Review

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Unraveling the Intricate Mechanisms: Regulation and Secretion of Glucagon in Response to Nutrient Composition

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Abstract: Glucagon was initially regarded as a hyperglycemic substance while recent research reveals its broader role in metabolism, encompassing effects on glucose, amino acids (AA), and lipid metabolism. Notably, the interplay of glucagon with nutrient intake, particularly AA, and non-nutrient components, is central to its secretion. In the context of type 2 diabetes (T2DM), fasting and postprandial hyperglucagonemia have long been linked to the disease's development and progression. However, recent studies have brought to light the positive impact of glucagon-agonists on lipid metabolism and energy homeostasis. This review delves into the intricate relationship and underlying mechanisms between glucagon and nutrient composition, which may hold promise in devising novel therapeutic approaches for T2DM management.

Keywords: gucagon; glucose; lipid; amino acid; hyperglucagonemia

1. Introduction

Glucagon, a 29-amino acid peptide, was discovered in 1921 (1), and was described as hyperglycemia substance due to contaminants in pancreatic extracts (2) in 1923. In 1948 it was established that glucagon is released from pancreatic α cells and later to a lesser extent, from brainstem neurons (3, 4). It is widely recognized that fasting and postprandial hyperglucagonemia play a crucial role in both the development and progression of type 2 diabetes (T2DM). However, researchers recently reshaped the role of glucagon in metabolism confirming that the biology of glucagon is more comprehensive and extends beyond hepatic hyperglycemic actions to exert effects on glucose, amino acids (AA) and lipid metabolism.

The hyperglycemic effect in T2DM is undisputedly present as demonstrated by glucagon receptor antagonists in humans which, however, induced hepatic side effects (5-7). The positive effects of glucagon-agonists on lipid metabolism, energy homeostasis and reduction of liver fat have been emphasized with the development of glucagon/GLP-1 co-agonists as well as GLP-1/GIP/glucagon triple agonists which are currently in clinical development/trials (8-10).

Nutrients, especially amino acids (AA), and non-nutrient components, stimulate glucagon secretion directly through sensory transporters and receptors or indirectly through their effects on cellular metabolism. Indeed, increasing protein intake, and thereby glucagon release, has shown positive effects in studies with orally treated T2DM patients (11, 12). We therefore review studies addressing the role of glucagon regulation by food intake. We describe the complex interplay of glucagon with glucose, protein/amino acid and lipid/fatty acid metabolism as well as secretion of insulin and other hormones to provide a mechanistic background. Our hypothesis is that the powerful stimulation of glucagon by AA and other food components may be exploited in the treatment of T2DM.

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2. Glucagon actions and regulation

Glucagon was viewed primarily as the counter-regulatory hormone of insulin in the control of glucose homeostasis in the decades after its discovery (13, 14). Nowadays, glucagon is considered as a pleiotropic hormone metabolic actions of which include insulin secretion (15), regulation of lipid and AA metabolism, an increase of energy expenditure, modulation of food intake and satiety, and weight loss in both animals and humans (16-18). Furthermore, glucagon has been demonstrated to play a role in the regulation of bile acid metabolism, encompassing processes such as bile acid synthesis, uptake, and efflux (19).

The secretion of glucagon by α-cells is primarily associated with occurrences of hypoglycemia, acting as a safeguard against low blood sugar levels. Additionally, it is also stimulated by elevated circulating levels of AAs and fatty acids, as well as in response to adrenergic stimulation and circulating incretins (20). It is well established that β-cell derived secretory products including insulin, zinc and gamma-aminobutyric acid (GABA) GABA inhibit glucagon secretion(21). Meanwhile, it is potently suppressed by somatostatin (22), GLP-1, amylin, leptin, fatty acids and ketone bodies and stimulated by GIP and vagal stimulation. Meier et al. demonstrated that GLP-2 also stimulates glucagon secretion in healthy subjects (23) (Figure 1). And some medications such as furosemide or acetylsalicylic acid may influence prostaglandin (PG) synthesis, mainly PGE, which in turn control glucagon release. In this regard, details of glucagon receptor function are helpful to understand their linkage to metabolic effects of glucagon.

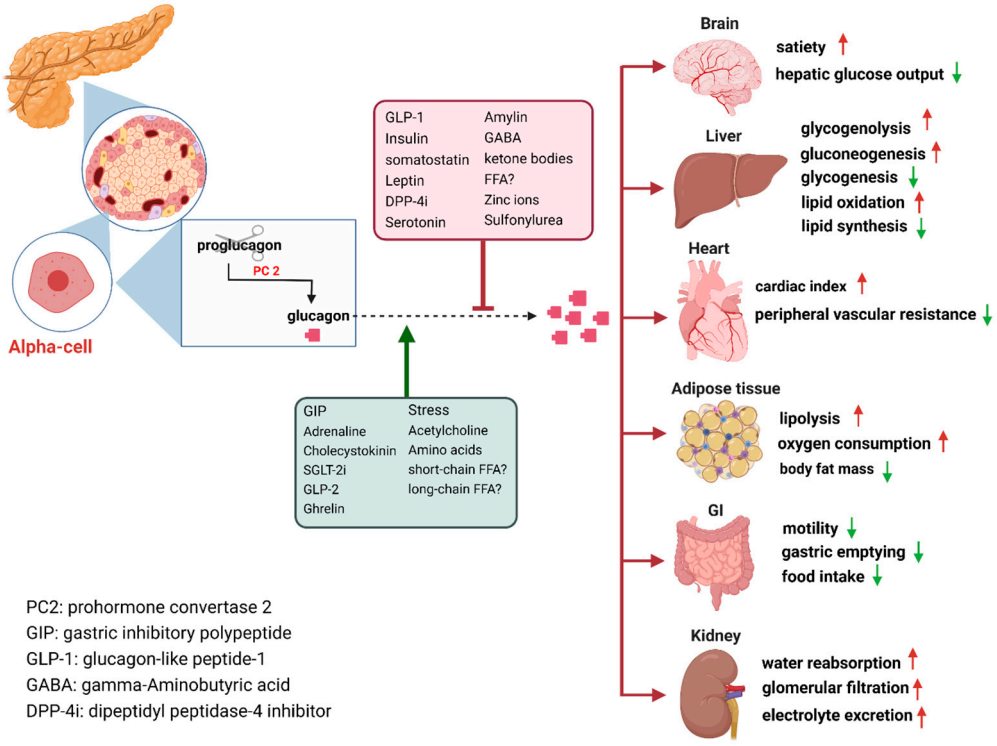

Figure 1. Glucagon receptors on multiple organs and the stimulation/inhibition of glucagon release (this graph is generated with www.biorender.de).

Glucagon receptors (GCGRs) belong to the class B of seven transmembrane (7TM) protein receptors known to activate adenylyl cyclase through Gαs-coupled proteins, which is accompanied by an increase in cellular cyclic AMP levels and activation of protein kinase A (PKA) (24, 25). Recently, GCGRs were also shown to activate the IP3 -pathway via Gq and activation of INSP3R1 in liver cells (26). GCGRs are highly expressed in the liver as well as multiple extrahepatic tissues (Figure 1), which play an essential role in glucose, AA, lipid and energy metabolism (21, 27, 28).

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How are GCGRs regulated?

In 1996 research in broilers demonstrated that reduced glucagon binding was closely associated with reduced lipolysis, implying that downregulation of cell surface GCGRs is the mechanism by which glucagon induced desensitization of its ability to stimulate lipolysis in adipocytes(29). It has been reported that dihydroxy bile acids like chenodeoxycholic acid (CDCA) induce GCGR desensitization(30). A study in 2006 demonstrated that CDCA stimulated phosphorylation and heterologous desensitization of the GCGRs through protein kinase C (PKC) activation (31). Given that glucagon is one of several peptide hormones that increase glucose levels, Leibiger et al. investigated the broader implications of autocrine secretion-biosynthesis coupling, using glucagon as an example. Their study demonstrated that glucagon, through signaling via GCGR, PKC, and PKA, upregulates the expression of its own gene, providing evidence for a more widespread applicability of positive autocrine feedback (32).

Downstream signaling pathways of GCGRs involve activation of Gs and Gq, leading to the formation of intracellular cAMP and inositol 1,4,5-trisphosphate (IP3) with subsequent release of intracellular Ca2+. Activation of either Gs-induced protein kinase A (PKA) or Gq's effect on Ca2+/calmodulin-dependent protein kinase can lead to the phosphorylation of cAMP response element binding protein (CREB). The metabolic effects of glucagon depend on its concentration, spatial features (mitochondrial vs. cytosolic effects), and substrate dependency. Thus, for ureagenesis and gluconeogenesis plasma concentrations of substrates are important, i.e. AAs and free fatty acids, respectively. It was described in previous studies that inositol triphosphate receptor 1 (INSP3R1) is the isoform primarily responsible for mitochondrial calcium signaling in hepatocytes and knocking down InsP3R1 reduced glucose production in isolated hepatocytes (33, 34). Perry et al. (26) implied that InsP3R1 activation and Ca2+/calmodulin-dependent protein kinase II (CAMKII) activity are important for the acute effect of glucagon in stimulating hepatic glucose production (HGP).

3. Glucagon and glucose metabolism

Glucose inhibits glucagon release upon oral or i.v. administration while hypoglycemia increases the secretion of glucagon to elevate hepatic glucose output by stimulation of glycogenolysis and gluconeogenesis, and additionally inhibits glycogenesis and glycolysis and induces ketone production through multiple mechanisms and thereby protects against hypoglycemia (24, 35, 36). Glucagon acts in concert with cortisol, growth hormone and the adrenergic hormones which also increase hepatic glucose output in hypoglycemia. Remarkably, the blockade of GCGRs does not impair the counter-regulation against hypoglycemia (7, 37, 38).

Although the level of glucagon rises rapidly in the early stage of fasting, circulating glucagon concentrations drop to postprandial levels upon prolonged fasting (see below), with persistently decreasing glycaemia due to glycogen depletion (39). Glucagon physiologically regulates the early phases of fasting in non-diabetic animals and humans which is relevant in real life since prolonged fasting is an unusual state in industrialized societies.

The inhibition of glucagon by increases of blood glucose is important to control blood glucose and is therefore finely tuned with insulin release. However, with the development of impaired glucose tolerance and T2DM, glucagon continues to induce glucose production during hyperglycemia in fasted and pre-prandial conditions (40-42). Both, fasting and postprandial hyperglucagonemia were proposed to trigger metabolic disturbances in obese and/or prediabetic subjects (43, 44). In 1970s, Unger et al. proposed that T2DM may not only the consequence of relative or absolute insulin deficiency, but also of glucagon excess in dogs (45). Reaven et al. later showed that hyperglucagonemia persists throughout the day in both obese and non-obese patients with T2DM, despite significant elevations in plasma insulin and glucose levels. These factors are traditionally expected to inhibit glucagon secretion under normal conditions (46). Recent studies have confirmed that most patients with T2DM exhibit abnormally high fasting plasma glucagon levels that do not appropriately decrease, and in some cases, may even increase after oral glucose tolerance test (OGTT) or carbohydrate intake (40, 42, 47-49). This failure to suppress glucagon secretion significantly contributes to post-prandial hyperglycemia by increasing hepatic glucose production in

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T2DM patients. Some researchers suggest that the difficulty in controlling glucagon could signal an initial problem with α-cells in the pancreas before insulin deficiency develops. This might involve reduced α-cell response to glucose and/or a lack of insulin's suppressive effect within the pancreatic islets (21, 43, 50, 51). However, i.v. administration of glucose was reported to inhibit glucagon release similarly in people with or without diabetes. Since manifest diabetes is associated with increased fasting plasma glucose in the presence of increased plasma glucagon, the inhibition of glucagon release is partially lost. However, as further increases of glucose by i.v. infusion still inhibit glucagon release, the response appears to be maintained but right shifted(52).

What are the explanations for this difference (oral vs i.v glucose)?

A careful comparison of oral or i.v. glucose in healthy people showed greater suppression of glucagon by i.v. than by oral glucose (53). Since the i.v. glucose does not stimulate incretins, this cannot be explained by suppression of glucagon by GLP-1. However, as GIP was stimulated by oral but not i.v. glucose, one might postulate that GIP stimulated glucagon and thereby attenuated the effect of glucose, and possibly due to the glucagonotropic action of GLP-2 (53). In people with T2DM, i.v. glucose dose dependently suppressed glucagon even at elevated basal plasma glucose while oral glucose caused an initial stimulation of glucagon which was not explained by the levels of incretins hormones (54, 55). On the other hand, studies in isolated α-cells and pancreatic islets also provided evidences. Notably, glucose unexpectedly stimulates glucagon release in isolated α-cells via mechanisms which involve KATP-channels and Ca2+-induced depolarization (56) which pointed to indirect and possibly paracrine mechanisms of inhibition of glucagon release in vivo. In addition, glucose inhibits glucagon release in intact mouse and human islets apparently by paracrine mechanisms involving somatostatin release which lost their function in islets from diabetes patients (57-59). This would suggest that deficient stimulation of somatostatin release by glucose or insulin from delta-cells in islets accounts for the hyperglucagonemia in diabetic conditions. Meanwhile, somatostatin independent mechanisms have also been proposed (59-61). Recently, hyperglycaemia-induced Na+-dependent reduction of ATP production and thus mitochondrial impairment was proposed as a primary mechanism (62, 63).

An important observation is that hyperglucagonemia in the fasting state and in response to arginine disappears upon prolonged infusion of insulin in insulin-dependent patients. This is compatible with a dysregulation of α-cells due to hyperglycemia and/or insulin deficiency and not a primary defect of α-cell function (62, 64). This observation would suggest that glucose-induced dysregulation of energy metabolism in islets drives hyperglucagonemia. This raises the question, whether α -cell dysfunction also involves an exaggerated response to AA or fat, which may also involve mitochondrial mechanisms.

How does hypoglycemia stimulate glucagon secretion?

Normally, hypoglycemia triggers a counter-regulatory response in the α-cells which does not happen in many T1DM and some T2DM patients. The comprehensive mechanisms how glucose regulates glucagon secretion remain unclear. It has been claimed that CNS and hepatoportal sensors (i.e. hypoglycemia activated gastrointestinal neurons in the brainstem and in several hypothalamic nuclei) contribute to the control of glucagon(65, 66). Recent studies also questioned whether the primary role of glucagon is to elevate glucose concentrations(67). While a fast's beginning is marked by an initial surge in glucagon levels along with a decrease in blood glucose, an extended fast exceeding three days triggers a gradual reduction in circulating glucagon levels. Surprisingly, these levels eventually normalize to what is typically seen after a meal, even in the presence of consistently low blood glucose(39). Furthermore, the administration of glucagon in hypoglycemic individuals who have been fasting for more than 3 days does not produce any significant changes in glycaemia, possibly due to depleted glycogen stores(68).

Two recent studies claimed that Irak4 (Interleukin-1 receptor associated kinase-4) controls hypoglycemia-induced glucagon secretion by modulating hypothalamic Il-1β signaling, and Agpat5 activation in AgRP (agouti-related protein) neurons leads to hypoglycemia-induced glucagon

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secretion (69, 70). It is established that sodium-glucose co-transporter 1 (SGLT-1) and the generation of reactive oxygen species (ROS) released from β-cells, are involved in increasing α-cell proliferation and glucagon secretion (71). The role of glucagon in contributing to hyperglycemia in diabetes was reinforced by observing lower glucose levels in mice with knockout of the glucagon receptors (GCGRs) (72), as well as improved glucose and HbA1c in humans treated with acute and prolonged pharmacological receptor antagonisms (GRAs) in clinical and preclinical trials, although accompanied with hepatic side effects such as increases in transaminases, liver fat accumulation and dyslipidemia in addition to α-cell hyperplasia (73-75).

The reason why counter-regulation fails in diabetic patients is not fully understood. Interestingly, inhibiting mitochondrial ATP production or pharmacologically acti-vating KATP channels with diazoxide mimics the dysregulation of glucagon secre-tion (63). These observations collectively suggest that the glucagon secretion defect in diabetic patients may stem from disrupted mitochondrial metabolism, though the exact mechanisms remain unclear.

It has been proposed that glucagon increases with the onset of obesity and fatty liver as a consequence of hepatic glucagon resistance (76) and insulin resistance due to inappropriate regulation of glucagon by fasting and a static glucagon/insulin ratio (77). Normally fasting induces an increase glucagon/insulin ratio which leads a predominant glucagon signaling to increase hepatic intracellular concentrations of the second messenger cAMP and downstream PKA and CREB signaling pathways, increasing hepatic glucose output (78). One study highlighted the role of nutrient signaling via mTOR complex 1 (mTORC1) regulation to control glucagon secretion and α-cell mass (79). However, one recent study in mice demonstrated that chronic hyperglucagonemia can improve glucose homeostasis by downregulating hepatic GCGR expression, inducing hepatic "glucagon resistance", and enhancing insulin secretion (80).

4. Glucagon and amino acid metabolism

In addition to its established glucose-regulatory effects, glucagon powerfully regulates hepatic AA turnover by increasing activities of necessary transporters and enzymes in the urea cycle through cAMP-PKA-CREB protein (81, 82). In fact, there is evidence to suggest that the impact of glucagon signaling may vary between fasting and post-prandial conditions (83). Glucagon activates the transcription of AA transporters located on the hepatocyte membrane, thus allowing increased AA uptake and substrate availability for ureagenesis (84). In turn, AAs enhance glucagon secretion from α cells (85). This generates a glucagon and AA feedback loop, referred to as "liver-α-cell axis", which might be as important for metabolism as the glucagon-glucose loop (86-90). In line with this, genetic, GCGR-antibody or pharmacological inhibition of glucagon signaling leads to α-cell hypersecretion and hyperplasia as well as a decrease of hepatic AA transporters and gene expression involved in AA metabolism, resulting in dramatically increased plasma concentrations of some but not AAs (86, 89, 91). Recently, decreased AAs levels were reported to reduce target of rapamycin (mTOR) signaling in α-cells and suppress α-cell proliferation (92). At the hormonal level, protein-rich meals acutely increase glucagon secretion depending on the kinetics of AA plasma levels (93, 94). If excess AA, i.e. more than can be utilized for protein synthesis, are taken up with meals or liberated by proteolysis upon fasting, they are used as energy substrates (95). Since muscle and other organs cannot handle the amino groups, they delaminate the AA and take the carbon moiety for Krebs cycle and use primarily alanine to shuttle the amino groups to the liver. Alanine therefore predominates within 15 glucogenic AA (96) and is preferentially taken up by the liver in the presence of elevated glucagon for glucose production (known as glucose–alanine cycle or Cahill cycle) (97-99). This glucose–alanine cycle is dysregulated in dysglycaemia in humans with obesity and T2DM, as exemplified by heightened splanchnic (that is, viscera and liver) alanine uptake. One recent study demonstrated that alanine transport and aminotransferase (ALT) isoform expression (ALT and ALT2) were remarkably higher in obese, prediabetes and overt diabetic mouse models and in individuals with metabolic diseases. In addition, a GCGR antagonist reduced hyperglycemia accompanied by blunting of increased ALT/ALT2 activity in mice (98).

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Glucagon regulates not only ureagenesis, but also affects renal nitrogen excretion (93). Glucagon affects fluid and solute transport in the distal tubule and collecting duct by increasing hepatic cAMP secretion, which, in turn, influences proximal tubule reabsorption of urea. This interaction increases the fractional excretion of urea, sodium, potassium and phosphates. After oral protein loading, there was a significant correlation between GFR and urinary urea nitrogen excretion rate (93). Furthermore, branched chain amino acids (BCAA) do not induce an increase in renal hemodynamics (100). A post-prandial increase in plasma glucagon could potentially counteract amino acid and insulin-stimulated mTORC1 activation, leading to the suppression of protein synthesis in the liver. After ingesting a protein-rich diet, the liver shows increased rates of translation initiation and protein synthesis compared to fasted animals. A hypothesis suggests that glucagon resistance, a molecular phenomenon affecting the physiological effects of glucagon on glucose, AA, and lipid metabolism, may contribute to the development of T2DM and metabolic diseases (94). In the healthy liver, glucagon binds to the hepatic GCGR and increases AA metabolism through urea production. Glucagon may influence several steps in this process, including various biochemical shunts in the carbamoyl phosphate and urea cycles. In subjects with liver diseases, such as NAFLD, GCGR resistance may affect this liver-α cell axis, as reduced hepatic GCGR expression or impaired GCGR signaling leads to decreased urea production, which in turn leads to hyperaminoacidemia and subsequent compensatory hyperglucagonemia. Furthermore, hepatic steatosis may impair glucagon-dependent enhancement of AA catabolism in mice and humans with NAFLD (101). A recent study reported that elevated plasma levels of total AAs associate with hyperglucagonemia in NAFLD independently of glycemic control (102). This hypothesis may explain the hyperglucagonemia frequently observed in obesity, fatty liver and T2DM. However, the relative contributions of alpha to beta-cell cross talk and diminished inhibition of glucagon secretion by insulin resistance or deficiency and increased AA stimulation is presently unclear. Both, fatty liver and insulin resistance correlate well with glucagon levels in plasma (103).

Most circulating AAs have been shown to potently stimulate both glucagon and insulin secretion in animals and humans, although not all AAs are identical in their glucagontropic effects (104, 105). The purpose of this increase in glucagon release is believed to prevent hypoglycemia after protein intake, as AAs also stimulate insulin secretion. As early as the 1970s, Unger had found that alanine infusion induced an increase in glucagon secretion accompanied with very little stimulatory effect on insulin secretion in dogs. Lysine contributed to a lesser extent to α-cell secretion, while BCAAs had no effects on glucagon secretion whereas they elicit a significant insulin response (104, 106-108). Nevertheless, However, other studies have reported that BCAAs stimulate the secretion of both insulin and glucagon, particularly with oral administration resulting in greater and more prolonged secretion of both hormones (109, 110). Later in 1974 arginine was proven to enhance both insulin and glucagon secretion which support separate glucose and arginine receptors on both α and β cells in rodents, or via direct plasma membrane depolarization and Ca2+ influx in the α-cell (111, 112). In mice, leucine intake indeed improved glycemic control while some studies observed negative effects on glucose homeostasis for methionine and BCAAs (113-115). Furthermore, studies in animal and human isolated α cells indicated L-glutamine is a positive modulator of glucagon release and could regulate α cell proliferation and mass via mTOR-dependent nutrient sensing (116, 117). In the following decades, clinical studies often utilized specific AAs, although the effects of individual AAs on glucagon secretion remain an area of controversy. Primarily arginine, is established as an α-cell secretagogue, which induces significant increases in circulating glucagon and insulin regardless of ambient glycaemia (9). Recent research demonstrated that BCAAs directly raised intracellular Ca2+ levels in α cells, leading to increased plasma glucagon levels in diabetic mice. This suggests that disordered BCAA catabolism in pancreatic islet cells contributes to postprandial hypersecretion of glucagon in diabetes (118). Additionally, a separate study showed that glycine ingestion resulted in a slight decrease in serum glucose and increased insulin and glucagon concentrations in healthy human subjects (119, 120). Galsgaard et al. proposed that defective glucagon signaling to the liver results in hyper-aminoacidemia, which further stimulates the secretion of glucagon, possibly resulting in hyperplasia of α cells (121). However, the precise number of amino acids involved in the

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glucagon and amino acid feedback loop remains unclear, with glutamine suggested as a potential candidate (116). Additionally, which AAs are capable of stimulating glucagon secretion directly from pancreatic α-cells or via increasing GCGR signaling remains mysterious. The Holst group also found that alanine, arginine, cysteine, and proline are involved in the acute regulation of the liver-α-cell axis through administering AA mixtures in vivo in mice(105).Ingested whey protein in healthy participants induced hyperglucagonemia while suppressing free fatty acids, and showed that physiological hyperglucagonemia can override the hepatic actions of insulin, and postprandial hyperglucagonemia evolved to concurrently and synergistically collaborate with insulin to regulate glucose, AA, and nitrogen metabolism(122). Epidemiological evidence indicates that plant protein-based nutrition, with lower methionine and BCAAs and higher arginine content, is inversely associated with mortality and T2DM (123). There are studies indicating that soy protein normalized fasting hyperglucagonemia and improved glucagon resistance in obese Zucker (fa/fa) rats through inducing increase GCGR recycling to the membrane of adipocytes and its ligand-binding and G-protein selectively(123-126). Our recent study showed that plant protein, with lower methionine and BCAAs but higher arginine content, leads to greater postprandial increases in glucagon compared to animal protein. As a result, it requires higher insulin levels to control glucose metabolism, which seems to be associated with the rate of amino acid appearance in patients with T2DM(127).

5. Glucagon and lipid metabolism

Glucagon is recognized for its potent hypolipidemic effects. In humans, intravenous glucagon administration reduces plasma cholesterol, total esterified fatty acids, hepatic synthesis of triglycerides, and apolipoproteins by stimulating β-oxidation and lipolysis in the liver (128, 129). Furthermore, glucagon also reduces hepatic lipid accumulation and decrease hepatic lipid secretion through the inhibition of lipogenesis in the liver (130). In addition, glucagon stimulates lipolysis in the white adipose tissue, increased ketone-body production and fatty acid oxidation in humans and rats (24) (131, 132).

It is established that increased GCGR signaling has been linked to improved lipid metabolism. In 1979, studies explored glucagon's role in the direct short-term regulation of hepatic free fatty acid (FFA) metabolism. They found that physiological concentrations of glucagon increased ketogenesis and reduced triglyceride synthesis from palmitate in hepatocytes from fed rats at FFA concentrations of 1.0 mM or lower (133). Glucagon could modulate FFA metabolism by both intrahepatic and extrahepatic mechanisms, i.e. glucagon reduces de novo fatty acid synthesis by inhibiting malonyl-CoA formation. This occurs after an increase in intracellular cAMP activates PKA, which phosphorylates and inactivates acetyl-CoA carboxylase (ACC) (134).

As GCGR are expressed on β-cells and may stimulate insulin through both GLP-1R and GCGR, one may speculate that intra-islet regulation of insulin by glucagon might contribute to its effect on lipid metabolism. As discussed above, GCGR antagonists (e.g., LY2409021, Volagidemab) have been considered as glucose-lowering therapy in T2DM patients but resulted in lipid disorders, whereas glucagon/GLP-1 receptors co-agonism improved dyslipidemia and reduced hepatic steatosis which brought up discussions regarding to the relationship between glucagon signaling and lipid metabolism (5, 135, 136).

It remains unclear how glucagon promotes hepatic mitochondrial fat oxidation and to what extent glucagon influences lipolysis in adipose tissue, especially in humans. Previous study confirmed that InsP3R-I is essential due to reduced glucose production with knockdown InsP3R-I in isolated hepatocytes (34). The recent discovery from Perry and co-workers(26) is quite impressive, which reported glucagon stimulates intrahepatic lipolysis through InsP3R-I/CAMKII-dependent activation with increased hepatic acetyl-CoA. In addition, glucagon stimulates hepatic mitochondrial oxidation through InsP3R-I-mediated calcium signaling. The INSP3R1–ATGL pathway appears to play a central role in the regulation of hepatic lipid metabolism in response to glucagon. This is supported by the fact that plasma non-esterified fatty acid levels did not change significantly in both INSP3R1-LKO mice and the control mice during glucagon infusion (26). Those results explain many

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of these actions since the cAMP/PKA mediated effects were transcriptional and did not explain the acute metabolic actions of glucagon.

In turn, the capability of FFAs to regulate glucagon secretion remains in debate although they are insulin secretagogues under some circumstances, and increased FFAs levels might be correlated with T2DM (21, 137-139). Early research in 1974 had shown that elevation of plasma FFA suppressed glucagon levels in people, which are supported by the following clinical studies (138, 140-142). Experiments on isolated rodent islets, an α-cell line, and human islets have shown that FFAs (oleate or palmitate) stimulate glucagon secretion. This occurs through signaling via fatty acid G-protein coupled receptors, β-oxidation of fatty acids, and activation of L-type Ca2+ channels. Additionally, it involves relieving the inhibitory paracrine action of somatostatin secreted from δ-cells (143-145). Some studies observed that palmitate stimulates glucagon secretion in a glucose-dependent manner. This means glucagon is secreted when the glucose concentration is below 10mM but not at 16.7mM (144, 146). Wang et al. reported long-chain FFA (linoleic acid) acutely stimulated glucagon secretion by activation of G protein coupled receptor 40 (GPR40) and phospholipase C to increase Ca2+ release and associated Ca2+ influx through Ca2+ channels in primary cultured rat pancreatic islets (147) (Figure 2). Similar effects have been observed in rat islets with oleic acid (148). The Danish group reported that short-term exposure to FFAs directly increases glucagon release from α-cells. The stimulatory action depends on the chain length, degree of unsaturation, and spatial configuration of FAs in isolated mouse islets and alpha TC1-6 cells. Saturated fatty acids (SFA) were found to be more effective than unsaturated fatty acids (USFA) in stimulating glucagon secretion (144, 149), and later new data indicated that prolonged exposure (up to 3 days) to palmitate and oleate leads to excessive lipid accumulation and induces time- and dose-dependent hyperglucagonemia in both isolated islets and alpha TC1-6 cells through oxidation (150, 151). Long-term culture of a clonal α-cell line with palmitate increased glucagon release and expression, likely through activation of the AMPK pathway (152). Conversely, insulin's inhibitory effect on glucagon release was impaired after prolonged exposure to free fatty acids (FFAs) due to palmitate-induced insulin resistance, involving defects in the IRS-1/PI3K/Akt pathway (152). In rat islets, chronic exposure to fatty acids resulted in increased glucagon release but decreased glucagon content without altering glucagon gene expression (153, 154). Glucolipotoxicity conditions, such as combining palmitate with high glucose levels, can induce apoptosis in rodent α-cells (155). In clinical studies, intravenous or oral administration of a lipid emulsion did not alter glucagon secretion, but only oral lipid ingestion elicited a clear insulin response with increased GIP and GLP-1 concentrations(156). In other studies, no difference in glucagon secretion was observed after a high-fat or low-fat diet intake in both never-obese and post-obese women (157). However, an alternative study noted a slight but higher glucagon response to C4-dietary oil compared to C18-olive oil in overweight subjects with T2DM (158). Furthermore, a sharp increase of plasma glucagon concentrations was observed in healthy men after the ingestion of long-chain fatty acids (olive oil and C8 fatty acids) while no increase with short-chain fatty acid (C4) (159). Another study reported 6 months mono-unsaturated fatty acids (MUFA) intake contributes to larger post-lunch glucagon responses compared to a control meal in healthy young subjects (160). Interestingly, a recent study revealed that palmitate can cause a switch to a glucagon-secreting phenotype in intestinal GLP-1 secreting cells, suggesting the potential of fatty acids to induce extra-pancreatic glucagon (161). Moreover, both long- and short-chain FFA increase GIP concentrations which might be one potential reason to stimulate glucagon release in human(162, 163). Overall, the data from animals and humans support the hypothesis that chronic elevation of fatty acids may contribute to α-cell deregulation in T2DM.

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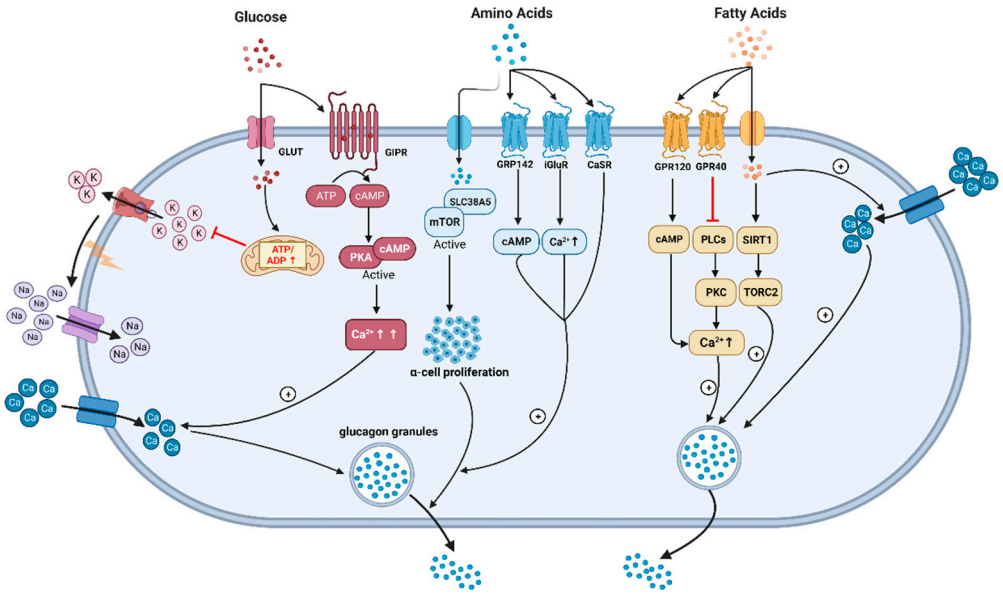

Figure 2. The underlying mechanisms of glucagon stimulation to glucose, amino acids, and fatty acids (this graph is generated with www.biorender.de).

Conclusion

In summary, glucagon plays a crucial role in glucose, AA and lipid metabolism, with its secretion tightly regulated by glucose levels and other factors. In patients with impaired glucose tolerance and T2DM, dysregulation of glucagon secretion leads to elevated fasting plasma glucagon concentrations even during hyperglycemia. The underlying mechanisms involve factors like incretins, somatostatin release, α cell dysfunction, hyperglycemia, and insulin deficiency. The exact mechanisms driving these dysregulations are still under investigation, presenting potential avenues for therapeutic intervention in diabetes management.

Furthermore, glucagon's impact extends beyond glucose regulation, influencing hepatic amino acid turnover and lipid metabolism. The "liver-α-cell axis" links amino acids to glucagon secretion, contributing to metabolic imbalances in conditions like obesity and T2DM. Specific AAs, such as arginine and branched-chain amino acids, directly influence plasma glucagon levels. Moreover, glucagon's potent hypolipidemic effects are attributed to its role in hepatic β-oxidation, lipolysis, and inhibition of lipogenesis. However, the complex interplay between glucagon and fatty acids requires further elucidation to understand its implications in metabolic diseases.

Overall, investigating the intricate mechanisms governing glucagon's diverse functions is vital to unraveling its role in metabolic disorders like T2DM. Advancing our understanding of glucagon's regulatory pathways may offer new opportunities for developing targeted therapies to restore metabolic balance and improve patient outcomes.

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