Effects of oral administration of levothyroxine sodium on concentrations of plasma lipids, concentration and composition of very-low-density lipoproteins, and glucose dynamics in healthy adult mares

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Objective—To evaluate glucose and lipid metabolism in healthy adult horses administered levothyroxine sodium (L-T_4).

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 between 7 AM and 8 AM in the morning grain meal for 2 weeks. Four additional horses remained untreated. Serum concentrations of nonesterified fatty acids, triglyceride (TG), total cholesterol (TC), and very-low-density lipoprotein (VLDL) were measured and composition of VLDL examined in samples obtained between 8 AM and 9 AM at weeks 0, 2, 4, 6, and 8. Glucose dynamics were assessed by use of a combined IV glucose-insulin tolerance test (IVGITT) conducted before and at the end of the 8-week treatment period. Data for each combined IVGITT were interpreted by use of the minimal model.

Results—Plasma TG, TC, and VLDL concentrations significantly decreased over time in treated horses. At the completion of the 8-week treatment period, mean plasma VLDL concentration was 46% of the mean value for week 0 in treated horses. Insulin sensitivity significantly increased (> 2-fold) in treated horses, but glucose effectiveness and net insulin response were not affected. Levothyroxine sodium significantly increased the rate of insulin disposal.

Conclusions and Clinical Relevance—Administration of L-T_4 decreases blood lipid concentrations, improves insulin sensitivity, and increases insulin disposal in horses. Levothyroxine sodium may have potential as a treatment for horses with reduced insulin sensitivity. (*Am J Vet Res* 2005;66:1032–1038)

Levothyroxine sodium (L-T_4) is commonly prescribed for use as a treatment in horses with presumed hypothyroidism, and clients anecdotally report favorable responses, including weight loss, increased activity, improved fertility, and fewer or milder episodes of laminitis. However, observed responses to L-T_4 are not consistent, and observations may be strongly influenced by the client's perception.

Beneficial effects of L-T_4 treatment may be mediated by alterations in energy metabolism. Humans with hyperthyroidism have a lower body mass index and lower serum concentrations of **triglyceride (TG)**, **total cholesterol (TC)**, and **very-low-density lipoprotein (VLDL)**.[1,2] In horses, VLDL is the primary transporter of TG in the blood, and changes in VLDL concentration or composition reflect alterations in lipid metabolism.[3] Plasma VLDL concentrations increase significantly in horses after thyroidectomy.[4,5] Glucose dynamics may also be affected by L-T_4 administration in horses. Administration of **thyroxine (T_4)** to rats increases insulin sensitivity by increasing the rate of glucose disposal and increasing glucose-stimulated insulin secretion.[6]

In the study reported here, we tested the hypothesis that L-T_4 administration significantly alters lipid and glucose metabolism in healthy adult horses. Our specific objectives were to measure concentrations of blood lipids and the concentration and composition of VLDL in horses treated with L-T_4 in accordance with an incrementally increasing dosing regimen. Glucose dynamics were measured by use of a combined **IV glucose-insulin tolerance test (IVGITT)** conducted at the beginning and end of the 8-week treatment period to determine the effect of L-T_4 administration on glucose metabolism in healthy adult horses.

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

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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 mares 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. Horses were observed daily, and complete physical examinations were performed weekly. Body weight was measured at the beginning of the study (before administration of L-T_4; week 0) and at the end of the 8-week study period. Blood samples were collected before and at 2-week intervals throughout the study. Samples for lipid and lipoprotein analysis were collected in EDTA-coated tubes, immediately chilled on ice, and refrigerated until processed.

Isolation of plasma VLDL—Low-speed centrifugation (1,000 × *g* for 20 minutes at 4°C) was used to obtain plasma. Plasma samples (6 mL) were then placed in a fixed-angle rotor[c] for ultracentrifugation (112,000 × *g* for 18 hours at 10°C). Fractions (1 mL) that had a density of < 1.006 g/mL were isolated from each tube. Duplicate samples were collected and stored at –70°C until compositional analysis was performed.

Analysis of plasma VLDL components—Concentrations of TG, **phospholipid (PL)**, TC, and **free cholesterol (FC)** in VLDL were measured in duplicate by use of the respective enzymatic colorimetric reagents[d-g] in an automated discrete analyzer.[h] Protein content of VLDL was analyzed by use of bovine serum albumin standards and a spectrophotometer[i] in accordance with modifications[7] of a method described elsewhere.[8] Intra-assay **coefficient of variation (CV)** was < 5% for duplicate analyses of samples. Plasma VLDL concentrations were calculated by summing concentrations of lipid (ie, TG, TC, and PL) and protein components.

Analysis of other plasma lipids—Concentrations of TG and TC in plasma were measured in duplicate by use of the respective enzymatic colorimetric reagents[d,f] in an automated discrete analyzer.[h] Plasma **nonesterified fatty acid (NEFA)** concentrations were measured by use of an in vitro enzymatic colorimetric test kit.[j]

Combined IVGITT—Two IVGITTs were conducted during the study (before [week 0] and at the completion [week 8] of the study). Horses were weighed the day before each test. Each horse was confined to its stall during each testing period but had ad libitum access to mixed-grass hay and water during the test. At approximately 8 AM on the test day, a 14-gauge polypropylene catheter was inserted into the left jugular vein. An injection cap and infusion set[k] (length, 30 cm; ID, 0.14 cm) were attached to the catheter. Testing was initiated at approximately 10 AM. A bolus (150 mg of glucose/kg) of a 50% dextrose solution[l] was infused into each horse via the infusion line and catheter; this was followed by infusion of heparinized saline (0.9% NaCl) solution, regular insulin[m] (0.1 U/kg), and another infusion of heparinized saline solution. Blood samples were collected via the catheter at 1, 5, 15, 25, 35, 45, 60, 75, 90, 105, 120, 135, and 150 minutes after insulin injection. At each time point, 3 mL of blood was withdrawn from the infusion line and discarded. A 6-mL blood sample was then collected, followed by infusion of 5 mL of heparinized saline solution. Half of each blood sample was transferred to tubes containing sodium fluoride and potassium oxalate, which were immediately cooled on ice and then refrigerated. The remaining half of each blood sample was placed in glass tubes containing no anticoagulant; these samples were allowed to clot at 22°C for 1 hour. Serum was subsequently harvested after low-speed centrifugation. Plasma and serum samples were stored at –20°C until further analysis.

Plasma glucose and serum insulin concentrations—Glucose concentrations were measured by use of a colorimetric assay[n] on an automated discrete analyzer.[h] Insulin concentrations were determined by use of a radioimmunoassay[o] that has been validated for equine insulin.[9] Each sample was assayed in duplicate; intra-assay CV was < 5% for the glucose and insulin assays.

Interpretation of combined IVGITT data using the minimal model—Values for **glucose effectiveness** (S_G) and **insulin sensitivity** (S_I) were calculated for each IVGITT test by use of commercially available software.[p,q] Values for S_G and S_I were calculated in accordance with the minimal model[10] by use of methods described elsewhere.[11,12] **Net insulin response (NIR)** was calculated from IVGITT data. We elected to determine the NIR instead of the acute insulin response to glucose because insulin was administered at the beginning of the test instead of after a delay of 20 minutes for a modified glucose-insulin test reported in another study.[12] Accordingly, the following equation was used for calculations:

$$NIR = \int (I_t - I_0) \times dt,$$

where I_t represents the insulin concentration at time t, I_0 represents the insulin concentration at time 0, and dt represents the change in time. The equation was integrated to determine values for $0 < t < 10$ minutes.

Calculation of insulin disposal rate—Serum insulin concentrations were plotted against time. Insulin concentrations were converted to natural logarithmic values, and linear regression analysis was then used to calculate an **insulin disposal rate (IDR)** for each combined IVGITT.

Statistical analysis—Mean plasma concentrations of TG, TC, NEFA, and VLDL were compared by use of a repeated-measures ANOVA.[r] When significance was established, the Bonferroni test for multiple comparisons was used to compare differences of least squares means for weeks 2, 4, 6, and 8 with values for week 0. Relationships between plasma lipid concentrations and dosage were evaluated within each group by use of Pearson correlation coefficients. Significance was defined at $P < 0.05$.

Results

In the study reported here, horses tolerated L-T_4 well, other than agitation observed during the 2 weeks that L-T_4 was administered at the rate of 96 mg/d. Results of physical examinations, changes in body weight, and effects of L-T_4 on blood concentrations of thyroid gland hormones for the horses in this study have been reported.[13]

Plasma concentrations of TG, TC, and VLDL were significantly altered over time in treated horses, compared with values for horses that did not receive L-T_4 (**Table 1**). We detected weak correlations between L-T_4 dosage and concentrations of TG (r, 0.43; $P = 0.006$) and TC (r, –0.25; $P = 0.022$) in treated horses. Plasma VLDL concentrations were not significantly correlated

Table 1—Mean ± SD plasma lipid concentrations 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.

		Time (wk)				
Variable	**Group**	**0**	**2**	**4**	**6**	**8**
TG (mg/dL)	Untreated	19.13 ± 1.36	23.63 ± 3.46	16.38 ± 6.16	26.63 ± 1.69*	30.25 ± 14.20*
	Treated	23.94 ± 4.61	23.81 ± 5.15	21.63 ± 6.71	23.81 ± 2.93	19.13 ± 5.63*
TC (mg/dL)	Untreated	79.50 ± 10.38	79.63 ± 12.83	85.25 ± 1.67	76.75 ± 8.35	90.88 ± 13.57*
	Treated	76.56 ± 10.26	79.75 ± 8.19	83.31 ± 11.79*	67.94 ± 7.54*	62.19 ± 17.18*
NEFA (µmol/L)	Untreated	343.08 ± 60.76	79.45 ± 34.41	161.53 ± 20.29	107.19 ± 30.61	158.81 ± 56.79
	Treated	348.39 ± 84.22	108.87 ± 62.53	229.06 ± 95.56	185.08 ± 83.40	262.85 ± 93.45
VLDL (mg/dL)	Untreated	19.10 ± 1.98	27.18 ± 5.96	21.61 ± 10.56	33.78 ± 3.64*	36.45 ± 25.99*
	Treated	35.76 ± 18.63	28.63 ± 7.12	17.68 ± 8.68*	28.75 ± 5.10	16.38 ± 2.40*

*Within a row, mean differs significantly ($P < 0.05$) from value for week 0 (before administration of L-T_4 was initiated).

TG = Triglyceride. TC = Total cholesterol. NEFA = Nonesterified fatty acid. VLDL = Very-low-density lipoprotein.

Table 2—Mean ± SD values for percentage of total mass composition of VLDL for 4 untreated horses and 8 horses treated with L -T_4 for 8 weeks in accordance with an incrementally increasing dosing regimen.

		Time (wk)				
Variable	**Group**	**0**	**2**	**4**	**6**	**8**
TG (%)	Untreated	60.98 ± 2.35	50.50 ± 16.71	63.45 ± 4.54	67.78 ± 3.49	58.23 ± 2.27
	Treated	64.48 ± 1.65	61.05 ± 6.27	66.28 ± 2.77	67.61 ± 1.69	64.05 ± 3.62
Protein (%)	Untreated	13.75 ± 2.74	19.03 ± 8.25	14.70 ± 3.65	12.05 ± 1.21	20.93 ± 2.46
	Treated	9.85 ± 1.30	11.81 ± 3.46	11.50 ± 2.18	10.68 ± 1.50	15.95 ± 3.10
Phospholipid (%)	Untreated	16.40 ± 1.09	19.83 ± 5.31	13.85 ± 0.68	12.78 ± 0.76	13.15 ± 0.48
	Treated	16.35 ± 1.42	17.10 ± 1.71	14.29 ± 0.31	13.59 ± 0.85	12.99 ± 0.24
TC (%)	Untreated	8.95 ± 0.82	10.85 ± 3.78	8.00 ± 0.54	7.45 ± 1.72	7.80 ± 0.22
	Treated	9.29 ± 0.89	10.13 ± 1.61	7.99 ± 0.64	8.08 ± 0.67	7.01 ± 0.84

See Table 1 for key.

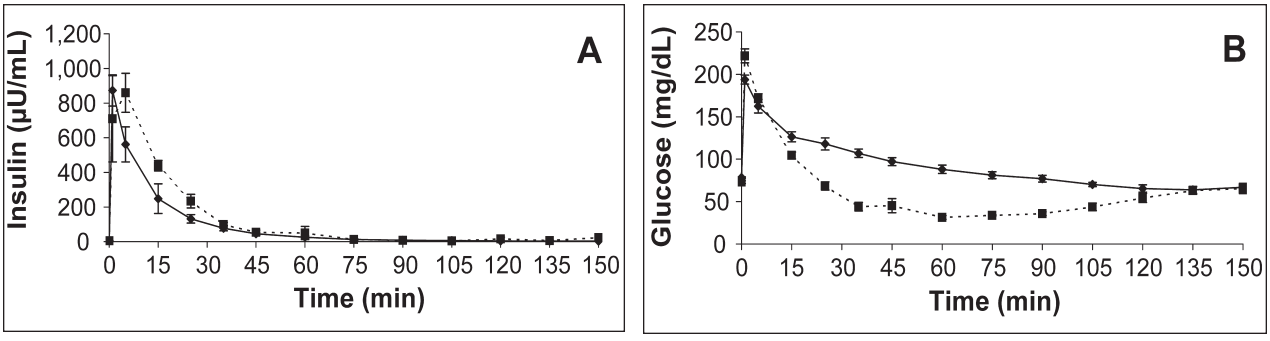

Figure 1—Mean ± SEM serum insulin concentrations (A) and plasma glucose concentrations (B) for 4 untreated horses during a combined IV glucose-insulin tolerance test (IVGITT) conducted before (diamonds) and at the end (squares) of an 8-week treatment period. Time 0 = Time of insulin injection.

(r, 0.44; $P = 0.053$) with L-T_4 dosage, but by week 8, mean plasma VLDL concentration significantly decreased to 46% of the value for week 0 in horses that were administered L-T_4. Plasma TG and VLDL concentrations in untreated horses were significantly higher at weeks 6 and 8, compared with concentrations for week 0. A transient increase in mean plasma TC concentration was detected at week 4 in treated horses, but concentrations at weeks 6 and 8 were significantly lower than the value for week 0. Mean plasma TC concentration in untreated horses was significantly higher at week 8 than for week 0. Administration of L-T_4 did not alter plasma NEFA concentrations or VLDL composition (**Table 2**).

Combined IVGITTs were successfully performed in all horses. During testing, 4 horses appeared restless and began to sweat when plasma glucose concentrations decreased to < 40 mg/dL. Affected horses

returned to normal without intervention. Combined IVGITT results for untreated and treated horses were plotted (**Figures 1 and 2**).

Data for 3 of 4 untreated horses and all 8 treated horses were successfully interpreted by use of the minimal model. Mean S_I increased significantly (P = 0.01) by > 2-fold in horses that received L-T_4 but did not differ significantly between the 2 testing periods in untreated horses (**Table 3**). The S_G and NIR were not affected by L-T_4 administration. Mean IDR increased significantly (P = 0.01) in treated horses, with blood insulin concentrations decreasing at a median rate of 9.4%/min (range, 7.2 to 11.3%/min) after injection at week 8, compared with 7.3%/min (range, 4.9 to 8.4%/min) at the beginning of the study.

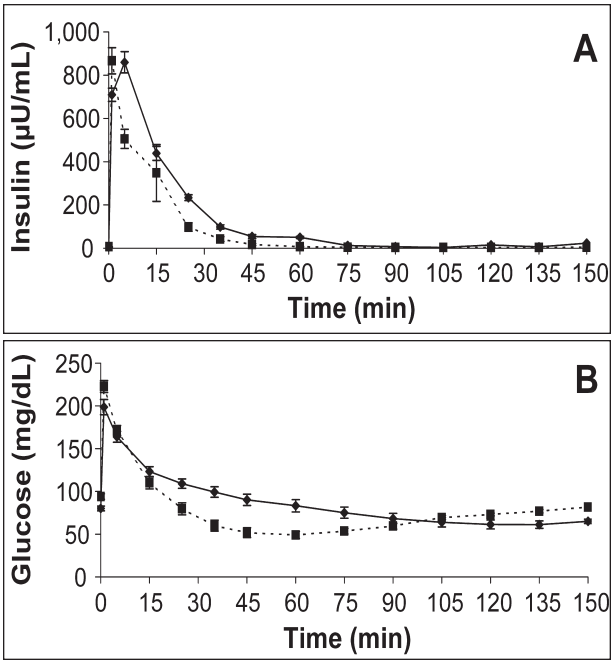

Figure 2—Mean ± SEM serum insulin concentrations (A) and plasma glucose concentrations (B) for 8 treated horses during a combined IVGITT conducted before (diamonds) and at the end (squares) of an 8-week treatment period. Horses were administered levothyroxine sodium PO for 8 weeks (weeks 1 through 8) in accordance with an incrementally increasing dosing regimen of 24, 48, 72, and 96 mg of levothyroxine sodium/d, with each dose administered for 2 weeks. *See* Figure 1 for key.

Discussion

Levothyroxine administration caused a significant decrease in plasma VLDL, TG, and TC concentrations in horses by 8 weeks after initiating treatment. This is the opposite response to the significant increase in plasma VLDL concentrations detected after thyroidectomy in horses.[4,5] Significantly higher VLDL-TG fractional catabolic rates have been detected in hyperthyroid humans, indicating that VLDL is cleared from the blood more rapidly.[1] Lower plasma concentrations of TC, **low-density-lipoprotein cholesterol (LDL-C)**, high-density-lipoprotein cholesterol, and apolipoprotein B are also associated with hyperthyroidism in humans, and these concentrations increase further with thyroid-suppressive treatments.[2,14] Lower plasma LDL-C concentrations are attributed to an increased abundance of hepatic LDL receptors and activity in treated patients.[15] Thyroid gland hormones also stimulate fatty acid consumption within hepatocytes by increasing rates of mitochondrial β-oxidation and noncoupled thermogenesis.[16] Acceleration of fatty acid consumption within the liver may reduce the availability of TG for VLDL synthesis. Lower VLDL concentrations may also result from accelerated clearance of lipoproteins from circulation. Triglyceride carried by VLDL is catabolized at a faster rate in hyperthyroid humans.[1]

Plasma TG, TC, and VLDL concentrations increased in untreated horses as the study progressed. Blood VLDL concentrations increase with time in a linear manner when horses are deprived of feed.[17] Feed intake was not measured in the study reported here, but horses appeared to be consuming feed normally throughout the study. Untreated horses may have become more stressed as the study progressed because their stalls and drylots were located adjacent to those of the treated horses. Treated horses became agitated after week 6 and were often seen trotting around their enclosures and interacting with adjacent horses. Stress is a risk factor for development of hyperlipemia in horses, a metabolic disorder characterized by increases in plasma lipid concentrations.[18]

Plasma NEFA concentrations were not significantly altered by L-T_4 administration in the study reported

Table 3—Median (range) values for glucose effectiveness (S_G), insulin sensitivity (S_I), and net insulin response (NIR) for 3 of 4 untreated horses and 8 horses treated with L -T_4 PO for 8 weeks in accordance with an incrementally increasing dosing regimen.

Variable	Time (wk)	Untreated	Treated
S_G ($\times 10^{-2}$/min)	0	2.65 (2.18–2.58)	1.37 (0.70–3.66)
	8	2.28 (1.65–3.14)	2.28 (1.65–3.14)
S_I ($\times 10^{-4}$ [L/mU]/min)	0	0.60 (0.09–12.33)	1.00 (0.50–2.36)
	8	2.22 (2.05–2.50)	2.48 (0.85–4.40)*
NIR ([mU/min]/L)	0	117.20 (81.93–163.16)	122.94 (102.52–152.31)
	8	144.57 (96.10–188.96)	101.36 (76.89–223.76)
IDR (%/min)†	0	6.54 (6.34–7.74)	7.34 (4.91–8.44)
	8	7.34 (6.89–10.03)	9.43 (7.17–11.28)*

*Within a variable within a treatment group, value differs significantly ($P < 0.05$) from the value for week 0. †Insulin disposal rate (IDR) was calculated for all 12 horses included in the study.

here. This finding was unexpected because an increase in norepinephrine-induced lipolysis and an increase in plasma NEFA concentrations have been detected in rats treated with **triiodothyronine** (T_3).[19] Increased rates of lipolysis and excessive energy expenditure are also associated with hyperthyroidism in humans and are accompanied by increased turnover rates for glycerol, NEFA, and ketone bodies.[20]

Lipoprotein composition also was unaffected in treated horses. Alterations in VLDL composition have been detected following thyroidectomy in horses.[4,5] Percentage of FC significantly increased during the 6 weeks after thyroidectomy, whereas TC content transiently decreased at 4 weeks after surgery and then returned to presurgical concentrations in 1 study.[5] Higher VLDL-TG clearance rates and lower VLDL-TG concentrations have been reported[1] in hyperthyroid humans, but the TG content of VLDL was not determined in that study. To our knowledge, specific alterations in VLDL composition have not been reported in humans treated with T_4.

Blood lipid concentrations were significantly altered over time by L-T_4 administration but were only weakly correlated with L-T_4 dosage in the study reported here. However, these results must be interpreted within the context of our experimental design because treatment periods were consecutive and not preceded by washout intervals. Thus, effects of L-T_4 may have been cumulative as the study progressed.

Data from each combined IVGITT performed in the study were successfully interpreted by use of the minimal model, except for data from 1 untreated horse. Problems were encountered with the placement and maintenance of the catheter in that horse, and that horse was more fractious than the other horses. Horses must remain calm during a combined IVGITT to avoid potential confounding effects of stress. Other horses in this study were quieter (ie, better temperament) and were not disturbed by the procedure as long as hay was available for consumption.

The minimal model was developed by investigators[10] for use in evaluating glucose-insulin test data collected from humans.[12] This model has been used to interpret data for a modified glucose-insulin test in horses,[11] and application of the minimal model to data collected from horses is described in detail in that report. Values for S_G and S_I for horses in the study reported here tended to be higher than those reported for another study.[11] Observed discrepancies were attributed to differences in the types of horses included in the studies (Thoroughbred geldings were used in that other study,[11] whereas Quarter Horse-type mares were used in our study).

Lower plasma glucose concentrations were detected in both groups of horses during a combined IVGITT performed at the end of the study. Because treated and untreated horses were affected, weight loss, change in diet, seasonal changes, reproductive cycle, and stress were considered as potential contributing factors. However, weight loss was considered less likely to be a factor because untreated horses did not have a change in median body weight. Blood glucose concentrations increase when cortisol and epinephrine are released in response to stress.[21] It is possible that stall confinement or testing procedures may have stressed horses to a greater extent at the beginning of the study. Horses may have become acclimated to their surroundings and blood collection procedures as the study progressed.

Insulin sensitivity increased in treated horses. When plots of serum insulin concentrations were examined qualitatively, the insulin peak for week 8 was wider than the peak detected for week 0 in untreated horses, whereas a narrower insulin peak was evident after 8 weeks in treated horses (Figure 1). Analysis for the minimal model confirmed that S_I and IDR significantly increased in treated horses but did not differ significantly between testing times in untreated horses. However, it should be mentioned that fewer horses were included in the untreated group and glucose dynamics varied widely among horses in this group, particularly at the beginning of the study. For example, S_I values ranged from 0.09×10^{-4} to 12.33×10^{-4} (L/mU)/min in untreated horses for week 0. Comparisons therefore focused on differences between testing times within the same group, and nonparametric statistical tests were used because data were not normally distributed. Larger group sizes or use of a crossover design should be considered for future studies.

Insulin sensitivity significantly increased by > 2-fold in horses treated with L-T_4 for 8 weeks at a daily dosage that ranged from 46 µg of L-T_4/kg (24 mg of L-T_4/d) to 225 µg of L-T_4/kg (96 mg of L-T_4/d). Alterations in glucose metabolism have been detected in rats treated with T_4, but results differ markedly between studies,[6,22] potentially because of differences in the dosage selected or duration of treatment. In 1 study,[6] it was reported that rats treated with 375 µg of T_4/kg/d for 30 days had increased S_I as evidenced by higher rates of glucose disposal and an increase in glucose-stimulated secretion of insulin. In contrast, no differences in S_I were detected by use of an **IV glucose tolerance test (IVGTT)** when rats were treated with low (100 µg of T_4/kg/d) or high (600 µg of T_4/kg/d) dosages of T_4 for 2 weeks; however, a reduction in secretory capacity of insulin was detected.[22] Reversible apoptosis of pancreatic β-cells has also been detected in rats treated with 600 µg of T_4/kg/d for 5 days.[23]

Alterations in S_I may result from changes in abundance of **glucose transporter (GLUT)** protein within adipose or skeletal muscle tissues.[24,25] Amounts of GLUT-1 and GLUT-4 increased by 87% and 90%, respectively, when mouse 3T3-L1 adipocytes were treated with T_3 for 3 days[24]; these cells also had a 3.6-fold increase in glucose uptake. However, opposite results were detected in rats treated with 500 µg of T_4/kg/d for 7 days.[25] Adipocytes harvested from treated rats had a significantly lower density of insulin receptors, reduced insulin-mediated glucose transport, and lower amounts of GLUT-4 within plasma membranes after stimulation by insulin.[25] Insulin-stimulated glucose uptake also significantly decreased in explants of slow and fast skeletal muscles obtained from young growing rats that received 2 mg of T_4/kg/d for 8 days.[26]

Insulin resistance is associated with unregulated hyperthyroidism in humans.[27,28] Higher **metabolic clearance rates of insulin (MCR-I)** have been detected in hyperthyroid patients, and S_I is reduced.[27] Insulin resistance and impaired glucose tolerance were also detected when hyperthyroid humans were compared to healthy control subjects by use of IVGTT and oral glucose tolerance test procedures.[28] Analysis of these findings suggests that the long-term effects of L-T_4 treatment in horses must be evaluated to ensure that the improvement in S_I detected after 8 weeks of treatment is sustained.

A higher IDR was detected in horses treated with L-T_4, compared with control horses, in the study reported here. Rats treated with T_4 for 5 days and then examined by use of IVGTT had lower serum insulin concentrations and significantly higher MCR-I values.[29] An increase in MCR-I has also been detected in hyperthyroid humans, and this variable has been positively correlated with serum free T_4 concentrations.[27] Increased insulin binding and a greater density of insulin receptors have been detected in adipocytes harvested from rats treated with T_4.[30]

Mild adverse effects were associated with injection of 0.1 U of regular insulin/kg during the combined IVGITT used in the study. Infrequently, horses appeared restless and began to sweat during the hypoglycemic phase of the test. However, each horse returned to normal without intervention. Although adverse effects were mild in this study, horses undergoing combined IVGITT should be monitored closely for clinical signs associated with hypoglycemia, and dextrose solution should be readily available in case treatment becomes necessary.

Levothyroxine is sometimes prescribed to promote weight loss in horses. Analysis of results of our study confirmed that L-T_4 reduced body weight in horses. Weight loss may result from increased energy expenditure and enhanced lipolysis.[31,32] Young rats treated with T_3 for 14 days failed to gain weight at the same rate as control rats, despite an increase in feed intake, and had a reduction of 50% in total body fat stores.[31] In another study,[32] rats treated with T_3 had a significant increase in energy expenditure accompanied by reductions in energy gain and gross efficiency. Treated rats had lower body lipid contents, increased serum NEFA concentrations, and increased rates of lipid oxidation.[32] Although body weight decreased in treated horses in the study reported here, body fat mass was not measured, so the specific tissues targeted by L-T_4 cannot be determined. Effects of L-T_4 on glucose dynamics may have been mediated through the reduction in body weight associated with treatment. Leaner horses have greater sensitivity to insulin and are less reliant on S_G for glucose disposal.[11] Effects of weight loss cannot be discerned from those of L-T_4 in the study reported here, but this issue should be considered when future studies are designed. Weight loss was probably exacerbated by limits placed on feed intake in this study. Horses were provided with the same ration of hay and grain throughout the study. Offering treated horses feed on an ad libitum basis may have increased feed intake to compensate for increased energy requirements.

Thyroxine was used to treat obesity in humans during the 1950s until adverse cardiovascular effects were recognized.[33] Tachycardia, arrhythmia, hypertension, left ventricular hypertrophy, increased left ventricular systolic function, increased stroke volume index, and decreased vascular resistance can result from overzealous T_4 administration or naturally developing hyperthyroidism.[34,35] Current protocols for treatment of obesity in humans rarely include T_4 supplementation unless there is concurrent hypothyroidism. However, low-dose administration of T_3 has been examined as a potential treatment for abdominal obesity in women.[36,37] In 1 study,[37] researchers treated 70 obese premenopausal women with T_3 at a low dosage (20 µg of T_3/d, PO) for 6 weeks. No adverse effects were reported, and reproductive health improved, but body weight and body mass index remained unaltered, and insulin variables measured by use of a euglycemic clamp were not affected by T_3 administration.[37]

To our knowledge, the safety of long-term L-T_4 administration in horses has not been determined. No adverse effects were detected in the horses during the 8-week duration of the study reported here, except for agitation during the final 2 weeks. However, heart rate was the only cardiovascular variable monitored.[13] In humans that require supplemental T_4, physicians titrate the dosage of T_4 to the point of suppression of thyroid-stimulating hormone while avoiding signs of thyrotoxicosis such as tachycardia and cardiac arrhythmias.[35] In a retrospective study,[35] humans treated with a mean dosage of 28 µg of T_4/kg/d for a mean duration of 9 years were compared with control subjects. Echocardiograms were performed on each patient, and the only cardiovascular abnormality detected in that study was a significantly higher left ventricular mass index. It was concluded that long-term administration of supplemental T_4 poses no threat to the health of patients provided that the dosage is appropriately managed. In the short-term study reported here, agitation was the only sign of thyrotoxicosis observed in horses.

Administration of L-T_4 significantly reduced body weight; decreased plasma concentrations of VLDL, TG, and TC; and altered glucose dynamics in healthy adult horses. Insulin sensitivity and IDR increased in horses treated with L-T_4 for 8 weeks, although stress may have influenced results. Additional studies are required to determine the long-term effects of L-T_4 on glucose metabolism, but analysis of our results suggests that this drug may have potential for use as a treatment for horses with reduced S_I. Levothyroxine sodium was tolerated well by the horses during the 8-week study, except for agitation observed during the 2 weeks that they received 96 mg of L-T_4/d. However, effects of long-term L-T_4 administration on health of horses were not evaluated.

a. DHI Forage Testing Laboratory, Ithaca, NY.
b. Thyro L, Lloyd Inc, Shenandoah, Iowa.
c. Type 50.4Ti ultracentrifuge rotor, Beckman Instruments Inc, Fullerton, Calif.
d. Triglyceride E, Wako Chemicals USA, Richmond, Va.
e. Phospholipids B, Wako Chemicals USA, Richmond, Va.
f. Cholesterol CII, Wako Chemicals USA, Richmond, Va.
g. Free cholesterol C, Wako Chemicals USA, Richmond, Va.

h. Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
i. UV-160, Shimadzu, Kyoto, Japan.
j. NEFA C, Wako Chemicals USA, Richmond, Va.
k. Butterfly, Abbott Laboratories, North Chicago, Ill.
l. Dextrose 50% injection, Abbott Laboratories, North Chicago, Ill.
m. Humulin R, Eli Lilly Co, Indianapolis, Ind.
n. Glucose, Roche Diagnostic Systems Inc, Somerville, NJ.
o. Coat-A-Count insulin, Diagnostic Products Corp, Los Angeles, Calif.
p. MinMod Millenium, version 5.10, Richard Bergman, University of Southern California, Los Angeles, Calif.
q. Stata 8, Stata Corp, College Station, Tex.
r. PROC MIXED, SAS, version 9.1, SAS Institute Inc, Cary, NC.

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