Assessment of the metabolic effects of hydrocortisone on llamas before and after feed restriction

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Objective—To evaluate the effects of administration of hydrocortisone on plasma concentration of insulin and serum concentrations of glucose, triglyceride, and nonesterified fatty acids (NEFAs) in llamas before and after feed restriction.

Animals—9 adult female llamas.

Procedure—Feed was withheld from llamas for 8 hours. Blood samples were collected before (0 minutes) and 120, 180, 240, and 300 minutes after IV injection of hydrocortisone sodium succinate (1 mg/kg) for determination of plasma insulin concentration and serum concentrations of glucose, triglyceride, and NEFAs. The llamas were then fed a limited diet (grass hay, 0.25% of body weight daily) for 21 days, after which the experimental procedures were repeated.

Results—Compared with llamas that were not feed-restricted, llamas after feed restriction had significantly higher plasma insulin concentration and serum concentrations of triglycerides and NEFAs. Feed-restricted llamas after hydrocortisone injection had a significantly smaller increase in serum glucose concentration, a decrease (rather than an increase) in serum concentration of NEFAs, and no change in blood concentrations of insulin or triglycerides.

Conclusions and Clinical Relevance—Short-acting glucocorticoid hormones did not appear to increase blood lipid concentrations in healthy llamas, regardless of ongoing fat mobilization. Thus, these hormones appear unlikely to be major direct contributors to diseases such as hepatic lipidosis or hyperlipemia. Although administration of hydrocortisone reduced serum concentration of fatty acids in feed-restricted llamas, its use has not been evaluated in sick camelids and cannot be considered therapeutically useful. (*Am J Vet Res* 2004;65:1002–1005)

Hepatic lipidosis,[1,4] hypertriglyceridemia,[1,2] and high blood concentrations of nonesterified fatty acids (NEFAs)[1,4] have been identified as indications of excess fat mobilization in New World camelids. The stimuli for excess fat mobilization have not been determined.

Ruminants and horses with similar conditions typically have some excess energy demand, such as pregnancy or lactation, and often have concurrent hypoglycemia. In contrast, llamas and alpacas may develop excess fat mobilization without the demand of pregnancy or lactation[2] and also often have concurrent normal to high blood glucose concentration.[1-3]

Elucidation of the particular mechanism of fat mobilization in camelids may allow development of novel treatments or prophylactic strategies. In camelids, the association between fat mobilization and hyperglycemia suggests a mechanism that involves either insulin deficiency or excessive action of energy-mobilizing hormones. Llamas are often easily stressed, and environmental stressors, particularly temperature, have been implicated in outbreaks of hepatic lipidosis[3]; consequently, the activity of stress hormones in these animals has received particular investigation. The activity of one such hormone, cortisol, increases with various physical manipulations in camelids.[5] The physiologic actions of cortisol in other species include mobilization of glycogen stores, inhibition of intracellular glucose utilization, potentiation of hormone-sensitive lipase activity, and promotion of lipoprotein production and release by the liver. Thus, the role of cortisol in the process of fat-mobilization deserves scrutiny.

In another investigation in alpacas,[6] fat-mobilizing effects of cortisol were not detected. Because cortisol may be more important as a potentiator of ongoing lipolysis rather than as the primary lipolytic stimulus, it was postulated that healthy alpacas did not have sufficient ongoing lipolysis (which may have been the result of suppression by endogenous insulin) to highlight any potentiating action of cortisol. The purpose of the study reported here was to evaluate the effects of administration of hydrocortisone on plasma insulin and serum concentrations of glucose, triglyceride, and NEFAs in llamas before and after feed restriction. Our hypothesis was that cortisol exerts a potent effect on fat mobilization in camelids in a lipolytic state, presumably in association with a muted insulin response.

Materials and Methods

Animals—Nine adult nonpregnant nonlactating female llamas from the university herd were catheterized[a] in the right jugular vein and acclimated to their stalls and handling areas in the university clinic for 72 hours. A physical examination and basic clinicopathologic tests were performed to ascertain health status. The 9 llamas were housed in groups of 3 to minimize separation stress. These llamas were also used in a study[7] of glucose tolerance conducted in sequence with the hydrocortisone trials.

Hydrocortisone challenge—This study was conducted with approval of the Institutional Animal Care and Use Committee of Oregon State University. Feed was withheld from the llamas for 8 hours overnight. By use of a simplified version of an established protocol,[6] a hydrocortisone challenge test was performed; blood samples were collected before (time 0) and 120, 180, 240, and 300 minutes after IV injection of hydrocortisone sodium succinate (1 mg/kg). Blood samples were divided between tubes containing no anticoagulant and tubes containing lithium heparin. Heparinized blood samples were immediately placed on ice and centrifuged in a refrigerated centrifuge within 20 minutes of collection to obtain plasma. Plasma was frozen at –70°C for ≤ 90 days before analysis. This is within the established stability period for insulin.[8] Blood in tubes containing no anticoagulant was allowed to clot, and samples were centrifuged within 1 hour for immediate analysis of sera. Plasma insulin concentration was measured by use of radioimmuno assay.[b] Serum concentrations of glucose, triglycerides, and NEFAs were measured by use of an automated analyzer.[c] All assays had been validated previously for scientific trials or routine diagnostic application.[2,4,9] The hydrocortisone challenge procedure was repeated after 21 days of feed restriction.

Feed restriction—Llamas were weighed daily and fed a restricted diet of hay; each llama received an amount equivalent to 0.25% of its body weight daily. This protocol has been used to induce hepatic lipidosis in pregnant and lactating llamas.[4] Blood samples were obtained every 2 to 3 days to monitor fat mobilization and health. At the conclusion of the study, llamas were fed a pelleted mineral supplement (250 g/llama) and grass hay ad libitum. A final health assessment was performed 7 days after the conclusion of this trial, and the llamas were returned to the herd the following day.

Statistical analyses—Mean ± SEM plasma concentration of insulin and serum concentrations of glucose, triglycerides, and NEFAs were each analyzed for changes over time and differences between values of the llamas before and after feed restriction by use of a 2-way ANOVA for repeated measures.[10,d] Differences between mean values were detected by use of a Tukey test. Values of $P < 0.05$ were considered significant in these comparisons.

Results

All 9 llamas appeared to be clinically normal during the period of feed restriction and maintained their appetites. Mean weight loss between the first and second study days was 4.9 ± 2.9% of body weight. After the study, all llamas rapidly regained weight and appeared normal on both physical and clinicopathologic examination.

Baseline serum glucose concentrations were not significantly different before (115.4 ± 5.7 mg/dL) and after (106.1 ± 18.0 mg/dL) feed restriction (**Figure 1**). Before feed restriction, mean serum glucose concentrations increased after administration of hydrocortisone and were significantly greater than the baseline value at all time points. At 300 minutes after administration of hydrocortisone, serum glucose concentration was 247.7 ± 58.4 mg/dL in llamas that were not feed-restricted. After the period of feed restriction, llamas also had an increase in serum glucose concentration. During the first 2 hours after administration of hydrocortisone, this increase was similar to that detected in llamas that were not feed-restricted; however, the magnitude of the increase in serum glucose concentration was significantly lower in the feed-restricted llamas at the final 3 time points. At 300 minutes after administration of hydrocortisone, serum glucose concentration was 194 ± 17.0 mg/dL in feed-restricted llamas.

Llamas that were not feed-restricted had significantly ($P < 0.022$) lower mean plasma insulin concentrations at all time points, compared with values obtained in feed-restricted llamas (**Figure 2**). Before feed restriction, plasma insulin concentration at 120 minutes after administration of hydrocortisone (2.32 ± 0.43 µU/mL) was significantly ($P = 0.017$) lower than the baseline value (2.65 ± 0.18 µU/mL); at 300 minutes after hydrocortisone administration, the value (2.67 ± 2.32 µU/mL) was significantly ($P = 0.008$) higher than the value at 120 minutes. After feed restriction, insulin concentrations did not change significantly from baseline (3.04 ± 0.34 µU/mL) during the experimental period.

Compared with values for llamas that were not feed-restricted, mean serum triglyceride concentrations after feed restriction were significantly higher at baseline (36.4 ± 13.1 mg/dL vs 18.9 ± 5.0 mg/dL; $P = 0.001$) and all subsequent time points ($P < 0.017$; **Figure 3**). In llamas that were not feed-restricted, serum
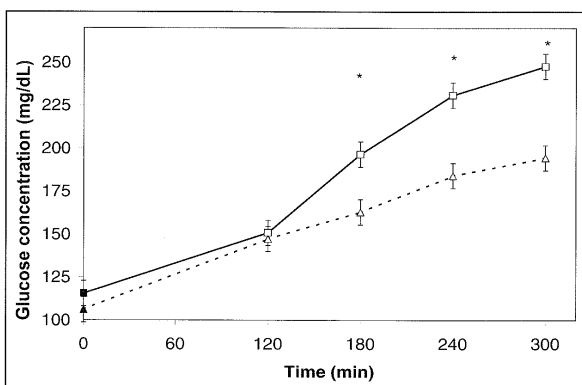
Figure 1—Mean ± SEM serum concentrations of glucose before and after IV administration of hydrocortisone sodium succinate (1 mg/kg) in 9 llamas that were (triangles) or were not (squares) feed-restricted. Hydrocortisone was administered immediately after obtaining the first blood sample (ie, at 0 minutes). Open symbols indicate values that are significantly ($P < 0.05$) different from baseline values for that experiment. *Time point at which values between experimental groups are significantly ($P < 0.05$) different.
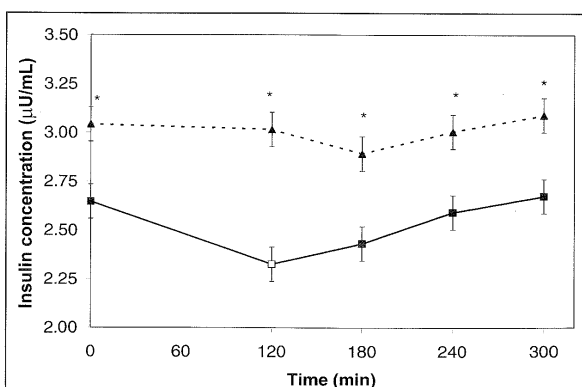
Figure 2—Mean ± SEM plasma concentrations of insulin before and after IV administration of hydrocortisone sodium succinate (1 mg/kg) in 9 llamas that were (triangles) or were not (squares) feed-restricted. Hydrocortisone was administered immediately after obtaining the first blood sample (ie, at 0 minutes). See Figure 1 for key.

triglyceride concentrations were significantly lower than the baseline value at 240 minutes (14.0 ± 2.4 mg/dL; $P = 0.022$) and 300 minutes (11.0 ± 2.3 mg/dL; $P < 0.001$) after hydrocortisone administration. After feed restriction, serum triglyceride concentration did not change after hydrocortisone injection.

Mean serum concentration of NEFAs at baseline was significantly ($P < 0.001$) higher in llamas after feed restriction than it was before feed restriction (0.70 ± 0.25 mEq/L vs 0.23 ± 0.10 mEq/L; **Figure 4**). Before feed restriction, serum concentration of NEFAs was significantly ($P = 0.009$) higher than the baseline value at 180 minutes (0.41 ± 0.20 mEq/L; $P = 0.014$) and 240 minutes (0.42 ± 0.15 mEq/L) after hydrocortisone injection. After feed restriction, serum concentration of NEFAs decreased significantly from the baseline value at 120 minutes after administration of hydrocortisone; similarly, low concentrations were maintained during the remainder of the experimental period. At 180, 240, and 300 minutes after administration of

Figure 3—Mean ± SEM serum concentrations of triglycerides before and after IV administration of hydrocortisone sodium succinate (1 mg/kg) in 9 llamas that were (triangles) or were not (squares) feed-restricted. Hydrocortisone was administered immediately after obtaining the first blood sample (ie, at 0 minutes). *See* Figure 1 for key.

Figure 4—Mean ± SEM serum concentrations of nonesterified fatty acids (NEFAs) before and after IV administration of hydrocortisone sodium succinate (1 mg/kg) in 9 llamas that were (triangles) or were not (squares) feed-restricted. Hydrocortisone was administered immediately after obtaining the first blood sample (ie, at 0 minutes). *See* Figure 1 for key.

hydrocortisone, serum concentrations of NEFAs were not significantly different in llamas with or without feed restriction.

Discussion

The results of the study reported here indicated that changes induced by administration of hydrocortisone in healthy llamas are similar to those induced in healthy alpacas, including an increase in serum glucose concentration and a decrease in serum triglyceride concentration.[6] Hydrocortisone injection also affected serum concentration of NEFAs and plasma concentration of insulin in llamas, which had not been detected previously. All of these responses to administration of hydrocortisone were considerably altered by feed restriction and the resultant increase in serum lipid concentration. Because reduced feed intake and the associated fat mobilization are common findings in sick camelids, the results of the feed restriction model may be relevant to that group.

The increase in serum glucose concentration after hydrocortisone injection was less in feed-restricted llamas than it was in llamas that were not feed-restricted, and this may have been associated with smaller body glycogen reserves. Other mechanisms by which cortisol can increase blood glucose concentration include promotion of protein catabolism, facilitation of gluconeogenesis, and inhibition of intracellular glucose phosphorylation and therefore inhibition of utilization. Although its contribution appeared to be small, protein catabolism might be expected to increase in feed-restricted camelids. The specific contributions of gluconeogenesis and altered intracellular metabolism were not evaluated in the study of this report.

Feed restriction eliminated the increase in plasma insulin concentrations detected after the hydrocortisone challenge. Nevertheless, feed-restricted llamas had higher plasma insulin concentrations throughout the trial, compared with llamas that were not feed-restricted. The lack of an insulin response to administration of hydrocortisone in llamas after feed restriction was likely because of the smaller increase in serum glucose concentration. Also, the higher concentrations of triglyceride and NEFAs in feed-restricted llamas may have indicated that lipid mobilization in those llamas is less sensitive to the effects of insulin. Relative insulin insensitivity could have been the result of changes in target cells; it is known that insulin binds to a cell-surface receptor to act and feed-restricted camelids might have downregulated insulin receptors, despite higher concentrations of circulating insulin. Also, these data suggested that either feed-restricted llamas have a muted insulin response to hyperglycemia or the measurable pancreatic response in camelids begins when blood glucose concentration nears or exceeds 200 mg/dL. Evidence for a muted insulin response to hyperglycemia in llamas has been reported.[7]

The initial decrease in plasma concentration of insulin after hydrocortisone injection (0 to 120 minutes) in llamas that were not feed-restricted is also not explained by known actions of hydrocortisone. It is more likely that the decrease in plasma insulin concentration and initial insignificant increases in serum
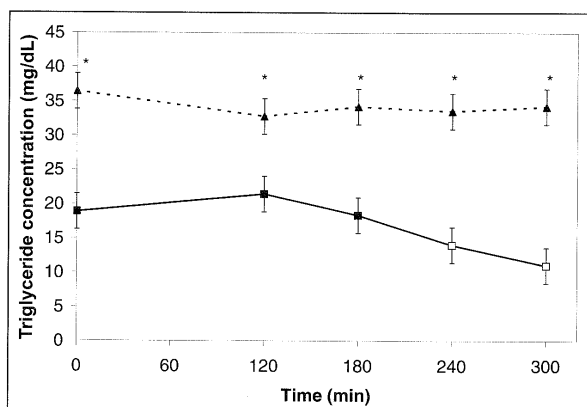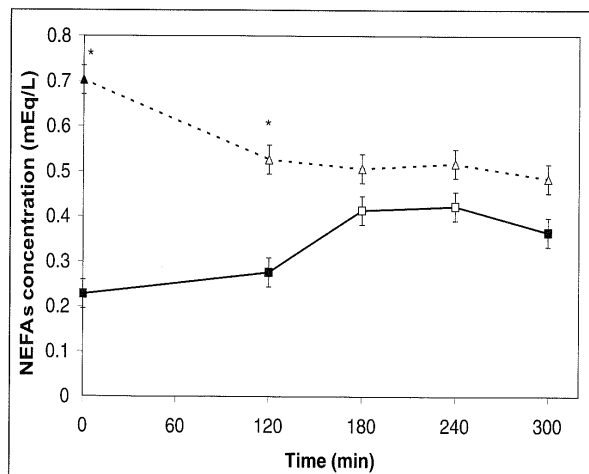
concentrations of triglyceride and NEFAs were continuations of the camelids' response to feed withdrawal that was performed during the preceding night. After 21 days of feed restriction, camelids were presumably more accustomed to the lack of feed and the effects of the overnight feed withdrawal were not as notable. Other potential causes of the decrease in plasma concentration of insulin after hydrocortisone injection in llamas that were not feed-restricted remain to be investigated, but may be of importance because reduced insulin action could play a role in syndromes of excess mobilization of fat or glucose in camelids.

The lack of an insulin response after hydrocortisone injection in feed-restricted camelids was helpful in the evaluation of the direct metabolic effects of hydrocortisone. In other species, hydrocortisone potentiates stimulation of hormone-sensitive lipase by other hormones and increases hepatic production and release of lipoprotein, although it may also stimulate peripheral lipoprotein breakdown. In those species, the lipid-mobilizing actions of hydrocortisone are antagonized by insulin, which inhibits hormone-sensitive lipase and enhances peripheral triglyceride clearance. Humans with diabetes are an exception; administration of glucocorticoids may increase concentrations of lipids in the blood of some individuals.[11] Results of a previous study[6] did not identify any lipid-mobilizing effects in healthy alpacas without feed restriction, possibly because of the strong concurrent insulin response to hyperglycemia and lack of preexisting lipolysis. In the study of this report, llamas that were not feed-restricted had a similar lack of lipid mobilization. Compared with llamas that were not feed-restricted, llamas after feed restriction had higher basal serum triglyceride concentrations and did not clear triglyceride after hydrocortisone injection, but there was no evidence that hydrocortisone increased circulating triglyceride concentration.

The changes in serum concentrations of NEFAs did not support a fat-mobilizing role for hydrocortisone in camelids. In llamas that were not feed-restricted, the increase in serum concentration of NEFAs was concurrent with the increase in serum glucose concentration (a known glucocorticoid effect), but it also was concurrent with an increase in plasma insulin concentration and a decrease in serum triglyceride concentration. Nonesterified fatty acids are detected in blood as the result of intracellular or intravascular lipolysis. In intracellular lipolysis, intracellular adipose stores are broken down under the influence of hormone-sensitive lipase; in intravascular lipolysis, circulating lipoprotein triglyceride is broken down under the influence of lipoprotein lipase. Under conditions of negative energy balance when both prehepatic (NEFAs) and posthepatic (triglycerides) fat may serve as important energy sources, both intracellular and intravascular lipolysis may be active. Without the application of regional clamp techniques, the particular source of NEFAs in

blood is impossible to determine. However, the lack of evidence for augmentation of intracellular lipolysis in llamas that are already in a lipolytic state (ie, after feed restriction) and the simultaneous (presumably insulin-mediated) clearance of triglyceride in llamas that are not feed-restricted have suggested that fat-mobilizing syndromes in camelids are associated with factors other than the action of glucocorticoids, at least within the limitations of our experimental model.

Although cortisol did not appear to increase concentrations of circulating lipids in llamas in our study, complex interactions between hormones cannot be ruled out. Glucocorticoids are known potentiators of lipolysis, and our experimental design may not have completely recreated conditions present in sick camelids. However, other primary lipolytic stimuli could be present in these camelids, and future efforts should be directed toward identifying these abnormalities. Furthermore, although a decrease in serum concentrations of fatty acids after administration of hydrocortisone was detected in the study of this report, it is unknown whether this agent would be useful as a treatment in clinical practice and its use cannot be recommended.

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