

# Evaluation of glucose tolerance and insulin sensitivity in llama crias

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**Objective**—To investigate glucose tolerance and insulin sensitivity in llama crias.

**Animals**—7 llamas (age range, 14 to 30 days).

**Procedure**—On each of 2 sequential days, crias were administered glucose (0.5 g/kg) via rapid IV injection. On 1 day (randomly determined for each cria), regular insulin (0.2 U/kg) or 0.9% NaCl solution (0.002 mL/kg) was administered IV 15 minutes after glucose administration. Blood samples were collected before (baseline) and at 5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes after glucose administration for determination of plasma glucose and insulin concentrations; fractional turnover rates and plasma half-life of glucose were calculated. The data were compared over time and between days (ie, between glucose treatments with and without insulin administration).

**Results**—A peak plasma glucose concentration of  $342 \pm 47$  mg/dL was detected at 5 minutes after glucose administration and llamas cleared glucose from plasma within 60 minutes; at 15 minutes, plasma insulin concentration attained a peak value of  $33 \pm 13$   $\mu$ U/mL (ie, triple the baseline value). During the 15- to 45-minute interval, fractional turnover rate of glucose was  $1.10 \pm 0.24\%$ /min and plasma half-life was  $65.7 \pm 13.4$  minutes. Insulin significantly increased glucose turnover and resulted in hypoglycemia within 75 minutes of administration.

**Conclusions and Clinical Relevance**—Healthy immature llamas have glucose tolerance and insulin sensitivity superior to that of adults. However, whether sick crias retain the pancreatic sufficiency and tissue responsiveness that are likely responsible for the rapid glucose clearance in healthy individuals is not known. (*Am J Vet Res* 2005;66:1013–1017)

We have known for many years that adult Old and New World camelids maintain higher blood glucose concentrations than most domestic ruminants, despite the fact that ruminants and camelids have digestive systems that prevent direct absorption of most dietary carbohydrate.<sup>1,2</sup> Various theories to explain this phenomenon have been put forward, but confirmatory studies have been lacking. Recently, it has been established that adult camelids have poor glucose tolerance,<sup>2-5</sup> due in part to poor insulin production and in part to insulin resistance.<sup>6,7</sup> These factors affecting glucose clearance likely also contribute to the high

blood glucose concentrations detected in clinically normal camelids. Potentially, the factors associated with poor glucose tolerance also make adult camelids susceptible to diabetes-like conditions when confronted with intake, production, or administration of excess carbohydrate; however, the fermentative digestive system of mature camelids should make development of such conditions unlikely by limiting entry of dietary carbohydrate into the blood.

In contrast to adults, immature camelids receive a milk diet that is high in carbohydrate<sup>8</sup> and have a digestive tract that should allow absorption of intact sugars. Whether crias assimilate glucose better than adult llamas remains unknown, but recommendations for feeding orphaned camelids often include provision of supplemental carbohydrate, and sick or dehydrated crias often are treated PO or IV with fluids containing glucose.<sup>9-12</sup> These treatments could have adverse health effects in animals with poor glucose tolerance.

Differences in carbohydrate utilization between immature and adult camelids have scarcely been investigated. In alpacas, glucose phosphorylation in the liver of fetuses is more effective than that in the liver of adults,<sup>9</sup> but it is not known if this carries through to the postnatal period or if it has clinical or physiologic importance. In theory, a higher rate of glucose phosphorylation would trap glucose within cells, whereas a lower rate could facilitate glucose transport back into the bloodstream. However, crias are susceptible to a hyperosmolar disorder related to extreme hyperglycemia,<sup>10</sup> and in some instances, this is associated with administration of glucose-rich electrolyte solutions; this syndrome has not been described in adult camelids. Given the lack of knowledge about glucose homeostasis and the greater risk for glucose exposure in neonates, establishing some basic data would have a valuable impact on strategies for care of sick or orphaned crias. The purpose of the study reported here was to investigate glucose tolerance and insulin sensitivity in healthy llama crias.

## Materials and Methods

**Animals**—Seven nursing llama crias between 14 and 30 days of age (mean age,  $20 \pm 5$  days) were catheterized in the right jugular vein and acclimated to their stalls and handling areas in the university clinic for 24 hours. A physical examination and basic clinicopathologic assessments were performed for each cria to ascertain health. Crias were housed with their dams, and each pair was kept in a separate stall. Six of the 7 llamas were privately owned and used in this study after informed consent was received from the owner. The remaining cria was from the university herd. This study was conducted with approval of the Institutional Animal Care and Use Committee of Oregon State University.

**Experimental procedures**—Two trials were performed on each cria. The trials were performed on subsequent days

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in an order that was determined randomly for each cria. For each trial, crias were prevented from nursing by introduction of a gate between them and their dams; the gate remained in place for the period from 15 minutes before initiation of the trial until its conclusion. To initiate each trial, crias received 0.5 g of glucose (50% solution)/kg via rapid IV injection (administration complete in < 10 seconds). Blood samples were collected immediately before glucose injection (baseline, time 0) and at 5, 15, 30, 45, 60, 90, 120, 180, and 240 minutes after glucose injection. The first 2 mL of each sample withdrawn from the catheter was discarded. The crias were additionally administered 0.2 U of regular insulin<sup>b</sup>/kg or an equivalent volume (0.002 mL/kg) of saline (0.9% NaCl) solution IV immediately after the 15-minute blood sample was collected. Blood samples were collected in tubes containing lithium heparin, which were placed on ice immediately and centrifuged in a refrigerated centrifuge within 15 minutes to obtain plasma. Plasma glucose concentrations were determined within 1 hour by use of an automated analyzer.<sup>c</sup> The remaining plasma obtained during the trial in which insulin was not administered was frozen at -70°C for as long as 40 days before determination of insulin concentration via radioimmunoassay.<sup>d</sup> This interval was within the established stability period for insulin.<sup>13</sup> All assays have been validated previously for scientific trials<sup>3</sup> or for diagnostic testing in our clinical laboratory.

The fractional turnover rate (glucose disappearance rate) *k* was calculated for each interval after insulin administration by use of the following formula<sup>14</sup>:

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

where  $\ln[\text{glucose}]_1$  represents the natural log of the concentration of glucose at the beginning of the interval and  $\ln[\text{glucose}]_2$  represents the natural log of the concentration at the end.

The fractional turnover rate of glucose was calculated over the interval from 15 to 45 minutes after glucose infusion. Plasma half-life of glucose was determined for this interval by use of the following formula<sup>14</sup>:

$$\text{Half life (min)} = 0.693/k \times 100.$$

Between trials, crias were allowed to nurse their dams and eat grass hay ad libitum. At the conclusion of the trial, crias and dams were returned to their herds.

**Statistical analyses**—Plasma concentrations of insulin, the calculated differences in plasma glucose concentrations from baseline, and the calculated glucose fractional turnover rate were analyzed for changes over time and differences between days (ie, between llamas receiving glucose treatments with and without insulin administration) by use of a 2-way ANOVA for repeated measures.<sup>15,c</sup> Differences between mean values were detected by use of a Tukey test. The fractional turnover rates and plasma half-life of glucose over the 15- to 45-minute interval were compared by use of the Student paired *t* test.<sup>16</sup> Comparisons were considered significant at values of *P* < 0.05.

## Results

For 45 minutes after IV administration of glucose (without subsequent treatment with insulin), mean plasma glucose concentration in the crias was significantly higher than the baseline value (Figure 1). The peak value of plasma glucose concentration in the crias that did not receive an insulin injection was detected at 5 minutes after glucose administration; the value was 342 ± 47 mg/dL (an increase above baseline value of

187 ± 40 mg/dL). From the 90-minute time point to the end of the trial, plasma glucose concentrations were lower than the baseline value but these differences were not significant.

In crias that did not receive an injection of insulin, fractional turnover rate of glucose was significantly higher than the final value (180- to 240-minute interval) for all intervals up to and including the 45- to 60-minute interval (Table 1). A peak value of the fractional turnover rate of glucose (2.04 ± 1.23%/min) was detected during the first interval (5- to 15-minute interval).

Plasma insulin concentrations increased after glucose administration and were significantly higher than the baseline value for 30 minutes in crias that did not receive an injection of insulin (Figure 2). A peak plasma insulin concentration was detected at 15 minutes after glucose administration; the value was 33 ± 13 μU/mL (triple the baseline concentration of 11 ± 4 μU/mL). From the 60-minute time point to the end of the trial, plasma insulin concentrations were lower than the baseline value but these differences were not significant. Two crias, the oldest and the youngest of the group (14 and 30 days of age, respectively), had peak values of plasma insulin concentration that were at least 33% lower than the mean value (12 and

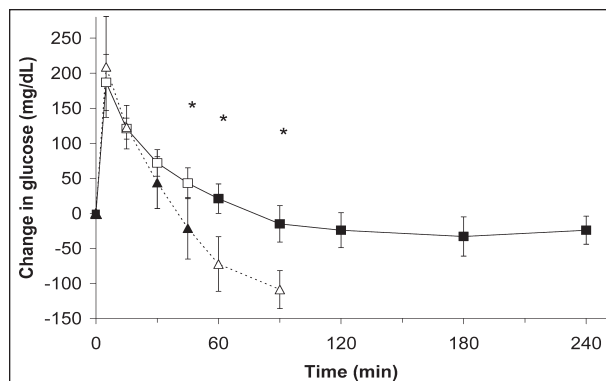


Figure 1—Mean ± SD changes in plasma glucose concentrations from baseline (time 0 [change = 0 at this time point]) values in 7 immature llamas administered glucose (0.5 g/kg) as an IV bolus followed by either an IV injection of saline (0.9% NaCl) solution (0.002 mL/kg; squares) or regular insulin (0.2 U/kg; triangles) 15 minutes later. Solid symbols represent values that are significantly (*P* < 0.05) different from the baseline value of that treatment group. \*Values significantly (*P* < 0.05) different between treatments at this time point.

Table 1—The fractional turnover rate of glucose (%/min) in 7 immature llamas administered glucose (0.5 g/kg) as an IV bolus followed by either an IV injection of saline (0.9% NaCl) solution (0.002 mL/kg) or regular insulin (0.2 U/kg) 15 minutes later.

Interval after glucose administration	Sham treatment	Insulin treatment
5 to 15 minutes	2.04 ± 1.23	2.10 ± 2.26
15 to 30 minutes	1.29 ± 0.34	2.21 ± 0.70
30 to 45 minutes	0.90 ± 0.44	2.68 ± 1.07
45 to 60 minutes	0.79 ± 0.54	3.62 ± 2.63
60 to 90 minutes	0.74 ± 0.32	1.62 ± 0.82
90 to 120 minutes	0.22 ± 0.33	ND
120 to 180 minutes	0.12 ± 0.19	ND
180 to 240 minutes	-0.11 ± 0.12	ND
ND = Not done.		

22  $\mu\text{U/mL}$ , respectively). Of the 7 crias, the oldest also had the lowest baseline plasma insulin concentration (4  $\mu\text{U/mL}$ ).

Plasma glucose concentrations in insulin-treated crias were significantly higher than the baseline value only at the 5- and 15-minute time points and significantly lower than the baseline value at the 60- and 90-minute time points; at 90 minutes after glucose administration, this trial was terminated in most crias because of hypoglycemia (Figure 1). Affected crias were administered a second bolus of glucose (0.25 g/kg) and reunited with their dams. At 45, 60, and 90 minutes after glucose administration, plasma glucose concentrations in insulin-treated crias were significantly lower than the values in crias that did not receive an insulin injection.

The fractional turnover rate of glucose increased sharply after insulin administration and was significantly ( $P = 0.002$ ) different from that of the crias that did not receive an insulin injection, although this difference could not be ascribed to specific intervals (Table 1). However, the fractional turnover rate for glucose calculated for the 15- to 45-minute interval was significantly ( $P = 0.001$ ) higher in insulin-treated crias ( $2.44 \pm 0.82\%/min$ ;) than it was in crias that were not treated with insulin ( $1.10 \pm 0.24\%/min$ ). The half-life of glucose was also significantly ( $P = 0.001$ ) different between the treatment groups: the value was  $31.0 \pm 9.7$  minutes in insulin-treated crias and  $65.7 \pm 13.4$  minutes in crias that were not treated with insulin. Whereas fractional turnover rate was highest in the 5- to 15-minute interval for all but 1 cria during the trial in which insulin was not administered, that interval had the highest rate for only 2 crias during the trial in which insulin was administered. Those 2 crias also had the greatest positive difference in plasma glucose concentration at the 5-minute time point in the trial in which insulin was administered, compared with their values at that time point in the trial in which insulin was not administered. Of the other crias in the trial in which insulin was administered, 3 had their highest fractional turnover rates of glucose in the 30- to 45-

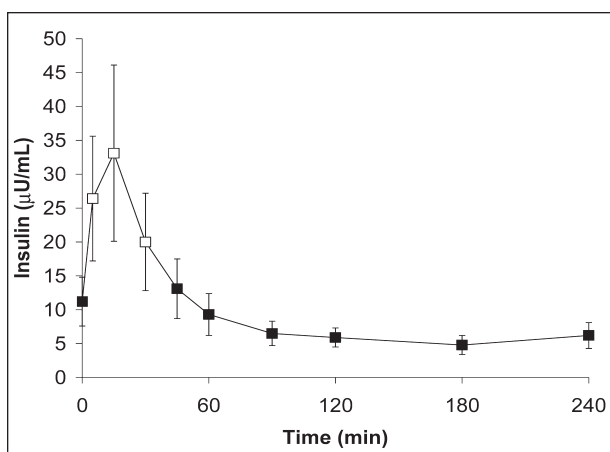


Figure 2—Mean  $\pm$  SD plasma insulin concentrations in 7 immature llamas administered glucose (0.5 g/kg) as an IV bolus (followed by an IV injection of saline solution [0.002 mL/kg]). Solid symbols represent values that are significantly ( $P < 0.05$ ) different from the baseline value of that treatment group.

minute interval and 2 had their highest fractional turnover rates of glucose in the 45- to 60-minute interval. From 30 minutes after glucose administration, the fractional turnover rate of glucose was higher after insulin administration for every interval in every cria, compared with the value in the same interval in the trial in which insulin was not administered.

## Discussion

Llama crias developed lower peak plasma glucose concentrations, had a stronger insulin response, were more sensitive to exogenous insulin, and cleared glucose from plasma more quickly than adult llamas and alpacas.<sup>2,7</sup> Peak plasma glucose concentrations were about 30% lower in crias than in adults tested under comparable circumstances (342 mg/dL vs approx 500 mg/dL), and the difference from baseline concentration was approximately half that determined in adults. These differences may have related to faster plasma glucose clearance in the 5-minute interval before blood samples were obtained or to differences in volume of distribution between immature camelids and adults. Glucose is hydrophilic and distributes quickly throughout the extracellular fluid compartment. Crias of this age (14 to 30 days) have less fat and less gastric fill than adults and hence have a proportionally large extracellular fluid compartment. This difference in camelids has not been estimated previously, but the apparent 2-fold difference in volume of distribution was similar to that determined in neonatal calves.<sup>17</sup> The higher volume of distribution should be kept in mind when treating crias for hypoglycemia and also when extrapolating drug dosages for use in immature camelids.

Even with lower peak plasma glucose concentrations, llama crias cleared glucose from plasma much more rapidly than adult camelids. The fractional turnover rate and half-life of glucose over the 15- to 45-minute interval (1.10%/min and 65.6 minutes, respectively) in llama crias that did not receive an insulin injection were similar to values in adult alpacas administered insulin during glucose tolerance testing ( $1.08 \pm 0.28\%/min$  and 69 minutes, respectively) and greater than values in adult alpacas that were not treated with insulin ( $0.63 \pm 0.06\%/min$  and 111 minutes, respectively) over the same interval.<sup>5</sup> Comparable published data from adult llamas are not available. Nonetheless, the fractional turnover rate during the 15- to 45-minute interval and half-life of glucose in crias that did not receive an insulin injection were still less than values in healthy cows (1.98%/min and 35 minutes, respectively) or dogs (2.76%/min and 25 minutes, respectively).<sup>14</sup>

The crias' strong insulin response was likely partially responsible for the fast glucose clearance. Adult camelids have low circulating concentrations of insulin and respond to exogenous glucose with a 2-fold increase in plasma insulin concentration.<sup>2,3</sup> Basal plasma insulin concentrations were approximately 2-fold higher in crias than in adults, but the crias had been allowed to feed until the onset of the trial. Stimulated values in crias were 3-fold higher than baseline or stimulated adult values. Peak plasma insulin concentra-

tions in crias were still considerably lower than values in adult sheep, cattle, and horses undergoing glucose tolerance tests (usually approx 50  $\mu\text{U/mL}$  on hay diets and  $> 100 \mu\text{U/mL}$  on hay-concentrate diets)<sup>5,18-21</sup> but nonetheless reflect a major difference from values in adult camelids.

The biologic importance of the difference in stimulated plasma insulin concentrations between neonates and adults is difficult to interpret. Higher plasma insulin concentrations in crias (despite the larger extracellular fluid compartment) could signify that crias produce more insulin per unit of body mass than adults. However, because neonates also typically have less skeletal muscle and adipose tissue than adults, this finding could also signify that insulin had a longer half-life in crias because of limited insulin receptors or, alternatively, that crias maximized the insulin effect by increasing the ratio of insulin to insulin receptors.

The reason for the stronger insulin response in crias, compared with that in adult camelids, remains unknown. Among other explanations, a difference in intracellular signaling is possible. Glucose must be trapped in cells through phosphorylation to undergo glycolysis and thereby stimulate insulin release. Results of previous research<sup>a</sup> suggest that there is a reduction in net phosphorylation of glucose in camelid hepatocytes from the fetal period to the adult period. A similar change in beta cells during the postnatal period would result in a decrease in glucose-stimulated insulin production and glucose tolerance. When that change occurs in hepatocytes or whether it occurs in camelid beta cells is unknown.

The greater insulin sensitivity of llama crias determined in our study suggests that crias have greater capacity than adults for cellular uptake of glucose. By having lower fat and muscle mass and a larger volume of distribution for the insulin, crias might have been expected to have lower whole-body insulin sensitivity. Insulin stimulates glucose uptake by interacting with insulin receptors and promoting movement of **glucose transporter (GLUT)-4** to the cell surface. The fact that insulin-treated crias had initial glucose turnover rates similar to those of monogastric animals strongly suggests that the insulin receptors and GLUT-4 are present at that time of life.

On the basis of the variation in fractional turnover rates of glucose after insulin administration, insulin sensitivity varied between crias in our study. Many factors may influence insulin sensitivity, including the number of insulin receptors, amount of GLUT-4, and activity of other hormones. These were not controlled in the present study beyond the crossover nature of the design (each cria received both treatments in a randomly determined order). However, it is important to point out that the general increase in fractional turnover rate of glucose after insulin administration suggested potency in all crias and that the persistence of the high turnover rates in insulin-treated crias, even as blood glucose concentrations decreased into the hypoglycemic range, represented an important physiologic difference from adult camelids.

These findings suggest that most healthy llama

crias have greater glucose tolerance than adult camelids and that administration of boluses of exogenous glucose may provide a useful energy source in crias with insufficient dietary caloric intake. However, higher glucose tolerance of crias likely depends on the pancreatic response, which was variable among crias. Previous clinical experiences also suggest that the pancreatic response in crias may be inadequate under some circumstances and may contribute to the hyperglycemic hyperosmolar disorder, which has been identified in crias but not in neonates of other domestic livestock species.<sup>10</sup> Factors that have been postulated to impair pancreatic sufficiency and glucose tolerance in crias include prematurity, illness, pancreatic exhaustion, and other stressors. On the basis of findings of the present study and previous clinical experiences, clinicians should monitor blood glucose concentrations before and after IV administration of boluses of glucose to crias, especially those that are sick, to avoid deleterious shifts in body water. Single boluses of 0.5 g of glucose/kg appear to be assimilated or excreted within 90 minutes in healthy crias.

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