Development of GIP ELISAs: The Next Level of Diabetes Management

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ABSTRACT

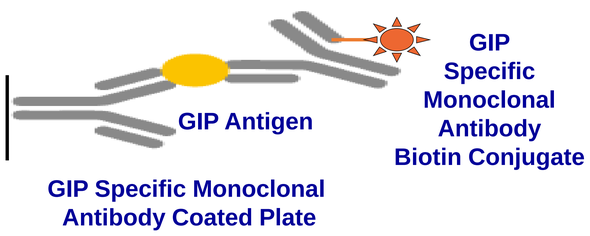
Background: To develop sensitive and specific ELISAs for the quantitative measurement of total and intact GIP in human plasma, and other biological fluids. Glucose-dependent insulinotropic polypeptide (GIP) is a peptide hormone consisting of 42 amino acids (1-42aa). This undergoes processing by a dipeptidyl peptidase-4 (DPP-4) and produces GIP (3-42aa) and GIP (3-30aa) fragments. The processed forms have higher affinity and very efficiently inhibits GIP receptor (GIPR) activity with no intrinsic activity. It has also been observed that the GIP response is dependent not only on meal size but also on meal composition. GIP has been found to have physiological and pharmacological relevance in the development of obesity and the pathogenesis of cardiovascular disease besides its involvement in type 2 diabetic pathophysiology. GIP acts in the entero-insular axis as an anabolic hormone that increases insulin levels, which in return increases glycogen and fatty acid synthesis and inhibits the breakdown of fat. GIP also has extra-pancreatic functions as well as roles in the stomach to reduce acid secretion by the parietal cells. On the bone, GIP has a dual effect as it causes the proliferation of osteoblasts as well as inhibits osteoclastic bone resorption. The widespread expression of GIP-R in the brain suggests that GIP might play an essential function in neuro-signaling mechanisms.

Methods: Highly specific and reproducible total GIP (AL-1013) and Intact GIP (AL-1022) ELISAs have been developed using specific monoclonal antibodies to help estimate the total and intact GIP concentrations in plasma in the respective immunoassays.

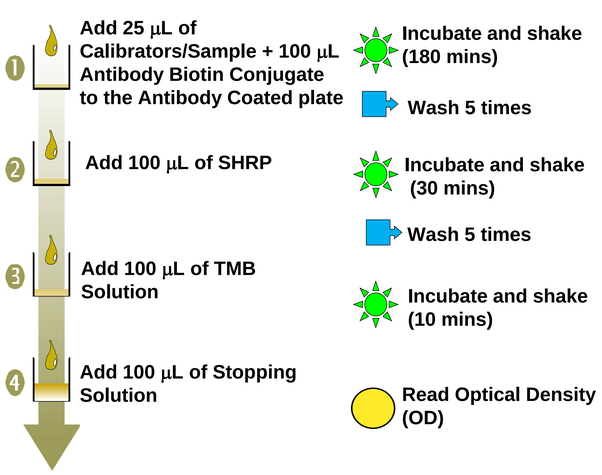
Results: Total and intact GIP ELISAs have been validated using well-characterized sample set and the peptide preparations. The total GIP ELISA detects 1-42aa, 3-42aa, 1-30aa, and 3-30aa in the sample. The intact GIP assay detects GIP (1-42aa) and the most abundant 3-42aa fragment equally in the human plasma. The intact GIP assay does not detect truncated C-terminal fragments(1-30aa). These assays did not cross-react to GRPP, Glucagon, Oxyntomodulin, GLP-1, and GLP-2. Method comparison between intact and total GIP assays using 337 samples yielded a slope of 0.817 (r=0.967, p < 0.0001). Both the assays are highly reproducible with the total coefficient of variation less than 10%. GIP concentrations were measured in both assays in lean and obese subjects on fat-rich diet at 30-minute intervals. Average concentrations in intact GIP assay at 0 minutes (baseline) in lean and obese subjects (n=10) were 115.6 and 88.5 pg/mL, respectively. Average concentrations in total GIP assay at 0 minutes (baseline) in lean and obese subjects (n=10) were 151.5 and 125.9 pg/mL, respectively. Both total and intact GIP had steep increase in concentrations up to 90 minutes and then flattened up to 180 minutes. The GIP results have been also compared to the mean plasma concentrations of Glucagon, GLP-1, C-Peptide between lean and obese subjects on fat-rich meal.

Conclusions: Highly sensitive and specific total and intact GIP ELISAs have been developed to reliably quantify these important endocrine and local regulators in physiological and pathophysiological studies for metabolic disorders.

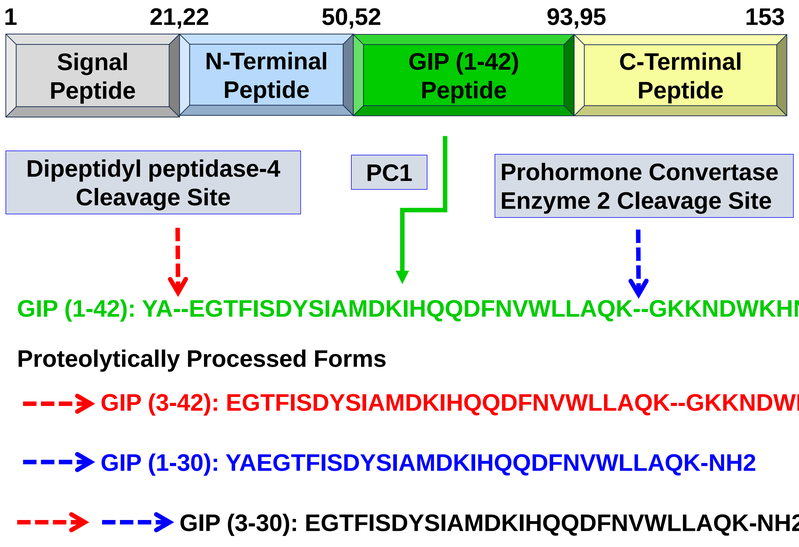
GIP ELISAs METHOD



Intact and Total GIP ELISAs Procedure



Pre-Pro-GASTRIC INHIBITORY POLYPEPTIDE PROCESSING



- GIP is synthesized in and secreted from K cells in the intestinal epithelium.
- There are four GIP molecular forms in circulation, GIP (1-42) GIP(3-42), GIP (1-30), and GIP (3-30).
- GIP secretion is primarily regulated by nutrients, especially fat.
- GIP(1–30) is the major biologically active fragment of GIP and is a GIP receptor agonist. It stimulates insulin secretion and reduces postprandial glycemic excursions.

RESULTS: ANALYTICAL CHARACTERISTICS

ELISA Reagents	Antibody Pair Specificity GIP Fragments	Dynamic Range (pg/mL)	Assay Calibration Traceability	Limit of Detection (pg/mL)
Total GIP AL-1013	1-42, 3-42, and 1-30	31-1000	1-42, and 3-42 Synthetic peptides	8.0
Intact GIP AL-1022	1-42, and 3-42	31-1000	1-42 and 3-42 Synthetic peptides	7.7

Intact and Total GIP Assays Imprecision

Reproducibility of the Total and Intact GIP assays was determined using samples in the low, mid, and high concentration ranges. The study included a total of 6 assays, 3 replicates of each sample per assay (n=18). Representative data were calculated based on EP10A-3 guidelines and are presented in the following tables below.

Total GIP ELISA

Sample	Mean Concentration (pg/mL)	Total SD (pg/mL)	Total CV %
Low	68.3	5.85	8.6
Mid	199.4	6.36	3.2
High	498.4	18.58	3.7

Intact GIP ELISA

Sample	Mean Concentration (pg/mL)	Total SD (pg/mL)	Total CV %
Low	37.7	3.49	9.3
Mid	107.2	4.72	4.4
High	284.2	11.14	3.9

Intact and Total GIP Assays Linearity of Dilution

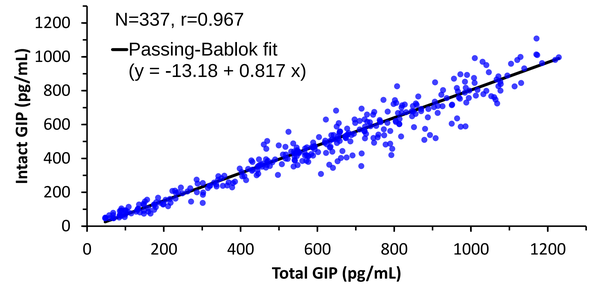
Total GIP ELISA Assay

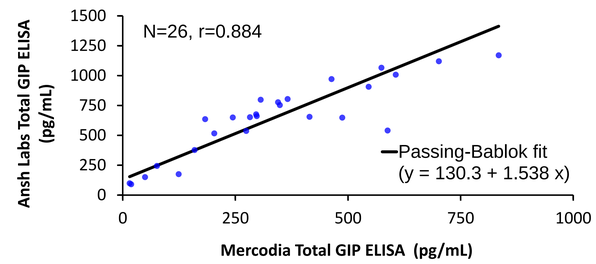
Sample ID	Dilution factor (1 in X)	Expected Value (pg/mL)	Observed Value (pg/mL)	% Recovery	Average % Recovery
Sample-1	Neat	665.80			92%
	2	332.90	320.97	96%	
	4	166.45	158.16	95%	
	8	83.23	73.77	89%	
	16	41.61	36.68	88%	
Sample-2	Neat	Over	N/A	N/A	90%
	2	558.80			
	4	279.40	269.33	96%	
	8	139.70	129.61	93%	
	16	69.85	60.27	86%	
	32	34.93	29.10	83%	
Sample-3	Neat	Over	N/A	N/A	104%
	2	Over	N/A	N/A	
	4	726.62			
	8	363.31	365.06	100%	
	16	181.66	176.83	97%	
	32	90.83	92.19	102%	
	64	45.41	46.19	102%	
	128	22.71	26.94	119%	

Intact GIP ELISA Assay

Sample ID	Dilution factor (1 in X)	Expected Value (pg/mL)	Observed Value (pg/mL)	% Recovery	Average % Recovery
Sample-1	Neat	362.25			88%
	2	181.13	161.54	89%	
	4	90.56	74.95	83%	
	8	45.28	36.01	80%	
	16	22.64	22.78	101%	
Sample-2	Neat	549.78			91%
	2	274.89	265.60	97%	
	4	137.45	129.04	94%	
	8	68.72	59.89	87%	
	16	34.36	27.33	80%	
	32	17.18	16.95	99%	
Sample-3	Neat	Over	N/A	N/A	97%
	2	862.98			
	4	431.5	428.33	99%	
	8	215.75	201.79	94%	
	16	107.87	101.11	94%	
	32	53.94	51.90	96%	
	64	26.97	27.94	104%	

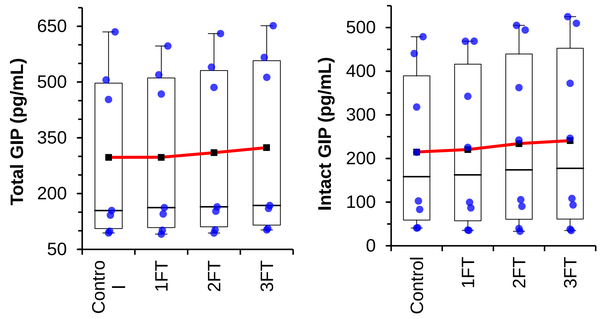
Method Comparison





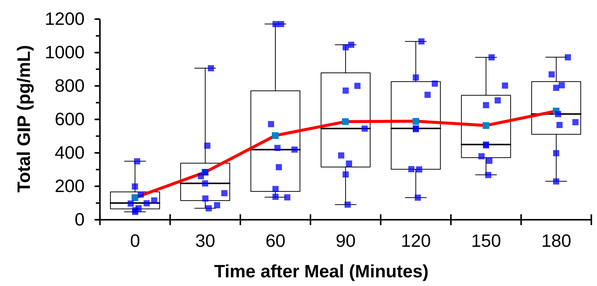
Plasma GIP Stability

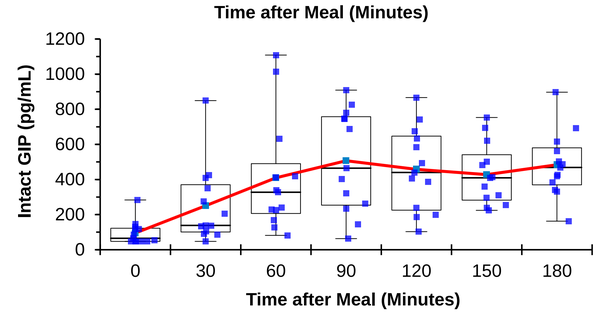
Multiple K2EDTA plasma samples were tested for their stability upon freeze and thaw stress cycles in Total and Intact GIP ELISA. The individual and mean concentrations have been plotted below.



Expected Ranges

Total and Intact GIP concentrations were measured in subjects, fasting (0 minutes) and between 30-180 minutes after meals at 30 minutes intervals. The GIP concentrations with time were analyzed and is presented below.





CONCLUSIONS

- Highly sensitive and specific Intact and Total GIP ELISAs have been developed to reliably quantify GIP fragments in circulation.
- The combination of these two ELISAs can now enable the measurement of bioactive 1-30aa GIP fragments in circulation.
- The stability of plasma specimen, low sample volume opens new avenue to re-analyze the residual samples from previous studies.