

Beginning of a new era in glucagon research: Breakthrough by the new glucagon assay

Incretin-based therapies were introduced a decade ago in the treatment of diabetes. They have dramatically changed the strategy of diabetes treatments and also reiterated the significance of glucagon and α -cells in the pathophysiology of diabetes. First, glucagon and α -cells have been extensively studied in the field of basic medical research. For example, our previous studies provided molecular evidence for the role of insulin signaling in α -cells in the regulation of glucagon secretion¹ and its involvement in the excessive secretion of glucagon in the hyperglycemic state². Furthermore, the strong association between glucagon and amino acids has recently been shown. Glucagon enhances amino acid metabolism in the liver, and amino acids enhance α -cell proliferation^{3,4}. Recent advances highlight the significance of α -cells in the maintenance of islet quality and quantity⁵. Together, glucagon and α -cells are now back under the spotlight.

In contrast to the progress in the field of basic medical research, clinical studies targeting glucagon were one step behind. The gap could be attributed to the unavailability of a reliable clinical assay for glucagon. Recent advances in molecular biology have been useful in addressing the problems of widely used conventional glucagon radioimmunoassay (RIA), including cross-reactivity of antibodies with proglucagon-related peptides and non-specific effects of serum impurities on the assay. Recently developed double-antibody “sandwich” enzyme-linked immunosorbent assay (ELISA) resolved these limitations. High-performance liquid chromatography results

have shown that this ELISA improves the accuracy of the assay, when compared with RIA⁶. Then, the ELISA is routinely applied to clinical diabetology, and is rapidly becoming the standard method for glucagon estimation. Hence, previous observations about glucagon are now being re-examined by the new system, which has led to publication of multiple reports reconfirming and/or discovering critical findings about the clinical significance of glucagon. *Journal of Diabetes Investigation* also contributes to the progress of clinical research about glucagon through publishing a number of clinical research papers.

One of the milestones in confirming the significance of glucagon is by Matsuo *et al.*⁷, who reported abnormal glucagon response to oral glucose tolerance tests in patients with type 2 diabetes. The study also compares the specificity of ELISA and RIA. The ELISA data recorded a significant difference in the glucagon response pattern to glucose load between patients with diabetes and healthy controls, whereas the RIA data did not show such a difference in glucagon response over time. Additionally, the absolute values of plasma glucagon levels estimated by ELISA were much lower than those estimated by RIA. Horie *et al.*⁸ also examined the glucagon response during the oral glucose tolerance test in young healthy volunteers, both men and women⁸. The data from that study showed significant suppression of glucagon levels in response to glucose load, thereby confirming the findings by Matsuo *et al.*⁷ Additionally, glucagon suppression was significantly larger in men as compared with that in women. The application of ELISA and physiological data from these studies^{7,8} has contributed significantly to the scientific progress in the field of glucagon research.

A study by Takahashi *et al.*⁹ reported a critical observation regarding the significance of glucagon unresponsiveness in hypoglycemia, observed in a patient with fulminant type 1 diabetes. The authors reported a sharp increase of glucagon in the arginine stimulation test just after the onset of the disease. However, the glucagon response of the same patient became defective at 6 months after the onset. Several research groups responded immediately to this report, and reported “preserved” glucagon response in their studies of patients with fulminant type 1 diabetes, and there were active discussions regarding this topic as well^{10–13}. These discussions are clinically important for interaction and dispersal of clinical data, experiences, and knowledge within the medical community. Usually, the introduction of new drugs and therapeutic approaches enhances the progress of both clinical medicine and basic research. These studies and discussions show that the new assay system could be a great catalyst for progress in the field of diabetes. Other reports also include important argument about diabetes in the context of glucagon dysregulation, in addition to insulin^{14,15}. These reports in the *Journal of Diabetes Investigation* left a significant scientific impact on the field of clinical diabetology, as they provided crucial evidence in the form of experimental data about the clinical significance of glucagon.

We also provided clinical evidence for the dysregulation of glucagon secretion in patients with type 1 diabetes, by estimating plasma glucagon levels by the new ELISA system¹⁶. The plasma glucagon levels were widely dispersed, and were not associated with the plasma glucose levels at the time of sampling. The glucagon levels were not elevated when the plasma glucose levels were low, and they were also not suppressed when the

*Corresponding author. Dan Kawamori

Tel: +81-6-6879-3743

Fax: +81-6-6879-3739

E-mail address: kawamori@dendmet.med.osaka-u.ac.jp

Received 3 April 2020; revised 7 April 2020;

accepted 9 April 2020

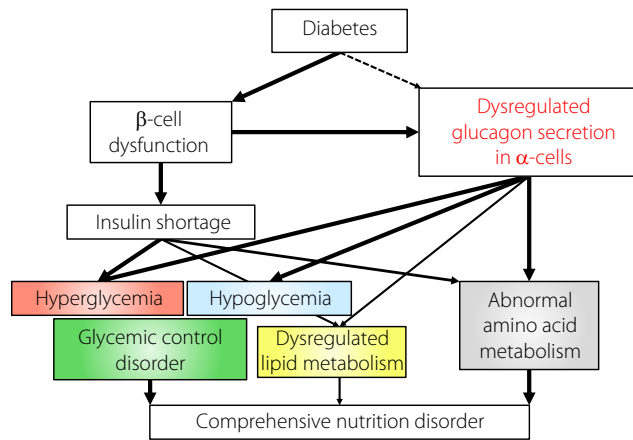


Figure 1 | New concept of diabetes as a “comprehensive nutrition disorder”, caused by both insulin and glucagon dysregulation. Pancreatic β -cell dysfunction and diabetes induce dysregulated glucagon secretion in α -cells in addition to systemic hyperglycemia caused by insulin shortage. Excessive glucagon secretion in the hyperglycemic state and defects in the hypoglycemic state exacerbate glycemic control disorder; both in hyper- and hypoglycemia. Excessive glucagon induces abnormal amino acid metabolism in the liver. Absolute and relative insulin shortage exacerbate glycemic control and abnormal amino acid metabolism. Dysregulation in insulin and glucagon also induces dysregulated lipid metabolism.

glucose levels were high. Importantly, glucagon levels in patients with hypoglycemia unawareness were significantly lower than those in patients without hypoglycemia unawareness, regardless of plasma glucose levels at the time of blood sampling. These data suggest a role for glucagon dysregulation in the pathophysiology of the disease: excessive (non-suppressible) secretion leading to exacerbation of hyperglycemia and unresponsiveness leading to hypoglycemic events. Additionally, the plasma glucagon levels were statistically associated only with serum blood urea nitrogen levels, but not with other parameters, such as glycemic control parameters and liver function markers. The annual comparison of glucagon levels in the same patients showed a significant correlation between both years¹⁷. We also confirmed a positive correlation between plasma glucagon levels and serum blood urea nitrogen levels, which established a functional link between glucagon and amino acid metabolism. Amino acids stimulate α -cell glucagon secretion and cellular proliferation, whereas glucagon enhances

amino acid metabolism in the liver^{3,4}. Thus, our clinical result could clinically validate the correlation between glucagon and amino acids found by basic glucagon studies¹⁸. From these findings, I propose a new concept of diabetes as a “comprehensive nutrition disorder”, caused by both insulin and glucagon dysregulation (Figure 1, see legend for details). The new glucagon assay is expected to be a powerful tool for clinical evaluation, and could be the beginning of a new era in glucagon research.

DISCLOSURE

The author declares no conflict of interest.

Dan Kawamori^{1,2,*} 

¹Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan, ²Medical Education Center, Osaka University Faculty of Medicine, Osaka, Japan

REFERENCES

1. Kawamori D, Kurpad AJ, Hu J, *et al.* Insulin signaling in alpha cells

modulates glucagon secretion in vivo. *Cell Metab* 2009; 9: 350–361.

2. Katsura T, Kawamori D, Aida E, *et al.* Glucotoxicity induces abnormal glucagon secretion through impaired insulin signaling in InR1G cells. *PLoS ONE* 2017; 12: e0176271.
3. Holst JJ, Wewer Albrechtsen NJ, Pedersen J, *et al.* Glucagon and amino acids are linked in a mutual feedback cycle: the liver-alpha-cell axis. *Diabetes* 2017; 66: 235–240.
4. Hayashi Y, Seino Y. Regulation of amino acid metabolism and alpha-cell proliferation by glucagon. *J Diabetes Investig* 2018; 9: 464–472.
5. Kawamori D. Alpha the versatile: guardians of the islets. *J Diabetes Investig* 2019; 10: 26–28.
6. Miyachi A, Kobayashi M, Mieno E, *et al.* Accurate analytical method for human plasma glucagon levels using liquid chromatography-high resolution mass spectrometry: comparison with commercially available immunoassays. *Anal Bioanal Chem* 2017; 409: 5911–5918.
7. Matsuo T, Miyagawa J, Kusunoki Y, *et al.* Postabsorptive hyperglucagonemia in patients with type 2 diabetes mellitus analyzed with a novel enzyme-linked immunosorbent assay. *J Diabetes Investig* 2016; 7: 324–331.
8. Horie I, Abiru N, Eto M, *et al.* Sex differences in insulin and glucagon responses for glucose homeostasis in young healthy Japanese adults. *J Diabetes Investig* 2018; 9: 1283–1287.
9. Takahashi N, Chujo D, Tsujimoto T, *et al.* Short-term changes in pancreatic alpha-cell function after the onset of fulminant type 1 diabetes. *J Diabetes Investig* 2018; 9: 636–637.
10. Murase-Mishiba Y, Bessho-Tachibana M, Imagawa A. “Preserved” glucagon secretion in fulminant type 1 diabetes. *J Diabetes Investig* 2019; 10: 186–187.
11. Takahashi N, Chujo D. Response to “Preserved” glucagon secretion in fulminant type 1 diabetes. *J Diabetes Investig* 2019; 10: 188–189.

12. Takahashi N, Chujo D, Kajio H, *et al.* Contribution of pancreatic alpha-cell function to insulin sensitivity and glycemic variability in patients with type 1 diabetes. *J Diabetes Investig* 2019; 10: 690–698.
13. Komada H, Hirota Y, Ogawa W. Glucagon secretions are impaired in patients with fulminant type 1 diabetes. *J Diabetes Investig* 2019; 10: 866–867.
14. Niwano F, Hiromine Y, Noso S, *et al.* Insulin deficiency with and without glucagon: a comparative study between total pancreatectomy and type 1 diabetes. *J Diabetes Investig* 2018; 9: 1084–1090.
15. Horie I, Haraguchi A, Ito A, *et al.* Impaired early-phase suppression of glucagon secretion after glucose load is associated with insulin requirement during pregnancy in gestational diabetes. *J Diabetes Investig* 2020; 11: 232–240.
16. Kawamori D, Katakami N, Takahara M, *et al.* Dysregulated plasma glucagon levels in Japanese young adult type 1 diabetes patients. *J Diabetes Investig* 2019; 10: 62–66.
17. Kawamori D, Katakami N, Takahara M, *et al.* Consistency of plasma glucagon levels in patients with type 1 diabetes after a 1-year period. *J Diabetes Investig* 2020; 11: 337–340.
18. Hayashi Y. Glutaminostatin: Another facet of glucagon as a regulator of plasma amino acid concentrations. *J Diabetes Investig* 2019; 10: 1391–1393.

Doi: 10.1111/jdi.13275