Ansh Labs Glucagon ELISA

Importance of reliable glucagon measurements

Insulin and glucagon are the central hormonal mediators of glucose homeostasis. Abnormalities in both hormones contribute to the pathophysiology of diabetes and understanding the role of glucagon is essential to optimizing the strategies for diabetes diagnosis and therapeutics. Persons with Type 2 Diabetes (T2D) have been reported to have elevated glucagon concentrations in both the fasting and prandial state. Drugs for diabetes are now available that reduce circulating levels of glucagon, e.g. dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists, and this is thought to contribute to their mechanism of action[1]. Therefore, accurate measurement of glucagon has increasing relevance in diabetes research and diabetes-related clinical trials and may eventually have a place in the clinical management of diabetic patients. To meet these current and emerging demands Ansh Labs has developed a specific Glucagon ELISA for measuring glucagon in the plasma of human subjects.

Reliability depends on specificity and sensitivity

The reliability of a glucagon measurement is dependent on the specificity and sensitivity of the assay. Although most antibodies used in assays have affinity for a specific antigen in the picomolar range, assays are sensitive to interference from matrix effects (components present in plasma such as globulins and other proteins) and cross reactivity from closely related molecular forms of the analytes[3].

Specificity (cross reactivity)

The capture and detection antibodies in the sandwich ELISA bind specifically to residues at the C- and N-terminus. Since molecular heterogenicity arises from differential tissue processing of proglucagon, specificity (cross reactivity) of the assays were evaluated against different molecular forms. In these validation studies the Ansh Labs Glucagon assay had virtually no cross reactivity with Glicentin, Oxyntomodulin GLP-1, GLP-2, MPGF and Mini-glucagon even at more than supraphysiologic concentrations of these analytes (Table. 1).

Molecular Forms	Concentration	Cross reactivity
Glucagon (53-81)	100 ng/mL	100%
Mini glucagon (51-61)	1000 pg/mL	< .002%
Oxyntomodulin (33-69)	1000 ng/mL	< .002%
GLP1 (92-128)	1000 pg/mL	< .002%
GLP1 truncated (98-128)	1000 ng/mL	< .002%
GLP1 truncated (100-128)	1000 ng/mL	< .002%
GLP2 (146-178)	1000 ng/mL	< .002%
Glicentin (21-89)	1000 ng/mL	< .002%
GRPP (21-50)	1000 ng/mL	< .002%
Major Pro Glucagon Fragment (92-178)	100 ng/mL	< .002%

Table 1. Specificity of Glucagon ELISA
Potentially cross-reacting proteins were tested in the assay at the concentrations shown. The numerical designations represent the amino acid positions relative to the preproglucagon protein as synthesized by the *GCG* gene. Percent cross reactivity was defined relative to that of the glucagon 100 ng/mL calibrator.

Sensitivity

Fasting glucagon circulates in the range of 10-20 pM (30-60 pg/mL) and can increase up to 10-fold in response to hypoglycemia. Differences among subjects can vary several fold, and previous reports suggest that the mean difference between diabetic and nondiabetic subjects differs 2-3-fold. These differences are present in both fasting and fed humans. Therefore, the sensitivity of the assay to efficiently capture that change is critical[4]. Ansh Labs Glucagon ELISA can measure the changes in glucagon level more precisely with relevance to physiology (fasting vs after meal) and

clinical conditions (normal vs T2D). Glucagon levels are suppressed after oral glucose challenge or mixed meal challenge. Higher post challenge glucagon levels are observed in Glucose intolerance and T2D subjects. (Figure 1, 2; unpublished).

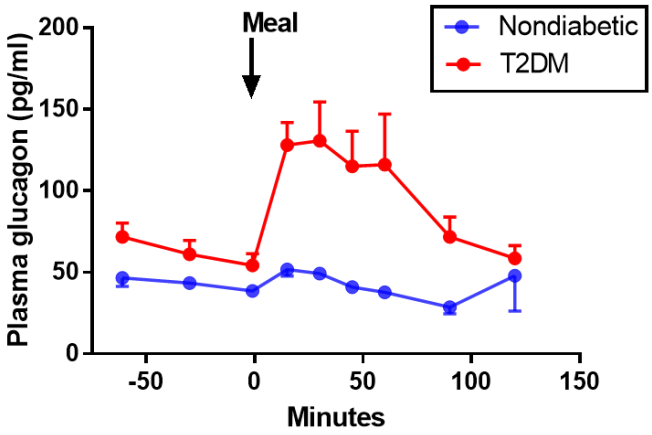

Figure 1: Plasma glucagon after a mixed nutrient meal in subjects with type 2 diabetes and nondiabetic controls.
Groups of 6 T2DM and nondiabetic subjects consumed 200 ml of a mixed nutrient liquid meal at time 0 and plasma samples collected for 2 hours. Plasma glucagon was assayed with the Ansh labs Glucagon ELISA (AL-157).

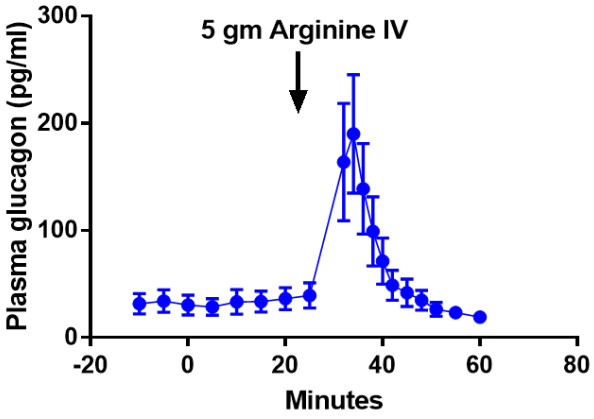

Figure 2: Fasting and arginine-stimulated glucagon secretion in healthy volunteers.
Healthy men and women have fasting blood collected for 40 minutes before intravenous administration of 5 gm of arginine over 1 minute and further blood sampling for 30 minutes. Plasma glucagon was assayed with the Ansh Labs glucagon ELISA (AL-157).

Incretins, such as GLP-1 and GIP, secreted by enteroendocrine cells stimulate insulin production and beta cell proliferation. Native GLP-1 and GIP have shorter half-life of 1-2 mins. GLP-1 analogues have been used for the treatment of T2D. GLP-1 analogues and GIP analogues suppress glucagon production in healthy individuals and T2D patients (Figure 3; unpublished).

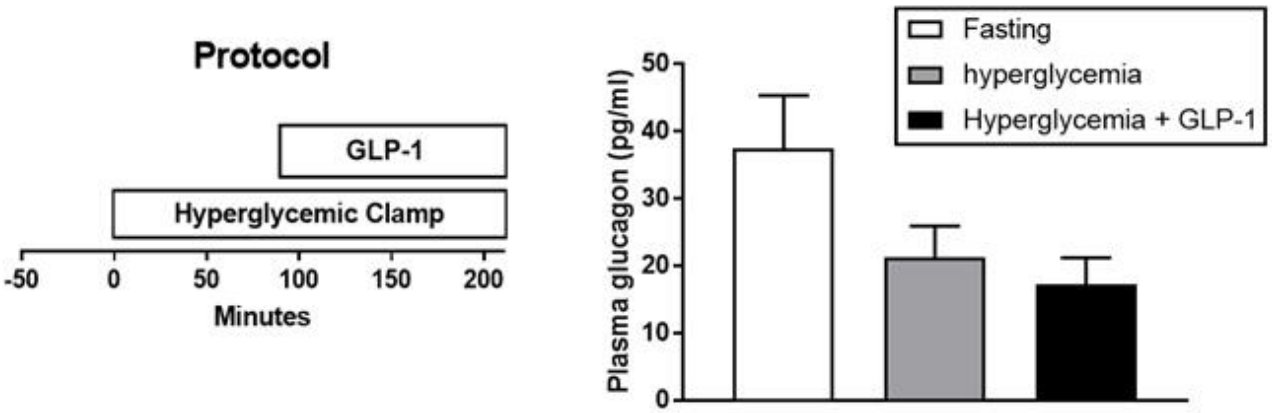

Figure 3: Plasma glucagon in response to fasting, hyperglycemia and hyperglycemia with intravenous GLP-1
Plasma glucagon concentrations in healthy subjects before and during an 8 mM hyperglycemic clamp and superimposed infusion of intravenous GLP-1 (2 pmol/kg/min). Plasma glucagon was assayed with the Ansh Labs Glucagon ELISA (AL-157).

References

1. Haedersdal et al, Mayo Clin Proc. 2018 Feb;93(2):217-239.
2. Muller et al, Physiol Rev. 2017 Apr;97(2):721-766.
3. Albrechtsen et al, J Diabetes Res. 2016; 2016: 8352957.
4. Faerch et al, Diabetes 2016 Nov; 65(11): 3473-3481.0