Effect of proteinase inhibition on glucagon-like peptide-2 concentrations in blood samples from healthy cats

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Background

- GLP-2 is an enteroendocrine hormone with intestinal protective and proliferative effects
- GLP-2 secretion is stimulated by enteral nutrition
  - GLP-2 is co-secreted with GLP-1 from intestinal endocrine L-cells
  - The peak secretion time for GLP-1 in cats and humans is between 30 - 75 minutes
  - Glucose-dependent insulinotropic polypeptide mediates GLP-2 secretion and is stimulated more by fat than other nutrients in cats
- GLP-2 is rapidly broken down ($T_{1/2}$ = 7 minutes in rats and humans) from the active form (1-33) GLP-2 to the inactive form (3-33) GLP-2 by the enzyme dipeptidyl peptidase IV (DPP-IV)
- Previous studies based on human GLP-2 found that use of the proteinase inhibitors Diprotin A and Aprotinin was the only viable method that delayed peptide degradation and resulted in increased measured concentrations
  - Diprotin A is a DPP-IV specific inhibitor
  - Aprotinin is a trypsin inhibitor
- While the sequence of GLP-2 in cats is unknown, GLP-2 is highly conserved, with 100% conservation of the N-terminal sequence across all studied mammalian species

Objective

- To determine the effect of sample collection with or without proteinase inhibition on measured pre- and post-prandial plasma GLP-2 concentrations in healthy cats.

Hypothesis

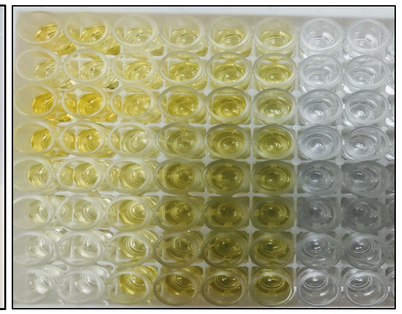
- Measured GLP-2 concentrations will be significantly greater in samples with proteinase inhibitors added.

Materials and Methods

- Study design: Prospective
- Population: 6 healthy, client-owned cats
  - Eligibility was based on physical exam including a body condition score of 4-6/9, and lack of abnormalities on blood work (CBC, serum chemistry profile)
  - Exclusions: <1 year of age, systemic or GI clinical signs, medications other than preventatives, prescription diets to control historical GI signs
- Sample collection:
  - After a ≥10 hour fast a pre-prandial blood sample was obtained
  - The cats were fed a standardized commercial diet (Protein 10.4 g/100 kcal, Fat 7.25 g/100 kcal, CHO 0.12 g/100 kcal) at ¼ resting energy requirements
  - A second, 1-hour post-prandial blood sample was obtained
- Sample handling:
  - Blood was collected into chilled EDTA tubes on ice
  - At the time of collection, half of each sample was immediately mixed with 10% volume per blood volume of the proteinase inhibitors Aprotinin and Diprotin A (ILE-PRO-ILE)
  - Samples were immediately centrifuged (temperature controlled), separated, and stored at -80^{o} C
- GLP-2 concentrations were assessed using commercial ELISA tests marketed for cats based on the human sequence of (1-33) GLP-2 (MyBiosource)
  - Monoclonal mouse capture antibody
  - Polyclonal rabbit detection antibody
  - Reported assay detection limits are 0.96 – 2.14 ng/ml with a sensitivity of 0.1 ng/ml in feline samples; however, results using this ELISA have not been published
- All samples were run in duplicate on two 96 well plates
  - Plate one consisted of samples without proteinase inhibitors and six random samples (three from plate one and three from plate two)
  - Plate two consisted of samples with proteinase inhibitors added and six random samples (three from plate one and three from plate two)

Data

- Population:
  - 5 spayed females, 1 castrated male
  - Breeds: 5 domestic short hair, 1 sphinx
  - Median age: 3.5 years (Range 1.5 - 6.9 years)
  - Median weight: 4.35 kg (Range 3.8 - 4.96 kg)
  - Median body condition score: 5/9 (Range 4 - 6/9)



Statistical Analysis

- Data were assessed for normality using the Shapiro-Wilk test
- The concentration of GLP-2 was compared between samples with and without proteinase inhibition using a paired t test
- $p < 0.05$ was considered significant

Data

- GLP-2 was detected in all samples
- Intra-assay variability was 3.1% in plate 1 and 2.56% in plate 2
- Inter-assay variability was 7.83%

Data

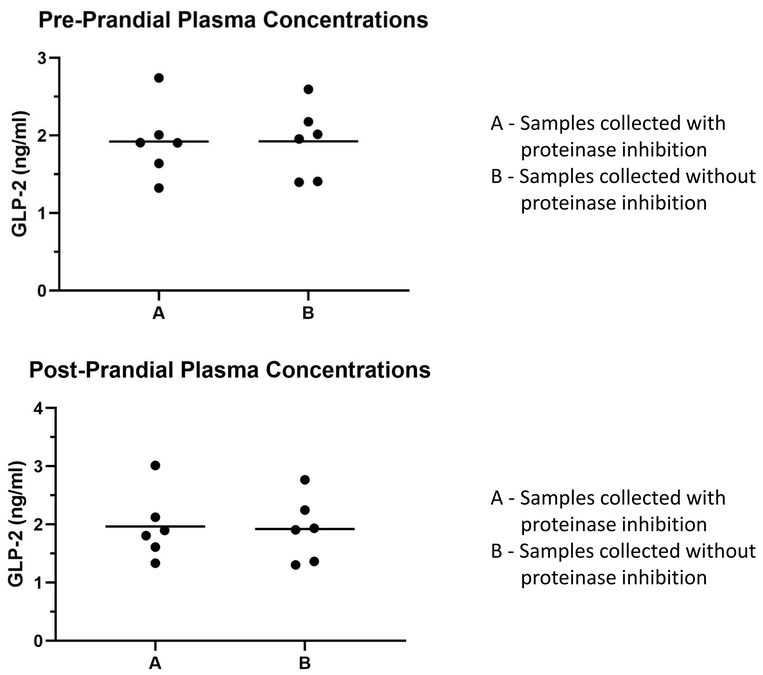


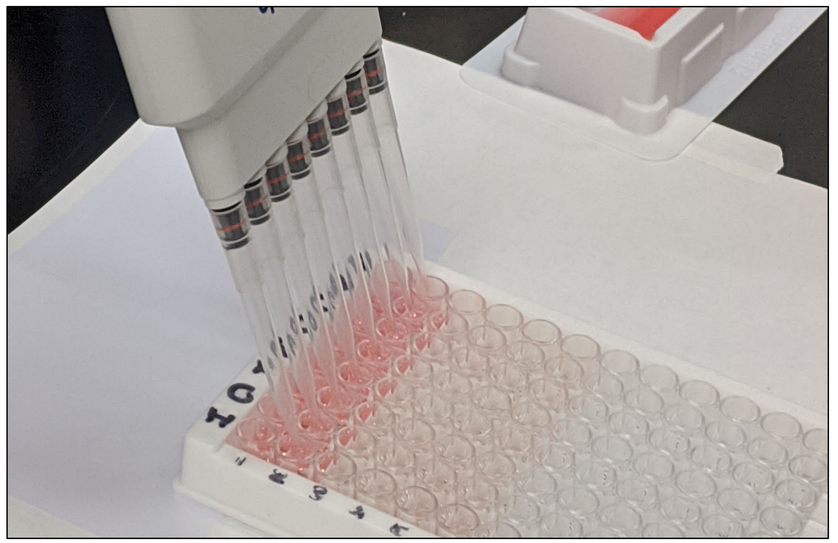
Figure 1. Individual cat GLP-2 concentrations in pre-prandial (top) and post-prandial (bottom) plasma samples from healthy cats.

Data

- There was no significant difference in GLP-2 concentration between samples with and without protease inhibitors:
  - Mean pre-prandial concentration with proteinase inhibition 1.92 ng/ml (SD = 0.47) vs 1.93 ng/ml (SD = 0.48) without proteinase inhibition (p = 0.96)
  - Mean post-prandial concentration with proteinase inhibition was 1.97 ng/ml (SD = 0.58) vs 1.92 (SD = 0.55) without proteinase inhibition (p = 0.55).

Discussion and Conclusions

- GLP-2 was successfully measured using a commercially available ELISA
- Addition of the proteinase inhibitors Aprotinin and Diprotin A at the time of sample collection did not affect measured concentration
- Additional studies are needed to determine whether results are due to differences in secretion or enzymatic GLP-2 degradation in cats compared to humans, other sample handling variables, or specificity of the ELISA to distinguish (1-33) vs (3-33) GLP-2
- The next step of this research is to sequence feline GLP-2 using immunoprecipitation, compared sample concentrations of (1-33) and (3-33) GLP-2 using high performance liquid chromatography and perform GLP-2 ELISA quantification on samples after GLP-2 has been precipitated out, which may provide further insight on the results that were observed.



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