Effect of withholding feed on concentration and composition of plasma very low density lipoprotein and serum nonesterified fatty acids in horses

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Objective—To measure and compare the concentration and composition of very low-density lipoprotein (VLDL) in plasma and selected lipids in serum of horses fed mixed grass hay ad libitum or denied feed for 36 hours.

Animals—4 healthy adult mares.

Procedure—Mares were either fed mixed grass hay ad libitum or denied feed for 36 hours beginning at 8:00 AM. Blood samples were collected every 2 hours during the study period and analyzed for nonesterified fatty acid (NEFA), triglyceride (TG), VLDL, and glucose concentrations and composition of VLDL.

Results—Withholding feed significantly increased mean serum concentrations of NEFA. By 36 hours, a 16-fold increase in mean serum NEFA concentration and 2-fold increase in mean plasma VLDL concentration, compared with baseline values, were detected. Mean plasma TG concentrations significantly increased with time in feed-deprived horses. Significantly lower overall mean plasma glucose concentrations were detected in feed-deprived horses. Mean percentage of protein in VLDL was significantly lower in feed-deprived horses. Plasma VLDL concentrations varied widely among horses in response to withholding feed. Plasma TG and VLDL concentrations remained unaltered in 2 horses.

Conclusions and Clinical Relevance—Withholding feed significantly increases blood lipid concentrations in horses, but individual horses respond differently. Serum NEFA concentrations were increased in all 4 horses denied feed, indicating mobilization of tissue triglyceride stores. Variation in plasma VLDL concentration in response to withholding feed suggests that its metabolism is strongly influenced by other, as yet undetermined, factors in horses. Differences in the plasma VLDL concentrations among horses in response to withholding feed may be used as an indication of susceptibility to the hyperlipemic syndrome of Equidae. (*Am J Vet Res* 2002;63:1018–1021)

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Reduction in feed intake frequently accompanies systemic disease, and horses are often denied feed during recovery from gastrointestinal disease or surgery. Physiologic consequences of withholding feed warrant particular attention in Equidae, because species in this family appear predisposed to the metabolic disorder referred to as hyperlipemia.[1] Hyperlipemic horses, ponies, or donkeys develop unregulated excessive accumulation of plasma **very low density lipoprotein (VLDL)** and have signs of depression, anorexia, and weakness.[1] Blood **triglyceride (TG)** concentrations in excess of 500 mg/dl are detected.[1] Reduced feed intake and stress are recognized risk factors for this disorder, but determinants of susceptibility among horses have not been fully elucidated.[2,3]

Hypertriglyceridemia (defined as blood TG concentrations in excess of 55 mg/dl[4]) during periods of withholding feed may serve as an indication of susceptibility to hyperlipemia. Naylor et al[5] reported that horses denied feed for 5 days developed hypertriglyceridemia, and variability in the response was observed among horses.[5] The objective of the study presented here was to evaluate plasma VLDL concentrations in response to withholding feed in healthy adult horses. More specifically, we sought to determine whether the effects of withholding feed on plasma VLDL and blood lipid concentrations differed among horses.

Materials and Methods

Animals—Four healthy mares were selected (2 Quarter Horses, 1 Thoroughbred, and 1 Arabian). Mean age was 13.5 ± 3.9 years (range, 8 to 17 years), mean body weight was 469 ± 18 kg (range, 445 to 484 kg), and body condition scores[6] ranged from 6 to 7 (on a scale of 1-9). Beginning 72 hours before sample collection, horses were enclosed in treatment pens measuring approximately 5 m × 15 m. Mixed grass hay (2.1% crude fat on a dry matter basis) and water were provided ad libitum during this acclimatization period.

Experimental study—The study protocol was approved by Purdue University Animal Care and Use Committee. A crossover design was used encompassing two 36-hour periods. Horses were randomly assigned to 1 of 2 treatment pens and either fed mixed grass hay ad libitum or denied feed for 36 hours beginning at 8:00 AM. Water was provided ad libitum in both pens. After 7 days at pasture, treatments were reversed. Blood samples were collected every 2 hours for 36 hours via an IV catheter inserted in the left jugular vein. Samples were collected in EDTA-coated tubes and stored immediately in ice. Blood samples collected to harvest sera were collected in uncoated glass tubes and allowed to clot at room temperature (approx 22 C) for 1 hour before centrifugation.

Isolation of plasma very low density lipoprotein—Low-speed centrifugation (1,000 × g) was used to separate

plasma and serum. Six-milliliter plasma samples were placed in a fixed-angle rotor[a] for ultracentrifugation at 112,000 × g for 18 hours at 10 C. A 1-ml fraction of density < 1.006 g/ml was isolated and analyzed. Chylomicrons might also be isolated at this density, but concentrations of this lipoprotein would be minimal when horses are being fed a hay diet.[7]

Analysis of plasma very low-density lipoprotein—The TG, **phospholipid (PL)**, **total cholesterol (TC)**, and **free cholesterol (FC)** components of VLDL were measured by use of enzymatic colorimetric reagents[b] in an automated discrete analyzer.[c] 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[a] in accordance with a modified method of the Lowry procedure.[8,9] Plasma VLDL concentrations were calculated by summing concentrations of lipid (TG, TC, PL) and protein components.

Analysis of plasma triglyceride and glucose and serum nonesterified fatty acid—Concentrations of plasma TG and glucose were measured by use of enzymatic colorimetric reagents[b,d] and the instrumentation already described. Glucose measurements were obtained through the enzymatic reactions of hexokinase coupled with glucose-6-phosphate dehydrogenase. Serum **nonesterified fatty acid (NEFA)** concentrations were measured by use of an in vitro enzymatic colorimetric test kit employing acyl CoA synthetase, acyl CoA oxidase, and ascorbate oxidase reactions.[b]

Statistical analysis—Data were analyzed by repeated-measures ANOVA by use of a computer software program.[e] Overall mean concentrations for fed and feed-deprived horses were also compared by use of Student *t*-tests. Data are reported as mean (± SEM) values. Significance was defined as values of $P < 0.05$.

Results

A significant treatment effect was detected for serum NEFA concentrations. Mean (n = 4) serum NEFA concentrations were higher during periods of withholding feed with a significant treatment × time effect observed (**Fig 1**). By 36 hours, a 16-fold increase in mean (± SEM) serum concentration of NEFA (513.32 ± 50.78 µmol/L) was detected in feed-deprived horses, compared with baseline (time = 0) values (31.96 ± 2.82 µmol/L). Overall mean (± SEM) serum concentration of NEFA was significantly higher in feed-deprived horses (299.89 ± 19.92 µmol/L), compared with fed control horses (46.35 ± 2.10 µmol/L).

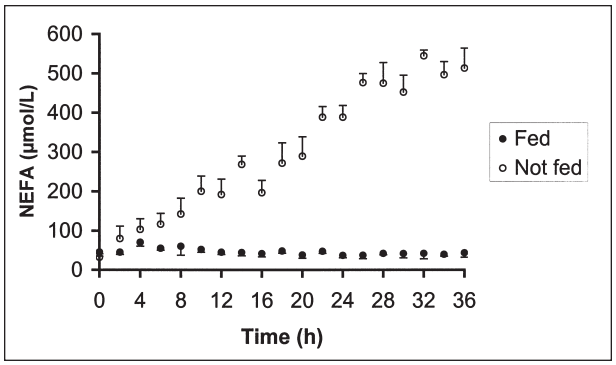

Figure 1—Mean ± SEM (n = 4) serum concentrations of nonesterified fatty acids (NEFA) in horses fed mixed grass hay ad libitum or denied feed for 36 hours. Treatment ($P < 0.001$) and treatment × time ($P < 0.001$) effects were significant.

Hypertriglyceridemia (blood TG concentrations > 55 mg/dl[4]) was detected after 36 hours of withholding feed (**Fig 2**). Overall mean plasma TG concentrations did not differ significantly between feed-deprived (67.00 ± 3.47 mg/dl) and fed (57.11 ± 1.41 mg/dl) horses, but a significant treatment × time effect was detected. Overall mean plasma glucose concentration was significantly higher in fed (109.37 ± 3.54 mg/dl) versus feed-deprived (86.94 ± 1.08 mg/dl) horses.

Overall mean plasma VLDL concentrations did not differ significantly between feed-deprived (45.77 ± 3.40 mg/dl) and fed (39.34 ± 1.40 mg/dl) horses, but concentrations increased with time in feed-deprived horses. At 36 hours, a 2-fold increase in mean (± SEM) plasma concentration of VLDL (83.55 ± 31.65 mg/dl) was detected in feed-deprived horses, compared with baseline (time = 0) values (40.08 ± 5.06 mg/dl). Mean per-

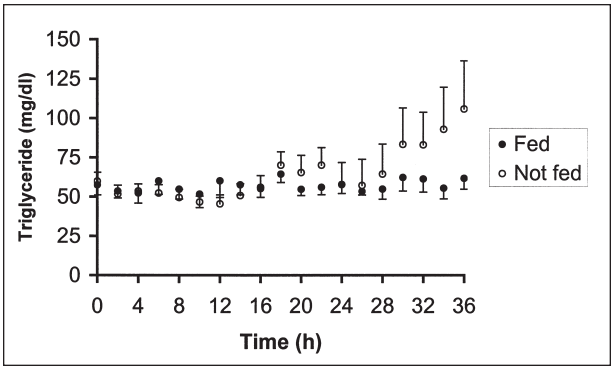

Figure 2—Mean ± SEM (n = 4) plasma concentrations of triglyceride in horses fed mixed grass hay ad libitum or denied feed for 36 hours. Treatment × time ($P = 0.007$) effect was significant.

Table 1—Overall mean ± SEM (n = 76) percentage of total mass composition of very low-density lipoprotein in 4 horses fed mixed grass hay ad libitum or denied feed for 36 hours

	Horses	
Variables	**Fed**	**Feed-deprived**
Triglyceride (%)	61.77 ± 0.35	62.29 ± 0.32
Total cholesterol (%)	6.80 ± 0.17	7.25 ± 0.24
Free cholesterol (%)	6.39 ± 0.06	6.46 ± 0.08
Phospholipid (%)	15.13 ± 0.13	15.54 ± 0.12
Protein (%)	16.30 ± 0.39	14.91 ± 0.41*

*Significant ($P < 0.05$) difference between treatments.

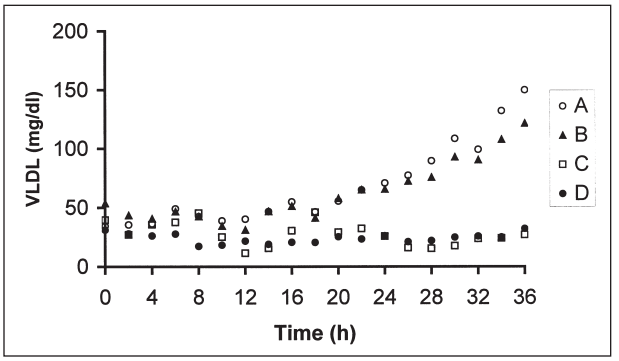

Figure 3—Plasma concentrations of very low density lipoprotein (VLDL) in 4 horses (A–D) denied feed for 36 hours.

centage protein was significantly lower in VLDL collected from feed-deprived horses (**Table 1**). Diurnal variation was not evident in data from fed or feed-deprived horses. No effect of sampling week was detected. Horses differed widely in their response to withholding feed. Plasma TG and VLDL (**Fig 3**) concentrations increased in 2 feed-deprived horses but remained unaffected by withholding feed in 2 other horses.

Discussion

Withholding feed induced a significant increase in serum NEFA concentrations reflecting an increase in release of fatty acids from tissue triglyceride stores. An increase in serum NEFA concentrations have been previously reported in feed-deprived horses[5,10] and ponies.[11] Serum NEFA concentrations for fed horses compared favorably with before-riding mean concentrations of 47 µmol/L reported in endurance horses.[10]

Hypertriglyceridemia has been previously reported in feed-deprived horses,[5] and numerous reports describe this phenomenon in feed-deprived ponies[11-13] and donkeys.[14] This is, however, the first report of an increase in plasma VLDL concentrations in feed-deprived horses. Baseline feed-deprived and fed horse plasma VLDL concentrations appear consistent with previously published values, although differences between methods hinder comparisons.[7,15,16] Plasma VLDL concentrations have been shown to increase in ponies denied feed for extended periods.[11-13] A 12-fold increase in plasma VLDL concentration was detected in a group of ponies denied feed for 8 days.[13] An increase in plasma VLDL concentrations reflect enhanced export of TG from the liver during periods of withholding feed.[1,7] Reduction in VLDL protein content is consistent with the previous finding that VLDL from hyperlipemic ponies is triglyceride-rich and contains lower percentages of protein and PL.[17]

Variation among horses in plasma VLDL concentrations in response to withholding feed was observed in our study and has been previously observed by others.[5,12,13] Naylor et al[5] reported that 1 of 6 horses denied feed for 5 days maintained plasma TG concentrations within reference range, while all other horses developed hypertriglyceridemia.[5] When ponies were denied feed for 8 days, 3 ponies developed 21- to 38-fold higher plasma TG concentrations, but 1 pony remained unaffected.[18] Wide variation between ponies was also reported in another group of 4 ponies denied feed for 8 days.[13] Plasma TG concentrations ranged from 5- to 29-fold higher than baseline, representing an almost 6-fold difference between highest and lowest values.[13] The lipid response to withholding feed may also differ significantly within the same animal. Morris et al[18] reported that 2 ponies that were hypertriglyceridemic after an 8-day fast had only minimal responses when subsequently denied feed for 88 hours.[18] Plasma VLDL concentrations vary among ponies during periods of withholding feed.[12] After a 5-day fast, VLDL concentrations ranged from 8- to 100-fold above baseline in feed-deprived ponies.[12]

Sex, breed, age, body composition, and endocrine status potentially influence plasma VLDL concentrations in response to withholding feed in horses. Susceptibility to hyperlipemia among donkeys is affected by sex, with females being at greater risk.[3] Pregnancy and lactation are contributing factors.[19] Effects of sex or breed have not been examined extensively in horses. Higher VLDL and TG concentrations were reported when Thoroughbreds were compared with Morgan horses, but differences were not significant.[20] Plasma lipoprotein concentrations do, however, differ markedly between horses and other species of the Equidae family, with significantly higher VLDL concentrations detected in ponies and donkeys.[21,22] Interestingly, horses that have an increase in plasma VLDL concentration in response to withholding feed in our study were of the Arabian and TB breeds. The two Quarter Horses included in our study remained unaffected.

Age has been identified as a risk factor for hyperlipemia in donkeys, but its effect on plasma VLDL concentrations has not been examined in horses.[3] Survival analysis of a donkey population revealed that older donkeys were at greater risk for development of hyperlipemia.[3] Body composition influences the blood lipid profile of donkeys and warrants further investigation in horses. When 3 groups of donkeys (thin, ideal, and obese body condition) were compared, significant differences in plasma VLDL (0.22, 0.40, and 0.71 mmol cholesterol/L, respectively) concentrations were detected.[22] Overweight donkeys were also found to be at higher risk for development of hyperlipemia.[3] A significant positive correlation between body condition score and resting blood insulin concentrations has been detected in donkeys, suggesting that tissue insulin sensitivity may be a determinant of susceptibility to hyperlipemia.[23]

Differences in the plasma VLDL concentrations among horses in response to withholding feed were observed in our study and may indicate predisposition to the metabolic disorder hyperlipemia. Further studies are required to determine the factors responsible for this variation.

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[a]Beckman Instruments Inc, Fullerton, Calif.
[b]Wako Chemicals USA, Richmond, Va.
[c]Cobas Mira, Roche Diagnostic Systems Inc, Somerville, NJ.
[d]Roche Reagents, Roche Diagnostic Systems Inc, Somerville, NJ.
[e]General linear model, SAS Institute, Cary, NC.

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