Assessment of the effects of exogenous long-acting insulin on glucose tolerance in alpacas

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Objective—To evaluate the effects of long-acting insulin on glucose clearance in alpacas.

Animals—8 adult castrated alpacas.

Procedure—On 2 days, food was withheld from alpacas for 8 hours. Alpacas were randomly allocated to receive an SC injection of long-acting insulin (0.4 U/kg) or saline (0.9% NaCl) solution 1 hour before the first of 3 administrations of glucose (at 60, 480, and 1,200 minutes after treatment) on day 1 and the alternate treatment and procedure on day 2. Plasma glucose concentration was determined before and 15, 45, 120, and 240 minutes after each glucose administration, and fractional turnover rates were calculated. The data were compared between alpacas with and without insulin administration and among the 3 glucose administrations for each day.

Results—Compared with sham-treated alpacas, insulin-treated alpacas had significantly lower blood glucose concentrations from 180 to 600 minutes after treatment; they also had glucose concentrations significantly below baseline values from 120 to 480 minutes, at which time the mean glucose concentration was in the hypoglycemic range. Also, mean fractional turnover of glucose was significantly higher in insulin-treated alpacas from 105 through 300 minutes.

Conclusions and Clinical Relevance—Compared with known effects of regular insulin in alpacas, the action of long-acting insulin was of slower onset but longer lasting; its administration may induce hypoglycemia, even in alpacas that receive glucose. To maintain the hypoglycemic effect, long-acting insulin may have to be administered more than once daily and blood glucose concentration should be monitored to avoid hypoglycemic complications in alpacas. (*Am J Vet Res* 2004;65:1688–1691)

Camelids have many unique physiological features including the ability to maintain higher blood glucose concentrations than other forestomach fermenting animals.[1,2] This relative hyperglycemia may be attributable to inherently low plasma concentrations of circulating insulin,[2-5] slow glucose clearance, and partial resistance of camelid cells to the effects of insulin,[6,7] all of which reduce the ability of camelid tissues to remove glucose from the bloodstream. The higher blood glucose concentrations detected in camelids generally are not pathologic, but a number of hyperglycemic disorders have been described, including stress hyperglycemia,[1] hyperosmolar disorder,[8] and diabetes mellitus with pancreatic atrophy.[9]

These hyperglycemic disorders likely develop in camelids when glucogenic stimuli overwhelm their ability to clear glucose or when exogenous glucose is administered. Because of the poor insulin response to hyperglycemia and partial insulin resistance in camelids, their ability to up regulate glucose clearance is limited. Impairment of clearance may also play a role in some instances. Because insulin also plays a role in the inhibition of fat mobilization, factors that impair insulin secretion or activity may also promote fat mobilization, hepatic lipidosis, and other disorders of lipid metabolism.

Insulin has been used to treat both hyperglycemic[8] and hyperlipemic[10] disorders in camelids. Although a variety of forms of insulin have been used clinically in camelids, only regular insulin has been studied scientifically. Administration of regular insulin enhances glucose clearance for < 1 hour.[6] Because repeated injections are impractical in some clinical situations, long-acting preparations of insulin are often used. These include zinc-insulin suspensions that have a slow rate of absorption and are associated with a prolonged duration of effects.[11,12] In humans, administration of long-acting insulin provides a low basal plasma concentration of insulin throughout the day with an onset of 4 to 8 hours, peak at 10 to 30 hours, and duration of 20 to 36 hours.[12] There is little information regarding the effects of this preparation in animals. The purpose of the study reported here was to evaluate the effects of long-acting insulin on glucose clearance in alpacas. The intention was to determine the time frame in which long-acting insulin is effective in lowering blood glucose concentrations and increasing glucose clearance in alpacas and thereby establish a treatment protocol for patients receiving glucose and insulin treatment.

Materials and Methods

Animals—Eight adult castrated male alpacas from the university herd were catheterized in the right jugular vein[a] and acclimated to their stalls and handling areas in the university clinic for 96 hours. A physical examination and basic clinicopathologic tests were performed to ascertain health. The same alpacas were also studied in a separate epinephrine trial 1 week previously.[13] All 8 alpacas were housed in groups of 2 or 3 to minimize separation stress and fed grass hay ad libitum.

Experimental design—This study was conducted with approval of the Institutional Animal Care and Use Committee of Oregon State University. For each alpaca, 2

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experiments were initiated 48 hours apart. The order in which the experiments were performed was determined randomly. Food was withheld for 8 hours prior to each experiment as well as during the experiments. For the first experiment, 4 alpacas received an SC injection of a crystalline suspension of human recombinant insulin with zinc.[b] The remaining 4 alpacas received a sham injection of (0.004 mL/kg) physiologic saline (0.9% NaCl) solution SC. For the second experiment, the 4 alpacas that had previously received an insulin injection were administered a sham injection and vice versa.

During each experiment, 3 glucose tolerance tests were performed in each alpaca; these were conducted at 60, 480, and 1,200 minutes after administration of the insulin or sham injection. Before beginning each of the 3 tolerance tests, a blood sample was collected from each alpaca into tubes containing lithium heparin. Immediately thereafter, glucose solution (0.5 g/kg) was administered to alpacas via rapid (< 15 seconds) injection through 1 lumen of the IV catheter. At 15, 45, 120, and 240 minutes after each glucose injection, blood samples were collected through the other lumen of the catheter; the first 5 mL of blood withdrawn was discarded before collection of each sample. Heparinized blood samples were placed on ice, and plasma was separated from erythrocytes within 20 minutes of collection. Samples were analyzed for glucose content by use of an automated chemistry analyzer.[c] The fractional turnover rate (k) of glucose was calculated for each interval by use of the following formula[14]:

$$k\ (\%/\text{min}) = (\ln[\text{glucose}]_1 - \ln[\text{glucose}]_2)/\text{interval}_{\text{min}} \times 100$$

where $\ln(\text{glucose})_1$ represents the concentration of glucose at the beginning of the interval and $\ln(\text{glucose})_2$ represents the concentration at the end.

After completion of the 2 experiments, the jugular catheters were removed, feed was provided, and the alpacas returned to their herd.

Statistical analyses—Plasma concentrations and fractional turnover rates of glucose were analyzed for changes over time and the effects of insulin administration by use of 2-way repeated-measures ANOVA.[15,d] Treatment and time were the 2 factors in the ANOVA. Differences between mean values for the same treatment at various time points and between treatments at the same time point were detected by use of the Tukey test. Comparisons were considered significant at values of $P < 0.05$. All data are expressed as mean ± SD.

Results

Plasma glucose concentration measurements—Plasma glucose concentration reached a peak in alpacas during both the insulin and sham injection experiments following glucose administration in each of the 3 tolerance tests (**Figure 1**). Peak concentrations were similar for all tolerance tests regardless of treatment or time, except that the mean ± SD peak plasma glucose concentration for insulin-treated alpacas (326.9 ± 47.1 mg/dL) was significantly ($P = 0.016$) lower than the peak concentration in sham-treated alpacas (365.8 ± 38.9 mg/dL) during the second tolerance test (at 495 minutes); immediately prior to administration of glucose at 480 minutes, insulin-treated alpacas had a significantly lower trough plasma glucose concentration than sham-treated alpacas. For each of the tolerance tests, plasma glucose concentrations declined from their peak values to near baseline values during each 4-hour test period.

Insulin-treated alpacas had significantly ($P = 0.003$ to 0.045) lower plasma glucose concentrations than those of untreated alpacas from 180 through 600 minutes after receiving the treatment injection. In insulin-treated alpacas, mean plasma glucose concentration was less than the baseline value (119.9 ± 22.5 mg/dL) at each sampling point during the last 120 minutes of the first tolerance test and at the initiation of the second tolerance test (480 minutes). Immediately prior to glucose administration at 480 minutes, plasma glucose concentration in insulin-treated alpacas was 86.9 ± 42.5 mg/dL (ie, 27.5% lower than the baseline value); at that same time point in the sham-treated alpacas, mean plasma glucose concentration was 130.8 ± 28.3 mg/dL (ie, 6.3% greater than the baseline value of 123.0 ± 16.3 mg/dL). At no time point did the mean

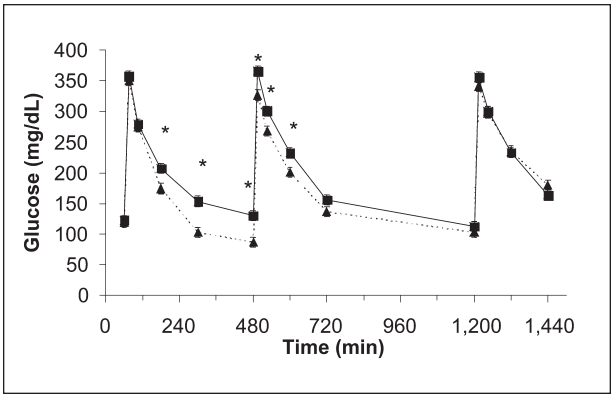

Figure 1—Mean ± SEM plasma glucose concentrations after repeated administration of glucose (0.5 g/kg, IV) in 8 alpacas that received an injection of human recombinant insulin with zinc (0.4 U/kg, SC; triangles) or a sham injection of saline (0.9% NaCl) solution (squares) 1 hour (0 minutes) before the first dose of glucose in a random crossover study. Glucose was administered at 60, 480, and 1,200 minutes. *Mean values for the 2 groups are significantly ($P < 0.05$) different at this time point.

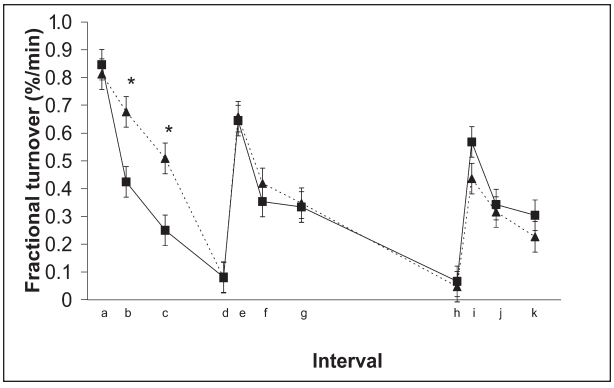

Figure 2—Mean ± SEM fractional turnover rates of glucose after repeated administration of glucose (0.5 g/kg, IV) in 8 alpacas that received an injection of human recombinant insulin with zinc (0.4 U/kg, SC; triangles) or a sham injection of saline solution (squares) 1 hour (0 minutes) before the first dose of glucose in a random crossover study. Glucose was administered at 60, 480, and 1,200 minutes. *Mean values for the 2 groups are significantly ($P < 0.05$) different at this time point. Intervals are indicated as follows: a = 75 to 105 minutes; b = 105 to 180 minutes; c = 180 to 300 minutes; d = 300 to 480 minutes; e = 495 to 525 minutes; f = 525 to 600 minutes; g = 600 to 720 minutes; h = 720 to 1,200 minutes; i = 1,215 to 1,245 minutes; j = 1,245 to 1,320 minutes; k = 1,320 to 1,440 minutes.

plasma glucose concentrations in sham-treated alpacas decrease to below the baseline value.

Glucose clearance—Mean fractional turnover rate of glucose differed significantly (P = 0.001) between treatment groups (**Figure 2**). Fractional turnover rate of glucose was significantly higher for the alpacas treated with insulin, compared with the alpacas treated with saline solution, during the intervals of 105 to 180 minutes (0.68 ± 0.33%/min vs 0.42 ± 0.19%/min; P = 0.002) and 180 to 300 minutes (0.51 ± 0.35%/min vs 0.25 ± 0.16%/min; P = 0.001). During these intervals, the mean fractional turnover rates in insulin-treated alpacas were 37.3% and 50.8% greater than those of the alpacas that did not receive insulin, respectively. In sham-treated alpacas, fractional turnover rates were similar for comparable intervals among the 3 tolerance tests. In insulin-treated alpacas, compared with rates calculated for comparable intervals in the third glucose test, fractional turnover rates were significantly higher during the first (0.82 ± 0.26%/min vs 0.44 ± 0.17%/min; P = 0.003) and second (0.68 ± 0.34%/min vs 0.32 ± 0.05%/min; P = 0.007) testing intervals after glucose administration in the first glucose tolerance test.

During the full experimental period, the mean fractional turnover rate was highest in both the insulin-treated (0.82 ± 0.26%/min) and sham-treated alpacas (0.85 ± 0.34%/min) during the 75- to 105-minute interval following the insulin or sham injection treatment. In the equivalent intervals of the second and third glucose tolerance tests (ie, the 495- to 525-minute and 1,215- to 1,245-minute intervals after the insulin or sham injection treatments, respectively), similar but progressively lesser peaks in fractional turnover rate were detected. During the remainder of each glucose tolerance test, fractional turnover rates decreased from these peak values until the end of the test period.

Discussion

The results of the study reported here have indicated that the administration of a long-acting formulation of insulin improved clearance of exogenous glucose in alpacas. In alpacas, the onset of this effect (within 3 hours) was faster than that reported in humans (4 to 6 hours), although the detectable duration of improved clearance of exogenous glucose was considerably shorter (as long as 10 hours in alpacas vs as long as 36 hours in humans).[12] However, in our study, alpacas received repeated IV administrations of glucose; because this was not included in the human study protocol, direct comparisons of findings of the 2 studies are difficult. Dairy cattle appear to have a similar onset of insulin action; plasma glucose concentrations decline from approximately 2 to 7 hours after insulin administration, and some suppression of plasma glucose concentration is detected for as long as 24 hours.[16] The duration of suppression of endogenous plasma glucose concentration after insulin administration in alpacas remains unknown, as we evaluated changes in endogenous and exogenous glucose in combination and also repeatedly administered glucose to the study animals.

Our data have also indicated a slower onset and longer duration of the effects of long-acting insulin in alpacas, compared with effects associated with administration of regular insulin. In alpacas, the onset of effects of regular insulin is immediate but the duration of action is only approximately 45 minutes.[6] If long-term effects are desired, a zinc-insulin suspension may be a more efficient treatment, although it may take as long as 3 hours for an effect to be detectable.

Unlike findings of another study[6] in which regular insulin was administered to alpacas, the administration of long-acting insulin induced hypoglycemia (reference range for plasma glucose concentration at the university Veterinary Diagnostic Laboratory, 88 to 151 mg/dL) after glucose administration during the first glucose tolerance test in the alpacas in the present study. In our study, although the difference in the fractional turnover rate of glucose after administration of long-acting insulin (compared with values in alpacas treated with saline solution) was of a lower magnitude than that detected after administration of regular insulin in alpacas[6] and was of a fairly short duration (relative to recommended dosing intervals in humans[12]), the decrease in plasma glucose concentration was of long duration. Insulin exerts a variety of hypoglycemic effects including increased glycolysis and glycogenesis and decreased gluconeogenesis and glycogenolysis.[17] Because of the relatively high ratio of glucose-6-phosphatase to hexokinase and glucokinase activities in camelid liver lysate, compared with the value in rat liver,[e] camelids have been claimed to have efficient gluconeogenic capabilities, although direct evidence of a high rate of glucose production is lacking. Our data have suggested that gluconeogenesis in alpacas was unable to maintain plasma glucose concentration after administration of a long-acting formulation of insulin, despite administration of supplemental exogenous glucose, the presence of enzyme systems that favor hepatic export of glucose, and an insulin-mediated glucose uptake process that appears to be inferior to that of many other domestic animals.[5-7,18] Potentially, previous perceptions about enhanced gluconeogenesis in alpacas need to be reevaluated in a more comprehensive research setting.

The results of our study suggested that once-daily administration of long-acting insulin might be insufficient for maintaining a prolonged action of exogenous glucose clearance. If long-acting insulin was administered in conjunction with exogenous glucose to sick alpacas, twice-daily administration may be more therapeutic. The hypoglycemia that we detected in the insulin-treated study alpacas suggested that the administration of long-acting insulin, which is used clinically to treat hyperlipemia and other disorders in camelids, should be accompanied by careful monitoring of plasma glucose concentrations and may necessitate administration of a glucose supplement. Although the effects of long-acting insulin on blood lipid concentrations were not assessed in our study, those data would be valuable additions to the investigation of the therapeutic benefits of this preparation in alpacas.

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

[b]Humulin U ultralente, Eli Lilly Co, Indianapolis, Ind.

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

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

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

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