Islet α cells and glucagon—critical regulators of energy homeostasis

Jonathan E. Campbell and Daniel J. Drucker

Abstract | Glucagon is secreted from islet α cells and controls blood levels of glucose in the fasting state. Impaired glucagon secretion predisposes some patients with type 1 diabetes mellitus (T1DM) to hypoglycaemia; whereas hyperglycaemia in patients with T1DM or type 2 diabetes mellitus (T2DM) is often associated with hyperglucagonaemia. Hence, therapeutic strategies to safely achieve euglycaemia in patients with diabetes mellitus now encompass bihormonal approaches to simultaneously deliver insulin and glucagon (in patients with T1DM) or reduce excess glucagon action (in patients with T1DM or T2DM). Glucagon also reduces food intake and increases energy expenditure through central and peripheral mechanisms, which suggests that activation of signalling through the glucagon receptor might be useful for controlling body weight. Here, we review new data that is relevant to understanding α-cell biology and glucagon action in the brain, liver, adipose tissue and heart, with attention to normal physiology, as well as conditions associated with dysregulated glucagon action. The feasibility and safety of current and emerging glucagon-based therapies that encompass both gain-of-function and loss-of-function approaches for the treatment of T1DM, T2DM and obesity is discussed in addition to developments, challenges and critical gaps in our knowledge that require additional investigation.

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Introduction

Glucagon is a peptide comprised of 29 amino acids that is secreted at low levels from pancreatic α cells in the basal non-fasting state; whereas during long-term fasting or in response to hypoglycaemia, secretion of glucagon is increased. Studies of normoglycaemic individuals and patients with diabetes mellitus reveal that glucagon is the primary glucoregulatory hormone that counteracts the metabolic consequences of excessive insulin action.[1] *GCG* encodes proglucagon—a 160 amino acid precursor polypeptide that is processed in a tissue-specific manner to yield multiple proglucagon-derived peptides (Figure 1). Enteroendocrine cells predominantly express neuroendocrine convertase 1 (also known as prohormone convertase 1, PC1; encoded by *PCSK1*), which generates glicentin (aa 1–69), oxyntomodulin (aa 33–69), glucagon-like peptide 1 (GLP-1; aa 78–107/108) and glucagon-like peptide 2 (GLP-2; aa 126–158). By contrast, neuroendocrine convertase 2 (also known as prohormone convertase 2; PC2) is expressed in α cells and liberates glucagon (aa 33–61) and the major proglucagon fragment, which contains unprocessed GLP-1 and GLP-2.[2]

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Competing interests
D.J.D. has served as a consultant for companies developing incretin-based therapies for the treatment of diabetes mellitus, including Arisaph Pharmaceuticals, Intarcia Therapeutics, MedImmune, Merck Research Laboratories, Novo Nordisk and Receptos. Neither D.J.D. nor his family members hold stock directly or indirectly in any of these companies. Preclinical studies in D.J.D.'s laboratory are supported, in part, by grants to Mount Sinai Hospital from Merck, Novo Nordisk and Sanofi. J.E.C. declares no competing interests.

Glucagon acts through the glucagon receptor (GCGR), which is a single G protein-coupled receptor expressed in the liver as well as multiple extrahepatic tissues, such as the brain, heart, kidney, gastrointestinal tract and adipose tissues (Figure 2). The hyperglycaemic properties of pancreatic extracts containing glucagon were first reported over 90 years ago,[3] and glucagon administration has been used for the acute treatment of hypoglycaemia since the 1950s.[4] Contemporary treatment strategies that utilize continuous administration of glucagon as a component of antidiabetic therapy to reduce the frequency of hypoglycaemia in patients with type 1 diabetes mellitus (T1DM), have engendered considerable interest. Furthermore, the central role of glucagon in the pathophysiology of hyperglycaemia continues to foster enthusiasm for attenuating glucagon action in the treatment of patients with T1DM and type 2 diabetes mellitus (T2DM). Here, we review emerging advances in α islet cell biology and discuss new concepts of glucagon action in the control of carbohydrate and energy metabolism. The therapeutic efforts directed at manipulating GCGR signalling for the treatment of metabolic disorders are also discussed.

Glucagon

Regulation of secretion

Glucagon is secreted in response to hypoglycaemia, with a robust secretory response that is triggered when levels of glucose decline below a key threshold, which differs slightly between different species.[5,6] Glucagon secretion also rises following increases in circulating levels

Key points

- Perturbation of normal homeostatic control of proglucagon processing can occur, which gives rise to production of glucagon-like peptide 1 in the inflamed or injured pancreas
- The plasticity and interconversion of α cells and β cells provides opportunities for cell replacement and differentiation strategies for the treatment of diabetes mellitus
- The central role of glucagon in maintenance of euglycaemia underscores the rationale for clinical strategies for simultaneous glucagon and insulin administration for therapy of type 1 diabetes mellitus (T1DM)
- Glucagon agonists reduce food intake and increase energy expenditure, which highlights pathways that could be targeted in the treatment of obesity
- Dysregulated glucagon secretion and increased hepatic glucose production in patients with T1DM or type 2 diabetes mellitus forms the basis of attempts to reduce glucagon action to treat these individuals
- Mechanism-based adverse events, such as cardiovascular effects, are mediated by glucagon receptor signalling and should be considered when developing therapeutic strategies directed at enhancing or attenuating glucagon action

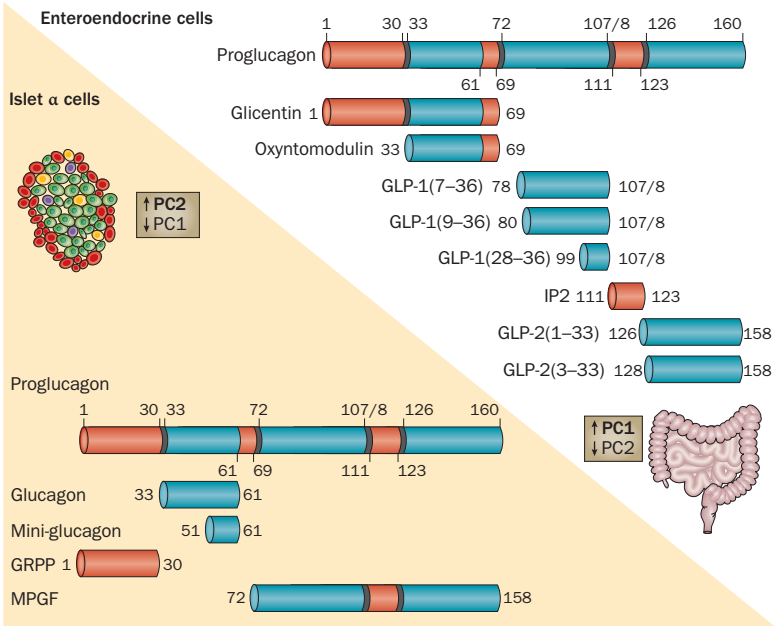

Figure 1 | Proglucagon-derived peptides liberated in the pancreas and gastrointestinal tract. A single mammalian gene gives rise to an identical precursor polypeptide, proglucagon, in enteroendocrine cells, brain and α cells. This prohormone undergoes differential cell-specific post-translational processing to yield different peptides, which themselves can undergo further enzymatic cleavage as shown. Abbreviations: GLP-1, glucagon-like peptide 1; GLP-2, glucagon-like peptide 2; GRPP, glicentin-related polypeptide; IP, intervening peptide; MPGF, major proglucagon fragment; PC1, prohormone convertase 1; PC2, prohormone convertase 2.

of amino acids and fatty acids, as well as in response to adrenergic stimulation and to some regulatory peptides.[7] The important role of multiple β-cell-derived secretory products, including insulin, zinc and γ-aminobutyric acid (GABA), in the suppression of glucagon secretion is widely appreciated[7] and might account for the failure of insulin alone to completely suppress glucagon secretion under conditions of impaired or lost β-cell function. Disruption of GLP-1 action, using antagonists or genetic manipulation, increases glucagon secretion in humans and rodents, respectively.[8] In addition, somatostatin is an essential negative regulator of cAMP-dependent glucagon secretion via the somatostatin receptor type 2 (SS2R); genetic disruption or transient antagonism of SS2R in rats results in increased glucagon secretion.[9] The potential of SS2R antagonism to correct defective glucagon secretion and reduce hypoglycaemia continues to be explored. Alternative approaches to restoring defective glucagon secretion, such as reducing the activity of K_{ATP} channels in α cells, are also being explored.[10]

The central nervous system (CNS) is an important sensor of ambient glucose levels and drives neural signals that augment glucagon secretion. Multiple regions and nuclei within the CNS, such as the hypothalamus and neurons positive for solute carrier family 2 facilitated glucose transporter member 2 (GLUT2) within the nucleus of the solitary tract of the medulla, contribute to the stimulation of glucagon secretion during hypoglycaemia via increased parasympathetic input.[11] By contrast, in humans, vagotomy leads to increased plasma levels of glucagon following consumption of a mixed meal, but not during intravenous glucose challenge,[12,13] which suggests that the vagus nerve conveys gut-derived inhibitory signals, either directly or indirectly, to α cells. Furthermore, the complexity of islet cholinergic innervation seems to be species-specific, as human (but not mouse) islet α cells express a range of molecules that are required for the synthesis, export and signalling of acetylcholine.[14] Counterintuitively, release of acetylcholine from stimulated α cells is implicated as a priming signal that enhances insulin secretion from adjacent β cells via muscarinic acetylcholine receptor M3.[15] Alternatively, intra-islet acetylcholine might target human δ cells via muscarinic acetylcholine receptor M1 to release somatostatin, thereby inhibiting insulin secretion.[14]

Whether glucose primarily controls glucagon release directly, via glucose sensing and metabolism in α cells, or indirectly, through β cell, δ cell or neural activity under normoglycaemic, hyperglycaemic or hypoglycaemic conditions, is difficult to precisely elucidate *in vivo*. The extrapolation of insights from elegant reductionist biology analysing glucose sensing in single α cells to whole animal physiology remains challenging. Although oral administration of glucose suppresses glucagon secretion in the normal postprandial state,[16] glucagon secretion can rise slightly following ingestion of mixed meals (depending on the composition of the meal) in healthy individuals.[17] Hyperglycaemia reduces glucagon secretion from the perfused pancreas or isolated islets; however, glucagon secretion unexpectedly rises in response to increases in glucose concentrations in isolated α cells.[18] Understanding how α cells integrate information from the gut, circulating levels of nutrients, metabolites, hormones, neural inputs and intra-islet signals to fine tune glucagon release in the fasting, postprandial and hypoglycaemic states, remains an important subject for investigation.

Challenges in accurate measurements

Glicentin, oxyntomodulin and glucagon each contain an identical sequence that corresponds to amino acids 33–61 of proglucagon (Figure 1). Thus, crossreactivity and non-specificity might be possible with assays utilizing antisera directed at residues within the core 29 amino acid glucagon

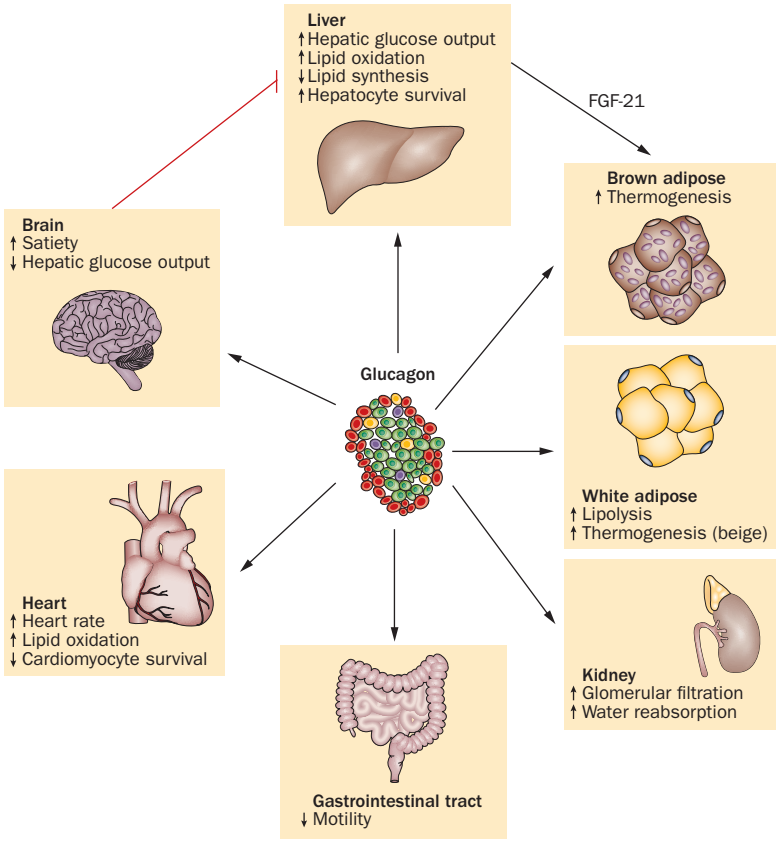

Figure 2 | Physiological actions of glucagon. Glucagon elicits direct biological actions through the glucagon receptor, which is expressed in the liver, brain, heart, kidney, white adipocytes, brown adipocytes and the gastrointestinal tract. Glucagon also regulates metabolism indirectly through neural signalling and by liberation of FGF-21 from the liver. Abbreviation: FGF-21, fibroblast growth factor 21.

sequence exists. Furthermore, glucagon is processed into truncated forms (amino acids 3–29, 18–29 and 19–29) by various endopeptidases (neprilysin, for example) and exopeptidases (such as dipeptidyl peptidase 4 [DPP4]), and these processed peptides can exhibit crossreactivity with some antisera.[19] Glucagon circulates at very low levels, which further challenges efforts to detect small changes in immunoreactive glucagon levels in response to physiological events such as refeeding. Evaluation of commercially available assays for measuring circulating levels of glucagon demonstrates that poor specificity and/or suboptimal sensitivity remain common problems.[19] Improved specificity, with minimal crossreactivity against oxyntomodulin and glicentin, has been achieved with newly developed enzyme-linked immunosorbent assays (ELISAs) that use separate monoclonal antibodies, which target each end of the glucagon peptide and have little crossreactivity to oxyntomodulin (<5%) or glicentin (<2%).[13] Circulating levels of glucagon in patients with renal impairment were raised when measured by a single-site-carboxyterminal-specific assay, yet normal when assayed with a specific two-site ELISA.[13] Interpretation of the existing literature reporting measurements of glucagon in plasma is problematic as a result of the widespread historical use of incompletely characterized assays that have suboptimal sensitivity and specificity.

Physiological fluctuations in secretion

Physiological levels of glucagon in healthy individuals are in the ranges of 6–12 pM (~20–40 pg/ml) following an overnight fast and 3–5 pM (~10–17 pg/ml) in the postprandial state.[13,19] Individuals with T2DM often exhibit a 50–100% increase in fasting levels of glucagon and manifest impaired suppression or inappropriate increases in glucagon levels following challenge with enteral but not intravenous glucose.[16] The clinical relevance of defective α-cell function in individuals with T2DM is highlighted by the frequent development of both fasting and postprandial hyperglucagonaemia, which leads to increased hepatic production of glucose and hyperglycaemia.[20] Despite the important inhibitory role of the β cell in physiological control of glucagon secretion, insulin replacement to correct dysglycaemia is frequently insufficient to restore normal glucagon dynamics in individuals with T1DM.[21] The failure of exogenous insulin to completely normalize glucagon levels might reflect challenges in achieving the necessary intra-islet concentrations of glucagon or the requirement for one or more additional inhibitory factors, such as zinc, GABA and/or somatostatin, that also suppress glucagon secretion.

Considerable evidence links the incretin hormone gastric inhibitory polypeptide (also known as glucose-dependent insulinotropic polypeptide; GIP) to control of α-cell function and glucagon secretion.[22,23] In patients with T2DM, GIP infusion increased postprandial levels of glucagon, despite a concomitant increase in plasma levels of insulin.[22] Similarly, infusion of GIP during a hyperglycaemic clamp impaired the suppression of plasma levels of glucagon in patients with T2DM.[23] Conversely, under conditions of hypoglycaemia, the actions of GIP to augment glucagon secretion might be beneficial in restoring euglycaemia. Of note, both mouse and human α cells have been reported to express a truncated, but biologically active, form of GIP (amino acids 1–30) that is capable of stimulating insulin secretion from β cells.[24] Although multiple laboratories have reported co-localization of GIP with glucagon by immunocytochemistry, transcriptomic analyses of purified islet-cell preparations have failed to demonstrate selective expression of *Gip* and *GIP* in mouse and human α cells, respectively.[25] Whether GIP produced from K cells is a physiologically important regulator of α-cell function remains uncertain, as the necessary preclinical studies have not been conducted and highly selective GIP receptor antagonists that are suitable for use in humans have not yet been developed.

Plasticity of islet α cells

Hormone secretion

Under normal physiological circumstances, α cells do not produce substantial amounts of GLP-1 or a detectable intestinal proglucagon-derived peptide profile; however, metabolic stress and pancreatic and/or islet injury or inflammation[26–28] can lead to induction of *PCSK1* expression and GLP-1 production in these cells.[8] Chemical ablation of β cells in rats resulted in induction of PCSK1 expression in α cells and increased levels of bioactive GLP-1 (amino acids 7–36) amide in pancreatic extracts.[26]

Exposure to inflammatory cytokines, such as IL-6, directly increased α-cell expression of PC1 and GLP-1 in mouse and human islets.[28] Moreover, acute intraperitoneal administration of IL-6 improved glucose tolerance and insulin secretion in wild-type, but not in *Glp1r*$^{-/-}$ mice.[28] Evidence for GLP-1 production and PC1 expression in islet α cells isolated from individuals without diabetes mellitus and from patients with T2DM was obtained using a combination of immunohistochemistry, western blotting and mass spectrometry.[27] Cultured human islet cells released bioactive GLP-1 in response to high levels of glucose and arginine; islets from donors with T2DM exhibited higher basal GLP-1 secretion than islets from healthy donors.[27] Nevertheless, levels of circulating GLP-1 are generally normal or modestly reduced in most individuals with T2DM.[29,30] Whether human α cells in their usual intra-islet location produce notable amounts of bioactive GLP-1 in the pancreata of normal individuals or patients with diabetes mellitus is challenging to ascertain; levels of GLP-1 in perfusates from pancreata of normal individuals or patients with diabetes mellitus have not been reported.

Islet cell phenotypes

Transdifferentiation of α cells into insulin-positive β cells has been described in studies of mice. Expression of the transcription factor gene *Pax4* in α cells induced a β-cell phenotype,[31] whereas elimination of *Pax4* in β cells resulted in increased numbers of α cells.[32] Near-complete destruction of β cells in a transgenetic mouse with β-cell-specific expression of the diphtheria toxin receptor (proheparin-binding EGF-like growth factor) resulted in spontaneous appearance of 'bihormonal' cells that stained positive for both insulin and glucagon.[33] These data suggested that α cells respond to environmental or metabolic cues that reflect depletion of β cells. Lineage-tracing experiments confirmed that the newly generated insulin-positive cells were derived from an α-cell population, as combined diphtheria-toxin-mediated ablation of both β cells and α cells prevented the emergence of these bihormonal cells.[33] Remarkably, despite the putative importance of α-cell–β-cell intra-islet communication, diphtheria-toxin-mediated destruction of 98% of murine α cells did not produce substantial disturbances in β-cell function or glucose homeostasis under normal or diabetic conditions.[34]

Transdifferentiation of mouse β cells to an α-cell phenotype did occur following transgenic β-cell-specific expression of a gain-of-function K_{ATP} channel, which markedly limited insulin secretion and induced severe diabetes mellitus.[35] Increased numbers of α cells, as well as β cells that coexpressed glucagon, were evident after 4 weeks of hyperglycaemia; this phenotype was reversed by treatment with glibenclamide.[35] Consistent with phenotypic evidence supporting plasticity and interconversion of islet cells, human α cells contain a high proportion of bivalent chromatin signatures at genes, such as *PDX1* and *MAFA*, that are active and normally assumed to function to maintain β-cell identity.[25] These findings revealed that a state of epigenetic flexibility in α cells exists, which enables genes to switch from transcriptionally repressed to active states.[25] Manipulation of histone methylation states with the histone methyltransferase inhibitor adenosine dialdehyde in human islets induced the expression of pancreas/duodenum homeobox protein 1 (PDX1) in α cells, which led to production of insulin and a 'β cell phenotype'.[25] Although substantial evidence supports the functional plasticity of rodent islet cells,[25,31,33,35] whether islet cells of individuals with diabetes mellitus exhibit similar plasticity is unclear. Delineation of therapeutic strategies and pathways for manipulating phenotypes of α cells and β cells to treat patients with diabetes mellitus requires considerable additional investigation.

Pathophysiology

Hyperglucagonaemia

Traditional dogma links increased plasma levels of glucagon to the inevitable development of hyperglycaemia; however, newly reported data has challenged this assumption. Administration of a single dose of the sodium/glucose cotransporter 2 (SGLT2) inhibitors empagliflozin or dapagliflozin increased both fasting and postprandial plasma levels of glucagon, which resulted in an increased glucagon:insulin ratio and enhanced endogenous glucose production in patients with T2DM.[36,37] Nevertheless, plasma levels of glucose in these individuals decreased as a result of the dominant effect of renal glycosuria.[36,37] Despite the reduction of glycaemia, plasma levels of glucagon remained high with sustained administration of SGLT2 inhibitors; however, the magnitude of the paradoxical increase in glucagon levels was attenuated with chronic drug administration.[36,37] The precise mechanisms that link SGLT2 inhibition to increased glucagon secretion are poorly understood, but probably reflect the actions of the SGLT2 transporter in islet α cells, which leads to enhanced glucagon biosynthesis and secretion when inhibited.[38]

In patients who have undergone metabolic surgery, glucagon levels can be raised following nutrient ingestion, despite major improvements in plasma levels of glucose and marked increases in levels of GLP-1.[13,39] Whether the increase in plasma levels of glucagon reflects disordered control of secretion from α cells, increased levels of circulating GLP-2, reduced clearance or *de novo* production of glucagon from enteroendocrine cells in the anatomically rearranged gastrointestinal tract is a subject of ongoing investigation.[13] Acute infusion of GLP-2 rapidly raises plasma levels of glucagon in both the fasting and postprandial states without any change in plasma levels of glucose in healthy individuals.[40] These actions of GLP-2 might be species-specific, as either gain or loss of signalling through the GLP-2 receptor (GLP-2R) has no effect on plasma levels of glucagon in mice.[41] Furthermore, disruption of glucose homeostasis or a state of hyperglucagonaemia have not been reported in patients with short bowel syndrome who have received long-term treatment with the GLP-2R agonist teduglutide.[42] Whether the rise in glucagon levels following administration of GLP-2 in healthy humans or patients with short bowel syndrome leads to increased hepatic production of glucose has not been examined. In addition, elucidation of the importance of endogenous GLP-2R signalling for glucagon secretion necessitates the development of selective GLP-2R antagonists that are suitable for use in humans.

Table 1 | Clinical trials of agents that regulate glucagon

Drug	Mechanism of action	Study duration	Current phase	Reference
Type 1 diabetes mellitus				
LY2409021	Selective GCGR antagonist	Single dose	Phase I	NCT01640834[104]
Type 2 diabetes mellitus				
MK-0893	Selective GCGR antagonist	4–13 weeks	Phase II	NCT00631488[105]
LY2409021	Selective GCGR antagonist	6–12 months	Phase II	NCT02111096[106]
PF-06291,874	Selective GCGR antagonist	28 days	Phase II	NCT02175121[107]
Ranolazine	Inhibition of α cell Na^+ channels	14 days	Phase I	NCT01843127[108]
LGD-6,972	Selective GCGR antagonist	28 days	Phase I	NCT02250222[109]
ISIS-GCGRRx	GCGR antisense siRNA	13 weeks	Phase II	NCT01885260[110]

Compounds were selected on the basis of a review of studies reported on https://clinicaltrials.gov/ since 2010. The active status of these drug development programmes is uncertain. Abbreviations: GCGR, glucagon receptor; siRNA, small interfering RNA.

Glucagon action in the brain

GCG is expressed predominantly in the brainstem and, to a lesser extent, in the hypothalamus;[43] however, the GCGR is widely expressed in the CNS,[44] and proglucagon-derived peptides, including glucagon, are transported to multiple distal regions of the CNS.[45] Classic pharmacological actions of glucagon in the brain include the induction of satiety, which supports the investigation of glucagon agonism for the treatment of obesity.[46,47] Surprisingly, infusion of glucagon into the mediobasal hypothalamus decreased hepatic glucose output in normoglycaemic rodents under conditions of a pancreatic clamp.[48] Thus, glucagon signalling in the mediobasal hypothalamus might serve to prevent the development of hyperglycaemia that is pursuant to sustained hepatic glucagon–GCGR signalling. Conversely, the inhibitory actions of glucagon in the CNS were attenuated in rats fed a high-fat diet. The mechanisms through which central GCGR signalling controls hepatic production of glucose rely on signalling through protein kinase A and intact hepatic vagal innervation.[48] Given the current limitations in selectively activating or blocking CNS action of glucagon in humans, the translational relevance of these findings is difficult to determine. Intracerebroventricular and systemic infusions of glucagon result in reduced appetite, which leads to weight loss with sustained administration.[46,49] Administration of glucagon also increased resting energy expenditure in healthy humans;[50] however, whether these effects are mediated by central and/or peripheral mechanisms needs to be investigated further.

Role of glucagon in hyperglycaemia

A glucagon-centred view of diabetes mellitus has re-emerged, and is supported by the requirement for glucagon in the development of hyperglycaemia in preclinical models of T1DM, T2DM and insulin deficiency.[4] Exogenous insulin fails to completely suppress glucagon secretion in patients with T1DM and T2DM,[4] which perhaps is reflective of the challenges of achieving required levels of intra-islet insulin and/or the importance of other β-cell products that are necessary for inhibition of α-cell function.[4] *Gcgr*$^{-/-}$ mice are not hyperglycaemic and demonstrate normal glucose tolerance, even after complete destruction of β cells;[51] restoration of hepatic *Gcgr* expression led to activation of gluconeogenesis and rapid reappearance of hyperglycaemia.[52] Nevertheless, *Gcgr*$^{-/-}$ mice also have compensatory upregulation of expression of GLP-1, oxyntomodulin and fibroblast growth factor 21 (FGF-21), which complicates elucidation of the mechanisms that contribute to euglycaemia in these animals.[53–55] Suppression of glucagon release with somatostatin in dogs that have been subjected to chemical destruction of their β cells or pancreatectomy alleviates hyperglycaemia.[56] Moreover, in patients with T1DM, treatment with somatostatin prevented development of hyperglycaemia and ketoacidosis following withdrawal of insulin.[57] The therapeutic potential of glucagon suppression using selective GCGR antagonists in patients with diabetes mellitus is currently being explored in clinical trials (Table 1).

The action of leptin in the suppression of glucagon secretion and normalization of glycaemic levels through CNS-dependent mechanisms in experimental models of T1DM has been proposed as additional evidence for the primacy of glucagon in the development of hyperglycaemia.[58] Nevertheless, administration of leptin rapidly reduces glucose levels in β-cell-depleted rats by 6 h; however, glucagon levels do not decrease until 24 h.[59] These findings illustrate the important role of glucocorticoids and the hypothalamic–pituitary axis (independent of glucagon) as critical downstream targets for leptin action in rodent models of leptin deficiency and T1DM.[59] The rapid development of hyperglycaemia in humans following total pancreatectomy, as well as in mice that have undergone β-cell destruction in the setting of ablation of α cells[34] also challenges the notion that glucagon is essential for the pathogenesis of hyperglycaemia in the context of insulin deficiency. Assessing the potential ability of human enteroendocrine cells to rapidly adapt to the loss of the entire pancreas, by generating bioactive mature glucagon (29 amino acids), requires careful analysis with highly specific glucagon immunoassays.

Glucagon as a therapeutic target

T1DM

Restoring defective glucagon secretion and/or development of systems to deliver exogenous glucagon continue to be explored as options for reducing development of severe hypoglycaemia in patients with T1DM. Intensive insulin therapy (INT), which is achieved with either multiple daily injections or with insulin pump therapy, reduces the development of microvascular and, to a lesser extent, macrovascular complications associated with T1DM. However, the incidence of complications associated with severe hypoglycaemia, including episodes of coma and seizure was threefold higher in the INT group than in the conventional treatment group.[60] Furthermore, study

participants allocated to INT experienced additional weight gain, with an increased number of patients becoming overweight or developing obesity. These and related observations have fostered efforts examining whether a combined glucagon–insulin regimen together with a closed-loop glucose sensor might mitigate some of the consequences of INT alone. Preliminary findings in a small group of patients with T1DM suggest that concomitant delivery of glucagon, triggered by the rate of decline or absolute level of glucose, attenuated or reversed the trend towards hypoglycaemia that is induced by infusion of rapid-acting insulin.[61]

The effectiveness of the bionic bihormonal pancreas was assessed in a 5-day randomized, cross-over study in patients with T1DM.[62] Mean glycaemia was reduced in adult participants, but not in adolescent participants, and all participants experienced considerably less frequent hypoglycaemic episodes with dual-glucagon-plus-insulin delivery relative to a traditional 'insulin-only' pump.[62] Optimization of adaptive individualized algorithms and wireless and pump hardware, as well as development of rapid-acting stable formulations of insulin and glucagon and enhanced glucose-sensing technology, is predicted to result in improved glucose control and decreased incidence of hypoglycaemic events.

T2DM

The primacy of hyperglucagonaemia in the pathophysiology of T2DM has invigorated efforts directed at reduction of glucagon action for the treatment of patients with this disease. The feasibility and success of this approach has been demonstrated in preclinical studies, in which antisense oligonucleotides to reduce hepatic *Gcgr* expression, the use of GCGR antagonists and antisera to immunoneutralize glucagon all effectively lowered glycaemia.[4,49] The rise in GLP-1 levels is a major contributor to the metabolic improvements that ensue from reductions in GCGR signalling in mice.[54,63] Genetic interruption[54,55,63,64] or pharmacological blockade of GLP-1 action[64] substantially attenuates the glucoregulatory benefits that arise from reducing glucagon action in experimental models of diabetes mellitus. Fasting increases the secretion of both glucagon and FGF-21; somewhat paradoxically, both activation and reduction of glucagon actions can trigger induction of FGF-21 expression.[55,65] Immunoneutralizion of FGF-21 impaired glucose tolerance in insulin-deficient *Gcgr*$^{-/-}$ mice[55] and resulted in deterioration of glucose control in insulin-deficient diabetic *Gcgr*$^{-/-}$ mice that were concomitantly treated with the GLP-1 receptor (GLP-1R) antagonist exendin (amino acids 9–39).[55] *Gcgr*$^{-/-}$ mice also exhibited marked increases in circulating levels of bile acids,[66] which might independently confer metabolic benefit through activation of nuclear and transmembrane receptors. Hence, reduction of GCGR signalling provides metabolic benefits beyond simple attenuation of glucagon-dependent increases in hepatic production of glucose.

Investigations of the efficacy and therapeutic potential of glucagon antagonism or reduction of GCGR expression are ongoing (Table 1). Treatment with a GCGR antisense oligonucleotide over 6 weeks reduced glucagon-stimulated hepatic production of glucose during a glucagon challenge test in healthy, non-obese male adults.[67] Additional studies of several small molecule GCGR antagonists performed over an increased time period have been completed (Table 1), the results of which have demonstrated robust glucose-lowering efficacies, with some GCGR antagonists producing reductions in HbA_{1c} levels comparable to, or greater than, metformin. The essential contribution of glucagon to the development of hyperglycaemia in experimental models of T1DM has also been demonstrated,[4,68] which has fostered clinical assessment of the efficacy of GCGR antagonists in the treatment of T1DM (Table 1). Importantly, the therapeutic efficacy of agents that reduce glucagon action might be offset by their potential liabilities.

Potential adverse effects

Germline disruption of *Gcgr* in mice leads to hyperglucagonaemia, which largely arises from hyperplastic α cells with dysregulated synthesis and secretion of proglucagon-derived peptides (including glucagon).[53] Even partial reduction of GCGR signalling, or antagonism of glucagon action, in adult rodents is sufficient to induce increased circulating levels of glucagon and GLP-1, as well as mild-to-moderate α-cell hyperplasia.[69–71] These findings are indicative of reduced hepatic GCGR signalling, as they are recapitulated by selective reduction or elimination of GCGR signalling in the liver.[72,73] Furthermore, restoration of hepatic GCGR expression in *Gcgr*$^{-/-}$ mice reversed the enhanced susceptibility to hepatic injury and considerably reduced plasma levels of glucagon.[74] The hyperplastic α cells that emerge in *Gcgr*$^{-/-}$ mice produce increased amounts of GLP-1 and do not express the molecular gene and protein signatures of fully differentiated α cells,[75] which suggests that these cells probably arise through a process that differs from that of the normal development of α cells. Whether primates respond to partial attenuation of GCGR signalling by increasing α-cell mass is not certain. However, individuals with *GCGR* mutations, including loss-of-function homozygous point mutations, demonstrated α-cell hyperplasia and hyperglucagonaemia, which suggests that the signals that link marked reduction of GCGR signalling to α-cell hyperplasia in humans are conserved.[76,77]

An increase in total pancreatic mass has been observed in rodents with interrupted GCGR signalling;[53,54,73] Additionally, individuals with an inactivating mutation in the *GCGR* gene can have an enlarged pancreas.[76] In rodents, enhanced GLP-1 action (an important contributor to metabolic benefits from reduced GCGR signalling[54]) does not induce α-cell hyperplasia,[78] but might promote a small increase in pancreatic[79] and intestinal[80] mass. Pancreatic enlargement secondary to administration of GLP-1 in rodents is due to increased protein synthesis in the exocrine pancreas; administration of a mammalian target of rapamycin (serine/threonine-protein kinase mTOR) protein synthesis inhibitor completely inhibited the GLP-1R-dependent increase in pancreatic mass in mice.[79] The relevance and importance of these findings for development of therapeutics that target the GCGR in humans requires additional study.

Table 2 | Physiological and pharmacological agents that reduce glucagon secretion

Biological agent	Drug	Direct action on α cells	Target(s) for glucagon regulation
Somatostatin	Octreotide	Yes	α cell
Amylin	Pramlintide	No	Central nervous system
GLP-1	GLP-1R agonists	No	β cell, δ cell
DPP4	DPP4 inhibitors	No	β cell, δ cell (via increases in levels of GLP-1)
Leptin	Metreleptin	Yes	Central nervous system
Insulin	Insulin	Yes	α cell

Abbreviations: DPP4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide 1; GLP-1R glucagon-like peptide 1 receptor.

Glucagon promotes hepatocyte survival, a reduction of hepatic lipid synthesis and an increase in fatty acid oxidation, as well as reduced circulating levels of cholesterol and triglycerides.[49,81] Disruption of GCGR signalling in mice resulted in impaired hepatic lipid oxidation, which predisposed these animals to hepatic steatosis and liver injury.[74,82] Although species-specific differences exist between humans and mice regarding how glucagon controls hepatic lipid metabolism, increased levels of circulating cholesterol and transaminase enzymes have been reported in as yet unpublished clinical trials of patients with diabetes mellitus treated with GCGR antagonists (Table 1). Elucidating the extent to which GCGR signalling can be safely inhibited in humans without incurring adverse effects requires careful clinical assessment.

Suppressing glucagon secretion

Several glucose-lowering therapies exert their actions in part through inhibition of glucagon secretion and subsequent reduction of hepatic glucose output (Table 2). Insulin robustly inhibits glucagon secretion in rodents and humans,[4] whereas α-cell-specific elimination of murine insulin receptors enhances glucagon secretion, which leads to impaired glucose tolerance and mild hyperglycaemia.[83] Amylin is a peptide hormone that is co-secreted with insulin from β cells.[84] Amylin and its associated analogues, such as pramlintide, also inhibit glucagon secretion, which contributes to reducing glycaemia in patients with T1DM and T2DM.[84] The glucagonostatic actions of amylin are probably indirect, as amylin does not inhibit glucagon secretion in isolated islets or in perfused rodent pancreas preparations.[85]

Incretin-based therapies (GLP-1R agonists and DPP4 inhibitors) reduce plasma levels of glucagon in patients with T2DM.[8] The mechanism by which GLP-1 inhibits glucagon secretion is controversial, as the majority of α cells in mice, primates and humans do not express the GLP-1R.[86,87] Both GLP-1R agonists and DPP4 inhibitors lowered glucose and glucagon levels in patients with T1DM who were negative for C peptide,[88,89] potentially via stimulation of somatostatin secretion. Co-infusion of exendin (amino acids 9–39) increased plasma levels of glucagon in the basal fasted state and prevented the glucagonostatic effects of the DPP4 inhibitor sitagliptin during a glucose tolerance test in patients with T2DM.[90] Hence, α cell glucagon secretion represents a physiologically essential component of endogenous GLP-1 action and a pharmacologically important target for the glucagoregulatory actions of incretin-based therapies.[8]

Enhancing glucagon and weight loss

Glucagon increases energy expenditure in both rodents and humans through induction of thermogenesis in brown adipose tissue.[46] Infusion of a low dose of glucagon, alone or in combination with GLP-1, increased the resting rate of energy expenditure, the respiratory exchange ratio, and carbohydrate and fat oxidation in healthy men.[91] The thermogenic effect of glucagon might be mediated through CNS control of sympathetic activity, as denervating brown adipose tissue or inhibiting β-adrengeric signalling considerably impairs the ability of glucagon to increase energy expenditure.[92]

The actions of glucagon to increase energy expenditure might be indirect, mediated in part through FGF-21. Whole-body inactivation of the *Gcg* gene in mice resulted in impaired thermogenesis, whereas administration of glucagon led to increased circulating levels of FGF-21 and improved thermogenic responses to adrenergic stimulation.[93] Glucagon rapidly increased levels of *Fgf21* mRNA transcripts and FGF-21 secretion from the liver and adipocytes in both normal and diabetic rodents and promoted lipolysis and increased circulating levels of FGF-21 in healthy individuals and patients with T1DM.[65,94] Furthermore, in *Fgf21*$^{-/-}$ mice, the actions of a selective GCGR agonist to increase energy expenditure and reduce body weight in obese diabetic mice were abolished.[65] Efforts to explore the possibility of utilizing glucagon to enhance satiety and increase energy expenditure to treat patients with obesity are ongoing.[50,95]

Co-agonism of GCGR and GLP-1R through simultaneous infusion of native glucagon and GLP-1 for 2 h reduced food intake and increased energy expenditure in healthy individuals.[91] Novel glucagon-containing co-agonists that mimic the anorectic and thermogenic actions of oxyntomodulin,[96] yet exhibit enhanced potency and optimized pharmacokinetic profiles *in vivo* have been developed.[97] A unique triagonist peptide that simultaneously activates the GIP receptor, GLP-1R and GCGR potently and rapidly induced weight loss and improved glucose tolerance in obese mice.[95] Studies of triagonist activity in *Gcgr*$^{-/-}$ mice implicated GCGR activity as crucial for increasing energy expenditure and for reduction of food intake, whereas the related incretin receptors mediated reduction of food intake (GLP-1R) and control of glycaemia (GLP-1R and GIP receptor). Nevertheless, GLP-1, glucagon and oxyntomodulin independently increase heart rate, although the mechanisms by which this occurs is incompletely understood.[98–100] In young normoglycaemic mice with ischaemic cardiac injury secondary to coronary artery ligation, unopposed glucagon agonism produced detrimental effects, such as increased infarct size and reduced survival.[101] In addition, specific reduction of GCGR signalling in cardiomyocytes resulted in a cardioprotective phenotype.[101] In rodents, GCGR agonists exert some of their favourable actions (such as enhanced energy expenditure), in part,

through induction of sympathetic nervous system activity.[8,102] Together, these findings suggest that the potential for using glucagon agonists to treat patients with obesity and diabetes mellitus requires careful scrutiny and exploration of dose-response relationships to minimize risk of adverse cardiovascular effects.

Conclusions

Considerable advances have been made in understanding the development, differentiation and plasticity of α cells. The majority of these discoveries were from young healthy rodents, which might not represent ideal models for adult patients with diabetes mellitus. Hence, additional studies using non-immortalized islets from these patients could be helpful in translating the findings from studies of α-cell function in rodents. New insights from embryonic and induced pluripotent stem cells provide new opportunities for studying the mechanisms that regulate functions of α cells.[103] Clinical trials to investigate the prophylactic use of glucagon alone, or together with insulin,[62] in patients with T1DM are encouraging and could provide opportunities for intensification of insulin action without further increasing rates of hypoglycaemia. The prospect of enhancing glucagon action in combination with one or more metabolically active peptide hormones for the treatment of diabetes mellitus and/or obesity is tantalizing; however, the potential adverse cardiovascular effects associated with these novel agents demands that their use be considered with additional scrutiny, especially in patients with pre-existing cardiovascular disease.

Reduction of glucagon action for the treatment of T2DM produces compelling improvements in glycaemic control in preclinical and human studies. However, when disrupted, the mechanism-based actions of glucagon can lead to increased plasma levels of cholesterol and transaminase enzymes, which suggests that the therapeutic window for reduction of GCGR signalling is narrow. Furthermore, the development of α-cell hyperplasia following reduction of glucagon action in preclinical studies implies that conducting studies to test the safety of sustained partial reduction of GCGR signalling to treat metabolic disorders in humans will be challenging. Finally, although GCGR is widely expressed in multiple extrahepatic tissues, including different regions of the CNS, our understanding of the contributions of direct and indirect actions of glucagon remains incomplete and will benefit from molecular dissection of glucagon action in distinct GCGR-positive cells. Taken together, the findings that GCGR signalling has a central role in the control of fuel homeostasis and body weight highlight the importance of understanding glucagon action for developing innovative therapies that improve the treatment of diabetes mellitus and obesity.

1. Cryer, P. E. Glycemic goals in diabetes: trade-off between glycemic control and iatrogenic hypoglycemia. *Diabetes* **63**, 2188–2195 (2014).
2. Baggio, L. L. & Drucker, D. J. Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132**, 2131–2157 (2007).
3. Pincus, I. J. & Rutman, J. Z. Glucagon, the hyperglycemic agent in pancreatic extracts; a possible factor in certain types of diabetes. *Arch. Intern. Med.* **92**, 666–677 (1953).
4. Unger, R. H. & Cherrington, A. D. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *J. Clin. Invest.* **122**, 4–12 (2012).
5. Cryer, P. E., Davis, S. N. & Shamoon, H. Hypoglycemia in diabetes. *Diabetes Care* **26**, 1902–1912 (2003).
6. Cryer, P. E. Mechanisms of hypoglycemia-associated autonomic failure in diabetes. *N. Engl. J. Med.* **369**, 362–372 (2013).
7. Gromada, J., Franklin, I. & Wollheim, C. B. α-cells of the endocrine pancreas: 35 years of research but the enigma remains. *Endocr. Rev.* **28**, 84–116 (2007).
8. Campbell, J. E. & Drucker, D. J. Pharmacology physiology and mechanisms of incretin hormone action. *Cell Metab.* **17**, 819–837 (2013).
9. Yue, J. T. *et al.* Amelioration of hypoglycemia via somatostatin receptor type 2 antagonism in recurrently hypoglycemic diabetic rats. *Diabetes* **62**, 2215–2222 (2013).
10. Zhang, Q. *et al.* Role of K_{ATP} channels in glucose-regulated glucagon secretion and impaired counterregulation in type 2 diabetes. *Cell Metab.* **18**, 871–882 (2013).
11. Lamy, C. M. *et al.* Hypoglycemia-activated GLUT2 neurons of the nucleus tractus solitarius stimulate vagal activity and glucagon secretion. *Cell Metab.* **19**, 527–538 (2014).
12. Plamboeck, A. *et al.* The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304**, G1117–G1127 (2013).
13. Wewer Albrechtsen, N. J. *et al.* Hyperglucagonaemia analysed by glucagon sandwich ELISA: nonspecific interference or truly elevated levels? *Diabetologia* **57**, 1919–1926 (2014).
14. Molina, J. *et al.* Control of insulin secretion by cholinergic signaling in the human pancreatic islet. *Diabetes* **63**, 2714–2726 (2014).
15. Rodriguez-Diaz, R. *et al.* α cells secrete acetylcholine as a non-neuronal paracrine signal priming beta cell function in humans. *Nat. Med.* **17**, 888–892 (2011).
16. Unger, R. H. Glucagon physiology and pathophysiology. *N. Engl. J. Med.* **285**, 443–449 (1971).
17. Gutniak, M., Grill, V. & Efendic, S. Effect of composition of mixed meals—low- versus high-carbohydrate content—on insulin, glucagon, and somatostatin release in healthy humans and in patients with NIDDM. *Diabetes Care* **9**, 244–249 (1986).
18. Le Marchand, S. J. & Piston, D. W. Glucose suppression of glucagon secretion: metabolic and calcium responses from α-cells in intact mouse pancreatic islets. *J. Biol. Chem.* **285**, 14389–14398 (2010).
19. Bak, M. J. *et al.* Specificity and sensitivity of commercially available assays for glucagon and oxyntomodulin measurement in humans. *Eur. J. Endocrinol.* **170**, 529–538 (2014).
20. Shah, P. *et al.* Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* **85**, 4053–4059 (2000).
21. Kramer, C. K., Borgono, C. A., Van Nostrand, P., Retnakaran, R. & Zinman, B. Glucagon response to oral glucose challenge in type 1 diabetes: lack of impact of euglycemia. *Diabetes Care* **37**, 1076–1082 (2014).
22. Chia, C. W. *et al.* Exogenous glucose-dependent insulinotropic polypeptide worsens post prandial hyperglycemia in type 2 diabetes. *Diabetes* **58**, 1342–1349 (2009).
23. Christensen, M. B., Calanna, S., Holst, J. J., Vilsboll, T. & Knop, F. K. Glucose-dependent insulinotropic polypeptide: blood glucose stabilizing effects in patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **99**, E418–E426 (2014).
24. Fujita, Y. *et al.* Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet α-cells and promotes insulin secretion. *Gastroenterology* **138**, 1966–1975 (2010).
25. Bramswig, N. C. *et al.* Epigenomic plasticity enables human pancreatic α- to β cell reprogramming. *J. Clin. Invest.* **123**, 1275–1284 (2013).
26. Nie, Y. *et al.* Regulation of pancreatic PC1 and PC2 associated with increased glucagon-like peptide 1 in diabetic rats. *J. Clin. Invest.* **105**, 955–965 (2000).
27. Marchetti, P. *et al.* A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets. *Diabetologia* **55**, 3262–3272 (2012).
28. Ellingsgaard, H. *et al.* Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and α cells. *Nat. Med.* **17**, 1481–1489 (2011).
29. Nauck, M. A., Vardarli, I., Deacon, C. F., Holst, J. J. & Meier, J. J. Secretion of glucagon-like peptide-1 (GLP-1) in type 2 diabetes: what is up, what is down? *Diabetologia* **54**, 10–18 (2010).
30. Faerch, K. *et al.* Glucagon-like peptide-1 (GLP-1) response to oral glucose is reduced in pre-diabetes, screen-detected tpe 2 diabetes and obesity, and influenced by sex: The ADDITION-PRO Study. *Diabetes* http://dx.doi.org/10.2337/db14-1751.
31. Collombat, P. *et al.* The ectopic expression of *Pax4* in the mouse pancreas converts progenitor cells into α and subsequently β cells. *Cell* **138**, 449–462 (2009).

32. Sosa-Pineda, B., Chowdhury, K., Torres, M., Oliver, G. & Gruss, P. The *Pax4* gene is essential for differentiation of insulin-producing β cells in the mammalian pancreas. *Nature* **386**, 399–402 (1997).
33. Thorel, F. *et al.* Conversion of adult pancreatic α-cells to β-cells after extreme β-cell loss. *Nature* **464**, 1149–1154 (2010).
34. Thorel, F. *et al.* Normal glucagon signaling and β-cell function after near-total α-cell ablation in adult mice. *Diabetes* **60**, 2872–2882 (2011).
35. Brereton, M. F. *et al.* Reversible changes in pancreatic islet structure and function produced by elevated blood glucose. *Nat. Commun.* **5**, 4639 (2014).
36. Ferrannini, E. *et al.* Metabolic response to sodium-glucose cotransporter 2 inhibition in type 2 diabetic patients. *J. Clin. Invest.* **124**, 499–508 (2014).
37. Merovci, A. *et al.* Dapagliflozin improves muscle insulin sensitivity but enhances endogenous glucose production. *J. Clin. Invest.* **124**, 509–514 (2014).
38. Bonner, C. *et al*. Inhibition of the glucose transporter SGLT2 with dapagliflozin in pancreatic α cells triggers glucagon secretion. *Nat. Med.* http://dx.doi.org/10.1038/nm.3828 (2015).
39. Jorgensen, N. B. *et al.* Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with type 2 diabetes and normal glucose tolerance. *Am. J. Physiol. Endocrinol. Metab.* **303**, E122–E131 (2012).
40. Meier, J. J. *et al.* Glucagon-like peptide 2 stimulates glucagon secretion, enhances lipid absorption, and inhibits gastric acid secretion in humans. *Gastroenterology* **130**, 44–54 (2006).
41. Bahrami, J., Longuet, C., Baggio, L. L., Li, K. & Drucker, D. J. The glucagon-like peptide-2 receptor modulates islet adaptation to metabolic stress in the ob/ob mouse. *Gastroenterology* **139**, 857–868 (2010).
42. Drucker, D. J. & Yusta, B. Physiology and pharmacology of the enteroendocrine hormone glucagon-like peptide-2. *Annu. Rev. Physiol.* **76**, 561–583 (2014).
43. Drucker, D. J. & Asa, S. Glucagon gene expression in vertebrate brain. *J. Biol. Chem.* **263**, 13475–13478 (1988).
44. Campos, R. V., Lee, Y. C. & Drucker, D. J. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* **134**, 2156–2164 (1994).
45. Jin, S.-L. C. *et al.* Distribution of glucagonlike peptide 1 (GLP-1), glucagon, and glicentin in the rat brain: an immunocytochemical study. *J. Comp. Neurol.* **271**, 519–532 (1988).
46. Habegger, K. M. *et al.* The metabolic actions of glucagon revisited. *Nat. Rev. Endocrinol.* **6**, 689–697 (2010).
47. Tschop, M. H. & DiMarchi, R. D. Outstanding Scientific Achievement Award Lecture 2011: defeating diabesity: the case for personalized combinatorial therapies. *Diabetes* **61**, 1309–1314 (2012).
48. Mighiu, P. I. *et al.* Hypothalamic glucagon signaling inhibits hepatic glucose production. *Nat. Med.* **19**, 766–772 (2013).
49. Ali, S. & Drucker, D. J. Benefits and limitations of reducing glucagon action for the treatment of type 2 diabetes. *Am. J. Physiol. Endocrinol. Metab.* **296**, E415–E421 (2009).
50. Tan, T. M. *et al.* Coadministration of glucagon-like peptide-1 during glucagon infusion in man results in increased energy expenditure and amelioration of hyperglycemia. *Diabetes* **62**, 1131–1138 (2013).
51. Lee, Y., Wang, M. Y., Du, X. Q., Charron, M. J. & Unger, R. H. Glucagon receptor knockout prevents insulin-deficient type 1 diabetes in mice. *Diabetes* **60**, 391–397 (2011).
52. Lee, Y. *et al.* Metabolic manifestations of insulin deficiency do not occur without glucagon action. *Proc. Natl Acad. Sci. USA* **109**, 14972–14976 (2012).
53. Gelling, R. W. *et al.* Lower blood glucose, hyperglucagonemia, and pancreatic α cell hyperplasia in glucagon receptor knockout mice. *Proc. Natl Acad. Sci. USA* **100**, 1438–1443 (2003).
54. Ali, S., Lamont, B. J., Charron, M. J. & Drucker, D. J. Dual elimination of the glucagon and GLP-1 receptors in mice reveals plasticity in the incretin axis. *J. Clin. Invest.* **121**, 1917–1929 (2011).
55. Omar, B. A. *et al.* Fibroblast growth factor 21 (FGF21) and glucagon-like peptide 1 contribute to diabetes resistance in glucagon receptor-deficient mice. *Diabetes* **63**, 101–110 (2014).
56. Dobbs, R. *et al.* Glucagon: role in the hyperglycemia of diabetes mellitus. *Science* **187**, 544–547 (1975).
57. Gerich, J. E. *et al.* Prevention of human diabetic ketoacidosis by somatostatin. Evidence for an essential role of glucagon. *N. Engl. J. Med.* **292**, 985–989 (1975).
58. Fujikawa, T., Chuang, J. C., Sakata, I., Ramadori, G. & Coppari, R. Leptin therapy improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice. *Proc. Natl Acad. Sci. USA* **107**, 17391–17396 (2010).
59. Perry, R. J. *et al.* Leptin reverses diabetes by suppression of the hypothalamic–pituitary–adrenal axis. *Nat. Med.* **20**, 759–763 (2014).
60. Nathan, D. M. *et al.* The diabetes control and complications trial/epidemiology of diabetes interventions and complications study at 30 years: overview. *Diabetes Care* **37**, 9–16 (2014).
61. El-Khatib, F. H., Russell, S. J., Nathan, D. M., Sutherlin, R. G. & Damiano, E. R. A bihormonal closed-loop artificial pancreas for type 1 diabetes. *Sci. Transl. Med.* **2**, 27ra27 (2010).
62. Russell, S. J. *et al.* Outpatient glycemic control with a bionic pancreas in type 1 diabetes. *N. Engl. J. Med.* **371**, 313–325 (2014).
63. Jun, L. S. *et al.* Absence of glucagon and insulin action reveals a role for the glucagon-like peptide-1 receptor in endogenous glucose production. *Diabetes* **64**, 819–827 (2014).
64. Gu, W. *et al.* Glucagon receptor antagonist-mediated improvements in glycemic control are dependent on functional pancreatic GLP-1 receptor. *Am. J. Physiol. Endocrinol. Metab.* **299**, E624–E632 (2010).
65. Habegger, K. M. *et al.* Fibroblast growth factor 21 mediates specific glucagon actions. *Diabetes* **62**, 1453–1463 (2013).
66. Yang, J. *et al.* Polyomic profiling reveals significant hepatic metabolic alterations in glucagon-receptor (GCGR) knockout mice: implications on anti-glucagon therapies for diabetes. *BMC Genomics* **12**, 281 (2011).
67. van Dongen, M. G. *et al.* First proof of pharmacology in humans of a novel glucagon receptor antisense drug. *J. Clin. Pharmacol.* **55**, 298–306 (2015).
68. Wang, M. Y. *et al.* Glucagon receptor antibody completely suppresses type 1 diabetes phenotype without insulin by disrupting a novel diabetogenic pathway. *Proc. Natl Acad. Sci. USA* **112**, 2503–2508 (2015).
69. Sloop, K. W. *et al.* Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors. *J. Clin. Invest.* **113**, 1571–1581 (2004).
70. Liang, Y. *et al.* Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in db/db mice. *Diabetes* **53**, 410–417 (2004).
71. Winzell, M. S. *et al.* Glucagon receptor antagonism improves islet function in mice with insulin resistance induced by a high-fat diet. *Diabetologia* **50**, 1453–1462 (2007).
72. Chen, M. *et al.* Absence of the glucagon-like peptide-1 receptor does not affect the metabolic phenotype of mice with liver-specific $G_s\alpha$ deficiency. *Endocrinology* **152**, 3343–3350 (2011).
73. Longuet, C. *et al.* Liver-specific disruption of the murine glucagon receptor produces α-cell hyperplasia: evidence for a circulating α-cell growth factor. *Diabetes* **62**, 1196–1205 (2013).
74. Sinclair, E. M. *et al.* Glucagon receptor signaling is essential for control of murine hepatocyte survival. *Gastroenterology* **135**, 2096–2106 (2008).
75. Vuguin, P. M. *et al.* Ablation of the glucagon receptor gene increases fetal lethality and produces alterations in islet development and maturation. *Endocrinology* **147**, 3995–4006 (2006).
76. Zhou, C., Dhall, D., Nissen, N. N., Chen, C. R. & Yu, R. A homozygous P86S mutation of the human glucagon receptor is associated with hyperglucagonemia, α cell hyperplasia, and islet cell tumor. *Pancreas* **38**, 941–946 (2009).
77. Sipos, B. *et al.* Glucagon cell hyperplasia and neoplasia with and without glucagon receptor mutations. *J. Clin. Endocrinol. Metab.* http://dx.doi.org/10.1210/jc.2014-4405.
78. Drucker, D. J. Incretin action in the pancreas: potential promise, possible perils, and pathological pitfalls. *Diabetes* **62**, 3316–3323 (2013).
79. Koehler, J. A. *et al.* Glucagon-like peptide-1 receptor agonists increase pancreatic mass by induction of protein synthesis. *Diabetes* **64**, 1046–1056 (2015).
80. Koehler, J. A. *et al.* GLP-1R agonists promote normal and neoplastic intestinal growth through mechanisms requiring Fgf7. *Cell Metab.* **21**, 379–391 (2015).
81. Xiao, C., Pavlic, M., Szeto, L., Patterson, B. W. & Lewis, G. F. Effects of acute hyperglucagonemia on hepatic and intestinal lipoprotein production and clearance in healthy humans. *Diabetes* **60**, 383–90 (2011).
82. Longuet, C. *et al.* The glucagon receptor is required for the adaptive metabolic response to fasting. *Cell Metab.* **8**, 359–371 (2008).
83. Kawamori, D. *et al.* Insulin signaling in α cells modulates glucagon secretion *in vivo*. *Cell Metab.* **9**, 350–361 (2009).
84. Riddle, M. C. & Drucker, D. J. Emerging therapies mimicking the effects of amylin and glucagon-like peptide 1. *Diabetes Care* **29**, 435–449 (2006).
85. Silvestre, R. A. *et al.* Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas. *Am. J. Physiol. Endocrinol. Metab.* **280**, E443–E449 (2001).
86. Richards, P. *et al.* Identification and characterisation of glucagon-like peptide-1 receptor expressing cells using a new transgenic mouse model. *Diabetes* **63**, 1224–1233 (2014).
87. Pyke, C. *et al.* GLP-1 receptor localization in monkey and human tissue: Novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* **155**, 1280–1290 (2014).
88. Dupre, J., Behme, M. T. & McDonald, T. J. Exendin-4 normalized postcibal glycemic

excursions in type 1 diabetes. *J. Clin. Endocrinol. Metab.* **89**, 3469–3473 (2004).
89. Farngren, J., Persson, M., Schweizer, A., Foley, J. E. & Ahren, B. Vildagliptin reduces glucagon during hyperglycemia and sustains glucagon counterregulation during hypoglycemia in type 1 diabetes. *J. Clin. Endocrinol. Metab.* **97**, 3799–3806 (2012).
90. Aulinger, B. A. *et al.* Defining the role of GLP-1 in the enteroinsulinar axis in type 2 diabetes using DPP-4 inhibition and GLP-1 receptor blockade. *Diabetes* **63**, 1079–1092 (2014).
91. Cegla, J. *et al.* Coinfusion of low-dose GLP-1 and glucagon in man results in a reduction in food intake. *Diabetes* **63**, 3711–3720 (2014).
92. Billington, C. J., Bartness, T. J., Briggs, J., Levine, A. S. & Morley, J. E. Glucagon stimulation of brown adipose tissue growth and thermogenesis. *Am. J. Physiol.* **252**, R160–R165 (1987).
93. Kinoshita, K. *et al.* Glucagon is essential for adaptive thermogenesis in brown adipose tissue. *Endocrinology* **155**, 3484–3492 (2014).
94. Arafat, A. M. *et al.* Glucagon increases circulating fibroblast growth factor 21 independently of endogenous insulin levels: a novel mechanism of glucagon-stimulated lipolysis? *Diabetologia* **56**, 588–597 (2013).
95. Finan, B. *et al.* A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nat. Med.* **21**, 27–36 (2015).
96. Pocai, A. Action and therapeutic potential of oxyntomodulin. *Mol. Metab.* **14**, 241–251 (2013).
97. Day, J. W. *et al.* A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat. Chem. Biol.* **5**, 749–757 (2009).
98. Mukharji, A., Drucker, D. J., Charron, M. J. & Swoap, S. J. Oxyntomodulin increases intrinsic heart rate through the glucagon receptor. *Physiol. Rep.* **1**, e00112 (2013).
99. Ussher, J. R. & Drucker, D. J. Cardiovascular actions of incretin-based therapies. *Circ. Res.* **114**, 1788–1803 (2014).
100. Ussher, J. R. *et al.* Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. *Mol. Metab.* **3**, 507–517 (2014).
101. Ali, S. *et al.* Cardiomyocyte glucagon receptor signaling modulates outcomes in mice with experimental myocardial infarction. *Mol. Metab.* **4**, 132–143 (2014).
102. Lockie, S. H. *et al.* Direct control of brown adipose tissue thermogenesis by central nervous system glucagon-like peptide-1 receptor signaling. *Diabetes* **61**, 2753–2762 (2012).
103. Rezania, A. *et al.* Production of functional glucagon-secreting α-cells from human embryonic stem cells. *Diabetes* **60**, 239–247 (2011).
104. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01640834?term=NCT01640834&rank=1 (2012).
105. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT00631488?term=NCT00631488&rank=1 (2015).
106. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02111096?term=NCT02111096&rank=1 (2015).
107. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02175121?term=NCT02175121&rank=1 (2015).
108. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01843127?term=NCT01843127&rank=1 (2014).
109. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT02250222?term=NCT02250222&rank=1 (2014).
110. US National Library of Medicine. *ClinicalTrials.gov* [online], https://clinicaltrials.gov/ct2/show/NCT01885260?term=NCT01885260&rank=1 (2014).

Author contributions

J.E.C. and D.J.D. researched data for the article, provided substantial contributions to discussions of the content, contributed equally to writing the article, and to reviewing and/or editing of the manuscript before submission.

ERRATUM

Islet α cells and glucagon—critical regulators of energy homeostasis

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