In the study the stability of GLP-1, GIP, GIP, Glucagon and Ghrelin was investigated by spiking these peptides into blood samples to assess the effects of endogenous and protease inhibitors. For GLP-1 instability, stable subject-specific peptides were used as an internal control MALDI-TOF MS analysis. The lack of half-life GLP-1 peptides (7-36A, 10-27), was found to be not only by GLP-1 activity but also by endo-endopeptidase activity.

Further digestion of the GLP-1 fragments was due to the intrinsic peptidase activities. Time-course kinetic analysis indicates that the substrate specificity of the intrinsic peptidase activity is related to sample type, and the serum intrinsic peptidase activity is broader than that in plasma samples. To stabilize these metabolic peptides, a 30s-based peptidase inhibitors was included into the blood collection tubes. Our results demonstrate that both GLP-1 and peptide inhibitors significantly increase the half-life of GLP-1, GIP, Glucagon and Ghrelin peptides.

**Methods and Procedure**

**Blood Collection and Plasma/ Serum Preparation**

Human blood from healthy individuals was directly drawn into a variety of plasma and serum tubes, including EDTA, P700*, and P800* tubes (BD, NJ) (*-For Heparin Plasma, and Serum tubes). The sample processes were followed as described previously (9).

**Peptides**

- GLP-1 (7-32):
  - HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-OH
- GIP (7-36A):
  - HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-OH
- Glucagon:
  - HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
- Ghrelin:
  - EFIAWLVKGR-OH

**Sample Preparation for Time-Course MALDI-TOF Mass Spectrometric Analysis**

- Specified peptides were spiked into thawed serum or plasma samples to a final ~ 40 fmol/μL/mL.
- Samples were incubated at RT in a time-dependent manner.
- At specified time period (0-96 h), the samples were processed with a final concentration of 1% AN and 2% TFA.
- The treated samples were analyzed by MALDI-TOF MS analysis.

**Time-Course Experiments**

- Specified peptides were spiked into a fresh serum or plasma samples to a final concentration of 1% AN and 2% TFA.
- Samples were incubated at RT in a time-dependent manner.
- At specified time period (0-96 h), the samples were processed with a final concentration of 1% AN and 2% TFA.

**Peptide purification**

- Prepared for extraction using Zip-Tip C18 (Millipore), followed by MALDI-TOF MS analysis.

**Stability and Kinetic Analysis**

Stability in BD™ P700 and P800: GLP-1 (7-36A) and GIP (3-42) are more stable than Glucagon and Ghrelin, respectively. The GIP (3-42) activity is not detectable.

GLP-1 in Serum

- The half-life of three GLP-1 peptides (GLP, GIP, Glucagon) was found to be not only by GLP-1 activity but also by endo-endopeptidase activity.

GLP-1 (7-36A), T1/2 = 2.36 h
- GLP (7-36A) and GIP (3-42) are more stable than Glucagon and Ghrelin, respectively.
- The GIP (3-42) activity is not detectable.

Incretin Peptides Are Stabilized in the Inhibited Plasma Samples

- GLP (7-32) and GIP (7-36A) are more stable than GLP (7-36A) and GIP (3-42) respectively.

GLP (7-32), T1/2 = 108 h
- GLP (7-32) and GIP (7-36A) are more stable than GLP (7-36A) and GIP (3-42) respectively.

GIP (7-36A), T1/2 = 5.6 h
- GIP (7-32) and GIP (7-36A) are more stable than Glucagon and Ghrelin, respectively.

GIP (7-32), T1/2 = 290 h
- GIP (7-32) and GIP (7-36A) are more stable than Glucagon and Ghrelin, respectively.

Mechanistic Analysis of GLP-1 Digestions G36A and AG36 in Serum

- GLP-1 is degraded by two N-terminal residues from G36A and AG36.
- The kinetics of degradation of GLP-1 peptides in serum and plasma samples are summarized in Table 1.

References

(2) Glucagon: HSQGTFTSDYSKYLDSRRAQDFVQWLMNT
(3) Yi, J.; Craft, D.; Gelfand, C. A., 2005, in press
(4) Add Control Peptide
(5) Specified peptides were spiked into thawed serum or plasma samples to a final ~ 40 fmol/μL/mL

**Conclusions**

- Intrinsic peptidase activities cause the instability of metabolic peptides:
  - GLP-1 in serum is destabilized by the intrinsic peptidase activities.

- The kinetic analysis provides a quantitative measurement of peptide stability (T1/2).

- The generated fragments are subject to further digestion by intrinsic peptidases.

- Peptide digestion caused by intrinsic peptidases follows a first-order reaction:

- The kinetic analysis provides a quantitative measurement of peptide stability (T1/2).

- Both BD P700 and P800 provide stabilization of GLP-1.

- P800 stabilizes also additional three metabolic peptides: GIP, Glucagon and Ghrelin.

- Time-course MALDI-TOF MS provides a feasible method for investigation of peptide biomarker stability under preanalytical processes.

**Summary of Metabolic Peptide Preservation**

- GLP-1 (7-32), T1/2 = 108 h
- GIP (7-36A), T1/2 = 5.6 h
- Glucagon, T1/2 = 290 h
- Ghrelin, T1/2 = 2.36 h

- GLP-1 is more stable than G36A and AG36 in the same sample.
- Further digestion of the GLP-1 fragments was due to the intrinsic peptidase activities.
- Time-course kinetic analysis indicates that the substrate specificity of the intrinsic peptidase activity is related to sample type.
- The serum intrinsic peptidase activity is broader than that in plasma samples.
- To stabilize these metabolic peptides, a 30s-based peptidase inhibitors was included into the blood collection tubes.
- Our results demonstrate that both GLP-1 and peptide inhibitors significantly increase the half-life of GLP-1, GIP, Glucagon and Ghrelin peptides.