Glucose tolerance testing in llamas and alpacas

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Objective—To determine blood glucose clearance in 2 species of New World camelids after IV challenge and to examine mechanisms of this clearance.

Animals—5 adult female llamas and 5 adult gelded alpacas.

Procedure—After food was withheld for 12 hours, camelids received 0.5 g of glucose/kg of body weight by rapid IV infusion. Serum concentrations of glucose, nonesterified fatty acids, cortisol, and insulin, and plasma concentrations of lactate were determined before and 0, 1, 2, 3, 4, 5, 15, 30, 60, 90, 120, 180, and 240 minutes after infusion. Ratios of insulin to glucose and insulin to cortisol were calculated for each time point.

Results—Postinfusion glucose concentrations were significantly higher in llamas than alpacas for the first 15 minutes and remained significantly higher than baseline values in both species for 180 minutes. Lactate and cortisol concentrations did not change significantly; nonesterified fatty acid concentrations decreased in both species 30 minutes after infusion. Baseline insulin concentrations were < 6 µU/ml in both species and increased only to 10.1 ± 0.7 µU/ml in llamas. Insulin concentrations did not change significantly in alpacas.

Conclusions and Clinical Relevance—Llamas and alpacas clear glucose more slowly than other domestic species after challenge, mainly because of a weak insulin response and slow cellular uptake. This response may impair the assimilation of exogenous glucose as well as make llamas and alpacas prone to diabetes-like disorders when an abundance of endogenous or exogenous glucogenic agents are present. (Am J Vet Res 2001;62:682–686)

For many years it has been recognized that New and Old World camelids typically have higher blood glucose concentrations than domestic ruminants.1-3 This is perplexing, because both ruminants and camelids are forestomach fermenters; dietary carbohydrate is not thought to escape gastric microbial fermentation, making these species dependent on gluconeogenesis to supply necessary substrate to glucose-dependent tissues. As a result, ruminants have adapted to supply most of their cellular energy needs with lipids and have little circulating carbohydrate. Camelids appear to differ from this scheme; however, whether this is attributable to increased carbohydrate uptake from the gastrointestinal tract, enhanced gluconeogenesis, poor glucose uptake by tissues, or some other mechanism remains unknown.

In recent years, several pathologic conditions have been identified in llamas and alpacas that appear to result from abnormal carbohydrate metabolism. These include hepatic lipidosis,4-6 pancreatitis,4-6 and pancreatic atrophy with diabetes mellitus.7,8 With each of these diseases, there are abnormal accumulations of energy substrate in blood or tissues. Some of these diseases resemble those of other domestic animals. Others appear to be unique to these cameld species, or the changes observed in affected camelids are contrary to those expected. For example, dairy cattle with hepatic lipidosis often have concurrent hypoglycemia,9 whereas camelids with similar histologic lesions are often hyperglycemic.10 These differences support the conclusion that camelids process carbohydrate differently than other animals.

Recent data suggest that Old World camelids respond differently than sheep or horses to IV glucose challenge. Camels eliminate glucose, secrete insulin, and suppress free fatty acid mobilization less than do other domestic large animals.11,12 Similar data obtained from New World camelids have been considered to be abnormal and used to diagnose diabetes mellitus,15 although reference values from healthy llamas are not available for comparison. It is possible that New World camelids resemble Old World camelids regarding poor insulin response to hyperglycemia and that high resting blood glucose concentrations, stress hyperglycemia, and poor postchallenge glucose clearance are physiologic processes rather than pathologic processes. It is also possible that physiologic differences in glucose and insulin dynamics are important to the development of several of the aforementioned syndromes, such as why camelids with hepatic lipidosis continue to mobilize adipose deposits despite apparently adequate glucose supply.16 The purposes of the study reported here were to determine glucose clearance in 2 species of New World camelids after IV challenge and examine mechanisms of this clearance.

Materials and Methods

Llamas and alpacas—Five adult (4- to 7-year-old) nonpregnant nonlactating female llamas weighing between 144 and 202 kg and 5 adult (3- to 7-year-old) castrated male alpacas weighing between 63 and 88 kg were used for this study. All camelids had been on pasture supplemented with orchard grass hay for several months. Camelids were acclimated to stalls and handling areas for 72 hours and deemed healthy on the basis of history, physical examination findings, and results of CBC and serum biochemical analysis. A 16-gauge double-lumen catheter was placed into the right jugular vein of each camelid the day before the trial. Camelids were housed in groups of 2 to 5 to minimize stress.

Sample collection and processing—This study was performed with approval of the Institutional Animal Care and

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Use Committee of Oregon State University. After food was withheld overnight for 12 hours, a 50% glucose solution was rapidly administered (<15 seconds) IV through 1 lumen of the catheter so that each camelid received a dose of 0.5 g of glucose/kg of body weight. All subsequent blood samples were withdrawn through the other lumen after discarding the first 5 ml of withdrawn fluid. Samples were drawn 1 minute before glucose injection and at 0, 1, 2, 3, 4, 5, 15, 30, 60, 90, 120, 180, and 240 minutes after glucose injection. Blood samples were placed on ice, and serum and fluoridated plasma were separated from erythrocytes within 20 minutes of collection. Plasma samples were analyzed for lactate content by use of an automated chemistry analyzer. Serum samples were analyzed for glucose and nonesterified fatty acid (NEFA) content using the same instrument, and for insulin and cortisol content by use of radioimmunassay. Glucose analysis was performed immediately after the trial; samples for NEFA and lactate concentrations were stored at −20°C and analyzed 24 hours later; samples for insulin and cortisol concentrations were stored at −70°C and analyzed 1 month later. After all blood collections, the jugular catheters were removed, feed was restored, and the camelids returned to their herd.

Assays to determine glucose and NEFA concentrations in camelid serum were validated previously for diagnostic use by the Oregon State University Veterinary Diagnostic Laboratory; the assay to determine cortisol concentration was validated in an earlier research trial. The assay to determine lactate concentrations was calibrated using lyophilized calibration serum and the automated chemistry analyzer to generate a standard curve, after which 2 control sera were analyzed to test the validity of the curve. The assay to determine insulin concentrations was validated for use in this trial. After addition of 6.6, 19, 58, 105, 200, and 400 µU of insulin/ml standard solutions to equal volumes of llama plasma containing 6.1 and 7.45 µU insulin/ml, mean recoveries were 104, 103, 107, 109, 104, and 100%, respectively. A test solution with an insulin concentration of 89.9 µU/ml was made by adding standard solution to llama plasma. The test solution was assayed unadulterated and after dilution with zero standard at ratios of 1:3, 1:1, and 3:1. Parallelism was tested by comparing the recovery to expected recovery based on a standard curve generated in the first assay. All insulin assays were performed in duplicate.

Statistical analyses—Plasma and serum concentrations of glucose, lactate, NEFA, cortisol, and insulin, and the insulin/glucose and insulin/cortisol ratios were each analyzed by use of 2-way repeated-measures ANOVA. Differences between mean values for the same species at different time points and for different species at the same time point were detected by use of the Tukey test. Comparisons were considered significant at P < 0.05. For

![Figure 1](image_url)

Figure 1—Mean ± SEM concentrations of glucose (A), nonesterified fatty acid (NEFA; B), cortisol (C), insulin (D), insulin/glucose ratio (E), and insulin/cortisol ratio (F) in 5 llamas (closed square) and 5 alpacas (closed triangle) before and after IV administration of 0.5 g of glucose/kg of body weight. Open symbols indicate means that are significantly (P < 0.05) different from baseline values for the respective species. Asterisks (*) denote time points when means for the different species are significantly (P < 0.05) different.
the ratios, the denominator was corrected to U/ml. All data are expressed as mean ± SEM.

Results

All camelids had a significant increase in serum glucose concentration immediately after infusion to 3 hours later (Fig 1A). Mean ± SEM glucose concentrations were 7.09 ± 14 mg/dl in llamas and 677 ± 14 mg/dl in alpacas immediately after infusion and approximately half those values at 30 minutes. Glucose concentrations remained >200 mg/dl at 180 minutes in both species and above baseline values at 240 minutes; by 240 minutes, the difference was no longer significant. Postinfusion glucose concentrations were significantly higher in llamas than in alpacas for the first 15 minutes.

Plasma lactate concentrations did not change significantly from baseline values of 4.2 ± 0.3 mg/dl in llamas and 4.6 ± 0.3 mg/dl in alpacas; differences in lactate response between llamas and alpacas were not detected. Serum NEFA concentrations decreased significantly from baseline values of 0.26 ± 0.02 mEq/L in llamas and 0.25 ± 0.02 mEq/L in alpacas starting at 30 minutes after infusion (Fig 1B). This effect lasted until the conclusion of the trial in llamas; however, there was an increase in NEFA concentrations toward baseline values after 180 minutes in alpacas. Trough values were 0.06 ± 0.02 mEq/L at 180 minutes in llamas and 0.12 ± 0.02 mEq/L at 60 minutes in alpacas.

Serum cortisol concentrations were significantly lower in alpacas (0.52 ± 0.13 µg/dl) than in llamas (1.04 ± 0.13 µg/dl) before infusion and from 60 minutes after infusion until conclusion of the trial (Fig 1C); these concentrations did not change significantly over the course of the trial in either species. In llamas, serum insulin concentrations increased significantly from baseline values of 5.6 ± 0.07 µU/ml and from values in alpacas at corresponding times from 30 minutes after infusion until conclusion of the study (Fig 1D). Peak values were 7.9 ± 0.07 µU/ml at 1 minute and 10.1 ± 0.07 µU/ml at 180 minutes. In alpacas, insulin concentrations did not change significantly throughout the trial. Baseline values in alpacas were 4.9 ± 0.07 µU/ml; peak values were 6.8 ± 0.07 µU/ml at 2 minutes and 6.4 ± 0.07 µU/ml at 15 and 30 minutes. Both species had an early insignificant peak on their insulin curves.

The insulin/cortisol ratio (Fig 1E) was significantly lower than baseline values of 4.4 ± 0.3 µU/mg in llamas and 3.7 ± 0.3 µU/mg in alpacas for the first 60 minutes in llamas and 120 minutes in alpacas before returning toward baseline values. The insulin/glucose ratio was significantly larger in llamas than in alpacas from 90 to 240 minutes.

The insulin/cortisol ratio (Fig 1F) was higher in alpacas (1,256 ± 34 µU/µg) than in llamas (768 ± 34 µU/µg) before infusion and at all time points after infusion; this difference was significant at 15 and 120 minutes, which were 2 peaks in the curve for alpacas that coincided with peak insulin concentrations (15 minutes) or trough cortisol concentrations (120 minutes). The insulin/cortisol ratio curve for llamas was relatively flat, with all data points falling in the range of 748 to 1,266 µU/µg. Except for the peak at 15 minutes (2,593 ± 34 µU/µg), all points on the alpaca curve fell in the range of 1,256 to 2,125 µU/µg.

Discussion

Results of our study revealed that llamas and alpacas clear glucose slowly after IV challenge. Despite some difficulty in comparing these results to previous trials performed on other herbivore species because of the variation between trials in glucose dose (the dose of 0.5 g of glucose/kg is commonly used in cattle),11,12 speed of administration, and timing of sample collection, it is clear that camelids have overtly slower clearance of glucose than other herbivores. In most other domestic herbivores, postinfusion serum glucose concentrations are indistinguishable from baseline values within 60 to 90 minutes, whereas the llamas and alpacas in our study had higher values than baseline through 180 minutes and remained hyperglycemic at the end of the study. Differences in peak and early plasma glucose values observed between the 2 camelid species were evident for only 15 minutes after infusion and were most likely attributable to a higher volume of distribution in the alpacas. Efforts to establish differences in the volume of distribution were confounded by an inability to distinguish exogenous from endogenous glucose and, thus, were not reported. Because of the design of this study, it also was not possible to determine whether the apparent differences in volume of distribution, as well as differences in insulin, cortisol, and antilipolytic response, were attributable to species or sex differences.

Glucose was injected rapidly so that early responses could be monitored; our results provide additional evidence of slow glucose clearance in New World camelids. Peak postinfusion glucose concentrations are expected to halve within 5 minutes of cessation of rapid infusion in horses11 but took 30 minutes to halve in llamas and alpacas. Failure of lactate concentrations to increase over the same period further confirmed that large amounts of glucose were not entering cells; overwhelming the body with glucose results in extrahepatic glycolysis and lactate production in other species.13,14 Slow glucose uptake suggests that alternate methods of clearance such as urinary excretion may be more important in camelids than in other species. It has been reported that slow blood clearance with concurrent glucose diuresis is of clinical importance in camelids with naturally occurring or iatrogenic hyperglycemia.15

Persistence of hyperglycemia was most likely attributable to a poor insulin response. In most species, there is a biphasic release of insulin in response to glucose challenge; a rapid release of pancreatic stores occurs immediately after challenge, and there is subsequent release of newly synthesized insulin approximately 1 hour later. The initial release of insulin is responsible for the rapid decrease in blood glucose and NEFA concentrations observed in other species and may result in up to a 4-fold increase in blood insulin concentrations over baseline values.16 The insulin/glucose ratio increases after challenge and may not return to baseline values until normal blood glucose concentrations are achieved.13,14 The camelids in our study had a decrease in the insulin/glucose ratio and closer to a 50% increase in insulin concentrations; insulin con-
centrations peaked well below fasted values for other species. The biphasic nature of the insulin response was observed, but neither component was especially strong, particularly in the alpacas. Thus, a poor insulin response to glucose challenge and a low fasting serum insulin concentration should be considered physiologic and not pathologic in llamas and alpacas.

Despite poor glucose clearance, the insulin response was sufficient to invoke significant decrease in serum NEFA concentrations. This decrease appeared similar in magnitude to that observed in other species and presumably was attributable to reduced mobilization and increased clearance. This was unexpected, because low fasting serum insulin concentrations and a poor insulin response to glucose challenge typically favor a lipolytic state. Other animals known to have a poor acute insulin response include camels, horses fed diets restricted in protein and energy, and lactating dairy cattle in the early postparturient period, fasted > 48 hours, with metabolic acidosis, or with ketonemia, all of which could be expected to facilitate fat mobilization or spare glucose for tissues that take up glucose in an insulin-independent manner (ie, mammary gland, brain) during periods of inadequate nutrition. Low fasting serum insulin concentrations and a poor insulin response possibly could be an adaptation in New World camelids (and similarly Old World camelids) to survive in harsh environments with limited and intermittent nutrient availability; muscle and liver cells must then rely on fats for energy, whereas glucose is spared for essential tissues. However, this suggests that llamas and alpacas are less able to shift their metabolism from fats to carbohydrates, and may suffer pathogenic consequences from carbohydrate excess: glucose suppresses the fat supply to cells without itself being taken up as a replacement. It also suggests that a metabolic defect exists in New World camelids with hepatic lipoidosis; lipolysis appears not to be suppressed in affected camelids even when glucose concentrations are within physiologic range or high, suggesting that affected camelids have a worse insulin response, or there is some interference with insulin activity. The metabolism of various energy substrates in camelids and how this metabolism is impaired under pathologic conditions warrants further investigation.

Lack of high cortisol concentrations and low insulin/cortisol ratios, especially in alpacas, make it unlikely that absolute or relative hypercortisolemia (often thought of as the cause of resting hyperglycemia in camelids) was a major inhibitor of glucose clearance in the camelids of our study. However, these findings may help explain why some camelids develop excessive lipolysis without obvious extraordinary energy requirements and despite an apparently adequate glucose supply. Physiologic control of lipolysis and glycogenolysis in mammals is achieved through the interactions of insulin, cortisol, glucagon, growth hormone, epinephrine, and other factors. Insulin is the strongest inhibitor of glucose and lipid mobilization, whereas the other factors primarily promote glucose and lipid mobilization. After a period of mobilization, an increase in blood insulin concentration halts mobilization and enhances tissue uptake of these energy substrates. On the basis of results of our study, it is logical to predict that high blood concentrations of cortisol, glucagon, growth hormone, or epinephrine would have a more pronounced effect on blood glucose concentration and lipolysis in camelids than in species that have a better insulin response. Thus, the physiologic limits of the pancreas may make camelids more susceptible to disruptions of the pathways of energy metabolism and the detrimental effects of such disruptions. Reports of such disruptions are plentiful; evidence of the hormonal basis for these disruptions is, for the most part, still lacking.

References


