Glucagon Receptor Signaling and Lipid Metabolism

Galsgaard, Katrine D; Pedersen, Jens; Knop, Filip K; Holst, Jens J; Wewer Albrechtsen, Nicolai J

Published in:
Frontiers in Physiology

DOI:
10.3389/fphys.2019.00413

Publication date:
2019

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Galsgaard, K. D., Pedersen, J., Knop, F. K., Holst, J. J., & Wewer Albrechtsen, N. J. (2019). Glucagon Receptor Signaling and Lipid Metabolism. Frontiers in Physiology, 10, [413]. https://doi.org/10.3389/fphys.2019.00413
Glucagon Receptor Signaling and Lipid Metabolism

Katrine D. Galsgaard1,2, Jens Pedersen1,3, Filip K. Knop2,4,5, Jens J. Holst1,2,* and Nicolai J. Wewer Albrechtsen1,6,7,*

1 Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 2 Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 3 Department of Cardiology, Nephrology and Endocrinology, Nordjylland Hospital Himmer, University of Copenhagen, Himmer, Denmark; 4 Clinical Metabolic Physiology, Steno Diabetes Center Copenhagen, Gentele Hospital, Hellerup, Denmark; 5 Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; 6 Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark; 7 Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Glucagon is secreted from the pancreatic alpha cells upon hypoglycemia and stimulates hepatic glucose production. Type 2 diabetes is associated with dysregulated glucagon secretion, and increased glucagon concentrations contribute to the diabetic hyperglycemia. Antagonists of the glucagon receptor have been considered as glucose-lowering therapy in type 2 diabetes patients, but their clinical applicability has been questioned because of reports of therapy-induced increments in liver fat content and increased plasma concentrations of low-density lipoprotein. Conversely, in animal models, increased glucagon receptor signaling has been linked to improved lipid metabolism. Glucagon acts primarily on the liver and by regulating hepatic lipid metabolism glucagon may reduce hepatic lipid accumulation and decrease hepatic lipid secretion. Regarding whole-body lipid metabolism, it is controversial to what extent glucagon influences lipolysis in adipose tissue, particularly in humans. Glucagon receptor agonists combined with glucagon-like peptide 1 receptor agonists (dual agonists) improve dyslipidemia and reduce hepatic steatosis. Collectively, emerging data support an essential role of glucagon for lipid metabolism.

Keywords: glucagon, lipid, liver, adipose tissue, alpha cell

INTRODUCTION

Glucagon is processed from its precursor, proglucagon, by prohormone convertase 2 and secreted from pancreatic alpha cells (Rouille et al., 1994). The role of glucagon in glucose metabolism has been intensively studied, and comprehensive reviews are found elsewhere (Jiang and Zhang, 2003; Ramnanan et al., 2011; Ahren, 2015; Holst et al., 2017a). In addition to regulating glucose metabolism, glucagon also seems important for minute-to-minute regulation of amino acid metabolism as part of the recently described liver-alpha cell axis (Solloway et al., 2015; Dean et al., 2017; Galsgaard et al., 2017; Holst et al., 2017b; Kim et al., 2017), in which amino acids stimulate glucagon secretion and glucagon in turn stimulates hepatic amino acid uptake and metabolism (ureagenesis) and, thus, circulating amino acid concentrations as well as increased hepatic NADH/NAD⁺ ratio. The actions of glucagon are mediated via the glucagon receptor,
α a seven transmembrane receptor coupled to Gq-proteins, which regulate adenylate cyclase (AC) and phospholipase C activities when activated (Wakelam et al., 1986; Jelinek et al., 1993; Aromataris et al., 2006). The glucagon receptor is primarily expressed in the liver, but it is also expressed in varying amounts in the central nervous system, kidneys, gastro-intestinal tract, heart (controversial), and pancreas (Svoboda et al., 1994).

Glucagon receptor expression has been reported in rat adipocytes (Svoboda et al., 1994; Hansen et al., 1995), where a lipolytic effect of glucagon may be of physiological relevance. As type 2 diabetic hyperglucagonaemia (Faerch et al., 2016) contributes to the hyperglycemic state of patients with type 2 diabetes (T2D) (Unger and Orci, 1975; Baron et al., 1987), inhibition of glucagon receptor signaling has been investigated as glucose-lowering therapy in T2D patients (Kazda et al., 2016; Kazierad et al., 2016, 2018; Vajda et al., 2017; Pettus et al., 2018). Interestingly, potential adverse effects of this therapeutic approach include increased low-density lipoprotein (LDL) plasma concentrations and increased hepatic fat accumulation (Guzman et al., 2017). Furthermore, hepatocyte studies have shown that glucagon stimulates beta-oxidation (Pegorier et al., 1989), inhibits lipogenesis and decrease triglyceride (TG) and very-low-density lipoprotein (VLDL) secretion (Guettet et al., 1988; Boe et al., 2003) emphasizing a potentially important role of glucagon in lipid metabolism.

**GLUCAGON MIGHT STIMULATE LIPOLYSIS IN ADIPOSE TISSUE IN RODENTS BUT NOT IN HUMANS**

Lipolysis in adipocytes depends on activation of AC and thereby increased protein kinase A (PKA) activity. PKA phosphorylates (hence activates) perilipins (Greenberg et al., 1991) and hormone-sensitive lipase (HSL) (Stralfors et al., 1984; Garten et al., 1988; Anthonisen et al., 1998), and two additional lipases, resulting in hydrolysis of TGs and release of glycerol and free fatty acids (FFAs), e.g., palmitate (Egan et al., 1992; Lass et al., 2006; Granneman et al., 2009; Shen et al., 2009; Wang et al., 2009; Figure 1). Circulating levels of FFAs and glycerol therefore reflect the rate of lipolysis (Schweiger et al., 2014). For glucagon to directly influence adipocyte function, its cognate receptor must be expressed. Glucagon receptor mRNA has been detected in rat adipocytes (Svoboda et al., 1994; Hansen et al., 1995), but to determine the physiological relevance of glucagon receptor mRNA expression, it is necessary to investigate whether the mRNA is actually translated into a functional receptor. Specific antibodies directed against the glucagon receptor are necessary in addressing this question, but development of specific antibodies against glucagon receptors has been challenging and the antibodies available are unspecific and therefore not suitable for receptor localization (van der Woning et al., 2016). As an example, one study reported localization of the glucagon receptor in rat adipocytes using a monoclonal antibody (Iwanij and Vincent, 1990) whereas another using autoradiography, glucagon receptors were not found to be expressed (Watanabe et al., 1998), and no studies have demonstrated presence of glucagon receptors on human adipocytes (Carranza et al., 1993). Clearly, future studies should investigate glucagon receptor expression using antibody and antibody-independent methods.

Glucagon has been reported to activate glucagon receptor signaling has been investigated as glucose-lowering therapy in T2D patients (Kazda et al., 2016; Kazierad et al., 2016, 2018; Vajda et al., 2017; Pettus et al., 2018). Interestingly, potential adverse effects of this therapeutic approach include increased low-density lipoprotein (LDL) plasma concentrations and increased hepatic fat accumulation (Guzman et al., 2017). Furthermore, hepatocyte studies have shown that glucagon stimulates beta-oxidation (Pegorier et al., 1989), inhibits lipogenesis and decrease triglyceride (TG) and very-low-density lipoprotein (VLDL) secretion (Guettet et al., 1988; Boe et al., 2003) emphasizing a potentially important role of glucagon in lipid metabolism.

**GLUCAGON MIGHT STIMULATE LIPOLYSIS IN ADIPOSE TISSUE IN RODENTS BUT NOT IN HUMANS**

Lipolysis in adipocytes depends on activation of AC and thereby increased protein kinase A (PKA) activity. PKA phosphorylates (hence activates) perilipins (Greenberg et al., 1991) and hormone-sensitive lipase (HSL) (Stralfors et al., 1984; Garten et al., 1988; Anthonisen et al., 1998), and two additional lipases, resulting in hydrolysis of TGs and release of glycerol and free fatty acids (FFAs), e.g., palmitate (Egan et al., 1992; Lass et al., 2006; Granneman et al., 2009; Shen et al., 2009; Wang et al., 2009; Figure 1). Circulating levels of FFAs and glycerol therefore reflect the rate of lipolysis (Schweiger et al., 2014). For glucagon to directly influence adipocyte function, its cognate receptor must be expressed. Glucagon receptor mRNA has been detected in rat adipocytes (Svoboda et al., 1994; Hansen et al., 1995), but to determine the physiological relevance of glucagon receptor mRNA expression, it is necessary to investigate whether the mRNA is actually translated into a functional receptor. Specific antibodies directed against the glucagon receptor are necessary in addressing this question, but development of specific antibodies against glucagon receptors has been challenging and the antibodies available are unspecific and therefore not suitable for receptor localization (van der Woning et al., 2016). As an example, one study reported localization of the glucagon receptor in rat adipocytes using a monoclonal antibody (Iwanij and Vincent, 1990) whereas another using autoradiography, glucagon receptors were not found to be expressed (Watanabe et al., 1998), and no studies have demonstrated presence of glucagon receptors on human adipocytes (Carranza et al., 1993). Clearly, future studies should investigate glucagon receptor expression using antibody and antibody-independent methods.

Glucagon has been reported to activate glucagon receptor signaling has been investigated as glucose-lowering therapy in T2D patients (Kazda et al., 2016; Kazierad et al., 2016, 2018; Vajda et al., 2017; Pettus et al., 2018). Interestingly, potential adverse effects of this therapeutic approach include increased low-density lipoprotein (LDL) plasma concentrations and increased hepatic fat accumulation (Guzman et al., 2017). Furthermore, hepatocyte studies have shown that glucagon stimulates beta-oxidation (Pegorier et al., 1989), inhibits lipogenesis and decrease triglyceride (TG) and very-low-density lipoprotein (VLDL) secretion (Guettet et al., 1988; Boe et al., 2003) emphasizing a potentially important role of glucagon in lipid metabolism.
Glucagon and Lipid Metabolism

**FIGURE 1** | Glucagon ensures energy supply by mobilizing lipids. In the fasting state, glucagon is secreted and insulin concentrations are not sufficient to inhibit lipolysis in adipocytes, where lipids are stored in lipid droplets consisting of a core of triglycerols (TG) and sterols esters coated with perilipins (P) (proteins restricting access to the lipid core). In response to an appropriate stimuli, e.g., epinephrine and possibly glucagon, AC found in the plasma membrane of the adipocyte is activated, leading to increased intracellular concentrations of cAMP stimulating protein kinase A (PKA) activity. PKA phosphorylates (hence activates) hormone sensitive lipase (HSL) and P. The phosphorylation of P results in dissociation of the protein CGI-58. CGI-58 activates adipose triglycerol lipase (ATGL), which converts TGs to diglycerols (DG). The phosphorylated P bind HSL and allows it to access the lipid droplet where it converts DGs to monoglycerols (MG). The monoglycerols are hydrolyzed by monoacylglycerol lipase (MGL), yielding free fatty acids (FFAs) and glycerol, which are released to the blood. FFAs may stimulate glucagon secretion, and glucagon in turn stimulates hepatic gluconeogenesis (using FFAs and glycerol as substrates), glycogenolysis, and beta-oxidation thus providing substrates for the liver to secure sufficient energy supply to metabolically active tissue. Enzymes are written in italic and arrows indicate stimulation.

(Gerich et al., 1976). Furthermore, infusion with saline only gave the same increase in FFA as compared to glucagon infusion. In another study glucagon was infused at 1.2 ng/kg × min (high but also relevant) together with somatostatin for 2 h, but there was no lipolytic effect of glucagon at insulin concentrations of 38 pM (Jensen et al., 1991). In contrast, a 2-h glucagon infusion at 1.3 ng/kg × min, during a mean insulin plasma concentration of 65 pM, increased the rate of appearance of labeled FFA and glycerol by 40 and 36%, respectively (Carlson et al., 1993). As glucagon receptors are expressed on beta
metabolic state in the hepatocytes may therefore be determined by release from adipocytes, but also by rate of uptake and re-esterification in other tissues. A lack of effect of glucagon on the free plasma pool of FFA and glycerol, does therefore not rule out that glucagon has a direct effect on lipid metabolism in adipocytes and hepatocytes (Figure 1).

**GLUCAGON STIMULATES HEPATIC BETA-OXIDATION AND INHIBITS LIPOGENESIS**

In hepatocytes, glucagon action increases the transcription factor cAMP responsive element binding (CREB) protein, which induces the transcription of carnitine acyl transferase I (CPT-1) (Longuet et al., 2008). CPT-1 enables catabolism of long-chain fatty acids by converting fatty acids to acyl-carnitines, which are transported into the mitochondria and subjected to beta-oxidation (Kim et al., 2000; Stephens et al., 2007). During beta-oxidation the fatty acids are degraded into acetate, which ultimately enters the citric acid cycle (DiMarco and Hoppel, 1975). Furthermore, through PKA-dependent phosphorylation, glucagon receptor signaling inactivates acetyl-CoA carboxylase, the enzyme catalyzing the formation of malonyl-CoA. Malonyl-CoA is the first intermediate in fatty acid synthesis and inhibits CPT-1 (i.e., inhibits beta-oxidation). By inhibiting the formation of malonyl-CoA, glucagon diverts FFAs to beta-oxidation rather than re-esterification into TGs (Figure 2). Periportal and perivenous hepatocytes receive different concentrations of substrates and oxygen and as a consequence periportal hepatocytes primarily mediate oxidative processes, including beta-oxidation, whereas perivenous hepatocytes preferentially mediate glucose uptake and lipogenesis (Jungermann, 1988; Guzman and Castro, 1989).

In hepatocytes, glucagon may bring about an energy-depleted state (increasing the AMP/ATP ratio) sufficient to activate AMP-activated kinase (Berglund et al., 2009), which phosphorylates acetyl-CoA carboxylase (Peng et al., 2012) and p38 mitogen-activated protein kinase, leading to transcriptional activation of peroxisome proliferator-activated receptor-α (PPARα) (Longuet et al., 2008). PPARα stimulates the transcription of genes involved in beta-oxidation including CPT-1, CPT-2, and acetyl-CoA oxidase (Patsouris et al., 2006), and the transcription of fibroblast growth factor 21, which is produced in the liver in response to glucagon (Xu et al., 2009; Cyphert et al., 2014). Glucagon also stimulates forkhead transcription factor A2 activity (FoxA2), which induces transcription of genes involved in beta-oxidation, such as CPT-1, very-, and medium- long-chain acyl-CoA dehydrogenase (Wolfrum and Stoffel, 2006; von Meyenn et al., 2013). Subsequent to activating its receptors on hepatocytes, insulin suppresses most of these pathways, and the metabolic state in the hepatocytes may therefore be determined by the insulin-glucagon ratio, rather than by the hormone concentrations per se (Parrilla et al., 1974). Insulin inhibits lipolysis in adipocytes and by reducing the amount of substrate (FFA and glycerol) reaching the liver may reduce (Perry et al., 2015) hepatic gluconeogenesis.

To investigate the physiological effects of glucagon in lipid metabolism, several studies have relied on glucagon receptor knockout (Gcgr−/−) mice or animals treated with GRA. In the livers of Gcgr−/− mice there is an increase in glycolysis and a decrease in gluconeogenesis and citric acid cycle activity, which results in decreased acetyl-CoA oxidation and acetyl-CoA accumulation. The accumulation of acetyl-CoA in the cytosol of hepatocytes results in increased lipogenesis. Supporting this, genes involved in lipogenesis, e.g., ATP citrate lyase and fatty acid synthase, were found to be upregulated in livers of Gcgr−/− mice at both the mRNA and protein level (Longuet et al., 2008; Yang et al., 2011), while CPT-1 and -2 levels, and other enzymes necessary for beta-oxidation, were downregulated (Yang et al., 2011). Hepatic beta-oxidation is essential for the production of both glucose and ketones since it provides the substrates acetyl-CoA and acetate and mitochondrial energy supply (ATP/NADH) needed for gluconeogenesis (Staehr et al., 2003). The hepatic gene expression profile changes markedly in response to fasting, and major differences have been reported in expression levels of genes involved in lipid metabolism between the fed and fasted state (Longuet et al., 2008; Zhang et al., 2011). Following a prolonged fast (16 h), wild-type mice had an increased hepatic expression of genes involved in beta-oxidation, such as CPT-1, CPT-2, and acetyl-CoA dehydrogenase, but this was not observed in Gcgr−/− mice, which displayed an impaired beta-oxidation in both the fasted and fed state (Longuet et al., 2008) and Gcgr−/− mice failed to change the hepatic energy state in response to fasting (Berglund et al., 2009). Furthermore, Gcgr−/− mice showed increased hepatic TG secretion and increased plasma concentrations of TG and FFA after a 16 h fasting period, but not after 5 h of fasting (Longuet et al., 2008). Others (Gelling et al., 2003) also found similar TG and FFA plasma concentrations in Gcgr−/− and wild-type mice after a short-term fast; they did, however, find increased plasma concentrations of LDL in Gcgr−/− mice. Glucagon thus seems to regulate hepatic metabolism in response to fasting by stimulating glucose-producing processes, including beta-oxidation. When challenged with a high fat diet (HFD) for 8 weeks, Gcgr−/− mice did not increase the amount of inguinal and epididymal fat, whereas the amount of both doubled in wild-type mice (Longuet et al., 2008). In line with this, others (Gelling et al., 2003) showed a decrease in white adipose tissue mass and an increase in lean body mass in Gcgr−/− compared to wild-type mice, without changes in bodyweight, food consumption, or energy expenditure and one group (Conarello et al., 2007) found that Gcgr−/− mice had lower amounts of white adipose tissue when fed both a HFD and a low fat diet compared to wild-type mice, and thus seemed to be resistant to diet-induced obesity. This could reflect an inability of Gcgr−/− mice to mobilize the hepatic lipid storage; instead adipocyte lipolysis (by catecholamines) maintain the energy supply to other metabolically active tissues.
FIGURE 2 | The effects of glucagon receptor signaling on hepatic lipid metabolism. Glucagon activates its cognate receptor, a seven transmembrane receptor coupled to a Gs protein, resulting in AC activity and cAMP production. The increase in intracellular cAMP activates protein kinase A (PKA), which phosphorylates acetyl-CoA carboxylase (ACC). Glucagon thus inhibits malonyl-CoA formation and the subsequent de novo fatty acid synthesis. When formed, the fatty acids are, after re-esterification, stored as triglycerides in and released from the hepatocytes in the form of very-low-density lipoprotein (VLDL). Thus, glucagon leads the free fatty acids toward beta-oxidation and decreases de novo fatty acid synthesis and VLDL release. cAMP accumulation in hepatocytes activates the cAMP responsive binding element (CREB) protein, which induces the transcription of carnitine acyl transferase-1 (CPT-1), and other genes needed for beta-oxidation. CPT-1 catalyzes the attachment of carnitine to fatty acyl-CoA, forming acyl-carnitine. The acyl-carnitines transverse the mitochondrial membrane mediated via the carnitine-acylcarnitine translocase (CACT). Once in the mitochondrial matrix, carnitine acyl transferase-2 (CPT-2) is responsible for transferring the acyl-group from the acyl-carnitine back to CoA. Carnitine leaves the mitochondria matrix through the carnitine-acylcarnitine translocase. During beta-oxidation, the fatty acid chains are degraded into acetate. Acetate reacts with CoA to yield acetyl-CoA, which reacts with oxaloacetate to form citrate that inhibits glycolysis through inhibition of pyruvate dehydrogenase and phosphofructokinase-1. Finally, citrate enters the citric acid cycle (TCA). Thus, glucagon increases fatty acid catabolism, inhibits glycolysis, and fuels the TCA cycle. By increasing AC activity glucagon increases the AMP/ATP ratio sufficient to activate AMP-activated kinase (AMPK), which phosphorylates ACC, leading to transcriptional activation of peroxisome proliferator-activated receptor-α (PPARα). PPARα stimulates the transcription of genes involved in beta-oxidation including CPT-1, CPT-2, and acetyl-CoA oxidase. Glucagon stimulates FoxA2 activity, which induces transcription of genes such as CPT-1, very-, and medium- long-chain acyl-CoA dehydrogenase. Enzymes and pathways inhibited by glucagon are shown in red, while enzymes and pathways stimulated by glucagon are shown in black.

IMPLICATIONS OF GLUCAGON RECEPTOR SIGNALING IN THE DEVELOPMENT OF STEATOSIS

Administration of GRAs has been associated with increased hepatic fat content (assessed as hepatic fat fraction measured by magnetic resonance imaging) and increased plasma concentrations of LDL (Guzman et al., 2017). Furthermore, subjects with endogenous glucagon deficiency (pancreatectomized subjects) (Dresler et al., 1991) and rats (Sloop et al., 2004) and diabetic (db/db) mice (Liang et al., 2004) treated with glucagon antisense oligonucleotide have increased hepatic fat. These data suggest that inhibition of glucagon receptor signaling results in hepatic lipid accumulation. In addition, Gcgr−/− mice may be prone to steatosis when challenged with a high fat diet (HFD) for 8 weeks (Longuet et al., 2008). However, a study involving a similar HFD diet for 12 weeks and mice with the same sex, gene modification, and background (C57BL/6J), showed that Gcgr−/− mice were protected from steatosis (Conarello et al., 2007). Of notice,
Acute administration of 30 µg/kg glucagon decreased FFA and TG plasma concentrations and reduced hepatic TG content and secretion in mice (Longuet et al., 2008). Chronic hyperglucagonemia (injection of 10 µg glucagon every 8 h for 21 days) had hypolipidemic effects in rats, evident by a 70 and 38% decrease in plasma concentrations of TGs and phospholipids, respectively (Guettet et al., 1988). Consistent with this, glucagon inhibited synthesis and secretion of TGs in cultured hepatocytes (Longuet et al., 2008), in perfused rat livers (Penhos et al., 1966; Heimberg et al., 1969), and decreased the synthesis of hepatic VLDL in rats (Eaton, 1973). In humans, hyperglucagonemia (56 ± 20 pM), during a pancreatic clamp, reduced hepatic lipoprotein particle turnover (Xiao et al., 2011), and glucagon administration increased hepatic beta-oxidation in humans (Prip-Buus et al., 1990). In diet-induced obese (DIO) mice, a once-weekly treatment with 70 nmol/kg glucagon/GLP-1 receptor co-agonist resulted in loss of fat mass, which in the same study was also found, although less pronounced in GLP-1 receptor knockout mice, and improved hepatic lipid metabolism and steatosis within 4 weeks (Day et al., 2009). Another glucagon/GLP-1 co-agonist (1.9 μmol/kg daily for 14 days) decreased acetyl-CoA and malonyl-CoA concentrations and increased CPT-1 mRNA in the livers of DIO mice, whereas a selective GLP-1 receptor agonist had no effect (Pocai et al., 2009). Both of these dual agonists reduced hepatic steatosis, increased HSL activity in adipocytes, and improved dyslipidemia in DIO mice (Day et al., 2009; Pocai et al., 2009). Supporting these data, other glucagon/GLP-1 receptor co-agonists have been reported to lower plasma concentrations of TG and cholesterol (Clemmensen et al., 2014), decrease hepatic fat content (Henderson et al., 2016), and reduce adipose mass in rodent models of T2D and obesity (Evers et al., 2017; Zhou et al., 2017). Importantly, acute administration of 25 nmol/kg glucagon/GLP-1 co-agonist decreased plasma concentration of TGs, cholesterol, and LDL in DIO mice within 1 h, whereas liraglutide (a pure GLP-1 receptor agonist) administration had no effect (More et al., 2017). In addition, hepatic synthesis of VLDL and palmitate, and fatty acid esterification decreased, while beta-oxidation and LDL receptors expression increased upon co-agonist, but not liraglutide, administration (More et al., 2017). The inhibitory effect on hepatic lipogenesis and stimulatory effect on beta-oxidation therefore seems to be mediated by glucagon receptor signaling. Several clinical studies are currently investigating the potential treatment of obesity and T2D using glucagon/GLP-1 co-agonists (Capozzi et al., 2018).

**REGULATION OF GLUCAGON SECRETION BY LIPIDS**

FFAs are under certain circumstances insulin secretagogues (Boden and Carnell, 2003) but their ability to stimulate glucagon secretion remains debated (Gerich et al., 1974; Bollheimer et al., 2004; Gromada et al., 2007). Some clinical studies found a suppression of glucagon secretion at increased FFA concentrations (Madison et al., 1968; Edwards and Taylor, 1970; Luyckx and Lefebvre, 1970; Gerich et al., 1974) whereas isolated alpha cells were shown to secrete glucagon in response to FFA stimulation (Gross and Mialhe, 1986; Collins et al., 2008). In isolated rat pancreatic islets, palmitate stimulated glucagon secretion (Gremlich et al., 1997; Dumonteil et al., 2000). Others found palmitate to stimulate glucagon secretion in a glucose-dependent manner using isolated pancreatic islets; increasing at glucose concentrations of 2.8, 5.6, and 10 mM (Olófsson et al., 2004) but not at 16.7 mM (Bollheimer et al., 2004). Medium and long-chain fatty acids (>C5) have been reported to stimulate glucagon secretion by activation of FFA receptor G protein-coupled receptor 40 (GPR40) (Wang et al., 2011; Kristinsson et al., 2017) and GPR119 (Hansen et al., 2012; Li et al., 2018), both present in the pancreatic islets (Briscoe et al., 2003). FFAs may also function as metabolic substrate and stimulate alpha cell secretion through beta-oxidation (Kristinsson et al., 2017; Briant et al., 2018). FFAs decrease secretion of somatostatin (Gromada et al., 2001), and may lower the tonic inhibition of somatostatin on alpha cells (Gromada et al., 2007; Müller et al., 2017). A clinical study investigating the effects of ingestion of lipids on hormone secretion, found no change in glucagon secretion after intravenous or oral administration of a lipid emulsion (3 ml/kg) (Lindgren et al., 2011), neither did glucagon plasma concentrations change upon a 300 min lipid infusion raising FFA plasma concentrations from 0.4 to 0.8 mM (Staehr et al., 2003). No difference in glucagon secretion was observed between subjects consuming a HFD or a low-fat diet for 2 weeks (Raben et al., 2001). In contrast to this, ingestion of long-chain fatty acids (olive oil and C8 fatty acids) lead to increased plasma concentrations of glucagon 40 min after, whereas no increase was observed after ingestion of short-chain fatty acids (C4), however, glucose-dependent insulinoceptive polypeptide (GIP) concentrations also increased upon ingestion of long-chain fatty acids and this may have caused an increase in glucagon secretion (Mandoe et al., 2015). Another study observed that a meal rich in mono-unsaturated fatty acids resulted in a larger glucagon response when compared to a control meal (Sloth et al., 2009). Others also observed an increase in glucagon concentrations upon fat-enriched meals (Radulescu et al., 2010; Niederwanger et al., 2014). The glucagon response observed upon a 90 min intraduodenal infusion of linoleic, oleic, and palmitic acids were significant lower than observed upon protein infusion (Ryan et al., 2013). Studies of ability of FFAs to stimulate glucagon secretion are complex, since FFAs are found in many forms and their stimulatory effect may vary (Radulescu et al., 2010) [as is the case for incretin secretion (Feltrin et al., 2004; Thomsen et al., 1999)]. Furthermore, the
increased glucagon concentrations reported in some studies may result from other proglucagon products (e.g., glicentin or oxyntomodulin), since measurements of plasma glucagon concentrations have been marred with problems regarding sensitivity and specificity (Wewer Albrechtsen et al., 2016), and further studies investigating the regulation of glucagon secretion by FFAs are needed.

CONCLUSION

Glucagon may, aside from its physiological actions on glucose and amino acid metabolism, also be important for lipid metabolism via effects on hepatic beta-oxidation and lipogenesis, and potentially increased lipolysis in adipocytes. A direct role of glucagon on adipocytes may be of importance in rodents, as glucagon stimulates lipolysis (Vaughn and Steinberg, 1963; Rodbell and Jones, 1966; Prigge and Grande, 1971; Manganiello and Vaughan, 1972; Lefebvre et al., 1973; Livingston et al., 1974), whereas in humans an adipocyte-dependent lipolysis of glucagon is more complex. In both rodents and humans, glucagon is a powerful regulator of hepatic lipid metabolism (Day et al., 2009; Xiao et al., 2011) as highlighted in studies using GRAs (Guzman et al., 2017). The clinical use of GRAs is further challenged by glucagon’s role in amino acid metabolism, and amino acid metabolism, also be important for lipid metabolism.

Consequently, glucagon agonism (Day et al., 2009). Emerging Investigator Grant – Endocrinology and Metabolism – Nordic Region (34250), and Excellence Programme (31526), NNF Project support in Endocrinology and Metabolism (NNF19OC0055001). All funding sources have been submitted. NNF Tandem Glucagon agonism (Day et al., 2009). Although glucagon agonism offers promise for lipid metabolism, further studies investigating the regulation of glucagon secretion by FFAs are needed.

CONCLUSION

Glucagon may, aside from its physiological actions on glucose and amino acid metabolism, also be important for lipid metabolism via effects on hepatic beta-oxidation and lipogenesis, and potentially increased lipolysis in adipocytes. A direct role of glucagon on adipocytes may be of importance in rodents, as glucagon stimulates lipolysis (Vaughn and Steinberg, 1963; Rodbell and Jones, 1966; Prigge and Grande, 1971; Manganiello and Vaughan, 1972; Lefebvre et al., 1973; Livingston et al., 1974), whereas in humans an adipocyte-dependent lipolysis of glucagon is more complex. In both rodents and humans, glucagon is a powerful regulator of hepatic lipid metabolism (Day et al., 2009; Xiao et al., 2011) as highlighted in studies using GRAs (Guzman et al., 2017). The clinical use of GRAs is further challenged by glucagon’s role in amino acid metabolism, and amino acid metabolism, also be important for lipid metabolism.

Consequently, glucagon agonism (Day et al., 2009). Emerging Investigator Grant – Endocrinology and Metabolism – Nordic Region (34250), and Excellence Programme (31526), NNF Project support in Endocrinology and Metabolism (NNF19OC0055001). All funding sources have been submitted. NNF Tandem Glucagon agonism (Day et al., 2009). Although glucagon agonism offers promise for lipid metabolism, further studies investigating the regulation of glucagon secretion by FFAs are needed.

CONCLUSION

Glucagon may, aside from its physiological actions on glucose and amino acid metabolism, also be important for lipid metabolism via effects on hepatic beta-oxidation and lipogenesis, and potentially increased lipolysis in adipocytes. A direct role of glucagon on adipocytes may be of importance in rodents, as glucagon stimulates lipolysis (Vaughn and Steinberg, 1963; Rodbell and Jones, 1966; Prigge and Grande, 1971; Manganiello and Vaughan, 1972; Lefebvre et al., 1973; Livingston et al., 1974), whereas in humans an adipocyte-dependent lipolysis of glucagon is more complex. In both rodents and humans, glucagon is a powerful regulator of hepatic lipid metabolism (Day et al., 2009; Xiao et al., 2011) as highlighted in studies using GRAs (Guzman et al., 2017). The clinical use of GRAs is further challenged by glucagon’s role in amino acid metabolism, and amino acid metabolism, also be important for lipid metabolism. Consequently, glucagon agonism (Day et al., 2009). Emerging Investigator Grant – Endocrinology and Metabolism – Nordic Region (34250), and Excellence Programme (31526), NNF Project support in Endocrinology and Metabolism (NNF19OC0055001). All funding sources have been submitted. NNF Tandem Glucagon agonism (Day et al., 2009). Although glucagon agonism offers promise for lipid metabolism, further studies investigating the regulation of glucagon secretion by FFAs are needed.
GLP-1/glucagon receptor peptide agonist in rodents and non-human primates. *Diabetes Obs. Metab.* 18, 1176–1190. doi: 10.1111/dom.12735

Hjorth, S. A., Adelhorst, K., Pedersen, B. B., Kirk, O., and Schwartz, T. W. (1994). Glucagon and glucagon-like peptide 1: selective receptor recognition via distinct peptide epitopes. *J. Biol. Chem.* 269, 30121–30124.

Holst, J. J., Holland, W., Gromada, J., Lee, Y., Unger, R. H., Yan, H., et al. (2017a). Insulin and glucagon: partners for life. *Endocrinology* 158, 696–701. doi: 10.1210/en.2016-1748

Holst, J. J., Wever Albrechtsen, N. J., Pedersen, J., and Knop, F. K. (2017b). Glucagon and amino acids are linked in a mutual feedback cycle: the liver-alpha-cell axis. *Diabetes* 66, 235–240. doi: 10.2373/diab16-0994

Honnor, R. C., Dhillon, G. S., and Londos, C. (1985). cAMP-dependent protein kinase and lipolysis in rat adipocytes. II. Definition of steady-state relationships with lipolytic and antilipolytic modulators. *J. Biol. Chem.* 260, 15130–15138.

Iwanij, V., and Vincent, A. C. (1990). Characterization of the glucagon receptor and its functional domains using monoclonal antibodies. *J. Biol. Chem. 265*, 21302–21308.

Jelinek, L. J., Lok, S., Rosenberg, G. B., Smith, R. A., Grant, F. J., Biggs, S., et al. (1993). Expression cloning and signaling properties of the rat glucagon receptor. *Science* 259, 1614–1616. doi: 10.1126/science.8384375

Jensen, M. D., Heiling, V. J., and Miles, J. M. (1991). Effects of glucagon on free fatty acid metabolism in humans. *J. Clin. Endocrinol. Metab.* 72, 308–315. doi: 10.1209/016-2-3-08

Jiang, G., and Zhang, B. B. (2003). Glucagon and regulation of glucose metabolism. *Am. J. Physiol. Endocrinol. Metab.* 284, E671–E678. doi: 10.1152/ajpendo.00492.2002

Jugermann, K. (1988). Metabolic zonation of liver parenchyma. *Semin. Liver Dis.* 8, 329–341. doi: 10.1055/s-2008-104554

Kazda, C. M., Ding, Y., Kelly, R. P., Garhyan, P., Shi, C., Lim, C. N., et al. (2016). Evaluation of efficacy and safety of the glucagon receptor antagonist LY2490021 in patients with type 2 diabetes: 12- and 24-week phase 2 studies. *Diabetes Care* 39, 1241–1249. doi: 10.2323/dci15-1643

Kazierad, D. J., Bergman, A., Tan, B., Erion, D. M., Somayaji, V., Lee, D. S., et al. (2016). Effects of multiple ascending doses of the glucagon receptor antagonist PF-06291874 in patients with type 2 diabetes mellitus. *Diabetes Obes. Metab.* 18, 795–802. doi: 10.1111/dobm.12176

Kazierad, D. J., Chidsey, K., Somayaji, V. R., Bergman, A. J., and Calle, R. A. (2018). Efficacy and safety of the glucagon receptor antagonist PF-06291874: a 12-week, randomized, dose-response study in patients with type 2 diabetes mellitus on background metformin therapy. *Diabetes Obes. Metab.* 20, 2608–2616. doi: 10.1111/dobm.13440

Kim, J., Okamoto, H., Huang, Z., Anguiano, G., Chen, S., Liu, Q., et al. (2017). Amino acid transporter Slc38a5 controls glucagon receptor inhibition-induced pancreatic alpha-cell hyperplasia in mice. *Cell Metab.* 25, 1348–1361.e8. doi: 10.1016/j战胜送丸.2017.05.006

Kim, J. Y., Hickner, R. C., Courttright, R. L., Dohm, G. L., and Hounard, J. A. (2000). Lipid oxidation is reduced in obese skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 279, E1039–E1045. doi: 10.1152/ajpendo.2000.279.5.E1039

Kristinsson, H., Sargsyan, E., Manell, H., Smith, D. M., Gopel, S. O., and Mitchell, M. L., Byrne, M. J., and Silver, J. (1996). Growth-hormone release by glucagon. *Lancet* 1, 289–290. doi: 10.1016/S0140-6736(96)91041-1

Leyte, A. S., and Lefebvre, P. J. (1970). Arguments for a regulation of pancreatic glucagon secretion by circulating plasma free fatty acids. *Proc. Soc. Exp. Biol. Med.* 133, 524–537. doi: 10.3874/jem.18.3011.3-1541

Lilenquist, J. E., Bomboy, J. D., Lewis, S. B., Smith-Ritchie, B. C., Felts, P. W., Lacy, W. W., et al. (1974). Effects of glucagon on lipolysis and ketogenesis in normal and diabetic men. *J. Clin. Invest.* 53, 190–197. doi: 10.1172/JCI107537

Lindgren, O., Carr, R. D., Deacon, C. F., Holst, J. J., Pacini, G., Mari, A., et al. (2011). Incretin hormone and insulin responses to oral versus intravenous lipid administration in humans. *J. Clin. Endocrinol. Metab.* 96, 2519–2524. doi: 10.1210/jc.2011-0266

Livingston, J. N., Cuatrecasas, P., and Lockwood, D. H. (1974). Studies of glucagon resistance in large rat adipocytes: 12SI-labeled glucagon binding and lipolytic capacity. *J. Lipid Res.* 15, 26–32.

Longuet, C., Sinclair, E. M., Maïda, A., Baggio, L. L., Maziaj, M. J., et al. (2008). The glucagon receptor is required for the adaptive metabolic response to fasting. *Cell Metab.* 8, 359–371. doi: 10.1016/j.cmet.2008.09.008

Lycklama, A. S., and Liu, C. (2017). Insulin and glucagon enhance lipolysis in vitro. *Diabetes* 65, 410–417. doi: 10.2327/dbi53.2.410
Sadry, S. A., and Drucker, D. J. (2013). Emerging combinatorial hormone therapies.

Frontiers in Physiology | www.frontiersin.org

Rouille, Y., Westermark, G., Martin, S. K., and Steiner, D. F. (1994). Proglucagon

Galsgaard et al. Glucagon and Lipid Metabolism

Ryan, A. T., Luscombe-Marsh, N. D., Saies, A. A., Little, T. J., Standfield, S., Rodbell, M., and Jones, A. B. (1966). Metabolism of isolated fat cells. 3. The similar

Sanchez-Garrido, M. A., Brandt, S. J., Clemmensen, C., Muller, T. D., DiMarchi, W. O., Robl, H., and Schwandt, P. (1989). Human glucagon and vasoactive

Perry, R. J., Camporez, J. G., Kursawe, R., Titchenell, P. M., Zhang, D., Perry, C. J., Sanchez-Garrido, M. A., Brandt, S. J., Clemmensen, C., Muller, T. D., DiMarchi, R. D., and Tschop, M. H. (2017). GLP-1/glucagon receptor co-agonism for treatment of obesity. Diabetologia 60, 1851–1861. doi: 10.1007/s00125-017-4354-9

Schade, D. S., and Eaton, R. P. (1975). Modulation of fatty acid metabolism by glucagon in man. I. Effects in normal subjects. Diabetes 24, 502–509. doi: 10.2337/diabetes.24.5.502

Schneider, S. H., Fineberg, S. E., and Blackburn, G. L. (1981). The acute metabolic effects of glucagon and its interactions with insulin in forearm tissue. Diabetologia 20, 616–621. doi: 10.1007/BF00257430

Schweiger, M., Eichmann, T. O., Taschner, U., Zimmermann, R., Zechner, R., and Lass, A. (2014). Measurement of lipolysis. Methods Enzymol. 538, 171–193. doi: 10.1016/B978-0-12-800280-3.00010-4

Shen, W. J., Patel, S., Miyoshi, H., Greenberg, A. S., and Kraemer, F. B. (2009). Functional interaction of hormone-sensitive lipase and perilipin in lipolysis. J. Lipid Res. 50, 2306–2313. doi: 10.1146/jlr.M900176-JLR200

Slavin, B. G., Ong, J. M., and Kern, P. A. (1994). Hormonal regulation of hormone-sensitive lipase activity and mRNA levels in isolated rat adipocytes. J. Lipid Res. 35, 1535–1541.

Sloop, K. W., Cao, J. X., Siesky, A. M., Zhang, H. Y., Bodenmiller, D. M., Cox, A. L., et al. (2004). Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. J. Clin. Invest. 113, 1571–1581. doi: 10.1172/JCI20991

Sloth, B., Due, A., Larsen, M. T., Holst, J., Heding, A., and Astrup, A. (2009). The effect of a high-MUFA, low-glycemic index diet and a low-fat diet on appetite and glucose metabolism during a 6-month weight maintenance period. Br. J. Nutr. 101, 1846–1858. doi: 10.1017/S0007114508377710

Solloway, M. J., Madjidi, A., Gu, C., Eastham-Anderson, J., Clarke, H., Kjavin, N., et al. (2015). Glucagon couples hepatic amino acid catabolism to mTOR-dependent regulation of alpha-cell mass. Cell Rep. 12, 495–510. doi: 10.1016/j.celrep.2015.06.034

Staehr, P., Holder-Nielsen, O., Landau, B. R., Chandramouli, V., Holst, J. J., and Beck-Nielsen, H. (2003). Effects of free fatty acids per se on glucose production, glucogenogenesis, and glycogenesis. Diabetes 52, 260–267. doi: 10.2337/diabetes.52.2.260

Stallknecht, B., Simonsen, L., Bulow, J., Vinten, J., and Galbo, H. (1995). Effect of training on epinephrine-stimulated lipolysis determined by microdialysis in human adipose tissue. Am. J. Physiol. 269(6 Pt 1), E1059–E1066. doi: 10.1152/ajpendo.1995.269.6.E1059

Stephens, F. B., Constantin-Teodosiu, D., and Greenhaff, P. L. (2007). New insights concerning the role of carnitine in the regulation of fuel metabolism in skeletal muscle. J. Physiol. 581(Pt 2), 431–444. doi: 10.1113/jphysiol.2006.125799

Stralfors, P., Bjorgell, P., and Belfrage, P. (1984). Hormonal regulation of hormone-sensitive lipase in intact adipocytes: identification of phosphorylated sites and effects on the phosphorylation by lipolytic hormones and insulin. Proc. Natl. Acad. Sci. U.S.A. 81, 3317–3321. doi: 10.1073/pnas.81.11.3317

Svensend, B., Larsen, O., Gabe, M. B. N., Christiansen, C. B., Rosenkilde, M. M., Drucker, D. J., et al. (2018). Insulin secretion depends on intra-islet glucagon signaling. Cell Rep. 25, 1127–1134.e2. doi: 10.1016/j.celrep.2018.10.018

Svoboda, M., Tastenov, M., Vertongen, P., and Robberecht, P. (1994). Relative quantitative analysis of glucagon receptor mRNA in rat tissues. Mol. Cell. Endocrinol. 105, 131–137. doi: 10.1016/0303-7207(94)01062-7

Thomsen, C., Rasmussen, O., Lousen, T., Holst, J. J., Fenselau, S., Schrezenmeir, J., et al. (1999). Differential effects of saturated and monounsaturated fatty acids on postprandial lipemia and incretin responses in healthy subjects. Am. J. Clin. Nutr. 69, 1135–1143. doi: 10.1093/ajcn/69.6.1135

Unger, R. H., and Orci, L. (1975). The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet 1, 14–16. doi: 10.1016/S0140-6736(75)92375-2

Vajda, E. G., Logan, D., Lasserter, K., Armas, D., Plotkin, D. J., Pipkin, D. J., et al. (2017). pharmacokinetics and pharmacodynamics of single and multiple doses of the glucagon receptor antagonist LGD-6972 in healthy subjects and subjects with type 2 diabetes mellitus. Diabetes Obes. Metab. 19, 24–32. doi: 10.1111/dom.12752

van der Woning, B., De Boeck, G., Blanchetot, C., Bobkov, V., Klarenbeek, A., Saunders, M., et al. (2016). DNA immunization combined with scFv phage display identifies antagonistic GCGR specific antibodies and reveals new epitopes on the small extracellular loops. MAbs 8, 1126–1135. doi: 10.1080/19420622.2016.1189050

Vaughn, M., Berger, J. E., and Steinberg, D. (1964). Hormone-sensitive lipase and monounaturated lipase activities in adipose tissue. J. Biol. Chem. 239, 401–409

Vaughn, M., and Steinberg, D. (1963). Effect of hormones on lipolysis and esterification of free fatty acids during incubation of adipose tissue in vitro. J. Lipid Res. 4, 193–199.
Vizek, K., Razova, M., and Melichar, V. (1979). Lipolytic effect of TSH, glucagon and hydrocortisone on the adipose tissue of newborns and adults in vitro. Physiol. Bohemoslov. 28, 325–331.

von Meyenn, F., Porstmann, T., Gasser, E., Selevsek, N., Schmidt, A., Aebersold, R., et al. (2013). Glucagon-induced acetylation of Foxa2 regulates hepatic lipid metabolism. Cell Metab. 17, 436–447. doi: 10.1016/j.cmet.2013.01.014

Wakelam, M. J., Murphy, G. J., Hruby, V. J., and Houslay, M. D. (1986). Activation of two signal-transduction systems in hepatocytes by glucagon. Nature 323, 68–71. doi: 10.1038/323068a0

Wang, H., Hu, L., Dalen, K., Dorward, H., Marcinkiewicz, A., Russell, D., et al. (2009). Activation of hormone-sensitive lipase requires two steps, protein phosphorylation and binding to the PAT-1 domain of lipid droplet coat proteins. J. Biol. Chem. 284, 32116–32125. doi: 10.1074/jbc.M109.006726

Wang, L., Zhao, Y., Gui, B., Fu, R., Ma, F., Yu, J., et al. (2011). Acute stimulation of glucagon secretion by linoleic acid results from GPR40 activation and [Ca2+]-i increase in pancreatic islet (alpha)-cells. J. Endocrinol. 210, 173–179. doi: 10.1530/JOE-11-0132

Xiao, C., Pavlic, M., Szeto, L., Patterson, B. W., and Lewis, G. F. (2011). Effects of acute hyperglucagonemia on hepatic and intestinal lipoprotein production and clearance in healthy humans. Diabetes 60, 383–390. doi: 10.2337/db10–0763

Xu, J., Stanislaus, S., Chinookoswong, N., Lau, Y. Y., Hager, T., Patel, J., et al. (2009). Acute glucose-lowering and insulin-sensitizing action of FGF21 in insulin-resistant mouse models—association with liver and adipose tissue effects. Am. J. Physiol. Endocrinol. Metab. 297, E1105–E1114. doi: 10.1152/ajpendo.00348.2009

Yang, J., MacDougall, M. L., McDowell, M. T., Xi, L., Wei, R., Zavadoski, W. J., et al. (2011). Polymeric profiling reveals significant hepatic metabolic alterations in glucagon-receptor (GCGR) knockout mice: implications on anti-glucagon therapies for diabetes. BMC Genom. 12:281. doi: 10.1186/1471-2164-12-281

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2019 Galsgaard, Pedersen, Knop, Holst and Wewer Albrechtsen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.