POSTER A-189

Development of Novel Specific and Sensitive ELISAs for Proglucagon-Derived Peptides*

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ABSTRACT

Objective: The aim of this study was to develop well characterized sensitive and specific ELISAs to quantitate Glucagon, Oxyntomodulin (OXM), and Glucagon-like peptide 1 (GLP-1) in biological fluids.

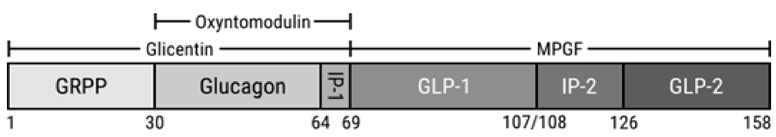
Relevance: Proglucagon, (PG) a 160aa peptide is cleaved from preproglucagon and the later is encoded by the glucagon gene (GCG) in humans. PG is a precursor of Glucagon, OXM, GLP-1 and several other peptides. These peptides arise by differential processing of PG. Glucagon, corresponding to PG residues (33-61aa), is formed in the alpha cells of the pancreas. Oxyntomodulin is a 37aa peptide hormone secreted by the gut endocrine L-cells post-prandially and shares identical amino acid sequence in the N-terminal to glucagon, with an extension of 8aa peptide in the C-terminus. Prohormone convertase 1/3 cleaves Proglucagon precursor into Oxyntomodulin, GLP-1/2 and GRPP upon nutrient ingestion. Oxyntomodulin is known to bind both the GLP-1 receptor and the glucagon receptor, but with lower affinity compared to GLP-1 and glucagon. Oxyntomodulin has been studied as a weight loss agent in obese patients via suppression of food intake and increase in energy expenditure. Glucagon has been studied for the treatment of hypoglycemia and glucagon receptor antagonists are under development for the treatment of type 2 diabetes. GLP-1 and GLP-2 receptor agonists appear to be promising therapies for the treatment of type 2 diabetes and intestinal disorders, respectively.

Methodology: Specific monoclonal antibody based ELISAs for glucagon (AL-157), oxyntomodulin (AL-139), and GLP-1 (AL-172) have been developed to measure their respective analyte in ≤50uL of the plasma. The glucagon assay is standardized to NIBSC code 69/194 v3.0 preparation and the other assays were gravimetrically calibrated to their corresponding pure peptides. These ELISAs were validated for their specificity to the Proglucagon fragments, specimen stability, and their circulating levels (fasting and non-fasting) in matched serum and plasma.

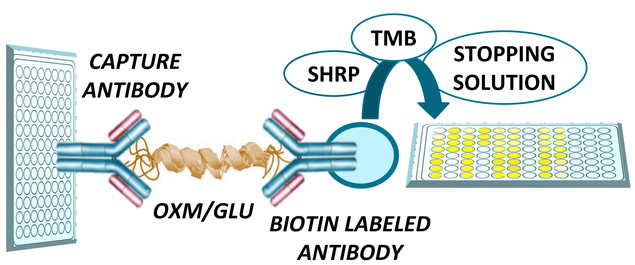
Validation: Glucagon, OXM, and GLP-1 ELISAs with a dynamic range of 20-300 pg/mL, 3-300 pg/mL, 15-600 pg/mL are highly specific to glucagon, OXM, and GLP-1, respectively. These assays did not cross-react to GRPP, Glucagon, OXM, GLP-1, and GLP-2 when assayed in their individual ELISAs. Proglucagon KO serum samples (n=3) in the OXM assay were non-detectable, whereas a concentration of 103-246 pg/mL was observed in the wild type mice (n=3). Median levels of Glucagon, OXM when studied in fresh/2-8°C/1FT/2FT drawn in EDTA plasma (no DPP-4) were 75.8/82.9/84.4/83.8 pg/mL and 353.8/342.4/389.3,409.9pg/mL, respectively. Median GLP-1 level (2 FT) on the same subjects was 235.2 pg/mL. Fasting/non-fasting (n=5) median Glucagon, OXM, and GLP-1 levels were 85.1/84.6, 215.3/645.9, 215.7/269.3 pg/mL, respectively.

Conclusions: Whole portfolio of easily accessible and standardized assays for Proglucagon-derived peptides are available to reliably quantitate these important endocrine and local regulators in physiological and pathophysiological studies for metabolic disorders.

METHODS



Assay Principle: One Step Sandwich" Immunoassay for Glucagon/Oxyntomodulin for human and other species



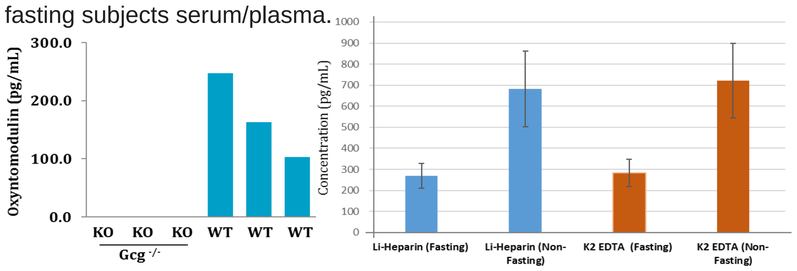
Assay	Range (pg/mL)	Sensitivity (pg/mL)
Glucagon	20-300	2.1
Oxyntomodulin	30-300	0.24

RESULTS

ANALYTICAL SPECIFICITY: Monoclonal antibody pair used in these assays cross-react to ***human, mouse and other species.*** *The assays are analyte specific and* does not cross-react to other closely related analytes.

Cross-Reactant	Concentration	% Cross-reactivity In Oxyntomodulin | Glucagon assays	
Glucagon (1-29)	1000 ng/mL	ND	100%
GLP-1 (7-36)	1000 ng/mL	ND	ND
GLP-1 (9-36)	1000 ng/mL	ND	ND
GLP-2 (1-34)	1000 ng/mL	ND	ND
GRPP	1000 ng/mL	ND	ND
MPGF	1000 ng/mL	ND	ND
Insulin	1000 pg/mL	ND	ND
C-peptide	1000 pg/mL	ND	ND
Thyroglobulin	1000 pg/mL	ND	ND
Oxyntomodulin (1-37)	100 pg/mL	100%	ND

Oxyntomodulin assay specificity was tested on multiple pro-Glucagon knockout mice, wild type mice, seven matched human fasting and non-fasting subjects serum/plasma.



LINEARITY OF DILUTION: Multiple dilutions of the plasma samples containing various Oxyntomodulin levels were diluted in calibrator A and recovery were calculated.

Dilution Levels	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
Neat	196.12	Neat	NA
1/2	98.06	95.21	97%
1/4	49.03	46.29	94%
1/8	24.51	22.19	91%
1/16	25.45	23.06	91%
Neat	105.75	Neat	NA
1/2	52.88	49.51	94%
1/4	26.44	23.86	90%
1/8	13.22	12.08	91%
1/16	6.61	5.86	89%
Neat	78.09	Neat	NA
1/2	39.05	37.26	95%
1/4	19.52	19.12	98%
1/8	9.76	9.46	97%
1/16	4.88	4.65	95%

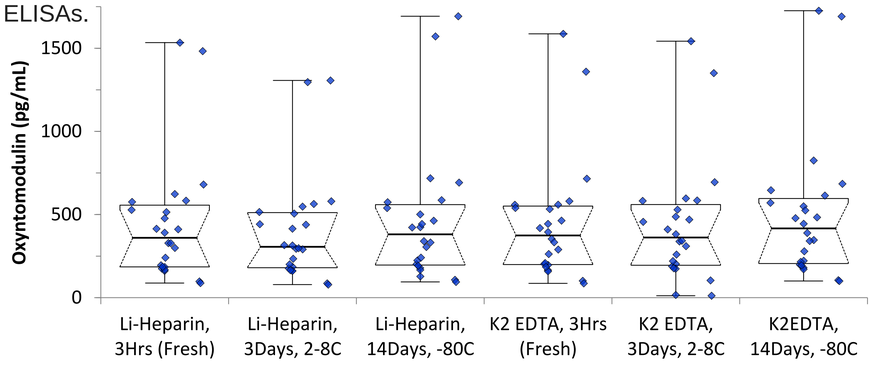
SPIKE RECOVERY: Known amounts of Oxyntomodulin were added to EDTA plasma specimens and spike recovery were calculated.

EDTA Sample	Endogenous Conc. (pg/mL)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	% Recovery
1	0.001	14.50	15.55	107%
		29.00	29.02	100%
		43.50	43.01	99%
2	12.761	26.62	26.54	100%
		40.48	39.23	97%
		54.35	50.39	93%
3	23.958	37.26	37.78	101%
		50.56	49.07	97%
		63.86	58.82	92%

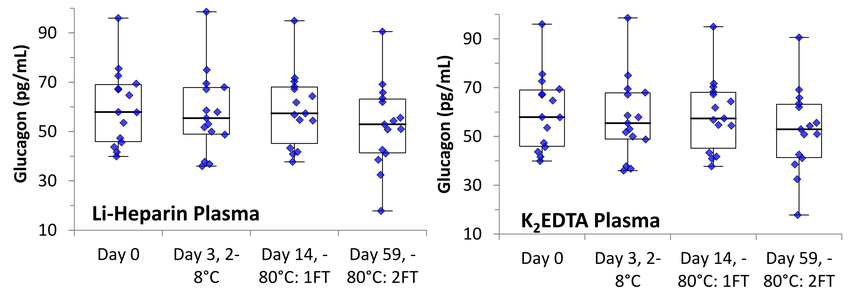
IMPRECISION: Reproducibility of the Oxyntomodulin ELISA assay was determined in a study using four K_2EDTA sample pools. The study included a total of 8 assays, four replicates of each per assay (n=32).

Sample	Mean conc.	Within run		Between run		Total	
	(pg/mL)	SD	%CV	SD	%CV	SD	%CV
Pool-1	13.072	0.408	3.12%	0.650	4.97%	0.767	5.87%
Pool-2	33.079	0.697	2.11%	1.260	3.81%	1.440	4.35%
Pool-3	111.957	2.571	2.30%	3.609	3.22%	4.431	3.96%
Pool-4	9.039	0.285	3.15%	0.358	3.96%	0.457	5.06%

SAMPLE STABILITY: K_2EDTA and Lithium-Heparin plasma specimens were aliquoted stored at 2-8°C and -80°C, for 3 and 14 days, respectively and compared to freshly drawn plasma samples in Oxyntomodulin and Glucagon ELISAs.

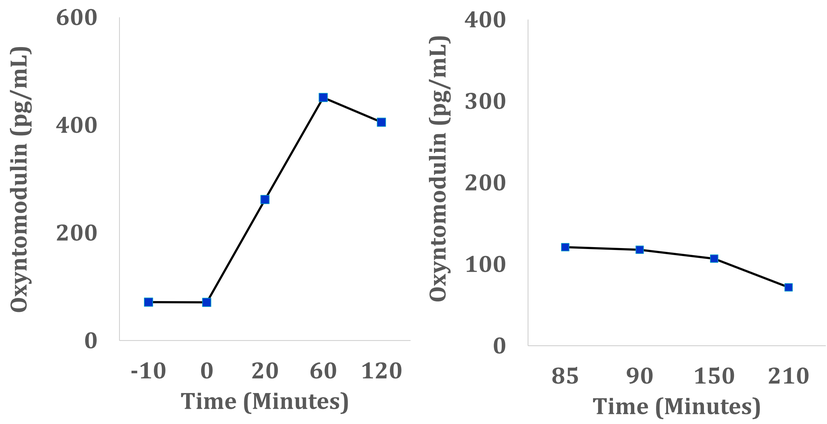


	1st Quartile	Median	'95% CI	3rd Quartile	IQR
Li-Heparin, 3Hrs (Fresh)	185	360	186to 528	556	371
Li-Heparin, 3Days, 2-8°C	180	306	185to 506	511	331
Li-Heparin, 14Days, -80°C	196	381	202to 539	559	363
K2 EDTA, 3Hrs (Fresh)	199	374	203to 540	551	352
K2 EDTA, 3Days, 2-8°C	195	362	203to 529	560	365
K2EDTA, 14Days, -80°C	206	417	215to 571	596	391



Li-Heparin	1st Quartile	Median	3rd Quartile	IQR	K_2EDTA	1st Quartile	Median	3rd Quartile	IQR
Day 0	45.9	57.9	69.1	23.1	Day 0	45.9	57.9	69.1	23.1
Day 3, 2-8°C	48.9	55.4	67.8	18.9	Day 3, 2-8°C	48.9	55.4	67.8	18.9
Day 14, -80°C: 1FT	45.2	57.4	68.1	22.9	Day 14, -80°C: 1FT	45.2	57.4	68.1	22.9
Day 59, -80°C: 2FT	41.4	52.9	63.2	21.8	Day 59, -80°C: 2FT	41.4	52.9	63.2	21.8

OXYNTOMODULIN RESPONSE: OGTT/GLP-1 INFUSION



CONCLUSIONS

- Specific monoclonal antibodies based ELISAs have been developed to measure pro-Glucagon peptides fragments.
- The Oxyntomodulin and Glucagon assays measures total Oxyntomodulin (1-37,3-37,4-37) and glucagon (1-29) in human and mouse plasma with no cross-reaction to glucagon and oxyntomodulin, respectively.
- Specimens collected in EDTA tubes are stable upon freezing at -80°C and does not require extraction procedure.
- Circulating Oxyntomodulin level increased with food intake and was not impacted by GLP-1 infusion.

*RESEARCH USE ONLY