week treatment period. Administration of thyroid hormones to rats increases metabolic rate, thermogenesis, and consumption of body fat stores as triglycerides are hydrolyzed to supply fatty acids.[14,15] Effects of L-T_4 administration on body weight may also have been exacerbated in the study reported here by the limitations placed on dietary intake. In rats, mean daily feed intake increased by 47% when they received daily IP injections of T_3 for 14 days.[15] The horses in our study were fed the same amount of feed throughout the treatment period, so a substantial increase in feed intake was not possible. It

is also conceivable that L-T_4 reduced appetite and therefore feed intake in treated horses. However, horses were monitored closely throughout the study, so it is unlikely that a change in appetite would have gone unnoticed.

Health complications were not detected in horses treated with L-T_4. Mean values for HR, RR, and body temperature did not differ significantly between groups. However, horses treated with 96 mg of L-T_4/d did become more agitated when they were handled during the final 2 weeks of the study. Treated horses were more difficult to catch during these weeks and appeared to be more excitable when observed in their stalls and pens. These effects were only associated with the dosage of 96 mg of L-T_4/d. This adverse effect is unlikely to be encountered by clinicians because the dosages commonly prescribed range from 24 to 48 mg of L-T_4/d. Differences in behavior were not detected at dosages of 24, 48, and 72 mg of L-T_4/d in this study, but treatment periods of 2 weeks may have been insufficient to fully evaluate this response.

Administration of L-T_4 in a small grain meal significantly altered serum concentrations of thyroid gland hormones in healthy horses. Horses were fed grain containing the drug for ease of administration and because L-T_4 is commonly administered in this manner. However, human patients are advised to take thyroid medications immediately after awakening and 30 minutes prior to eating the morning meal.[16] Fiber or bran products, including breakfast cereals and calcium-containing antacids, can inhibit absorption of L-T_4 from the intestines.[16] Analysis of the results of the study reported here indicated that L-T_4 was biologically active in horses when administered with a small grain meal, but maximal absorption may not have been attained under these conditions.

Concentrations of thyroid gland hormones were measured in serum samples obtained approximately 24 hours after administration of L-T_4. Therefore, serum

concentrations of these hormones may have been influenced by differences in rates of absorption or catabolism of L-T_4. Although the pharmacokinetics of L-T_4 have not been evaluated appropriately in horses, peak serum tT_3 and tT_4 concentrations were detected in horses 1 and 2 hours, respectively, after a single 10-mg dose of L-T_4 was administered in another study.[2] In dogs, serum tT_3, tT_4, fT_3, and fT_4 concentrations reportedly peak 4 to 6 hours after administration.[17] Samples were collected from horses in the study reported here when concentrations of thyroid gland hormones were likely to be at their lowest point during the day. Additional studies are required to determine the pharmacokinetics of L-T_4 and therefore the optimal time for monitoring serum concentrations of thyroid gland hormones.

Serum tT_4 concentrations were closely correlated with dosage in healthy horses in our study. However, serum tT_4 concentrations also reflected the cumulative effects of L-T_4 administered during the preceding weeks because washout intervals were not included in the study. It should also be mentioned that these results apply only to euthyroid healthy horses. Horses that are hypothyroid as a result of thyroidectomy or naturally developing disease may respond differently to L-T_4.

Serum TSH concentrations are preferentially monitored in hypothyroid human patients that are receiving L-T_4.[4] Because the dose of L-T_4 administered is determined by serum TSH concentrations in these patients, serum tT_4 concentrations may increase above the reference range during treatment.[18] Tachycardia, increased urinary excretion of sodium, and enhanced bone resorption have been reported in humans[18,19] that receive excessive amounts of L-T_4, but the only abnormality detected in appropriately managed patients is an increase in left ventricular mass index value, which does not affect cardiac function. Long-term effects of L-T_4 on health have not been examined in euthyroid or hypothyroid horses, so it is currently recommended that serum tT_4 concentrations be maintained close to the reference range during treatment. In our study, serum tT_4 concentrations were still within the reference range (2.5 to 37 ng/mL)[20] after 24 mg of L-T_4/d was administered for 2 weeks, but concentrations may have increased further with prolonged treatment. An interval of 4 weeks is recommended before serum tT_4 concentrations are assessed in humans after initiating L-T_4 replacement therapy.[16]

Free T_4 concentrations were measured after equilibrium dialysis by use of serum obtained approximately 24 hours after administration of L-T_4. Concentrations of this hormone increased over time in treated horses and were sufficiently correlated with dosage to warrant measurement. Serum fT_4 concentrations in horses treated with ≥ 48 mg of L-T_4/d exceeded the reference range of 19.5 ± 10.8 pmol/L (15 ± 8 pg/mL).[10] That range was developed on the basis of data collected from 40 healthy horses at pasture, and measurements were made after equilibrium dialysis.[10] Free T_4 concentrations may be preferentially monitored in horses receiving protein-bound drugs such as phenylbutazone because this drug displaces bound T_4 from plasma proteins and lowers serum tT_4 concentrations.[10] Serum tT_4 concentrations remain suppressed for 10 days after phenylbutazone treatment, whereas fT_4 concentrations return to the reference range within 3 days after discontinuing treatment.[10] Transient lowering of fT_4 concentrations as a result of administration of phenylbutazone is attributed to suppression of TSH secretion from the pituitary gland.[10]

Serum concentrations of tT_3 and fT_3 significantly increased over time but were weakly correlated with dosage in treated horses. When serum tT_3 and fT_3 concentrations were measured in dogs treated with L-T_4,[17] these concentrations increased by only 27% and 16%, respectively, when the dosage of L-T_4 was doubled. Serum tT_3 concentrations also remain within the reference range in some humans treated with L-T_4.[4] Analysis of these findings suggests that conversion of T_4 to T_3 may be under autoregulatory control.[4,17]

Serum TSH concentrations measured in horses of the study reported here were similar to those reported for horses in other studies.[5,6,12,21] Mean ± SD serum TSH concentrations of 0.10 ± 0.06 ng/mL,[6] 0.17 ± 0.12 ng/mL,[12] 0.25 ± 0.14 ng/mL,[21] and 0.40 ± 0.29 ng/mL[5] have been detected in healthy adult horses. In the study reported here, serum TSH concentrations ranged from 0.10 to 0.66 ng/mL in untreated horses and from 0.09 to 0.75 ng/mL in treated horses. Concentrations varied widely among horses, including those horses that were not treated. Samples must be obtained from horses more frequently after L-T_4 administration in future studies to determine the degree to which sample collection time influences results. Analysis of our findings indicated that L-T_4 significantly decreased serum TSH concentrations in adult euthyroid horses, but the variability observed in this study should discourage veterinarians from basing clinical decisions on a single measurement of this hormone. Release of TSH from the pituitary gland is pulsatile in rats, and a circadian rhythm has been detected.[22] Although samples were collected at approximately the same time of day in all horses, TSH concentrations may have varied according to when pulses were released from the pituitary gland.

Lower serum TSH concentrations may have been detected in horses treated with 96 mg of L-T_4/d had the duration of treatment been longer. In humans, TSH concentrations do not stabilize until 4 weeks after initiation of L-T_4 treatment.[4,16] Subtle differences in TSH concentrations may also be more difficult to discern in horses because the radioimmunoassay used was less sensitive than immunometric assays available in human medicine.[4] Horses treated with L-T_4 had a significant decrease in mean serum TSH concentration over time, which is the opposite response to the increase in mean serum TSH concentration detected in horses with primary hypothyroidism induced by administration of propylthiouracil.[5,6]

Serum rT_3 concentrations were significantly higher in treated horses after 8 weeks of L-T_4 administration. Reverse T_3 is believed to be devoid of hormonal activity but acts as a major competitive inhibitor of T_3 activity at the cellular level.[23] An increase in serum rT_3 concentrations likely resulted from L-T_4 being con-

verted to both T_3 and rT_3.[23] Mean serum rT_3 concentrations detected at the beginning of this study (0.74 ± 0.22 nmol/L) compare favorably with mean values of 0.40 ± 0.06 and 0.40 ± 0.16 nmol/L reported[6] for healthy horses. Lower concentrations of rT_3 have been associated with propylthiouracil-induced hypothyroidism in horses and are attributed to reduced production of T_4 by the compromised thyroid gland.[6] Increased rT_3 concentrations have been associated with feed deprivation in horses[11] and euthyroid sick syndrome in dogs[24] and humans,[25] which is associated with nonthyroidal illness. In these situations, peripheral 5′-deiodinase activity is inhibited, which results in increased conversion of T_4 to rT_3 and reduced rT_3 catabolism.[24]

It is generally accepted that serum tT_3 and tT_4 concentrations increase 2-fold by 2 or 4 hours after injection, respectively, in euthyroid horses.[1,26] At the beginning of the study reported here, horses of both groups had the expected increase in tT_3 concentration by 2 hours, but tT_4 concentrations were lower than anticipated. Responses also varied considerably in untreated horses for the 5 time points at which they were tested during the 8-week study period. Because our results document that healthy euthyroid horses have lower responses to TRH and that those responses vary with time, use of the 2-fold increase as a standard for diagnosis of thyroid gland dysfunction must be questioned. Interestingly, only a 1.7-fold mean increase in serum tT_4 over 4 hours was reported in one of the first reports[7] that described this procedure. Because lower responses were detected in our study, data were reexamined to identify specific horses with thyroid gland dysfunction that may have been inadvertently included in the study; however, we did not identify any such horses. It is also conceivable that repeated administration of TRH stimulated an immune response and that antibodies produced against this synthetic product inhibited its biological activity. Other factors that may also have affected results included improper preparation or storage of the hormone and poor injection technique.

Oral administration of L-T_4 blunted TSH responses to TRH injection in treated horses. Negative-feedback mechanisms likely responded to higher circulating fT_3 and fT_4 concentrations by downregulating pathways for TSH synthesis and secretion.[23] This effect was evident within 2 weeks after initiation of L-T_4 administration. At the beginning of the study, a 3-fold increase in serum TSH concentration was detected 2 hours after TRH injection, whereas an increase of only 13% was detected after 24 mg of L-T_4/d had been administered daily for 2 weeks. Responses remained low throughout the remainder of the study, but the TSH response to TRH administration was not affected in a dose-dependent manner. In human patients with thyroid cancer or goiter, L-T_4 is administered to stimulate this feedback mechanism, which lowers TSH secretion by the pituitary gland and ultimately reduces the size of the thyroid gland.[27]

Administration of L-T_4 was also associated with significant reductions in serum tT_3, tT_4, and fT_3 concentrations in response to TRH injection over time. Concentrations of thyroid gland hormones in the blood after TRH injection reflect function of the pituitary gland and thyroid gland.[23] Lower serum TSH concentrations detected in treated horses provided evidence that pituitary gland function was inhibited in treated horses. Analysis of results of this study indicated that healthy euthyroid horses treated with L-T_4 will not respond to TRH injection in a typical manner, so this test should not be used to assess thyroid gland function in horses recently treated with L-T_4. Human patients require an interval of 4 weeks after cessation of L-T_4 treatment before testing to allow pituitary thyrotrophs to recover from the suppressive effects of L-T_4.[19]

Analysis of results of the study reported here revealed that short-term administration of L-T_4 was not associated with adverse effects, other than agitation, when 96 mg of L-T_4/d was administered. Serum tT_4 concentrations were closely correlated with dosage in healthy horses in this study, but concentrations reflected the cumulative effects of L-T_4 because washout intervals were not included. Serum TSH concentrations decreased during L-T_4 administration but varied widely among horses and sample collection times. Blunted responses to TRH injection should be anticipated in horses that are being treated with L-T_4.

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