INCRETIN-SECRETING K AND L CELLS IN THE EQUINE SMALL INTESTINE

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INTRODUCTION

Laminitis is a common and debilitating disease of horses, resulting in significant suffering and economic loss *(Fig. 1)*. Research has identified hyperinsulinemia [for example, as seen in equine metabolic syndrome (EMS) *(Fig. 2)*] as a significant risk factor for laminitis.[1,2] The exact reason for hyperinsulinemia in susceptible horses is unknown, but one hypothesis pertains to the enteroendocrine axis and, specifically, the incretin hormones known as glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). GIP and GLP-1 are secreted by intestinal cells called K and L cells, respectively, and one of their chief functions is to stimulate insulin release from pancreatic β cells in response to ingesta.[3] It has been proposed that the intestinal lining of EMS-affected horses may be populated by an exceptionally high number of K and L cells, and hyperinsulinemia results from excessive incretin responses to feeding. Therefore, the objective of this study was to first assess the quantity and distribution of K and L cells in the small intestine of healthy horses.

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Figures 1 & 2: Depictions of equine metabolic syndrome (EMS). *Figure 1* shows abnormal hoof growth that resulted from chronic endocrinopathic laminitis as a consequence of EMS. *Figure 2* depicts the typical appearance of an EMS-affected horse, exhibiting regional adiposity. EMS is characterized by obesity, activated inflammation, dyslipidemia, and insulin resistance (IR), and affected horses are predisposed to pasture-associated laminitis when grazing rich grass.[4] Laminitis results from hyperinsulinemia (triggered by ingestion of nonstructural carbohydrates and exacerbated by IR), because insulin stimulates basal epithelial cell proliferation in the hoof via insulin-like growth factor receptors within the lamellae.[5] Once stimulated, the strength of attachment of proliferating basal epithelial cells to underlying basement membrane is decreased, placing the hoof-lamellar interface at risk of laminitic separation (causing pain, altered horn growth, and ultimately sinking or rotation of the distal phalanx, known as "founder").

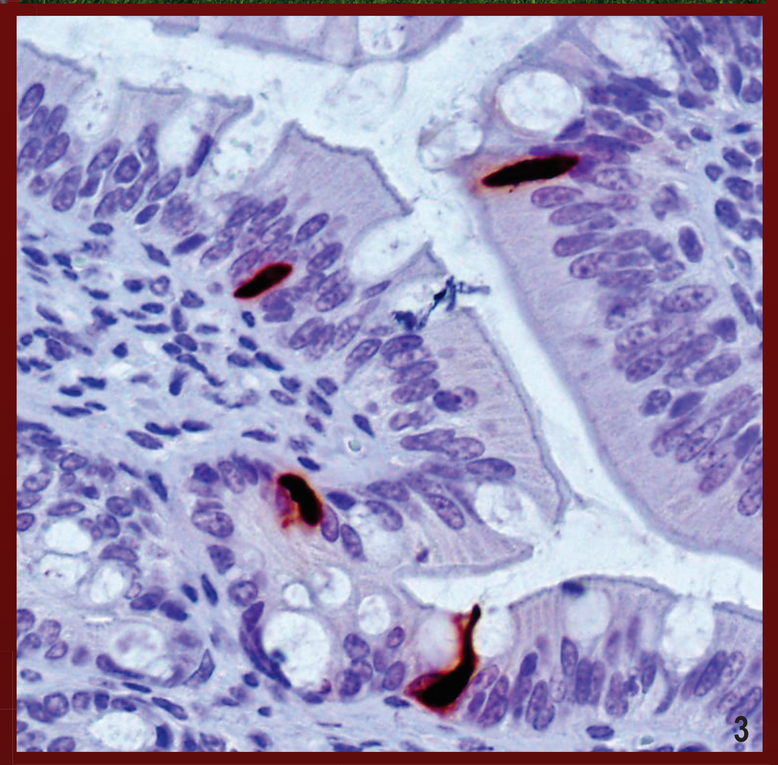

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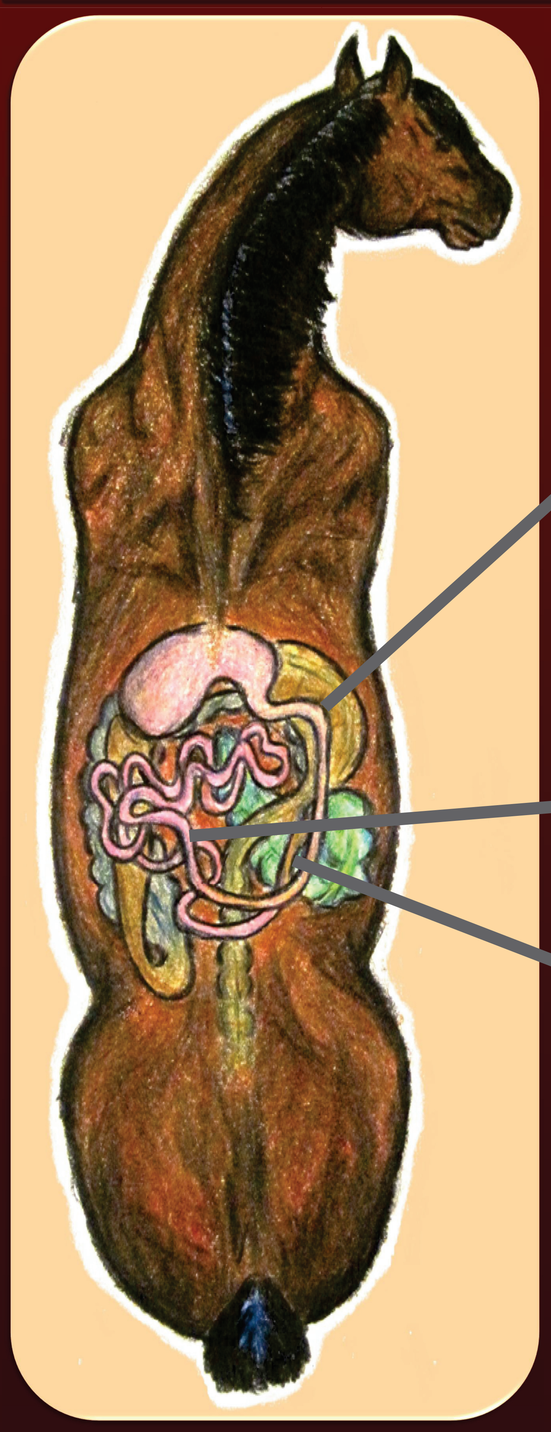
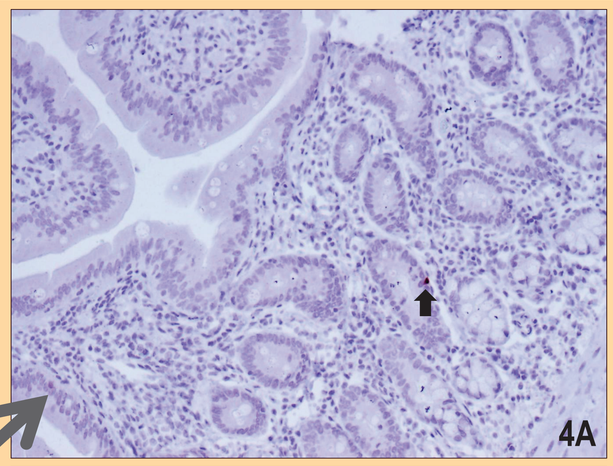
Figure 3: Microscopic appearance of GLP-1 positively labeled L cells in the ileal epithelium (400X).

METHODS

Three horses (two mares and one gelding, ranging in age from 5-19 years) without gross evidence of endocrine or gastrointestinal pathology were used in this study. Immediately following euthanasia (to preclude autolysis), two samples of the mucosa (one from the mesenteric and one from the antimesenteric region) were each collected from the duodenum, jejunum, and ileum. These samples were fixed in formalin, embedded in paraffin, sectioned to 5 μm thickness, and mounted on glass slides. The sections were then stained with anti-GIP or anti-GLP-1 antibodies to identify K cells or L cells *(Fig. 3)*. Briefly, the prepared sections were pretreated by a heat-induced antigen retrieval method using citrate buffer (0.01M, pH 6.0). The primary antibodies were rabbit anti-human GIP polyclonal antibody (1:300, Bioss, Woburn, MA) or mouse anti-[all mammals] GLP-1 monoclonal antibody (1:500, Abcam, Cambridge, MA). EnVision™+ system (Dako, Carpinteria, CA) was used for antigen detection and the immunoreactivity was visualized using AEC Chromogen (Biocare Medical, Concord, CA). Hematoxylin was used for counterstain. The slides were systematically viewed on a microscope at 200X magnification so the populations of positively-stained cells in crypts and villi could be identified and counted by a trained observer *(Fig. 4)*. The length of sample in each case was measured along the mucosal border for comparative purposes by digitally scanning the slides, then tracing the outline in an image editing program. Results were expressed as mean number of positive cells [(mesenteric + antimesenteric)/2] per unit length of intestinal segment.





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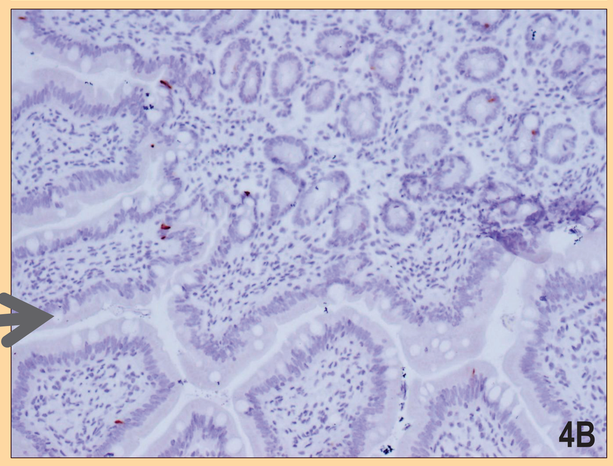

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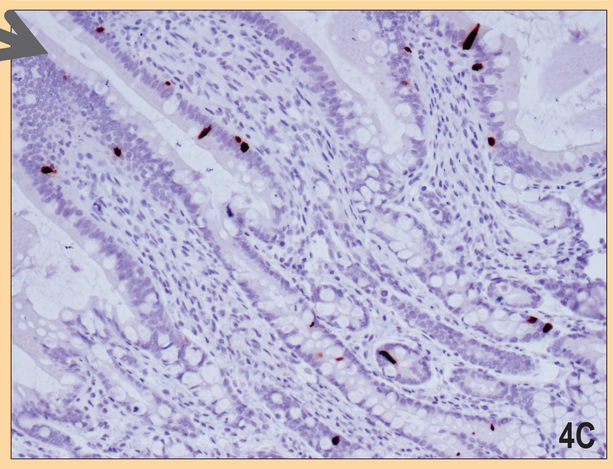

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Figure 4: Microscopic appearance of the duodenum *(A)*, jejunum *(B)*, and ileum *(C)* following GLP-1 immunohistochemistry to identify L cells (200X) alongside an anatomical schematic to illustrate locations of sample collection. A representative positive cell has been identified by an arrow in the cryptal epithelium of the duodenum; other images depict multiple positive cells throughout the mucosa. Notice the higher prevalence of L cells in crypts (versus villi) and the increasing number of L cells seen aborally from duodenum to ileum.

RESULTS and DISCUSSION

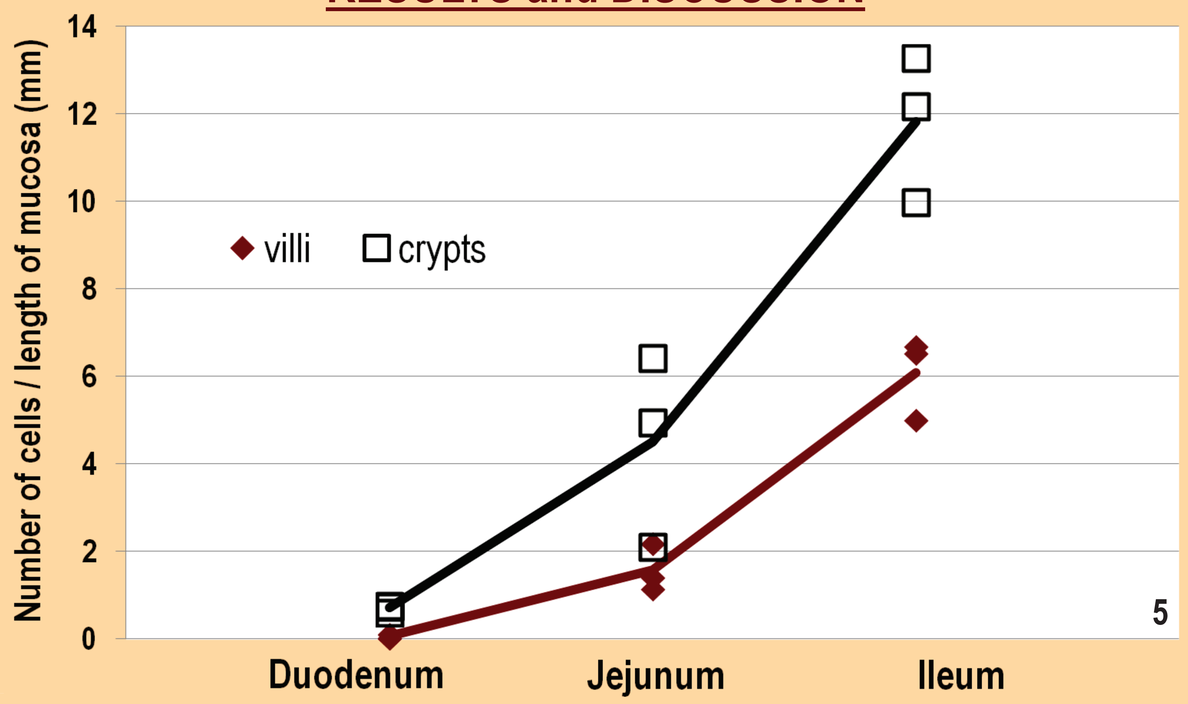

Figure 5: Quantification of L cells in sections from duodenum, jejunum, and ileum. Units are number of cells positively stained for GLP-1 per millimeter of mucosa, averaged between mesenteric and antimesenteric samples. Trend lines were obtained by averaging the values for the three horses in each segment of bowel and region of mucosa, illustrating the relative increase in L cell density exhibited by both villous and cryptal epithelium in more aboral segments.

Results of L cell quantification are depicted above *(Fig. 5)*. Increasing L cell density was evident in the more aboral aspects of the small intestine; this finding is consistent with previous studies in other species, which report the greatest L cell numbers in the ileum and the least in the duodenum.[6] L cells were also more numerous in the cryptal epithelium relative to the villi; this finding contrasts with earlier reports in other mammals, where no difference between the two regions of mucosa was observed.[6] Further research with greater numbers of normal horses is needed to verify the trends observed in this study. Research with horses affected by EMS is also necessary to investigate the possible role of an increased population of GLP-1 secreting cells as a feature of this condition. (Results for quantification of K cells are pending.)

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ACKNOWLEDGMENTS

Student support for this project was provided through the University of Missouri College of Veterinary Medicine Agricultural Experiment Station Funds via the Veterinary Research Scholars Program. Funding for sample handling and immunohistochemistry was provided by the Animal Health Foundation of Pacific, MO. Special thanks to Candace Kassel, Karen Clifford, and Don Connor for their assistance with staining, image capture, and poster design, respectively.