Effects of hydrocortisone on substrates of energy metabolism in alpacas

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Objective—To evaluate effects of hydrocortisone administration, with and without concurrent administration of insulin, on intermediary metabolism in alpacas.

Animals—8 adult castrated male alpacas.

Procedure—On each of 2 consecutive days, food was withheld from alpacas for 8 hours. Alpacas then were administered 1 mg of hydrocortisone sodium succinate/kg, IV (time 0). On 1 of the days, randomly assigned alpacas were also administered regular insulin (0.2 U/kg, IV) 120 minutes after hydrocortisone administration. Blood samples were collected at 0, 120, 135, 150, 165, 180, 210, 240, 300, and 360 minutes. Plasma concentrations of glucose and lactate and serum concentrations of triglycerides, cholesterol, nonesterified fatty acids, and β-hydroxybutyrate were determined. Data were compared between days. Additionally, serum insulin concentrations before and after hydrocortisone administration were determined for selected samples.

Results—Hydrocortisone administration induced hyperglycemia, hyperinsulinemia, a reduction in concentrations of triglycerides and cholesterol, and a reduction in triglyceride-to-cholesterol ratio. Subsequent insulin administration temporarily negated the hyperglycemic effects of hydrocortisone, induced temporary hyperlactemia, and augmented the reduction in blood triglycerides.

Conclusions and Clinical Relevance—A single dose of a short-acting corticosteroid does not increase blood lipid fractions in healthy alpacas, probably because of a competent endogenous insulin response. Corticosteroids may induce differing responses in camelids with depleted glycogen stores or an ineffective insulin response. Administration of insulin can effectively negate the hyperglycemic effects of hydrocortisone and augment lipoprotein clearance. Hence, insulin administration may be therapeutic for alpacas with hyperglycemia, hyperlipemia, or hyperketonemia. (*Am J Vet Res* 2002;63:1269-1274)

Stress with presumptive hypercortisolemia has been implicated as a contributor to many abnormal conditions in New World camelids. These conditions include benign transient hyperglycemia,[1] reproductive failure,[2] life-threatening gastric ulcers,[3] hepatic lipidosis and hyperlipemia,[4-6] and hyperosmolar syndrome.[7]

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Improvements in our understanding of the unique aspects of camelid anatomy and physiology enable us to more fully explain the pathogenesis of these conditions. However, our understanding of the physiologic effects of stress and hypercortisolemia is still rudimentary.

Camelids are remarkable among ruminating animals because they maintain resting blood glucose concentrations comparable to those found in many monogastric species despite minimal absorption of carbohydrate from the gastrointestinal tract.[1] This can be explained partially by their small amounts of circulating insulin,[8,9] slow glucose clearance, partial resistance to administration of insulin,[10,11] and the predominance of glucose-6-phosphatase over glucokinase and hexokinase.[a] These features suggest limited capacity for end-tissue carbohydrate uptake, and further suggest that camelids are poorly able to compensate for increases in glucogenic factors. Thus, they cannot increase glucose clearance to match increased mobilization, and persistent or extreme hyperglycemia ensues. Therefore, cortisol and other stress hormones could be expected to evoke more severe hyperglycemia in camelids than in species with greater insulin production and sensitivity. There is evidence that this happens and is clinically relevant.[7]

The effects of stress hormones and scarce insulin on fat metabolism in camelids are not as well understood. Llamas and alpacas presumably rely to a similar degree (or possibly even more so, as indicated by their slow glucose clearance) as ruminants on gastric microbial production of short-chain fatty acids to provide most of their energy needs. Longer-chain fatty acids and ketone bodies, whose release into the blood is inhibited by insulin in other species, are normally only found in low concentrations in fed camelids,[12] suggesting that the small amount of circulating insulin still inhibits adipose lipolysis in camelids. Whether stress hormones and negative energy balance, which often promote lipolysis in other species through inhibition of insulin secretion, would have a more profound effect in camelids is unknown. Camelids are susceptible to disorders that resemble pregnancy toxemia, ketosis, and hepatic lipidosis in cattle and sheep, but they often develop these disorders without belonging to 1 of the putative high-risk groups of pregnant or lactating animals. Furthermore, camelids also frequently have high blood concentrations of triglycerides, cholesterol, **nonesterified fatty acids (NEFA)**, and β-hydroxybutyrate.[4-6,13,14] Thus, although camelids develop biochemical abnormalities similar to those seen in anorectic ruminants (although to a smaller degree),[5,6] they also are susceptible to increases in circulating lipoproteins (presumably the very-low-density lipoprotein fraction).[4,6]

Another difference from ruminants is that camelids with abnormalities of fat metabolism are more likely to have hyperglycemia than hypoglycemia.[6,13] Concurrent hyperglycemia and hyperlipemia support a role of insulin deficiency or excessive amounts of stress hormones in the development of these conditions. Camelids with naturally developing and experimentally induced lipid disorders provide some evidence for insulin deficiency and excess amounts of corticosteroids, but the relative contribution of each hormone is unknown.[7,12]

The objective of the study reported here was to evaluate the effects of a short-acting corticosteroid on blood carbohydrate and lipid constituents in alpacas. Additionally, we wanted to test whether administration of insulin, which speeds glucose clearance after IV administration,[10,11] would also be effective for reducing hyperglycemia stimulated by corticosteroids or for reducing cortisol-mediated lipid mobilization. If hydrocortisone caused disruption in energy metabolism pathways, we would have a clue as to the origin of some metabolic disorders in camelids. If insulin were effective at controlling those deleterious effects, then it could serve an important role in the treatment of corticosteroid-induced disorders of energy metabolism in New World camelids.

Materials and Methods

Animals—Eight adult castrated male alpacas were used in the study. All alpacas had been maintained on pasture and provided supplemental orchard grass hay for several months preceding the study. Alpacas were acclimated to stalls and handling areas for 96 hours before the study began. All alpacas were judged to be healthy on the basis of medical history and results of physical examination, CBC count, and serum biochemical analysis. A 16-gauge double-lumen catheter[b] was inserted into the right jugular vein of each alpaca 2 days before onset of the study. Before and between experiments, alpacas were housed in groups to minimize stress. This study was conducted with approval of the Institutional Animal Care and Use Committee of Oregon State University.

Experimental design—For each alpaca, 2 experiments were performed on consecutive days. The order in which the experiments were performed was determined randomly. Food was withheld for 8 hours before each experiment as well as during the experiments. Before beginning each experiment, a blood sample was collected from each alpaca into tubes containing sodium fluoride without anticoagulant. Immediately thereafter, alpacas received hydrocortisone sodium succinate (1 mg/kg, IV) by rapid injection through 1 lumen of the jugular vein catheter (time 0). Subsequent blood samples were collected 120, 135, 150, 165, 180, 210, 240, 300, and 360 minutes after hydrocortisone injection. These samples were collected through the other lumen of the jugular vein catheter; the first 5 ml of blood withdrawn was discarded before collection of each sample.

In 1 of the 2 experiments, regular insulin (0.2 U/kg) was administered IV immediately following collection of the blood sample at 120 minutes. The time points for collection of blood samples and administration of insulin were selected on the basis of results of a preliminary study conducted on 2 of the alpacas 4 days before the study reported here. That preliminary study was conducted in accordance with the protocol used for the study reported here, and it revealed that this dose of hydrocortisone did not increase blood glucose concentrations for 120 minutes. In another study,[10] IV administration of regular insulin had its greatest effect within 90 minutes after administration.

After the conclusion of both experiments, the catheters were removed. Alpacas were then fed and returned to the herd.

Analysis of samples—Blood samples were allowed to clot, and serum was harvested and frozen. Fluoridated blood samples were placed on ice immediately, and plasma was harvested within 20 minutes after samples were collected. Plasma samples were analyzed immediately for glucose and lactate concentrations by use of an automated chemistry analyzer.[c] Serum samples were thawed and analyzed for triglyceride, cholesterol, NEFA, and β-hydroxybutyrate concentrations by use of the same analyzer. Serum samples obtained from alpacas before as well as 120 and 360 minutes after hydrocortisone administration also were analyzed for insulin content by use of a commercial radioimmunoassay kit.[d] All assays have been validated for use in blood samples obtained from camelids.[8]

Statistical analysis—Plasma and serum concentrations of glucose, lactate, triglycerides, cholesterol, NEFA, and β-hydroxybutyrate and the ratio of triglyceride to cholesterol were analyzed by use of 2-way repeated-measures ANOVA.[e] Treatment and time were the 2 factors in the ANOVA. The interaction between treatment and time also was tested to evaluate short-lived differences between treatments. Such analysis can be useful in studies such as this when differences may be transient. Differences between mean values for the same treatment at various time points and for each treatment at the same time point were detected by use of the Tukey honestly significant difference test.[15] Comparisons were considered significant at values of $P < 0.05$.

Results

Effects of restricting feed for 8 hours were mild. At time 0, all concentrations were within reference ranges, except for the concentration of NEFA, which was greater than the upper limit of the reference value (0.24 mEq/L) in 6 alpacas (maximum concentration, 0.68 mEq/L). These 6 alpacas typically had low triglyceride concentrations, and alpacas with higher triglyceride concentrations typically had low concentrations of NEFA (**Fig 1**). Each alpaca did not always have the same pattern of lipids prior to time 0 for each of the experiments.

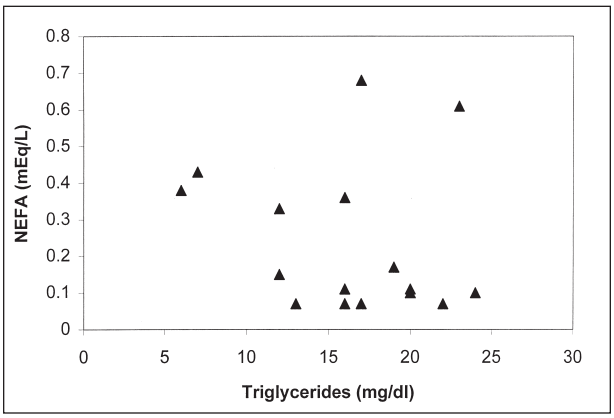

Figure 1—Plasma concentrations of triglycerides (reference range, 8.3 to 55.8 mg/dl) and nonesterified fatty acids (NEFA; reference range, < 0.24 mEq/L) in 8 alpacas on each of 2 consecutive mornings after 8 hours of feed deprivation.

Hydrocortisone administration induced hyperglycemia, hyperinsulinemia, and a reduction in concentrations of triglycerides and cholesterol. Subsequent insulin administration temporarily negated the hyperglycemic effects of hydrocortisone, induced temporary hyperlactemia, and augmented the reduction in triglyceride concentrations.

Glucose concentrations changed significantly with time ($P = 0.002$) and treatment ($P < 0.001$; **Fig 2**). There also was a significant ($P < 0.001$) interaction between time and treatment. Glucose concentration increased significantly in alpacas after hydrocortisone treatment, and they continued to increase for the duration of the experiment in alpacas administered hydrocortisone alone. Glucose concentration decreased for the initial 90 minutes after insulin injection, and then it increased again.

Lactate concentrations changed significantly ($P < 0.001$) with time, and there also was a significant ($P < 0.001$) interaction between time and treatment (**Fig 3**). Lactate concentrations did not differ significantly between treatments. Lactate concentration decreased over time for both treatments, except that it increased immediately after insulin injection.

Triglyceride concentrations decreased significantly ($P < 0.001$) for both treatments over the course of the study, and there was a significant ($P = 0.003$) interaction between time and treatment (Fig 4). Triglyceride concentrations did not differ significantly between treatments. Alpacas had an abrupt decrease in triglyceride concentration immediately after administration of insulin, and they also had lower nadir values. Triglyceride concentrations decreased (mean ± SD) 33.3 ± 8.2% at 30 minutes and 42.3 ± 12.0% at 60 minutes after insulin administration, but decreased only 6.8 ± 18.5% and 12.8 ± 14.9% at the same time points in alpacas that were administered hydrocortisone alone.

Cholesterol concentrations decreased significantly ($P < 0.001$) over time independent of treatment, although the change was small (**Fig 5**). The triglyceride-to-cholesterol ratio decreased significantly ($P < 0.001$) over time independent of treatment (**Fig 6**).

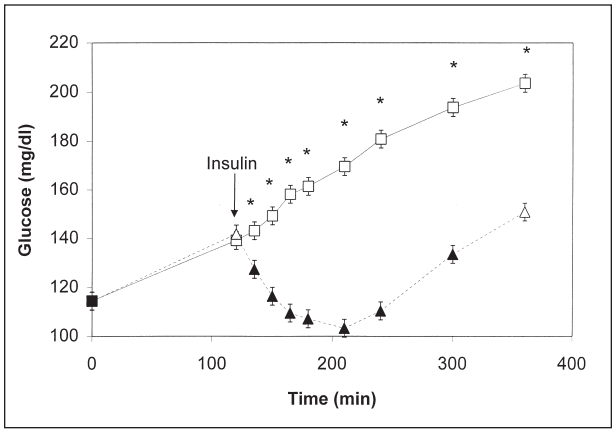

Figure 2—Mean ± SEM concentrations of glucose before and after IV administration of hydrocortisone sodium succinate (1 mg/kg) in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. Hydrocortisone was administered immediately after collection of the first blood sample (time 0), and insulin was administered (arrow) immediately after collection of the blood sample at 120 minutes. Mean values that differ significantly ($P < 0.05$) from baseline values for each treatment are indicated (open triangles and open squares, respectively). *Mean values differ significantly ($P < 0.05$) between treatments for this time point.

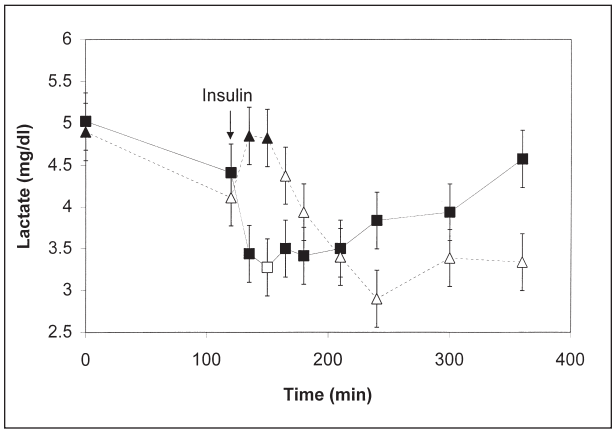

Figure 3—Mean ± SEM concentrations of lactate before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

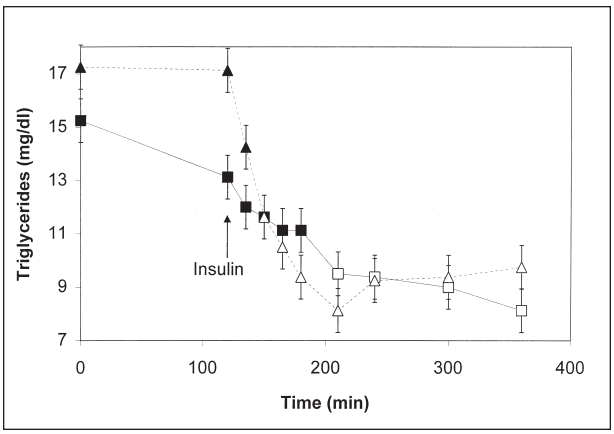

Figure 4—Mean ± SEM concentrations of triglycerides before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

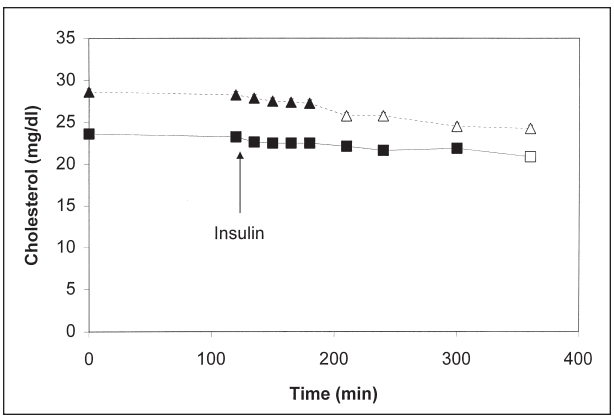

Figure 5—Mean ± SEM concentrations of cholesterol before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

The mean ± SD value for the ratio was 0.70 ± 0.34 and 0.68 ± 0.27 for the 2 treatments before hydrocortisone administration and 0.41 ± 0.17 and 0.45 ± 0.17 in alpacas without and with insulin treatment, respectively, at the conclusion of the experiment. Concentrations of β-hydroxybutyrate also decreased significantly ($P < 0.001$) at 165 minutes for both treatments (**Fig 7**). Although this decrease appeared to be of longer duration after administration of insulin, the concentrations did not differ significantly between treatments. Concentrations of NEFA did not change consistently or significantly during the experiments (**Fig 8**); patterns varied greatly among alpacas, with some having rapid decreases, others having increases, and still others having concentrations that did not change. In general, there appeared to be a slight overall reduction in concentrations of NEFA, with a decrease evident in the middle of the experiments.

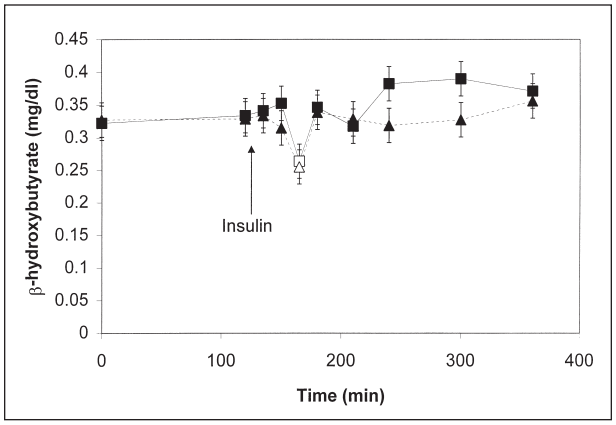

Figure 6—Mean ± SEM concentrations of triglyceride and cholesterol before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

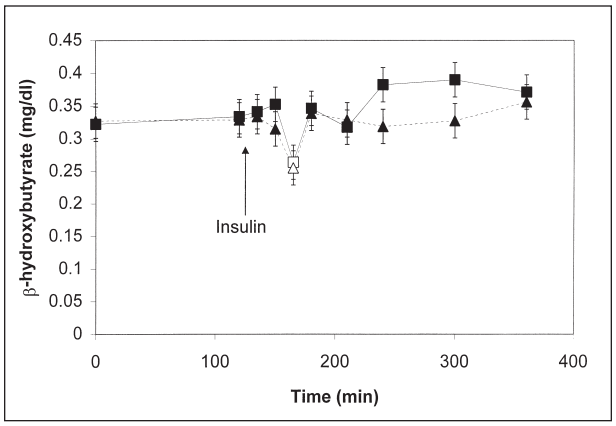

Figure 7—Mean ± SEM concentrations of β-hydroxybutyrate before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

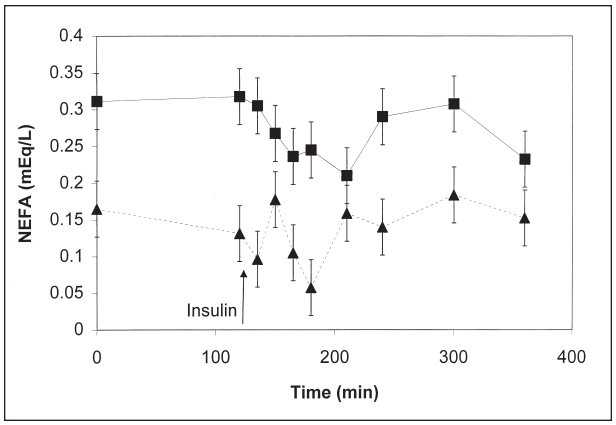

Figure 8—Mean ± SEM concentrations of NEFA before and after IV administration of hydrocortisone in 8 alpacas that were (triangle) or were not (square) subsequently administered insulin. *See* Figure 2 for key.

During the experiment in which alpacas received hydrocortisone but not insulin, 2 alpacas had increases in plasma insulin concentrations, with concentrations increasing from 3.0 and 3.4 U/L, respectively, before hydrocortisone administration to 9.5 and 7.4U/L, respectively, at the conclusion of the experiment. Samples from other alpacas were not considered suitable for analysis because of an unintentional delay between collection of blood samples and freezing of plasma.

Discussion

The most pronounced effects of hydrocortisone administration in the study reported here were on glucose concentrations. Corticosteroids increase glucose concentrations in other species by promoting gluconeogenesis (with glucagon) and glycogenolysis as well as inhibiting end-tissue utilization (primarily by inhibiting or reversing glucose phosphorylation).[16] The relative contribution of these mechanisms to hyperglycemia in alpacas remains unknown. The reduction in lactate concentrations could have been attributable to increased gluconeogenesis or decreased glycolysis; results of another study[a] suggest that hydrocortisone does not affect the enzymes of phosphorylation.

Hyperglycemia initially developed 90 to 120 minutes after injection of hydrocortisone, making it unlikely that cortisol is responsible for the stress-induced hyperglycemia occasionally seen after routine venipuncture, unless the process (herding, confinement, restraint, venipuncture) lasts 90 to 120 minutes. Other faster-acting hormones such as epinephrine may be more important physiologic contributors to the stress response. However, hypercortisolemia may be responsible for hyperglycemia in a sick camelid or any other camelid that has been subject to prolonged stress. With prolonged hypercortisolemia, hyperglycemia may be compounded as a result of insulin resistance or pancreatic exhaustion, which is a decrease in insulin production by chronically stimulated beta cells.

Administration of insulin counteracted the hyperglycemic stimulus of hydrocortisone. The transient increase in lactate concentration suggests that the acute response was mediated in part by enhanced uptake and utilization or via inhibition of gluconeogenesis. In another study,[a] investigators suggested that insulin enhances utilization by promoting phosphorylation. Effects on mobilization could not be determined in the study reported here. Hypoglycemic effects lasted only

90 minutes after IV administration of regular insulin, the same duration for improved clearance after administration of glucose that has been described for an identical insulin protocol.[10] These findings suggest that administration of insulin would be helpful in preventing or treating pathologic hyperosmolality and impaired tissue utilization of glucose in camelids with hypercortisolemia. Because of the short-lived action of regular insulin, frequent administration or use of longer-acting forms of insulin would be necessary to completely counteract the prolonged action of corticosteroids.

Withholding of food for 8 hours evoked only a mild lipolytic state, which was characterized in some alpacas by an increase in concentration of NEFA and in other alpacas by a higher mean triglyceride concentration. The reason for these differing responses was beyond the scope of this study. Hydrocortisone did not increase either type of lipid. Findings for other species suggest that hydrocortisone increases intracellular (breakdown of triglyceride stores) and intravascular (breakdown of circulating triglyceride) lipolysis, although not necessarily in adipose tissue,[17-20] and also increases hepatic production and output of lipoproteins.[21,22] The disproportionate decrease in triglyceride concentrations, compared with cholesterol concentrations, suggested selective removal of fat from triglyceride-rich lipoproteins rather than complete clearance of denser lipoproteins. This suggests that the increase in lipoprotein lipase activity predominated over lipoprotein production in these alpacas. During intravascular lipolysis, NEFA are produced locally in the blood.[17] Because some of these fatty acids move into tissues, changes in central venous components may not be seen. Changes in NEFA concentrations were erratic in our study. Although there appeared to be some relationship between decreases in triglyceride concentrations and appearance of NEFA in specific alpacas, these were not sufficiently consistent to prove an association. An effect would potentially have been more pronounced in camelids with higher baseline concentrations of triglycerides in which more triglyceride substrate would have been available for lipolysis.

Some of the reduction in triglyceride and cholesterol concentrations as well as the nonsignificant decrease in concentrations of NEFA may have been attributable to the increase in naturally released insulin. Hydrocortisone administration stimulates insulin secretion, probably through hyperglycemia.[23] In the 2 alpacas of our study, hydrocortisone administration induced hyperinsulinemia of similar or greater magnitude than that induced by administration of glucose.[8] Some of the established effects of insulin (lipoprotein clearance, reduction in ketogenesis, and intracellular lipolysis) were initially detected in the alpacas reported here at approximately the same time as the development of hyperglycemia. Compared with the effects of insulin administration on glucose concentrations, the difference in lipid fractions between the 2 treatments was small. This suggested that although the magnitude of the insulin response in camelids is small, compared with that in other species, it may be more important and more effective at regulating fat metabolism than carbohydrate metabolism. This would be compatible with findings from other species in which fat mobilization is extremely sensitive to small amounts of insulin.[24] The response to naturally released insulin was reinforced by administration of additional insulin. Improvement of hyperlipemia and ketonuria was observed after insulin administration in an ill llama.[4] Analysis of these data suggests that insulin administration may be therapeutic for camelids with hyperlipidemia or hyperketonemia.

The findings reported here may aid in our understanding and treatment of disorders of lipid metabolism in camelids. Although commonly reported abnormalities (increases in NEFA, β-hydroxybutyrate, triglyceride, and cholesterol concentrations) were not induced with a single dose of a short-acting corticosteroid, a role for corticosteroids in lipid disorders cannot be ruled out. In other species, effects of sustained hypercortisolemia differ from those observed after a single pulse,[21] promoting hyperlipidemia over clearance of triglycerides and eventually leading to pancreatic exhaustion. The role of the insulin response deserves further scrutiny. The response reported here in clinically normal alpacas may have been responsible for reductions in ketogenesis and triglyceridemia and may have helped prevent increases in concentrations of NEFA, whereas an inadequate response will allow accumulation of lipids in the blood and liver.[12] Factors that decrease the insulin response directly (by suppression or exhaustion of islet cells) or indirectly (by inhibiting hyperglycemia through glycogen depletion or lack of gluconeogenic precursors) are likely to promote a lipolytic or hyperlipemic state. Hence, the insulin response may be of similar importance, or even more importance, than lipolysis attributable to direct causes.

[a]Huaman J, Villavicencio M, Guerra R, et al. Effect of insulin and hydrocortisone on the activity of glycolytic and gluconeogenic enzymes of the alpaca liver (abstr). *Federation Proceedings* 1975;34:659.

[b]Long-term polyurethane catheter, MILA International Inc, Erlanger, Ky.

[c]Hitachi 717 serum biochemical analyzer, Boehringer Mannheim Corp, Indianapolis, Ind.

[d]Coat-A-Count insulin kit, Diagnostic Products Corp, Los Angeles, Calif.

[e]SigmaStat 2.0, SPSS Inc, Chicago, Ill.

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