Effects of long-term oral administration of levothyroxine sodium on serum thyroid hormone concentrations, clinicopathologic variables, and echocardiographic measurements in healthy adult horses

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Objective—To determine the effects of long-term oral levothyroxine sodium (L-T₄) administration on serum thyroid hormone concentrations, thyroid gland function, clinicopathologic variables, and echocardiographic examination measurements in adult euthyroid horses.

Animals—6 healthy adult mares.

Procedures—Horses received L-T₄ (48 mg/d) orally for 48 weeks. Every 4 weeks, physical examinations were performed; blood samples were collected for CBC, plasma biochemical analyses, and assessments of serum total triiodothyronine (tT₃) and thyroxine (tT₄) concentrations. Plasma creatine kinase MB activity and cardiac troponin I concentration were also measured. Echocardiographic examinations were performed before and at 16, 32, and 48 weeks during the treatment period.

Results—During the treatment period, mean body weight decreased significantly; heart rate varied significantly, but the pattern of variation was not consistent. Significant time effects were detected for certain clinicopathologic variables, but mean values remained within reference ranges. Cardiac troponin I was only detectable in 8 of 24 plasma samples (concentration range, 0.01 to 0.03 ng/mL). Serum creatine kinase MB activity did not change significantly over time. Compared with the pretreatment value, 5.4-, 4.0-, and 3.7-fold increases in mean serum tT₄ concentrations were detected at 16, 32, and 48 weeks, respectively. Some cardiac measurements changed significantly over time, but mean values remained within published reference ranges. Mean fractional shortening was lower than the pretreatment mean value at 16 and 32 weeks.

Conclusions and Clinical Relevance—In horses, long-term oral administration of 48 mg of L-T₄/d significantly increased serum tT₄ concentrations and did not appear to adversely affect health. (Am J Vet Res 2008;69:68–75)

In previous studies,¹² we determined that oral administration of L-T₄ significantly decreased body weight and increased insulin sensitivity in horses. These results supported the use of L-T₄ to treat obesity and insulin resistance in horses, but clinicians are concerned about negative health effects in horses receiving long-term treatment. Much of this concern stems from reports³⁻⁵ of cardiac abnormalities in humans that have been treated with L-T₄ for extended periods.

In a study by Biondi et al³, 20 humans who were receiving L-T₄ as a supplement after thyroidectomy (n = 10) or because of nodular or diffuse goiter (n = 10) were examined. These patients were receiving long-term treatment (1 to 9 years) with L-T₄ at dosages that suppressed plasma thyrotropin concentrations to values less than the limit of detection of the assay used. Twelve of 20 (60%) patients complained of heart palpitations. The treated group had tachycardia and left ventricular hypertrophy; left ventricular systolic function was enhanced and mean %FS was increased, compared with untreated control subjects. It was concluded that cardiac function can be markedly affected by long-term L-T₄ treatment. In humans treated with L-T₄ for 3 to 21 years at dosages that decreased TSH concentrations to less than the lower reference limit, left ventricular hypertrophy was detected and mean left ventricular mass index was 18.4% higher than that in healthy untreated individuals.⁴ However, no other cardiac abnormalities were detected in treated patients. It was concluded that long-term L-T₄ treatment can cause

<table>
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<th>Abbreviations</th>
<th>L-T₄</th>
<th>%FS</th>
<th>TSH</th>
<th>TRH</th>
<th>tT₃</th>
<th>tT₄</th>
<th>CKMB</th>
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<tr>
<td>Levothyroxine sodium</td>
<td>Percentage fractional shortening</td>
<td>Thyroid-stimulating hormone</td>
<td>Thyrotropin-releasing hormone</td>
<td>Total triiodothyronine</td>
<td>Total thyroxine</td>
<td>Creatine kinase MB</td>
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left ventricular hypertrophy but does not affect heart rate, blood pressure, or cardiac function in humans. When the study performed by Biondi et al. was repeated by another group of researchers, no differences were detected between treatment and control groups, with the exception of left ventricular mass index. This measurement was 27% higher in treated patients, compared with the healthy volunteers, but still within the reference range. Treated patients did not complain of heart problems; therefore, it was suggested that results of the first study were confounded by the inclusion of individuals with concurrent heart disease or hypertension. Discrepancies between results of the aforementioned studies highlight the controversy associated with long-term L-T₄ administration in humans.

Electrocardiographic abnormalities including atrial premature beats and atrial fibrillation have also been associated with L-T₄ administration in humans receiving the drug at thyrotropin-suppressive dosages. Detection of these arrhythmias has been facilitated by 24-hour Holter monitoring. Holter recordings have also revealed that L-T₄–treated patients can develop higher heart rates, decreased mean R-R intervals, and altered heart rate variability, which has been attributed to impaired sympathovagal balance.

When examining health risks associated with L-T₄ treatment, it is important to focus on the animals of interest because L-T₄ dosages vary markedly among species. Mean TSH suppressive dosages of L-T₄ administered to humans in the aforementioned studies were 0.16, 0.21, and 0.19 mg/d, compared with dosages of 24 to 96 mg/d that have been evaluated in horses. With the exception of agitation associated with the dosage of 96 mg of L-T₄/d, clinical signs of hyperthyroidism have not been associated with short-term administration of L-T₄ in horses. Tachycardia was not detected in horses when they were treated with L-T₄ at dosages ranging from 24 to 96 mg/d for 8 weeks; higher dosages of L-T₄ suppressed serum TSH concentrations over time, but ranges overlapped among groups, and the hormone was always detected in blood samples collected from treated horses.

The purpose of the study reported here was to determine the effects of long-term oral administration of L-T₄ on serum thyroid hormone concentrations, thyroid gland function, clinicopathologic variables, and echocardiographic examination measurements in adult euthyroid horses. We hypothesized that long-term L-T₄ administration would not be associated with alterations in general health or cardiac structure and function, as determined by echocardiographic examinations.

Materials and Methods

Animals—Six healthy mares of mixed breed and Quarter Horse body type were selected for use in the study. Mares were chosen as the experimental unit to reduce variability associated with differences in sex. Immature and old horses were also not selected. Mean ± SD age was 8 ± 2 years (range, 6 to 10 years), and mean body weight was 301 ± 38 kg (range, 443 to 550 kg) at the beginning of the study. Horses were housed in indoor stalls (2.75 × 3.5 m) from approximately 4 PM until 7 AM and then turned out on pasture for the remainder of the day. Each horse had access to grass hay in round bales when on pasture, and 2 flakes of mixed-grass hay (approx 4.5 kg) were provided in the stall when the horse returned at 4 PM. Each horse also received 0.3 kg of oats once daily in the morning (approx 7 AM). Water was provided ad libitum. Horses were transported to the veterinary teaching hospital on the Friday before each testing week and were housed in stalls (3.7 × 3.7 m) for 7 days. Four to 6 flakes of grass hay were fed each day during these periods. The study protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental design—Each day, the 6 horses were each given 48 mg (4 teaspoons) of L-T₄ mixed with 30 mL of water in their morning meal of oats during a 48-week treatment period. This was followed by a 6-week drug withdrawal period during which horses were orally administered 24 mg of L-T₄/d for 2 weeks, followed by 12 mg of L-T₄/d for 2 weeks, and then no treatment for 2 weeks. Horses were observed daily, and complete physical examinations were performed every 4 weeks during the treatment period. Rectal temperature, heart rate, and respiratory rate were recorded. Horses were weighed, echocardiographic examinations were performed, and TRH response tests were conducted prior to and every 16 weeks during the 48-week treatment period. Echocardiographic examinations were performed 5 to 9 weeks before beginning treatment and then at 16-week intervals (ie, weeks 16, 32, and 48). Treatment was initiated in November 2004 and testing performed after 16, 32, and 48 weeks occurred in March, June, and October, respectively. Treatment periods were staggered so that 2 horses were brought into the hospital each week; all horses were evaluated over a period of 3 weeks. Blood samples were collected for analysis prior to injection of TRH and weekly throughout the treatment and drug withdrawal periods. Frequently sampled IV glucose tolerance tests were also performed every 16 weeks, and biopsies of adipose and skeletal muscle tissues were performed prior to and at the completion of the 48-week treatment period. Results of that work have been published.

TRH response test—At approximately 9 AM on the selected test days, blood samples were collected prior to injection of 1.2 mg of TRH. Blood samples were subsequently collected 2 and 4 hours after TRH injection for assessment of serum T₄, and T₃ concentrations, respectively. On testing days, L-T₄ was not administered until after blood samples were collected at the 4-hour time point.

Blood collection—Blood was collected via jugular venipuncture into tubes containing EDTA, sodium heparin, or no anticoagulant. Low-speed centrifugation (1,000 × g for 20 minutes at 4°C) was used to obtain plasma or serum.

CBC and plasma biochemical analysis—A routine CBC was performed on freshly collected whole blood by use of an automated analyzer to provide WBC, RBC, and platelet counts; Hct; and hemoglobin concentration. For plasma biochemical analysis, concentrations of BUN, calcium, chloride, creatinine, glucose, potassium, sodium, phosphorus, total bilirubin, total carbon
dioxide, total protein, albumin, and globulin; activities of CK, aspartate transaminase, and γ-glutamyltransferase; and the anion gap were measured in duplicate in plasma samples containing heparin by use of an automated discrete analyzer with reagents provided by the manufacturer of the analyzer.

Measurement of thyroid hormones—Serum concentrations of T1 and T4 were measured by use of radioimmunoassays validated for use with equine sera. Samples obtained from the horses at all time points and after TRH injection and standards provided by the manufacturer were concurrently analyzed in duplicate. Results of duplicate analyses were examined, and an intra-assay coefficient of variability < 10% was required for acceptance of results.

Cardiac troponin 1 and CKMB measurements—Samples of plasma containing heparin or serum were also submitted to 2 laboratories for the measurement of cardiac troponin 1 concentrations and CKMB activities, respectively. Cardiac troponin 1 concentration was measured in equine plasma by use of an immunoassay and an automated analyzer. Creatine kinase MB activity was measured in equine serum by use of an immunoassay that utilized murine antibodies against human CKMB and an automated analyzer. The cardiac index was calculated by multiplying the measured CKMB activity (ng/mL) by 100 and dividing by the measured creatine kinase (U/L) activity; this measure has been previously used to evaluate myocardial damage.

Echocardiography—Horses were restrained in stocks and B- and M-mode echocardiographic examinations were performed by 1 investigator (BRB) with a 2.5-MHz phased-array sector transducer. The maximal depth was 30 cm, and the mean of 3 nonconsecutive cardiac cycles was calculated. A base-apex ECG was superimposed for timing, and scans were recorded on videotape. Echocardiographic examinations were performed according to standard procedures. Right and left ventricular outflow tracts were evaluated via the right parasternal window to obtain B-mode 2-dimensional measurements. The long-axis right ventricular outflow tract was visualized, and the inside diameter of the pulmonary artery was measured at end of systole. The view of the left ventricular outflow tract was then optimized, and a long-axis measurement of the aortic root diameter was made from inside edge to inside edge at the level of the sinus of Valsalva at the end of diastole.

A right parasternal short-axis view was obtained for M-mode measurements, and a 2-dimensional image of the left ventricle was displayed for guidance. All M-mode measurements were obtained at the chordal level. End-diastolic and end-systolic measurements of interventricular septum myocardial wall thickness, left ventricular free myocardial wall thickness, left ventricular internal diameter, and right ventricular internal diameter were obtained. Percentage fractional shortening was calculated by use of an equation as follows:

\[
\%FS = 100 \times \frac{LVIDd - LVIDs}{LVIDd}
\]

where LVIDd and LVIDs is the end-diastolic and end-systolic left ventricular internal diameter, respectively.

Statistical analysis—A repeated-measures ANOVA in a statistical program was used to assess variables. When significance was established, multiple comparisons were made of the differences of least squares means for weeks 16, 32, and 48 with the pretreatment mean value by use of the Bonferroni test. For baseline serum thyroid hormone concentrations, least squares means for weeks 49 to 54 were also compared with the pretreatment mean value. Correlations between CBC and plasma biochemical analysis results and time were evaluated by use of Pearson correlation coefficients. Coefficients of variation were calculated for the echocardiographic measurements obtained during 3 cardiac cycles. Mean ± SD values are reported. Nonparametric tests were used to assess cardiac troponin 1 concentrations, CKMB activities, and CKMB index values, and median (range) values are reported for these variables. Significance was defined as a value of \( P < 0.05 \).

Results

Mean ± SD body weight significantly \( (P < 0.001) \) decreased from 501 ± 37 kg before treatment with L-T4 to 452 ± 32 kg, 458 ± 35 kg, and 475 ± 28 kg at weeks 16, 32, and 48, respectively. Rectal temperature and

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretreatment</th>
<th>16</th>
<th>32</th>
<th>48</th>
<th>r</th>
<th>Reference range</th>
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<tbody>
<tr>
<td>Anion gap (mEq/L)</td>
<td>8.4 ± 6.4</td>
<td>13.0 ± 15.5*</td>
<td>15.6 ± 3.5*</td>
<td>16.1 ± 3.0*</td>
<td>0.85</td>
<td>6–19</td>
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<td>Creatinine (mg/dL)</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.3</td>
<td>1.6 ± 0.6*</td>
<td>1.5 ± 0.3*</td>
<td>0.66</td>
<td>0.9–1.8</td>
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<tr>
<td>Platelet count</td>
<td>166 ± 35</td>
<td>191 ± 69</td>
<td>207 ± 39*</td>
<td>208 ± 37</td>
<td>0.62</td>
<td>70–251</td>
</tr>
<tr>
<td>(platelets/μL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>2.6 ± 2.3</td>
<td>3.3 ± 1.2</td>
<td>3.2 ± 1.4</td>
<td>3.9 ± 0.9*</td>
<td>0.61</td>
<td>2.1–4.7</td>
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<tr>
<td>Sodium (mEq/L)</td>
<td>137 ± 6</td>
<td>137 ± 3</td>
<td>140 ± 4*</td>
<td>139 ± 3</td>
<td>0.53</td>
<td>134–141</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>38 ± 8</td>
<td>36 ± 6</td>
<td>43 ± 6*</td>
<td>40 ± 8</td>
<td>0.42</td>
<td>31–51</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>102 ± 5</td>
<td>101 ± 4</td>
<td>102 ± 5</td>
<td>98 ± 7*</td>
<td>−0.49</td>
<td>95–106</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>12.0 ± 0.9</td>
<td>12.4 ± 0.8</td>
<td>11.9 ± 0.9</td>
<td>11.4 ± 0.7*</td>
<td>−0.55</td>
<td>11.2–13.0</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>19 ± 4</td>
<td>18 ± 4</td>
<td>16 ± 5*</td>
<td>15 ± 1*</td>
<td>−0.75</td>
<td>9–20</td>
</tr>
</tbody>
</table>

*For a given variable, value is significantly \( (P < 0.05) \) different from the pretreatment mean value, as determined by an ANOVA for repeated measures. Post hoc analysis was performed by comparison of least squares mean values with the Bonferroni adjustment.
respiratory rate did not change over time. Heart rates ranged from 36 to 54 beats/min prior to treatment and from 30 to 60 beats/min during the 48-week period in which L-T₄ was administered. A significant (P = 0.028) time effect was detected for this variable, but treatment period mean values did not differ significantly from the pretreatment mean value. Certain clinicopathologic variables were associated with significant time effects (Table 1). Mean values were all within reference ranges for our laboratory. Cardiac troponin I was only detectable in 8 of 24 plasma samples, and concentrations ranged from 0.01 to 0.03 ng/mL. Serum CKMB activities and CKMB index values did not change significantly over time.

Serum tT₄ concentrations varied significantly (P = 0.008) with time, and the mean concentration at 32 weeks was significantly lower than the pretreatment mean (Figure 1). In contrast, serum tT₃ concentrations increased significantly (P < 0.001) over time, and mean values at 16, 32, and 48 weeks were significantly higher than the pretreatment mean (Figure 2). Serum tT₄ and tT₃ concentrations did not increase in response to TRH injection at weeks 16, 32, and 48, and these responses differed significantly from those detected prior to treatment (Table 2).

Time effects were significant for 8 of the echocardiographic measurements examined (Table 3), but mean values remained within 1 or more previously published reference ranges (Appendix). Of the variables with significant time effects, only the change in %FS was considered of potential clinical importance. Compared with the pretreatment mean value, mean %FS values were 20% and 15% lower after 16 and 32 weeks of L-T₄ treatment, respectively.

**Discussion**

In the present study, oral administration of L-T₄ at a dosage of 48 mg/d for 48 weeks significantly increased serum tT₄ concentrations, suppressed thyroid hormone responses to TRH, and induced weight loss but did not adversely affect general health or echocardiographic measurements in euthyroid adult horses. Although the horses lost weight in response to treatment, they remained healthy in appearance. Hyperthyroidism is rare in horses. Affected horses have profound weight loss, intermittent tachycardia, hyperactive behavior, and mild fever. With the exception of weight loss, horses in our study did not develop any of the clinical signs associated with hyperthyroidism in horses.

A significant time effect was detected for heart rate, but this appeared to be a result of month-to-month fluctuations, rather than tachycardia. We also failed to detect tachycardia in horses in our previous study, even

<table>
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<tr>
<th>Time (wk)</th>
<th>Fold increase*</th>
<th>Pretreatment</th>
<th>16</th>
<th>32</th>
<th>48</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum tT₄ concentration (2 hours after TRH injection)</td>
<td>4.4 ± 1.6</td>
<td>1.0 ± 0.27</td>
<td>1.2 ± 0.61</td>
<td>1.1 ± 0.11</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>Serum tT₃ concentration (4 hours after TRH injection)</td>
<td>1.7 ± 0.4</td>
<td>0.9 ± 0.11</td>
<td>1.0 ± 0.1</td>
<td>0.9 ± 0.11</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
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</table>

*The fold increase was calculated by dividing the concentration after TRH injection by the preinjection concentration. Within a row, value differs significantly (P < 0.05) from the pretreatment mean value.

Figure 1—Mean ± SD serum tT₃ concentration in 6 mares that were orally administered 48 mg of L-T₄/d for 48 weeks, 24 mg of L-T₄/d for 2 weeks (weeks 49 and 50), and then 12 mg of L-T₄/d for 2 weeks (weeks 51 and 52). Concentrations were assessed before (week 0) and every 16 weeks during treatment, every week during the drug withdrawal period (weeks 49 to 52), and also 7 and 14 days (weeks 53 and 54) after L-T₄ administration was discontinued. Time of sample collection significantly (P = 0.008) affected concentrations. *Value is significantly (P < 0.05) different from the pretreatment (week 0) value.

Figure 2—Mean ± SD serum tT₄ concentration in 6 mares that were orally administered 48 mg of L-T₄/d for 48 weeks, 24 mg of L-T₄/d for 2 weeks (weeks 49 and 50), and then 12 mg of L-T₄/d for 2 weeks (weeks 51 and 52). Concentrations were assessed before (week 0) and every 16 weeks during treatment, every week during the drug withdrawal period (weeks 49 to 52), and also 7 and 14 days (weeks 53 and 54) after L-T₄ administration was discontinued. Time of sample collection significantly (P = 0.008) affected concentrations. *Value is significantly (P < 0.05) different from the pretreatment (week 0) value.
after treatment with the highest dosage of L-T₄. Tachycardia has been an inconsistent finding in studies of long-term L-T₄ administration in humans. In their study, Biondi et al. detected a higher mean heart rate (84 beats/min) in patients treated with L-T₄ compared with the value for the control group (70 beats/min); however, heart rates have remained within reference range in other studies. The results of the present study have suggested that long-term administration of 48 mg of L-T₄/d is tolerated well by horses that are healthy at the onset of treatment. However, further studies are required to determine whether L-T₄ can be safely administered to horses with medical problems and whether it remains a safe treatment in horses that are facing physiologic challenges such as exercise or pregnancy.

Among the study horses, none of the clinicopathologic variables assessed were significantly altered by long-term L-T₄ administration, including plasma CKMB activity and cardiac troponin I concentration, which are 2 indicators of myocardial damage. Values of some variables were significantly correlated with time, and significant time effects were identified. However, none of these alterations were judged to be clinically important because mean values remained within reference ranges. The clinicopathologic alterations may reflect subtle changes in metabolism induced by L-T₄ or the diet consumed, but differences were small and unlikely to be clinically relevant. Anion gap increased over time, but the mean value was low prior to treatment, and this finding cannot be explained. Only the mean BUN concentration changed progressively; the value decreased from 19 ± 4 mg/dL to 15 ± 1 mg/dL over the 48-week treatment period. It is conceivable that this reduction reflected a decrease in protein intake or an effect of L-T₄ on protein metabolism.

The horses were judged to be euthyroid at the beginning of the study on the basis of physical examination findings and serum tT₃ and tT₄ concentrations. Prior to L-T₄ treatment, serum tT₃ and tT₄ concentrations increased in all horses in response to administration of exogenous TRH. It has been reported that serum tT₃ and tT₄ concentrations should increase >2-fold when measured 2 and 4 hours after TRH administration, respectively. Five of the 6 horses had this response for tT₃, but 1 horse had only a 1.9-fold increase in the concentration of this hormone following TRH injection. Evaluation of the mean tT₄ response among the 6 horses revealed a 1.7-fold increase at 4 hours after TRH, which was a lower than expected value. This result was consistent with the 1.6-fold increase in tT₄ detected in horses in our previous study and with findings of other studies. This indicates that administration of exogenous TRH does not consistently result in a >2-fold increase in serum tT₃ or tT₄ concentration in healthy euthyroid horses.

Mean serum tT₃ and tT₄ concentrations did not increase in response to exogenous TRH when horses in the present study were assessed after 16, 32, and 48 weeks of L-T₄ treatment. These results are consistent with those of our previous study in horses in which serum tT₃ and tT₄ concentrations did not increase in response to TRH administration after 4 weeks of L-T₄ treatment. It is possible that exogenous L-T₄ suppresses the hypothalamic–pituitary gland–thyroid gland axis over time in horses. However, there is some question as to whether horses can develop tolerance to exogenous TRH over time. In our previous study, the influence of TRH on serum tT₃ and tT₄ concentrations in control horses decreased over time when testing was performed 5 times at 2-week intervals. However, no evidence of tolerance was detected when the same compound was used to perform TRH response tests in 10 horses that were evaluated at the beginning and after 1 and 2 months of another study. In hindsight, our study
design would have been strengthened by including a control group and retesting horses once L-T$_4$ treatment had been discontinued to assess the return of normal thyroid gland function.

In the horses of the present study, serum thyroid hormone concentrations were significantly altered by L-T$_4$ treatment, but only T$_4$ concentrations increased over time. Levothyroxine sodium administered at a dosage of 48 mg/d increased serum T$_4$ concentrations to values that were greater than laboratory (10 to 40 ng/mL) and published reference ranges. Mean serum T$_4$ concentrations were 1.5- to 2-fold greater than the upper limit of the laboratory reference range when L-T$_4$ was administered, and then values decreased steadily during the drug withdrawal period. Serum T$_3$ concentrations returned to approximately 20 ng/mL by the completion of the study, which suggested that the drug withdrawal strategy was successful.

Serum T$_3$, concentrations detected during the treatment period in the present study were lower that those detected in treated horses in our previous study. Serum T$_3$, and free T$_3$, concentrations were higher at the completion of that study after horses had been treated with L-T$_4$ at dosages ranging from 24 to 96 mg/d. Levothyroxine may exert different effects on serum T$_3$, concentrations, depending on the dosage used or duration of treatment. Low serum T$_3$, concentrations could develop as L-T$_4$ is converted into free T$_3$, and negative feedback mechanisms are activated. However, higher dosages of L-T$_4$ might also have an opposite effect if L-T$_4$ is converted into T$_3$, by deiodinases. Levothyroxine returns both T$_4$ and T$_3$ concentrations to values that are within reference ranges in humans with primary hypothyroidism and increases mean serum T$_3$, concentration and the mean free T$_3$ index when given to euthyroid humans at a thyrotropin-suppressive dosage. Efforts of L-T$_4$, on serum free T$_3$, and T$_3$, concentrations in horses warrant further investigation, and serum thyrotropin concentrations should be measured over time to assess feedback responses.

A transient decrease in %FS was the only echocardiographic finding with potential clinical relevance. Mean %FS values were 20% and 13% lower than the mean pretreatment value at weeks 16 and 32, respectively, and then returned to the pretreatment value at week 48, which suggested that any effects of L-T$_4$ on cardiac contractility were transient. If the decrease in %FS in horses was caused by L-T$_4$ administration, this would be contrary to the treatment response detected in humans. Biondi et al reported that mean ± SD %FS was 38 ± 7% in patients treated long term with L-T$_4$, compared with 34 ± 4% in a control group of age- and sex-matched individuals.

Other echocardiographic measurements with significant time effects may have resulted from variability in the behavior and resting heart rate of the study horses or techniques used to perform the examinations. Horses sometimes became agitated during the echocardiographic procedure and images were more difficult to acquire at these times. Attempts were made to acclimatize the study horses to procedures, but each horse resisted the procedure on at least 1 occasion during the study. Unfortunately, these behavior problems were not recorded at the time that evaluations were performed. Mean coefficients of variation ranged from 1% to 10% for echocardiographic measurements obtained during 3 cardiac cycles in the present study.

Only ECG tracings recorded by the ultrasound machine were examined in the study reported here, and more extensive investigations involving the use of 24-hour Holter monitoring are required to determine whether L-T$_4$ affects conduction within the heart in horses. In the study by Biondi et al, the prevalence of atrial premature beats was higher in humans that were receiving long-term L-T$_4$ treatment than in healthy persons (100% vs 60%). Hypertension has also been associated with untreated hyperthyroidism in humans; therefore, blood pressure measurements should be included in future studies. Finally, it has been reported that long-term L-T$_4$ administration lowers exercise tolerance in humans; additional studies are required to assess athletic performance in L-T$_4$-treated horses.

Overall, the results of the present study have indicated that oral administration of 48 mg of L-T$_4$/d for 48 weeks does not adversely affect cardiac structure or function in horses. This conclusion contrasts with reports of the development of left ventricular hypertrophy in humans that have been treated long term with L-T$_4$. There may be several explanations for this discrepancy. First, the L-T$_4$ dosage selected for our study may be lower than the dosages evaluated in humans after interspecies differences are accounted for. This is relevant because left ventricular hypertrophy has only been associated with L-T$_4$ administration at dosages sufficient to suppress serum TSH concentrations to values less than the lower reference limit or the assay’s limit of detection. In another study in humans, L-T$_4$ dosages were adjusted for individuals by targeting a serum TSH concentration of 0.1 mU/mL; as a result, left ventricular mass improved or returned to reference values. Unfortunately, serum thyrotropin concentrations were not measured in the present study. Second, cardiac structure may have remained unaffected in horses because the duration of treatment was short, compared with that used in other studies. Humans treated with L-T$_4$ on a daily basis for periods of 1 to 9 years, 3 to 21 years, and 2.9 to 23 years developed increased left ventricular mass compared with healthy persons. The duration of treatment must therefore be considered when prescribing L-T$_4$ to animals of any species. It is still possible that L-T$_4$ treatment could alter cardiac structure in horses over time with long periods of treatment. Nevertheless, administration of 48 mg of L-T$_4$/d in feed for 48 weeks significantly increased serum T$_4$, concentrations and suppressed thyroid hormone responses to TRH in healthy adult horses, without adversely affecting general health and cardiac structure or function.

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c. P2161 Sigma Chemical Co, St Louis, Mo.
d. ADVIA 120 Hematology System, Bayer HealthCare LLC, Tarrytown, NY.
e. Hitachi 911, Boehringer Mannheim Corp, Indianapolis, Ind.
References


Appendix appears on the next page
### Appendix

Published reference ranges for echocardiographic measurements in adult horses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Long et al&lt;sup&gt;12&lt;/sup&gt; (range)</th>
<th>Slater and Heritgage&lt;sup&gt;16&lt;/sup&gt; (range)</th>
<th>Marr et al&lt;sup&gt;15&lt;/sup&gt; (range)</th>
<th>Patterson et al&lt;sup&gt;13&lt;/sup&gt; (range)</th>
<th>Buhl et al&lt;sup&gt;14&lt;/sup&gt; (mean ± SE)</th>
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<tr>
<td>Body weight (kg)</td>
<td>432–648</td>
<td>454–620</td>
<td>–</td>
<td>–</td>
<td>477–540</td>
</tr>
<tr>
<td>No. of horses</td>
<td>27</td>
<td>16</td>
<td>–</td>
<td>78</td>
<td>8</td>
</tr>
<tr>
<td>2-dimensional echocardiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2DARD (cm)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>7.5–9.8</td>
<td>6.77 ± 0.02</td>
</tr>
<tr>
<td>2DPAD (cm)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>5.2–8.0</td>
<td>5.51 ± 0.02</td>
</tr>
<tr>
<td>M-mode echocardiography</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSd (cm)</td>
<td>2.4–3.7</td>
<td>2.6–3.1</td>
<td>–</td>
<td>2.3–3.7</td>
<td>2.56 ± 0.01</td>
</tr>
<tr>
<td>IVSs (cm)</td>
<td>3.3–5.6</td>
<td>3.9–5.2</td>
<td>–</td>
<td>3.2–5.2</td>
<td>3.50 ± 0.02</td>
</tr>
<tr>
<td>LVFWd (cm)</td>
<td>3.0–5.4</td>
<td>3.1–4.2</td>
<td>–</td>
<td>3.2–4.6</td>
<td>3.47 ± 0.02</td>
</tr>
<tr>
<td>LVFWs (cm)</td>
<td>10.5–13.4</td>
<td>9.9–12.2</td>
<td>8.0–13.0</td>
<td>9.7–13.5</td>
<td>11.47 ± 0.05</td>
</tr>
<tr>
<td>LVIDd (cm)</td>
<td>6.1–8.7</td>
<td>6.4–9.0</td>
<td>4.3–7.9</td>
<td>5.8–9.1</td>
<td>7.66 ± 0.04</td>
</tr>
<tr>
<td>%FS</td>
<td>29–47</td>
<td>–</td>
<td>32–55</td>
<td>26–45</td>
<td>–</td>
</tr>
</tbody>
</table>

–– = Not reported.

2DPAD = Two-dimensional inside diameter measurement of the pulmonary artery at end of systole obtained from the right parasternal long-axis view of the right ventricular outflow tract. 2DARD = Two-dimensional aortic root diameter measurement taken from inside edge to inside edge at the level of the sinus of Valsalva at the end of diastole from the right parasternal long-axis view of the left ventricular outflow tract. IVSd and IVSs = End-diastole and end-systole interventricular septum myocardial wall thickness, respectively. LVFWd and LVFWs = End-diastole and end-systole left ventricular free myocardial wall thickness, respectively. LVIDd and LVIDs = End-diastole and end-systole left ventricular internal diameter, respectively. RVIDd and RVIDs = End-diastole and end-systole right ventricular internal diameter, respectively.