

# Therapeutic Use of Glucagon: The Future of Diabetes Mellitus Therapy

Norikiyo Honzawa, Kei Fujimoto \*

Division of Diabetes, Metabolism and Endocrinology, Department of Internal Medicine, Jikei University Daisan Hospital, Japan

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## ABSTRACT

The etiology of type 2 diabetes has long been centered on insulin. Recently, however, glucagon has been attracting attention. It has been reported that glucagon receptor or pancreatic  $\alpha$  cell-deficient mice do not deteriorate glucose intolerance after pancreatic beta cells are destroyed with streptozotocin and insulin secretion is depleted. This suggests that glucagon and glucagon receptors are crucial for glucose intolerance in diabetes mellitus, and the elucidation of glucagon signaling is still awaited. This review will begin with an overview of the history of glucagon. In the nearly 100 years since the discovery of glucagon, it has become clear that glucagon plays a role in blood glucose in type 2 diabetes as much or more than insulin. This is due in large part to the advent of the sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method, which allows accurate measurement of glucagon by using N- and C-terminal antibodies. Next, the mechanism of action of glucagon on blood glucose levels will be outlined. Glucagon increases blood glucose levels by enhancing gluconeogenesis and glycogenolysis. Glucagon also enhances lipolysis, which indirectly contributes to weight loss and improved blood glucose levels. Recently, mice with defective or decreased glucagon secretion have been developed, and the effects of glucagon are becoming clearer in vivo. Mice with elevated glucagon secretion have also appeared, and their glucose tolerance improvement through lipolysis and weight loss is also attracting attention. Research on glucagon is not limited to animal experiments but has already reached the stage of clinical application. Drugs that suppress glucagon have side effects such as liver damage, but they are effective in improving blood glucose. Drugs that increase glucagon also have hypoglycemic effects resulting from weight loss. Thus, glucagon has undergone a dizzying evolution over the past decade. The day may soon come when drugs that target glucagon will dominate diabetes treatment.

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### \*Correspondence:

Kei Fujimoto  
fuji@jikei.ac.jp

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**Abbreviations:** ATGL: Adipose Triglyceride Lipase; cAMP: Cyclic Adenosine Monophosphate; CREB: Camp Responsive Element Binding; CRTC2: CREB Regulated Transcription Co-Activator 2; FoxO1: Forkhead Box Protein O1; HSL: Hormone-Sensitive Lipase; KLF15: Kruppel-Like Factor 15; PGC1a: Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-a; RIA: Radioimmunoassay; LC-MS/MS: Liquid Chromatography/Mass Spectrometry

## INTRODUCTION

Type 2 diabetes has long been considered to be caused by relative insulin deficiency and insulin resistance; however, Unger's "bi-hormonal theory" proposes that glucagon is as important as or more important than insulin alone [1,2]. In the past 10 years, glucagon has been the focus of much attention. Its mode of secretion and receptor signaling are being elucidated [3-6]. In 2023, glucagon will celebrate the 100<sup>th</sup> anniversary of its discovery. This review will outline the history of glucagon, the measurement system issues that delayed glucagon research, whether glucagon is good or bad for blood glucose, and drugs for diabetes that target the glucagon pathway.

### History of Glucagon

In 1921, Banting and Best found that using the acid ethanol method to intravenously administer pancreatic extract to a pancreatectomized diabetic dog (Marjorie) reduced blood glucose levels [7]. This is the famous story of the discovery of insulin. In contrast, the story of the discovery of glucagon is less well-known. In 1923, Kimball and Murlin discovered glucagon, a substance that induces glycogenesis, in pancreatic extracts. It is derived from "the mobilizer of glucose" [8]. In 1956, Bromer et al. succeeded in purifying glucagon and subsequently decoded its amino acid sequence. Interestingly, unlike insulin, its amino acid sequence was found to be identical in humans, bovine, and porcine.

In 1959, Unger et al. established a Radioimmunoassay (RIA) system to measure glucagon levels using glucagon antibodies [9]. This was the first report of a system that allowed measurement of the human peptide hormone. In 1972, Wunsch et al. succeeded in synthesizing glucagon, followed by the determination of its molecular structure in 1983 by Blundell et al. The results of this research advanced glucagon research. However, unlike insulin, glucagon was not considered to be involved in the onset and progression of diabetes and was not recognized until 1975.

In 1889, the "uni-hormonal theory" was proposed by Von Mering and Minkowski. It is hypothesized that all metabolic disorders are secondary to an absolute or relative deficiency of insulin. Banting and Best demonstrated this in 1922 [7]. In 1975, Unger et al. proposed the "bi-hormonal theory" that glucose intolerance is caused by abnormal secretion of both glucagon and insulin [1,2]. In other words, the absolute or relative deficiency of insulin and the paradoxical secretion of glucagon cause glucose excess, resulting in the development of glucose intolerance. This "bi-hormonal theory" was an epoch-making hypothesis that

is still supported today.

In 2010 and 2011, it was reported that glucose tolerance did not worsen when pancreatic  $\beta$ -cells were destroyed with streptozotocin in glucagon receptor- or pancreatic  $\alpha$ -cell-deficient mice, and insulin secretion was depleted [10,11]. Also in 2011, transient expression of glucagon receptors in the liver of glucagon receptor-deficient mice in which pancreatic  $\beta$ -cells were destroyed using adenovirus caused blood glucose levels to rise again [12]. These results suggested that the cause of hyperglycemia is not only insulin deficiency but also the presence of glucagon is highly involved. Based on this, the "glucagoncentric theory" was proposed by Unger et al. in 2012 [13]. Thus, after almost 100 years, the importance of glucagon is being recognized and a glucagon renaissance is underway.

### Glucagon and Measurement Issues

Glucagon is a peptide hormone with a molecular weight of 3,450 Da and 29 amino acids. Glucagon is encoded by the proglucagon gene, which is a glucagon precursor. Mature glucagon is produced from proglucagon cleaved by Prohormone Convertases (PC) 1/3 in pancreatic  $\alpha$ -cells [14]. However, glucagon-related peptides, such as glicentin (1-61) and oxyntomodulin are also simultaneously produced. The presence of these glucagon-related peptides later made glucagon measurement difficult and was the reason that glucagon research did not progress. The RIA method established in 1959 was the main method for glucagon measurement, but cross-reactivity with glucagon-related peptides was a problem. In 1969, Unger et al. produced a C-terminal antibody that recognized the C-terminal amino acid sequence of glucagon [15]. This was considered the world standard for RIA, but it was reported to cross-react with several peptides, including glicentin (1-69) [16]. To solve this problem, a sandwich Enzyme-Linked Immunosorbent Assay (ELISA) method using both N- and C-terminal antibodies to glucagon was recently developed [17]. This method theoretically avoids cross-reactivity of glucagon with oxyntomodulin, which has the same N-terminal amino acid sequence, and glicentin (1-61), which has the same C-terminal amino acid sequence. Holst et al. reported that Mercodia's sandwich ELISA is the most accurate way to measure glucagon [18]. However, it is impossible to fully prevent cross-reactivity with these immunoassays. A glucagon measurement system using Liquid Chromatography/Mass Spectrometry (LC-MS/MS) was developed in 2017, breaking away from immunoassays [19]. Glucagon can now be accurately measured. However, despite its extremely high specificity, the measurement duration is long and the cost is high; therefore, sandwich

ELISA remains mainstream for glucagon measurement. Thus, glucagon can now be accurately measured, approximately 100 years after its discovery. In contrast, glucagon concentrations up to around 2010 may be inaccurate. Previous studies on glucagon may need to be rechecked.

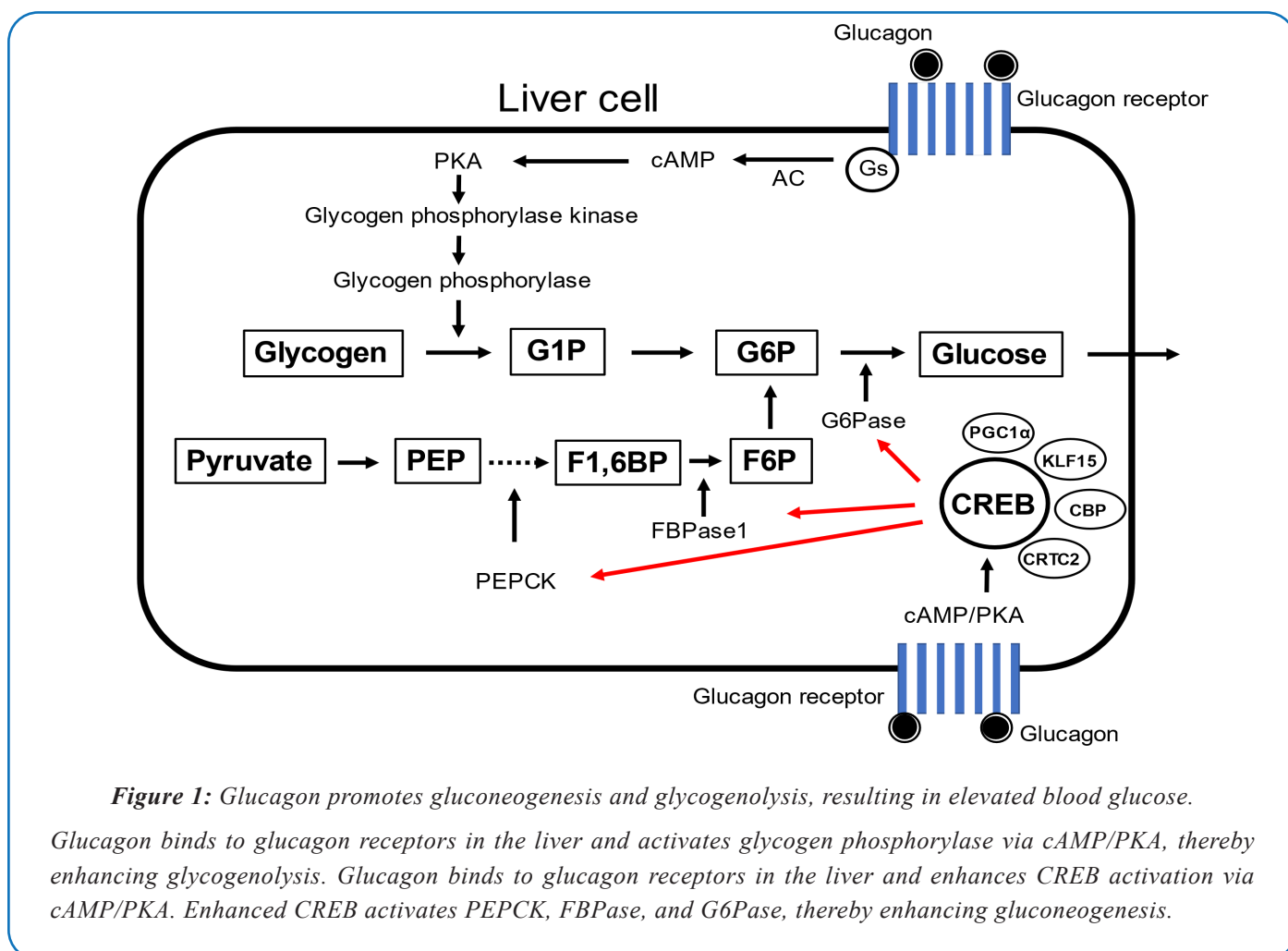
### Glucagon Increases Gluconeogenesis and Glycogenolysis in the Liver and Raises Blood Glucose (acute response) (Figure 1)

Glucagon increases glycogenolysis and glycogenesis, thereby increasing glucose production and release in the liver, and raising blood glucose [20]. Glucagon also inhibits gastrointestinal peristalsis. These effects have clinical applications in the emergency treatment of hypoglycemia. The glucagon-responsive rapid blood glucose elevation during hypoglycemia reaches its peak in approximately 15 minutes with duration of approximately three hours secondary to glycogenolysis. Other known effects of glucagon include decreased appetite, increased pulse rate, increased glomerular filtration rate, and accelerated water reabsorption from the collecting duct, increased lipolysis,

and amino acid metabolism.

The rate-limiting enzymes in glycogenolysis, the main action of glucagon, are glycogen phosphorylase and glycogen synthase [21]. Glucagon degrades glycogen into glucose by activating the former and inactivating the latter. This activates the Cyclic Adenosine Monophosphate (cAMP) sensor Protein Kinase A (PKA), resulting in the phosphorylation and activation of glycogen phosphorylase kinase. Activated glycogen phosphorylase kinase phosphorylates and activates glycogen phosphorylase. Consequently, glycogen is converted from Glucose 1-phosphate (G1P) to Glucose 6-phosphate (G6P). This is also converted to glucose by glucose 6-phosphatase (G6Pase) and then released outside the liver cells, resulting in an increase in blood glucose levels.

Glucagon also works to promote gluconeogenesis, which is the process of synthesizing glucose from lactic and pyruvic acids. The rate-limiting enzymes for gluconeogenesis are Phosphoenolpyruvate Carboxylase (PEPCK), Fructose biphosphatase 1 (FBPase1), and glucose 6-phosphatase (G6Pase). As mentioned above, the binding of glucagon



**Figure 1:** Glucagon promotes gluconeogenesis and glycogenolysis, resulting in elevated blood glucose.

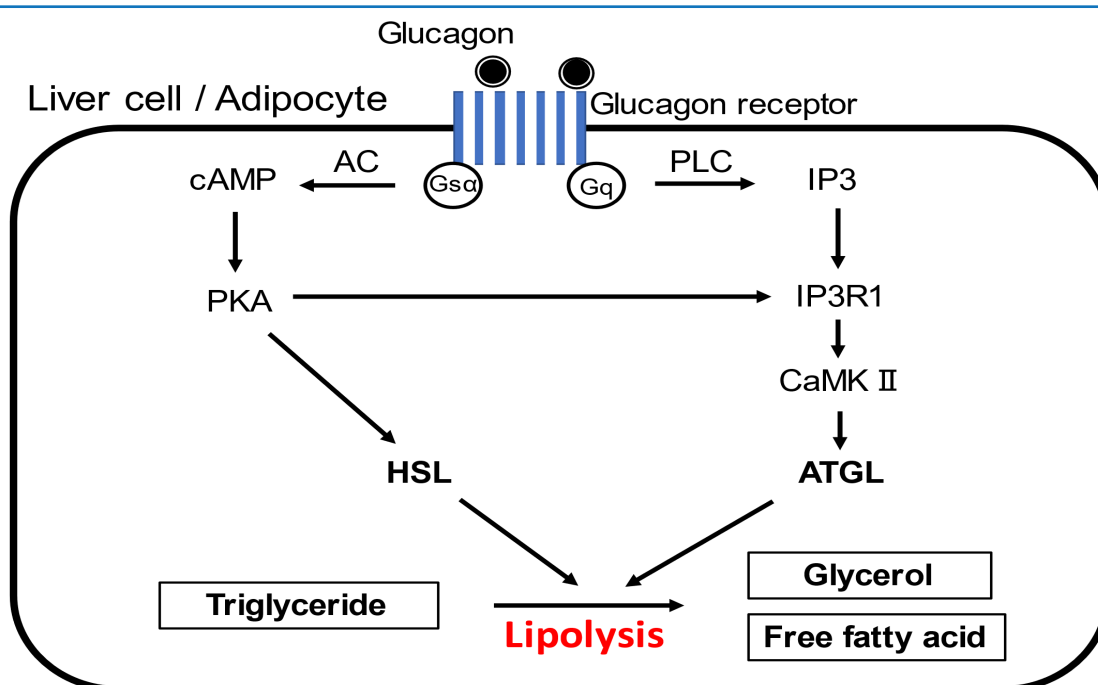
Glucagon binds to glucagon receptors in the liver and activates glycogen phosphorylase via cAMP/PKA, thereby enhancing glycogenolysis. Glucagon binds to glucagon receptors in the liver and enhances CREB activation via cAMP/PKA. Enhanced CREB activates PEPCK, FBPase, and G6Pase, thereby enhancing gluconeogenesis.

to hepatocyte glucagon receptors induces the transcription of G6Pase and PEPCK genes via the cAMP/PKA pathway. Additionally, PKA phosphorylates cAMP-Responsive Element Binding Protein (CREB). CREB, together with coactivators such as CREB binding protein (CBP), CREB Regulated Transcription Co-activator (CRTC2), and Forkhead box protein O1 (FoxO1), enhances the expression of glycogenic enzymes [21,22]. In addition, glucagon also enhances protein catabolism and provides amino acids necessary for glycogenesis.

Peroxisome proliferator-activated receptor gamma coactivator 1- $\alpha$  (PGC1 $\alpha$ ) and Kruppel-Like Factor 15 (KLF15) are involved in this pathway [23,24]. CREB is a master regulator of glucagon signaling and regulates the gene expression of glycogenic rate-limiting enzymes. As an acute response, glucagon promotes glycogenolysis and gluconeogenesis at the levels of translation, transcription, and enzymatic reactions, leading to an increase in blood glucose levels.

### Glucagon Increases Lipolysis and Indirectly Decreases Blood Glucose (chronic response) (Figure 2)

Adipocytes also contain glucagon receptors [25]. In adipocytes, glucagon enhances lipolysis and is thought to chronically act on weight loss, resulting in improved glucose tolerance. Glucagon activates Hormone Sensitive Lipase (HSL), which is the rate-limiting enzyme in lipolysis [26]. HSL is also activated by catecholamines and glucagon. When glucagon binds to glucagon receptors in adipocytes, it phosphorylates HSL via the cAMP/PKA pathway from the G $\alpha$  protein. When HSL is phosphorylated from its inactivated to its active form, it hydrolyzes triglycerides into glycerol and free fatty acids. This results in weight loss. There are other rate-limiting enzymes for lipolysis in addition to HSL. Adipose Triglyceride Lipase (ATGL) is a lipid droplet-localized protein that was reported in 2004 [27]. It is relatively abundant in adipose tissue. Recently, a similar mechanism has been considered in the liver, where glucagon activates Phospholipase C (PLC) via the G $q$  protein upon binding to the hepatocyte glucagon receptor. PLC increases inositol 1,4,5-triphosphate (IP $_3$ ), a second messenger, and activates Ca $^{2+}$ /calmodulin-dependent protein kinase II (CaMKII) when IP $_3$  binds to the IP $_3$  receptor (IP $_3$ R) [28]. Consequently, ATGL is activated and enhances lipolysis in the liver. Thus, glucagon enhances lipolysis in



**Figure 2:** Glucagon promotes lipolysis leading to weight loss.

*In hepatocytes and adipocytes, glucagon binding to glucagon receptors activates HSL via the cAMP/PKA pathway. Activated HSL enhances lipolysis. Glucagon enhances PLC and activates ATGL via IP and CaMKII. ATGL enhances lipolysis, resulting in weight loss.*

adipocytes and hepatocytes. As such, insulin resistance is reduced and glucose tolerance is improved.

## Current Status of Glucagon Research

Many biological effects of glucagon have recently been reported in rodents. In 2009, Hayashi et al. generated a Glucagon-EGFP knockin mouse ( $Gcg^{gfp/gfp}$ ) that is functionally deficient in the preproglucagon gene encoding the glucagon protein [29]. Interestingly, 2-weeks-old  $Gcg^{gfp/gfp}$  mice showed a decrease in blood glucose levels, whereas the same mice at 2-3 months old showed weight gain, and the hypoglycemic effect was abolished. These findings suggest that the suppression of lipolysis and reduction in activity associated with decreased glucagon secretion may be responsible for these effects. Also in 2010, Hancock et al. generated mice deficient in pancreatic  $\alpha$ -cells (Arx-deficient mice) [10]. These mice have reduced glucagon secretion because of suppressed pancreatic  $\alpha$ -cell development and differentiation. Three-month-old Arx-deficient mice had decreased blood glucose levels, but no changes in body weight. Additionally, in 2011, Lee et al. generated  $GcgrKO$  mice deficient in whole-body glucagon receptors [11].  $GcgrKO$  mice do not show glucagon effects because of glucagon receptor deficiency. Six-week-old  $GcgrKO$  mice had lower blood glucose levels, but no changes in body weight. The age at which the chronic phase response to glucagon begins to appear remains controversial. Blood glucose levels are influenced by both glucagon and compensatory hormones such as insulin. Various factors besides glucagon are also involved in weight gain. Therefore, it is difficult to explain glucose tolerance and body weight solely in terms of glucagon. However, results have so far been obtained in glucagon-decreased mice generally associated with decreased gluconeogenesis, reduced lipolysis, and weight gain. However, the results are generally associated with decreased gluconeogenesis, lipolysis, and weight gain in glucagon-decreased mice. Regarding blood glucose levels, it has been suggested that decreased glucagon secretion *in vivo* contributes to improved blood glucose.

Nadejda et al. created the pancreatic  $\alpha$ -cell-specific Tuberous Sclerosis Knockout ( $\alpha$ TSCKO) mouse, a mouse model of sustained activation of pancreatic  $\alpha$ -cell mTOR, in 2021 [30]. Two-month-old  $\alpha$ TSCKO mice lost weight but did not show remarkable changes in blood glucose levels. Additionally, chronic hyperglucagonemia resulted in decreased expression of glucagon receptors in the liver. Thus, chronic hyperglucagonemia contributes to the improvement of insulin resistance as a result of increased lipolysis and weight loss. On the other hand, the decrease in

hepatic glucagon receptor expression may be considered to have suppressed the increased blood glucose levels. Patients with glucagonoma, a neuroendocrine tumor, show weight loss and do not have elevated blood glucose.

These results indicate that both reduced and increased glucagon secretion in mice work directly or indirectly to improve blood glucose levels. Acutely, glucagon binds to glucagon receptors in the liver, resulting in increased blood glucose levels via enhanced gluconeogenesis and glycogenolysis. Chronically, hyperglucagonemia increases metabolism promotes lipolysis and decreases body weight. It is also suggested that chronic hyperglucagonemia may induce the downregulation of hepatic glucagon receptors and reduce the increased blood glucose.

## Therapeutic Agents Targeting Glucagon

The development of hypoglycemic agents to inhibit hepatic gluconeogenesis and glucose release by blocking glucagon receptors and glucagon signaling is underway. LY2409021, a glucagon receptor antagonist, showed marked improvement in blood glucose levels when administered to patients with type 2 diabetes; however, side effects such as weight gain, increased blood pressure, fatty liver, and serum lipid levels, caused the discontinuation of its development [31-33]. In contrast, REMD2.59, a glucagon receptor-neutralizing antibody, demonstrated a marked improvement in blood glucose levels in a mouse model of diabetes mellitus [34]. The adverse effects observed with LY2409021 were absent. REMD2.59 helped with weight loss and fatty liver. Additionally, drugs are being developed to improve weight loss and glucose tolerance by activating glucagon. Glucagon has recently been expected to be useful when activated with other molecules. Cotadutide, a GLP-1/glucagon co-agonist, showed remarkably greater weight loss and stronger hypoglycemic effects than GLP-1 receptor agonists [35-38]. Cotadutide has been shown to decrease liver enzymes, such as AST and ALT, in addition to weight loss. It is being tested in a clinical trial regarding improving Nonalcoholic Steatohepatitis (NASH). Additionally, GLP-1/GIP/glucagon receptor triagonists are being developed [39-40]. The drug was associated with lower HbA1c levels and weight loss compared to the control group. The mechanism is thought to be via an increase in fibroblast growth factor 21 (FGF-21), which improves glucose and lipid metabolism. Drugs that activate glucagon signaling will likely aim to expand indications to include obesity and NASH through weight loss effects and to lower blood glucose by improving insulin resistance. Interestingly, glucagon improves blood glucose by enhancing glucagon signaling, leading to lipolysis and weight loss. In contrast, inhibition of glucagon

signaling also contributes to glycemic improvement by decreasing hepatic gluconeogenesis. Future considerations should include therapeutics targeting glucagon.

## CONCLUSION

Since its discovery in 1923, glucagon has never been recognized regarding glucose intolerance, as insulin has been the focus. However, the challenge of measurement systems, which was a barrier to glucagon research, was resolved in the last decade. In 2012, the “glucagonocentric theory” was proposed. Glucagon is now in the spotlight as much as or more than insulin regarding glucose intolerance. The primary effects of glucagon are to increase blood glucose via hepatic gluconeogenesis and hepatic glucose release and to decrease body weight mainly through lipolysis. These detailed mechanisms of action are also becoming clear from studies of mice with decreased/increased glucagon secretion. Inhibition of glucagon secretion directly improves blood glucose levels, while increased glucagon secretion indirectly contributes to blood glucose reduction through lipolysis and weight loss effects. Based on these results, drugs, such as LY2409021 and REMD2.59, which aim to improve blood glucose by decreasing glucagon signaling, and drugs such as GLP-1/glucagon coactivators, and GLP-1/GIP/glucagon receptor triagonists, which increase glucagon signaling and result in weight loss, are being applied clinically. It is possible that glucagon will change diabetes therapy in the future.

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## CONFLICT OF INTEREST

K.F. received honorarium from Sanofi K.K, Merck Sharpe & Dohme, Taisho Pharmaceutical, Eli Lilly Japan KK, TERUMO Corporation, ARKRAY, Astellas Pharma, AstraZeneca, Nippon Boehringer Ingelheim, Mitsubishi Tanabe Pharma Corporation, Ono Pharmaceutical, Novo Nordisk Pharma, Kissei Pharmaceutical, Sumitomo Dainippon Pharma, Kowa Pharmaceutical, Takeda Pharmaceutical, Daiichi Sankyo, Chugai Pharmaceutical, Abbot Japan, Otsuka Pharmaceutical and Kyowa Hakko Kirin. R.N. received honorarium from Sanofi K.K, Nippon Boehringer Ingelheim, Takeda Pharmaceutical, Kissei Pharmaceutical, Novartis Pharma, Eli Lilly Japan KK, Novo Nordisk Pharma, MSD, Astellas Pharma, Taisho Pharmaceutical, Ono Pharmaceutical.

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