Effect of hypothyroidism on kinetics of metabolism of very-low-density lipoprotein in mares

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Objective—To compare kinetics of the metabolism of very-low-density lipoprotein (VLDL) apolipoprotein B (apoB) before and after thyroidectomy in mares.

Animals—5 healthy adult mares.

Procedure—Thyroidectomy was performed in euthyroid mares. Kinetics of VLDL apoB metabolism were measured before and after thyroidectomy by use of a bolus IV injection of 5,5,5-$^2H_3$ (98%) leucine (5 mg/kg) and subsequent isolation of labeled amino acid from plasma and VLDL. Labeled leucine was quantified by use of gas chromatography-mass spectrometry. Production rate (PR), delay time, and fractional catabolic rate (FCR) were calculated for the 2 forms of equine VLDL, apoB-48 VLDL, and apoB-100 VLDL. Plasma lipid concentrations were measured, and VLDL composition was determined.

Results—Physical appearance of horses was not altered by thyroidectomy. Significantly lower mean blood concentrations of thyroid hormones and nonesterified fatty acids were detected following thyroidectomy. Mean percentage of free cholesterol in VLDL was significantly higher after thyroidectomy. Mean plasma VLDL concentration or kinetics of apoB-48 or apoB-100 were not significantly altered by thyroidectomy. Mean ± SEM PR was significantly lower (8.70 ± 1.61 mg/kg/d) and mean delay time significantly longer (1.58 ± 0.12 hours) for apoB-48 VLDL in euthyroid mares, compared with values for thyroidectomized mares (16.15 ± 2.24 mg/kg/d and 0.93 ± 0.10 hours, respectively).

Conclusions and Clinical Relevance—Hypothyroidism did not significantly alter plasma VLDL concentrations or kinetics of VLDL apoB metabolism. Metabolism of apoB-48 VLDL differed significantly from that of apoB-100 VLDL in euthyroid mares. (*Am J Vet Res* 2003;64:1052–1058)

Detection of subnormal serum concentrations of **triiodothyronine (T_3)** and **thyroxine (T_4)** in horses with a syndrome characterized by obesity, redistribution of adipose tissue to the neck and tail-head regions, and laminitis has resulted in numerous anecdotal reports of hypothyroidism in horses. Unfortunately, diagnostic testing is commonly limited to the measurement of serum concentrations of total thyroid hormones, which are influenced by other factors including age, concurrent disease, climate, training, diet, or medications.[1,2] Stimulation of the hypothalamic-pituitary-thyroid axis by IV administration of **thyrotropin-releasing hormone (TRH)** can be used to confirm a diagnosis of hypothyroidism in horses, but it is rarely performed because of the relative expense and inconvenience of this test.[3] To our knowledge, an abnormal TRH response has not been reported in horses with the aforementioned syndrome.

If hypothyroidism contributes to the development of obesity and redistribution of adipose tissue in horses, then altering the thyroid hormone status of healthy horses would be expected to affect lipid metabolism. In a 67-week study[4] of thyroidectomized horses, serum **total cholesterol (TC)** concentrations increased after surgical removal of the thyroid gland, but hypothyroid horses did not become obese or develop abnormal deposits of adipose tissue.[4] In another study[5] conducted by our laboratory group, significantly higher plasma concentrations of **very-low-density lipoprotein (VLDL)** were detected in thyroidectomized horses. Those horses also remained normal in appearance during that 4-week study period. Significantly increased plasma concentrations of VLDL have been detected in obese humans with hypothyroidism.[6] **Production rate (PR)** for VLDL was higher and lipoproteins were cleared at a slower rate when this group of hypothyroid humans was compared with a group of euthyroid obese control individuals.[6]

The study reported here was conducted to investigate the relationship between hypothyroidism and lipid metabolism in horses. Metabolism of VLDL was selected for study based on the findings of our previous study[5] and because of the importance of this lipoprotein in the transport of **triglyceride (TG)**. Very-low-density lipoprotein represents 24% of total lipoprotein mass in horses, compared with 15% for **low-density lipoprotein (LDL)** and 61% for **high-density lipoprotein (HDL)**.[7] Although VLDL is not the predominant lipoprotein in horses, it carries almost 90% of plasma TG.[8] Hepatic synthesis of VLDL facilitates transport of hydrophobic TG through the aqueous environment of the blood by incorporation of TG into the lipoprotein core.[9] As VLDL circulates, lipases located on endothelial surfaces hydrolyze TG and liberate fatty acids for local use or storage.[10]

Metabolism of VLDL can be measured by deter-

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mining kinetics of **apolipoprotein (apo)**B.[11-13] Apolipoprotein B provides the framework on which VLDL particles are constructed and represents approximately 45% of the protein mass of lipoproteins.[7] In horses, the liver secretes 2 forms of VLDL, apoB-48 VLDL and apoB-100 VLDL.[7,11] Stable isotopes of amino acids have been used to label apoB and assess VLDL metabolism in humans.[12,13] In addition, radioactive-labeled methionine has been added to the media of equine primary hepatocytes, and labeled apoB has subsequently been recovered from VLDL secreted by cells.[11] Variables used to assess kinetics of apoB include PR, which is a measure of VLDL secretion from the liver, and **fractional catabolic rate (FCR)**, which represents the loss of VLDL from the blood.[13] Delay time is also included in the model and represents the interval between removal of labeled amino acid from the blood and release of labeled apoB (within VLDL) from the liver.[13]

The objectives of the study reported here were to use labeling techniques for stable isotopes to assess VLDL metabolism in horses and determine whether hypothyroidism significantly altered VLDL apoB kinetics in horses. We hypothesized that induction of hypothyroidism would significantly alter VLDL metabolism and, therefore, contribute to the development of obesity and redistribution of adipose tissue in horses.

Materials and Methods

Animals—Five healthy mares (4 Quarter Horses and 1 Arabian) were selected for use in the study. Mares were selected as the experimental unit to reduce variability associated with differences attributable to sex. Immature and older horses were also avoided. Horses ranged from 5 to 18 years (mean ± SD, 11.6 ± 6.6 years) and weighed 445 to 491 kg (mean, 467 ± 20 kg). Horses were housed in grass paddocks except for the 3-day period when they were hospitalized for thyroidectomy and two 48-hour periods when they were housed in stalls during infusions of stable isotopes. Mixed grass-alfalfa hay (2.1% crude ether extract on a dry-matter basis) was fed twice daily, and water was provided for ad libitum consumption. Grain or supplements were not offered. The study protocol was approved by the Purdue University Animal Care and Use Committee.

Experimental design—Infusion studies to examine kinetics of apoB were performed before and 6 weeks after thyroidectomy. The 5 horses were evaluated in 2 groups (1 group of 3 horses followed by a second group of 2 horses 3 months later). Procedures were performed within 48 hours for all horses in a group. Selected variables for blood lipids were measured ≤ 72 hours before thyroidectomy and 4, 5, and 6 weeks after thyroidectomy. Physical examinations were performed on horses prior to each infusion of stable isotopes.

Measurement of thyroid hormones—Serum T_3 and T_4 concentrations were measured before and 10 days after thyroidectomy by use of radioimmunoassays[a,b] validated for use with equine sera.[14,15] Samples were analyzed in duplicate along with standards provided by the manufacturer. Results of duplicate analyses were examined, and an intra-assay coefficient of variability of < 5% was required for acceptance of results. Interassay variability was assessed by use of duplicate serum control samples[c] that contained known concentrations of canine T_3 and T_4. Values for these control samples were compared among runs of each assay, and variability of < 10% was required for acceptance of results.

Thyroidectomy—General anesthesia was induced by IV administration of xylazine hydrochloride and ketamine hydrochloride and maintained by administration of isoflurane in oxygen. Each horse was positioned in dorsal recumbency, and the cricoid cartilage was identified by palpation. A 6-cm incision that extended caudally from the cricoid cartilage was made through the skin on the ventral midline. Subcutaneous tissues were dissected, and the sternothyrohyoideus muscles were bluntly separated. Dissection was continued along the lateral aspects of the trachea until the thyroid gland was exposed. Associated blood vessels were ligated, the thyroidal isthmus was transected, and each lobe was removed. Subcutaneous tissues were sutured with 2-0 polyglactin 910[d] in a simple continuous pattern, and the skin was closed with surgical staples.

Infusion of $^2H_3$-labeled leucine—Methods used to measure apoB kinetics in horses were adapted from those used to examine lipoprotein metabolism in humans.[12,13] Horses were housed in 5 × 15-m treatment pens. At approximately 7 AM on the day of an infusion, a 14-gauge polypropylene catheter was inserted into each of the jugular veins. At 9 AM, 5,5,5-$^2H_3$ (98%) leucine[e] (5 mg/kg) dissolved in 120 mL of sterile saline (0.9% NaCl) solution was infused IV during a period of approximately 1 minute. Blood samples (10 mL; 18 samples/horse) were collected for analysis of free plasma amino acid concentrations. Samples were collected immediately before (time 0 [baseline]) and 5, 10, 15, 20, 30, and 45 minutes and 1, 1.25, 1.5, 1.75, 2, 2.5, 3, 4, 5, 6, and 12 hours after infusion. Blood samples (50 mL; 20 samples/horse) for VLDL isolation were collected immediately before (time 0 [baseline]) and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 7, 8, 10, 12, 16, 20, and 24 hours after infusion.

Isolation of plasma VLDL—Low-speed centrifugation (1,000 × *g* for 20 minutes at 4°C) was used to obtain plasma. For each time point, two 6-mL plasma samples were placed in a fixed-angle rotor[f] for ultracentrifugation (112,000 × *g* for 18 hours at 10°C). A 1-mL fraction that had a density of < 1.006 g/mL was isolated from each tube and pooled for each time point (total aliquot, 2 mL). It has been established[7,11,12] by use of density-gradient ultracentrifugation and gel-filtration chromatography techniques that equine VLDL is found at the same flotation density (< 1.006 g/mL) as human VLDL. One-milliliter VLDL samples obtained ≤ 72 hours before thyroidectomy and 4, 5, and 6 weeks after surgery were isolated in accordance with the aforementioned procedure. Samples were stored at –70°C until compositional analysis was performed 8 weeks after thyroidectomy. Composition of fractionated VLDL was expected to remain stable at this storage temperature.[16]

Isolation of apoB-48 and apoB-100—For each time point, lipid was removed from 2-mL aliquots of VLDL by overnight incubation in a solution of ethanol:ether (3:1 [vol:vol]) at –20°C. Apolipoproteins were separated by use of SDS-PAGE performed in accordance with the method of Laemmli.[17] The apoB-48 and apoB-100 bands were excised from gels, and amino acids were liberated by acid hydrolysis through addition of 1 mL of 6 N hydrochloric acid and heating for 24 hours at 110°C. Proteins were precipitated by addition of 15% sulfosalicylic acid, and free amino acids were isolated from 200 µL of precipitated plasma. Amino acids were purified by cation-exchange chromatography by use of hydrogen-form resin.[g] *N*-heptafluorobutyryl isobutyl ester derivatives were prepared from previously purified amino acids, as described elsewhere.[18] Gas chromatography-mass spectrometry analyses were performed by use of electron ionization on a tandem mass spectrometer[h] equipped with a 15-m capillary column.[i] The instrument scanned the range from 210 to 370 atomic mass units. Peak areas of the ions of

mass/charge 282 and 285 (ie, L-leucine and ^{2}H-leucine, respectively) provided quantitative results. Ratios of tracer (^{2}H-leucine) to tracee (leucine) were plotted against time.

Data were inserted into a compartmental model established in another study[13] as a representation of apoB metabolism in humans, but it was simplified to represent VLDL metabolism in horses (**Fig 1**). Software[j] was used to model data. The FCRs for apoB were derived from modeled data. Production rates were calculated under the assumption that VLDL metabolism remained in a steady state throughout the 24-hour study period. Apolipoprotein PRs were calculated by use of the following equation:

$$PR = FCR \times \text{plasma apoB concentration}$$

Results for PR were reported as the number of milligrams per kilogram per day, assuming a mean plasma volume of 48 mL/kg for each horse.[19]

Analysis of plasma VLDL components—Concentrations of TG, **phospholipid (PL)**, TC, and **free cholesterol (FC)** in VLDL were measured in duplicate by use of enzymatic colorimetric reagents[k-n] in an automated discrete analyzer.[o] Lipoprotein lipase, phospholipase D, and cholesterol oxidase, respectively, were the principal reagents of the TG, PL, and cholesterol assays used. Protein content of VLDL was analyzed by use of bovine serum albumin standards and a spectrophotometer[p] in accordance with modifications of the method described by Lowry et al.[20,21] Intra-assay coefficients of variation were < 5% for duplicate analyses of samples. Plasma VLDL concentrations were calculated by summing concentrations of lipid (ie, TG, TC, and PL) and protein components.

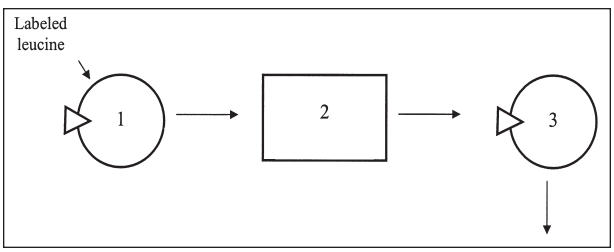

Figure 1—Multicompartmental model for apolipoprotein (apo)B-48 or apoB-100. Compartment 1 represents the plasma amino acid forcing function. Compartment 2 is an intracellular delay component representing the synthesis of apoB and assembly of lipoproteins in the liver. Compartment 3 represents very-low-density lipoprotein (VLDL) in the plasma. Triangles indicate compartments from which samples were obtained.

Concentrations of apoB-48 and apoB-100 in VLDL samples collected before and 6 weeks after thyroidectomy were determined by use of gel electrophoresis. Human VLDL samples with known concentrations of apoB-100 were serially diluted and loaded onto gels as standards. Bands were compared by use of scanning digital densitometry[q] performed in accordance with established methods.[22] Chromogenicity of human apoB-100 and equine apoB bands was assumed to be equal.[22]

Analysis of other plasma lipids—Concentrations of plasma TG and TC were measured by use of enzymatic colorimetric reagents[k,m] and the instruments[o] described previously. Plasma **nonesterified fatty acid (NEFA)** concentrations were measured by use of an in vitro enzymatic colorimetric test kit[r] that involved reactions for acyl CoA synthetase, acyl CoA oxidase, and ascorbate oxidase.

Statistical analysis—Mean serum concentrations of thyroid hormones for samples obtained before and after thyroidectomy were compared by use of a paired *t* test. Serum T_4 concentrations were reported as 0.1 µg/dL when results were below the limit of detection for the assay. Data for hypothyroid horses (weeks 4, 5, and 6 after thyroidectomy) were compared with baseline (euthyroid) data; this anlaysis was performed with a repeated-measures ANOVA by use of a computerized statistical program.[s] Mean values for apoB-48 and apoB-100 kinetics were compared within the same time period by use of a paired *t* test. Significance was defined as values of $P < 0.05$.

Results

Physical appearance of horses was not altered as a result of hypothyroidism during the 6-week study period. Physical examinations conducted during the study revealed that mean ± SEM heart rate (27 ± 1 beats/min) and rectal temperature (36.9 ± 0.1°C) were significantly lower in hypothyroid horses examined 6 weeks after thy-

Table 1—Mean ± SEM plasma lipid concentrations in 5 horses before and after thyroidectomy

Variable	Euthyroid (baseline)	Time after thyroidectomy (wk) 4	5	6
VLDL (mg/dL)	32.20 ± 6.96	53.24 ± 9.57	52.29 ± 13.21	44.97 ± 15.39
Triglyceride (mg/dL)	32.84 ± 4.97	42.46 ± 6.28	43.82 ± 8.74	36.44 ± 9.43
Total cholesterol (mg/dL)	74.94 ± 6.08	71.69 ± 7.11	79.34 ± 5.76	77.28 ± 4.74
NEFA (µmol/L)	259.21 ± 32.06	109.38 ± 0.42*	114.68 ± 3.22*	196.64 ± 8.03

*Within a variable, value differs significantly ($P < 0.05$) from baseline value (repeated-measures ANOVA).
VLDL = Very-low-density lipoprotein. NEFA = Nonesterified fatty acid.

Table 2—Mean ± SEM values for composition of the total mass of VLDL in 5 mares before and after thyroidectomy

Variable	Euthyroid (baseline)	Time after thyroidectomy (wk) 4	5	6
Triglyceride (%)	59.56 ± 2.12	62.03 ± 0.74	59.99 ± 1.67	59.14 ± 3.04
Protein (%)	22.11 ± 2.68	18.97 ± 1.14	20.14 ± 2.05	21.82 ± 3.91
Phospholipid (%)	11.13 ± 0.90	12.61 ± 0.44	12.71 ± 0.59	11.54 ± 1.37
Total cholesterol (%)	7.20 ± 0.38	6.39 ± 0.35*	7.16 ± 0.15	7.50 ± 0.34
Free cholesterol (%)	4.12 ± 0.33	4.98 ± 0.20*	5.39 ± 0.05*	5.82 ± 0.34*

See Table 1 for key.

roidectomy, compared with values determined before surgery (38 ± 1 beats/min and 37.8 ± 0.1°C, respectively).

Mean ± SEM serum concentrations of T_3 and T_4 (0.53 ± 0.06 and 2.30 ± 0.30 µg/dL, respectively) before surgery were within established reference ranges for horses,[15] but they decreased significantly (T_3, 0.25 ± 0.005 µg/dL; T_4, 0.1 µg/dL) after thyroidectomy. Serum T_4 concentrations were 0.1 µg/dL (lowest limit of detection) in all 5 mares at 10 days after thyroidectomy.

Table 3—Mean ± SEM values for kinetics of metabolism of VLDL apolipoprotein (apo)B in 5 horses before and after thyroidectomy

Status	apoB	Production rate (mg/kg/d)	Fractional catabolic rate (pools/h)	Delay time (h)
Euthyroid				
	apoB-100	16.15 ± 2.24[a]	0.70 ± 0.08	0.93 ± 0.10[a]
	apoB-48	8.70 ± 1.61[b]	0.67 ± 0.13	1.58 ± 0.12[b]
Hypothyroid				
	apoB-100	18.01 ± 6.19	0.82 ± 0.37	1.00 ± 0.17
	apoB-48	9.88 ± 4.94	0.68 ± 0.31	1.63 ± 0.34

[a,b]Within a column, values with different superscript letters differ significantly ($P < 0.05$; paired *t* test).

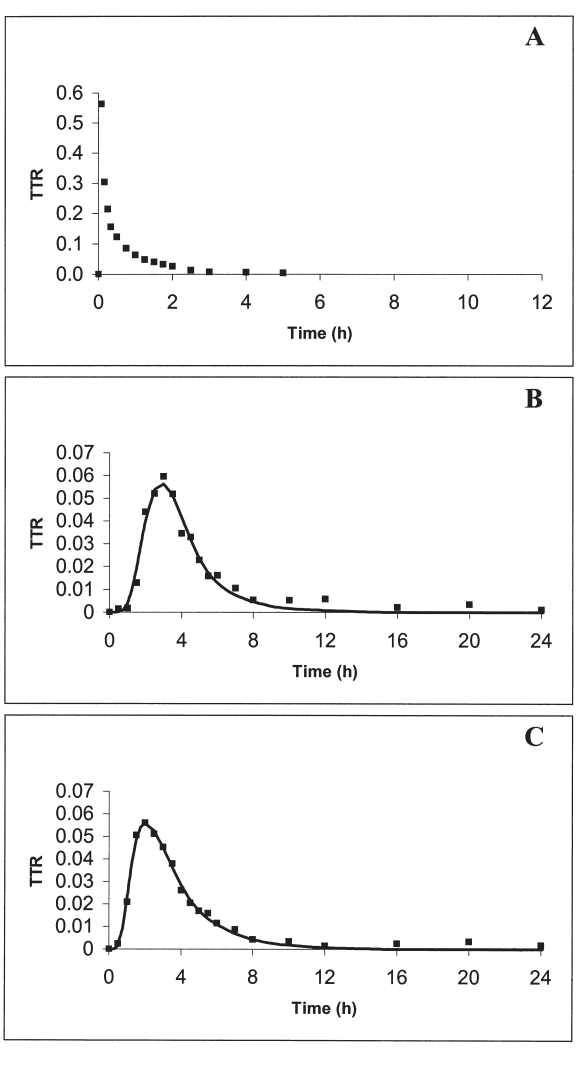

Figure 2—Tracer-to-tracee ratio (TTR) for labeled leucine in plasma (A), VLDL apoB-48 (B), and VLDL apoB-100 (C) in samples obtained from a representative euthyroid horse. Observed values (symbols) and model predicted values (lines) are indicated.

Of the blood lipids measured, only plasma NEFA concentrations were significantly affected by thyroidectomy; concentrations lower than those at baseline were detected 4 and 5 weeks after thyroidectomy (**Table 1**). Composition of VLDL was significantly altered by thyroidectomy (**Table 2**). There was a significant reduction in mean percentage total mass of TC observed 4 weeks after thyroidectomy and a significant increase in mean percentage of FC in thyroidectomized horses.

Thyroidectomy did not significantly alter mean plasma VLDL concentration, PR, FCR, and delay time for apoB-48 VLDL or apoB-100 VLDL (**Table 3**). However, metabolism of plasma VLDL apoB-48 differed significantly from that of VLDL apoB-100 in euthyroid horses. Mean PR was significantly lower, and mean delay time, which represented intracellular lipoprotein assembly and secretion, was significantly higher for VLDL apoB-48 than for VLDL apoB-100. The VLDL apoB-48 and VLDL apoB-100 kinetics also differed, but not significantly, in hypothyroid horses. Mean FCR did not differ between VLDL apoB-48 and VLDL apoB-100. Ratios of tracer (ie, ^{2}H-leucine) to tracee (ie, leucine) detected in plasma and VLDL apoB-48 and VLDL apoB-100 amino acids after IV injection of labeled leucine were calculated (**Fig 2**).

Discussion

Horses in the study reported here retained the same physical characteristics after thyroidectomy as those that were observed before surgery. This finding is consistent with results of other studies[5,23] of similar duration. By 10 days after surgery, thyroidectomized mares had serum T_3 and T_4 concentrations significantly lower than those at baseline, and serum T_4 concentrations were below the limit of detection of the assay. Serum T_4 concentrations reportedly are undetectable in thyroidectomized horses, even as long as 67 weeks after thyroidectomy.[4] Regeneration of thyroid tissue, a phenomenon documented[4,24,25] in other mammals after partial thyroidectomy, was not detected. Although this phenomenon cannot be excluded here, equine thyroid glands are large and easily distinguished from surrounding tissues, making partial removal unlikely.[26]

Mean heart rate and mean body temperature were significantly lower than baseline values at 6 weeks after thyroidectomy. These findings are consistent with the thyroidectomy-induced hypothyroid state observed in horses in other studies.[4,5,23] Subnormal body temperatures detected in the horses of the study reported here were attributed to hypothyroidism, because ambient temperatures were high in the summer months when this study was performed.

Significantly lower plasma NEFA concentrations were detected in hypothyroid mares, potentially resulting from reduced activity of hormone-sensitive lipase. Humans supplemented with thyroxine have increased activity of hormone-sensitive lipase.[27] In that same study, hypothyroidism induced only minor alterations in plasma VLDL concentration and composition.

Mean plasma VLDL concentration increased 40 to 65% in this study after thyroidectomy, but this was not a significant increase. This finding contrasts with the significant 9-fold increase in mean plasma VLDL concentration observed by our laboratory group 4 weeks after thyroidectomy in another study.[5] When lipid concentrations detected in healthy euthyroid horses are compared between studies, a marked discrepancy between reported baseline euthyroid plasma TG and VLDL concentrations is evident.[5] Mean ± SEM blood concentrations of TG and VLDL were reported as 17.0 ± 4.1 and 6.5 ± 1.1 mg/dL, respectively, for euthyroid mares in our other study,[5] compared with 32.8 ± 5.0 and 32.2 ± 7.0 mg/dL in the study[5] reported here. Differences in the diet consumed by horses may have contributed to this discrepancy. In the study reported here, horses were given access to a pasture for grazing and provided supplemental hay. In contrast, horses in our previous study[5] were housed in dirt paddocks and exclusively fed a commercial pelleted feed that contained 4% crude fat as a proportion of total digestible energy; feeding of that diet began 4 weeks prior to collection of baseline euthyroid samples. Significantly lower plasma TG concentrations have been detected in euthyroid horses consuming high-fat diets.[28-32] It should be mentioned, however, that the feed consumed by horses in our other study had a lower fat content than many of the high-fat diets examined in the other cited studies. Other factors, such as the exclusive consumption of pelleted feed without access to forages or variability among horses, may have contributed to the discrepancies observed between studies.

Alterations in VLDL composition were limited to differences in cholesterol content after thyroidectomy. The observed increase in FC content of VLDL may have resulted from reduced activity of plasma lecithin-cholesterol acyl transferase. This enzyme catalyzes the esterification of FC as it is transferred from VLDL to HDL during lipolysis mediated by lipoprotein lipase.[10] An explanation for the transient reduction in percentage of TC in VLDL is not readily apparent. This finding suggests that cholesterol ester content of VLDL decreased in horses after thyroidectomy. Availability of cholesterol for VLDL synthesis may have been reduced.[33] In other mammals, including humans, reduced activity of cholesterol ester transfer protein may explain the observed reductions in VLDL-cholesterol ester, but this enzyme is not active in horses.[34] In humans, cholesterol ester transfer protein transfers cholesterol esters from HDL to VLDL in exchange for TG.[34] Composition of VLDL isolated from euthyroid horses in the study reported here compare favorably with that in other reports.[7,8] In 1 of those studies,[7] VLDL isolated from healthy Thoroughbred horses contained 57% TG, 21% protein, 13% PL, and 9% cholesterol.

Concentrations of apoB in the plasma fraction that had a density of < 1.006 g/mL (ie, VLDL) varied among horses for both euthyroid and post-thyroidectomy sampling times. Variation may have been of analytic or biological origin. Direct measurement of equine apoB-48 and apoB-100 by immunoassay instead of scanning of gels to assess band density may have reduced analytic variability. Unfortunately, assays available at the time of this study did not distinguish between apoB-48 and apoB-100 and were not validated for use in horses. If biological in origin, variation in apoB concentrations may reflect the effects of other undetermined factors. Based on the wider variation observed in hypothyroid lipid variables, it can also be postulated that the lack of thyroid hormones permits other factors to influence lipid metabolism to a greater extent. Additional studies are required to examine some of these potential confounding factors, such as body composition or glucose-insulin status.

Differences in stages of the reproductive cycle were also not accounted for in the study reported here. Effects of reproductive hormones on the lipoprotein pattern in horses have not been established, but higher plasma VLDL and HDL concentrations have been detected in pregnant or lactating ponies.[35] Increased plasma VLDL and total apoB concentrations have been detected in women receiving estrogen therapy.[36,37]

Contamination of VLDL samples with chylomicrons was also considered a potential source of variation. Chylomicrons are TG-rich lipoproteins with a density < 1.006 g/mL that are synthesized by intestinal tissues in response to ingestion of lipids.[9] The existence of this type of lipoprotein has not been confirmed in equine plasma, and in several studies,[7,10,38] higher concentrations of lipoproteins with a density < 1.006 g/mL were not detected when mares were fed hay, compared with concentrations in mares deprived of feed.[7,10,38]

Kinetics of apoB metabolism were successfully measured in horses. Variables of VLDL-TG metabolism have been measured in healthy and hyperlipidemic ponies.[t] Mean PR and FCR of VLDL-TG in healthy ponies were 6.6 ± 4.4 mmol/h (58.5 ± 39.0 mg/dL/h) and 0.48 ± 0.25 pools/h, respectively.[t] Kinetic variables of apoB and VLDL-TG cannot be directly compared, however, because removal of lipid from VLDL takes place in the circulation prior to clearance of lipoproteins from the blood.[39] Furthermore, monoexponential slope calculations for radioactivity decline (eg, those used in the aforementioned study[t] of ponies) and underestimate FCR content by > 50%, compared to model-derived rates.[40]

Thyroid hormone status did not significantly alter mean PR, FCR, or delay time for VLDL apoB in mares, a finding consistent with studies[6,41] on hypothyroidism in nonobese humans. Wide variation among horses and the low number of horses included in our study hampered statistical comparisons. Initial power calculations performed to estimate sample size were based on the 9-fold increase in plasma VLDL concentrations observed after thyroidectomy of horses in our other study.[5] Significant differences may become apparent if more horses are examined and potential sources of variation are identified and controlled.

Kinetics of apoB metabolism have been measured in hypothyroid nonobese humans supplemented with T_4.[41] In that study, thyroid hormone status did not affect apoB PR and FCR. Caution should be applied when comparing results from studies conducted in humans with those from studies conducted in horses, because

equine lipoproteins, although similar in flotation density and structure, may not be metabolized in the same manner as lipoproteins in humans. In horses, examination of primary hepatocytes has confirmed that VLDL is synthesized by the liver to facilitate TG export.[11] However, the synthesis of 2 forms of apoB by the liver in horses in the absence of activity of cholesterol ester transport protein and differences between species with respect to LDL and HDL metabolism are likely to influence VLDL metabolism in horses. Metabolism of LDL and HDL differs markedly between horses and humans, with HDL being the largest constituent of total lipoprotein mass in horses.[7] Cholesterol is primarily transported within HDL in horses (60% of plasma TC), whereas it is primarily transported within LDL (70 to 75% of plasma TC) in humans.[42]

Examination of VLDL apoB kinetics provides in vivo measures of hepatic-origin apoB-48 metabolism. In horses, apoB-48 is primarily synthesized within the liver and incorporated into VLDL with only minimal contributions from intestinal sources.[11] In contrast, humans exclusively synthesize apoB-48 within intestinal tissues and incorporate this apolipoprotein into chylomicrons.[11] Studies[43,44] involving the infusion of stable isotopes have been used to evaluate the metabolism of intestinal-origin apoB-48 in humans. Mean ± SEM PR of apoB-48 lipoproteins was 6.5% of the PR recorded for VLDL apoB-100 (1.3 ± 0.9 and 20.1 ± 6.5 mg/kg/d, respectively).[44] In contrast, a PR of 31% was detected for a truncated apoB form (apoB-43.7) produced by the liver in humans with the hereditary disorder, hypobetalipoproteinemia.[45] In the euthyroid mares reported here, mean PR for VLDL apoB-48 was 55% of the PR for VLDL apoB-100 (8.7 ± 1.6 and 16.2 ± 2.2 mg/kg/d, respectively). Production rates for both forms of equine apoB were close in magnitude to those of human hepatic-origin apoB-100.[44]

Mean delay time was longer and PR slower for VLDL apoB-48 in euthyroid horses, compared with values for VLDL apoB-100. Because both types of apoB originate from the same organ (ie, liver) in horses, possible explanations for this finding include a higher rate of intracelullar degradation of apoB-48, slower rate of assembly of VLDL apoB-48, or delayed secretion of lipoprotein into the circulation. In the study reported here, FCRs for both forms of equine apoB were almost equal in euthyroid horses. However, a slower mean FCR has been detected for apoB-48 in humans.[44] Measured FCR for apoB-48 and apoB-100 in euthyroid horses ranged between 16.0 and 17.0 pools/d. In contrast, mean FCR for VLDL apoB-48 in humans is 5.0 pools/d, compared with 6.7 pools/d for VLDL apoB-100.[44] Therefore, VLDL appears to be catabolized at a faster rate in horses than in humans.

Analysis of results of the study reported here suggests that healthy euthyroid horses produce VLDL apoB-48 and VLDL apoB-100 at differing rates, but VLDL clearance rates are the same for both types of lipoprotein. These findings may indicate different roles for apoB-48, perhaps in providing a reserve capacity for TG export. Ponies with the metabolic syndrome hyperlipemia develop a significantly higher ratio of VLDL apoB-48 to VLDL apoB-100 at a time when TG export from the liver is being maximized.[46] The VLDL apoB-48 isolated from hyperlipemic ponies is also enriched with TG, a feature that may further improve the efficiency of hepatic export of TG.[46] Production rates for apoB-48 and apoB-100 also differed, but not significantly, in hypothyroid horses in our study.

It was assumed prior to modeling of data that horses would remain in a steady state during the infusion procedure. Influences of factors, such as stress, reduced feed intake, or an undetected effect of the infused stable isotope, may have invalidated this assumption. Stress was minimized in this study by selecting calm horses, frequently handling the horses, and placing them in stalls the night before the infusion procedure. Collection of blood samples from a catheter inserted in a jugular vein minimized stress associated with sample collection. Hay was provided ad libitum during the infusion procedure, and horses were allowed to move around the stalls after the first 2 hours. Plasma VLDL concentrations varied minimally in horses fed hay ad libitum during a 36-hour period.[38] Infusion of deuterated leucine has not been associated with adverse effects in humans,[12,13,39,43,44] and none of the horses had adverse reactions to the isotope.

In conclusion, kinetics of VLDL apoB-48 and VLDL apoB-100 were successfully measured in horses. Metabolism of VLDL apoB-48 differs significantly from that of VLDL apoB-100. Hypothyroidism did not significantly alter kinetic variables of VLDL apoB metabolism in this study, but additional studies involving larger numbers of horses are required to reduce the effects of variability among horses. Results of such studies must be examined before it can be conclusively determined whether an association exists between hypothyroidism and altered lipid metabolism in horses.

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[a]Coat-A-Count Total T3, Diagnostic Products Corp, Los Angeles, Calif.
[b]Coat-A-Count Canine T4, Diagnostic Products Corp, Los Angeles, Calif.
[c]K9CON, Diagnostic Products Corp, Los Angeles, Calif.
[d]Vicryl, Ethicon Inc, Somerville, NJ.
[e]5,5,5-D3 L-leucine, Cambridge Isotope Laboratories Inc, Andover, Mass.
[f]Type 50.4Ti ultracentrifuge rotor, Beckman Instruments Inc, Fullerton, Calfi.
[g]50W-X8 hydrogen-form resin, Bio-Rad Laboratories, Hercules, Calif.
[h]Finnigan GCQ tandem mass spectrometer, Thermo Finnigan, San Jose, Calif.
[i]DB-1 capillary column, J&W Scientific, Folsom, Calif.
[j]CONSAAM, SAAM Institute Inc, Seattle, Wash.
[k]Triglyceride E, Wako Chemicals USA, Richmond, Va.
[l]Phospholipids B, Wako Chemicals USA, Richmond, Va.
[m]Cholesterol CII, Wako Chemicals USA, Richmond, Va.
[n]Free cholesterol C, Wako Chemicals USA, Richmond, Va.
[o]Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
[p]DU640 spectrophotometer, Beckman Instruments Inc, Fullerton, Calif.
[q]Kodak digital science 1D image analysis, Eastman Kodak Co, Rochester, NY.
[r]NEFA C, Wako Chemicals USA, Richmond, Va.
[s]SAS, version 8e, SAS Institute Inc, Cary, NC.
[t]Watson TDG. *Lipoprotein metabolism in the horse*. PhD Thesis, Department of Veterinary Medicine, University of Glasgow Veterinary School, Glasgow, UK, 1991;146–167.

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