

# Lack of glucagon receptor signaling and its implications beyond glucose homeostasis

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## Abstract

Glucagon action is transduced by a G protein-coupled receptor located in liver, kidney, intestinal smooth muscle, brain, adipose tissue, heart, pancreatic  $\beta$ -cells, and placenta. Genetically modified animal models have provided important clues about the role of glucagon and its receptor (Gcgr) beyond glucose control. The PubMed database was searched for articles published between 1995 and 2014 using the key terms glucagon, glucagon receptor, signaling, and animal models. Lack of Gcgr signaling has been associated with: i) hypoglycemic pregnancies, altered placentation, poor fetal growth, and increased fetal–neonatal death; ii) pancreatic glucagon cell hyperplasia and hyperglucagonemia; iii) altered body composition, energy state, and protection from diet-induced obesity; iv) impaired hepatocyte survival; v) altered glucose, lipid, and hormonal milieu; vi) altered metabolic response to prolonged fasting and exercise; vii) reduced gastric emptying and increased intestinal length; viii) altered retinal function; and ix) prevention of the development of diabetes in insulin-deficient mice. Similar phenotypic findings were observed in the hepatocyte-specific deletion of *Gcgr*. Glucagon action has been involved in the modulation of sweet taste responsiveness, inotropic and chronotropic effects in the heart, satiety, glomerular filtration rate, secretion of insulin, cortisol, ghrelin, GH, glucagon, and somatostatin, and hypothalamic signaling to suppress hepatic glucose production. Glucagon ( $\alpha$ ) cells under certain conditions can transdifferentiate into insulin ( $\beta$ ) cells. These findings suggest that glucagon signaling plays an important role in multiple organs. Thus, treatment options designed to block Gcgr activation in diabetics may have implications beyond glucose homeostasis.

## Key Words

- ▶ pancreas
- ▶ glucagon cells

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## Introduction

Glucagon is a 29-amino acid polypeptide secreted by the  $\alpha$  or glucagon cell of the islet of Langerhans in response to hypoglycemia, arginine, gastric inhibitory polypeptide (during ambient reduced glucose levels), gastrin, and potassium chloride. Glucagon was initially discovered as

a contaminant of pancreatic extracts with glucogenic properties or properties that mobilize glucose in 1923. It took more than 20 years for Sutherland and de Duve to establish that glucagon is secreted by pancreatic  $\alpha$ -cells. Between 1959 and 1962, Unger *et al.* developed a RIA

making it possible to investigate the physiology of glucagon and its role in various disorders (Unger *et al.* 1959, 1962, Gromada *et al.* 2007).

As a counter-regulatory hormone, glucagon maintains blood glucose levels by activating glycogenolysis and gluconeogenesis. In addition, glucagon reduces triglyceride, very LDL release, and cholesterol levels and stimulates fatty acid oxidation (Eaton 1973, Longuet *et al.* 2008). Beyond glucose homeostasis, glucagon elicits significant extra-hepatic effects in tissues such as kidney, heart, adipose tissue (white and brown), gastrointestinal tract, thyroid, and CNS (Lefebvre 1995, Burcelin *et al.* 1996, Porte *et al.* 1998, Kieffer & Habener 1999, Kinoshita *et al.* 2014).

Glucagon action is transduced by a G protein-coupled receptor (GCGR/*Gcgr*) that is a member of the class II GCGR superfamily of seven transmembrane spanning receptors that are coupled via GTP-binding proteins to adenylyl cyclase resulting in an increase in cAMP production. cAMP activates signaling pathways that cause an increase in gluconeogenesis, glycogenolysis, and fatty acid oxidation. In addition, glucagon controls glucose, energy, and lipid metabolism at least in part via AC/cAMP-independent signals including p38MAPK, IP3/DAG/Ca, peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ ), and fibroblast growth factor 21 (FGF21)-dependent pathways (Berglund *et al.* 2010, Habegger *et al.* 2010, 2013, Rodgers 2012, Cyphert *et al.* 2014).

Binding sites for glucagon have been identified in liver, kidney, intestinal smooth muscle, brain, adipose tissue, heart, pancreatic islet  $\beta$ -cells, and placenta (Ouhilal *et al.* 2012). *Gcgr* gene expression is positively regulated by glucose and negatively regulated by glucagon and agents that increase intracellular cAMP (Quesada *et al.* 2008).

This review will focus on the role of glucagon and glucagon signaling in fetal growth, pancreatic development, and glucose and lipid homeostasis in genetically modified animal models that have been demonstrated to provide important clues about the role of glucagon in health and disease. Animal models provide an invaluable tool to study the underlying mechanisms associated with glucagon action; however, they have the disadvantage that genetic manipulation could lead to lifelong adaptations that can skew results. Thus, some of the findings may not necessarily translate into human disease.

The PubMed database was searched for articles published between 1995 and 2014 using the key terms glucagon, glucagon receptor, and animal models. Articles obtained from this search are discussed in this review. A brief summary of all the known metabolic changes that

have been identified from genetically modified animal models with altered *Gcgr* expression is provided in Fig. 1.

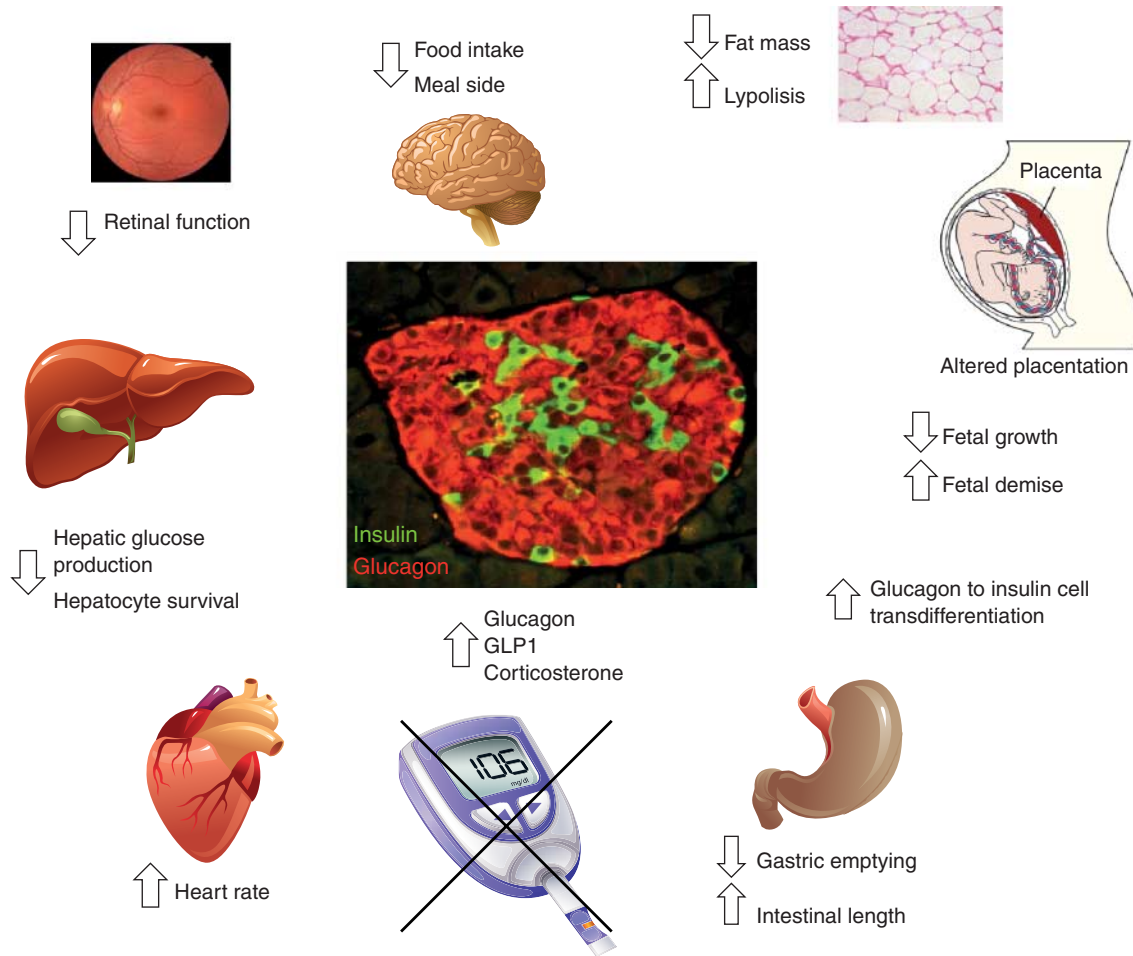
### Role of glucagon on pregnancy maintenance and fetal growth

Disruption of the glucagon receptor gene (*Gcgr*) during pregnancy is associated with maternal hypoglycemia, hyperglucagonemia, abnormalities of placentation, poor fetal growth, and increased fetal and early postnatal death. *Gcgr*<sup>-/-</sup> placentas are characterized by extensive mineralization, fibrinoid necrosis, narrowing of the vascular channels, and a thickened interstitium associated with trophoblast hyperplasia. In addition, lack of glucagon placental signaling down-regulates genes that control growth, adrenergic signaling, vascularization, oxidative stress, and G protein-coupled receptors (Ouhilal *et al.* 2012).

### Role of glucagon in pancreatic development and pancreatic islet morphology

During fetal development, glucagon is required for early insulin or  $\beta$ -cell differentiation and to mature a subset of glucagon cells (Vuguin *et al.* 2006). In rodent models, disruption of the *Gcgr* gene is associated with an increase in the number of pancreatic islets and an increase in the number of somatostatin cells without altering insulin cell mass. Lack of glucagon signaling is also associated with a profound glucagon cell hyperplasia. A subset of those glucagon cells coexpress markers of immature islet endocrine cells such as insulin, PDX1, and glucose transporter 2 (Vuguin *et al.* 2006). Similar to the *Gcgr*<sup>-/-</sup> model, glucagon cell expansion is observed in the majority of the models in which there is reduced or absent *Gcgr* receptor signaling, such as inactivation or a reduction in glucagon and/or its receptor by genetic manipulation, immuno-blockade, or treatment with antisense oligonucleotides (Gelling *et al.* 2003, Sloop *et al.* 2004, Conarello *et al.* 2007, Winzell *et al.* 2007, Gu *et al.* 2009, Hayashi *et al.* 2009, Lee *et al.* 2011, Longuet *et al.* 2013). In those studies,  $\alpha$  or glucagon cell expansion is accompanied by elevated plasma glucagon levels. One exception has been the study by Liang *et al.* (2004), which demonstrated that a reduction in *Gcgr* expression using an antisense oligonucleotide is not accompanied by changes in  $\alpha$  or glucagon cell number; however, glucagon levels were nonetheless significantly increased.

Interestingly, specific inactivation of the *Gcgr* gene in the hepatocyte recapitulates the phenotype observed in

**Figure 1**

Studies using the *Gcgr* knockout mouse model (*Gcgr*<sup>-/-</sup>) have revealed important insights into the role of glucagon signaling in pancreatic and extra-pancreatic tissues.

*Gcgr*<sup>-/-</sup> mice. Specifically, both *Gcgr*<sup>-/-</sup> mice and the liver-specific *Gcgr*<sup>-/-</sup> mice have similar reductions in fasting glucose and improved glucose tolerance and insulin sensitivity, and glucagon cell hyperplasia and hyperglucagonemia, suggesting that an independent circulating factor produced by the lack of *Gcgr* signaling in liver can increase glucagon cell proliferation (Longuet *et al.* 2013).

### Role of glucagon in food intake and body composition

Glucagon has beneficial effects on food intake, body fat mass, and energy expenditure (Habegger *et al.* 2010, Heppner *et al.* 2010). In addition, glucagon has a satiety effect by decreasing meal size through a combination of

peripheral and central actions (Heppner *et al.* 2010). Consistent with a role in modulating food intake, glucagon also appears to affect the regulation of body weight by promoting weight loss in physiological and pathological doses as observed in patients with glucagonoma (Schulman *et al.* 1957, Bloom & Polak 1987). In addition, rodent models and *in vitro* studies have demonstrated that glucagon increases energy expenditure through activation of brown adipose tissue (Billington *et al.* 1991).

Disruption of the *Gcgr* gene is associated with a significant decrease in total adipose tissue, which is compensated for by an increase in lean body mass. The changes in body composition are not accompanied by a change in growth rates, food intake, resting O<sub>2</sub> consumption, and energy expenditure when compared with WT

littermates (Gelling *et al.* 2003, Vuguin *et al.* 2006). Notably, during other pathological states, such as insulin deficiency and/or hyperglucagonemia, glucagon increases energy expenditure and thermogenesis. This increase in thermogenesis involves targeting of brown adipose tissue (Yahata & Kuroshima 1987) and white adipose tissue lipolysis (Lefebvre 1975).

When exposed to a high-fat diet, disruption of the *Gcgr* gene is associated with a decrease in consumption of fat, 30% less weight gain, and protection from high-fat-induced hepatic steatosis when compared with WT littermates (Conarello *et al.* 2007). In contrast, it has been demonstrated that *Gcgr*<sup>-/-</sup> mice have enhanced susceptibility to Jo2-induced liver injury by increasing the apoptotic rate (Sinclair *et al.* 2008). Moreover, glucagon is essential for hepatocyte survival via regulation of cAMP-dependent pathways that decrease caspase activity (Sinclair *et al.* 2008).

### Role of glucagon in glucose and lipid homeostasis

Glucagon plays a central role in the response to hypoglycemia by stimulating gluconeogenesis and glycolysis and opposing the insulin effects. Its main action on the liver is mediated by the activation of adenylyl cyclase and the protein kinase A signaling pathway (Quesada *et al.* 2008). Glucagon stimulates changes to lower the energy state by activating AMPK signaling in the liver, thereby improving the efficiency by which the liver converts gluconeogenic substrate into glucose following glucagon stimulation (Berglund *et al.* 2009). The first-line biguanide drug for the treatment of diabetes 'metformin' has been shown to antagonize the effect of glucagon in the liver by increasing AMP levels (Miller *et al.* 2013). Glucagon has also been shown to have an inhibitory effect on insulin secretion. It has been recently shown that glucagon stimulated signaling, via cAMP-PKA-CREB, and the subsequent hepatic production of kisspeptin 1 suppresses insulin secretion (Song *et al.* 2014). In addition to its effect on the liver, glucagon can suppress hepatic glucose production by acting through the mediobasal hypothalamic region of the brain, suggesting that glucagon can limit its own direct stimulatory effect in the liver (Mighiu *et al.* 2013).

The lipolytic effect of glucagon in humans has been challenged (Gravholt *et al.* 2001). In animal models, glucagon has potent hypolipidemic actions (Eaton 1973, Guettet *et al.* 1991, Bobe *et al.* 2003). Glucagon decreases triglyceride and very-LDL release by the liver (Guettet *et al.*

1989, Bobe *et al.* 2003), reduces plasma cholesterol (Guettet *et al.* 1988, 1989, 1991), and increases  $\beta$ -oxidation (Prip-Buus *et al.* 1990). Glucagon action on lipid metabolism is mediated through AMPK-, p38 MAPK-, PPAR $\alpha$ -, Foxa2-, and FGF21-dependent mechanisms (Longuet *et al.* 2008, Berglund *et al.* 2010, von Meyenn *et al.* 2013). In addition, glucagon plays a central role in fatty acid oxidization during prolonged fasting and in response to exercise (Longuet *et al.* 2008, Berglund *et al.* 2010).

In rodent models, disruption of the *Gcgr* gene is associated with lower blood glucose levels during the day and the development of hypoglycemia during a prolonged fast, increased plasma LDL, and, in female rodents, decreased levels of triglycerides (Gelling *et al.* 2003).

### Role of glucagon in the hormonal milieu

Glucagon does not seem to play an important role in insulin action but induces glucose-stimulated insulin release (Gelling *et al.* 2003). Similarly, glucagon action stimulates its own secretion in isolated rat and mouse glucagon cells by increasing cAMP levels and stimulating somatostatin release (Shimatsu *et al.* 1983, Ma *et al.* 2005). In humans, glucagon also has a variety of neuroendocrine effects including the stimulation of GH and cortisol secretion and inhibition of ghrelin secretion (Arafat *et al.* 2005).

Disruption of the *Gcgr* gene is associated with hyperglucagonemia and elevated glucagon-like peptide 1 (GLP1) levels, with normal insulin and lactate levels. *Gcgr*<sup>-/-</sup> mice have a twofold increase in corticosterone during fasting, low corticosterone levels in the afternoon, and an increased responsiveness to epinephrine when compared with WT littermates. Female *Gcgr*<sup>-/-</sup> mice display a small decrease in insulin-like growth factor1 levels (Gelling *et al.* 2003). Consumption of a high-fat diet does not alter levels of plasma glucagon, GLP1, triglycerides, non-esterified free fatty acids, or corticosterone in *Gcgr*<sup>-/-</sup> mice when compared with WT littermates (Conarello *et al.* 2007).

### Role of glucagon in satiety and gastric emptying

Glucagon has been shown to evoke a marked delay in gastric emptying (Jonderko *et al.* 1989). These anti-motility effects on the gastrointestinal tract (esophagus, stomach, and small and large intestines) are observed when glucagon is administered to humans in pharmacological doses (Patel *et al.* 1979). Glucagon also controls

meal size and satiation in both humans and rodents (Geary 1990, Geary *et al.* 1992).

In rodent models, disruption of the *Gcgr* gene is associated with decreased gastric emptying (Conarello *et al.* 2007), increased length of the intestines (by 20%) due to an increased rate of crypt neogenesis and crypt bifurcation, and an increase in the number of L and LK cells/villi compared with WT littermates (Grigoryan *et al.* 2012).

### Role of glucagon in retinal function

In rodent models, disruption of the *Gcgr* gene is associated with a late-onset loss of retinal function, loss of visual acuity, and eventual death of retinal cells (Umino *et al.* 2006). These retinal changes were observed at 10 months of age and correlated directly with the degree of hypoglycemia.

### Role of glucagon in taste

Glucagon and its receptor are coexpressed in a subset of mouse taste receptor cells that express T1R3 taste receptor implicated in sweet and/or umami taste (Elson *et al.* 2010). No major alterations in taste have been described in *Gcgr*<sup>-/-</sup> mouse models.

### Role of glucagon in cardiac contractility

Glucagon exerts positive inotropic and chronotropic effects in the ventricular myocardium by activation of cardiac adenylate cyclase leading to increased cAMP formation (MacLeod *et al.* 1981, Mery *et al.* 1990, Gonzalez-Munoz *et al.* 2008).

In rodent models, disruption of the *Gcgr* gene is associated with a diminished parasympathetic tone, leading to higher heart rates during the light phase and a modest elevation in the heart rate in response to atropine (Mukharji *et al.* 2013).

### Role of glucagon in renal blood flow

Glucagon exerts a positive effect on renal blood flow and glomerular filtration rate, and increases sodium, chloride, potassium, and inorganic phosphorus clearance ratios (Elrick *et al.* 1958, Bailly *et al.* 1980, Denis *et al.* 2003). No major alterations in renal function have been described in *Gcgr*<sup>-/-</sup> mouse models.

### Role of glucagon in $\beta$ (insulin) cell function

Transgenic mice were engineered that overexpress the *Gcgr* in insulin cells using the rat insulin II promoter (RiP-*Gcgr*) to determine the functional role of *Gcgr* receptor in  $\beta$ -cell function. Overexpression of *Gcgr* in  $\beta$ -cells increased glucagon-stimulated insulin release and significantly increased  $\beta$ -cell volume, suggesting a role for *Gcgr* receptor in increased insulin cell competency (Gelling *et al.* 2009). These data are strengthened by the findings of low levels of PDX1, GLUT2, and MafA, molecules involved in the regulation of insulin expression, in insulin cells of *Gcgr*<sup>-/-</sup> mice (Vuguin *et al.* 2006).

### Role of glucagon in the development of diabetes in insulin-deficient mice

Elevated glucagon:insulin ratio has been shown to accelerate gluconeogenesis and fatty acid oxidation leading to the formation of ketone bodies (Vons *et al.* 1991). Hyperglycemia and elevated ketone bodies are the main component of diabetic ketoacidosis (Eledrisi *et al.* 2006). Disruption of the *Gcgr* gene in an insulin-deficient diabetic rodent model is accompanied by an asymptomatic, benign, non-catabolic state when followed for 6 weeks (Conarello *et al.* 2007, Lee *et al.* 2011), suggesting that 'other factors' contribute to the normalization of the catabolic state. Similarly, it has been demonstrated that a low amount of  $\alpha$ -cell (2% of the normal  $\alpha$ -cell mass) is sufficient to prevent the metabolic dysregulation observed in diabetes (Thorel *et al.* 2011).

Disruption of the *Gcgr* gene increases circulating level of FGF21 and GLP1, which promote glucose tolerance independently of insulin level. Thus, FGF21 and GLP1 seem to be the major players in preventing the developing of diabetes in *Gcgr*<sup>-/-</sup> diabetic mice (Omar *et al.* 2014).

### $\alpha$ (glucagon) cell transdifferentiation as a potential treatment for diabetes

It has been recently demonstrated that, in certain situations, newly formed  $\beta$ -cells can originate from cells that previously expressed glucagon, a phenomenon called transdifferentiation. Such situations include extreme  $\beta$ -cell loss, increased expression of *Pax4* in  $\alpha$ -cells, forced PDX1 expression, epigenomic manipulation, or the use of the peptide caerulein after treatment with alloxan (Collombat *et al.* 2009, Liu & Habener 2009, Thorel *et al.* 2010, Yang *et al.* 2011, Bramswig *et al.* 2013, Piran *et al.* 2014).



## Antagonizing glucagon action as a potential treatment for diabetes

If alterations in glucagon secretion are indeed the cause of hyperglycemia and other metabolic complications in diabetic patients, suppression of glucagon signaling can be viewed as an important therapeutic option. Potent peptide antagonists, glucagon-neutralizing antibodies, small-molecule glucagon receptor antagonist, and receptor antisense oligonucleotides have been used in animal models to control hyperglycemia but their use in humans have been limited by their side effects as well as the limited mode of delivery (Johnson *et al.* 1982, Brand *et al.* 1994, Qureshi *et al.* 2004, Estall & Drucker 2006). Recently, four novel peptide-based glucagon analogs have been developed that are resistant to DPP4 degradation and thus display substantial abilities to suppress glucagon action in different animal models (O'Harte *et al.* 2013). All analogs inhibit glucagon-induced insulin secretion *in vitro*, and in rodents, analogs inhibited glucagon-induced hyperglycemia and the insulinotropic response (O'Harte *et al.* 2013).

## Conclusion

It has been suggested that, in states of insulin deficiency, excess glucagon secretion plays a major role in the metabolic perturbations associated with diabetes, such as hyperglycemia and ketonuria. Thus, inhibition of glucagon receptor signaling represents a possible option for the treatment of diabetes. Animal models have demonstrated that the physiological processes regulated by glucagon and its receptor are much broader than expected. Glucagon plays important roles in pancreatic development, insulin cell function, and metabolic response to prolonged fasting, exercise, lipid metabolism, hepatic energy state, hepatocyte survival, meal size and satiety, gastric emptying, intestinal length, as well as visual acuity, placentation, and cardiac contractility. In addition, under some extreme metabolic conditions of insulin deficiency, glucagon or  $\alpha$ -cells possess the capacity to transdifferentiate into insulin cells. Therefore, antagonizing glucagon action as a therapy for diabetes may improve glucose and insulin levels but in addition may have several unintended consequences that could further compromise the regulatory response to an altered metabolic state.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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