Anti–glucagon-like peptide-1 immunoreactivity in samples of blood and ileum obtained from neonatal and adult alpacas

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Objective—To compare numbers of L cells in intestinal samples and blood concentrations of glucagon-like peptide (GLP)-1 between neonatal and mature alpacas.

Sample—Intestinal samples from carcasses of 4 suckling crias and 4 postweaning alpacas for immunohistochemical analysis and blood samples from 32 suckling crias and 19 healthy adult alpacas for an ELISA.

Procedures—Immunohistochemical staining was conducted in accordance with Oregon State University Veterinary Diagnostic Laboratory standard procedures with a rabbit polyclonal anti–GLP-1 primary antibody. Stained cells with staining results in ileal tissue were counted in 20 fields by 2 investigators, and the mean value was calculated. For quantification of GLP-1 concentrations, blood samples were collected into tubes containing a dipeptidyl peptidase-4 inhibitor. Plasma samples were tested in duplicate with a commercial GLP-1 ELISA validated for use in alpacas.

Results—Counts of stained cells (mean ± SD, 50 ± 18 cells) and plasma GLP-1 concentrations (median, 0.086 ng/mL; interquartile range, 0.061 to 0.144 ng/mL) were higher for suckling alpacas than for postsuckling alpacas (stained cells, 26 ± 4 cells; plasma GLP-1 concentration, median, 0.034 ng/mL; interquartile range, 0.015 to 0.048 ng/mL).

Conclusions and Clinical Relevance—Older alpacas had lower numbers of L cells in intestinal tissues and lower blood concentrations of GLP-1 than those in neonates. These findings suggested that there may be a decrease in the contribution of GLP-1 to insulin production in adult alpacas, compared with the contribution in neonates. (Am J Vet Res 2013;74:1409–1414)
Microbial fermentation in the forestomach converts carbohydrate into volatile fatty acids, which are then readily absorbed across the gastric mucosa. Thus, it is possible that maturing camels have a lower quantity of intact carbohydrates that pass into the intestinal lumen, which leads to a decrease in enteroendocrine cell populations or that pass into the intestinal lumen, which leads to a decrease in GLP-1 release. This could play a role in the poor insulin response of adult alpacas to pathological and experimentally induced hyperglycemia. Therefore, the purpose of the study reported here was to investigate whether there are differences in blood concentrations of GLP-1 and ileal populations of GLP-1 immunoreactive L cells between suckling and adult alpacas.

Materials and Methods

Animals—For immunohistochemical analysis, tissue samples were collected from the carcasses of 4 suckling alpaca crias (age, 1 day, 4 days, 4 weeks, and 5 weeks) and 4 postweaning alpacas (age, 10 months, 3 years, 4 years, and 8 years) submitted to the Oregon State University College of Veterinary Medicine Diagnostic Laboratory for necropsy. These alpacas had recently died or been euthanized for a variety of causes, primarily musculoskeletal disorders for the suckling crias and dermatologic disorders as part of a herd culling program for the postweaning alpacas, unrelated to disorders of energy metabolism or gastrointestinal disease.

For measurement of blood concentrations of GLP-1, blood samples were collected from 19 healthy adult (≥2 years old) alpacas and 32 suckling crias (≤1 month old). Eleven of the adults were part of a university research herd, and 8 adults and all the crias were client-owned animals. All adult alpacas had unlimited access to feed, and crias were housed with their dams and had unrestrained suckling. Clients provided informed consent for use of their animals in the study, and all components of the study involving live animals were conducted with the approval of the Oregon State University Institutional Animal Care and Use Committee.

Immunohistochemical analysis—Sections of ileum and skeletal muscle from the diaphragm were collected from each alpaca carcass. Duodenal samples were not collected because preliminary studies revealed that rapid autolysis rendered them unusable. The skeletal muscle tissue obtained from the diaphragm, which is generally devoid of GLP-1, and tissue samples incubated without the primary antibody were used as negative control samples.

Samples of pancreas and ileum were collected from mice euthanized as part of an unrelated research project at the same institution. These tissues were used as positive control samples because they have anti–GLP-1 immunoreactivity in several species, and the specific primary antibody used in the study had been tested in murine tissues.

Samples were fixed in neutral-buffered 10% formalin for 24 hours, embedded in paraffin, cut at a thickness of 5 μm, and placed on staining system slides. For each alpaca, a single slide was made that contained a small sample of diaphragm and 2 to 4 sections of ileum. Slides were incubated for 1 hour at 60°C, deparaffinized, and hydrated through 2 rinses in xylene, 2 rinses in 100% ethanol, 1 rinse in 80% ethanol, and a final rinse with tap water. Inmate tissue peroxidase activity was blocked by incubating slides in 3% H2O2 TBST wash buffer for 10 minutes. Slides were loaded into a staining system slide holder; nonspecific proteins were blocked by incubating slides for 10 minutes in a serum-free protein block. Slides then were incubated with primary rabbit polyclonal anti–GLP-1 antibody (200 μL/well) diluted in antibody diluent for 30 minutes at room temperature (approx 22°C) in a sealed humidity chamber to prevent air-drying of the tissue sections. Negative control samples were incubated with a universal negative control solution for rabbit primary antibodies.

After incubation, slides were washed in TBST wash buffer 3 times (5 min/wash) and incubated in the chamber with secondary antibody (horseradish peroxidase polymer anti-rabbit) for another 30 minutes.
Slides were washed in TBST wash buffer 3 times (5 min/wash), rinsed in deionized water, and coated by incubation with a stain for 5 minutes. Slides were rinsed with deionized water, incubated with Gill hematoxylin stain for 5 minutes, rinsed again with deionized water, and briefly immersed in TBST wash buffer to enhance the color of the hematoxylin stain. Slides were dehydrated, and a cover slip was applied. Stained cells in alpaca ileal tissue were counted in 20 fields at 400X magnification by 2 investigators who were not apprised of the source of the tissue samples, and the mean value for each alpaca was calculated.

Analysis of plasma concentrations of GLP-1—All blood samples were collected in the morning approximately 2 to 4 hours after the morning feeding for the adults and during a period of suckling activity for the crias. For client-owned alpacas, collection of blood samples for the study was timed to coincide with collection of blood samples for management or regulatory purposes, such as for determination of immunoglobulin concentrations, testing to determine parentage, or testing needed for completion of a health certificate.

Each blood sample was collected from a jugular vein into a tube containing a dipeptidyl peptidase-4 inhibitor, and tubes were then immediately placed on ice. Plasma was separated by centrifugation (1,200 X g for 10 minutes) within 2 hours after collection and frozen at –80°C until analysis.

All plasma samples were tested in duplicate with a commercial competitive GLP-1 ELISA that was validated for use in camelids. Briefly, the matrix effect of camelid plasma was assessed by determining dilutional parallelism and the interassay coefficient of variation of standard samples reconstituted in camelid plasma. Known concentrations of GLP-1 reconstituted in camelid plasma had a high degree of parallelism, and the interassay coefficient of variation was <12%.

An extraction-free protocol was used for the ELISA. Briefly, 25 µL of anti–GLP-1 antibody solution was added to each well of a 96-well plate, and the plates were incubated for 1 hour at room temperature. After incubation, samples and standards were added in duplicate (50 µL/well), and plates were incubated at room temperature for 2 hours. Then, a competitive biotinylated GLP-1 tracer was added to each well, and plates were incubated overnight at 4°C. The following day, plates were washed 5 times with wash buffer, streptavidin–horseradish peroxidase was added to each well (100 µL/well), and plates were incubated at room temperature for 1 hour. Plates were again washed 5 times, 3,3’,5,5’-tetramethylbenzidine substrate was added, and plates were incubated at room temperature for 30 to 60 minutes, after which 100 µL of a stop solution (2N HCl) was added to each well. Plates were analyzed with a spectrophotometer at 450 nm.

Statistical analysis—Mean cell counts obtained by immunohistochemical analysis were compared between suckling and older alpacas with a randomization test to detect differences in means, whereby all possible combinations of the data set were tested to estimate the likelihood that the observed difference in means was a chance occurrence. Plasma GLP-1 concentrations were compared between crias and adults by use of the Mann-Whitney rank sum test. A value of P < 0.05 was considered significant for all analyses.

Results

Cells containing GLP-1 immunoreactivity were identified on the basis of dark, red staining of the cytoplasm (Figure 1). Negative control samples did not have cell staining, whereas positive control samples had evidence of cell staining. Stained cells were identified in the crypts and villi of the mucosal epithelium of the ileum obtained from all alpacas. Most stained cells were teardrop shaped, with the apical portion appearing to project into the gastrointestinal lumen. All suckling crias had significantly (P = 0.014) higher numbers of stained cells (mean ± SD, 50 ± 18 cells; range, 36 to 76 cells) than any of the postsuckling alpacas (mean, 26 ± 4 cells; range, 21 to 31 cells). The youngest cria (1 day old) had the highest mean cell count (76 cells), and the oldest alpaca (8 years old) had the lowest mean cell count (21 cells).
Plasma GLP-1 concentrations of crias and adult alpacas were compared. Plasma GLP-1 concentrations for suckling crias (median, 0.086 ng/mL; interquartile range, 0.061 to 0.144 ng/mL) were significantly (P < 0.001) higher than those for adult alpacas (median, 0.034 ng/mL; interquartile range, 0.015 to 0.048 ng/mL).

Discussion

Suckling alpacas had a significantly higher number of GLP-1 immunoreactive L cells in the ileum than did postsuckling alpacas. It is not known whether the health status of the alpacas affected these results. There was no plausible reason for an increase in the number of L cells in the neonates, and the adults typically did not have disorders that affected appetite, which made such an effect unlikely but still possible. Analysis of the results suggested that there was a decrease in the number of L cells coinciding with the change in diet and digestive function from suckling crias to adult alpacas. Other species undergo similar dietary and physiologic changes, but the degree of change in the small intestinal environment of omnivores and animals with simple stomachs may be less than that in alpacas. Comparative data from other species are sparse. Although enteroendocrine cell populations have been measured in various species, there is little information concerning age-related changes, and most studies have involved only mature animals. Data from calf of various ages are equivocal. In 1 study,17 it was suggested that calves have higher densities for a number of enteroendocrine cells than do adult cattle, but in another study18 in which investigators specifically compared tissue GLP-1 immunoreactivity at various times from fetus through adult, results suggest a decrease throughout the gastrointestinal tract from the fetal state through weaning, with a slight rebound increase with maturation. Adult cows have slightly higher densities of L cells in ileal tissues than do 1- to 2-week-old suckling calves. The postnatal decrease in cattle could possibly explain the high L cell counts in the youngest cria in the present study, but the fact that the older crias still had counts higher than those of the adult alpacas could represent an important difference between alpacas and cattle.

Adult alpacas in the present study had plasma GLP-1 concentrations that were lower than those found in adults of several other species. The concentrations in the fed alpacas reported here were similar to those in fasting humans in some studies22,23 but 20% to 65% lower than those in fed nonlactating dairy cows24 or goats25 and one-third to one-tenth lower than those detected in fed lactating dairy cows,24 goats,25 or humans after a meal29 or oral glucose tolerance test.33 To our knowledge, there is only 1 report26 of any population of healthy mammals that had lower blood concentrations of GLP-1 than those in healthy adult camelds, and that study involved a small control group (n = 7) of fasting humans of various ages. The role of feeding was not investigated in the present study, but GLP-1 concentrations change little (approx 10%) with feeding in weaned and ruminating sheep and cattle.26,27,37 Thus, feeding was not considered to be a major factor in these alpacas. On the basis of these comparisons, adult alpac-
is a more important insulin secretagogue in camelids; however, we did not test this hypothesis.

The potential role of GLP-1 in camelids is also supported by the experimental use of synthetic analogues. Glucagon-like peptide-1 acts directly on beta islet cells in the pancreas via binding to specific G-protein-coupled receptors, leading to increased cytosolic cAMP concentrations, opening of voltage-gated calcium channels, and thus an increase in insulin exocytosis.11 Neonatal and adult camelids appear to have similar pancreatic islet populations,38 suggesting the presence of similar target tissue. Stimulation of adult alpaca islet cells in vivo with an exogenous GLP-1 mimetic appears to restore the insulin response to neonatal concentrations.11 These findings support that some extrapancreatic factors, such as declining GLP-1 production, may play a role in the declining insulin response.

Results of the present study may prove useful when devising management and treatment strategies for camels with energy metabolism disorders. Currently, little is known about the effects of various diets on the longevity of L cells or stimulation of incretins in camelids. Certain diets may be particularly beneficial or harmful; thus, the substrates entering the small intestines may potentially promote or compromise the function and quantity of L cells and potentially retard or accelerate the relative decrease in glucose regulation that develops with maturation in camelids. Specific nutrients can affect blood GLP-1 concentrations in ruminants,26,39–41 but whether they would have similar effects in camelids is unknown. For sick camelids with disorders of energy metabolism, administration of insulin is currently one of the cornerstones of treatment but has limitations, including the risk of harmful iatrogenic hypoglycemia.8 The main justification for the use of insulin in these camelids is the low circulating insulin concentration.1,4–6 On the basis of results for the present study, the same argument could be made for medications that augment incretin function. Incretin mimetics (including GLP-1 agonists) and dipeptidyl peptidase-4 antagonists are being widely used as alternatives to insulin for the treatment of humans with insulin-independent diabetes mellitus. Such agents have been limited use in camelids,11 but they may prove to be safe, effective treatments when an increase in insulin activity is desired. Furthermore, their use may be justified on the basis of low endogenous concentrations.

References


