Effects of high doses of levothyroxine sodium on serum concentrations of triiodothyronine and thyroxine in horses

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OBJECTIVE
To investigate the effect of high doses of orally administered levothyroxine sodium (LT_4) on serum concentrations of triiodothyronine (T_3) and thyroxine (T_4) in euthyroid horses.

ANIMALS
12 healthy adult horses.

PROCEDURES
10 horses initially received water (vehicle) or 240 mg (5X treatment) or 480 mg (10X treatment) of LT_4, and blood samples were collected at baseline (0 hours) and 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48, 72, 96, and 120 hours after treatment to measure serum T_3 and T_4 concentrations. Three horses then received 480 mg of LT_4 for 14 days, and T_4 concentration was measured on days 0, 14, 21, 28, and 35. Changes in T_3 and T_4 concentrations were compared over time and among treatments.

RESULTS
One-time administration of LT_4 resulted in variable but significant increases in both T_3 and T_4 concentrations for up to 120 hours; however, T_3 and T_4 concentrations rarely exceeded reference intervals with either treatment. Prolonged administration of 480 mg of LT_4 resulted in a 15-fold increase in T_4 concentration after 14 days, but concentration returned to day 0 values within 21 days after LT_4 administration was discontinued.

CONCLUSIONS AND CLINICAL RELEVANCE
In euthyroid horses, administration of a high dose of LT_4 resulted in mild increases in thyroid hormone concentrations; however, prolonged administration of high doses of LT_4 resulted in markedly increased thyroid hormone concentrations that returned to pretreatment values within 3 weeks after discontinuation of LT_4 administration. These results indicated complex kinetics of LT_4 and suggested a possible saturation of T_4 excretion in euthyroid horses. (*Am J Vet Res* 2019;80:565–571)

The hormones T_3 and T_4 are involved in virtually all metabolic processes in horses, with their primary action being to promote oxygen consumption.[1-3] Effects of these hormones are through their actions on cells, both directly (which results in rapid modulation of cellular activities) and indirectly through genomic stimulation (which results in the upregulation and downregulation of protein synthesis). Thyroid hormones enhance protein and lipid anabolism and catabolism, stimulate basal metabolic rate, and regulate body heat production.[1] Secretion of thyroid hormones is regulated by thyroid-stimulating hormone from the pituitary gland, and secretion of thyroid-stimulating hormone in turn is regulated by thyrotropin-releasing hormone from the hypothalamus.[4] After they are secreted, T_3 and T_4 in the bloodstream are primarily bound to proteins; however, the free fractions of T_3 and T_4 are the metabolically active forms, with free T_3 being the most active.[4] Although thyroid hormones are involved in many metabolic processes and stimulate organ growth and maturation in horses, they are not an essential requirement for life because thyroidectomized horses have only limited clinical signs, which include cold intolerance and hair coat abnormalities (coarse hair, mild alopecia, and delayed shedding).[5-9]

Naturally occurring hypothyroidism is rare in adult horses, and the existence of primary hypothyroidism in adult horses has been debated.[1] However, extrapolation of the clinical signs described for humans or dogs with hypothyroidism has suggested a possible association between hypothyroidism and weight gain, low fertility, and impaired lipid metabolism in horses. More recently, those clinical signs have been associated with EMS rather than with actual hypothyroidism.[10] The pathophysi-

ABBREVIATIONS

EMS	Equine metabolic syndrome
LT_4	Levothyroxine sodium
T_3	Triiodothyronine
T_4	Thyroxine
tT_3	Total triiodothyronine
tT_4	Total thyroxine

ology of EMS is still unclear, but insulin dysregulation has been found to be at the center of this disorder.[11] Insulin dysregulation encompasses hyperinsulinemia and insulin resistance in peripheral tissues; therefore, treatment options for EMS are aimed at reducing hyperinsulinemia and improving sensitivity to insulin.[12,13] Management of hyperinsulinemia is mainly achieved by dietary modifications to limit ingestion of nonstructural carbohydrates. On the other hand, insulin sensitivity can be improved by exercise and weight loss.[14,15] Unfortunately, exercise is often contraindicated for horses with laminitis, and efforts in such animals are usually concentrated on weight loss. Successful weight loss can be achieved by dietary modification; however, a weight loss–resistant phenotype has been described that necessitates pharmaceutical intervention with metformin or thyroid hormone analogs.[16,17] Metformin possibly blunts postprandial insulin responses, and it has been suggested that LT_4 (the synthetic analog of T_4) can improve carbohydrate and fat metabolism and accelerate weight loss.[10,13,18]

Long-term administration of LT_4 to euthyroid horses has resulted in weight loss with no adverse effects, which suggests that even if horses with EMS do not have hypothyroidism, thyroid hormone supplementation could be beneficial.[19,20] In addition, after receiving LT_4 for 8 weeks, euthyroid horses had improvements in insulin sensitivity and insulin disposal.[21] Although increasing the concentrations of thyroid hormones could be beneficial in horses with EMS, there is conflicting evidence regarding the dose and duration of treatment to obtain effects. In one study,[19] an increase in the T_4 concentration was observed only after administration of high doses for 16 weeks, whereas in another study,[22] an increase in the T_4 concentration was observed 30 minutes after administration of a low dose. Effects of thyroid hormones at replacement doses have been described,[22] but to our knowledge, the short-term effects of supraphysiologic doses of LT_4 have not been reported. Therefore, the purpose of the study reported here was to investigate effects of 1-time and prolonged administration of high doses of LT_4 on serum concentrations of T_3 and T_4 in adult euthyroid horses.

Materials and Methods

Horses

The study population consisted of 12 adult horses of various breeds donated to or purchased by the Purdue University Veterinary Teaching Hospital. Eleven horses had been donated because of problems unrelated to the gastrointestinal or endocrine systems (nonlaminitic lameness [n = 5], recurrent airway obstruction [3], heart murmur [1], behavioral disorder [1], and infertility [1]), and 1 horse had been purchased for use in exercise physiology studies. The population comprised 8 mares and 4 geldings; median age was 15 years. There were 4 Quarter Horses, 3 Thoroughbreds, 2 Standardbreds, 1 Appaloosa, 1 Hanoverian, and 1 mixed-breed horse. All horses were systemically healthy as determined by results of physical examination; none of the horses had clinical signs consistent with EMS or pituitary pars intermedia dysfunction, and none had received any treatments within the 4 weeks preceding the study. The procedures were approved by the Purdue University Animal Care and Use Committee.

Procedures

The study was conducted in 2 phases. The first phase of the study was designed to investigate the short-term effects of 1-time administration of 2 doses of LT_4 on serum concentrations of T_3 and T_4. Ten horses were housed in stalls and allowed to acclimatize for 24 hours, during which they had free access to mixed-grass hay and water. Then, a catheter was aseptically placed in a jugular vein. One hour after the catheter was placed, horses were randomly assigned (dice roll) to receive water (vehicle treatment), 240 mg of LT_4[a] (5 times the daily recommended dose of 48 mg) in water (5X treatment), or 480 mg of LT_4 (10 times the daily recommended dose of 48 mg) in water (10X treatment) through a nasogastric tube (5 horses/group). Blood samples (4 mL/sample) were then collected via the IV catheter immediately before (0 hours; baseline) and 0.5, 1, 2, 4, 6, 8, 10, 12, 18, 24, 48, 72, 96, and 120 hours after treatment and placed in heparinized tubes. After a 14-day washout period, the procedures were repeated, so each horse received 2 of the 3 treatments.

The second phase of the study was designed to investigate the effects of prolonged administration of a high dose of LT_4 on serum concentrations of T_4. For that purpose, 3 horses were again housed in stalls and allowed to acclimatize for 24 hours as described previously. Then, horses received 480 mg of LT_4/d orally for 14 days. The LT_4 dose was divided and provided twice daily mixed with grain. The dose of 480 mg was based on results of the first phase of the study. Blood samples were collected via venipuncture into heparinized tubes immediately before (day 0) and 14 (end of treatment), 21, 28, and 35 (7, 14, and 21 days after end of treatment, respectively) days after the start of treatment.

Measurement of serum T_3 and T_4 concentrations

All blood samples were centrifuged (2,000 × *g* for 10 minutes at 4°C), and serum was harvested and used to measure tT_3 and tT_4 concentrations. The tT_3 concentration was measured with a solid-phase competitive chemiluminescent immunoassay on a chemical analyzer.[b] Analytic sensitivity of the assay was 19 ng/dL, and calibration range was 40 to 600 ng/dL. Mean coefficient of variation of the assay was 6.5%, mean intra-assay precision for equine samples was 5.5%, and mean percentage recovery for equine samples was 94.7%. The tT_4 concentration was also measured with a solid-phase competitive chemiluminescent immunoassay by use of the same chemical analyzer.[b] Analytic sensitivity of the assay was 0.3 µg/dL, and calibration range was 1.0 to 24 µg/dL. Mean coefficient of varia-

tion of the assay was 7.6%, mean intra-assay precision for equine samples was 8.7%, and mean percentage recovery for equine samples was 102.7%.

Data analysis

Normal distribution was determined by use of the Shapiro-Wilk test. Mean ± SD was calculated for normally distributed data, and median and range were calculated for nonnormally distributed data. For the first phase of the study, horses were grouped on the basis of treatment (vehicle, 5X, or 10X); however, each horse received only 2 of the 3 treatments. Initial (baseline and day 0 for the first and second phases, respectively) tT_3 and tT_4 concentrations were compared among treatments by use of a 1-way ANOVA, and changes in tT_3 and tT_4 concentrations resulting from LT_4 administration were compared by means of a 2-way repeated-measures ANOVA and Tukey post hoc test, when relevant. For the second phase of the study, changes in tT_4 concentrations resulting from LT_4 administration were compared by use of a 1-way ANOVA and Dunnett post hoc test. Statistical analyses were performed with commercially available software.[c] Values of $P < 0.05$ were considered significant.

Results

All horses tolerated both phases of the study well. The only adverse effects, which were observed in all horses, were an increase in nervous behavior and reluctance to stand still at day 14 of the second phase of the study.

At baseline, the tT_3 concentration of 2 horses (1 for the vehicle treatment and 1 for the 10X treatment) was lower than the lower limit of the reference interval (30 to 80 ng/dL), but there were no significant differences ($P = 0.11$) in baseline tT_3 concentrations among treatments. Similarly, the baseline tT_4 concentration of 2 other horses (1 for the vehicle treatment and 1 for the 10X treatment) was lower than the lower limit of the reference interval (1.0 to 3.0 µg/dL), but there were no significant differences ($P = 0.93$) in baseline tT_4 concentrations among treatments. None of the horses had baseline tT_3 or tT_4 concentrations greater than the upper limit of the reference intervals.

One-time administration of LT_4 resulted in an increase in tT_3 concentration **(Figure 1)**; the concentration was significantly different from the baseline concentration at 4, 8, and 120 hours for the 10X treatment. Compared with concentrations for the vehicle treatment, tT_3 concentration was significantly higher at 8 and 120 hours for the 5X treatment and at 4, 8, 12, and 120 hours for the 10X treatment. Administration of LT_4 resulted in tT_3 concentrations transiently greater than the reference interval in 2 horses for the 5X treatment and 1 horse for the 10X treatment.

One-time administration of LT_4 also resulted in an increase in tT_4 concentration **(Figure 2)**. The concentration was significantly ($P = 0.02$) different from the

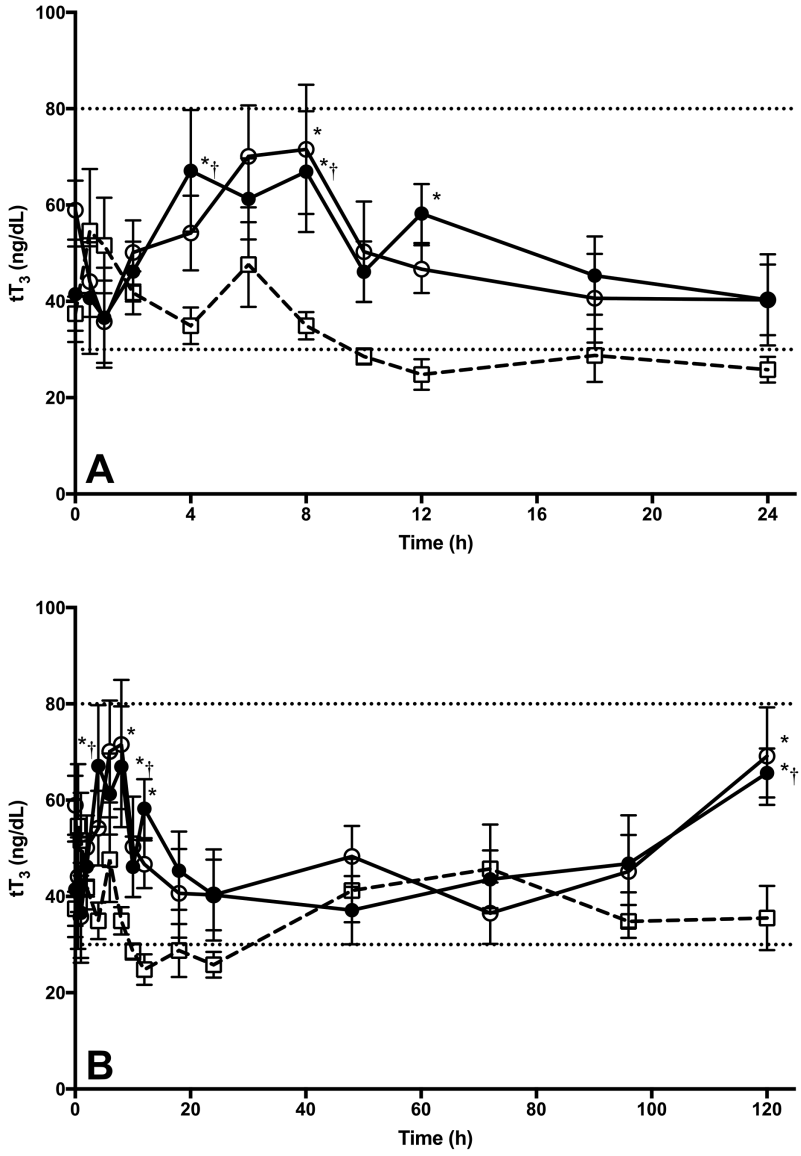

Figure 1—Mean ± SD serum concentration of tT_3 for 5 horses over 24 (A) and 120 (B) hours after receiving 1 dose of water (vehicle treatment; squares and dashed line), 240 mg of LT_4 (white circles and solid line), or 480 mg of LT_4 (black circles and solid line) by nasogastric intubation. Baseline (0 hours) was immediately before LT_4 administration. The dotted lines indicate the reference interval. *Within a time point, value differs significantly ($P < 0.05$) from the value for the vehicle treatment. †Within a treatment, value differs significantly ($P < 0.05$) from the baseline value.

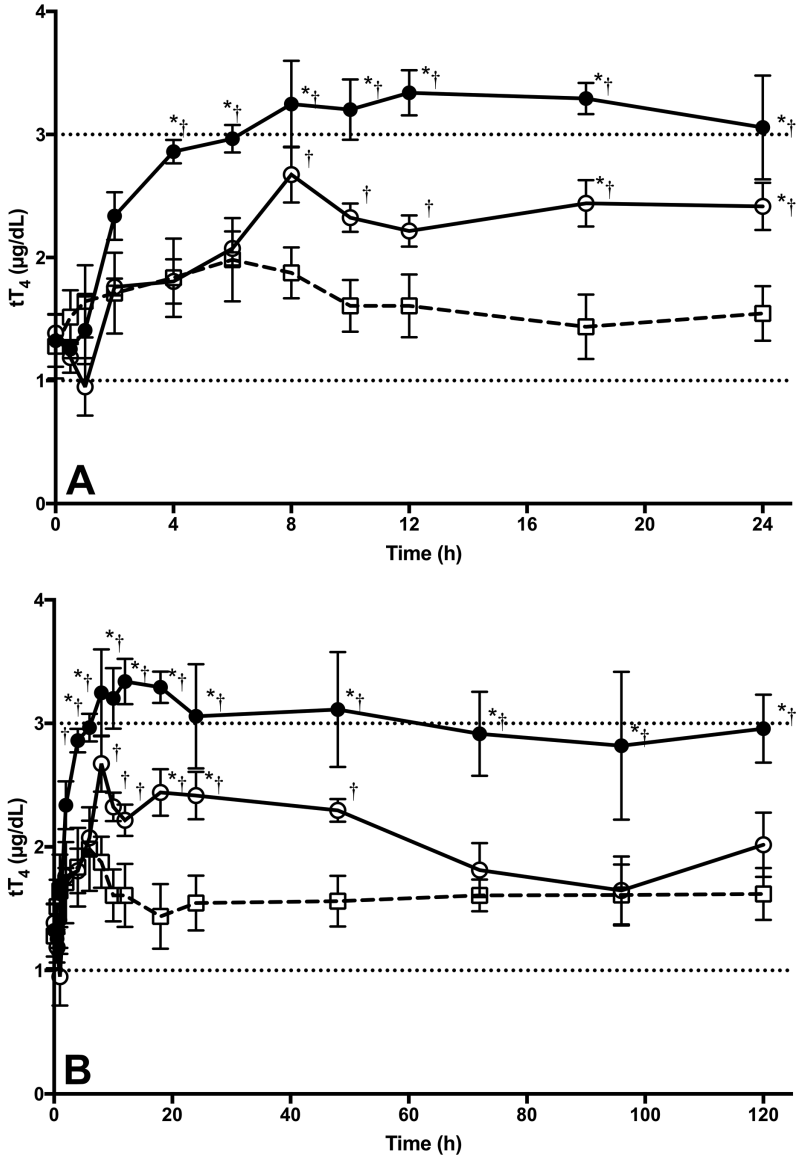

Figure 2—Mean ± SD serum concentration of tT_4 for 5 horses over 24 (A) and 120 (B) hours after receiving 1 dose of water (vehicle treatment; squares and dashed line), 240 mg of LT_4 (white circles and solid line), or 480 mg of LT_4 (black circles and solid line) by nasogastric intubation. ***See*** Figure 1 for remainder of key.

baseline concentration from 8 to 48 hours for the 5X treatment and from 2 to 120 hours for the 10X treatment. Compared with concentrations for the vehicle treatment, the tT_4 concentration was significantly higher from 18 to 24 hours for the 5X treatment and from 4 to 120 hours for the 10X treatment. Administration of LT_4 resulted in tT_4 concentrations transiently greater than the reference interval in 1 horse for the 5X treatment but continuously greater than the reference interval in 5 horses for the 10X treatment.

Administration of LT_4 for 14 days in the second phase of the study resulted in a marked increase in tT_4 concentration **(Figure 3)**. The concentration on day 14 was significantly different from the concentration on day 0, but the concentration on day 21 (7 days after cessation of administration) was not significantly different from the concentration on day 0. Discontinuation of treatment resulted in a return to tT_4 concentrations to within the reference interval by day 35 (21 days after cessation of administration).

Discussion

The main result of the study reported here was that although 1-time administration of high doses of LT_4 resulted in a significant increase in serum tT_3 and tT_4 concentrations, those increases were mild and rarely resulted in concentrations above the reference intervals. In contrast, administration of a high dose of LT_4 for 14 days resulted in a marked increase in the tT_4 concentration, which returned to preadministration concentrations gradually when treatment was discontinued.

The limited effect of LT_4 administration on tT_3 concentrations has been previously described.[20,23] In 1 of those studies,[20] administration of LT_4 at a lower dose (48 mg/d) but for a longer period (48 weeks) resulted in a decrease in tT_3 concentrations by 32 weeks, with mean values as low as 20 ng/dL. On the other hand, in another study,[22] a lower dose of LT_4 (10 mg, once) resulted in marked increases in tT_3 concentrations within 2 hours, with mean values as high as 100 ng/dL. These changes were not detected after 1-time administration in the study reported here because, despite the higher doses used in the present study, mean values remained within the reference intervals, and only 3 horses had values transiently and mildly greater than the reference intervals. The effect of LT_4 administration on tT_3 concentrations depends on dose and duration of LT_4 administration, with possible paradoxical effects. On the one hand, LT_4 could decrease tT_3 concentrations because LT_4 would be converted to free T_4 and activate negative feedback on thyroid-stimulating hormone secretion, but on the other hand, LT_4 could increase tT_3 concentrations because most of tT_3 comes from peripheral deiodination of tT_4.[24,25] Measurement of free T_3 concentrations in the present study and in other studies might have been a more accurate estimation of the effect of LT_4 administration; however, total and free fractions supposedly are correlated in healthy subjects.[1,20,26]

In 1 study,[22] LT_4 administration (10 mg, once) induced an increase in tT_4 concentration by 1 hour after

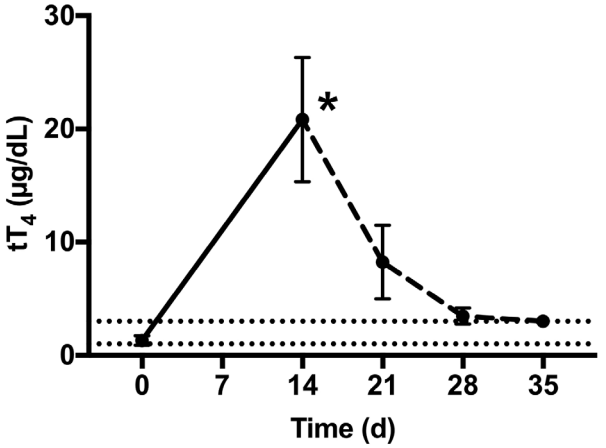

Figure 3—Mean ± SD serum concentration of tT_4 for 3 horses after receiving 480 mg of LT_4/d mixed with grain for 14 days (solid line) and after discontinuation of treatment (dashed line). Day 0 was immediately before the start of administration. Notice that days 21, 28, and 35 correspond to days 7, 14, and 21 after cessation of treatment, respectively. The dotted lines indicate the reference interval (1 to 3 µg/dL). *Value differs significantly ($P < 0.05$) from the value for day 0.

administration, and the increase was sustained for 24 hours. Consistent with results of that study,[22] a more moderate but acute increase in tT_4 concentration was detected in the study reported here. The increases detected in that other study[22] were mild, with a mean peak concentration of 2.0 µg/dL, whereas in the present study, a mean peak of 3.5 µg/dL was achieved for the 10X treatment. This difference could be attributable to the dose used (10 mg vs 480 mg); however, it could be expected that a 48-fold increase in drug dose would result in larger changes. A possible explanation for a limited dose effect is the poor absorption of LT_4 after oral administration to horses. In human medicine, fiber and bran cereal decrease oral absorption of thyroid hormones.[27,28] Considering the diet of horses, it could be expected that orally administered LT_4 would be poorly absorbed, which suggests that even large increases in drug doses would result in only minimal increases in blood hormone concentrations and that for maximal effects, food should be withheld from horses prior to administration of thyroid hormones. In the present study, the difference between the 5X and the 10X treatments was less than could be expected, which suggested a possible saturation of LT_4 absorption when administered to horses that did not have food withheld prior to LT_4 administration. This poor absorption could also explain the reason that no results are detected during the first few weeks of administration and only limited increases in concentrations are evident, even with long-term administration to horses.[20,23] However, even if LT_4 is absorbed in horses from which food has been withheld, administering the drug to horses with an empty stomach is impractical in most on-farm settings. Further investigations of LT_4 absorption in horses from which food has been withheld prior to LT_4 administration are warranted, even if the practice would potentially have limited clinical relevance.

Despite the fact there is poor drug absorption of LT_4, long-term administration of LT_4 has been used clinically for the treatment of various conditions. Immune-mediated thyroid gland disease has been reported in humans and in dogs but is rare in horses; therefore, long-term treatment has mainly been recommended for euthyroid horses with obesity-related endocrine disorders such as EMS.[13,29,30] In those cases, common practice has been to adapt the dose of LT_4 on the basis of tT_4 concentrations.[1] In the study reported here, administration of 480 mg of LT_4/d for 14 days resulted in a 15-fold increase in the tT_4 concentration, with a mean value of approximately 21 µg/dL. These results are in agreement with those of another study[20] in which administration of 48 mg of LT_4/d resulted in a 5-fold increase in the tT_4 concentration to achieve a mean value of approximately 9 µg/dL within 16 weeks.

The tremendous differences in concentrations achieved between 1-time administration and administration of LT_4 for 14 days indicated that thyroid hormones have extremely complicated kinetics. Furthermore, these results suggested that there would be a saturation effect of LT_4 excretion and that horses could physiologically manage a high dose of LT_4 for a short time but not for prolonged periods of administration.

The awareness of EMS is increasing, and LT_4 is mainly used for treatment of obesity in horses.[10] Although the precise mechanism of EMS has not been determined, obesity is one of its most common yet nonessential traits.[31] In euthyroid horses, prolonged treatment with LT_4 results in weight loss and improved insulin sensitivity.[19] Within 16 weeks after the start of treatment, substantial weight loss associated with an increase in insulin sensitivity, as measured via a frequently sampled IV glucose tolerance test, has been reported.[19] These findings suggest a beneficial effect for the treatment of obesity in horses with insulin dysregulation in the context of EMS. A positive correlation between thyroid gland function and insulin sensitivity has been described for humans, and the administration of low doses of T_3 has been recommended for specific cases of obesity and human metabolic syndrome.[32–34] The effect of an increased dose of LT_4 on glucose and insulin dynamics was beyond the scope of the study reported here; however, the data reported here support the use of a higher dose of LT_4 for prolonged periods in horses because it resulted in a significant increase in tT_4 concentrations. In addition, there are possible effects on weight loss. Although no clinical complications were observed in the present study, a more thorough investigation of the safety of higher doses is warranted.

In the study reported here, 4 horses had low thyroid hormone concentrations at baseline, which suggested that those horses could have been misdiagnosed with hypothyroidism on the basis of analysis of

a sample obtained at a single time point. Measurement of thyroid hormones has been both physiologically and technically challenging, which has led to a false-positive diagnosis of hypothyroidism in horses and also has led to some authors questioning the validity of the reference intervals used.[1] Therefore, a low hormone concentration at a single time point would be a weak indicator of hypothyroidism because factors such as weather, diet, feed availability, exercise, drug administration, stage of the reproductive cycle, and time of day have all been found to decrease thyroid hormone concentrations.[35–40]

In addition to differences between measurement of total or free fractions of thyroid hormone concentrations when hormonal protein binding can be altered by disease state, drugs, or other hormones, measurement by direct methods reportedly underestimates T_4 concentrations, compared with concentrations obtained by use of dialysis or ultrafiltration methods.[38,41–43] In the present study, different, although not significantly so, initial (baseline) results obtained from the same horses were in agreement with the low diagnostic value of a result obtained at a single time point when assessing thyroid gland function. Stimulation tests to assess thyroid gland function have been described and could have been used in the study reported here to confirm the absence of hypothyroidism; however, given the low prevalence of hypothyroidism in horses, the limited description of those tests, and the poor availability of drugs such as synthetic thyroid-stimulating hormone, stimulation tests were not undertaken.[44–46]

Results of the present study indicated that 1-time administration of LT_4, even at high doses, induced limited increases in serum tT_3 and tT_4 concentrations but that prolonged administration of high doses of LT_4 significantly increased the serum tT_4 concentration, which could be relevant in horses with weight loss resistance. Further studies are warranted to evaluate the effect of high doses of LT_4 on insulin dynamics of horses.

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Footnotes

a. Thyro-L powder, Lloyd Inc, Shenandoah, Iowa.
b. Immulite 2000 analyzer, Siemens Healthcare Diagnostics, Llanberis, Wales.
c. Prism, GraphPad Software Inc, La Jolla, Calif.

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