Metabolic changes and induction of hepatic lipidosis during feed restriction in llamas

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Objectives—To determine whether feed restriction induces hepatic lipidosis (HL) in llamas and to evaluate the metabolic changes that develop during feed restriction.

Animals—8 healthy adult female llamas.

Procedure—Llamas were fed grass hay at a rate of 0.25% of their body weight per day for 13 to 28 days. Llamas were monitored by use of clinical observation, serum biochemical analyses, and ultrasound-guided liver biopsies.

Results—All 8 llamas lost weight and mobilized fat. Five llamas developed HL, including 4 that were nursing crias. During the period of feed restriction, mean serum concentration of bile acids and activities of aspartate aminotransferase (AST), sorbitol dehydrogenase (SDH), and γ-glutamyl transferase (GGT) were significantly higher in llamas that developed HL, compared with llamas that did not. Mean insulin-to-cortisol concentration ratios were lower in llamas with HL before and up to 7 days of feed restriction, compared with those that did not develop HL.

Conclusions and Clinical Relevance—HL in llamas may be induced by severe feed restriction, particularly in the face of increased energy demand. Llamas with weight loss attributable to inadequate dietary intake may develop biochemical evidence of hepatopathy and HL. Increases in serum concentration of bile acids and activities of GGT, AST, and SDH may indicate the development of HL in llamas and identify affected animals for aggressive therapeutic intervention. (*Am J Vet Res* 2001;62:1081–1087)

Hepatic lipidosis (HL) is a morphologic change of the liver associated with severe clinical disease and high mortality in camelids.[1] It appears to result from metabolic disturbances attributable to energy deficits and other as yet undefined factors. Clinical syndromes associated with HL are well described in several other species, including cats, cows, ponies, and humans. There is evidence that pathophysiologic mechanisms leading to the accumulation of fat in hepatocytes differ among species.[2-7] However, all mechanisms appear to be related to negative energy balance, especially in animals that are lactating or in the latter stages of pregnancy. In cattle and cats, obesity is another risk factor. Clinical signs associated with HL vary among species and range from no abnormal signs or slightly reduced productivity to high mortality.[1,4,8]

The etiopathogenesis of HL in camelids is not clear. Hepatic lipidosis has been reported in camelids with an acute or prolonged illness or in camelids that have died with few or no other gross or histologic lesions. Infectious agents, toxins, and trace mineral deficiencies have not been identified as the cause of HL in many of these cases.[1] A common factor in many but not all cases of HL in camelids is a history of recent weight loss or anorexia. This is similar to HL in cats, which most often develops during periods of prolonged anorexia and severe weight loss.[2,8] The association of HL with obesity, lactation, and pregnancy is not as strong in camelids as in other species; only about half of the camelids with HL were pregnant or lactating, and most were not obese.[1]

On the basis of information about naturally occurring HL in camelids, we hypothesized that the cause of HL was multifactorial, with negative energy balance imposed by feed restriction being 1 of the most important contributing factors. The purpose of the study reported here was to determine whether HL could be induced in llamas by feed restriction alone and whether lactation increased the likelihood of developing HL. An additional purpose was to evaluate metabolic changes that developed with feed restriction to determine whether alterations in serum biochemical variables associated with metabolic changes could be used to diagnose HL.

Materials and Methods

Llamas—Eight healthy adult female llamas that were part of the Oregon State University College of Veterinary Medicine teaching herd were used in this study. These llamas were removed from pasture and housed in stalls for 2 days of acclimation prior to the study. None of the llamas were pregnant at the time of the study, but 6 were lactating; crias ranged in age from 3.5 to 17 weeks at the onset of feed restriction. Two of the llamas were considered overweight, with a body score of 4 or 5 on a scale of 1 to 5; a body score of 3 represents optimal condition. One of the obese llamas was lactating, and 1 was not. Three llamas were thin, with body scores of 1.5 to 2; 2 of these llamas were lactating. The 3 llamas with optimal body scores (3 to 3.5) were lactating.

Llamas in this teaching herd were treated with anthelminthics 4 times a year, using an alternating schedule of fenbendazole, pyrantel, ivermectin, and clorsulon. Fecal

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flotations were examined regularly for parasite ova, but ova were not detected.

Study design—All procedures were approved by the Institutional Animal Use and Care Committee at Oregon State University and were in compliance with the Animal Welfare Act. During the 2-day acclimation period in stalls, llamas were provided grass hay and fresh water ad libitum. In addition, on days –2 and 0, llamas were weighed, and blood was obtained via jugular venipuncture for baseline CBC, serum biochemical analyses, and insulin and cortisol assays. Two llamas were assessed at any 1 time, and llamas were kept in adjacent stalls with porthole windows during the acclimation and feed restriction periods so they could see each other to minimize social stress. On day 0 of the trial immediately prior to initiation of feed restriction, llamas were restrained in a chute and sedated with xylazine hydrochloride (1.5 mg/kg of body weight, IM) and butorphanol (0.05 mg/kg, IM). The liver was located by use of ultrasonography, 2% lidocaine was used for local anesthesia of the skin and subcutaneous tissue, and liver biopsy specimens were obtained, using an 18-gauge biopsy needle.[a] Duplicate specimens were placed in neutral-buffered 10% formalin and frozen at –20 C.

A pilot study was performed prior to this study, using an overweight lactating llama fed hay at 1.0% of its body weight per day and an overweight nonlactating llama fed hay at 0.5% of its body weight per day. Although these llamas initially lost weight, body weight stabilized after 5 days of feed restriction. Thus, we concluded that feed restriction at 0.5 to 1.0% of body weight was not sufficient to induce HL. Therefore, during the feed restriction period in this study, llamas were fed hay at 0.25% of their body weight per day on a dry-matter basis irrespective of lactation status.

During feed restriction trials, llamas were kept in stalls without bedding and fed a weighed amount of mature posthead grass hay (crude protein, 8.3%; acid detergent fiber, 42.7%; and neutral detergent fiber, 69.6%; all values on a dry matter basis) each day. Water was provided ad libitum. Crias were kept with their dams and allowed to nurse freely except for 1 hour during each day of the feed restriction trial. During this hour, dams were allowed to eat their daily ration of hay while crias were moved into separate stalls and fed grain and grass hay. Nonlactating llamas were allowed to eat their daily ration of hay throughout the day.

Body weights of all llamas were recorded every morning before feeding. Blood was collected into tubes with and without EDTA every other day for 1 week from all adult llamas, then every 1 to 2 days until the end of the trial. Adult llamas and crias were observed several times a day for signs of weakness and depression or prolonged periods of recumbency. A second ultrasound-guided liver biopsy specimen was obtained from each adult llama when results of serum biochemical analyses indicated evidence of hepatopathy or, in llamas without evidence of hepatopathy, after 21 to 28 days of feed restriction.

Feed restriction trials were terminated when llamas developed signs of depression or became recumbent for prolonged periods, when results of serum biochemical analyses were consistent with hepatopathy and results of liver biopsy revealed evidence of hepatic lipidosis, or after 21 to 28 days in llamas without evidence of hepatopathy. After feed restriction was terminated, each llama was kept in a bedded stall, provided grass hay and water ad libitum, and observed and weighed daily for at least 4 days. Four of the llamas that developed HL were maintained in this manner for 5 to 12 days, at which time an additional ultrasound-guided liver biopsy specimen was obtained.

Data collection—Body weights and clinical observations were recorded daily. Complete blood counts were performed immediately on anticoagulated blood, using an automated hematology analyzer.[b] Blood without anticoagulant was allowed to clot, and serum was separated and analyzed within 3 hours of collection. An automated chemistry analyzer[c] was used to measure serum concentrations of BUN, creatinine, glucose, total protein, albumin, total bilirubin, total cholesterol, triglycerides, bile acids, calcium, phosphorus, potassium, sodium, chloride, **β-hydroxybutyrate (β-HB)**, and **nonesterified fatty acids (NEFA)** and activities of **aspartate transaminase (AST)**, **creatine kinase (CK)**, **γ-glutamyl transferase (GGT)**, and **sorbitol dehydrogenase (SDH)**. Serum insulin and cortisol concentrations were measured by use of commercially available radioimmunoassays[d,e] validated for use with camelid serum.[9,10] Liver biopsy specimens were processed routinely for histologic evaluation, evaluated by a single observer (SJT), and graded according to the number of lipid vacuoles in hepatocytes. Selected frozen sections were stained with oil red O to confirm the presence of lipid in hepatocytes.

Statistical analyses—Data were analyzed by use of a 2-way ANOVA for repeated measures[11,f]; main effects were HL status (positive or negative) and day. Multiple comparisons between means were performed by use of a Tukey test. For all tests, $P < 0.05$ was considered significant.

Results

Llamas were monitored for periods ranging from 23 to 33 days; 5 llamas developed HL, and 3 did not (**Table 1**). Feed restriction lasted for 21 to 28 days in llamas that did not develop HL and 13 to 21 days in llamas that did. All llamas lost weight after feed restriction trials began. Weight loss ranged from 9.5 to 27.7% of initial body weight. Mean weight loss for all llamas was 18.6% of initial body weight, and percentage of weight loss was not significantly different between those llamas that developed HL and those that did not. The llamas that developed HL generally had a greater decrease in weight during the first week of feed restriction, but percentage of body weight lost during this period was not significantly different between the 2 groups.

Four of the 5 llamas that developed HL were lactating, and 2 of the 3 unaffected llamas were lactating. The 4 affected lactating llamas had younger crias (age range, 3.5 to 7 weeks) than did the 2 unaffected lactating llamas (9.5 to 17 weeks). All crias gained weight slowly but steadily throughout the study.

Degree of lipid accumulation in hepatocytes of 2 of

Table 1—Initial characteristics of female llamas that did or did not develop hepatic lipidosis as a result of feed restriction

Llama	Body condition score*	Lactation status	Age of cria (wk)
Hepatic lipidosis			
1	3.5	+	6.0
2	3.5	+	7.0
3	3.0	+	3.5
4	4.0	+	7.0
5	2.0	–	NA
Unaffected			
1	5.0	–	NA
2	1.5	+	17.0
3	1.5	+	9.5

*On a scale of 1 to 5; 3 is optimal.
NA = Not applicable.

the 5 llamas with HL was mild (< 30% hepatocytes affected). Two other affected llamas had a moderate degree of lipid accumulation (31 to 60% hepatocytes affected); distribution of lipid accumulation was primarily centrilobular. The fifth affected llama had severe hepatic lipidosis with 70% of hepatocytes affected. Lipid accumulation was again primarily centrilobular in distribution. This fifth affected llama was lactating, had a body condition score of 3 at the start of the study, and did not develop clinical signs of HL. Liver biopsy specimens obtained from 4 of the 5 affected llamas (1 with mild HL, 2 with moderate HL, and 1 with severe HL) 5 to 12 days after the termination of the feed restriction trial revealed no evidence of hepatic lipid accumulation in 2 of the llamas and mild HL in the other 2. This latter group included the llama that had developed severe HL during the feed restriction trial.

There was biochemical evidence of fat mobilization

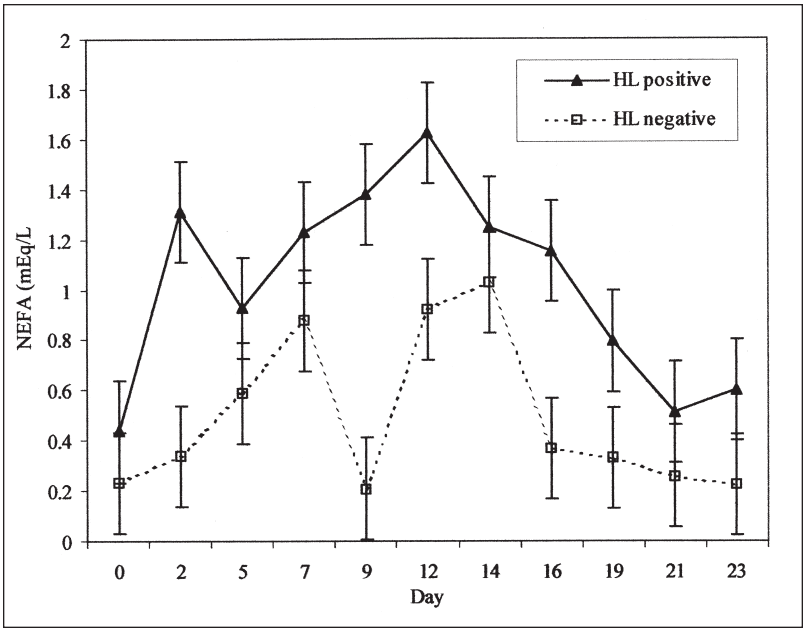

Figure 1—Mean ± SEM serum concentrations of nonesterified fatty acids (NEFA) in female llamas that did (n = 5) or did not (3) develop hepatic lipidosis (HL) during a period of feed restriction. Llamas were fed hay at 0.25% of their body weight per day on a dry-matter basis beginning on day 0. Feed restriction was terminated between days 13 and 21 for affected llamas and days 21 and 28 for unaffected llamas.

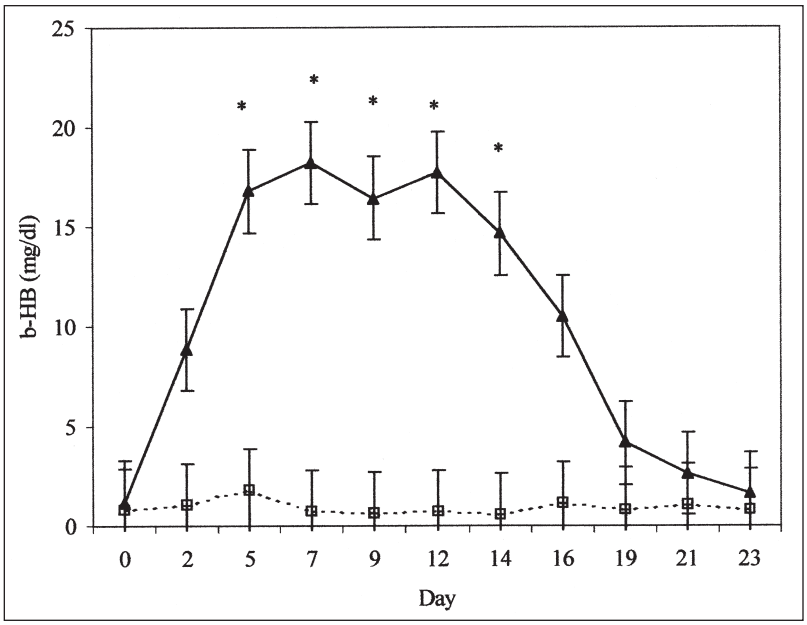

Figure 2—Mean ± SEM serum concentrations of β-hydroxybutyrate (β-HB) in female llamas that did or did not develop HL during a period of feed restriction. *Values significantly ($P < 0.05$) different between groups. *See* Fig 1 for key.

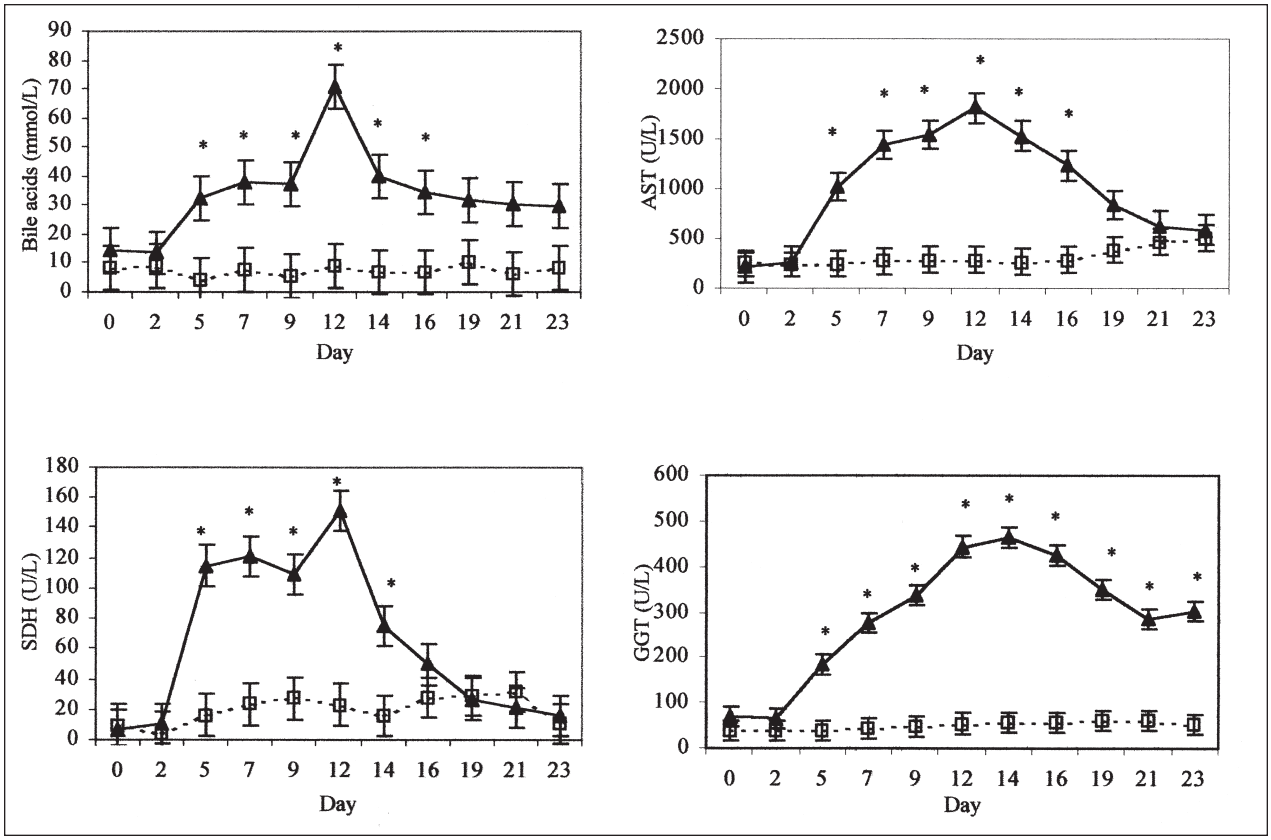

Figure 3—Mean ± SEM serum concentrations of bile acids and activities of aspartate transaminase (AST), sorbitol dehydrogenase (SDH), and γ–glutamyl transferase (GGT) in female llamas that did or did not develop HL during a period of feed restriction. *See* Fig 1 and 2 for key.

for all 8 llamas; serum NEFA concentration increased by day 2 and remained high in most llamas throughout the feed restriction trial (**Fig 1**). However, there were no significant differences in mean NEFA concentrations between affected and unaffected llamas. Serum β-HB concentration also increased in all llamas by day 2 of the trial, although mean concentration in the unaffected llamas was not significantly greater than baseline until day 5 (**Fig 2**). Llamas with HL had a significantly higher overall mean (± SEM) β-HB concentration (10.24 ± 2.12 mg/dl) than did unaffected llamas (0.92 ± 2.66 mg/dl). In addition, β-HB concentration was significantly higher in affected llamas at all times except days 0, 2,16,19, 21, and 23. The latter 4 days were timepoints at which all llamas were fed ad libitum.

Mean overall activities of AST and SDH and concentration of bile acids were significantly higher in llamas with HL than in unaffected llamas (**Fig 3**). However, values were not significantly different between groups on days 0 and 2 for all 3 analytes and on days 16, 19, 21, and 23 for SDH and days 19, 21, and 23 for AST. Mean overall GGT concentration was also significantly higher in affected llamas, with no difference between groups on days 0 and 2.

Patterns of changes in serum activities of AST, SDH, and GGT and concentration of bile acids were somewhat different among affected llamas, although each analyte generally increased during the period of feed restriction. Activities of AST in all affected llamas were greatly increased (approx 4-fold) by day 5, compared with baseline values, peaked around day 12, and decreased rapidly after feed restriction was terminated. However, activities had returned to baseline at the end of the study in only 2 of 5 affected llamas. Activities of SDH followed a similar pattern, with pronounced increases (15- to 20-fold) by day 5, a peak on day 12, and a rapid decrease after termination of feed restriction. Values for all affected llamas returned to baseline by day 23. Serum GGT activity increased more slowly, reached a peak on day 14 to 16, and decreased more slowly following termination of the trial. Activity of GGT had not returned to baseline in any llama at the end of the study. Serum bile acid concentrations also increased gradually to a peak concentration on day 12. In most llamas, bile acid concentrations decreased rapidly after termination of feed restriction, then gradually and irregularly decreased. Values had returned to baseline in 3 of 5 affected llamas by the end of the study. Serum levels of AST, SDH, GGT, and bile acids did not increase significantly in unaffected llamas.

Mean overall cholesterol concentration was significantly higher in llamas with HL (148.13 ± 12.3 mg/dl), compared with unaffected llamas (69.94 ± 15.3 mg/dl). Values did not differ between groups on days 0, 2, 5, and 7. Overall triglyceride concentrations were not different between the 2 groups.

Mean overall insulin concentration of llamas with HL (3.62 ± 0.30 μU/ml) was not significantly different

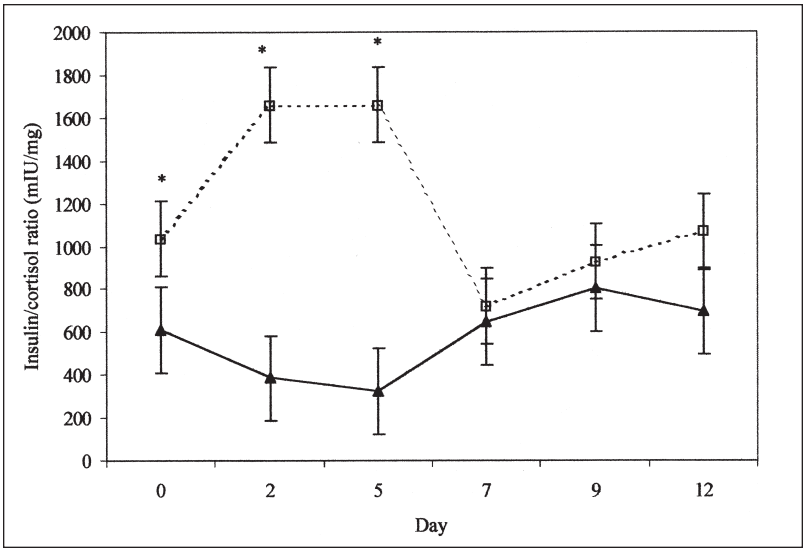

Figure 4—Mean ± SEM serum insulin-to-cortisol concentration (insulin/cortisol) ratio in female llamas that did or did not develop HL during a period of feed restriction. *See* Fig 1 and 2 for key.

from that of unaffected llamas (4.4 ± 0.38 μU/ml), and insulin concentration did not significantly differ between groups on any day. Similarly, although mean overall cortisol concentration was higher in llamas with HL (0.93 ± 0.09 μg/dl), compared with the unaffected group (0.56 ± 0.12 μg/dl), this difference was not significant ($P = 0.05$). The overall insulin-to-cortisol concentration ratio was less in affected llamas (552.5 ± 141.7 μU/μg), compared with unaffected llamas (1,081.7 ± 182.9 μU/μg), but the difference in ratio was significant only on days 0, 2, and 5 (**Fig 4**).

For some biochemical variables, there were no significant differences in values between affected and unaffected llamas, but mean values for all llamas changed over time. For example, mean BUN for all llamas was less on day 19, compared with days 2, 5, and 7. Total protein concentration was greater on days 19 and 21 than on all earlier days, and glucose concentration was less on day 12 than on day 0. Serum concentrations of creatinine, albumin, total bilirubin, calcium, phosphorus, potassium, sodium, and chloride, activity of CK, and results of CBC did not change over time, nor were there significant differences between groups on any day.

Discussion

The study reported here was undertaken to determine whether HL could be induced in llamas solely by feed restriction, which was used to simulate anorexia. In naturally occurring HL, anorexia and weight loss are consistent findings, with other stressors and disease states playing undetermined roles.[1] Our results indicate that feed restriction alone did not consistently result in HL; an additional metabolic demand, such as nursing a young cria, was required to induce HL in most cases. Initial body condition did not appear to be a predisposing factor. Only 1 of the 5 llamas that developed HL was not lactating, and that llama became partially anorectic and did not always eat the full daily allotment of hay, thus self-imposing a more severe feed restriction. The clinical course of disease development in that llama resembled that of naturally occurring HL in camelids. In the natural disease, there is often no apparent cause for onset of clinical disease. Even the increased metabolic demand of nursing a cria did not result in HL in 2 of llamas. However, these llamas had older crias (9.5 to 17 weeks old) than the 4 lactating llamas that did develop HL. Given that New World camelids evolved in an environment where food is not consistently plentiful, it is not surprising that they can adapt readily to reduced feed intake under most conditions.

All llamas in the study mobilized fat, as evidenced by increased serum NEFA concentrations by the second day of feed restriction and weight loss. Weight loss and NEFA concentration increases were most pronounced in llamas with the youngest crias and least pronounced in the 2 thin llamas (body condition scores of 1) with older crias. These thin llamas had the least fat to mobilize, and their crias were probably obtaining a greater part of their caloric intake from solid food rather than milk. Hyperketonemia, as evidenced by increased serum β-HB concentrations, developed in all the feed-restricted llamas between days 5 and 14, with higher mean values in the affected llamas, compared with the unaffected group. This suggests that llamas have the necessary enzymes for ketone production during periods of fat mobilization.

Development of HL in these llamas was not associated with clinical signs typically seen in cats and ponies with HL and in most camelids with naturally occurring HL.[1,2,8,12] Affected llamas did not develop signs of depression, nor did they become lethargic or recumbent. In addition, only 1 became partially anorectic during the study. No abnormal neurologic signs were seen in any of the llamas. In contrast, 26% (8/31) of camelids with naturally occurring HL had abnormal neurologic signs.[1] Clinically, the affected llamas in our study behaved more like dairy cows with HL than cats or camelids with HL. Most dairy cows

with mild to moderate hepatic fat accumulation have a subclinical syndrome, which may be associated with variable reproductive performance or milk production.[4] The lack of clinical signs of disease may reflect that the HL that developed in the llamas of the present study was generally mild to moderate. In 87.5% (27/31) of camelids with naturally occurring HL, accumulation of lipid hepatocytes was severe and diffuse.[1] Similar to subclinical HL seen in high-producing dairy cows, the accumulation of lipid in hepatocytes of the llamas in the present study was largely reversible. When affected llamas were returned to normal diets (ie, grass hay ad libitum) for 5 to 12 days, 2 had no visible hepatic vacuoles and 2 had only few scattered lipid vacuoles in liver biopsy specimens.

Increases in serum activities of AST, SDH and GGT and concentration of bile acids occurred in llamas that developed HL. Of these analytes, SDH appeared to be the marker that both increased when liver damage was occurring and decreased most reliably to baseline values when feed restriction and, presumably, liver damage were terminated. The fact that these analytes were significantly increased in llamas with HL, compared with baseline values, but not in unaffected llamas supports their use as markers of hepatopathy in llamas as in other species.[13] Concentrations of these analytes also were abnormal in camelids with naturally occurring HL.[1] No change in total bilirubin concentration was detected in this study, indicating that this analyte is an insensitive predictor of hepatopathy in llamas in contrast to cats[3] and cattle.[5]

Increases in serum lipid concentrations during periods of feed restriction indicate that llamas have the ability to increase hepatic lipid output in response to negative energy balance and an influx of fatty acids into the liver. This is in contrast to dairy cows, in which the capacity for export of fatty acids from the liver in the form of very-low-density lipoproteins is limited.[14] The greater magnitude in total cholesterol increase in llamas with HL, compared with unaffected llamas, may reflect a greater requirement for fat attributable to impaired carbohydrate availability in affected llamas, a different distribution of circulating lipoproteins between affected and unaffected llamas, or other factors such as hormonal influences on the liver. Development of hypercholesterolemia without severe hypertriglyceridemia in llamas with HL, which are all mobilizing fat, suggested that much of the mobilized triglyceride was used for energy by cells. In contrast, hypertriglyceridemia is a common finding in camelids with naturally occurring HL, suggesting impaired tissue uptake.[1]

In the present study, baseline serum insulin concentrations and insulin response to feed restriction varied among animals. This did not appear to be related to body condition at the onset of the study nor to lactation status. Baseline insulin-to-cortisol concentration ratio was slightly but not significantly lower in llamas that went on to develop HL, suggesting that HL is more likely to develop in llamas with a hormonal bias toward lipolysis. Stress and the presumptive accompanying increase in serum cortisol concentration have been identified as contributing factors in the development of naturally occurring HL in camelids as well as other diseases of abnormal energy metabolism.[1] Traditionally, we believed that camelids were stress-prone animals and that cortisol played a pivotal role in the development of HL. Results of the present study as well as some of our recent experiences with naturally occurring HL in camelids suggest an equal if not greater role for insulin. Thus, determination of insulin concentration and the insulin-to-cortisol concentration ratio may be useful for identifying camelids at risk of HL. The possible role of insulin in development of HL is further complicated by the fact that insulin resistance rather than insulin deficiency may be a predisposing factor. Nonalcoholic-induced hepatic lipidosis in humans is strongly associated with both obesity and noninsulin dependent diabetes mellitus, conditions in which insulin resistance plays a prominent role.[7,15]

In addition to the differences in severity and reversibility of hepatic lesions, there were also other important differences between the llamas in the present study with experimentally induced HL and llamas with naturally occurring HL. None of the affected llamas in the present study developed hyperglycemia during feed restriction, and some actually became mildly hypoglycemic by day 12. Although hypoglycemia is a common feature in cattle with HL,[5] 53% (9/17) of camelids with naturally occurring HL were hyperglycemic and 47% (8/17) were normoglycemic at the time of blood sampling.[1] The minimal change in serum total protein concentration in affected llamas in the present study contrasts with results of a previous study[1] reporting on the naturally occurring disease. In that study, 72% (13/18) of affected camelids were hypoproteinemic. These differences may be attributable to the relative homogeneity of our study population, which comprised predominantly lactating females that developed HL because energy intake did not meet demand. The study population in the previous report was heterogenous; HL developed in those camelids as a result of a variety of conditions. In some cases of naturally occurring HL, it appears likely that inadequate dietary protein and impaired tissue uptake of glucose and triglyceride rather than simple dietary energy deficiency may be important risk factors for disease development. This is similar to the situation in humans, in whom development of HL has been associated with severe dietary protein deficiency.[7] Thus, it is important to recognize that results of the present feed restriction study may not be applicable to all camelids with HL. However, our results do provide information on the development of HL under a specific set of conditions.

[a]Bard-Biopty/Cut biopsy needle, CR Bard Inc, Covington, Ga.
[b]Serono-Baker 9000 hematology analyzer, Serono-Baker Diagnostics, Allentown, Pa.
[c]Hitachi 717 biochemical analyzer, Boehringer-Manheim, Indianapolis, Ind.
[d]Coat-A-Count insulin kit, Diagnostic Products Corp, Los Angeles, Calif.
[e]Coat-A-Count cortisol kit, Diagnostic Products Corp, Los Angels, Calif.
[f]SigmaStat Statistical Software, SPSS Inc, Chicago, Ill.

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